Antineoplastic drugs in veterinary oncology: excretion in dogs, contamination of the environment and exposure assessment of people at risk

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Antineoplastic drugs in veterinary oncology: excretion in dogs, contamination of the environment and exposure assessment of people at risk

Chemotherapeutica in de veterinaire oncologie: excretie door honden, besmetting van de omgeving en evaluatie van de blootstelling bij mensen die een contactrisico lopen

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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door

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Voor Erik (1969 – 2010)

i carry your heart(i carry it in my heart) *~ E. E. Cummings – 95 Poems, 1958*

Toujours et toujours.

Contents

Preface

Preface

The use of chemotherapy to treat cancer in a veterinary patient was reported for the first time in 1946.[1] By the late 1970s, use of antineoplastic drugs became accepted in veterinary oncology. Since then, veterinary medical oncology has been advancing at an ever-increasing rate. However, antineoplastic drugs have been associated with several adverse health effects in human patients.[2] By now, numerous studies have reported surface contamination with anticancer drugs in human oncology centers and uptake of these compounds in exposed personnel. This is important since increased genotoxic responses have been associated with occupational exposure.[3,4] Moreover, carcinogenic and teratogenic effects, and adverse reproductive outcomes have been reported in personnel occupationally exposed to anticancer drugs in human oncology. In response, guidelines for safe handling of antineoplastic drugs have been established, implemented and improved in human and veterinary oncology. The veterinary guidelines are largely based on knowledge transferred from human oncology. Yet, veterinary oncology differs from human oncology in several ways. The use of chemotherapy in veterinary medicine is not as widespread as in human oncology. Moreover, the drug doses are, in general, much lower compared to human medicine. On the other hand, pet-animals may not always comply with rigorous guidelines. Consequently, it is of interest to evaluate the efficacy of the current veterinary safe handling guidelines. This can be achieved by monitoring potential sources of exposure to anticancer drugs, such as excretion products and environmental contamination, by determining whether uptake of these compounds occurs in people at risk of exposure and by evaluating the biological effects of exposure.

Studies assessing occupational exposure to antineoplastic drugs have focused almost exclusively on personnel in human oncology. Thus, little is known about occupational exposure to anticancer drugs in veterinary oncology. This thesis attempts to address this gap in current knowledge. In **chapter 1.1**, we review the monitoring of occupational exposure to antineoplastic drugs, focusing on veterinary oncology. In **chapter 1.2**, we describe potential harmful effects associated with antineoplastic drugs and we discuss the risk assessment of long-term low dose exposure to these compounds.

Chapter 2 describes the development and validation of inductively coupled plasma-mass spectrometry assays. **Chapter 2.1** focuses on the determination of platinum originating from carboplatin in human urine and canine urine, feces and saliva, while **chapter 2.2** describes the assays for the detection of platinum originating from carboplatin in canine sebum and cerumen.

Chapter 3 focuses on the monitoring of potential sources of exposure to platinum originating from carboplatin, in veterinary oncology. In **chapter 3.1**, we describe the excretion of platinum in urine, feces, saliva, sebum and cerumen of pet-dogs treated with carboplatin. **Chapter 3.2** presents the results of environmental monitoring of platinum in

veterinary and human oncology centers. In **chapter 3.3**, we discuss the surface contamination with platinum in homes of veterinary and human oncology patients. **Chapter 3.4** outlines the monitoring of platinum excretion in pet-dogs and the evaluation of platinum surface contamination, when a sustained drug delivery formulation of carboplatin is administered to pet-dogs.

As it turned out that platinum could be monitored in excretion products of pet-dogs and in surface samples collected in veterinary oncology centers and homes of veterinary cancer patients, the next step was to investigate potential sources of exposure when other antineoplastic drugs were administered to pet-dogs. Platinum was detected at levels markedly lower than most assays can achieve. Therefore, we chose to develop and use high performance liquid chromatography-mass spectrometric methods for the determination of doxorubicin and its metabolite doxorubicinol, and epirubicin and its metabolite epirubicinol, at as sensitive levels as could be accomplished. **Chapter 4** presents the results of the monitoring of doxorubicin, epirubicin, and their C-13 metabolites in canine excretion products, and environmental samples collected in veterinary oncology centers and homes of treated pet-dogs.

Because environmental contamination with platinum, doxorubicin, epirubicin, and their C-13 metabolites was detected in veterinary oncology centers and homes of treated pet-dogs, we have evaluated the uptake of these compounds by monitoring their presence in urine samples of veterinary personnel and owners (**Chapter 5**). Doxorubicin, doxorubicinol, epirubicin and epirubicinol have cytotoxic and cardiotoxic potency. To evaluate the biological reactivity of the platinum species present in urine of pet-dogs treated with carboplatin, the *ex vivo* DNA-binding potency of the platinum was assessed.

Exposure to antineoplastic drugs is an inevitable risk for veterinary oncology personnel and owners of treated pet-dogs. We think that the knowledge obtained from studies monitoring exposure in this field, can be used to adapt the guidelines on safe handling of antineoplastic drugs, treated animals and related wastes to the reality and needs of veterinary medical oncology. We hope that the studies in this thesis contribute to the further diminishing of exposure to antineoplastic drugs, in all populations at risk.

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Chapter 1

Introduction

Chapter 1.1

Antineoplastic drugs in veterinary oncology - Part 1:

Occupational exposure

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Submitted for publication

Abstract

Since the late 1970s the use of chemotherapy has been accepted in veterinary medicine as a genuine treatment of cancer. It has been gaining popularity the past few decades, leading, however, also to an increasing number of people at risk of exposure to these antineoplastic drugs. It is well established that there are risks associated with the preparation and administration of, and treatment with, antineoplastic drugs. However, it is still difficult to demonstrate a clear and straightforward relationship between low dose exposure to these drugs and harmful effects. In the first part of this review we discuss the environmental and biological monitoring of exposure to antineoplastic drugs, focusing on veterinary medicine. In the second part we will review the risk assessments of long-term low dose exposure.

Introduction

The era of modern cancer chemotherapy started in the 1940s with the first *recorded* treatment of human cancer with a nitrogen mustard compound.[1] The treatment only resulted in temporary regressions, but it led to the development of more selective and effective agents that revolutionized cancer treatment.

Veterinary oncology has closely followed the advances in human oncology. The first veterinary cancer patient treated with chemotherapy was reported in 1946.[1] However, it took until the late 1970s before the use of chemotherapy in veterinary oncology became accepted. Nowadays, chemotherapy has become the treatment of choice in the management of systemic hematopoietic tumors in dogs and cats.[2] At first, the knowledge in veterinary oncology was based on transference from the human literature or retrospective studies.[3,4] However, prospective clinical trials, applied to companion animal populations, testing new antineoplastic compounds or attempting to optimize the dose and dose intervals of chemotherapeutic drugs already in use, are nowadays becoming standard in veterinary oncology.[4-7] Concurrent with the advancement of veterinary oncology, companion animals are increasingly regarded as full members of the family, thus leading to the tendency of owners to ask for the newest and most promising treatments for their pets.[8] As cancer is one of the five main causes of mortality in companion animals, cancer treatment has become increasingly important.[8,9]

Despite their intertwined history, veterinary and human oncology differs in several ways. The objectives of chemotherapy in veterinary practice are: induction of remission of sensitive tumors, eradication of occult micro metastases and palliative therapy for metastatic cancer.[10,11] In contrast to human oncology, veterinarians and their clients are generally less willing to accept a high degree of side-effects, resulting in lower doses of the same drugs than those used in human oncology.[12] Even though the use of cancer chemotherapy in veterinary medicine is not as widespread as in human oncology and even though the drug doses are much lower compared to human medicine, concern has been raised about the excretion and spread of antineoplastic drugs by treated pets. This concern regards the handling of the treated pet at home by the owners. Clearly, pet-animals may potentially lack to comply with rigorous safety guidelines. There are dozens of studies showing the uptake of antineoplastic drugs by healthcare workers such as nurses and pharmacy technicians.[13-15] Uptake of these drugs by veterinary personnel and owners of treated pets is therefore plausible. However, few studies so far have focused upon occupational exposure of veterinarians and assisting personnel to anticancer drugs.[16-22] To our knowledge, so far only our research group has investigated the exposure of owners and other care-takers in close contact with companion animals receiving chemotherapy.[23]

This first part of the review will be exploring the main body of literature on occupational exposure to antineoplastic drugs most commonly used in veterinary oncology. First, we will

review the various sources of exposure in veterinary oncology. Second, we will examine the assessment of occupational exposure to antineoplastic drugs reported in the literature.

Sources of exposure in veterinary oncology

There are several sources of exposure to antineoplastic drugs in veterinary medicine, with different subpopulations at risk (see Figure 1). These sources are: preparation of antineoplastic drugs, handling of antineoplastic drugs and its wastes, and handling of treated animals and their excreta.

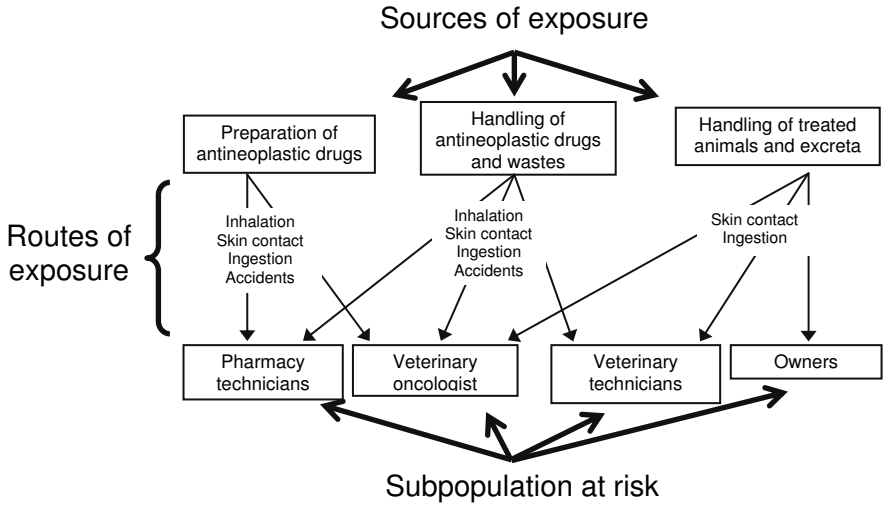

Figure 1 Schematic representation of exposure to antineoplastic drugs in veterinary oncology: sources of exposure, routes of exposure and subpopulations at risk

Preparation of antineoplastic drugs

Exposure may occur when touching drug-contaminated vials, work surfaces, floors and final drug products. Preparation of the antineoplastic drug by reconstituting lyophilized, powdered or concentrated liquid drugs, expelling air from syringes that contain these drugs, crushing tablets to make oral liquid doses or counting out individual uncoated tablets can result in exposure as well. Furthermore, handling of contaminated wastes may also lead to exposure. Routes of exposure are: inhalation (of vapors, droplets or particles), skin contact (when touching contaminated surfaces or direct contact with spilled drugs), ingestion (from hand-to-mouth contact) and accidental injections.[24-27] The subpopulations at risk are the pharmacy technicians, the veterinary oncologists (if they prepare the drugs) and veterinary technicians (e.g. when disposing of wastes).

Table 1 Antineoplastic drugs commonly used in dogs in the Netherlands and Flanders

	Main indications	Dosage	Regimen
CBL	Low grade lymphoma Metronomic chemotherapy	0.1 – 0.2 mg/kg PO s.i.d. or 20 mg/m² PO or 4 mg/m² PO s.i.d.	SA therapy: lifelong low dose s.i.d. or pulse therapy every 2 weeks
CP	Lymphoma Hemangiosarcoma	125 mg/m² PO s.i.d. for 2 days or 250 mg/m² PO/IV	CHOP or COP protocols VAC protocol
CCNU	T cell lymphoma or rescue lymphoma protocol Mast cell tumor Histiocytic carcinoma Brain tumors	60 – 90 mg/m² PO	SA therapy: every 3 weeks (until cumulative dose: 1000 mg/m²) Combination therapy: with vinblastine every 5 weeks (until cumulative dose: 1000 mg/m²)
Doxo	Lymphoma Osteosarcoma Mammacarcinoma Soft tissue sarcoma Hemangiosarcoma	30 mg/m² IV Dogs < 15 kg: 1 mg/kg IV	CHOP protocols SA therapy: every 3 weeks (6x) Combination therapy with carboplatin: every 6 weeks (3-6x)
Epi	Lymphoma Osteosarcoma Mammacarcinoma Soft tissue sarcoma Hemangiosarcoma	30 mg m² IV Dogs < 15 kg: 1 mg/kg IV	CHOP protocols SA therapy: every 3 weeks (6x) Combination therapy with carboplatin: every 6 weeks (3-6x)
MTN	Transitional cell carcinoma	5-6 mg/m² IV	SA therapy: every 3 weeks (6x)
VBL	Mast cell tumor	2 – 4 mg/m² IV	SA therapy: every week for 8 weeks + every 2 weeks for 8 weeks, or every week for 12 weeks Combination therapy: with CCNU every 5 weeks (minimally 6x)
VCR	Lymphoma Transmissible venereal tumor	0.5 – 0.7 mg/m² IV	CHOP or COP protocols SA therapy: every week (2-8x)
CaPt	Osteosarcoma Several carcinomas	300 mg/m² IV Dogs < 15 kg: 10 mg/kg	SA therapy: every 3 weeks (6-12x) Combination therapy with doxorubicin/epirubicin: every 6 weeks (3x)

CaPt: carboplatin; CBL: chlorambucil; CCNU: lomustine; CP: cyclophosphamide; Doxo: doxorubicin; Epi: epirubicin; IV: intravenous; MTN: mitoxantrone; PO: per os; SA: single (antineoplastic) agent; VBL: vinblastine; VCR: vincristine

Handling of antineoplastic drugs and its wastes

Priming of the intravenous (IV) set with a drug-containing solution or administration of the antineoplastic drug can result in exposure. This may also occur when handling drug-contaminated wastes, cleaning of the drug preparation or administration areas or disposing of personal protective equipment. Routes of exposure are: inhalation, skin contact, ingestion and accidental injections.[24-27] The veterinary oncologists and the veterinary technicians are the subpopulations at risk.

Handling of treated animals and its excreta

Handling of the animals can result in skin contact with sebum and saliva. During care-taking of the pet-animal, the disposal of the animal's excreta (e.g. urine, feces, vomit) might

be necessary. Contact with these excretion products may result in exposure of care-takers to antineoplastic drugs or the metabolites of these compounds. Whether sebum, saliva, urine, or feces contain antineoplastic drugs depends on the pharmacokinetics of the administrated drug. Each drug has its own unique disposition, metabolism, elimination, and excretion profile. We have, therefore, described the pharmacokinetics of antineoplastic drugs commonly used in veterinary medicine below. Routes of exposure are mainly skin contact and ingestion.[24-27] The subpopulations at risk are the veterinary oncologists and veterinary technicians. However, after administration the animal is usually sent home with its owners. This reduces the risk of exposure of veterinary personnel, but creates a whole new care-taking subpopulation at risk: the owners and other care-takers.

Table 2 Antineoplastic drugs commonly used in cats in the Netherlands and Flanders

	Main indications	Dosage	Regimen
CBL	Low grade lymphoma Metronomic chemotherapy	0.1 – 0.2 mg/kg PO s.i.d.	SA therapy: lifelong
CP	Lymphoma	250 mg/m² PO	Modified COP protocol: every 3 weeks (16x), on the day after vincristine
Doxo	Lymphoma Mammacarcinoma Fibrosarcoma Hemangiosarcoma	22.5 mg/m² or 1 mg/kg IV	SA therapy: every 3 weeks (6x) Combination therapy with carboplatin: every 7 weeks (3x)
Epi	Lymphoma Mammacarcinoma Fibrosarcoma Hemangiosarcoma	22.5 mg/m² or 1 mg/kg IV	SA therapy: every 3 weeks (6x) Combination therapy with carboplatin: every 7 weeks (3x)
VCR	Lymphoma	0.5 – 0.7 mg/m² IV	Modified COP protocol: on week 1, 2, 3 and 4. After induction: every 3 weeks (16x)
CaPt	Mammacarcinoma	210 mg/m² IV	SA therapy: every 4 weeks (6x) Combination therapy with doxorubicin/epirubicin: every 7 weeks (3x)

CaPt: carboplatin; CBL: chlorambucil; CP: cyclophosphamide; Doxo: doxorubicin; Epi: epirubicin; IV: intravenous; MTN: mitoxantrone; PO: per os; SA: single (antineoplastic) agent; VCR: vincristine

Pharmacokinetics of antineoplastic drugs

We have focused our review on the antineoplastic agents commonly used in veterinary practice (see Table 1 and 2). Table 3 summarizes the pharmacokinetic properties of these compounds. One relevant study per antineoplastic drug and per species is provided. We have focused on research describing excretion routes, namely via urine and feces, whenever possible.

Table 3 Pharmacokinetic parameters of the antineoplastic drugs commonly used in veterinary oncology

		Dose	Last PK time point (analyte)	V_d	C_{max}	CL_T	$t_{1/2\,el.}$ (h)	Billiary excretion	Urinary excretion	Ref
CBL	Mice	10 mg/kg IV	4 h (CBL)	0.180 ± 7.1x10^{-3} L/kg	-	0.591 ± 3.5x10^{-3} L/(h kg)	0.3 ± 0.02	-	-	28
	Rats	40 mg/kg SC	16 h ([^{3}H] CBL)	-	0.040 μmoles/mL	-	2.4	-	-	29
	Dogs	?	?	?	?	?	?	?	?	
	Cats	?	?	?	?	?	?	?	?	
	Men	30 mg PO	24 h (CBL)	-	0.508 ± 0.205 μg/mL	0.43 ± 0.18 L/(h kg)	1.2 ±0.5	-	-	30
CP	Mice	100 mg/kg IP	4 h (CP)	-	41.1 ± 1.8 μg/mL	0.12 ± 4.8x10^{-3} L/h	0.28 ± 0.02	-	-	31
	Rats	-	6 h (CP)	1.06 ± 0.0318 L/kg	C_0= 94.2 ± 2.78 μg/mL	0.416 ± 0.031 L/(h kg)	1.78 ± 0.086	-	-	32
	Dogs	250 mg/m^2 PO	24 h (CP)	-	1.16 ± 1.80 μg/mL	327.7 ± 206.9 L/h	0.78 ± 0.39	-	-	33
	Cats	?	?	?	?	?	?	?	?	
	Men	500 – 1000 mg/m^2 IV	24 h (CP)	0.49 ± 0.1 L/kg	± 20 – 25 μg/mL	4.7 ± 0.6 L/h	4.8 ± 1.1	-	19.1 ± 4.5% dose (over 24h)	34
CCNU	Mice	20 mg/kg IP	± 3.7 h (CCNU)	35.6 L/kg	-	27.8 L/(h kg)	0.667 – 1.27	-	-	35
	Rats	9.8 mg/kg IV	3 h (CCNU)	4.49 ± 0.11 L/kg	1.87 ± 0.35 μg/mL	11.6 ± 1.21 L/(h kg)	0.30 ± 0.13	-	-	36
	Dogs	15 mg/kg IV	6 h ([^{14}C] CCNU)	-	± 1500 CPM/mL	-	± 0.8	-	67.9 – 84.3% dose (over 48 h)	37
	Cats	?	?	?	?	?	?	?	?	
	Men	15 mg/kg PO	24 h (CCNU)	-	1.33 ± 1.25 μg/mL	-	3.30 ± 1.79	-	-	38
Doxo	Mice	6 mg/kg IV	8 h (Doxo)	-	-	0.052 ± 0.017 L/h	10.3 ± 4.0	-	-	39
	Rats	4 mg/kg IV	10 h (Doxo)	-	-	1.60 ± 0.23 L/(h kg)	-	14.3 ± 1.70% dose (over 10 h)	6.17 ± 1.7% dose (over 10 h)	40
	Dogs	30 mg/m^2 IV	± 6 h (Doxo)	-	-	46.5 ± 28.5 L/h	19.8 ± 22.3	-	-	39
	Cats	?	?	?	?	?	?	?	?	
	Men	25 – 72 mg/kg IV	48 h (Doxo)	1758.2 ± 850 L	-	44.5 ± 18.1 L/h	28.5 ± 10.7	-	-	41
Epi	Mice	0.85 mg/kg IV	24 h (Epi)	-	1.17 – 8.03 μg/mL	-	0.15 – 0.348	-	-	42
	Rats	4 mg/kg IV	10 h (Epi)	2.18 ± 0.18 L	-	8.81 ± 3.65 L/(h kg)	-	20.4 ±5.59% dose (over 10 h)	3 – 5% dose (over 10 h)	40
	Dogs	?	?	?	?	?	?	?	?	
	Cats	?	?	?	?	?	?	?	?	
	Men	90 mg/kg IV	24 h (Epi)	10.8 – 12.6 L	1.11 – 1.42 μg/mL	76.2 – 83.4 L/h	7.77	-	-	43

Table 3 Continued

		Dose	Last PK time point (analyte)	V_d	C_{max}	CL_T	$t_{1/2\,el.}$ (h)	Billiary excretion	Urinary excretion	Ref
MTN	Mice	1 mg/kg IV	24 h (MTN)	113 ± 42.8 L/kg	0.25 ± 0.051 μg/mL	268 ± 89.9 L/(h kg)	0.28 ± 0.03	-	-	44
	Rats	0.5 mg/kg IV	4 h (MTN)	1.1 ± 0.187 L/kg	± 0.5 μg/mL	7.08 ± 0.41 L/(h kg)	-	6.08 ± 2.32% dose after 180 min	-	45
	Dogs	5 mg/kg IV	48 h ([^{14}C] MTN)	26.6 ± 4.9 L/kg	7.5 ± 2.6 μg/mL	0.606 ± 0.02 L/(h kg)	28.1 ± 7.1	3.0 ± 0.1% dose (over 48 h)	2.4 ± 0.6% dose (over 48 h)	46
	Cats	5 mg/m^2 IV	120 h (MTN)	4.512 L/kg	-	3.51 L/(h kg)	1.51	-	-	47
	Men	12 mg/m^2 IV	120 h ([^{14}C] MTN and MTN)	1875 ± 670 L/m^2	-	0.034 ± 0.014 L/(h m^2)	42.6 ± 19.96	13.6 – 24.8% dose (over 120 h, [^{14}C] MTN)	5.2 – 7.9% dose (over 120 h)	48
VBL	Mice	1 mg/kg IV	31 h (VBL)	-	± 0.100 μg/mL	10.1 ± 0.5 L/(h kg)	2.1 ± 0.3	Feces: ± 80% dose (over 96 h, incl. metabolites)	10 – 15% dose (over 96 h, incl. metabolites)	49
	Rats	0.6 mg/kg IV	48 h ([^{3}H] VBL)	11.5 ± 3.78 L/kg	± 0.100 μg/mL	1.49 ± 0.91 L/kg	7.5 ± 2.8	40 – 50% dose (over 48 h, dose: 0.9 mg/kg)	-	50
	Dogs	0.15 mg/kg IV	Day 9 ([^{3}H] VBL)	-	± 0.060 – 0.150 μg/mL	-	3 – 5	Feces: 30.1 – 36.1% dose (over 9 days)	12.1 – 16.8% dose (over 9 days)	51
	Cats	?	?	?	?	?	?	?	?	
	Men	3 mg/m^2 IV	72 h ([^{3}H] VBL)	-	-	33.1 ± 10.9 L/(h m^2)	29.2 ± 11.2	-	-	52
VCR	Mice	0.5 mg/kg IV	24 h ([^{3}H] VCR)	3.81 L/kg	2.10 μg/mL	3.3x10^{-3} L/h	85.6	-	-	53
	Rats	0.1 mg/kg IV	4 h ([^{3}H] VCR)	-	± 0.440 μg/(mL kg)	-	1.3	Feces: 70.1 ± 5.4 μg/kg (over 72 h, males)	17.1 ± 2.2 μg/kg (over 72 h, males)	54
	Dogs	0.5 mg/kg IV	4 h ([^{3}H] VCR)	-	± 0.150 μg/(mL kg)	-	1.3	27% dose (over 4 h, females)	-	54
	Cats	?	?	?	?	?	?	?	?	
	Men	2 mg IV	24 h (VCR)	12.8 ± 2.2 L/kg	-	34.1 ± 4.6 L/h	19.4 ± 3.6	-	-	55
CaPt	Mice	80 mg/kg IV	Day 10 (Total Pt)	-	57.6 – 76.1 μg/mL	-	49.5	23.3 μg/mL (at 4 h, males)	92.6 ± 0.3% dose (over 72 h)	56
	Rats	20 mg/kg IV	1 h (Total Pt, CaPt)	0.363 ± 0.018 L/kg (total Pt)	± 0.300 μmol/mL	0.56 ± 0.02 L/(h kg) (CA)	0.43 ± 0.032 (total Pt)	0.7% dose (over 6 h), total Pt	88.6 ± 3.9% dose (over 4 h, total Pt)	57
	Dogs	150 mg/m^2 IV	24 h (Pt)	33.8 ± 2.8 L/m^2	-	6.51 ± 1.01 L/(h m^2)	3.65 ± 0.293	-	31.7 ± 7.8% dose (over 4 h)	58
	Cats	200 mg/m^2 IV	6 h (Total Pt)	0.630 ± 0.250 L/kg	23 ± 7 μg/mL	0.36 ± 0.20 L/(h kg)	1.3 ± 0.37	-	-	59
	Men	170 – 500 mg/m^2 IV	96 h (CaPt)	16 ± 31 L	22 – 63 μg/mL	4.4 ± 0.84 L/h	3.0 ± 0.5	Non renal CL= 1.58 ± 0.5 L/h	71 ± 5.7% dose (over 48 h)	60

CaPt: carboplatin; CBL: chlorambucil; CCNU: lomustine; CP: cyclophosphamide; CPM: counts per minute; Doxo: doxorubicin; Epi: epirubicin; IV: intravenous; MTN: mitoxantrone; Pt: platinum, SC: subcutaneous; VBL: vinblastine; VCR: vincristine; -: not specified; ?: no studies were found

Alkylating agents

No studies were found regarding pharmacokinetics of chlorambucil in dogs or cats (see Table 3). In rats, depending on the labeled compound used in the study, chlorambucil and metabolites were mainly excreted via urine (up to 60% over 24 h, dose: 8 mg/kg subcutaneous)[61] or feces (up to 36% of the dose, over 72 h, dose: 25 mg/kg per os).[62] The major route of excretion of chlorambucil and metabolites in mice was via urine (up to 55% dose over 48 h, dose: 19 mg/kg intraperitoneal), with some excretion via feces (up to 12% dose over 48 h).[63] Excretion of chlorambucil in dogs and cats may therefore occur via both urine and feces.

Warry et al. (2011) [64] describe pharmacokinetic parameters of cyclophosphamide in dogs, but they did not investigate its excretion (see Table 3). In men, up to 20% of the administered dose was excreted via urine, over 24 h (dose: 500 – 1000 mg/m^2 intravenous (IV)).[34] Looking at the cumulative excretion of cyclophosphamide and its metabolites, up to 50% of the dose is excreted via urine in men (dose: 20 mg/kg IV).[65] Knobloch et al. (2010) [22] found a median concentration of about 400 μg/L cyclophosphamide in urinary samples of dogs directly after treatment, but they could not detect concentrations above the lower limit of detection (0.25 μg/L) in the samples from day 1 to day 3 after administration of the drug. This can be explained by the short elimination half-life of cyclophosphamide in dogs. However, the presence of cyclophosphamide at concentrations lower than 0.25 μg/L cannot be excluded. In dogs, approximately 11% of the total amount of cyclophosphamide and metabolites excreted in urine (over 24 h) consists of unchanged cyclophosphamide.[66] This is of importance, since cyclophosphamide is an inactive pro-drug.[67] Fecal excretion of cyclophosphamide and its metabolites in dogs and cats cannot be excluded. Elimination of cyclophosphamide and metabolites via urine in cats should be considered.

Lomustine and metabolites are mainly excreted via urine in dogs, with up to 85% of the dose over 48 h after IV administration of 15 mg/kg lomustine (see Table 3).[68] No information on the pharmacokinetic properties of lomustine in cats could be found. Even though the metabolism of dogs and cats differs, urinary excretion in cats seems likely, and fecal excretion is a possibility in both cats and dogs.

Cytotoxic antibiotics

The pharmacokinetics of doxorubicin in dogs has been described (see Table 3).[39] Directly after administration a median concentration of approximately 355 μg/L doxorubicin was quantified in urinary samples of dogs, and up until day 21 after treatment low amounts could be detected (but not in samples gathered after that time point; limit of detection: 0.5 μg/L).[22] This is explained by the long elimination half-life of doxorubicin in dogs. Since it is mainly excreted via bile in rats[40] (see Table 3), doxorubicin could also be excreted via feces in dogs. Because the urinary excretion of doxorubicin in dogs lasts up until day 21 after

administration, fecal excretion may also persist until at least day 21. In cats, the elimination of doxorubicin via urine and feces should be considered, up until at least day 21 as well.

No studies were traced regarding pharmacokinetics of epirubicin in dogs or cats (see Table 3). In rats, epirubicin is mainly excreted via bile and to a lesser extent via urine (see Table 3).[40] Consequently, both routes of elimination should be considered in dogs and cats. Epirubicin has different pharmacokinetic properties than doxorubicin, resulting in a shorter elimination half-life in mice,[69] rats[40] and men.[43] Consequently, excretion up until day 21 after administration is less likely in dogs and cats.

The elimination of mitoxantrone in dogs seems to occur quite evenly via urine and bile, though only small amounts were detected after 48 h (see Table 3),[46] which reflects the long elimination half-life of mitoxantrone in dogs. Excretion via urine and feces could, therefore, occur at least up until day 21 after administration. The elimination half-life in cats is much shorter.[47] No data on mitoxantrone excretion in cats could be found, but excretion via urine and feces seems obvious, though probably not up until day 21.

Vinca alkaloids

Vinblastine and metabolites are mainly excreted via feces in dogs (30 – 36% of the dose), with still a substantial amount being excreted via urine (12 – 17% dose, with dose: 0.15 mg/kg IV) (see Table 3).[51] A median concentration of around 145 μg/L could be detected in urinary samples of dogs, directly after administration (limit of detection: 0.25 μg/L).[22] Low amounts can be detected up until day 7 to 9 after administration.[22,51] Feces is also the major route of excretion, in mice, rats and men.[49,50,70] As a result, fecal elimination of vinblastine in cats seems likely, although urinary excretion should also be considered, up until at least day 9 after administration.

The cumulative excretion of vincristine and its metabolites via bile is approximately 27% of the dose (0.5 mg/kg IV) in dogs over 4 h after administration (see Table 3).[54] Immediately after administration a median concentration of about 55 μg/L could be detected in urinary samples of dogs.[22] Five days after administration no levels above the lower limit of detection could be measured in urine (limit of detection: 0.5 μg/L). The biliary cumulative excretion in rats is comparable with that of dogs.[54] Additionally, some elimination via urine occurs in rats. Therefore, elimination via feces seems likely in cats, while urinary excretion should still be considered, as well, at least up until 4 days after administration.

Platinum-containing drugs

Up to 40% of the administered carboplatin dose (150 mg/m^2 IV) is excreted via urine during the first 4 h in dogs (see Table 3),[58] and up to 80% of the dose over 96 h (when measuring platinum, dose: 3 to 24 mg/kg IV).[71] Urine is also the main route of elimination in mice and

rats, with only low amounts found in bile or feces.[56,57,72] The elimination half-life in mice, rats and men can be rather long, varying from 49.5 h in mice up to 7.5 days and even 4.5 years in men, due to tissue binding.[56,60,72,73] This explains the trace amounts of platinum found in urine and feces of dogs up to 21 days after administration of carboplatin.[23] Given these data, urinary elimination of carboplatin seems likely in cats. Furthermore, excretion via feces up until day 21 in cats is conceivable.

Some research regarding excretion of antineoplastic drugs via other routes has been performed in men, demonstrating the salivary excretion of cyclophosphamide, doxorubicin and epirubicin.[74-76] Our research group has monitored platinum in urine, feces, saliva, sebum and cerumen in dogs treated with carboplatin, up until day 21 after administration.[23,77,78] The analytical methods using inductively coupled plasma mass spectrometry (ICPMS) can detect amounts well below the limit of detection of most methods of the experiments described here (e.g. limit of detection for urine: 7.50 ng/L). Therefore, the lack of measurable concentrations of antineoplastic drugs in excretion products, as described by Knobloch et al. (2010),[22] does not necessarily mean that no drug is present. As a result, both urine and feces of pet-animals treated with any of the antineoplastic drugs described in this review can be considered as a potential source of exposure, with maximal elimination during the first few days after administration, while the presence of low amounts of antineoplastic drugs in other excretion products or after those first few days cannot be excluded.

Assessment of occupational exposure to antineoplastic drugs

Since the early 1980s, guidelines for safe handling of antineoplastic drugs have been established, implemented and improved in human oncology.[79] This has led to a reduction in occupational exposure.[27] In response, guidelines have been developed by the European College of Veterinary Internal Medicine – Companion Animals (ECVIM-CA), focused on handling of antineoplastic drugs, treated animals and related wastes in veterinary oncology.[80] However, these guidelines have been based on knowledge transferred from human oncology and experimental animals. But pharmacokinetics is species-dependent and measures might, therefore, not be adequate. Consequently, assessment of occupational exposure to antineoplastic drugs in daily routine veterinary oncology has become imperative. The assessment of occupational exposure involves: monitoring of surface contamination (environmental monitoring), evaluation of uptake of the antineoplastic drugs by the exposed population (biomonitoring: biomarkers of exposure), and determination of biological effects (biomonitoring: biomarkers of effect and of health risks).

Table 4 Environmental monitoring of antineoplastic drugs

	Monitoring of	Assay	Samples[a]	Results	Remarks	*Ref*
CisPt	Workplace	HPLC-UV	13/16	<0.03 ng/cm² – 1.45 ng/cm²	-	*81*
CisPt	Drug vials	Adsorptive voltammetry	12/12	0.2 – 99 ng/pad	-	*82*
CP	Workplace	GC-MS	42/185	<7.5x10^{-3} – 5.11 ng/cm²	Human oncology units	*83*
Pt		Voltammetry	147/147	<0.1x10^{-3} – 2.7 ng/cm²		
CP	Workplace	GC-MS	2/2	5.74 – 10.5 ng/cm²	Human pharmacy	*84*
CaPt		ICP-MS	2/2	39.3 – 63.5 ng/cm²		
CisPt			-	-		
CP	Drug vials	GC-MS	3/30	<5 – 39 ng/vial		
CaPt		ICP-MS	30/30	7 – 251 ng/vial		
CisPt		ICP-MS	4/30	<1 – 9 ng/vial		
CP	Gloves	GC-MS	0/1	< 1nM		
CaPt		ICP-MS	1/1	38 ng/pair gloves		
CisPt		ICP-MS	1/1	28 ng/pair gloves		
CP	Workplace	GC-MS	260/400	<0.06 – >100 ng/cm²	Human oncology units	*85*
CP	Drug vials	HPLC-MS/MS	48/48	88 – 6.98x10^{4} ng/vial	Study 1:Human pharmacy	*86*
CP		GC-MS/MS	48/54	<16 – 8.78x10^{3} ng/vial	Study 2: Human pharmacy	
Pt		Voltammetry	-/368	BV – 3.59 ng/cm²	Study 3: Production unit	
CP	Workplace	GC-MS/MS	14/28	<0.1 ng/mL – 6.55 ng/cm²	Human oncology units	*87*
	Gloves		4/7	<0.1 ng/mL – 9.21x10^{3} ng/glove		
CP	Primary packaging	LC-MS/MS	75/100	<0.04 – 190 ng/sample	Human pharmacy	*88*
	Drug vials		20/20	4.8 – 130 ng/sample		
	Tablet packages		24/24	0.2 – 3.5 ng/sample		
	Workplace: surfaces		49/49	2.2x10^{-3} – 0.87 ng/cm²		
	Workplace: other		13/14	<0.04 – 2.1x10^{3} ng/sample		
CP	Workplace	GC-MS	15/15	0.22 – 16 ng/cm²	Human pharmacies	*89*
	Gloves		-/12	<5 – 5.99x10^{3} ng/glove		
Pt	Workplace	ICP-MS	15/15	0.05 – 1.3 ng/cm²		
	Gloves		12/12	3 – 102 ng/glove		
CP	Workplace	GC-MS/MS	220/228	<3.3x10^{-3} – 0.0323 ng/cm² (without CSTD)	Human pharmacies	*90*
			104/114	<3.3x10^{-3} – 9.8x10^{-3} ng/cm² (with CSTD)		
Pt	Workplace	Voltammetry	12/12	± 2.5x10^{-4} – 6.73 ng/cm²	Veterinary oncology units	*17*
	Gloves		6/12	<4.8 – 534 ng/pair		
Pt	Workplace	ICP-MS	108/124	<5x10^{-5} – 5.76 ng/cm²	Human pharmacies	*91*

Table 4 Continued

	Monitoring of	Assay	Samples[a]	Results	Remarks	*Ref*
CP	Workplace	GC-MS/MS HPLC-UV	197/249	<0.2 – >10 ng/cm^2	Human oncology unit	*92*
CP	Workplace	LC-MS/MS	204/229 78/104	<0.05x10^{-3} – 6.3 ng/cm^2 <0.05x10^{-3} – 5.7 ng/cm^2	Human oncology units Human pharmacy	*93*
Pt	Workplace	Voltammetry	996/1008	<0.025x10^{-3} – 23.1 ng/cm^2	Human pharmacies	*94*
CP	Workplace Gloves	GC-MS	127/127 102/136 15/22 6/27	9.5x10^{-3} – 27 ng/cm^2 (without CSTD) <6.0 ng/sample – 4.4 ng/cm^2 (with CSTD) <6.0 – 3.2x10^3 ng/pair (without CSTD) <6.0 – 740 ng/pair (with CSTD)	Human pharmacy	*95*
CP	Workplace	HPLC-MS/MS	13/62 45/81	<0.10 – (0.4 ± 0.4 ng/cm^2) <0.10 – (18.1 ± 51 ng/cm^2)	Human oncology units Human pharmacies	*96*
Pt	Workplace	Voltammetry	12/27	<0.50 ng/mL HCl – 4.61 ng/cm^2	Veterinary oncology unit	*21*
CP **Pt**	Drug vials	GC-MS Voltammetry	60/70 302/302	<3 – 912 ng/vial 0.1 – 3.71x10^3 ng/vial	Human pharmacies	*97*
CP	Workplace	GC-MS/MS	1/3 2/10	<0.02 – 0.07 ng/cm^2 <100 – 740 ng/sample	Human oncology unit	*98*
CP **Pt** **Doxo** **Epi**	Workplace	HPLC-MS/MS ICP-MS HPLC-MS/MS HPLC-MS/MS	142/238 60/241 0/- 0/-	<10 – 1.6x10^4 ng/sample < 1– 714 ng/sample <LOQ <LOQ	Human oncology units LOQ not disclosed LOQ not disclosed	*15*
CP	Workplace	GC-MS	89/114 77/114	<0.01 – 158 ng/cm^2 (without CSTD) <0.01 – 17.2 ng/cm^2 (with CSTD)	Human pharmacies	*99*
CP	Workplace	GC-MS	22/48 13/24	<0.10 ng/mL NaOH – 6.1 ng/cm^2 < 0.10 ng/mL NaOH – 26.2 ng/cm^2	Human oncology units Human pharmacies	*100*
CP **Pt**	Workplace	GC-MS	± 57/116 ±70/116	<6.0 ng/sample – 0.85 ± 1.2 ng/cm^2 <10 ng/sample – 0.32 ± 0.38 ng/cm^2	Human pharmacies	*101*
Pt	Workplace Gloves	Voltammetry	44/44 88/88 45/45	0.07x10-3 – 0.592 ng/cm2 0.07x10-3 – 110 ng/cm2 0.01 – 729 ng/pair	Before HIPEC After HIPEC Inner and outer gloves	*102*

Table 4 Legend

[a]: number of positive samples per total number of samples

CaPt: carboplatin; CisPt: cisplatin; CP: cyclophosphamide; CSTD: closed-system drug transfer device; Doxo: doxorubicin; Epi: epirubicin; GC: gas chromatography; (HP)LC: (high pressure) liquid chromatography; ICP: inductively coupled plasma; MS: mass spectrometry; Pt: platinum; UV: ultra violet detection; -: not specified

Environmental monitoring of occupational exposure to antineoplastic drugs

A compound can enter the body by several routes: inhalation, ingestion and dermal contact.[24-26] Environmental monitoring can identify potential sources and routes of exposure, and can offer insight into the effectiveness of hygiene measures.[103] As a result, more informed and appropriate risk reduction strategies can be developed. However, evidence of presence of antineoplastic drugs in the environment, does not necessarily imply that the drugs under investigation are able to enter the body (see Figure 2).

Many researchers have monitored environmental contamination of pharmacies and hospitals with antineoplastic drugs, but research has rarely focused on environmental contamination of workplaces in veterinary oncology. Table 4 summarizes relevant studies describing environmental monitoring in human and veterinary oncology, performed since 2000.

Since the implementation of the guidelines in human oncology, a reduction in occupational exposure could be observed.[27,79] However, research demonstrates that environmental contamination continues to occur (see Table 4). Most studies showed environmental contamination, but varied largely between <1 – 10 ng/cm^2 (surfaces), <100 – 700 ng/pair gloves and <10 – 4000 ng/vial, with maximal levels of 158 ng/cm^2 (surface), 9.21x10^3 ng/glove and 6.98x10^4 ng/vial being reported. The variation of contamination between studies might be explained by differences in methodologies (e.g. use of closed-system drug-transfer devices), amount of processed antineoplastic drugs, and differences in (adherence to) guidelines in the monitored oncology units, pharmacies, and factories that supply the antineoplastic agents.

Only 2 studies with focus on environmental contamination in veterinary oncology units, have been reported since 2000.[17,21] The contamination described by the authors was comparable to the levels reported for the human oncology units. Yet, considerably lower amounts are annually processed in veterinary oncology units. This implies that the hygiene measures in the investigated units were either not sufficient, or not adequately implemented. Furthermore, we have previously published preliminary results indicating platinum surface contamination at the homes of pet-dogs, after administration of carboplatin.[23] Environmental samples showed some increase in platinum contamination on nearly all monitored surfaces, including those unreachable for the dog (0.24-30.1 pg/cm^2). These findings indicate that transference of platinum by the owners occurred.

Table 5 Urinary excretion of antineoplastic drugs by exposed workers

Assay		Samples[a]	Results		Statistical significance	Remarks	Ref
			Exposed	Control group			
CP	GC-MS/MS	12/12	20 – 9.14x10^{3} ng/24 h	-	-		104
CP Pt	HPLC-MS/MS ICP-MS	18/62 3/62	<0.5 ng/L – 10.0 ng/mL <1x10^{-3} ng/L – 1.3 ng/mL	- -	- -		105
CP Pt	GC-MS/MS ICP-MS	0/73	<0.3 ng/mL Cfr. control	- -	- -		106
Pt	-	NA	NA	0.99 – 4.48 ng/g creatinine	-	Reference values	107
Pt	Adsorptive voltammetry	NA	NA	< 9x10^{-4} – 0.065 ng/mL	-	Reference values	108
CP Doxo Epi Pt	GC-MS HPLC HPLC Voltammetry	106/1415 34/1752 45/1752 -/670 -/306 -/158	<0.04 – 0.76 ng/mL <9.9x10^{-3} – 0.127 ng/mL <0.011 – 0.182 ng/mL 0.7 – 65 ng/g creatinine 1.2 – 78 ng/g creatinine 2.7 – 64 ng/g creatinine	- - - 0.9 – 72 ng/g creatinine	- - - $P < 0.001$ $P < 0.001$ $P < 0.001$	 Cycle 1 Cycle 2 Cycle 3 Control= after holidays	13
Pt	ICP-MS	NA	NA	2x10^{-3} – 0.036 ng/mL	-	Reference values	109
CP Pt	GC-MS ICP-MS	- -	<0.3 ng/g 9.8 – 55.4 ng/g creatinine 9.8 – 142 ng/g creatinine	- <4.3x10^{-3} – 25.0 ng/g creatinine	- $P < 0.01$ $P < 0.01$	 Positive pressure isolator Negative pressure isolator	89
CP	GC-MS/MS	61/717 7/294	<0.1 ng/mL – 1250 ng/24 h <0.1 ng/mL – 45 ng/24 h	- -	- -	At 1997 At 2000	14
CP	GC-MS	42/60	<0.04 – 900 ng/mL	-	-		110
CP	LC-MS/MS	0/44	<0.01 ng/mL	-	-	Urine spot sampling	111
CP	GC-MS	18/27	<0.1 ng – 170 ng/24 h <0.1 ng – 15 ng/24 h	- -	- -	Without CSTD With CSTD	95
CP	HPLC-MS/MS	2/67	<0.015 – 0.079 ng/mL	<0.015 ng/mL	-		96
CP	LC-MS/MS	0/35	<0.4 ng/mL	-	-		112
Doxo Epi	HPLC-FL	2/56 4/56	<0.6 – 33.9 ng/mL <1.2 – 84.1 ng/mL	- -	- -		113

Table 5 Continued

Assay		Samples[a]	Results		Statistical significance	Remarks	Ref
			Exposed	Control group			
CP Doxo Epi	HPLC-MS/MS	124/806 4/430 6/430	<0.02 – 6.11 ng/mL <0.10 – 0.834 ng/mL <0.10 – 0.765 ng/mL	- - -	- - -		114
CP Pt Doxo Epi	HPLC-MS/MS ICP-MS HPLC-MS/MS HPLC-MS/MS	0/207	<0.2 ng/mL <$4x10^{-4}$ ng/mL <0.3 ng/mL <0.2 ng/mL	- - - -	- - - -		15
CP	GC-MS/MS	90/276	ND – 463 ng/24 h	-	-	LOD not disclosed	100
CP	GC-MS	7/40	<0.1 – 1.2 ng/mL	-	-		115
CP Pt	GC-MS ICP-MS	3/17 0/17	<0.1 ng – 52 ng/24 h <2.0 ng	-	-		101

[a]: number of positive samples per total number of samples

CaPt: carboplatin; CisPt: cisplatin; CP: cyclophosphamide; CSTD: closed-system drug transfer device; Doxo: doxorubicin; Epi: epirubicin; FL: fluorescence detection; GC: gas chromatography; (HP)LC: (high pressure) liquid chromatography; ICP: inductively coupled plasma; LOD: limit of detection; MS: mass spectrometry; Pt: platinum; -: not specified; NA: not applicable; ND: not disclosed

Biomonitoring of occupational exposure to antineoplastic drugs

Human biological monitoring, also known as biomonitoring, serves as the intermediate step between environmental exposure and adverse health effects. The development of a disease like cancer is the result of several biochemical and biological changes in an organism. Biomonitoring measures biological markers (biomarkers) that characterize different events that occur between initial exposure and the onset of disease (see Figure 2).[116,117] It measures the amount of a compound, such as antineoplastic drugs, directly in a person's body (e.g. human tissue or fluid samples).[103,116,117] This can be done by detecting biomarkers of exposure: the substance itself, its metabolites, or its biological reaction products such as protein or DNA adducts. As a result, biomonitoring data take several processes into account: uptake variability related with different exposure routes (e.g. inhalation, skin contact), metabolism, bioaccumulation, and excretion.[103] Consequently, the measured internal body concentration is much more relevant than mere environmental exposure data. However, the presence of biomarkers of exposure in tissue or fluid samples does not necessarily imply evidence of harm.[103,116,117] Therefore, biological effects along the pathway from exposure to disease should be monitored by using biomarkers of biological effect (e.g. chromosomal aberrations and micronuclei).[103,116,117] On the other hand, biomarkers of biological effects are often non-specific.[116,117] An elevated level of such a biomarker could also be caused by other events such as smoking and ageing. Preferably, biomarkers should be combined with the determination of the presence of the substance or its metabolites in the organism. Moreover, evidence of biological effects does not equal adverse health effects either.[116] There is currently only one biomarker that has been validated as a biomarker of health risk: chromosomal aberrations in lymphocytes.[118] However, the first prospective studies supporting a correlation between micronuclei frequency in lymphocytes and cancer risk are starting to emerge.[119,120] Thus, estimation of health risks using biomonitoring can only be done by using several biomarkers that identify absorption of the substance, that determine biological events along the route from exposure to development of cancer, and that indicate possible health risks. In addition, biomonitoring should be evaluated in conjunction with environmental contamination data and epidemiological data.[103]

Table 5 and 6 present some relevant studies reporting on biomonitoring in human oncology, performed since 2000. Our research group has investigated the urinary excretion of platinum in owners of pet-dogs treated with carboplatin.[23] The preliminary results did not show an increase in urinary platinum levels of the owners, during treatment of their pet-dog with carboplatin. No other studies with focus on biomonitoring in veterinary oncology since 2000 could be traced.

Research shows that antineoplastic drugs can be found in urine of workers exposed to these agents (see Table 5). Reported levels varied largely between <1 – 6 ng/mL urine, with a maximal level of 900 ng/mL (cyclophosphamide). The workers investigated in the referenced studies all used protective measures to reduce exposure to antineoplastic drugs.

Table 6 Biomarkers in samples of workers occupationally exposed to antineoplastic drugs

	Assay	Samples[a]	Subjects[b]	c	Results		*P*-value[d]	Units (scoring)	Remarks	Ref
					Exposed	Control				
LY	MN	247 (exposed) 60 (controls)	100 (exposed) 60 (controls)	-	$1x10^{-3}$ – 0.050	$3x10^{-3}$ – 0.032	$p = 0.29$	% MN/ 1000 cells (1000 BN cells/subject)	Human oncology units Human pharmacies	121
LY	SCGE	50 (exposed) 20 (controls)	50 (exposed) 20 (controls)	+ + +	17.5 ± 1.99 81.5 ± 4.31 14.3 ± 2.16	12.6 ± 0.82 76.0 ± 3.70 9.78 ± 0.91	$p < 0.05$ $p < 0.05$ $p < 0.05$	Tail length (μm) (50 comets/sample) % Tail DNA (50 comets/sample) Tail moment (50 comets/sample)	Human oncology units	122
LY	CA	20 (exposed) 18 (controls)	20 (exposed) 18 (controls)	+	1.15 ± 0.65	0.49 ± 0.56	$p = 0.021$	% CA (200 metaphases/sample)	Human oncology units	104
LY	SCGE MN	59 (exposed) 44 (controls)	30 (exposed) 22 (controls)	+ -	46.5 ± 0.09 3.53 ± 0.59	42.7 ± 0.10 3.09 ± 0.37	$p < 0.01$ $p > 0.05$	Tail length (μm) (100 cells/sample) % MN/1000 cells (1000 BN cells/subject)	Human oncology units	123
LY	CA	339 (uncontrolled) 147 (controlled) 14 ISO (controlled) 94 (controls)	339 (uncontrolled) 147 (controlled) 14 ISO (controlled) 94 (controls)	+ - -	2.32 ± 0.19 1.77 ± 0.21 0.79 ± 0.39	1.72 ± 0.25	$p < 0.05$ (increase) $p > 0.05$ $p < 0.05$ (decrease)	% CA (100 metaphases/sample) % CA (100 metaphases/sample) % CA (100 metaphases/sample)	Uncontrolled vs. controls Controlled vs. controls ISO controlled vs. controls Human oncology units	124
LY	SCGE	19 (exposed) 18 (controls)	19 (exposed) 18 (controls)	+	4.5 – 13.6	3.5 – 10.3	$p = 0.004$	Tail length (μm)(100 cells/sample)	Human oncology unit	125
LY LY BEC	SCGE MN MN	120 (exposed) 120 (controls)	60 (exposed) 60 (controls)	+ + +	13.67± 2.37 6.53 ± 1.15 2.66 ± 0.83	6.21 ± 0.92 3.11 ± 1.09 1.86 ± 0.62	$p < 0.05$ $p < 0.05$ $p < 0.05$	Tail length (μm)(100 cells/subject) % (MN/1000 cells)(1000 BN cells/subject) % (MN/1000 cells)(5000 cells/subject)	Human oncology unit	110
LY	CA	76 (exposed) 72 (controls)	76 (exposed) 72 (controls)	+	11.2 ± 4.39	3.04 ± 2.76	$p < 0.0001$	% CA (100 metaphases/sample)	Human oncology units	126
LY	MN SCGE	83 (exposed) 73 (controls)	83 (exposed) 73 (controls)	+ +	13.1 ± 6.70 1.16 ± 0.82	6.70 ± 4.01 0.77 ± 0.47	$p < 0.0001$ $p < 0.001$	‰ (MN/1000 cells)(1000 BN cells/subject) % Tail DNA (100 comets/subject)	Human oncology units	127
LY	SCGE	109 (exposed) 46 (controls)	109 (exposed) 46 (controls)	+ -	0.764 ± 0.121 0.312 ± 0.253	0.711 ± 0.089 0.253 ± 0.237	$p = 0.02$ $p = 0.239$	Log[tail length (μm)(100 cells/sample)] Log[tail moment (100 cells/sample)]	Human oncology units	128

Table 6 Continued

	Assay	Samples[a]	Subjects[b]	[c]	Results		*P*-value[d]	Units (scoring)	Remarks	Ref
					Exposed	**Control**				
LY BEC LY BEC	SCGE SCGE MN MN	60 (exposed) 152 (controls)	30 (exposed) 76 (controls)	- - - +	10.7 ± 7.04 12.0 ± 6.1 8.73 ± 4.06 0.85 ± 0.68	11.2 ± 8.67 13.8 ± 9.80 8.04 ± 4.10 0.48 ± 0.45	$p > 0.05$ $p > 0.05$ $p = 0.63$ $p = 0.042$	% (comets/1000 cells)(50 comets/sample) % (comets/1000 cells)(50 comets/sample) % (MN/1000 cells)(1000 BN cells/subject) % (MN/1000 cells)(at least 2000 cells/subject)	Human oncology unit Human pharmacy	129
LY	SCGE MN CA	50 (exposed) 50 (controls)	50 (exposed) 50 (controls)	+ + +	14.5 ± 0.16 – 23.4 ± 0.74 10 – 33 0.5 – 5.0	12.5 ± 0.08 – 15.42± 0.13 1 – 12 0 – 1	$p < 0.001$ $p < 0.001$ $p < 0.001$	Tail length (μm)(50-100 comets/subject) ∑MN/1000 cells (1000 BN cells/subject) % CA (200 cells/ subject)	Human oncology units	130
LY	SCGE	66 (exposed) 52 (controls)	66 (exposed) 52 (controls)	- -	53.1 ± 7.32 $2.54x10^3 \pm 652$	53.1 ± 7.50 $2.52x10^3 \pm 715$	$p = 0.96$ $p = 0.86$	% Tail DNA (100 cells/sample) Comet tail moment (100 cells/sample)		96
LY	CA	17 (high exposure) 46 (low exposure) 46 (controls)	17 (high exposure) 46 (low exposure) 46 (controls)	+ -	0.47 ± 0.80 0.11 ± 0.31	0.13 ± 0.40	$p = 0.02$ $p = 0.56$	% CA chromosome 5 or 7 (200 cells/subject) % CA chromosome 5 or 7 (200 cells/subject)	Human oncology units Human pharmacies	131
LY	CA MN	20 (exposed) 20 (controls)	20 (exposed) 20 (controls)	+ +	1.85 ± 1.56 9.4 ± 4.35	0.32 ± 0.67 1.85 ± 0.3	$p = 0.024$ $p < 0.05$	% CA (100 metaphases/subject) % MN/ 2000 cells (2000 BN cells/subject)	Human oncology unit	132
LY	*SCGE*	*52 (exposed)* *52 (controls)*	*52 (exposed)* *52 (controls)*	+	*2.73 ± 0.28*	*1.67 ± 0.14*	$p < 0.05$	*% Tail DNA (150 cells/subject)*	*Human oncology unit*	115

[a]: number of samples
[b]: number of subjects
[c]: global result
[d]: only the *P*-value was used to determine statistical significance in the studies reported here
BEC: Buccal epithelial cells, BN: binucleated, CA: chromosomal aberrations, LY: lymphocytes, MN: micronucleus, SCGE: single cell gel electrophoresis (also known as comet assay), +: positive result, -: negative result, results are expressed in range or average ± SD

Apparently, implementation of guidelines is not sufficient to eradicate uptake of antineoplastic drugs completely.

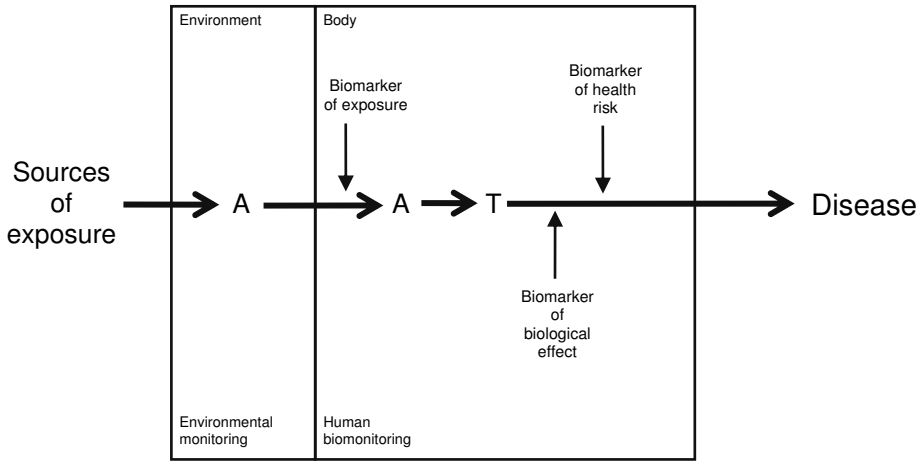

Figure 2 Schematic representation of environmental and human biological monitoring of antineoplastic drugs, with A: antineoplastic drug and T: target (e.g. DNA, proteins)

The majority of studies using genotoxicity biomarkers (14 out of the 16 studies described here) report a statistically significant increase of biomarkers, correlated with occupational exposure to antineoplastic drugs. However, some of these studies, using more than one biomarker, also reported the absence of significant differences between exposed workers and their controls. These non-significant biomarker levels were obtained using other biomarkers (e.g. comet assay vs. micronucleus test), other types of investigated cells (e.g. lymphocytes vs. buccal epithelial cells), or using different criteria to evaluate the same biomarker (e.g. tail length vs. tail moment in the comet assay) (see Table 6). Furthermore, there are some methodological problems associated with human biomonitoring. For example, the use of the *p*-value to determine statistical significance of a correlation is fiercely debated.[133] Statisticians strongly recommend additional use of confidence interval or the Bayes factor, but most published studies continue to rely solely on the *p* -value to determine statistical significance (see Tables 5 and 6). As a result, statistically significant correlations may not be significant when the confidence interval or the Bayes factor is determined. Moreover, the sample sizes of most studies are small, weakening the reported results and making interpretation of the data much more difficult, if even possible. Still, the uptake of antineoplastic drugs and an elevated presence of biomarkers in workers occupationally exposed to these compounds has been a rather consistent finding since the first study by Falck et al. (1979).[134] The inconsistency between and within studies, as evidenced in Table 6, can be explained by the transient nature of biological effects. Each biomarker and each

sampled individual might have its own pace regarding induction of genotoxicity and potential DNA-repair. All in all, the results seem to support the hypothesis that occupational exposure to antineoplastic drugs is correlated with some genotoxic effect, even if only transiently. As with environmental contamination, a reduction of occupational exposure can be observed these past decades, probably due to improvement and implementation of the guidelines.[135]

Conclusion

While the use of chemotherapy has been gaining popularity in veterinary medicine these past few decades, research focusing on occupational exposure in veterinary oncology has been rare. Reviewing environmental and biological monitoring in human oncology revealed that the implementation of guidelines since the early 80s has led to a reduction in occupational exposure to antineoplastic drugs, yet it is also clear that total eradication of exposure is apparently impossible, at this moment. Chemotherapy in veterinary medicine is different from chemotherapy in human oncology. Its use is by far less widespread in veterinary practice and the used dosages are much lower. Besides, animals are not like humans. They do not follow guidelines voluntarily. And they have owners, who are a potentially exposed population as well. The tailored investigation of exposure to antineoplastic drugs and associated risks in veterinary oncology has, therefore, become mandatory.

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Chapter 1.2

Antineoplastic drugs in veterinary oncology – Part 2: Adverse health effects and risk assessment

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Abstract

Chemotherapy is increasingly used in veterinary oncology as a cornerstone of the treatment of cancer in companion animals. As a result, the number of workers and owners exposed to antineoplastic drugs is rising. In the first part of the review, which is published separately, we explored the body of literature on occupational exposure to these compounds, with focus on veterinary medicine. It has become evident that occupational exposure occurs in veterinary medicine, despite implementation of guidelines regarding safe handling of antineoplastic drugs. In this second part, we will review potential harmful effects associated with the use of antineoplastic drugs in veterinary oncology. Furthermore, we discuss the risk assessment of long-term low dose exposure to antineoplastic drugs and point out the difficulties related with answering what seems a simple question: what risks are involved with exposure of veterinary personnel and owners to antineoplastic drugs?

Introduction

Cancer is an ancient disease that has been around since dinosaurs walked the earth.[1] Past civilizations have attempted to treat cancer in numerous ways for 5000 years, but malignant tumors remained incurable, except when complete surgical excision was possible, until about 60 years ago.[2] By then, modern cancer chemotherapy emerged and led to the development of 14 categories of antineoplastic drugs, representing over 50 different compounds. By 2007 cancer mortality had declined to 50% of what it was in 1990.[3] Half of the decline is attributed to advances in treatment, while the other half is attributed to prevention and early diagnosis.

Ironically, it has become evident that the curative treatment modalities also can cause the disease. The first indication that antineoplastic drugs might cause cancer was found by Haddow et al. (1948),[4] but it has been confirmed by many researchers since.[5,6] Today, antineoplastic drugs are associated with several harmful effects, such as genotoxicity, carcinogenicity, embryotoxicity and fertility problems.[5-8] Because workers in human and veterinary oncology can be long-term exposed to low doses of antineoplastic drugs, this might result in one or more of these adverse health effects. When this realization first dawned in the 1970s, efforts were focused on discovering whether occupational exposure occurred. Falck et al. (1979) [9] were the first to report mutagenic activity of urine from nurses handling antineoplastic drugs. Since then, numerous studies have described environmental contamination with antineoplastic drugs,[10,11] uptake of these compounds in occupationally exposed workers,[12,13] and the induction of genotoxic responses.[14,15] As a result, guidelines have been developed and implemented since the 1980s regarding the safe handling of antineoplastic drugs in human oncology.[15] These have served as a basis for the guidelines used in veterinary oncology.[16] It has become clear that, even though implementation of protective measures and guidelines has resulted in diminishing the exposure to antineoplastic drugs, total eradication of exposure is apparently impossible, at this moment.[14,17] As chemotherapy is gaining popularity in veterinary oncology, exposure to these drugs is an inevitable risk for an increasing number of people. This leads to the obvious question: what risk is involved with this exposure?

In the first part of this review we explored the main body of literature on occupational exposure to the antineoplastic drugs generally used in veterinary oncology. In this second part we will review the health risks associated with antineoplastic drugs and we will attempt to interpret this information in a risk assessment.

Genotoxicity, carcinogenicity, embryotoxicity and fertility problems associated with antineoplastic drugs

Antineoplastic drugs suppress cell proliferation and cause cell death, either by binding to DNA, RNA, proteins or specific molecular targets, or by inhibition of the production of these macromolecules. However, antineoplastic drugs do not only exert these effects on cancerous cells, but also on normal ones. This lack of selectivity may cause detrimental effects to normal tissues, which can lead to carcinogenesis, embryotoxicity and fertility problems. Most data on these effects of antineoplastic drugs have been derived from animal studies. Information on the effect of individual agents on humans is limited, as most reports are obtained from exposure to multiple drugs.[7] The International Agency for Research on Cancer (IARC) has classified several antineoplastic drugs as possibly, probably or definitely carcinogenic to humans (see Table 1).[18-21]

The literature on harmful effects of antineoplastic drugs at high doses is very extensive.[5-8,22] An exhaustive review of the literature is beyond the scope of this work. We have focused our review on the antineoplastic agents most commonly used in veterinary practice (see Table 1 and 2 in the first part of this review). First, we will describe the adverse health effects associated with high dose exposure to these compounds. Secondly, we will discuss the adverse health effects associated with occupational exposure to antineoplastic drugs.

Table 1 Antineoplastic drugs classified by the IARC [19-21]

Group 1 Carcinogenic to humans	Group 2A Probably carcinogenic to humans	Group 2B Possibly carcinogenic to humans
Azathioprine	Doxorubicin	Bleomycin
Busulfan	Carmustine	Dacarbazine
Chlomaphazine	Cisplatin	Daunomycin
Chlorambucil	Lomustine	Mitomycin
Cyclophosphamide	Nitrogen mustard	Mitoxantrone
Etoposide	Procarbazine	Streptozotocin
Melphalan	Teniposide	
Semustine		
Tamoxifen		
Thiotepa		
Treosulphan		
Certain combined chemotherapy		

Effects of high-dose exposure to antineoplastic drugs

Table 2 summarizes genotoxic, carcinogenic, embryotoxic and gonadotoxic effects associated with these agents as observed in *in vitro* experiments, *in vivo* experiments with rodents, or studies in human patients. Only reference to original articles is provided. We limited the number of referred original articles to one relevant study per clearly distinguished and evaluated effect in the table. For detailed outline of the various effects, we refer the reader to some excellent reviews [5-8,22-26]

Genotoxic effects of antineoplastic drugs at high-dose exposure

The major types of DNA damage are: base damage, clustered lesions, single and double strand breaks, intra- and inter-strand cross-links, DNA-protein cross-links, and interference with nucleotide metabolism and DNA synthesis.[27,28] The cell with a damaged genome has three choices: remove the DNA lesions, continue functioning in spite of damage, or activate the apoptotic pathway. Cells have developed intricate response mechanisms, collectively called the DNA-damage response (DDR).[28] These mechanisms include detection of DNA lesions, signaling of the presence of these lesions, initiation of cell cycle checkpoints, execution of DNA-damage repair, and activation of cell-death in the event of unrepairable DNA-damage (see Figure 1). The cell has several pathways to repair DNA-damage at its disposal: direct repair, base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), DNA double strand break repair via homologous recombination (HR) or non-homologous end joining (NHEJ), translesion DNA synthesis (TLS), Fanconi anemia pathway (FA) and post replication repair (PRR).[27-29]

Antineoplastic drugs have different modes of action, leading to different types of DNA lesions and DDRs. In general, **alkylating agents** interact non-specifically with DNA, causing irreversible covalent lesions.[22,30] Bifunctional alkylating agents such as chlorambucil, cyclophosphamide, and lomustine contain two reactive groups that can bind to separate DNA bases to form inter-strand cross-links in addition to the formation of monoadducts.[26,31] Other secondary lesions can also be formed, such as mismatches, strand breaks, and protein-DNA cross-links.[26] The DNA-damage can induce mutations and/or block important cellular processes, e.g. DNA transcription and replication (which may result in cell death).[31] Different mechanisms are involved in repair of primary lesions (direct repair and NER) and secondary lesions (NER, MMR, HR, NHEJ, TLS and FA).[26,31]

Cytotoxic antibiotics (e.g. doxorubicin, epirubicin, mitoxantrone) reversibly inhibit topoisomerase II enzymes.[22,32-34] Topoisomerase II is a cellular enzyme which transiently creates and reseals DNA double strand breaks. This enables the control of DNA structure by maintaining of the correct superhelical state and resolving of intertwined DNA strands.[22,24,35] Hence, topoisomerase II is crucial for the relaxation of DNA supercoils generated by cellular processes (e.g. transcription, recombination, replication), and for the condensation and

segregation of chromosomes during mitosis.[24,35] Inhibition of this enzyme leads to stabilization of the enzyme-DNA covalent cleavage intermediates.[22,32,33] Cells are unable to undergo chromosome segregation, when the cellular amount of cleavage complexes become too low. As a result, the cells eventually die due to mitotic crisis.[24] On the other hand, if the level becomes too high, the DNA repair mechanisms can transform these complexes to permanent double strand breaks.[22,24] Accumulation of double strand breaks can lead to chromosome translocations and other DNA aberrations.[24] If the accumulation is overwhelming, they trigger apoptotic pathways and kill the cell, or result in mutations.[24,36] The double strand breaks can be repaired by the HR and NHEJ pathways.[37] Additionally, doxorubicin, epirubicin and mitoxantrone can form drug-DNA adducts.[38-40] These adducts are covalently bound to one DNA strand and strongly stabilize the local DNA region via noncovalent hydrogen bonds with the complementary DNA strand.[38,41] As a result, the adducts act much like an inter-strand cross-link. Subsequently, DNA strand breaks are formed, posing as physical blocks for DNA transcription and replication.[38,41] The breaks are repaired by NER and HR pathways.[38,41]

Vinca alkaloids (e.g. vincristine and vinblastine) are microtubule destabilizers that bind preferentially to free tubulin, but also at the end of the microtubule.[23,42] As a result, formation of a functional spindle during mitosis is impaired. This results in mitotic delay, a state that cannot be sustained.[42,43] Consequently, the cell undergoes cell death or escapes the mitotic arrest via mitotic slippage.[42-44] Prolonged mitotic arrest can lead to DNA damage.[45,46] In addition, chromosome segregation errors can occur, [46,47] which in itself can cause chromosome breaks. [48] Furthermore, emerging evidence indicates that antimitotic drugs can also induce DNA strand breaks.[46,47] The cell can respond to these types of DNA damage, at least in some situations, by p53 induction, cell cycle arrest or apoptosis.[46,47,49]

Platinum (Pt)-containing compounds (e.g. carboplatin) form Pt-DNA adducts resulting in intra- and inter-strand cross-links between adjacent nucleobases.[50-52] This leads to a distortion of the DNA-structure.[50,52] As a result, DNA-adducts block DNA replication and transcription, ultimately leading to cell division arrest and cell death.[50-52] Pt-DNA adducts can be repaired by the following pathways: NER, MMR, TLS and HR.[53,54] The cell may eventually resolve unrepairable DNA-damage by inducing cell death.[53,54]

Though limited, studies investigating the genotoxic effects of antineoplastic drugs used as single agents in human patients have been reported. For example, chlorambucil induces a significant increase in sister chromatid exchanges after single agent therapy (number of patients: 10).[55] Sanderson et al. (2001) [56] showed significant increases in the frequency of mutant 6-thioguanine-resistant peripheral blood lymphocytes, measured at several time points after administration of single agent cyclophosphamide in four out of six multiple sclerosis patients, using each patient as their own control. A significant increase in nine oxidatively modified DNA bases in peripheral blood mononuclear cells of 15 cancer patients was observed after administration of single agent doxorubicin.[57]

Table 2 Harmful effects associated with the antineoplastic drugs commonly used in veterinary oncology

	Mode of action		Genotoxicity	Carcinogenicity	Teratogenicity	Male gonadal dysfunction	Female gonadal dysfunction	*Ref*
CBL	Alkylation [58]	In vitro:	+[a]	+[a]	+[a]	?	?	*59-61*
		Rodents:	+[a]	+[a]	+[a]	+[a]	+[a]	*62-67*
		Men:	+[b d f]	+[a c] / -[a]	+[g] / -[g]	+[c]	+[b g]	*55,59,68-79*
CP	Alkylation [58]	In vitro:	+[a]	+[a]	+[a]	+[a]	+[a]	*80-85*
		Rodents:	+[a]	+[a]	+[a]	+[a]	+[a]	*63,86-91*
		Men:	+[a f]	+[b d e]	+[g]	+[c]	+[a b]	*56,73,92-100*
CCNU	Alkylation [101]	In vitro:	+[a]	+[a]	+[c]	?	?	*60,102,103*
		Rodents:	+[a]	+[a]	+[a] / -[a]	+[a]	+[a]	*104-107*
		Men:	+[c f]	+[d]	?	+[d]	+[b]	*69,73,99,108-110*
Doxo	Topoisomerase II inhibition [33] and DNA adduct formation [38]	In vitro:	+[a]	+[a]	+[a]	+[a f]	+[a]	*80,111-114*
		Rodents:	+[a]	+[a] / -[a]	+[a]	+[a]	+[a]	*32,115-119*
		Men:	+[a d f]	+[d]	+[c g] / -[d]	+[c f] / -[c]	+[c f] / -[c]	*57,68,113,120-127*
Epi	Topoisomerase II inhibition [33] and DNA adduct formation [39]	In vitro:	+[a]	+[a]	+[a]	?	?	*128-130*
		Rodents:	+[a]	+[a] / -[a]	+[h]	+[a]	?	*131-134*
		Men:	+[b f]	+[b]	+[c g] / -[a]	+[c]	+[c d] / -[c]	*125,135-141*

Table 2 Continued

	Mode of action		Genotoxicity	Carcinogenicity	Teratogenicity	Male gonadal dysfunction	Female gonadal dysfunction	*Ref*
MTN	Topoisomerase II inhibition [142] and DNA adduct formation [40]	In vitro:	+[a]	+[a]	?	?	?	*60,143*
		Rodents:	+[a]	?	?	+[h]	?	*144,145*
		Men:	+[a c f]	+[a c]	+[g] / -[a]	+[c]	+[a]	*146-152*
VBL	Interaction with cytoskeleton [23]	In vitro:	+[a]	+[a]	+[a]	+[a]	+[a]	*153-157*
		Rodents:	+[a]	-[a]	+[a]	+[a] / -[a]	+[a]	*86,158-162*
		Men:	+[c f]	+[c]	-[c]	+[c d]	+[e]	*163-169*
VCR	Interaction with cytoskeleton[23]	In vitro:	+[a]	+[a]	+[a]	+[a]	+[a]	*60,170-174*
		Rodents:	+[a]	?	+[a]	+[a]	+[a]	*86,175-177*
		Men:	+[c f d]	+[c]	-[c]	+[c d]	+[d] / -[c]	*108,121,152,166,178-181*
CaPt	Covalent DNA binding [182]	In vitro:	+[a]	+[a]	?	+[a]	?	*183-185*
		Rodents:	+[a]	?	+[a] / -[a]	+[a]	+[a]	*186-189*
		Men:	+[a b f]	+[b] / -[a]	-[g]	+[a]	+[c]	*138,186,190-194*

+: results showing an effect; -: results showing no effect; +/-: contradicting results; ?: no studies were found; [a]: compound is used as single agent; [b]: compound is a strong independent risk factor; [c]: effect after combination therapy; [d]: effect after combination therapy with one or more alkylating agents; [e]: effect after combination therapy with RT; [f]: in human cells (in vitro); [g]: case report; [h]: little information on statistical or clinical significance
CaPt: carboplatin; CBL: chlorambucil; CCNU: lomustine; CP: cyclophosphamide; Doxo: doxorubicin; Epi: epirubicin; MTN: mitoxantrone; VBL: vinblastine; VCR: vincristine

Carcinogenic effects of antineoplastic drugs at high-dose exposure

Whenever the DDR fails, this may result in incorporation of DNA lesions into the genome. DNA damage is necessary for tumor initiation, but more is needed for tumor promotion. Hanahan and Weinberg (2000 and 2011) [195,196] propose that most types of human tumors are a result of six essential modifications in cell physiology: self-sufficiency in growth signals, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (see Figure 1). According to Vineis et al. (2010) [197] DNA changes are important, but may be secondary epiphenomena, whereas tissue disruption is critical.

Since combination chemotherapy protocols are generally used, establishing a correlation between tumorigenesis in humans and administration of a specific antineoplastic drug has been difficult. Still, some antineoplastic drugs have provided evidence supporting a carcinogenic effect in people when used in single agent therapy. For example, single agent chlorambucil is clearly correlated with acute leukemia in men.[198] Furthermore, cyclophosphamide is a strong, independent risk factor for bladder tumors in patients treated with combination chemotherapy, resulting in an approximately four-fold increase in risk.[100] Additionally, topoisomerase II inhibitors are correlated with specific chromosomal aberrations, such as translocations to 21q22, inv(16), t(15;17), and t(9;22) chromosome bands,[199] in therapy-related acute myeloid leukemia and myelodysplasia.

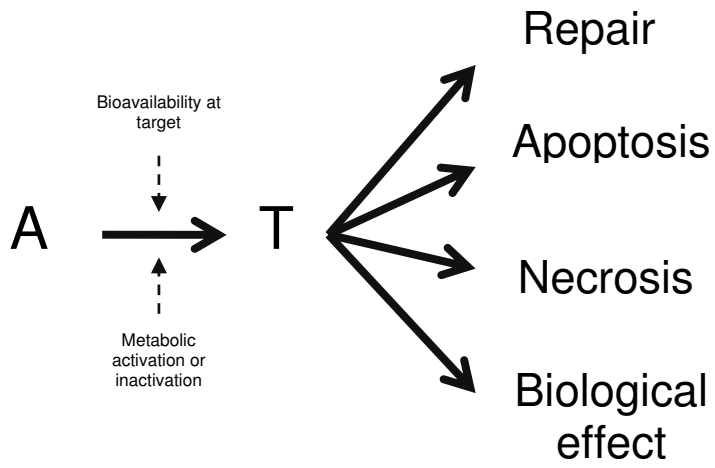

Figure 1 Schematic presentation of the interaction between a genotoxic agent (A) and its target (T)

Embryotoxic effects of high-dose exposure to antineoplastic drugs

In utero exposure of embryo's to antineoplastic drugs may lead to direct and indirect genotoxic effects, induction of somatic mutations, carcinogenesis, teratogenesis, induction of functional deficits (e.g. neurological deficits) and embryolethality.[200] The DDR consists of DNA damage detection, several DNA repair systems (e.g. BER, NER, MMR, DBS repair) and cell cycle checkpoints and apoptosis.[201] However, the expression of the DDR varies between developmental stages. When exposure occurs during the preimplantational stage, this generally results in embryolethality.[200,202] During implantation or organogenesis, chemical exposure can lead to congenital malformations.[200,202] Alternatively, exposure during the fetal period may lead to induction of functional deficits or cancer.[200,202] Furthermore, the type of compound and placental transfer are of importance as well.[203] Literature on transplacental passage of antineoplastic drugs is scarce. Van Calsteren et al. (2010, 2011) [204-206] investigated transplacental passage in mice and baboon models. In mice, the fetal plasma concentrations for doxorubicin (dose: 9 mg kg^{-1}), epirubicin (dose: 11 mg kg^{-1}), vinblastine (dose: 6 mg kg^{-1}) and carboplatin (dose: 50 mg kg^{-1}) averaged 5% (n = 8), 5% (n = 8), 14% (n = 6) and 117% (n = 6) of maternal plasma concentrations, respectively. Fetal plasma concentrations of doxorubicin (dose: 25-50 mg m^{-2}), epirubicin (dose: 100-200 mg m^{-2}), vinblastine (dose: 6-12 mg m^{-2}), 4-hydroxy-cyclophosphamide (dose cyclophosphamide: 500-1000 mg m^{-2}) and carboplatin (target area under curve (AUC) 6) averaged 8% (n = 6), 4% (n = 8), 19% (n = 9), 25% (n = 6) and 58% (n = 7) of maternal plasma concentrations in baboons, respectively. Doxorubicin, epirubicin and vinblastine could not be detected in cerebrospinal fluid, while the concentration of 4-hydroxy-cyclophosphamide measured in cerebrospinal fluid of one baboon fetus was approximately 63%. In humans, Karp et al. (1983) [207] reported fetal tissue levels of doxorubicin to be 10 times higher than the maternal plasma concentration 15 h after administration (dose: 25 mg m^{-2}, therapeutic abortion) in liver, kidney and lung, but not in brain, intestines or muscle. Others could not detect doxorubicin in amniotic fluid, fetuses or cord blood plasma.[208,209] Arango et al. (1994) [210] found a cisplatin concentration of 40 μg L^{-1} in blood of a neonate (on the first day of life), three days after administration to the mother (Dose: 100 mg m^{-2}).

Embryotoxic effects of antineoplastic drugs in humans are mostly described in case reports.[98,138,151,167,203,211,212] Nonetheless, evidence suggests that administration of antineoplastic drugs during pregnancy is associated with increased frequency of embryotoxic effects, mainly during the first trimester of gestation. The following embryotoxic effects have been reported: spontaneous abortion,[123,138,213] stillbirth,[123,138] neonatal death,[138] malformations,[123,138,214] fetal complications,[123] and prematurity.[123,138]

Male gonadotoxic effects of high-dose exposure to antineoplastic drugs

Spermatogonial stem cells show a high degree of mitotic activity, resulting in an increased susceptibility for DNA lesions due to DNA replication errors.[29] However, other

spermatogenic cells (e.g. secondary spermatocytes, spermatids, spermatozoa) do not synthesize DNA. Yet, high levels of DNA damage have been detected in these cells after genotoxic insult. This is expected to be the result of different processes. Some essential cellular events are lost during spermatogenesis (e.g. apoptosis).[29] Alterations of translation and/or transcription of DNA repair genes may occur.[29] Moreover, in elongating spermatids condensing of the chromatin restricts the access of DNA repair proteins to the DNA, limiting the ability to repair a damaged genome.[215] The most common types of DNA lesions arising during spermatogenesis are: loss of a base, chemical alteration of a base, single or double strand breaks, intra- and inter-strand cross-links and protein-DNA cross-links.[216] Human male germ cells use several strategies to repair damaged DNA, namely BER, NER, MMR, HR, NHEJ and PRR.[29,215] Additionally, if gametogenic DNA repair was unable to resolve the DNA lesions, or when the damage occurred in spermatozoa, the lesions may be repaired during fertilization (before the zygote is formed).[215] However, when a damaged genome remains unrepaired, this may result in a decrease of conception rates, impairment of embryonic development, increase in number of spontaneous abortions, impairement of growth and development of offspring, and an increased incidence of childhood carcinogenesis.[29,216]

Antineoplastic drugs may induce male gonadal dysfunction at all stages of life in men.[217] Some of these drugs can damage sensitive spermatogonial stem cells, resulting in temporary oligozoospermeria or azoospermia.[217] When the damage is severe, the pool of spermatogonial cells may be depleted completely, resulting in permanent infertility. Mild and transient male gonadal dysfunction has been reported in men after carboplatin single agent therapy.[193] The authors measured follicle-stimulating hormone (FSH) levels before treatment (n = 44) and up to 12 months after end of treatment (n varies between 32 at five weeks and eight at 12 months, dose: 400 mg m^{-2}). The FSH levels returned to normal at 12 months after end of treatment. No others studies reporting male gonadotoxicity associated with single agent chemotherapy protocols could be traced. However, the replacement of alkylating agents by nonalkylating agents in chemotherapy protocols results in a clinically significant reduction of gonadal dysfunction in men. For example, van der Kaaij et al. (2007) [125] reported an 8% increase in FSH level, when men were treated with nonalkylating chemotherapy protocols (doxorubicin, bleomycin, vinblastine, dacarbazine [ABVD-protocol]; epirubicin, bleomycin, vinblastine, prednisone [EBVP-protocol]; n = 101) In contrast, men treated with alkylating chemotherapy regimens (mechlorethamine, vincristine, procarbazine, prednisone [MOPP-protocol]; MOPP with doxorubicin, bleomycin, vinblastine [MOPP/ABV-protocol]; bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone [BEACOPP-protocol]; n = 192) demonstrated a 60% elevation of FSH concentrations ($P < 0.001$). Moreover, the recovery of fertility occurred in 82% of patients treated with nonalkylating protocols, while recovery occurred in about 50% of the men treated with alkylating regimens ($P < 0.001$), with a median recovery time of 18 and 27 months after end of treatment, respectively (ABVD and

EBVP: n = 107; MOPP, MOPP/ABV and BEACOPP: n = 185). In addition, exposure of male mice to antineoplastic drugs (e.g. cyclophosphamide: dose: 150 mg/kg and n = 550) has resulted in transgenerational genome-wide destabilization, as evidenced by increased rates of somatic and F_1 germ-line mutations.[218] Elevated mutation rates in F_1 germ-line has also been demonstrated after paternal exposure to ethylnitrosourea in mice (dose: 150 mg/kg, n = 403).[219]

Female gonadotoxic effects of high-dose exposure to antineoplastic drugs

The most important causes of DNA lesions in oocytes are loss of a base, base modification, single or double strand breaks, and inter-strand cross-links.[220] The human oocyte uses several mechanisms to repair a damaged genome: direct repair, BER, NER, MMR, HR and NHEJ.[29,220] In addition, mammalian female germ cells have cell cycle check points at their disposal.[29] Finally, if the DDR falls short, apoptosis may occur.[29,220] There are three main stages of susceptibility during oocyte development: fetal development, birth and puberty.[221] Exposure to chemicals during these stages may lead to impairment of oocyte growth and development. In addition, the zygote's ability to repair DNA after fertilization is dependent on the DNA repair proteins of maternal origin.[201,220]

In women, exposure to antineoplastic drugs may damage maturing follicles and cause ovarian fibrosis.[222,223] As a result, FSH secretion increases, inducing additional follicles to enter the maturation pathway. Recurrence of chemotherapy administration may eventually lead to depletion of the ovarian reserve. Female gonadal dysfunction can therefore be characterized by reversible, partial or permanent amenorrhea, hormonal disturbance without amenorrhea, subfertility or infertility.[222,223] The likelihood of female gonadotoxicity is influenced by the type of compound, the cumulative dose and the age of the patient.[223] The ovarian reserve diminishes as the age of women progresses. Alkylating agents (e.g. cyclophosphamide and chlorambucil) are associated with the highest risk of induction of ovarian toxicity.[223] For example, 26 % of women treated with, in general, single agent chlorambucil developed amenorrhea after treatment (n = 27, age: 6-75 years, mean duration of follow-up: 4.3 years).[79] Appenzeller et al. (2008) [224] reported persistent amenorrhea in 18% and transient amenorrhea in 12% of women treated with single agent cyclophosphamide for systemic lupus erythematosus (SLE)(n = 57, dose: 0.75 mg/body surface area, group A). On the other hand, 20% of SLE patients treated with single agent cyclophosphamide at 0.5 mg m^{-2} reported transient amenorrhea, while no-one reported persistent amenorrhea (n = 50, group B). Furthermore, the control group consisting of women with SLE, but never treated with cyclophosphamide, did not report transient or sustained amenorrhea (n = 50). The mean age at start of treatment was 20 and 21.8 years for groups A and B respectively, while the controls had a similar age distribution. In addition, single agent mitoxantrone has been correlated with transient and persistent amenorrhoea (respectively 27% and 17.3% of 391 patients, age: 45 years or younger).[151] Moreover, exposure to antineoplastic drugs may also

effect the next generations of offspring, as evidenced by exposure of female mice to doxorubicin (dose: 10 mg/kg, n = 237) by Kujjo et al. (2011).[117] The authors described chromosomal abnormalities, malformations, and increased rates of neonatal death, especially in the fourth and sixth generation.

Effects of occupational exposure to antineoplastic drugs

We can state that all of the antineoplastic drugs explored in this review exert genotoxicity, carcinogenicity, embryotoxicity and/or gonadotoxicity to varying degrees of severity, with cancer being the most fatal side-effect. These results are, however, obtained from studies in which the subjects were patients, thus exposed to high amounts of one or more of these agents. In veterinary oncology, on the other hand, people are exposed for longer or shorter periods at a time to relatively low levels of antineoplastic drugs, via handling of antineoplastic drugs, treated pet-animals or associated wastes.

Acute health effects of occupational exposure to antineoplastic drugs

Acute health effects in workers, primarily nurses, exposed to antineoplastic drugs, have been reported in the literature. These include general systemic symptoms (e.g. hair loss, malaise), allergic reactions, symptoms of infection (e.g. fever), acute irritation (e.g. eye irritation, skin rash), gastrointestinal symptoms (e.g. nausea, diarrhea), respiratory effects (e.g. cough, burning sensation in the respiratory system), cardiac symptoms (chest pain, elevated blood pressure), musculoskeletal problems (e.g. lumbago), and central nervous system symptoms (e.g. headaches, dizziness).[225,226] Valanis et al. (1993) [225] described significant associations between skin contact in nurses and a small increase in acute symptoms regarding general, allergic, gastrointestinal, infectious and cardiac effects ($p < 0.05$, n with skin contact = 1,063; n without skin contact = 283, n controls = 738). Skin contact during handling of excreta of patients was also significantly associated with these acute health effects, though this might not be the result of contact with antineoplastic drugs as such. Constantinidis et al. (2010) [226] found that the number of antineoplastic drugs handled and spillage accidents was positively correlated with several health effects such as skin irritation, respiratory problems, and adverse effects on the central nervous system ($P < 0.05$, n = 353). However, the authors did not report using a control group. The evidence is insufficient to determine the risk of acute effects associated with exposure to antineoplastic drugs.

Genotoxic effects of occupational exposure to antineoplastic drugs

As discussed in the first part of this review, increases in the presence of biomarkers such as chromosomal aberrations and micronuclei has been a rather consistent finding for the past 30 years in workers occupationally exposed to antineoplastic drugs.[14]

Embryotoxic and gonadotoxic effects of occupational exposure to antineoplastic drugs

Adverse reproductive outcomes (e.g. infertility, spontaneous abortions and congenital malformations) and poor neonatal outcome (e.g. premature delivery and low birth weight) have been reported when workers are occupationally exposed to antineoplastic drugs (see Table 3), although not all studies reported significant associations with the handling of antineoplastic drugs or associated wastes. Furthermore, Shortridge et al. (1995) [227] found a correlation between current handling of antineoplastic drugs and menstrual dysfunction in nurses between 30 – 45 years old (prevalence odds ratio (OR): 1.6; CI: 1.3 – 1.9; n = 1,458). Constantinidis et al. (2010) [226] also reported disorders of the menstrual cycle in nurses exposed to antineoplastic drugs (20-23%, n = 353, mean age: 35.4 ± 6.92 years), though no correlation with the handling of these compounds or the use of a control group was described. Quansah et al. (2010) [228] reviewed observational studies that investigated the association between reproductive outcomes and occupational exposure to antineoplastic drugs in female nurses, reported between 1966 and 2009. After analyzing the data, the authors reported a moderate and consistent association between spontaneous abortion and occupational exposure to antineoplastic drugs (summary OR: 1.35; 95% CI: 0.91-2.01).[228] This is in agreement with the meta-analysis of Dranitsaris et al. (2005) [229] who reported a small increased risk for spontaneous abortion in female staff exposed to these compounds (OR: 1.46, 95% CI: 1.11 – 1.92). Quansah et al. (2010) [228] did not find a significant publication or small study bias. In conclusion, the risk of spontaneous abortion may be increased when low-dose exposure to antineoplastic drugs occurs.

Table 3 Adverse reproductive outcomes associated with occupational exposure to antineoplastic drugs

Adverse reproductive outcome	Positive association (statistically significant)	No association
Reduced fertility	*230*	
Ectopic pregnancy	*231*	
Spontaneous abortion	*228,229,232-235*	*226,236-239*
Still birth		*232,236,240*
Congenital malformation	*237,240*	*236,238,240*
Premature delivery	*236*	*238,240*
Reduced birth weight	*236*	*240*

Carcinogenic effects of occupational exposure to antineoplastic drugs

Evidence emerges, substantiating the correlation between genotoxic responses and cancer risk.[241-243] However, it is still difficult to establish an association between low-dose exposure and cancer. The literature on tumorigenesis in workers occupationally exposed to antineoplastic drugs (e.g. nurses in human oncology) is scarce. We traced 3 studies, each focusing on nurses and reporting a significant association between cancer and occupational exposure to antineoplastic compounds.[238,240,244] However, the number of patients described in these studies are small. Skov et al. (1992) [238] based their evaluation on 2 cases, while Gunnarsdottir et al. (1997) [244] used 7 cases. Ratner et al. (2010) [240] observed a significant elevated risk for rectal cancer (14 cases) and a non-significant elevated risk for breast cancer (87 cases). They did not find a significant association with leukemia (3 cases) or other types of cancer, nor did they find an elevated risk between 'all cancer types' and occupational exposure. Taken together, there is insufficient evidence to determine the risk of cancer after occupational exposure to antineoplastic drugs.

In conclusion, we can confirm that high dose exposure to the antineoplastic drugs described in this review can induce one or more adverse health effects (e.g. cancer and embryotoxicity). Long-term exposure of workers to relatively low doses of these drugs, has been associated with acute health effects, adverse reproductive outcomes and cancer, although validity issues related with most observational studies limit the significance of these associations. Consequently, a question naturally arises: what are the risks associated with these harmful health effects?

Risk assessment of occupational exposure

There is no simple correlation between exposure to an antineoplastic drug and health risks. The development of cancer is a very complex and only partly understood process, with both environmental and genetic factors contributing to its induction. Furthermore, several aspects of occupational exposure complicate any possible correlation with cancer or other adverse health effects. Moreover, the methodological problems of the majority of the reported observational studies on effects of occupational exposure to antineoplastic drugs, limit the significance of the described associations.

Pathways to cancer

According to the single hit, single target theory, a single molecule of a mutagen (or a carcinogen) reaching its intracellular target (e.g. DNA) could induce mutation, cancer, fertility problems and embryotoxicity, when the damage is left unrepaired.[245] However,

cancer is relatively rare during an average human lifetime. One would expect on the basis of this theory, that cancer would have a much higher incidence. Even though a single molecule of a mutagen could induce cancer, it seems that most of the times more is needed. As mentioned before, Hanahan and Weinberg (2000 and 2011) [195,196] propose that most types of human tumors are a result of six essential modifications in cell physiology (see Figure 2).

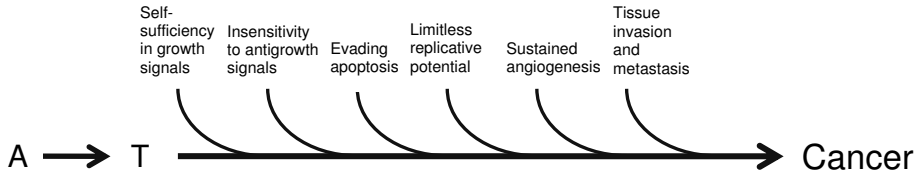

Figure 2 The six hallmarks of cancer, as described by Hanahan and Weinberg (2000, 2011),[195,196] with A: genotoxic agent and T: its target

The most fundamental characteristic of cancer cells is their ability to maintain proliferation. This is in part the result of their manipulation of production and release of growth-promoting signals.[196,246] The cancer cells can achieve this by producing the growth factor ligands themselves (autocrine stimulation), by stimulating cells in the tumor-associated stroma to produce growth factors (paracrine stmulation), by increasing the receptor proteins on the surface of the tumor cell making them hyperresponsive, by activating one or more of the signaling pathways downstream of these receptors, or by disrupting the negative feedback systems that regulate growth signal production. In addition, sustaining chronic proliferation also depends on the ability of the tumor cell to evade antigrowth signals.[196,247] Programmed cell death (e.g. apoptosis, necrosis) is part of the mechanisms ensuring normal tissue homeostasis. Cancer cells develop an array of strategies limiting or circumventing this, such as eliminating the sensor that signals overwhelming or irreparable damage in the apoptotic circuitry, increasing the expression of antiapoptotic regulators, or downregulating proapoptotic factors.[196,248] Furthermore, to be able to produce macroscopic tumors, cancer cells need to acquire unlimited replicative potential. Consequently, the tumor cells must overcome telomere shortening in order to avoid apoptosis and cell senescence (a nonproliferative but viable cell state).[196,249] Additionally, the development of macroscopic tumors requires sustenance (e.g. oxygen and nutrients) and the ability to dispose of carbon dioxide and metabolic wastes. This is provided by the neovasculature of the tumor. The sprouting of new blood vessels from existing ones (angiogenesis) is almost always activated during tumor progression.[196,247] Cancer cells achieve this by deregulating factors that induce or inhibit angiogenesis. Moreover, most types of human cancer spawn pioneer cells from primary tumor masses, during tumor development.[195] These pioneer cells invade

neighboring tissues, enter nearby blood and lymphatic vessels, and travel to distant sites in the body, where they may extravasate and create new colonies.[195,250] This involves activation of extracellular proteases and changes in the physical coupling of the tumor cells to their microenvironment. Hanahan and Weinberg (2011) [196] also describe two emerging hallmarks of cancer, namely: the ability to reprogram the cellular energy metabolism and the ability to escape immunological destruction. Furthermore, while the tumor develops, a distinct tumor microenvironment is created progressively which enables primary, invasive and metastatic growth.[196,197]

All in all, a multiplicity of changes at cellular and tissue level is required to allow malignant growth. Moreover, there are not only defense mechanisms preventing disruption of genomic and tissue integrity, but cells also have the DDR.[28,195] Thus, an affected cell can become cancerous, but can also repair DNA damage or succumb due to apoptosis or necrosis (see Figure 1). This multiplicity of defenses may explain why cancer does not occur as often as may be expected on the basis of the single hit, single target theory.

Aspects of occupational exposure

Low dose exposure

There is sufficient evidence that some antineoplastic drugs cause harmful effects at high doses, e.g. cancer and embryotoxicity (see Table 2). But occupational exposure is, in general, limited to relatively low levels. Regarding mutagenesis and carcinogenesis, public policies have assumed a cautionary attitude since the 1970s.[251,252] When considering low dose exposure, the linear extrapolation to low doses is advocated in dose-response models.[253] This linear model assumes that any dose is harmful and therefore all measures should be taken in order to avoid exposure as much as possible. This attitude is based more on qualitative assessment then on systematic measurement of effects at very low doses (quantitative assessment).[253,254] So far, little research has been performed to identify the shape of the dose-response curve for carcinogenesis, especially in the low dose range.[255] However, determining the nature of responses at low doses has its difficulties.[253,256] Measurements of exposures at workplaces are frequently close to the lower limit of quantification of analytical methods, and results obtained in this range are less precise. Additionally, there is a lack of statistical power in measuring small differences and quantifying effects at low levels. Simply put, the effects of long-term low dose exposure are largely unknown.

Limitations of studying occupational exposure

Since exposing people to varying levels of antineoplastic agents just for the sake of knowledge is unethical, environmental and biological monitoring studies rely on epidemiology. However, epidemiological studies have several inherent drawbacks.[257] In

epidemiology, identifying the exposure to more discrete widespread risk factors, analyzing the complex interaction between environmental exposure and genetic susceptibility of the host, and quantifying the relative contribution of different etiological agents is limited, if even possible. The reports mentioned earlier, describing acute effects, reproductive outcomes and cancer after occupational exposure to antineoplastic drugs, are based on observational studies. To allow interpretation and comparison of the results of observational studies a valid comparison group should be selected, sample sizes should be adequate, the definition of the critical window of exposure and the assessed end points should be homogenous among compared studies, and questions regarding the strength and consistency of the associations or potential confounding factors should be addressed.[228,258] Unfortunately, many observational studies reported in the literature, have one or more methodological problems.[228,258] For example, Quansah et al. (2010) [228] reviewed observational studies focusing on the correlation between occupational exposure of nurses to anesthetic gases, antineoplastic drugs and shift work, and adverse reproductive outcomes, reported between 1966 and 2009. They evaluated the studies in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[259] Of the 924 identified articles, only 25 met the inclusion criteria (e.g. English article, a case-control or cross-sectional or cohort design, sufficient information to estimate the association, original, focusing on female nurses and occupational exposures) and were included in their systematic review and meta-analysis. Of these, six articles investigated the correlation between reproductive outcome and occupational exposure of nurses to antineoplastic drugs. As described above, the authors found a moderate, consistent correlation between occupational exposure to antineoplastic drugs in nurses and spontaneous abortion.[228] Still, the significance of this association was limited due to the methodological issues of the reviewed studies. In many observational studies reported in the literature, inaccurate classification and assessment of exposure occurs.[228,258] The classification of outcomes is also an important methodological challenge.[228,258] Furthermore, acquiring sufficient sample sizes is often a major issue. [228,258] Because the strength of a study largely depends on its sample size,[228,258,260] especially exposures that occur in small disciplines, such as veterinary medicine, are not well covered by epidemiological research.

In addition, cancer is a complex disease. Consequently, an individual case can be the result of multifactorial causes.[261] As a result, most causal factors have relatively weak effects. Yet, our methods and study designs were developed to detect strong risk factors.[261] Moreover, cancer is a disease that has both genetic and environmental components and the investigated occupational risk factor is only one of many possible genotoxic agents.[260,261] However, at this moment we are not able to tease the multitude of causal relationships apart. Furthermore, when interpreting results from epidemiologic studies it should be kept in mind that population risks are estimations. Nevertheless, individual risks are often equated to population risks. Hence, these individual risks are treated as being high.

All these limitations complicate the interpretation of the data. Exposure to high doses of antineoplastic drugs can induce adverse health effects, but quantitative assessment of the effects of low dose exposure is rare and difficult to interpret. The multifactorial nature of cancer and the multiplicity of tissue and cellular defenses against genotoxicity and carcinogenicity further obscure potential causality. Besides, our methodology is actually not able to detect weak risk factors, as exposure to low doses of antineoplastic agents – among many other potentially mutagenic or carcinogenic agents – probably are. Even when a population risk can be estimated, interpretation is difficult. As a result, environmental and biological monitoring can reveal exposure and observe biological effects, but it cannot clarify what this means.

Conclusion

In conclusion, occupational exposure remains an issue in human and veterinary oncology. Environmental monitoring has demonstrated that surface contamination still occurs. Biological monitoring of workers exposed to antineoplastic drugs in human oncology revealed that uptake of these drugs continues to transpire as well, in spite of guidelines intended to prevent this. In addition, occupational exposure has been associated with induction of genotoxic responses (e.g. chromosomal aberrations, micronuclei). There are a lot of hurdles to be cleared in occupational risk assessment regarding exposure to antineoplastic drugs. Current knowledge is not able to quantify whether and how existent occupational exposure to antineoplastic drugs can lead to health risks. Science is, on the other hand, able to provide an insight into the dynamics of exposure within a (sub)-population. Furthermore, it is able to check whether adequate measures are taken.

Future perspectives

Veterinary cancer chemotherapy has been gaining popularity for the past few decades, and it is unlikely that this trend will stop. As a result, exposure to antineoplastic drugs is an inevitable risk for an increasing number of people. As long as antineoplastic drugs with potentially harmful effects are used while the risks involved with low dose exposure remain unknown, the environmental contamination with and uptake of these compounds should be kept at as low as reasonable achievable levels. Occupational exposure assessment (e.g. environmental monitoring and biomonitoring) is, therefore, crucial to evaluate exposure in veterinary oncology. This assessment should also focus on owners and other care-takers of pet-animals treated with antineoplastic drugs, since they are an exposed subpopulation as well. The findings of the exposure assessment could then be used to adjust the safe handling guidelines to the reality and needs of veterinary cancer chemotherapy.

Meanwhile, the complexities of cancer, and the limitations of epidemiological studies necessitate conceptual and methodological rethinking of (exposure) science, so that one day scientific knowledge might give a clear and straightforward answer to the question: what are the risks involved with long-term low dose exposure to antineoplastic drugs?

List of Abbreviations

AUC: area under the curve

ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine

BEACOPP: bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone

BER: base excision repair

DDR: DNA-damage response

EBVP: epirubicin, bleomycin, vinblastine, prednisone

FA: Fanconi anemia

FSH: follicle-stimulating hormone

HR: homologous recombination

MMR: mismatch repair

MOPP: mechlorethamine, vincristine, procarbazine, prednisone

MOPP/ABV: MOPP with doxorubicin, bleomycin, vinblastine

NER: nucleotide excision repair

NHEJ: non- homologous end joining

PRR: post replication repair

TLS: translesion DNA synthesis

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Chapter 2

Development and validation of bioanalytical assays

Chapter 2.1

Determination of platinum originating from carboplatin in human urine and canine excretion products by inductively coupled plasma-mass spectrometry

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Abstract

We present highly sensitive, rapid methods for the determination of Pt originating from carboplatin in human urine and canine urine, feces and oral fluid. The methods are based on the quantification of Pt by inductively coupled plasma mass spectrometry, and allow quantification of 7.50 ng L^{-1} Pt in human and canine urine (in 15 μL of matrix), 15.0 ng L^{-1} Pt in canine oral fluid (in 15 μL of matrix), and 0.105 ng g^{-1} Pt in canine feces (in 5 μg of matrix). Sample pretreatment mainly involved dilution with appropriate diluents. The performance of the methods fulfilled the most recent FDA guidelines for bioanalytical method validation. Validated ranges of quantification were 7.50 to $1.00x10^4$ ng L^{-1} Pt in human and canine urine, 0.105 to 30.0 ng g^{-1} Pt in canine feces and 15.0 to $1.00x10^4$ ng L^{-1} Pt in oral fluid. Canine urine and oral fluid cannot be easily obtained. Therefore, we also investigated the validity of the usage of human matrix samples for the preparation of calibration standards and quality control samples as alternatives, to be used in future clinical studies. The assays are used to support biomonitoring studies and pharmacokinetic studies in pet dogs treated with carboplatin.

Introduction

Carboplatin(*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) is a second generation Pt containing compound, that is markedly less emetogenic and toxic to the kidneys and the nervous system, as compared to the first generation compound cisplatin (*cis*-diamminedichloridoplatinum(II)).[1-4] One of the treatment strategies of solid tumors in companion animals is the administration of platinum (Pt) containing compounds as a single agent or in combination therapies.[3-7]

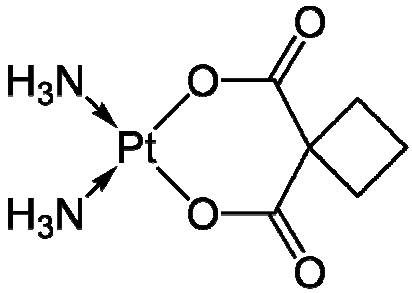

Figure 1 Molecular formula of carboplatin (Mw 371.249 g mol^{-1})

Little is known about the pharmacokinetics of carboplatin in dogs. Studies in dogs demonstrated the elimination of carboplatin via urine.[8,9] Furthermore, low amounts of cisplatin were found in bile following intraveneous administration.[10] Thus, we may assume that some excretion of carboplatin does occur via feces. As both cisplatin and carboplatin are found to be excreted in human saliva, salivary excretion of carboplatin in dogs is a plausible assumption.[11,12] The elimination of carboplatin might lead to the contamination of the environment in which the treated animal is held. Therefore, people, such as veterinarians, veterinary personnel, and owners of treated dogs, may come in contact with antineoplastic drugs via the animal or its surroundings. This has led to the development of guidelines for veterinary practices handling antineoplastic drugs and to the formulation of recommendations for the owners, regarding the handling of their treated pet, based on knowledge transferred from human oncology.[13-17] Thus far few studies have focused upon occupational exposure of veterinarians and assisting personnel to antineoplastic drugs.[18-20] To our best knowledge, no research has been performed regarding the exposure of owners and others in close contact with companion animals receiving chemotherapy.

To be able to evaluate the effectiveness of the protective measures described in the guidelines implemented in veterinary oncology, we have developed and validated highly sensitive and rapid ICPMS (inductively coupled plasma mass spectrometry) assays for the determination of Pt in human urine and canine excretion products. We opted for the development of assays using ICPMS due to its applicability to a wide variety of sample

matrices, including both biological and surface samples, and its enormous sensitivity.[21-26] ICPMS has shown a 2600-fold gain in LLOQ for carboplatin, compared to our former used graphite-furnace atomic-absorption-spectrometry (GF-AAS) methods.[27,28]

In this article we describe the development and validation of assays, according to most recent FDA guidelines on bioanalytical method validation.[29]

The clinical applicability of the assays was demonstrated by analysis of samples obtained from a canine patient treated with carboplatin.

Experimental

Chemicals

Carboplatin reference standard, used for preparation of calibration solution and quality control (QC) samples, was obtained from Calbiochem (San Diego, CA, USA). Iridium Chloride ICP standard, containing 1,000 mg L^{-1} iridium in 7% HNO_3, used for internal standardisation, was purchased from Merck (Darmstadt, Germany). Nitric acid (HNO_3) 70% Ultrex II ultrapure reagent was obtained from Mallinckrodt Baker (Philipsburg, NJ, USA). Ammonium EDTA and Triton X-100 (4-oxtylphenol polyethoxylate) were from Sigma-Aldrich (St. Louis, MO, USA). Water used for the ICPMS analysis was sterile water for irrigation (Aqua B. Braun Medical, Melsungen, Germany). A multi-element solution containing 10 mg L^{-1} of Ba, Be, Ce, Co, In, Mg, Pb, Th, Tl (VAR-TS-MS) in 5% HNO_3 was purchased from Inorganic Ventures/IV Labs (Lakewood, NJ, USA). Drug-free urine from healthy volunteers was used. Drug-free canine urine, feces and oral fluid from healthy dogs were used. Van Linde Gas Benelux (Schiedam, the Netherlands) provided argon gas (4.6) of 99.996% purity.

Instrumentation

Analyses were performed on an ICP-quadrupole-mass spectrometer (Varian 810-MS) equipped with a 90° reflecting ion mirror (Varian, Mulgrave, Victoria, Australia). The sample introduction system consisted of a Micromist glass low flow nebulizer (sample uptake 0.12 mL min^{-1}), a peltier-cooled (4°C) double pass glass spray chamber, a quartz torch, and a nickel sampler and skimmer cone (Varian). The spray chamber was cooled to reduce the vapour loading on the plasma, increasing the available energy for atomisation and ionisation of the elements of interest. Sample transport from the SPS-3 autosampler (Varian) to the nebulizer was perfomed using a peristaltic pump. The instrument was cooled by using a Kühlmobil 142 VD (Van der Heijden, Dörentrup, Germany). Data were acquired and processed using the ICPMS Expert Software version 1.1 b49 (Varian). Further data handling was performed using Excel 2003 (Microsoft, Redmond, WA, USA). All

measurements were carried out in a dedicated temperature-controlled, positively pressurised environment in order to maintain optimum instrument performance and minimise contamination. All solutions were prepared using plastic pipettes (VWR International B.V., Amsterdam, The Netherlands) and 10 mL (Plastiques-Gosselin, Hazebrouck Cedex, France) and 30 mL (Sarstedt AG&Co, Nümbrecht, Germany) polypropylene tubes. Prior to method development, all sample pretreatment devices were checked thoroughly for Pt contamination and appeared to be suitable for Pt analyses.

Determination of Pt by ICPMS

To optimise the ICPMS signal for the high masses and to reduce the formation of oxides and doubly charged ions, a solution containing 5,000 ng L^{-1} of Th, In, Ce, Ba, and Pt was used. Typically this 5,000 ng L^{-1} solution gave readings of ^{115}In: 2.40 x 10^6 counts per second (cps); ^{232}Th: 5.80 x 10^5 cps and ^{194}Pt: 1.60 x 10^5 cps. The production of CeO^+ was less than 1% of the total Ce^+ counts. The formation of doubly charged $[Ba]^{2+}$ was less than 2%. Instrument settings are summarised in Table 1. The performance was checked daily, using a multi-element solution. Other than a daily torch alignment, there was no need to tune any of the other instrumental parameters.

For the detection of Pt, three isotopes ^{194}Pt (abundance 33%), ^{195}Pt (33.8%) and ^{196}Pt (25.2%) were monitored[30]. All three monitored Pt isotopes can be subject to the interference of hafnium(Hf)-oxides[31]. But, because of low oxide formation and low observed Hf counts in all the analysed samples, these oxides were insignificant and no corrections were necessary. The interference of Hg on ^{196}Pt was corrected on-line by monitoring ^{202}Hg. In order to monitor unanticipated isobaric interferences, the ^{194}Pt /^{195}Pt and ^{196}Pt/^{195}Pt ratios were measured for all samples. When ratios were similar to those reported for natural Pt, it proved that the isotopic signals reflected the Pt content of the sample with no other spectral interference. The Pt isotope used for calculation of the validation parameters was ^{194}Pt. The detection mode for all isotopes was based on peak jumping with peak dwell times of 50 ms, 25 scans per replicate and three replicates per sample. The total measurement time for one sample during validation procedures was 3.30 minutes.

Iridium (Ir) was used as internal standard. It is expected that, because of its similar mass and ionisation potential, the behaviour of Ir will accurately reflect that of Pt in a way that it will respond similar to matrix effects and possible plasma fluctuations. Internal standardisation was performed on each replicate using ^{191}Ir. Quantification was based on the mean concentration of three replicates analysed against a calibration curve (as described below) using weighted linear regression analysis.

Table 1 ICPMS instrument settings

Flow parameters (L min^{-1})	
Plasma flow	18.0
Auxiliary flow	1.65
Sheath gas	0.25
Nebuliser flow	1.05
Torch alignment (mm)	
Sampling depth	5.00
Ion optics (volts)	
First extraction lens	-12.0
Second extraction lens	-220
Third extraction lens	-230
Corner lens	-240
Mirror lens left	37.0
Mirror lens right	35.0
Mirror lens bottom	20.0
Entrance lens	5.00
Fringe bias	-3.00
Entrance plate	-50.0
Detector focus	-500
Pole bias	0.00
Other	
RF power (kW)	1.30
Pump rate (mL min^{-1})	0.28
Stabilization delay (s)	40.0

Preparation of reagents

A 1% (*v*/*v*) HNO_3 solution in water, used for primary urine and fecal homogenate dilutions, was prepared. A 0.01% (*g*/*v*) ammonium EDTA and Triton X-100 mixture in water (0.01% EDTA/Triton X) was prepared for primary dilutions of oral fluid samples. Drug-free fecal homogenates were prepared (1:3, feces:water). Solutions containing 1% *(v/v)* drug-free human urine in 1% HNO_3, 1% *(v/v)* drug-free canine fecal homogenate in 1% HNO_3 and 1% *(v/v)* drug-free human oral fluid in 0.01% EDTA/Triton X were prepared for preparation of calibration standards and for dilution of samples exceeding the upper limit of quantification. Reagents were prepared freshly before use.

Preparation of stock solutions, calibration standards, and quality control samples

For this method, samples were diluted to reduce contamination of the sample introduction system and to minimize matrix effects. As a compromise between high sensitivity of the method and low contamination of the sample introduction system, we chose to dilute the samples 100-fold. A carboplatin stock solution containing 400 mg L^{-1} Pt in water was

prepared to obtain working solutions with concentrations ranging from 50.0 to 5.00x10^3 ng L^{-1} Pt in 1% HNO_3. For human and canine urine, working solutions were diluted with drug-free human urine:1% HNO_3 (1:100, *v*/*v*) to obtain calibration standards ranging from 75.0 to 1.00x10^4 ng L^{-1} Pt in human urine (corresponding to 0.75 to 100 ng L^{-1} Pt in 1:100 *v*/*v* diluted matrix). For canine feces, working solutions were diluted with drug-free fecal homogenate:1% HNO_3 (1:100, *v*/*v*) to obtain calibration standards ranging from 1.05 to 30.0 ng g^{-1} Pt in canine feces (corresponding to 0.0105 to 0.30 ng g^{-1} Pt in 1:100 *v*/*v* diluted matrix). For oral fluid, 1% HNO_3 was not chosen as a diluent, because it can precipitate proteins, and thereby block the nebulizer. Therefore, working solutions were diluted with drug-free human oral fluid:0.01% EDTA/Triton X (1:100, *v*/*v*) to obtain calibration standards ranging from 150 to 1.00x10^4 ng L^{-1} Pt in oral fluid (corresponding to 1.50 to 100 ng L^{-1} Pt in 1:100 *v*/*v* diluted matrix). Calibration standards were analysed without further dilution.

Another carboplatin stock solution, prepared from a separate weighing, was diluted with water in order to obtain working solutions with Pt concentrations ranging from 500 to 5.00x10^6 ng L^{-1}. Drug-free human and canine urine, were spiked with these working solutions to obtain quality control (QC) samples at four concentration levels (100, 225, 1.00x10^3, and 7.00x10^3 ng L^{-1} Pt). Drug-free canine fecal homogenates were also spiked at four concentration levels (1.05, 1.50, 3.00, and 21.0 ng g^{-1} Pt). Drug-free human and canine oral fluid, were spiked with these working solutions to obtain QC samples at four concentration levels (150, 225, 1.00x10^3, and 7.00x10^3 ng L^{-1} Pt). QC samples were processed according to the sample pretreatment methods described below.

For all matrices, two additional concentration levels were prepared. The first (7.50 ng L^{-1} in human and canine urine, 0.105 ng g^{-1} in canine feces and 15.0 ng L^{-1} in canine oral fluid) was prepared to validate the ability to quantify samples below the lower limit of quantification (<LLOQ). These samples were diluted tenfold prior to analysis. The second (300 ng g^{-1} feces, 1.00x10^5 ng L^{-1} for the other matrices) was prepared to validate the ability to quantify samples originally exceeding the upper limit of quantification (>ULOQ). Prior to analysis, for human and canine urine and canine fecal homogenates, these samples were diluted 100-fold with 1% HNO_3 and then 100-fold with urine:1% HNO_3 (1:100, *v*/*v*) or 100-fold with fecal homogenate:1% HNO_3 (1:100, *v*/*v*). For human and canine oral fluid these samples were diluted 100-fold with 0.01% EDTA/Triton X and successively 100-fold with oral fluid:0.01% EDTA/Triton X (1:100, *v*/*v*).

An internal standard solution of 1.00x10^4 ng L^{-1} Ir was prepared from an Ir reference solution of 1,000 mg L^{-1}. To 2 mL calibration standard or diluted QC sample, 20 µL of internal standard solution was added (final internal standard concentration 100 ng L^{-1}).

Sample pretreatment

Human and canine urine samples were collected in 30 mL polypropylene tubes (Sarstedt AG&Co, Nümbrecht, Germany). Canine fecal samples were collected in 250 mL polypropylene vessels (Sarstedt AG&Co, Nümbrecht, Germany). Canine oral fluid samples were collected using the Salivette® system (Sarstedt AG&Co, Nümbrecht, Germany). All samples were stored at –20 °C until analysis. Prior to analysis, urine samples were thawed, whirl mixed and subsequently diluted 100-fold with 1% HNO_3 solution. Canine fecal samples were thawed, weighed, homogenised (1:3 feces:water) and whirl mixed. The samples were first centrifuged at 2,100 *g* for 5 min. The supernatant was centrifuged at 10,300 *g* for 5 min and subsequently diluted 100-fold with 1% HNO_3 solution. The resulting solution was filtered using a 0.2 μm filter (Sartorius minisart, Sarstedt). A procedure similar to the pretreatment of urine samples was followed for canine oral fluid samples. However, these were diluted with a 0.01% EDTA/Triton X solution and if necessary successively with a oral fluid: 0.01% EDTA/Triton X solution (1:100, *v*/*v*).

To each 2 mL diluted sample, 20 μL of internal standard solution was added. Subsequently, diluted samples were transferred to autosampler tubes.

Validation

For the human and canine urine assay, full validation according to the FDA guidelines[29] was, as applicable for ICPMS, performed. For canine feces and oral fluid, a partial validation was carried out. According to the FDA guidelines a partial validation is sufficient to test the method, when a change in matrix with the same analyte is concerned or when a change in species with the same matrix and the same analyte is concerned.

Matrix effect and effectiveness of internal standardisation

To test the effect of human urine and canine urine, feces and oral fluid on the detector response, the following matrices: 0, 0.5, 1, 1.5, 2, 5, 10 % human urine, canine urine, fecal homogenate and oral fluid in 1% HNO_3 or 0.01% EDTA/Triton X, depending on the matrix, were spiked with the same amount of carboplatin. The amount of Pt spiked into the different matrices was 5.00 ng L^{-1} for human urine, 40.0 ng L^{-1} for canine urine, 50.0 ng L^{-1} for canine oral fluid and 15.0 ng g^{-1} for canine feces.

Signals at *m*/*z* of 194 were monitored. Pt concentrations were calculated against a calibration curve in 1% human and canine urine, 1% canine fecal homogenate and 1% canine oral fluid in 1% HNO_3 or 0.01% EDTA/Triton X, depending on the matrix. The Ir signal was used as internal standard.

Table 2 Deviation from theoretical concentration (DEV %) for carboplatin standards in human urine and human oral fluid, and relative standard deviations (RSD %) (n=3) for calibration standards in human urine

Matrix	Nominal Pt concentration (ng L^{-1})	Pt concentration in final matrix after 1:100 dilution (ng L^{-1})	DEV (%) from nominal concentration	RSD (%)
Human urine	75.0	0.75	-0.51	0.03
validation	150.0	1.50	-1.96	0.10
of canine and	500.0	5.00	-3.41	0.31
human urine)	$1.00x10^3$	10.0	1.65	0.18
	$2.50x10^3$	25.0	2.40	1.87
	$7.50x10^3$	75.0	-1.65	2.07
	$1.00x10^4$	100	-1.45	2.24
Human oral fluid	150	1.50	0.83	[a]
validation	500	5.00	0.52	[a]
of canine oral fluid)	$1.00x10^3$	10.0	1.56	[a]
	$2.50x10^3$	25.0	1.72	[a]
	$5.00x10^3$	50.0	1.24	[a]
	$7.50x10^3$	75.0	-1.48	[a]
	$1.00x10^4$	100	0.76	[a]

[a] a partial validation was performed for canine oral fluid. Therefore no relative standard deviations for carboplatin standards was calculated

Limit of quantification

The analyte response at the LLOQ should be at least 5 times the response in a blank sample.[29] The acceptance criteria require that the LLOQ is determined with a precision less than 20% and that the mean value deviates no more than 20% from the actual value.[29]

Carry-over

To evaluate and minimise the effect of carry-over, we studied the signals of blank readings following the ULOQ calibration sample (30.0 ng g^{-1} Pt for canine feces and $1.00x10^4$ ng L^{-1} Pt for all the other matrices, diluted 100-fold) and we optimised the rinse-time.

Linearity

Some canine matrices cannot be easily obtained in large quantities, whereas the human matrices can be readily collected. Therefore, calibration standards were prepared using human urine, canine fecal homogenates and human oral fluid.

Seven non-zero calibration standards in a dynamic range of 75.0-$1.00x10^4$ ng L^{-1} of Pt in human urine, of 350-$1.00x10^4$ ng L^{-1} of Pt in canine fecal homogenate, and of 150-$1.00x10^4$ ng L^{-1} of Pt in human oral fluid were processed, diluted 100-fold, and single measurements

were performed. For the assays in human and canine urine, calibration samples were analysed in three separate analytical runs. As a partial validation was carried out for canine feces and oral fluid, calibration samples for these matrices were analysed in one analytical run. The FDA guidelines require deviations of within ±20% for the LLOQ and ±15% for other concentrations.[29]

Accuracy and precision

Accuracy and precision of the method were determined by assaying QC samples at six concentration levels, with different dilution factors. For human and canine urine, accuracy, and within- and between-run precision data were assessed by analysing five replicates of each sample in three analytical runs. For canine fecal homogenates and oral fluid, accuracy and within-run precision data were determined by analysing five replicates of each sample in one analytical run. Accuracy was expressed as a percentage of the nominal concentration and it had to be within 80-120% for the LLOQ and within 85-115% for the other concentrations. Within- and between-run precisions were calculated by analysis of variances (ANOVA) for each test concentration using the analytical run as the grouping variable. The precision should not exceed ±20% for the LLOQ and ±15% for the other concentrations.[29]

Table 3 Deviation from theoretical concentration (DEV %) for carboplatin standards in canine feces

Matrix	Nominal Pt concentration (ng g^{-1})	Pt concentration in final matrix after 1:100 dilution (ng L^{-1})[b]	DEV (%) from nominal concentration	RSD (%)
Canine feces	1.05	3.50	-4.14	[a]
	1.50	5.00	1.18	[a]
	3.00	10.0	3.86	[a]
	6.00	20.0	-1.41	[a]
	15.0	50.0	-2.84	[a]
	22.5	75.0	0.07	[a]
	30.0	100	1.20	[a]

[a] a partial validation was performed for canine feces. Therefore no relative standard deviations for carboplatin standards was calculated

[b] Calculation: Pt concentration in final matrix after 1:100 dilution (ng L^{-1})= nominal Pt concentration (ng g^{-1})*(1000/3) *(1/100)
(with 1000/3 equaling the amount of feces (in g) in 1 L fecal homogenate)

Inter-exchangeability of human and canine matrices

As canine urine and oral fluid cannot be collected in large quantities, we investigated the inter-exchangeability of human urine and oral fluid with the respective canine matrices for the preparation of QC samples. During the development of the assays, QC samples in both canine and human matrices were prepared, accuracy and precision were investigated and mean and relative standard deviations of both types of QC samples were compared.

Specificity

From six individual batches of drug-free human and canine urine, samples containing neither analyte nor internal standard (blank), and samples containing 7.50 ng L^{-1} Pt and 100 ng L^{-1} internal standard were prepared and diluted tenfold. These samples were prepared in order to determine whether endogenous compounds interfered at the masses selected for Pt or internal standard in the most concentrated matrix used (tenfold dilution). Similar solutions were prepared using six individual batches of drug-free canine fecal homogenate and oral fluid, however the samples containing Pt and internal standard were spiked with carboplatin in order to obtain a concentration of 0.105 ng g^{-1} and 15.0 ng L^{-1} Pt respectively. Ir was added in order to obtain a concentration of 100 ng L^{-1}. All samples were analysed in one analytical run. The signal of any interfering peak at *m*/*z* 194 in blank solutions was not allowed to exceed 20% of the response of the LLOQ standard. The response of any interfering peak at *m*/*z* 191 in the blank solution should not exceed 5% of the response of 100 ng L^{-1} internal standard. Accuracies of the samples spiked with Pt at the LLOQ standard level had to be within 80-120% of the nominal value.[29]

Cross analyte/internal standard interference test

Interference of the internal standard solution on the *m*/*z* 194 and interference of Pt on *m*/*z* 191 had to be assessed. Drug-free human and canine urine, canine fecal homogenate and oral fluid were spiked with carboplatin at ULOQ standard level and after a 100-fold dilution, the Ir signal at *m*/*z* 191 was monitored. The response of the interfering peak at *m*/*z* 191 should be less than 5% of the response of 100 ng L^{-1} internal standard. Drug-free human and canine urine, canine fecal homogenate and oral fluid were diluted 1:100, and spiked with 100 ng L^{-1} Ir. The response of the interfering peak at *m*/*z* 194 should be less than 20% of the response of the LLOQ standard.

Application of ICPMS assay

The methods described here are used to support clinical pharmacokinetic studies to monitor Pt originating from carboplatin in human urine and canine urine, feces and oral fluid. An example of the analysis of canine urine, feces and oral fluid samples of a pet dog treated with carboplatin, 300 mg/m^2 as a 30 min infusion, is described. The samples were collected on the day of administration up until day 3 postinfusion and processed according to the methods described above.

Table 4 Accuracy and precision for each quality control sample in human urine and canine urine and oral fluid (n=5)

Matrix	Nominal Pt concentration in matrix (ng L^{-1})	Dilution factor	Within-run precision (%)	Between-run precision (%)	Accuracy (%)
Human urine	7.50	10	9.70	0.00	106
	75.0	100	7.02	3.87	97.5
	225	100	3.26	4.57	98.0
	$1.00x10^3$	100	2.34	2.25	95.7
	$7.00x10^3$	100	2.61	10.73	97.2
	$1.00x10^5$	10	2.95	5.01	92.8
Canine urine	7.50	10	5.35	2.89	100
	75.0	100	5.80	0.00	99.7
	225	100	3.95	0.00	99.6
	$1.00x10^3$	100	2.63	1.63	97.9
	$7.00x10^3$	100	2.72	0.99	94.5
	$1.00x10^5$	10	3.03	1.74	96.3
Canine oral fluid	15.0	10	4.32	[a]	94.4
	150	100	3.71	[a]	99.6
	225	100	5.01	[a]	99.7
	$1.00x10^3$	100	2.20	[a]	102
	$7.00x10^3$	100	1.35	[a]	98.9
	$1.00x10^5$	10	1.99	[a]	100

[a] a partial validation was performed for canine oral fluid. Therefore no between-run precision was calculated

Results and discussion

Validation

Matrix effect and effectiveness of internal standardisation

Figure 2 shows the effect of the different matrices. Significant signal suppression occurs with increasing human urine concentration. Compared to 1% HNO_3, a 10% human urine solution caused an ion suppression of 30%. A similar effect was observed for canine urine. Compared to 0.01% EDTA/Triton X, a 10% canine oral fluid solution caused a signal suppression of 7%. Increasing canine fecal matter, on the other hand, does not have a significant effect on Pt signal. Compared to 1% HNO_3, a 10% canine fecal homogenate solution caused an ion suppression of 5%. Some signal suppression occurs with increasing canine oral fluid. For all matrices, the internal standard corrected well for the matrix effect.

Limit of quantification

Pt can be present in drug-free human and canine matrices. Several studies have detected Pt in human body fluids originating from humans who have never been treated with chemotherapeutic agents.[32-34] We thoroughly screened the different matrices used in these

assays for the presence of Pt before preparation of calibration standards and QC samples. The natural Pt background was found to be lowest in human and canine urine, while canine fecal homogenate rendered the highest background signals (data not shown). For human and canine urine the LLOQ of the assay was set at a Pt concentration of 75.0 ng L^{-1} when using a standard 100-fold dilution. For canine feces, the LLOQ of the assay was set at a Pt concentration of 1.05 ng g^{-1} when using a standard 100-fold dilution. For canine oral fluid, the LLOQ of the assay was set at a Pt concentration of 150 ng L^{-1} when using a standard 100-fold dilution.

Our standard procedure involved a 100-fold dilution to reduce contamination of the sample introduction system and cones. As the internal standard was able to correct for matrix influences, we could validate the analysis of samples which were only diluted tenfold against a calibration curve prepared using a 100-fold dilution, for human urine and canine urine, feces and oral fluid. A reduction of the dilution factor was done to lower the LLOQ to respectively 7.50 ng L^{-1} for human and canine urine, 0.105 ng g^{-1} for canine feces and 15.0 ng L^{-1} for canine oral fluid.

Signal-to-noise (S/N) ratios at the LLOQ level exceeded 5 during all experiments, which was in accordance with the requirement.[29] S/N ratios were dependent on the batches of the drug-free matrices used. The acceptance criteria, which required that the LLOQ was determined with a precision less than 20% and that the mean value deviated no more than 20% from the actual value, were easily met (see Table 2 and 3).[29]

Carry-over

A 55-s rinse time with 1% HNO_3 between two samples was required to avoid a memory effect from the preceding high concentration sample and to achieve a blank signal <20% of the LLOQ standard signal.

Linearity

In table 2 and 3, deviations for human urine, canine feces and human oral fluid and relative standard deviations (RSD) for human urine are presented. Deviations from the nominal concentration were between –4.14 and 3.86% for all concentration levels, which all met the requirements.[29] RSDs for the calibration samples in human urine were less than 2.24%. Correlation coefficients were higher than > 0.9998.

Accuracy and precision

Accuracy and precision data are summarised in Table 4 and 5. It can be seen that at all QC concentration levels, the data were within the limits for bioanalytical method validation.[29] The different dilution factors of the QC samples did not affect the performance of the method.

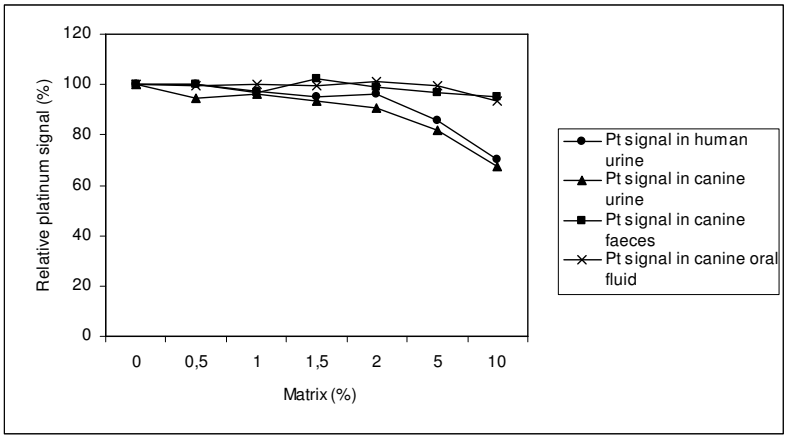

Figure 2 Matrix effect of human and canine urine, canine fecal homogenate and oral fluid. The left y-axis depicts ^{194}Pt signals relative to the signal of Pt in 1% HNO_3 or 0.01% EDTA/Triton X, depending on the matrix (● depicts Pt signal in human urine; ▲ depicts Pt signal in canine urine; ■ depicts Pt signal in canine feces; x depicts Pt signal in canine oral fluid)

Inter-exchangeability of human and canine matrices

The data summarised in Table 6 show that the canine and human QC samples are all inter-exchangeable. This allows us to use human urine and oral fluid for the preparation of calibration standards and quality control samples in future clinical studies.

Table 5 Accuracy and precision for each quality control sample in canine feces (n=5)

Matrix	Nominal Pt concentration in matrix (ng g^{-1})	Dilution factor	Within-run precision (%)	Between-run precision (%)	Accuracy (%)
Canine feces	1.05	10	2.62	[a]	106
	1.50	100	3.29	[a]	102
	3.00	100	1.72	[a]	98.1
	6.00	100	1.80	[a]	98.6
	15.0	100	0.98	[a]	102
	22.5	10	4.30	[a]	101

[a] A partial validation was performed for canine feces. Therefore no between-run precision was calculated

Specificity

Blank samples from six individual batches did not show interferences from endogenous material at the *m/z* selected for Pt with a response >20% of the LLOQ standard signal. The response of interfering peaks at *m/z* 191 did not exceed 5% of the response of 100 ng L^{-1}

internal standard. Deviations from the nominal concentrations at the LLOQ level were between –4.75 and 0.95% (data not shown).

Table 6 Mean and standard deviation for each quality control sample in human and canine biological matrix (n=5)

Matrix	Nominal Pt concentration in matrix (ng L^{-1})	Dilution factor	Mean Pt concentration in canine matrix (ng L^{-1})	Mean Pt concentration in human matrix (ng L^{-1})	Mean canine vs human matrixa (%)	RSD canine matrix[b] (%)	RSD human matrix[b] (%)
Urine	7.50	10	7.50	7.95	5.82	5.88	9.04
	75.0	100	75.0	73.0	2.20	5.60	7.74
	225	100	224	220	1.62	3.93	5.05
	$1.00x10^3$	100	$0.98x10^3$	$0.96x10^3$	2.28	2.97	3.02
	$7.00x10^3$	100	$6.61x10^3$	$6.79x10^3$	2.81	2.85	9.44
	$1.00x10^5$	10	$0.96x10^5$	$0.93x10^5$	3.73	3.36	5.16
Oral fluid	15.0	10	14.21	13.8	2.99	4.32	6.29
	150	100	149	147	1.61	3.71	5.74
	225	100	224	224	0.01	5.01	1.86
	$1.00x10^3$	100	$1.02x10^3$	$1.01x10^3$	1.26	2.20	2.64
	$7.00x10^3$	100	$6.92x10^3$	$6.96x10^3$	0.64	1.35	2.03
	$1.00x10^5$	10	$1.00x10^5$	$1.01x10^5$	1.17	1.99	1.96

[a] Means were compared using the following formula: (x-y)/y*100, with x= mean human matrix and y= mean canine matrix

[b] RSDs were calculated using: SD/mean*100

Cross analyte/internal standard interference test

The response of the interfering peak at *m/z* 194 was less than the maximum allowed 20% of the response of the LLOQ standard.

Application of ICPMS assay

The clinical applicability of the assays was demonstrated by analysis of canine urine, feces and oral fluid samples obtained from a pet dog treated with carboplatin. A Pt concentration versus time profile for Pt originating from carboplatin in canine urine, fecal homogenate and oral fluid is presented in Figure 3.

Conclusion

Highly sensitive ICPMS assays for the reliable and fast determination of Pt originating from carboplatin in human and canine urine, canine feces and oral fluid were developed. Subsequently, the assays were validated according to current FDA guidelines. The validated range was from 75.0 to $1.00x10^4$ ng L^{-1} Pt in human and canine urine, 1.05 to 30.0 ng g^{-1} Pt in canine feces and 150.0 to $1.00x10^4$ ng L^{-1}in canine oral fluid. The LLOQ was lowered from

75.0 to 7.5 ng L^{-1} for Pt in human and canine urine, from 1.05 to 0.105 ng g^{-1} for Pt in canine feces and from 150 to 15.0 ng L^{-1} Pt in canine oral fluid by including a tenfold dilution into the validation procedures. A dilution factor of 10^4 was validated, resulting in excellent accuracy and precision data. The assay is now successfully applied to support pharmacokinetic studies of pet dogs treated with carboplatin and biomonitoring studies of people in close contact with pet dogs receiving carboplatin.

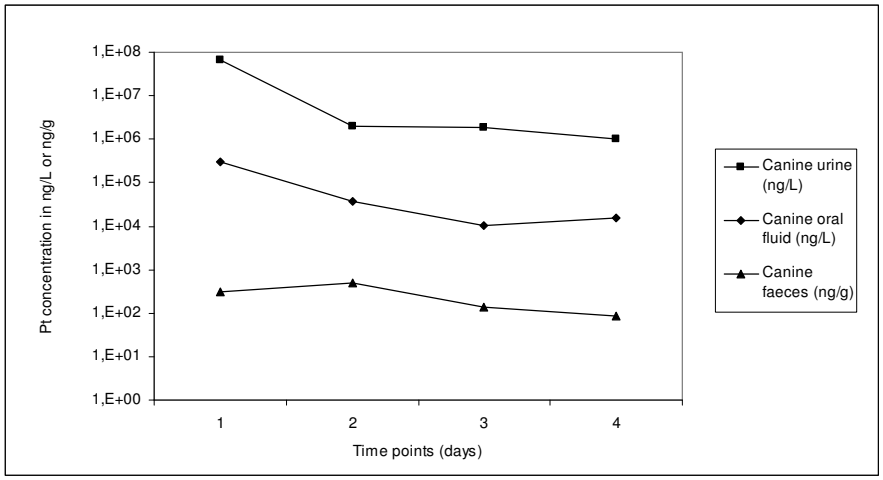

Figure 3 A Pt concentration versus time plot in urine, feces and oral fluid of a pet dog treated with carboplatin (300 mg/m^2) (■ depicts Pt concentration in ng/L in canine urine; ♦ depicts Pt concentration in ng/g in canine feces; x depicts Pt concentration in ng/L in canine oral fluid)

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Chapter 2.2

Determination of platinum originating from carboplatin in canine sebum and cerumen by inductively coupled plasma mass spectrometry

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Abstract

We present highly sensitive, reliable methods for the determination of platinum originating from carboplatin in canine sebum and cerumen. The methods are based on the measurement of platinum by inductively coupled plasma mass spectrometry and allow quantification of 0.15 pg platinum per cm^2 body surface in canine sebum and of 7.50 pg platinum per sampled ear canal. The sample pretreatment procedure involved extraction of wipe samples followed by dilution with appropriate diluents. The performance of the methods, in terms of accuracy and precision, fulfilled the most recent FDA guidelines for bioanalytical method validation. Validated ranges of quantification were 15.0 to $1.00x10^4$ ng L^{-1} for platinum in canine sebum extraction solution (corresponding to 15.0 pg per wipe sample or 0.15 pg cm^{-2}) and 7.50 to $1.00x10^4$ ng L^{-1} for platinum in canine cerumen extraction solution (corresponding to 7.50 pg per sampled external acoustic meatus). Canine matrices may not always be obtained in sufficient quantities. Therefore, we also confirmed the legitimacy of the application of human matrix samples for the preparation of calibration standards and quality control samples as alternatives, to be used in future clinical studies. The assays are used to support human biomonitoring studies and pharmacokinetic oncology studies in pet dogs treated with carboplatin.

Introduction

Pt containing compounds are an important class of chemotherapeutics. Carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato) platinum(II)) is a second generation Pt containing compound (Figure 1). Due to its less severe side effects as compared to cisplatin this compound is gaining popularity in veterinary oncology.

Several investigations have underlined the importance of skin contact as a route of exposure to hazardous substances, including antineoplastic drugs.[1-3] This has led to the development of guidelines for veterinary practices and owners, regarding the handling of pets treated with these drugs.[4-6] Questions are now arising about the justification of those guidelines and recommendations. Thus far it is not known which matrices should be of concern. Therefore, in addition to the assays we developed for the measurement of carboplatin in excretion products such as urine, faeces, and oral fluid, we now have developed assays to monitor the excretion of platinum originating from carboplatin in the less studied excretion products, sebum and cerumen, in dogs. Because contact with these excretion products is inevitable when handling animals, it is relevant to know the extent and duration of excretion of platinum originating from carboplatin in these matrices. To our knowledge, the excretion of Pt containing compounds in sebum and cerumen has never been monitored, neither in humans nor in companion animals.

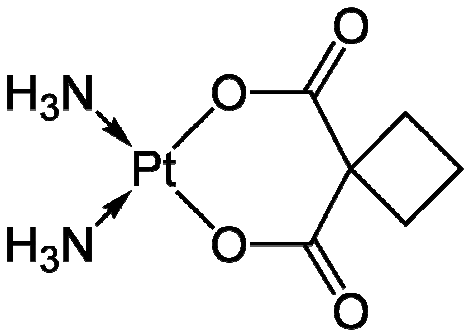

Figure 1 Molecular formula of carboplatin (Mw 371.249 g mol-1)

As carboplatin is a hydrophilic compound, we can assume that excretion via sebum and cerumen will yield lower levels as compared to urinary and fecal excretion. Therefore, the use of an ultra sensitive analytical technique such as Inductively Coupled Plasma Mass Spectrometry (ICPMS) is essential. ICPMS can be applied to a wide range of sample matrices including those of biological and environmental origin.[7-12] Moreover, sample pretreatment methods are usually straightforward and simple. For all these reasons usage of ICPMS and therefore monitoring of Pt originating from carboplatin seemed the most suitable approach.

In this article we describe the development and validation of ICPMS assays for platinum, according to most recent FDA guidelines on bioanalytical method validation.[13]

The clinical applicability of the assays was demonstrated by analysis of samples obtained from a pet dog treated with carboplatin.

Experimental

Chemicals

Carboplatin reference standard, used for preparation of calibration solution and quality control (QC) samples, was obtained from Calbiochem (San Diego, CA, USA). Iridium Chloride ICP standard, containing 1,000 mg L^{-1} iridium in 7% HNO_3, used for internal standardisation, was purchased from Merck (Darmstadt, Germany). Nitric acid (HNO_3) 70% and HCl 35% Ultrex II ultrapure reagent were obtained from Mallinckrodt Baker (Philipsburg, NJ, USA). Water used for the ICPMS analysis was sterile water for irrigation (Aqua B. Braun Medical, Melsungen, Germany). A multi-element solution containing 10.0 mg L^{-1} of Ba, Be, Ce, Co, In, Mg, Pb, Th, Tl (VAR-TS-MS) in 5% HNO_3 was purchased from Inorganic Ventures/IV Labs (Lakewood, NJ, USA). Drug-free human sebum from healthy volunteers was used. Drug-free canine sebum and cerumen from healthy dogs was used. Van Linde Gas Benelux (Schiedam, the Netherlands) provided argon gas (4.6) of 99.996% purity.

Instrumentation

Analyses were performed on an ICP-quadrupole-mass spectrometer (Varian 810-MS) equipped with a 90° reflecting ion mirror (Varian, Mulgrave, Victoria, Australia). The sample introduction system consisted of a Micromist glass low flow nebuliser (sample uptake 0.12 mL min^{-1}), a peltier-cooled (4°C) double pass glass spray chamber, a quartz torch, and a nickel sampler and skimmer cone (Varian). The spray chamber was cooled to reduce the vapour loading on the plasma, increasing the available energy for atomisation and ionisation of the elements of interest. Sample transport from the SPS-3 autosampler (Varian) to the nebuliser was perfomed using a peristaltic pump. The instrument was cooled by using a Kühlmobil 142 VD (Van der Heijden, Dörentrup, Germany). Data were acquired and processed using the ICPMS Expert Software version 1.1 b49 (Varian). Further data handling was performed using Excel 2003 (Microsoft, Redmond, WA, USA). All measurements were carried out in a dedicated temperature-controlled, positively pressurised environment in order to maintain optimum instrument performance and minimise contamination. All solutions were prepared using plastic pipettes (VWR International B.V., Amsterdam, The Netherlands) and 10 mL (Plastiques-Gosselin, Hazebrouck Cedex, France) and 30 mL (Sarstedt AG&Co, Nümbrecht, Germany)

polypropylene tubes. Prior to method development, all sample pretreatment devices were checked thoroughly for Pt contamination and appeared to be suitable for Pt analyses.

Determination of Pt by ICPMS

To optimise the ICPMS signal for the mid range masses and to reduce the formation of oxides and doubly charged ions, a solution containing 5,000 ng L^{-1} of Th, In, Ce, Ba, and Pt was used. Typically this 5,000 ng L^{-1} solution gave readings of ^{115}In: 2.40 x 10^6 counts per second (cps); ^{232}Th: 5.80 x 10^5 cps and ^{194}Pt: 1.60 x 10^5 cps. The production of CeO^+ was less than 1% of the total Ce^+ counts. The formation of doubly charged Ba^{2+} was less than 2%. Instrument settings are summarised in Table 1. The performance was checked daily. Other than a daily torch alignment, there was no need to tune any of the other instrumental parameters. The conditions as depicted in Table 1 were kept constant and only replacement of consumable parts such as torch, nebuliser and cones required additional tuning of the instrument settings. Thus, the signals never deviated more than 15% of the values for In, Th, Pt, doubly charged, and oxides as mentioned above.

For the detection of Pt, three isotopes ^{194}Pt (abundance 33%), ^{195}Pt (33.8%) and ^{196}Pt (25.2%) were monitored[14]. All three monitored Pt isotopes can be subject to the interference of hafnium(Hf)-oxides[15]. However, because of low oxide formation and low observed Hf counts in all the analysed samples, these oxides were insignificant and no corrections were necessary. The interference of Hg on ^{196}Pt was corrected on-line by monitoring ^{202}Hg. In order to monitor unanticipated isobaric interferences, the ^{194}Pt /^{195}Pt and ^{196}Pt/^{195}Pt ratios were measured for all samples. When ratios were similar to those reported for natural Pt, it proved that the isotopic signals reflected the Pt content of the sample with no other spectral interference. The Pt isotope used for calculation of the validation parameters was ^{194}Pt. The detection mode for all isotopes was based on peak jumping with peak dwell times of 968 ms, 25 scans per replicate and three replicates per sample. The total measurement time for one sample during validation procedures was 3.3 minutes.

Iridium (Ir) was used as internal standard. It is expected that, because of its similar mass and ionisation potential, the behaviour of Ir will accurately reflect that of Pt in a way that it will respond similar to matrix effects and possible plasma fluctuations. Internal standardisation was performed on each replicate using ^{191}Ir. Quantification was based on the mean concentration of three replicates analysed against a calibration curve using weighted linear regression analysis. By using weight factors the calibration points with higher deviations will not have a major influence on the calibration curve function.

Table 1 ICPMS instrument settings

Flow parameters (L min^{-1})	
Plasma flow	18.0
Auxiliary flow	1.65
Sheath gas	0.25
Nebuliser flow	1.05
Torch alignment (mm)	
Sampling depth	5.00
Ion optics (volts)	
First extraction lens	-12.0
Second extraction lens	-220
Third extraction lens	-230
Corner lens	-240
Mirror lens left	37.0
Mirror lens right	35.0
Mirror lens bottom	20.0
Entrance lens	5.00
Fringe bias	-3.00
Entrance plate	-50.0
Detector focus	-500
Pole bias	0.00
Other	
RF power (kW)	1.30
Pump rate (mL min^{-1})	0.28
Stabilization delay (s)	40.0

Assay development

Wipe material and sampling method

Sebum sampling was performed by wiping a body surface area of 10x10 cm on both the coat and the skin of the sampled dog. All wipe samples were collected using a uniform sampling procedure by wiping the predefined body surface thrice. Cerumen sampling was performed by wiping the accessible part of the ear canal. We used Kimtech Science precision wipes (Kimberley-Clark Professional, Irving, Tx, USA). Wipe samples were stored in 50 mL disposable polypropylene flasks (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA) at -20 °C until further processing.

Extraction procedure

One percent HCl (*v*/*v*) was used as extraction solvent. Prior to analysis, 10 mL of extraction solvent was added to the sample and vessels were kept in an ultrasonic bath at 40 °C for 60 min. Then, samples were filtered (Sartorius minisart, Sarstedt, 0.2 μm) in order to remove particles which could block the nebuliser, or could interfere with the analysis. Afterwards Ir was added as internal standard (100 ng L^{-1}, 10 μL per mL sample). Two millilitres of sample were introduced directly into the ICPMS.

Validation

For canine sebum and cerumen, a partial validation was carried out. According to the FDA guidelines, a partial validation is sufficient to test the method, when a change in matrix with the same analyte is concerned or when a change in species with the same matrix and the same analyte is concerned. We have developed and validated a method earlier for the determination of carboplatin in surface samples by ICPMS.[16] A partial validation was performed for canine sebum and cerumen.

Table 2 Deviation from theoretical concentration (DEV %) for carboplatin standards in 1% HCl

Matrix	Nominal Pt concentration (ng L^{-1})	Pt concentration in final matrix after 1 :100 dilution (ng L^{-1})	Mean Pt concentration back calculated(ng L^{-1})	DEV (%) from nominal concentration
1% HCl (used in the validation of canine sebum and cerumen)	150	1.50	1.51	0.87
	500	5.00	5.21	4.14
	$1.00x10^3$	10.0	9.65	-3.48
	$2.50x10^3$	25.0	24.3	-2.75
	$5.00x10^3$	50.0	48.6	-2.91
	$7.50x10^3$	75.0	73.2	-2.46
	$1.00x10^4$	100	99.6	-0.36

A partial validation was performed for sebum and cerumen. Therefore no relative standard deviations for carboplatin standards was calculated.

Matrix effect and effectiveness of internal standardisation

In order to test the matrix effect of canine sebum and cerumen extraction solutions on the detector response and to validate the use of Ir as internal standard, the extraction solutions were diluted with 1% HCl to 0, 1, 10, 20, 50 and 100 % extraction solution. These were then spiked with carboplatin in order to obtain solutions containing 50.0 ng L^{-1} Pt. The signals at *(m/z)* of 194 were monitored. Pt concentrations were calculated against a calibration curve in 1% HCl using Ir as internal standard.

Limit of quantification

The LLOQ was defined as the concentration at which the analyte response was at least five times the response of a blank wipe sample.[13] Furthermore, the LLOQ, when spiked on blank wipes, had to be determined with a precision less than 20% and the mean value was not allowed to deviate more than 20% of the actual value. The LLOQ was determined using six samples from individual batches of drug-free canine sebum and cerumen.

Carry-over

To evaluate and minimise the effect of carry-over, we studied the signals of blank readings following the analysis of the ULOQ calibration sample ($1.00x10^4$ ng L^{-1} Pt in the canine sebum and cerumen extraction solutions) and we optimised the rinse-time.

Linearity

A carboplatin stock solution containing 400 mg L^{-1} Pt in water was prepared to obtain working solutions with concentrations ranging from 50.0 to $5.00x10^3$ ng L^{-1} Pt in water. For sebum samples, working solutions were diluted with 1% HCl (*v*/*v*) to obtain calibration standards ranging from 1.50 to 100 ng L^{-1} Pt. For cerumen samples, working solutions were diluted with 1% HCl (*v*/*v*) to obtain calibration standards ranging from 0.75 to 100 ng L^{-1} Pt. Calibration standards were injected directly into the ICPMS. An internal standard solution of $1.00x10^4$ ng L^{-1} Ir was prepared from an Ir reference solution of 1,000 mg L^{-1}. Before analysis, 20 µL of Ir internal standard solution was added to 2 mL of each calibration standard (final internal standard concentration 100 ng L^{-1}). The seven non-zero calibration standards were processed and analysed in one analytical run. The calibrations were back-calculated from the responses. Deviations from the nominal concentration were evaluated.

The FDA guidelines require deviations of within ±20% for the LLOQ and ±15% for other concentrations [13].

Precision

Quality control (QC) samples were prepared to obtain information on the recovery and precision of the extraction method and Pt analysis. Therefore, another carboplatin stock solution, prepared from a separate weighing, was diluted with water in order to obtain working solutions with Pt concentrations ranging from 500 to $5.00x10^6$ ng L^{-1}. For drug-free human and canine sebum, the working solutions were further diluted to obtain spiking solutions with concentrations ranging from 30.0 to $2.00x10^3$ ng L^{-1}. Tissues with drug-free human and canine sebum were spiked with these solutions serving as QC samples at the following concentration levels: $1.50x10^{-2}$, $2.50x10^{-2}$, 0.10, and 1.00 ng Pt on the tissues, corresponding to 1.50, 2.50, 10.0, and 100 ng L^{-1} Pt in the final solution after extraction with 10 mL 1% HCl. For drug-free human sebum and canine cerumen, the working solutions were further diluted to obtain spiking solutions with concentrations ranging from 15.0 to $2.00x10^3$ ng L^{-1}. Tissues with drug-free human sebum and canine cerumen were spiked with these solutions serving as QC samples at the following concentration levels: $0.75x10^{-2}$, $2.50x10^{-2}$, 0.10, and 1.00 ng Pt on the tissues, corresponding to 0.75, 2.50, 10.0, and 100 ng L^{-1} Pt in the final solution after extraction with 10 mL 1% HCl. These tissues were processed as described earlier. To 2 mL diluted QC sample, 20 µL of internal standard solution was

added (final internal standard concentration 100 ng L^{-1}). Five replicates of each sample were analysed in one analytical run. Accuracy was expressed as a percentage of the nominal concentration and it had to be within 80-120% for the LLOQ and within 85-115% for the other concentrations. Within-run precisions were calculated by analysis of variances (ANOVA) for each test concentration using the analytical run as the grouping variable. The precision should not exceed ±20% for the LLOQ and ±15% for the other concentrations.[13]

Inter-exchangeability of human and canine matrices

Canine sebum and cerumen can not be collected in large quantities. On the other hand, human sebum can be more easily obtained. For future clinical studies, it would be more convenient to prepare quality control samples using the human alternative. Therefore, we assessed the legitimacy of using human sebum for the preparation of QC samples as a substitute for canine sebum. The collection of human cerumen samples is subjected to the same limitations as the collection of canine cerumen samples. As sebum and cerumen share most of their constituents,[17-20] we evaluated the inter-exchangeability of canine cerumen QC samples with human sebum QC samples instead. Calibration standards were prepared using 1% HCl. Both human and canine QC samples were measured using this calibration curve.

Selectivity

From six individual batches of drug-free canine sebum wipe tissues, samples containing neither analyte nor internal standard (blank), and samples containing 1.50 ng L^{-1} Pt and 100 ng L^{-1} internal standard were prepared and injected directly into the ICPMS. The same procedure was followed for drug-free canine cerumen wipe tissue. These were spiked with with carboplatin in order to obtain concentrations of 0.75 ng L^{-1} Pt and were injected directly into the ICPMS. These samples were prepared to determine whether endogenous compounds interfered at the masses selected for Pt or internal standard. All samples were analysed in one analytical run. The signal of any interfering peak at *m/z* 194 in blank solutions was not allowed to exceed 20% of the response of the LLOQ standard. The response of any interfering peak at *m/z* 191 in the blank solution should not exceed 5% of the response of 100 ng L^{-1} internal standard. Accuracies of the samples spiked with Pt at the LLOQ standard level had to be within 80-120% of the nominal value.[13]

Cross analyte/internal standard interference test

Interference of the internal standard solution on the *m/z* 194 and interference of the carboplatin solution on *m/z* 191 had to be assessed. Drug-free canine sebum and cerumen wipe tissues were spiked with carboplatin at ULOQ standard level and the Ir signal at *m/z*

191 was monitored. The response of the interfering peak at *m/z* 191 should be less than 5% of the response of 100 ng L^{-1} internal standard.[13]

Drug-free canine sebum and cerumen wipe samples were prepared and spiked with Ir in order to obtain a concentration of 100 ng L^{-1}. The response of the interfering peak at *m/z* 194 should be less than 20% of the response of the LLOQ standard.[13]

Application of ICPMS assay

The described analytical methods are used to support clinical pharmacokinetic studies to monitor Pt originating from carboplatin in canine sebum and cerumen. An example of the analysis of sebum wipe samples from the coat and skin, and cerumen wipe samples of a pet dog treated with carboplatin, 300 mg/m^2 as a 30 min intravenous infusion, is given here. The samples were collected on the day of administration until day 3 postinfusion and processed according to the methods described above.

Results and discussion

Validation

Matrix effect and effectiveness of internal standardisation

Some signal suppression occurs with increasing canine sebum extraction solution concentration (see Figure 2). Compared to 1% HCl, an extract of 10 mL of canine sebum caused an ion suppression of 10%. The internal standard corrected well for this matrix effect.

Increasing canine cerumen extraction solution caused a minor signal enhancement (see Figure 2). Compared to 1% HCl, an extract of 10 mL of canine cerumen caused a signal enhancement of 1.5%. Again, the internal standard corrected well for this matrix effect.

Limit of quantification

For canine sebum, the LLOQ of the assay was set at a Pt concentration of 1.50 ng L^{-1} in 1% HCl, corresponding to 15.0 pg per wipe sample or 0.15 pg cm^{-2} taking into account a body surface of 10x10 cm. For canine cerumen, the LLOQ of the assay was set at a Pt concentration of 0.75 ng L^{-1} in 1% HCl, corresponding to 7.50 pg per wipe sample or per sampled acoustic meatus.

Signal-to-noise (S/N) ratios were dependent on the batches of the drug-free matrices used. S/N ratios at the LLOQ level exceeded 5 during all experiments, which was in accordance with the requirement.[13] The acceptance criteria were easily met.

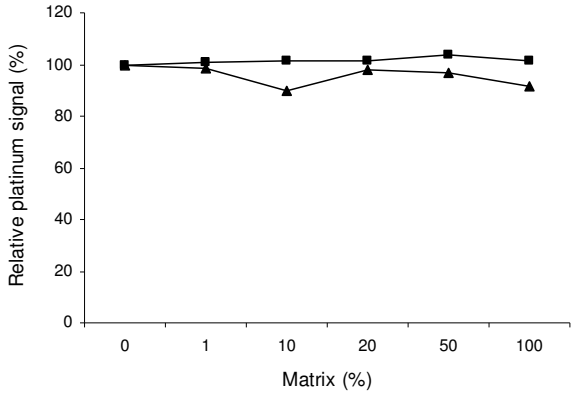

Figure 2 Matrix effect of canine sebum (▲) and cerumen (■) extraction solution. The left y-axis depicts ^{194}Pt signals relative to the signal of Pt in 1% HCl

The lower S/N ratio observed in cerumen wipe samples is probably due to the environment created by the acoustic meatus that shields the ear canal from external influences and thereby, Pt contamination.

Carry-over

A 55-s rinse time with 1% HNO_3 between two samples was required to avoid a memory effect from the preceding high concentration sample and to achieve a blank signal <20% of the LLOQ standard signal.[13]

Linearity

In table 2, deviations for 1% HCl are presented. Deviations from the nominal concentration were between -3.48 and 4.14% for all concentration levels, which all met the requirements of within ±20% for the LLOQ and ±15% for other concentrations.[13] Correlation coefficients were higher than > 0.9998.

Accuracy and precision

Accuracy and precision data are summarised in Table 3. It can be seen from Table 3 that, at all QC concentration levels, the data were within the limits for bioanalytical method validation.[13] The data summarised in Table 4, show that the canine and human QC samples are all inter-exchangeable. In future clinical studies, human sebum can be used to prepare QC samples as an alternative for canine sebum and cerumen.

Table 3 Mean, within run precision and accuracy for each quality control sample in human and canine biological matrix (n=5)

Matrix	Nominal Pt concentration in matrix (ng L^{-1})	Mean Pt concentration in canine matrix	Mean Pt concentration in human matrix	Mean canine vs human matrix[b] (%)	Within run precision canine matrix[c] (%)	Within run precision human matrix[c] (%)	Accuracy canine matrix (%)	Accuracy human matrix (%)
Sebum	30.0	31.7	29.74	6.73	10.9	5.21	106	99.2
	50.0	51.6	50.10	2.96	2.10	6.69	103	100
	200	208	189	9.08	11.1	1.81	104	94.6
	$2.00x10^3$	$1.91x10^3$	$1.87x10^3$	2.01	1.71	0.43	95.5	93.5
Cerumen[a]	15.0	16.3	17.4	6.38	2.77	8.34	109	116
	50.0	54.5	55.4	1.86	4.36	8.35	109	111
	200	212	192	9.47	2.37	3.84	106	96.1
	$2.00x10^3$	$2.15x10^3$	$1.9x10^3$	11.7	1.18	2.12	108	95.1

A partial validation was performed for canine sebum and cerumen. Therefore no between-run precision was calculated.

[a] Human sebum quality control samples were used.

[b] Means were compared using the following formula: *(x-y)/y*100*, with x= mean human matrix and y= mean canine matrix

[c] Within run precisions were calculated using*: SD/mean*100*

Selectivity

Blank samples from six individual batches did not show interferences from endogenous material at the *m/z* selected for Pt with a response >20% of the LLOQ standard signal. The response of interfering peaks at *m/z* 191 did not exceed 5% of the response of 100 ng L^{-1} internal standard. Deviations from the nominal concentrations at the LLOQ level were between 8.15 and 11.3% (data not shown).

Cross analyte/internal standard interference test

The response of the interfering peak at *m/z* 194 was less than the maximum allowed 20% of the response of the LLOQ standard.

Application of ICPMS assay

The clinical applicability of the described assays was demonstrated by analysis of canine sebum and cerumen wipe samples obtained from a pet dog treated with carboplatin. A Pt versus time profile for Pt in canine sebum and cerumen is presented in Figure 3.

The results show that Pt can be found in canine sebum and cerumen at concentrations above the LLOQ. However, these results are only preliminarily and should be confirmed in future studies.

The presence of Pt in sebum samples might partly be explained by external contamination of the skin and the coat of the pet dog via its surroundings or via contact with its urine and saliva. However, this cannot explain the presence of Pt in cerumen. We may assume that the ear canal shields the cerumen from external contamination. As cerumen samples also contained measurable Pt levels, the excretion of Pt in cerumen and, probably, in sebum seems plausible. Either way, Pt at concentrations above the LLOQ of the described methods can be found in sebum and cerumen of a client-owned pet dog treated with carboplatin. Therefore, handling and caressing the animal may lead to exposure of veterinary personnel, owners and others in close contact with treated animals.

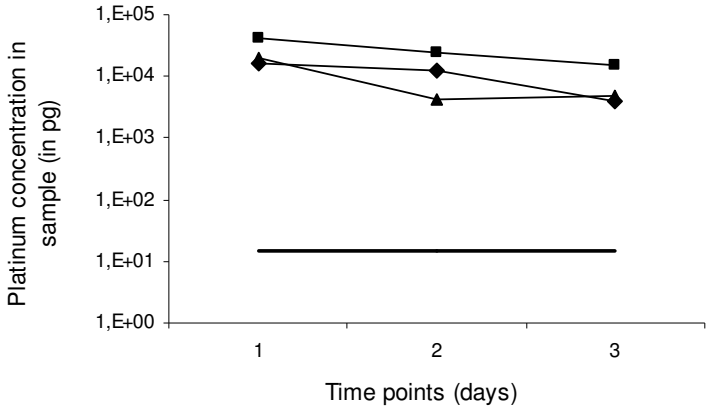

Figure 3 A Pt concentration versus time plot in canine sebum (of the coat: ♦; of the skin: ■) and cerumen (▲) of a pet dog treated with carboplatin (300 mg/m²). The LLOQ of the sebum assay (0.15 pg Pt cm^{-2} body surface) is delineated in the figure as a horizontal line.

Pt concentrations in cerumen and sebum were substantially lower than Pt concentrations in urine, feces and saliva of the same pet dog (data not shown). Whether these amounts represent a health risk for people remains a question to be answered.

Conclusion

Highly sensitive ICPMS assays for the reliable and fast determination of Pt originating from carboplatin in canine sebum and cerumen, were developed. Subsequently, the assays were validated according to current FDA guidelines. The validated range was from 15.0 to $1.00x10^4$ ng L^{-1}in canine sebum extraction solution and 7.50 to $1.00x10^4$ ng L^{-1} Pt in canine cerumen extraction solution, corresponding to 0.15 pg Pt cm^{-2} body surface and 7.50 pg Pt

peracoustic meatus, respectively. The assay is now successfully applied to support pharmacokinetic studies of patients treated with carboplatin.

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Chapter 3

Platinum: excretion in dogs and environmental contamination

Chapter 3.1

Inductively coupled plasma mass-spectrometric determination of platinum in excretion products of client-owned pet-dogs

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Submitted for publication

Abstract

Background: Residues of antineoplastic drugs in canine excretion products may represent exposure risks to veterinary personnel, owners of pet-dogs and other animal care-takers.

Objective: The aim of this study was to measure the extent and duration of platinum excretion in pet-dogs treated with carboplatin.

Methods: Samples were collected before and up to 21 days after administration of carboplatin. We used validated, ultra sensitive inductively coupled plasma-mass spectrometry assays to measure platinum in canine urine, feces, saliva, sebum and cerumen.

Results: Urine is the major route of elimination of platinum in dogs. In addition, excretion occurs via feces and saliva, with the highest amounts eliminated during the first five days. The amount of excreted platinum decreased over time but was still quantifiable at 21 days after administration of carboplatin.

Conclusion: Increased platinum levels were found in all measured excretion products up to 21 days after administration of carboplatin to pet-dogs, with urine as the main route of excretion. These findings may be used to further adapt current veterinary guidelines on safe handling of antineoplastic drugs and treated animals.

Introduction

Because the use of antineoplastic drugs in veterinary oncology has been gaining popularity the past decades, the question arises whether these drugs may pose health risks to owners or veterinary personnel handling the pet-dogs. In order to keep exposure at "as low as reasonable achievable" levels, guidelines have been developed, and implemented, on safe handling of antineoplastic drugs, treated animals and associated wastes in veterinary oncology.[1] These also apply to owners of treated pet-animals, since the dog is usually sent home after treatment. The veterinary safe handling guidelines are largely based on knowledge from human oncology. Hence, the period of risk during which measures are taken is, in general, based on the pharmacokinetic behavior of antineoplastic drugs as observed in humans and experimental animals. However, the pharmacokinetics of compounds is species dependent. To address the question whether the guidelines are completely adequate to diminish exposure in veterinary oncology, it is relevant to investigate the pharmacokinetics of the antineoplastic drugs used in pet-animals.

Table 1 Pharmacokinetics of carboplatin in dogs

Dose	Compound	Period (h)	n	V_d (L/m^2)	C_{max} (μg/mL)	CL_T ($L/(h\ m^2)$)	$t_{1/2\ el}$ (h)	Urinary excretion (% dose)	*Ref*
12 mg/kg IV	Carboplatin	96	3	5.7 ± 0.4	83 ± 7	5.4 ± 0.2	1.2 ± 0.4	37 ± 15 (over 24 h)	2
12 mg/kg IV	Pt	96	3	5.1 ± 0.4	93 ± 14	5.2 ± 0.3	0.9 ± 0.1	61 ± 9 (over 96 h)	2
150 mg/m² IV	Pt	24	3	34 ± 2.8	-	6.5 ± 1.0	3.7 ± 0.29	31.7 ± (over 4 h)	3
250 mg/m² IV	Carboplatin	8	9	4.3 ± 0.58	-	4.3 ± 0.44	0.79 ± 0.06	-	4

C_{max}: peak plasma concentration of a drug after administration, CL_T: total body clearance, n: number of subjects, period: period of sample collection, Ref: reference, $t_{1/2\ el}$: biological half-life, V_d: volume of distribution, -: not available

An example of an antineoplastic agent frequently used in pet-animals is carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)), a second generation platinum (Pt)-containing compound. It is used in pet-dogs for the treatment of numerous solid tumors, especially osteosarcomas and several carcinomas, e.g. thyroid carcinoma and anal sac carcinoma.[5,6] The pharmacokinetics of carboplatin in dogs has only been studied in a limited number of cases, mainly using canine plasma (see Table 1). Literature indicates that urine is the principal route of excretion of Pt and carboplatin, but some excretion occurs via feces as well.[7] Furthermore, human research has demonstrated that Pt originating from carboplatin can be excreted in saliva.[8] Therefore, to evaluate whether the current veterinary safe handling guidelines are adequate for carboplatin, we have monitored Pt amounts in urine, feces and saliva of treated pet-dogs. The coat and skin of the dogs may be contaminated with Pt due to an excretory process or as the result of transfer from contaminated surfaces. Since contact with the skin and the coat of pet-dogs is rather inevitable when handling them, we have also monitored Pt levels in samples collected from treated pet-dogs. To determine the source of potential Pt contamination, we have monitored Pt in cerumen of pet-dogs treated with carboplatin. We hypothesize that the ear canal forms a sheltered environment.

Thus, potential increases in Pt levels in cerumen would likely be the result of an excretion process.

Materials and Methods

Patients and chemotherapy regimes

Client-owned pet-dogs treated with single agent carboplatin[ab] or carboplatin in combination with doxorubicin or epirubicin, were eligible for this study. In a normal canine single agent carboplatin protocol, the drug is delivered every three weeks for in total six to twelve times. In a combination protocol with doxorubicin or epirubicin, the anthracycline and carboplatin are alternately delivered every three weeks, so the dog receives carboplatin once in six weeks. Great heterogeneity exists in the patient population, with regard to body weight and body surface area, due to the limited number of eligible dogs. Dogs were enrolled in the study between October 2009 and May 2012. The characteristics of the dogs are presented in Table 2. A signed informed consent form was obtained from the owners. The study was reviewed by the Netherlands Cancer Institute Independent Medical Ethical Evaluation Committee.

Table 2 Characteristics of pet dogs treated with carboplatin

Dog	Age[a]	Breed	Gender	Tumor type	Administered dose[a,b]	Cumulative dose[a]
1	9	Airedale Terrier	F	Transitional cell carcinoma	270	270
2	10	German Shepherd	M	Thyroid carcinoma	350	350
3	7	West Highland white terrier	F	Lung carcinoma	72	72
4	2	Bullmastiff	M	Osteosarcoma	460	460
5	4	xRottweiler	M	Squamous-cell carcinoma	300	300
6	10	xLabrador Retriever	M	Adenocarcinoma	210	210
7	8	Irish Setter	F	Thyroid carcinoma	270	270
8	7	Boxer	M	Squamous-cell carcinoma	390	390
9	11	Jack Russell Terrier	M	Thyroid carcinoma	100	100
10	11	Bernese Mountain Dog	M	Sarcoma of the nasal cavity	350	1060

[a]: age at treatment (in years), administered dose at sample collection (in mg), cumulative dose at sample collection (in mg)
[b]: Dose was calculated using 300 mg m^{-2} (when body weight is above 15 kg), else: 15 mg kg^{-1}
F: female, M: male, x: cross-breed

Sample collection

All samples were collected by the pet owners prior to treatment and on the day of drug administration up until day 21 post-infusion, using a new pair of nitrile gloves[c] for each sample, as described earlier.[9,10] Urine samples were collected in 30 mL polypropylene tubes[d] using voided urine. Canine fecal samples were collected in 50 mL polypropylene tubes.[e] Canine saliva samples were collected by letting the dog chew on Salivette® cotton swabs[f]

and by inserting the swab between the teeth and the cheek of the dog, both while holding the swab, until the swab was soaked with saliva or the dog persistently resisted sampling. Saliva production was stimulated by exercise or the presentation of food. Sebum sampling was performed by wiping a body surface area on both the coat (of the thorax) and the skin (of the lower abdomen) of the dog. All wipe samples were collected using a uniform sampling procedure by wiping a predefined body surface of 10x10 cm three times. Cerumen sampling was performed by wiping the accessible part of the ear canal. We used Kimtech Science precision wipes.[g] Wipe samples were stored in 30 mL disposable polypropylene tubes. The owners stored the samples at –20 °C, until the samples were transported to the laboratory. All samples were then stored at –20 °C until analysis.

Determination of Pt by inductively coupled plasma-mass spectrometry

We recently developed and validated assays for the determination of Pt originating from carboplatin in canine urine, feces, oral fluid, sebum and cerumen.[9,10] The methods are based on the quantification of Pt by inductively coupled plasma mass spectrometry (ICPMS), and allow lower limits of quantification (LLOQ) of 7.50 ng L^{-1} Pt in canine urine (in 15 µL of matrix), 15.0 ng L^{-1} Pt in canine oral fluid (in 15 µL of matrix), and 0.105 ng g^{-1} Pt in canine feces (in 5 µg of matrix), 0.15 pg cm^{-2} Pt in canine sebum (in 15 µL of extraction solution) and 7.50 pg per sampled external ear canal (in 15 µL of extraction solution).[9,10] Sample pretreatment mainly involved dilution with appropriate diluents. In brief, urine samples were thawed, whirl mixed and then diluted 100 times with 1% HNO_3 (*v*/*v*) solution. Canine fecal samples were thawed, weighed, homogenized (1:3 feces: ultrapure water[h]) and whirl mixed. The samples were then centrifuged at 2,100 *g* for five min. The supernatant was centrifuged at 10,300 *g* for five min and subsequently diluted 100-fold with 1% HNO_3 solution. The resulting solution was filtered using a 0.2 µm filter.[i] A procedure similar to the pretreatment of urine samples was followed for canine oral fluid samples. However, these were diluted with a 0.01% (*g*/*v*) ammonium EDTA[j] and Triton X-100[k] solution (in water) and if necessary successively with a oral fluid: 0.01% EDTA/Triton X solution (1:100, *v*/*v*). To each 2 mL diluted sample, 20 µL of internal standard solution was added. Subsequently, diluted samples were transferred to autosampler tubes. Sebum and cerumen wipes were extracted with 10 mL 1% HCl[l] (*v*/*v*), and vessels were kept in an ultrasonic bath at 40 °C for 60 min. Then, samples were filtered using a 0.2 µm filter. Afterwards internal standard was added (iridium,[m] 100 ng L^{-1}, 10 µL per mL sample). Two mL of sample were introduced directly into the ICPMS.[n]

Results

Due to pollution by car exhaust catalysts, Pt is ubiquitous in the environment.[11] By measuring Pt background in healthy untreated pet-dogs, and in the treated pet-dogs prior to the first treatment with carboplatin, we were able to distinguish a rise in Pt levels, as a result of administration of carboplatin, from normal Pt background variation.

Table 3 Excretion of Pt in urine, feces and saliva of pet-dogs treated with carboplatin

Day	Urine		Feces		Saliva	
	Mean[a] (range)[a,b]	n	Mean[a] (range)[a,b]	n	Mean[a] (range)[a,b]	n
Pretreatment	<LLOQ	1	0.2	3	<LLOQ	2
1	55.9 (0.3 – 138.1)	9	659 (17.3 – 1641)	3	66 (299 – 1017)	2
2	13.2 (0.07 – 82.8)	7	1518 (21.4 – 4328)	7	212 (36 – 388)	2
3	4.4 (0.06 – 28.4)	8	796 (51.4 – 2022)	7	150	1
4	1.7 (0.02 – 4.6)	4	269 (17.6 – 605)	6	64 (15 – 112)	2
5	0.8 (0.03 – 1.2)	6	108 (13.7 – 367)	5	3.7 (0.5 – 6.9)	2
6	0.9 (0.7 – 1.4)	5	25.0 (14.0 – 44.5)	4	2.7 (2.2 – 3.2)	2
7	0.4 (0.01 – 0.8)	5	34.9 (7.88 – 112)	7	2.6 (0.2 – 5.0)	2
8	0.4 (0.2 – 0.6)	3	17.6 (9.16 – 26.1)	2	1.7 (1.1 – 2.4)	2
9	0.4 (0.4 – 0.5)	3	15.1 (10.4 – 17.8)	3	2.3 (0.4 – 4.1)	2
10	0.3 (0.05 – 0.5)	5	24.1 (15.5 – 31.9)	5	-	0
11	0.2 (0.2 – 0.3)	3	31.0	1	1.2	1
12	0.3 (0.6 – 0.5)	4	12.7 (5.1 – 18.7)	3	0.6	1
13	0.3 (0.006 – 0.7)	7	30.5 (6.0 – 90.9)	5	0.2	1
14	0.2	2	14.1 (8.6 – 19.6)	2	0.7	1
15	0.2 (0.2 – 0.2)	2	7.6 (6.4 – 8.9)	2	-	0
16	0.07 (0.02 – 0.1)	3	8.7 (5.6 – 10.5)	3	1.0	1
17	0.4 (0.07 – 0.7)	3	12.3 (7.6 – 17.0)	2	-	0
18	0.2 (0.002 – 0.3)	4	7.1 (5.8 – 8.6)	3	0.6 (0.3 – 0.9)	2
19	0.1 (0.003 – 0.3)	6	15.9 (13.4 – 18.3)	2	0.4	1
20	0.2 (0.1 – 0.4)	3	7.4 (7.0 – 7.8)	2	-	0
21	0.1[c]	1	18.6	1	-	0

[a]: in mg L^{-1} (urine), ng g^{-1} (feces) or μg L^{-1} (saliva)
[b]: minimum – maximum concentrations
[c]: collected on day 22 instead of day 21
n: number of samples
-: not available for measurement

Pt levels in urine, feces and saliva of pet-dogs treated with carboplatin

Pt levels were determined in 98 urine samples collected at several time points before and after administration of carboplatin in ten pet-dogs (see Table 3 and Figure 1). Median Pt level in urine samples on day 1 (after administration) is 30.8 mg L^{-1}. The median urinary Pt concentration rapidly declines to 1.1 mg L^{-1} on day 4. Afterwards, median Pt concentration decreases more slowly in urine, to 0.1 mg L^{-1} (day 20).

Pt levels were monitored in 78 fecal samples of eight treated pet-dogs collected at different time points before and after administration of carboplatin (see Table 3 and Figure 1). The pretreatment sample of dog 2 showed a higher than usual background level of Pt. Median Pt level on day 1 is 318 ng g^{-1} feces. Median Pt concentration then raises to 1178 ng g^{-1} feces (day 2). The median amount of Pt falls rapidly after achieving maximal levels to 43.8 ng g^{-1} feces. Pt levels in feces continue to decrease at a slower rate to day 21 (median: 29.0 ng g^{-1}).

Saliva was difficult to obtain. One dog did not allow sampling. The others did, but sampling did not always generate enough saliva. Twenty-seven samples from two dogs, collected at different time points before and after administration of carboplatin, were monitored (see Table 3 and Figure 1). The highest median amount of Pt excreted via saliva was on day 1 (658 μg L^{-1}). The levels of Pt declined rapidly at first (day 4: median of 64 μg L^{-1}), and then more gradually until day 18 (median of 0.591 μg L^{-1}).

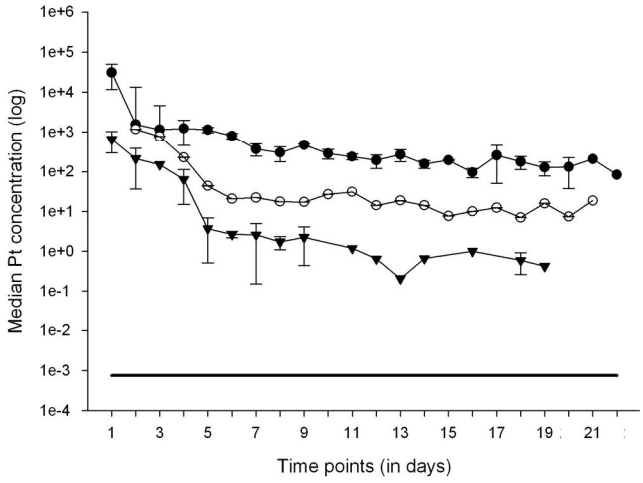

Figure 1 Depiction of median Pt concentrations in urine (●, μg L^{-1}, n = 10), feces (○, μg kg^{-1}, n = 8) and saliva (▼, μg L^{-1}, n = 2) of pet-dogs treated with carboplatin, up to 22 days after administration. The LLOQ of the urine assay ($7.5x10^{-4}$ μg L^{-1}) is delineated in the figure as a horizontal line. Error bars are based on standard error*2.

Pt levels in sebum and cerumen of pet-dogs treated with carboplatin

Ninety-four samples from seven dogs, collected on various time points before and after administration of carboplatin, were available for monitoring (see Table 4 and Figure 2). Of the two dogs with higher than usual Pt background levels, one lived in the garden and only rarely entered the house, while the other lived in a city. Median Pt concentration was highest on day 1 (367 pg cm^{-2}). The median Pt level declines until day 4 to 86.2 pg cm^{-2}, and more slowly until day 21 (4.8 pg cm^{-2}).

Table 4 Excretion of Pt in sebum and cerumen of pet-dogs treated with carboplatin

Day	Sebum (skin)		Sebum (coat)		Cerumen	
	Mean[a] (range)[a,b]	n	Mean[a] (range)[a,b]	n	Mean[a] (range)[a,b]	n
Pretreatment	0.3 (0.2 – 0.5)	6	<LLOQ	6	<LLOQ	6
1	560 (105 – 1948)	6	35.0 (1.4 – 161)	8	3497 (2.3 – 19878)	6
2	258 (111 – 516)	7	55.9 (2.6 – 120)	6	1517 (34.8 – 4901)	7
3	166 (4.64 – 665)	7	33.2 (0.9 – 65.9)	6	1543 (76.2 – 4737)	8
4	106 (18.0 – 335)	7	31.4 (0.5 – 125)	7	411 (1.1 – 1395)	7
5	102 (7.8 – 338)	6	90.1 (1.0 – 555)	7	703 (54.8 – 2442)	6
6	39.8 (2.1 – 110)	6	6.0 (1.2 – 19.8)	5	526 (23.8 – 2257)	7
7	45.0 (0.5 – 126)	5	5.6 (0.3 – 19.2)	7	460 (11.6 – 1935)	7
8	65.0 (2.3 – 124)	4	2.9 (2.3 – 3.6)	2	596 (13.1 – 1713)	4
9	42.4 (1.6 – 106)	4	7.4 (0.4 – 19.3)	4	353 (50.8 – 1083)	4
10	14.5 (2.2 – 23.8)	4	3.3 (0.7 – 6.1)	4	536 (105 – 1203)	4
11	19.8	1	3.6 (0.2 – 5.4)	3	828 (30.3 – 1627)	2
12	4.1 (1.5 – 8.5)	3	2.1 (0.3 – 4.7)	4	372 (120 – 981)	4
13	20.1 (1.2 – 30.5)	3	2.1 (0.4 – 4.0)	5	354 (32.2 – 1128)	5
14	8.36	1	3.81 (3.6 – 4.0)	2	563 (201 – 925)	2
15	5.1 (0.9 – 10.4)	4	1.8 (0.3 – 5.3)	4	408 (128 – 1178)	4
16	6.4 (1.10 – 16.0)	4	1.5 (0.6 – 2.7)	4	465 (71.6 – 1351)	4
17	3.9 (2.1 – 5.7)	2	2.6 (0.8 – 4.4)	2	115	1
18	5.0 (3.1 – 6.2)	3	2.4 (0.3 – 5.9)	4	382 (30.6 – 1375)	4
19	5.4 (1.0 – 9.8)	6	1.9 (0.8 – 3.2)	6	174 (35.1 – 482)	5
20	2.0 (1.6 – 2.3)	2	2.0 (1.7 – 2.5)	2	547 (106 – 988)	2
21	4.8 (2.6 – 7.0)	2	1.3 (0.2 – 2.3)	3	90.1 (62.0 – 113)	3
22	0.8	1	0.6	1	248	1

[a]: in pg cm^{-2} (sebum) or pg per sample (cerumen)
[b]: minimum – maximum concentrations
n: number of samples

A total of 102 sebum samples of the coat were monitored in eight dogs, at several time points prior to the treatment with carboplatin and post-infusion (see Table 4). Maximal median amount of Pt was found on day 2 (49.7 pg cm^{-2}). The median Pt level falls quite slowly to 7.2 pg cm^{-2} on day 5 and, afterwards, even slower to 1.4 pg cm^{-2} on day 21.

Pt levels were monitored in 103 cerumen samples from eight dogs, collected at different time points prior to treatment and after administration of carboplatin (see Table 4). Highest median level of Pt was found on day 3 (689 pg per sample). Afterwards, median Pt levels gradually decline to 95.1 pg per sample on day 21.

Discussion

We report increased levels of Pt in several canine excretion products, up to at least three weeks after administration of carboplatin to pet-dogs. To our knowledge, this is the first article describing Pt excretion in feces, saliva, sebum and cerumen of pet-dogs treated with carboplatin.

Pt was mainly excreted via urine in pet-dogs in the present study. This is in agreement with the literature. Gaver et al. (1988) [2] and Page et al. (1993) [3] also described urine as the primary route of Pt excretion when carboplatin is administered to dogs (see Table 1). Our results demonstrate that Pt was excreted to a lesser extent in feces and saliva of treated pet-dogs. Fecal excretion[7,12,13] of carboplatin has been reported in mice and rats, while salivary excretion[8] has been described in humans, though urine is the main route of excretion in these species as well.[12,14] Furthermore, we were able to detect traces of Pt in canine sebum and cerumen samples. No reports regarding these excretion products could be traced.

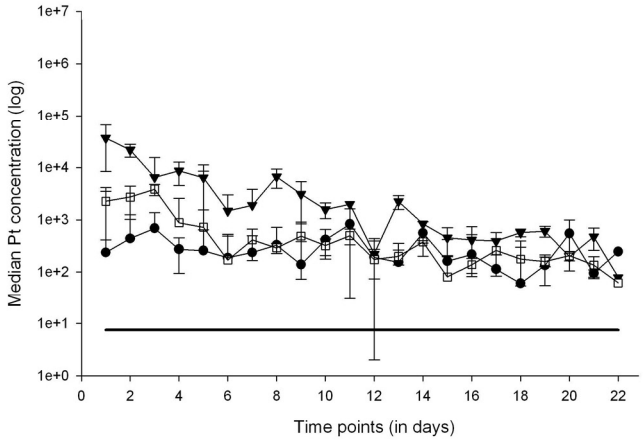

Figure 2 Depiction of median Pt concentrations in sebum of the skin (▼, pg per 100 cm^2, n= 7), sebum of the coat (□, pg per 100 cm^2, n= 8) and cerumen (●, pg/sample, n= 8) of pet-dogs treated with carboplatin, up to 21 days after administration. The LLOQ of the cerumen assay (7.5 pg per sample) is delineated in the figure as a horizontal line. Error bars are based on standard error*2.

As mentioned above, we theorized that the external ear canal is a sheltered environment, thereby reducing the contribution of potential external Pt contamination. Therefore, the marked increase in Pt levels in cerumen after administration of carboplatin indicates that transdermal excretion of Pt occurred. This may be the result of secretion of Pt into sebum and cerumen. However, this is rather unlikely given the time lapse between sebum production and its excretion onto the skin surface (on average eight days in humans, though unknown in dogs).[15] Transcellular transport, on the other hand, would require transition through the virtually impenetrable intracellular matrix of the keratinocytes.[16] Intercellular

transport across the skin involves interaction with hydrophobic and hydrophilic environments,[16] but carboplatin is a hydrophilic compound[17,18] with an affinity for hydrophobic environments.[17] Moreover, both carboplatin and Pt are polar compounds, and very polar solutions have been reported to permeate the stratum corneum.[16] Even though this applies to external-to-internal trans-dermal transport, intercellular transport of Pt still seems the most probable route for its excretion into sebum and cerumen in dogs.

The Pt concentrations detected in sebum of the coat are roughly 2-20 times lower than what is found in sebum of the skin. This difference in Pt levels may be the result of common dog behavior (e.g. licking), droplet formation when urinating or topical histological differences of the skin (e.g. variation in number of sebaceous glands, thickness of epidermis and stratum corneum).[19]

Our findings demonstrate that Pt is excreted in pet-dogs up until at least 21 days after administration of carboplatin. However, the duration of sample collection in the current study, only covered the first three weeks after dosing. Based on reports in the literature, Pt excretion may occur over longer periods of time. For example, prolonged excretion of Pt has also been reported in humans, with urinary Pt levels measurable up to 17 years after treatment with Pt-containing compounds.[20,21] This can be explained by the Pt retention that occurs in various organs (e.g. liver, kidney) and major tissues such as skin, bone and muscle.[20,22-24] From these distinct body compartments, Pt is then slowly released into the bloodstream,[21] probably as the result of normal cell turn-over.[24-26] Another process likely contributing to the prolonged excretion of Pt, is the persistent circulation of Pt in blood due to protein binding.[27]

The guidelines on handling antineoplastic drugs, treated animals and associated wastes in veterinary oncology list five days as being an expected period of risk for carboplatin.[1] This is in accordance with the period of fast elimination reported here. Moreover, the guidelines consider all excreta, namely urine, feces, saliva and vomit, as potentially hazardous. However, our findings indicate that sebum may be a potential source of exposure by dogs. Furthermore, the results demonstrate that low levels of Pt are excreted via all routes, up to at least three weeks after administration of carboplatin.

It is not clear whether the Pt levels reported in this article represent true health risks. Occupational exposure of nurses and pharmacy personnel to antineoplastic drugs in human oncology centers is associated with increased genotoxic responses.[28] However, doses given to pet-animals are relatively low compared to human oncology patients. Thus, we may conclude that the exposure of owners and veterinary personnel via canine excretion products is lower than the occupational exposure encountered in human oncology. Nevertheless, Pt-containing compounds have been associated with an increased risk of cancer in human patients[29,30] and any exposure to carcinogenic compounds could, theoretically, result in adverse health effects.[31] Importantly, using the ICPMS, we do not distinguish between carboplatin and its (active) metabolites (e.g. serum albumin adducts,

cisplatin).[27,32] Consequently, we are unable to provide information on the biological activity of the measured Pt. We are, therefore, presently investigating the biological activity of the Pt measured in canine urine.

Conclusion

The present study demonstrates that not only urine is of importance considering the safe-handling of pet-dogs treated with carboplatin. Other excretion products, such as saliva and sebum, also contain increased Pt levels after administration of carboplatin. Furthermore, Pt could be measured in urine, feces, saliva, sebum and cerumen of pet-dogs, up to at least 21 days after administration of carboplatin. These findings may be used to further adjust the veterinary guidelines on safe handling of pet-dogs treated with antineoplastic drugs.

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Footnotes

[a] Carboplatin, Hospira Benelux, Brussels, Belgium
[b] Carbosin, Teva Pharmachemie, Haarlem, the Netherlands
[c] Klinion, Medeco, Oud-Beijerland, the Netherlands
[d] Sarstedt AG&Co, Nümbrecht, Germany
[e] Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA
[f] Sarstedt AG&Co, Nümbrecht, Germany
[g] Kimberley- Clark Professional, Irving, TX, USA
[h] Aqua B. Braun Medical, Melsungen, Germany
[i] Sartorius minisart, Sarstedt AG&Co, Nümbrecht, Germany
[j] Sigma-Aldrich, St. Louis, MO, USA
[k] Sigma-Aldrich, St. Louis, MO, USA
[l] Mallinckrodt Baker, Philipsburg, NJ, USA
[m] Merck, Darmstadt, Germany
[n] Varian 810-MS, Mulgrave, Victoria, Australia

Chapter 3.2

Environmental monitoring of platinum in veterinary and human oncology centers using inductively coupled plasma-mass spectrometry

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Abstract

Objective: Determination of surface contamination with platinum-containing antineoplastic drugs in veterinary and human oncology centers.

Methods: Inductively coupled plasma-mass spectrometry was used to measure platinum levels in surface samples.

Results: In veterinary and human oncology centers, 46.3% and 68.9% of the sampled surfaces demonstrated platinum contamination, respectively. Highest platinum levels were found in the preparation rooms (44.6 pg cm^{-2}) in veterinary centers, while maximal levels in human centers were found in oncology patient-only toilets (725 pg cm^{-2}). Transference of platinum by workers outside areas where antineoplastic drugs were handled was observed in veterinary and human oncology centers.

Conclusion: Only low levels of platinum contamination attributable to carboplatin were found in the sampled veterinary oncology centers. However, dispersion of platinum outside areas where antineoplastic drugs were handled was detected in veterinary and human oncology centers. Consequently, not only personnel, but also others may be exposed to platinum.

Introduction

Falck et al. (1979) [1] were the first to report the mutagenicity of urine of workers occupationally exposed to antineoplastic drugs. Since then numerous studies have been performed using environmental and biological monitoring methods to evaluate occupational exposure to these drugs.[2-6] Research demonstrated that the implementation of appropriate staff training, guidelines on safe handling of antineoplastic drugs, and protective measures was able to diminish the exposure, although a certain amount of exposure cannot be avoided.[4,7-10] Most of the studies have focused on workers in human oncology. However, there are other populations that are occupationally exposed to antineoplastic drugs as well.[11] One of the fields at risk is veterinary medicine. In response, guidelines on safe handling of antineoplastic drugs have been developed by the European College of Veterinary Internal Medicine of Companion Animals (ECVIM-CA).[12] These guidelines are based on knowledge acquired in human oncology. Usage of chemotherapy in veterinary oncology is still not as widespread as in human oncology. In addition, veterinarians and their clients are generally less willing to accept a high degree of side effects, resulting in lower doses of the same drugs than those used in human oncology.[13] On the other hand, animals do not always comply with the strict measures necessary for safe handling of antineoplastic drugs and related wastes. Thus, extrapolating knowledge on the excretion of antineoplastic drugs from human patients to veterinary patients is difficult. However, research on occupational exposure in veterinary oncology is scarce.[11,14-18]

The objective of this study was to evaluate the efficacy of the established guidelines by monitoring of the occupational exposure of workers in veterinary oncology centers. To achieve this objective, we measured the surface contamination by platinum (Pt) originating from carboplatin in five veterinary oncology centers and a veterinary pharmacy using inductively coupled plasma mass spectrometry (ICPMS). To be able to place the data in a broader perspective we also evaluated the Pt surface contamination originating from Pt-containing drugs in two human oncology centers. Furthermore, the Dutch human oncology centers perform annual surface monitoring of antineoplastic drugs to evaluate potential exposure of workers. We compared the Pt surface contamination in another human hospital as measured during annual surface monitoring, with the Pt levels detected in the current study.

Material and Methods

Monitoring of surface contamination in veterinary oncology centers

The wipe samples were collected in five veterinary oncology centers (sites 1 to 5a) and one veterinary pharmacy (site 5b) in the Netherlands and Flanders. The chosen facilities provide a representation of the diversity in veterinary oncology centers in the Netherlands and

Flanders, in terms of size and amount of carboplatin[ab] handled annually. Table 1 describes the characteristics of the centers. One of those centers (site 5a) was associated with a veterinary pharmacy (site 5b), where the antineoplastic drug applications were prepared. Hence, the pharmacy was included in the study.

Each center consisted of a preparation room with one biological safety cabinet (BSC), apart from site 1 and the pharmacy, which used a laminar air flow (LAF) hood. Apart from site 1, which uses a separate room for administration, the centers administered the antineoplastic drugs in the consultation room. Centers used the PhaSeal® closed system drug transfer device[c] (CSTD) or the Tevadaptor® drug-handling device[d] (DHD). Samples were taken at locations that were prone to contamination, such as surfaces in the administration and preparation rooms, but also at other locations, such as surfaces in the canteen.

Table 1 Characteristics of the sampled veterinary oncology centers

	Site					
	1	2	3	4	5a	5b
Total amount of Pt processed, annually (in mg) [a]	9538	0 – 1200[c]	5600	[b]	6350	6350
Number of years in service[a]	26	4	8	8	4	<1

[a]: at time of environmental monitoring, with amount of Pt in mg
[b]: not available
[c]: estimation, depends on number of treated patients, annually

Monitoring of surface contamination in human oncology centers

The wipe samples were taken in one inpatient (site 1) and one outpatient (site 2) oncology department of a human hospital in the Netherlands. The selected facilities provide a representation of medium-sized human oncology departments in the Netherlands. Table 2 delineates the characteristics of the departments.

Table 2 Characteristics of the sampled human oncology centers

	Site	
	1	2
Type	Inpatient	Outpatient
Total amount of Pt processed, annually (in g)[a]	43	175
Number of years in service[a]	5	< 1

[a]: at time of environmental monitoring

Each department consisted of a preparation room, an administration room with adjacent patient-only sanitary facilities, a waiting room and reception. The antineoplastic drugs were prepared in the pharmacy of the hospital and then transported to the medication room of each department, where the preparations were stored before administration to the patient. Depending on the department, a canteen, non-restricted sanitary facilities and rooms for other patients were present. Samples were collected at locations that were likely to be

contaminated, such as in the administration and preparation rooms, but also at other locations, such as the canteen.

Wipe sampling procedure

The surface monitoring was announced in advance. The same person performed the sampling by wiping a surface area of 10x10 cm. If a 10x10 cm area could not be sampled, the complete top of the device was wiped and the area was estimated. The wiping procedure involved wiping the predefined surface three times in different directions. A new pair of disposable, nitrile gloves[e] was used for each wipe sample. The wiping tissue[f] was moistened with 500 µL pure water[g] before wiping the surface. During each visit, two procedural blank samples were prepared by moistening the wiping tissues with 500 µL water, followed by storing of the wipes in30 mL polypropylene tubes.[h] The collected surface wipe samples were stored also stored in 30 mL polypropylene tubes. After the visit, all samples were stored at -20 °C until further processing.

Analytical procedures

Pt analyses were performed as reported elsewhere.[19] In brief, extraction was accomplished by adding 10 mL 1% HCl solution (*v*/*v*) to the wipe samples, followed by ultrasonification for 60 min at 40 °C. The extraction solution was then filtrated using 0.20 µm filters.[i] When Pt concentrations appeared to be higher than the highest concentration validated ($1.00x10^4$ ng L^{-1} in extraction solution), the filtrated solution was further diluted using 1% HCl until the Pt concentration was within the validated range. Subsequently, 10 µL internal standard solution (iridium,[j] 100 ng L^{-1}) is added to each mL sample, to measure Pt concentrations. The extraction solutions are then introduced directly into the ICPMS.[k] The lower limit of quantification (LLOQ) of the assay is 0.500 ng L^{-1} Pt (corresponding to 0.050 pg Pt cm^{-2} of the sampled surface).[19] The assay was validated according to the FDA guidelines.[20]

Results

Contamination was calculated in pg cm^{-2}. The presence of Pt in the environment is ubiquitous, due to pollution by car exhaust catalysts.[21] Below a threshold of 1.00 ng L^{-1} Pt, corresponding to 0.100 pg cm^{-2}, it was not possible to address the source of contamination.[19] Consequently, only results 'at or above the threshold' are described.

Monitoring of Pt surface contamination in veterinary oncology centers

The wipe samples were collected in five veterinary oncology centers between October 2009 and February 2011. Surface contamination of all standard locations is presented in Table 3. None of the procedural blank samples prepared in each center, contained concentrations of Pt exceeding the LLOQ level.

Table 3 Pt contamination in veterinary oncology centers in the Netherlands and Flanders

Sampled surface	Pt contamination (in pg cm^{-2})					
	Site					
	1	2	3	4	5a	5b
Waiting room: floor	[a]	[a]	0.1	0.1	[b]	0.7
Reception: floor	[a]	[a]	[b]	[a]	[b]	[b]
Consultation room:						
Floor	[b]	[a]	0.1	0.1	[b]	[b]
Table	0.1	[a]	[a]	[a]	[b]	[a]
Door handle	0.4	[a]	[a]	[a]	[b]	[a]
Handle phone	0.1	[b]	[a]	0.1	[b]	1.4
Mouse computer	0.2	[b]	0.1	[a]	[b]	[a]
Door handle refrigerator	0.1	[a]	[b]	[b]	[b]	[b]
Waste bin top	2.7	[a]	0.1	[b]	[b]	1.7
Administration room						
Floor	0.4	[b]	[b]	[b]	[b]	[b]
Table	[b]	[b]	[b]	[b]	[b]	[b]
Door handle	0.4	[b]	[b]	[b]	[b]	[b]
Waste bin top	0.3	[b]	[b]	[b]	[b]	[b]
Preparation room						
Floor	0.5	[a]	8.9	0.2	0.2	[b]
Work bench	2.0	0.1	0.2	2.1	0.4	0.4
Door handle	[a]	[a]	[c]	[a]	[a]	[b]
Door handle refrigerator	[a]	[a]	44.6	[a]	0.3	[b]
Waste bin top	0.1	0.1	[a]	[b]	[a]	[b]
Transport box	[b]	[b]	[b]	[b]	1.1	[b]
Canteen						
Floor	[b]	[a]	0.1	0.1	[a]	0.1
Table	[a]	[a]	[a]	[a]	[a]	[a]
Kitchen surface	[a]	[a]	[a]	[a]	[a]	[b]

[a]: Below threshold (0.1 pg cm^{-2})
[b]: Sample was not available

Forty-six percent of the surface samples contained levels that were at or above the threshold. There was variation in the level of contamination between the centers. Center 2 showed the lowest overall Pt contamination, with only 13.3% of the wipe samples containing Pt at or above the set threshold. Center 1 showed the highest overall Pt contamination with 63.2% of the samples containing Pt at levels at or above the threshold. The highest level of contamination was detected in the preparation rooms. Pt was found in all wipe samples taken from the middle of the BSC workbench. Some samples from the consultation and administration rooms contained Pt above the threshold level, including samples taken from

the computer mouse. We detected Pt contamination above the threshold on the floors of the canteen and the waiting room at three centers.

Table 4 Pt contamination in two human oncology centers in the Netherlands

Sampled surface	Pt contamination (in pg cm^{-2}) Site	
	1	2
Entrance: door handle	0.1	[a]
Non-restricted area: patient room: door handle	1.3	[b]
Waiting room: floor	4.3	1.4
Reception		
Floor	1.0	1.6
Handle telephone	[a]	[a]
Computer mouse	0.1	0.1
Preparation room		
Floor	[b]	[b]
Door handle	[b]	[b]
Work bench	0.1	0.1
Transport box	[a]	[a]
Outer packaging infusion bag	[b]	[a]
Administration room		
Floor next to bed/chair patient	10.8	9.2
Infusion pump	1.4	0.3
Bell	3.9	[b]
Control system bed/chair patient	1.4	0.3
Chair	0.7	[b]
Door handle	[a]	1.0
Table	0.1	0.1
Waste bin top	0.4	0.2
Patient-only sanitary facilities		
Toilet: door handle	[b]	2.3
Toilet: seat	725	0.6
Toilet: cover	350	[b]
Toilet: floor	0.3	4.8
Shower	0.4	[b]
Sluice room		
Work bench	1.4	[b]
Floor	271	[b]
Canteen		
Floor	1.5	2.5
Table	0.1	0.1
Kitchen surface	[a]	[b]

[a]: Below threshold (0.1 pg cm^{-2})
[b]: Sample was not available

Monitoring of Pt surface contamination in human oncology centers

The wipe samples were collected in two human oncology centers in May 2011.
Surface contamination of all standard locations is presented in Table 4. Mean and maximum Pt concentrations at human and veterinary oncology units are presented in Table 5. None of the procedural blanks prepared in each center contained levels of Pt exceeding the LLOQ.

At 69% of all sampled surfaces Pt at levels above the threshold were found. There was variation in the level of contamination between the oncology centers, especially with regard to the patient-only sanitary facilities. The samples collected in the patient-only toilet, the sluice room and on the floor next to the patient's bed (inpatient department) or chair (outpatient department) showed the highest level of Pt contamination. Some samples from the waiting room, reception and the canteen contained Pt levels above the threshold.

Table 5 Summary of Pt surface contamination

	n[a]	Samples[b]	Mean[c]	Median[c]	Min[c]	Max[c]	SD[c]
Veterinary oncology centers	5	33/71	3.2	0.4	0.1	44.6	9.7
Veterinary hospital pharmacy	1	4-sep	0.5	0.4	0.2	1.1	0.1
All veterinary facilities	6	37/80	1.9	0.2	0.1	44.6	7.4
Human oncology centers	2	31/45	45.1	1.37	0.103	724.5	148.1
Human hospital pharmacies[d]	7	108/124	145.3	3.30	0.105	5760	636.8

[a]: number of sampled centers

[b]: number of samples above threshold per total number of samples

[c]: expressed in pg cm^{-2}

[d]: based on data previously reported by Brouwers et al. (2007) [19]

Min: minimal concentration, Max: maximal concentration, SD: standard deviation

Discussion

This article describes environmental contamination by Pt originating from Pt-containing antineoplastic drugs at veterinary and human oncology centers in the Netherlands and Flanders.

Monitoring of surface contamination with antineoplastic drugs in veterinary oncology centers is rarely reported in the literature. Meijster et al. (2006) [11] measured environmental contamination with carboplatin in two veterinary oncology centers in the Netherlands. They sampled 12 surfaces in the administration and preparation rooms, and detected contamination in all samples, ranging between approximately 0.3 – 6730 pg cm^{-2}. Kandel-Tschiederer et al. (2010) [15] measured Pt surface contamination at the preparation room, administration room and canteen of one German veterinary oncology center. The authors reported Pt contamination ranging between 20 – 4610 pg cm^{-2} in 12 out of 28 samples. The Pt contamination detected in our study ranged between 0.1 – 44.6 pg cm^{-2}, with the highest level of contamination generally found in the preparation rooms. The majority of the contaminated surfaces had Pt levels roughly 10-1000 times lower than the results reported by Meijster et al. (2006) and Kandel-Tschiederer et al. (2010).[11,15] The two veterinary centers monitored by Meijster et al. (2006), [11] were evaluated by our study as well (site 1 and site 5b). Since then, however, the veterinary oncology centers changed their working procedures. Nowadays, the veterinary centers use the PhaSeal® CSTD or the Tevadaptor® DHD when handling antineoplastic drugs. Kandel-Tschiederer et al. (2010) [15] also describe

the introduction of the PhaSeal® CSTD, which led to a marked decrease in Pt contamination. This reduction of surface contamination after introducing a CSTD has been reported in human oncology centers as well.[22,23] Thus, our results confirm that the use of a CSTD or a DHD can significantly diminish contamination of surfaces in veterinary oncology centers.

The variation in surface contamination between veterinary oncology centers reported in this article can be correlated with the amount of Pt processed annually in the centers. However, there also seems to be an influence of cleaning and working procedures as evidenced by the similar Pt levels found in site 1 and 3, since site 1 processed a considerably higher amount of carboplatin. Site 1 uses a laminar air flow (LAF) hood and has a separate room for administration, while site 3 uses a BSC and does not have a separate administration room. All in all, this indicates that extensive protective measures and rigorous compliance to guidelines diminishes contamination of surfaces in veterinary oncology centers markedly.

The environmental contamination by Pt in the human oncology centers ranged from 0.1 to 725 pg cm^{-2}. This is comparable with the Pt levels at surfaces in human oncology centers reported in the literature.[24,25] Furthermore, this Pt contamination in the human oncology centers is up to 16 times higher than the levels found at the veterinary oncology centers, and up to 8 times lower than the Pt contamination found in Dutch hospital pharmacies, published previously by our research group (see Table 5).[19] Our findings indicate that hospital admission of human patients treated with Pt-containing compounds leads to a generally higher Pt surface contamination of the oncology center. Consequently, the difference in environmental contamination by Pt between veterinary and human oncology centers, as found in the current study, is likely the result of differences in total amounts of Pt-containing antineoplastic drugs handled annually and the result of sending the treated pet home after dosing.

In both human and veterinary oncology centers, wipe samples obtained from e.g. the computer mouse, the floor at the canteen and the handle of the door of a non-restricted patient room, demonstrate that transference of Pt may occur. The monitoring of antineoplastic drugs in areas outside the rooms where these compounds or their wastes (e.g. patient's excreta) are handled or outside the sanitary facilities for treated patients, has rarely been reported in the literature. Some contamination of these surfaces has been described in a human hospital pharmacy (e.g. dressing room)[26] and some human oncology centers (e.g. writing desk)[27]. The transference of antineoplastic drugs to surfaces that are not expected to be contaminated (e.g. computer mouse) in areas where antineoplastic drugs are handled, is more frequently investigated and contamination of these surfaces has been reported.[19,28,29] In veterinary oncology, dispersion of carboplatin or Pt by workers has also been reported.[11,15] As a result, surfaces deemed 'safe' may be contaminated, resulting in the potential exposure of workers, patients and visitors.

Table 6 Pt contamination at a different human oncology center in the Netherlands, as measured during annual surface monitoring

Sampled surface	Pt contamination (in pg cm^{-2})
LLOD (in pg cm^{-2})	3
Amount of Pt handled in previous year [a]	1154.1
Hospital pharmacy	
preparation room: floor in front of LAF hood 1	40
preparation room: floor in front of LAF hood 2	40
adjacent room: waste bin	970
adjacent room: outer surface transport box 1	4
adjacent room: outer surface transport box 2	10
Inpatient oncology department 1	
Drug room: workbench	[b]
Sluice room: floor	10
Team post: floor	10
Team post: computer mouse	[b]
Patient's room: infusion pump	[b]
Patient's room: edge of bed 1	[b]
Patient's room: floor	50
Patient's room: toilet seat	20
Patient's room: nightstand	[b]
Toilet personnel: door handle	[b]
Inpatient oncology department 2	
Sluice room: floor	4
Drug room: floor	[b]
Drug room: workbench	[b]
Drug room: inner surface transport box	[b]
Drug room: outer surface transport box	[b]
Team post: floor	[b]
Team post: computer mouse	[b]
Team post: workbench	[b]
Patient's room: edge of bed 1	[b]
Patient's room: floor	[b]
Patient's room: toilet seat	[b]
Patient's room: toilet floor	50
Patient's room: chair for visitors	[b]
Toilet personnel: door handle	[b]
Summary	
n samples [c]	11/29
Mean	109.8
Median	20.0
Minimum	4
Maximum	970
Standard deviation	258.9

[a]: Expressed in grams

[b]: Below lower limit of detection (LLOD): method uses atomic emission spectroscopy

[c]: Number of samples above LLOD per total number of samples

The Pt contamination measured at another Dutch human hospital during an annual surface monitoring is up to 16 times higher than the Pt levels at the human oncology centers found during our investigation (see Table 6). The cut-off values for evaluation of surface contamination used in human oncology centers in the Netherlands are: <100 pg cm^{-2} (only an annual surface monitoring is required), 100 – 10,000 pg cm^{-2} (risk assessment, followed by surface monitoring and implementation of measures if necessary), and >10,000 pg cm^{-2} (implementation of measures, followed by surface monitoring).[30] Most surfaces at the human oncology centers measured in the current study had Pt levels lower than 100 pg cm^{-2} and none were higher than 1000 pg cm^{-2}, while none of the levels measured in veterinary oncology centers exceeded 100 pg cm^{-2}. The measurements during annual surface monitoring of Dutch human oncology centers are carried out using analytical techniques that are not as sensitive, but less expensive than the method reported in this article. The less sensitive assays suffice for monitoring in areas where contamination can be expected. However, with regard to the areas that should not be contaminated (e.g. canteen) our results indicate that contamination with Pt does occur and that the contamination levels are not detectable using the less sensitive analytical assays. The presence of contamination on these surfaces indicates failure of working procedures, according to the criteria set by the human oncology centers in the Netherlands. Therefore, to ensure a more accurate depiction of the actual transference of antineoplastic drugs, the use of an ultra-sensitive assay (e.g. ICPMS) could be considered, for specific surfaces. This may also be considered for surface monitoring in veterinary oncology centers.

Conclusion

Even though we only found low levels of Pt contamination attributable to carboplatin in the sampled veterinary oncology centers, we did discern a contamination pattern. The amount of antineoplastic drugs annually handled is of importance, as can be expected. The data also indicate that protective measures, working and cleaning procedures, and the use of a CSTD or a DHD can greatly influence environmental contamination. Highest contamination can be found in the drug preparation rooms, but there is evidence that transference outside the areas where antineoplastic drugs and their wastes are handled occurs. Therefore, not only those who actively work with antineoplastic drugs, but others as well might be exposed.

The level of contamination is higher in the human oncology centers than in the veterinary oncology centers, as can be expected on the basis of the amounts of antineoplastic drugs annually handled. As in the veterinary oncology centers, spreading of Pt outside the areas where antineoplastic drugs and their wastes are handled occurs, which might result in exposure of workers, patients and visitors.

ICPMS allows detection of Pt at ultra-sensitive levels, thus providing a more accurate evaluation of exposure routes and working procedures. However, by only measuring Pt we are unable to distinguish between the Pt-containing compounds or their (active) metabolites.

We are, therefore, presently monitoring Pt levels in the urine of veterinary personnel and we are evaluating the biological activity of the Pt found in urine samples of treated pet-dogs, to further investigate potential clinical implications of the observed contaminations.

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Footnotes

[a] Carboplatin, Hospira Benelux, Brussels, Belgium
[b] Carbosin, Teva Pharmachemie, Haarlem, the Netherlands
[c] Carmel Pharma AB, Göteborg, Sweden
[d] Teva Medical, Netanya, Israel
[e] Klinion, Medeco, Oud-Beijerland, the Netherlands
[f] Kimtech Science precision wipes, Kimberley-Clark Professional, Irving, Tx, USA
[g] Aqua B. Braun Medical, Melsungen, Germany
[h] Sarstedt AG&Co, Nümbrecht, Germany
[i] Sartorius minisart, Sarstedt AG&Co, Nümbrecht, Germany
[j] Merck, Darmstadt, Germany
[k] Varian 810-MS, Mulgrave, Victoria, Australia

Chapter 3.3

Environmental monitoring of platinum in homes of canine and human cancer patients using inductively coupled plasma-mass spectrometry

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Abstract

Objective: Monitoring platinum surface contamination in homes of canine and human cancer patients treated with carboplatin.

Methods: Contamination was measured using inductively coupled plasma mass spectrometry.

Results: Before treatment, platinum contamination due to normal background variation was observed in 16.5% and 10.9% of the wipe samples in the homes of pet-dogs and human patients respectively. After administration of carboplatin, Pt contamination was detected in 71.5 and 61.0% of the wipe samples, with median platinum concentrations of 0.9 and 0.4 pg cm^{-2}, respectively. The surface contamination in the homes of treated pet-dogs decreased over time, after end of treatment.

Conclusion: Platinum surface contamination increased after administration of carboplatin to veterinary and human cancer patients, with contamination in homes of treated pet-dogs at somewhat higher levels than those in the homes of human cancer patients. Furthermore, dispersion of platinum by owners of pet-dogs, human cancer patients or other family members was detected.

Introduction

In modern European, American and Asian societies, many companion animals are nowadays regarded as full family members.[1] As a result, owners are inclined to ask for the newest and most promising medical treatments for their pets. In veterinary oncology several treatment strategies can be used, such as surgery, radiation therapy, chemotherapy, targeted therapy with small molecules, and new experimental treatments, including anti-angiogenic therapy and immunotherapy.[1,2] The past decades, chemotherapy has been gaining popularity in veterinary oncology, particularly for lympho- and myeloproliferative diseases, mast cell tumors and osteosarcoma.[2] Nearly all of the owners who decided for medical treatment for the cancer of their pet have a positive perception of the treatment. They regard it as being worthwhile, the discomfort of their animal being manageable and acceptable. In the unlikely event of having another companion animal bearing a tumor in the future, they would opt for this treatment again.[1,3,4] As a result, the usage of chemotherapy in veterinary medicine will probably continue to increase. In veterinary medicine, quality of life of the treated animals has priority over treatment outcome, so the antineoplastic drugs used are similar to human oncology, but are administered at a lower dose per kg body weight.

Since the 1970s concerns have been raised regarding the exposure to antineoplastic drugs of workers in human - and to some extent veterinary – oncology.[5-8] Potential health risks have been recognized (e.g., adverse reproductive outcomes, cancer),[9] which has led to the establishment of guidelines to ensure safe handling of antineoplastic drugs and related wastes. Implementation of appropriate staff training, guidelines, and protective measures can diminish the exposure, though total exclusion of exposure cannot be achieved.[5,10-13] On top of guidelines to ensure safe handling of drugs and wastes, veterinary oncology guidelines also describe the safe handling of treated pets.[14] Since pet-dogs are generally sent home right after administration of the antineoplastic drugs, owners are instructed about handling their pets according to these guidelines. However, the veterinary guidelines are largely based on knowledge transferred from human oncology. Furthermore, most pharmacokinetic studies in humans focus on the first few days after administration of the drug.[15-17] In view of the differences between human and veterinary oncology, such as lower drug doses being used in pets and possible different pharmacokinetics of the drugs, extrapolation of data is delicate.[18]

In the present article, we describe the determination of surface contamination with platinum (Pt) originating from carboplatin in nine homes of canine oncology patients. We also monitored the Pt surface contamination in seven homes of human cancer patients treated with carboplatin, to be able to place the data into a broader perspective.

Table 1 Characteristics of pet-dogs treated with carboplatin

Dog	Age[a]	Breed	Gender	Tumor type	Dose[a, b]	Cumulative dose[a]
1	9	Airedale terrier	F	Transitional cell carcinoma	270	270
2	2	Bullmastiff	M	Osteosarcoma	460	460
3	4	xRottweiler[b]	M	Squamous-cell carcinoma	300	300
4	8	Irish setter	F	Thyroid carcinoma	270	270
5	7	Boxer	M	Squamous-cell carcinoma	390	390
6	11	Jack Russell terrier	M	Thyroid carcinoma	100	100
7	10	German shepherd	M	Thyroid carcinoma	350	350
8	7	West highland white terrier	F	Lung carcinoma	72	72
9	10	xLabrador retriever	M	Adenocarcinoma	210	210

[a]: age at treatment in years, doses in mg
[b]: Dose was calculated using 300 mg m^{-2} (when body weight is below 15 kg), else: 15 mg kg^{-1}
F: female, M: male, x: cross-breed

Table 2 Characteristics of pet-dogs, formerly treated with carboplatin

Dog	Age[a]	Breed	Gender	Tumor type	Cumulative dose [a,b]	Time[c]
7	10	German shepherd	M	Thyroid carcinoma	2100	261
8	7	West highland white terrier	F	Lung carcinoma	814	108
9	10	xLabrador retriever	M	Adenocarcinoma	510	162
10	10	xBelgian Malinois	M	Adenocarcinoma of the anal sac	1200	135
11	9	Beagle	F	Transitional cell carcinoma	1800	124
12	7	German longhaired pointer	M	Thyroid carcinoma	1620	456

[a]: age at start treatment in years, dose in mg
[b]: Dose was calculated using 300 mg m^{-2} (when body weight is above 15 kg), else: 15 mg kg^{-1}
[c]: Time period in days, between end of treatment and sample collection
F: female, M: male, x: cross-breed

Material and Methods

Monitoring of Pt surface contamination in the homes of veterinary cancer patients

Wipe samples were taken at the homes of nine families that had their pet-dog treated with carboplatin,[ab] and at the homes of seven families with pet-dogs formerly treated with carboplatin. The characteristics of the dogs and the families are presented in Tables 1 to 4. Several veterinary oncology centers in the Netherlands and Flanders participated in the study by informing owners of eligible dogs about this investigation. Every family acted as its own control whenever possible, by allowing environmental monitoring prior to the first course with carboplatin, to check the Pt contamination of other sources than chemotherapy, such as car exhaust catalysts.[19] Pt was monitored preferably three weeks after the first administration. When monitoring the homes of formerly treated pet-dogs, samples were collected during one visit after the last administration of carboplatin. Depending on the family, the time period between last course and environmental monitoring was 3-15 months.

Table 3 Characteristics of the families with treated pet-dogs

Home	Location	Number of family members	Children still at home	Main living area dog	Number of pretreatment samples	Number of samples
1	Town	2	No	Family room	13	13
2	Town	3	Yes	Family room	12	12
3	Village	5	Yes (1 adult)	Garden	11	13
4	Village	3	Yes (adult)	Family room	11	12
5	Village	2	No	Family room	13	14
6	City	1	No	Entire home	0	22
7	Country side	2	No	Family room	13	13
8	Town	1	No	Entire home	12	11
9	City	2	No	Family room	0	13

Table 4 Characteristics of the families with formerly treated pet-dogs

Home	Location	Number of family members	Children still at home	Main living area dog	Days[a]	Number of pretreatment samples	Number of samples
7	Country side	2	No	Family room	261	13	14
8	Town	1	No	Entire home	108	12	11
9	City	2	No	Family room	162	0	13
10	Town	2	No	Family room	135	0	12
11	City	2	Yes (adult)	Family room	124	0	14
12a	Village	3?	Yes	Family room	456	0	9
12b	Village	2	No	Family room	456	0	7

[a]: Time period in days, between end of treatment and sample collection

Table 5 Characteristics of human patients treated with carboplatin

ID	Age[a]	Gender	Tumor	Dose[a]	Cumulative dose[a]	Days[a]
1	70	F	Ovarian carcinoma	373	373	21
2	54	F	Ovarian carcinoma	695[b]	5310	21
3	31	M	Seminoma	1140	1140	21
4	45	F	Mammary carcinoma	400	3480	1
5	80	F	Non-small cell lung carcinoma	350	690 (136)	21
6	66	F	Endometrial carcinoma	700	700	20

[a]: age at treatment in years, dose in mg, time period between end of treatment and sample collection in days

[b]: first administration of carboplatin of the second round of treatment, 2 years after the first treatment with carboplatin

[c]: administration of: carboplatin (cisplatin)

Monitoring of Pt surface contamination in the homes of human cancer patients

The wipe samples were collected in the homes of seven families that had a family member treated with carboplatin. The characteristics of the patients and the families are presented in Tables 5 and 6. Two human oncology centers, both located in Amsterdam, participated in the study by informing eligible patients about this investigation. Here as well, families acted

as their own control whenever possible, by allowing environmental monitoring prior to the first course with carboplatin. Preferably Pt was monitored three weeks after the first administration. However, some patients had received one or more courses of Pt-containing compounds, before sample collection (see Table 5).

Table 6 Characteristics of the families with treated human patients

Home	Location	Number of family members	Children still at home	Number of pretreatment samples	Number of samples
1	Village	1	No	13	13
2	Town	3	Yes	0	15
3a	Village	3	Yes	18	6
3b	Town	2	No	19	8
4	City	1	No	0	3
5	City	1	No	0	6
6	City	2	No	5	8

Table 7 Pt levels at the homes of pet-dogs treated with carboplatin in the Netherlands and Flanders, prior to treatment

Sampled surface	Pt levels (in pg cm^{-2}) Homes						
	1	2	3	4	5	7	8
Resting place	[a]	[a]	[a]	[a]	[a]	[a]	0.1
Area for feeding and drinking	[a]	[a]	[b]	[a]	[a]	[a]	0.1
Kitchen							
Door handle	[a]	[a]	[a]	[a]	[b]	[a]	[a]
Work surface	[a]	[a]	0.4	[a]	[a]	[a]	1.1
Floor	[a]	[a]	[a]	[a]	[a]	[a]	0.1
Door handle refrigerator	[a]	0.3	[a]	[b]	[a]	[a]	0.1
Family room							
Table	0.1	[b]	0.1	[a]	[a]	[b]	[a]
Door handle	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Small table	[a]	[a]	[a]	[a]	[a]	[a]	1.8
Floor	[b]	[a]	0.2	[a]	[a]	[a]	[a]
Remote control	[a]	[a]	[b]	[a]	[a]	[a]	0.1
Handle telephone	[a]	[a]	[a]	[a]	[a]	[a]	0.1
Computer mouse	[a]	[b]	[a]	[b]	[a]	[a]	[b]
Waste bin top	[a]	[a]	[b]	[b]	[a]	[a]	0.3

[a]: Below LLOQ (0.05 pg cm^{-2})
[b]: Sample was not available

Wipe sampling

Platinum surface monitoring was announced in advance. Sampling was performed by the same person. A surface area of 10x10 cm was wiped, unless it was not possible to take a 10x10 cm sample. For these surfaces, the complete top of the device was sampled, while the

surface area was estimated. All wipe samples were collected using a uniform sampling procedure: the predefined surface was wiped three times in different directions. A new pair of nitrile gloves[c] was used for each sample. Before wiping, each wipe[d] was moistened with 500 µL pure water.[e] Two procedural blank samples were prepared for each visit, by moistening the wipe with 500 µL water, without further use. Samples were stored in 30 mL polypropylene tubes[f] after wiping at -20 °C until further processing.

Analytical procedures

Pt analyses were performed as described elsewhere.[20] Briefly, extraction was performed by adding 10 mL 1% HCl[g] solution (*v*/*v*) to the wipe tissues. This was followed by ultrasonification for 60 min at 40 °C. The subsequent extraction solution was filtrated using 0.20 µm filters.[h] If Pt concentrations appeared to be higher than the highest concentration validated (1.00x104 ng L^{-1} extraction solution), the filtrated solution was diluted further with 1% HCl until the Pt concentration was within the validated range. The solutions are introduced directly into the inductively coupled plasma-mass spectrometer (ICPMS),[i] after addition of iridium[j] (100 ng L^{-1}, 10 µL per mL sample) as internal standard, to measure Pt concentrations. The lower limit of quantification (LLOQ) of the assay is 0.50 ng L^{-1} Pt (corresponding to 0.05 pg Pt cm^{-2} of the sampled surface).[20] The used method was fully validated according to the FDA guidelines.[21]

Table 8 Pt levels at the homes of pet-dogs treated with carboplatin in the Netherlands and Flanders, after the first course

Sampled surface	Pt levels (in pg cm^{-2}) Home									
	1	2	3	4	5	6a	6b	7	8	9
Resting place	7.2	1.6	1.3	1.1	1.1	[b]	0.1	1.5	1.1	2.0
Area for feeding and drinking	5.9	9.2	[b]	0.3	0.4	0.4	1.8	20.1	1.3	0.2
Kitchen										
Door handle	3.7	1.5	0.4	[a]	[a]	66.1	[a]	1.1	0.6	[a]
Work surface	0.2	0.1	[a]	[a]	[a]	0.4	[a]	[a]	0.3	[a]
Floor	35.1	13.4	0.6	0.3	0.3	1.4	6.4	2.5	2.3	1.4
Door handle refrigerator	0.4	2.0	0.4	[a]	[a]	[a]	[a]	147.3	0.4	0.9
Family room										
Table	2.6	[b]	0.1	[a]	[a]	[b]	[b]	[b]	0.2	[a]
Door handle	1.9	1.2	[a]	[a]	[a]	2.0	[a]	0.3	[b]	[a]
Small table	5.3	0.1	0.1	[a]	[a]	[b]	[b]	1.3	0.3	1.0
Floor	[b]	1.2	0.3	0.3	0.3	3.0	2.5	6.2	[b]	[b]
Remote control	1.0	0.8	[a]	[a]	[a]	1.0	[a]	0.2	0.2	0.1
Handle telephone	0.5	0.1	[a]	0.1	0.1	0.2	[a]	0.4	0.1	[a]
Computer mouse	0.6	[b]	[a]	[b]	[a]	1.7	[b]	0.8	[b]	0.1
Waste bin top	0.8	3.3	0.6	[b]	[a]	0.8	[a]	1.3	0.3	0.1

[a]: Below LLOQ (0.05 pg cm^{-2})
[b]: Sample was not available

Results

Monitoring of Pt surface contamination in the homes of veterinary cancer patients, during treatment with carboplatin

The wipe samples were collected between October 2009 and May 2012. Results of Pt surface contamination are presented in Tables 7, 8 and 12. None of the procedural blanks prepared at the visits contained quantifiable levels of Pt. Before the first course with carboplatin, 16.5% of the samples contained Pt levels at or above the LLOQ level. These were samples collected from floors, but also from other surfaces (see Table 7). The Pt background varied between families. Three weeks after the first course with carboplatin, 71.5 % of the samples contained levels at or above the LLOQ. The number of contaminated wipe samples and location of the contamination varied considerably between families. In 3 families all collected samples were contaminated. Samples showing high contamination were wipe samples of the floor, but also a sample of the kitchen and the refrigerator door handle (see Table 8).

Table 9 Pt levels at the homes of pet-dogs, formerly treated with carboplatin in the Netherlands and Flanders

Sampled surface	Pt levels (in pg cm^{-2}) Home						
	7	**8**	**9**	**10**	**11**	**12a**	**12b**
Time[c]	261	108	162	135	124	456	456
Resting place	3.0	0.2	0.9	0.1	[a]	[a]	[a]
Area for feeding and drinking	4.8	0.5	0.1	[a]	0.3	[a]	[a]
Kitchen							
Door handle	2.7	[b]	[a]	[a]	[a]	[b]	[a]
Work surface	[a]	0.1	[a]	[a]	[a]	[a]	[a]
Floor	0.6	2.2	[a]	[a]	0.3	[a]	[a]
Door handle refrigerator	[a]	[a]	[a]	[a]	[a]	[a]	[b]
Family room							
Table	0.2	0.2	[a]	[a]	[a]	[a]	[b]
Door handle	[a]	[a]	[a]	[a]	[a]	[a]	[b]
Small table	1.4	[a]	[a]	[a]	[a]	[b]	[b]
Floor	1.2	[b]	[b]	[a]	[a]	[b]	[b]
Remote control	0.1	0.1	[a]	[a]	[a]	[b]	[b]
Handle telephone	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Computer mouse	0.3	[b]	[a]	[b]	[a]	[a]	[a]
Waste bin top	0.1	[a]	[a]	[b]	[a]	[b]	[b]

[a]: Below LLOQ (0.05 pg cm^{-2})
[b]: Sample was not available
[c]: Time period in days, between end of treatment and sample collection

Monitoring of Pt surface contamination in the homes of veterinary cancer patients, formerly treated with carboplatin

Surface samples were collected in December 2011. Platinum contamination of all standard locations is presented in Table 9 and 12. None of the procedural blanks prepared at the visits contained quantifiable levels of Pt. Three to fifteen months after the last administration of carboplatin, 26.3% of the samples contained Pt amounts at or above the LLOQ. The number of contaminated wipe samples and location of the contamination varied considerably between families. Samples showing high contamination were mainly wipe samples of the floors (see Table 9). At two homes other surfaces showed contamination as well. However, one of these homes had a relatively high Pt background before the first course with carboplatin (see Table 7, home 8). Another home was sampled before first administration of carboplatin (see Table 7, home 7). Three homes were also monitored three weeks after the first course (homes 7, 8 and 9 in Table 8 and 9). Family 12a had their dog stay over at the home of relatives (family 12b) for a few days after each administration, since they had young children and wanted to avoid exposure as much as possible. Homes 12a and 12b were sampled 456 days after the last infusion of carboplatin, and none of the wipe samples contained Pt levels exceeding the LLOQ.

Monitoring of Pt surface contamination in the homes of human cancer patients, during treatment with carboplatin

Surface sampling was performed between October 2010 and May 2011. Surface contamination is presented in tables 10 to 12. None of the procedural blanks prepared at the visits contained quantifiable levels of Pt. Before first administration of carboplatin, 10.9% of the samples contained Pt levels at or above the LLOQ. These were mainly collected from surfaces of handles and remote control (see Table 10). The Pt background varied between families. In samples collected at the homes of human patients after administration of carboplatin, 61.0% of the samples contained Pt levels at or above the LLOQ. There is some variation with regard to the location of the Pt contamination, between families. Samples showing high contamination were wipe samples of door handles and the handle of the telephone (see Table 11). There was no obvious difference in Pt levels of wipe samples collected 3 weeks after the first course, or after several courses of carboplatin.

Discussion

The presence of contamination in oncology centers and pharmacies with antineoplastic drugs, has long been recognized.[5-8] Since pet-dogs and human oncology patients are treated on an outpatient basis whenever possible, excretion of the administered antineoplastic drugs might, therefore, give rise to contamination of their homes. To our best knowledge,

this study is the first to monitor environmental contamination with Pt originating from carboplatin in domestic homes of human and canine cancer patients.

Table 10 Pt levels at the homes of human patients treated with carboplatin in the Netherlands, prior to treatment

Sampled surface	Pt levels (in pg cm^{-2}) Home						
	1	2	3a	3b	4	5	6
Shower/tub	[b]	[b]	[b]	[a]	[b]	[b]	[b]
Night table	[a]	[b]	0.1	[a]	[b]	[b]	[b]
Chair	[a]	[b]	[a]	[a]	[b]	[b]	0.8
Toilet							
Seat	[a]	[b]	[a]	[a]	[b]	[b]	[b]
Cover	[b]	[b]	[a]	0.1	[b]	[b]	[b]
Floor	[b]	[b]	[a]	[a]	[b]	[b]	[a]
Door handle	[a]	[b]	[a]	[a]	[b]	[b]	[b]
Kitchen							
Door handle	[b]	[b]	[a]	[a]	[b]	[b]	[b]
Work surface	[a]	[b]	[a]	[a]	[b]	[b]	[b]
Floor	[a]	[b]	[a]	[a]	[b]	[b]	[b]
Door handle refrigerator	[a]	[b]	[a]	[a]	[b]	[b]	[b]
Family room							
Table	[a]	[b]	[a]	[a]	[b]	[b]	[b]
Door handle	[a]	[b]	0.4	[a]	[b]	[b]	[b]
Small table	[a]	[b]	[a]	[a]	[b]	[b]	[a]
Floor	[a]	[b]	[a]	[a]	[b]	[b]	[a]
Remote control	0.2	[b]	[a]	0.1	[b]	[b]	[b]
Handle telephone	[a]	[b]	[a]	0.1	[b]	[b]	0.9
Computer mouse	[b]	[b]	[a]	[a]	[b]	[b]	[b]
Waste bin top	[b]	[b]	[a]	[a]	[b]	[b]	[b]

[a]: Below LLOQ (0.05 pg cm^{-2})
[b]: Sample was not available

The Pt levels exceeding the LLOQ in some of the wipe samples collected prior to first administration with carboplatin is most likely caused by variation in environmental pollution with Pt by car exhaust catalysts.[19] The high pretreatment Pt background found at home 8 of a treated pet-dog, is probably the result of pollution due to exhausts of airplanes. This house was located under important landing- and departure- zones of Amsterdam airport.

Comparison of the Pt background levels at the homes of pet-dogs with Pt levels after carboplatin therapy indicates that there is a substantial increase in Pt surface contamination of the homes after treatment with carboplatin. There is considerable variation in the amount of Pt contamination after carboplatin administration, between homes of treated pet-dogs. Factors that could affect the environmental contamination with Pt after administration of carboplatin are: the administered dose, individual differences in elimination of Pt, the presence and frequency of vomiting, diarrhea and in house urinating, differences in the implementation of and compliance to the veterinary guidelines, and differences in cleaning

procedures. There is also substantial variation in the type of contaminated surfaces between the families, though floors, and especially the floor of the area where the feeder and water bowl are located, seem to be more prone to contamination. Furthermore, surfaces that were not accessible to the pet-dog, such as the computer mouse and the handle of the telephone, were monitored. The findings reported here, demonstrate that there is dispersion of Pt by the owners. One family kept their dog outside the house. The number of contaminated surfaces and the level of contamination at their home were in general lower, when compared to the homes of pet-dogs treated with a similar amount of carboplatin. There was, however, still an increase in Pt levels detected after administration of carboplatin. Moreover, one pet-dog was already treated with carboplatin, but kept in his bench while environmental monitoring was performed. When analyzing the Pt levels in the wipe samples, it was obvious that transference of Pt had occurred (see home 9a, Table 8). The amounts of Pt in the surface samples at the same home collected three weeks after dosing were considerably lower (see home 9b, Table 8). This supports the observation that family members disperse Pt. Since gloves were only worn when handling the dog or its wastes during the first five days after administration of carboplatin some skin contact with Pt may have occurred.

Table 11 Pt levels at the homes of human patients treated with carboplatin in the Netherlands, after treatment

Sampled surface	Pt levels (in pg cm^{-2}) Home						
	1	2	3a	3b	4	5	6
Shower/tub	[b]	[b]	[b]	[b]	[b]	[b]	[b]
Night table	0.1	[a]	[b]	[b]	[b]	[b]	[b]
Chair	0.1	0.1	0.2	0.9	[b]	[b]	[a]
Toilet							
Seat	0.8	0.2	1.1	2.3	[b]	3.4	[a]
Cover		[b]	[b]	[b]	[b]	45.6	[b]
Floor		[b]	2.3	1.7	[b]	[b]	[a]
Door handle	1.7	[a]	[b]	[b]	[b]	13.0	[b]
Kitchen							
Door handle	[b]	0.4	[b]	[b]	[b]	[b]	[b]
Work surface	[a]	0.1	[b]	[b]	[b]	[b]	[b]
Floor	0.1	0.1	[a]	0.1	[b]	[b]	[a]
Door handle refrigerator	[a]	[a]	[b]	[b]	[b]	4.1	[b]
Family room							
Table	0.1	[a]	[b]	[a]	[b]	[b]	[a]
Door handle	1.5	[a]	[a]	[a]	[b]	[b]	[a]
Small table	[a]	[a]	[b]	[a]	[b]	[b]	[a]
Floor	0.1	0.1	[b]	[b]	[b]	1.1	[b]
Remote control	0.4	0.2	[b]	[b]	0.2	0.5	[b]
Handle telephone	0.3	1.5	[b]	[b]	1.3	[b]	[a]
Computer mouse	[b]	[b]	0.2	0.2	0.5	[b]	[b]
Waste bin top	[b]	[a]	[b]	[b]	[b]	[b]	[b]

[a]: Below LLOQ (0.05 pg cm^{-2})

[b]: Sample was not available

The observation that Pt levels in homes, 3-15 months after treatment were lower than during treatment indicates that Pt levels decrease over time. This might be a consequence of cleaning. The amount of Pt contamination, the type of contaminated surfaces, and the duration of the return to normal Pt background varied between the families. That variation is probably subject to the same factors as mentioned earlier. Some samples showed Pt contamination of surfaces inaccessible to the dog, such as the remote control and the computer mouse. This indicates that skin contact with Pt was possible, from the first administration of carboplatin up to at least the day of sample collection, in some of the families.

Table 12 Summary of Pt surface contamination above LLOQ at the homes of treated pet-dogs, formerly treated pet-dogs and human oncology patients

	n homes	n samples[a]	Mean[b]	Median[b]	Minimum[b]	Maximum[b]
Pet-dogs						
Before treatment	7	14/85	0.3	0.1	0.1	1.7
During treatment	9	88/123	4.7	0.9	0.1	147.3
After end of treatment	7	21/80	0.9	0.3	0.1	4.8
Human patients						
Before treatment	4	jun-55	0.3	0.2	0.1	0.9
During treatment	7	36/59	2.2	0.4	0.1	45.6

[a]: number of samples above LLOQ per total number of samples
[b]: Pt levels above LLOQ, expressed in pg cm^{-2}

To be able to evaluate the environmental contamination measured at the homes of treated pet-dogs, Pt levels at the homes of human cancer patients treated with carboplatin were also monitored. The Pt background prior to the first administration was comparable to the levels found at the homes of pet-dogs. After administration of carboplatin, an increase in Pt levels was observed, although the rise is clearly lower than what was detected at the homes of pet-dogs. An explanation for this difference could be that none of the pet-dogs could access a garden at will, resulting in contamination of the home whenever vomiting, diarrhea or urination occurred unexpectedly. Furthermore, our method can only be applied to smooth surfaces. Fransman et al. (2005,2007) [22,23] have previously reported that excised sections of bed sheets used by human cancer patients can contain traces of antineoplastic drugs. Their findings range between lower than LLOQ and $3.1x10^4$ pg cm^{-2}, depending on the investigated compound. The authors did not investigate Pt levels, however.

The types of contaminated surfaces vary between families, but it is obvious that contamination is not restricted to the surfaces that are only used by the patient.

Conclusion

It is obvious that administration of carboplatin gives rise to increased Pt levels at the homes of treated pet-dogs and human oncology patients. Moreover, the pattern of contamination indicates that family members can have skin contact with contaminated surfaces. Additionally, our results show that Pt levels were on average 0.7 – 30-fold higher at the homes of treated pet-dogs. For comparison, the concentrations described in this article are 10-1000 times lower than the Pt surface contamination found in most oncology centers and pharmacies.[24,25] It seems that the guidelines, implemented by the owners and human patients, are sufficient to keep Pt contamination of their homes at low levels. The potential uptake of these low levels of Pt by the owners of pet-dogs is a point of discussion. We are currently monitoring Pt levels in the urine of owners and we are evaluating the biological activity of the Pt found in urine samples of treated pet-dogs, to further investigate potential clinical implications of the observed contaminations.

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Footnotes

[a] Carboplatin, Hospira Benelux, Brussels, Belgium
[b] Carbosin, Teva Pharmachemie, Haarlem, the Netherlands
[c] Klinion, Medeco, Oud-Beijerland, the Netherlands
[d] Kimtech Science precision wipes, Kimberley-Clark Professional, Irving, Tx, USA
[e] Aqua B. Braun Medical, Melsungen, Germany
[f] Sarstedt AG&Co, Nümbrecht, Germany
[g] Mallinckrodt Baker, Philipsburg, NJ, USA
[h] Sartorius minisart, Sarstedt AG&Co, Nümbrecht, Germany
[i] Varian 810-MS, Mulgrave, Victoria, Australia
[j] Merck, Darmstadt, Germany

Chapter 3.4

Sustained drug delivery of carboplatin: excretion in dogs and surface contamination

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Abstract

Background: Drug delivery systems can improve pharmacological properties, but may alter the pharmacokinetics of the carrier-mediated compound.

Objective: To evaluate drug release of an intramuscular carboplatin sustained drug delivery formulation in pet-dogs and assess surface contamination.

Animals: Pet-dogs treated with a carboplatin sustained drug delivery formulation.

Methods: Measurement of platinum in canine excretion products and surface samples using inductively coupled plasma-mass spectrometry.

Results: Increased platinum levels were detected in canine urine, feces, sebum and cerumen, up to at least four weeks after administration. Platinum contamination at the homes of pet-dogs was detected in 35.7 % of the wiped surfaces (median concentration 1.1 pg cm^{-2}). Transference of platinum by owners was evident.

Conclusion: The findings indicate that five days of implementing safe handling guidelines is not sufficient when a sustained drug delivery formulation of carboplatin is administered. Though platinum surface contamination was low, skin contact with contaminated surfaces may have occurred.

Introduction

Cisplatin (*cis*-diamminedichloridoplatinum(II)) was discovered by serendipity in the 1960s by Barnett Rosenberg[1] and soon proved to have a broad spectrum of antitumor activity and a remarkably high response and cure rate in several solid tumors.[2] However, the use of cisplatin is hampered by side-effects (e.g. nephrotoxicity, ototoxicity and resistance). As a result, carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) was introduced into the clinic in the mid 1980s.[3] Carboplatin is considerably less nephrotoxic, less neurotoxic, and less toxic for the gastrointestinal tract, compared with cisplatin.[4-6] For carboplatin, however, myelosuppression proved to be the dose-limiting toxicity. New drug-development efforts try to find platinum (Pt)-containing compounds or formulations that exhibit superior efficacy, lower toxicity, or improved pharmacological properties. One way of enabling this, is by using a sustained drug delivery system (sDDS).[7,8] Conventional methods of drug administration (e.g. intravenous injection), result in a 'peak and trough' pattern of plasma drug concentrations between doses, while sDDSs allow long-term administration of drugs at therapeutic doses.[9,10] Consequently, the frequency of drug administration is decreased in general, and the adverse side-effects may be reduced or even eliminated. Furthermore, association of drugs with lipids (e.g. liposomes) or polymers (e.g. polyethylene glycol) might increase intratumoral drug concentrations, due to the abnormal characteristics of solid tumors, such as leaky vasculature and impaired lymphatic drainage.[11] Moreover, active targeting of tumor cells can be achieved by linking the polymer with ligands, e.g. antibodies.[10] By now, several Pt-containing drugs, including carboplatin, have been associated with liposomal formulations and co-polymers in human and veterinary oncology.[12-16] The pharmacokinetics of these formulations, however, is more complicated than that of the standard formulations.[17] Prolonged release from drug carrier formulations will likely result in prolonged excretion of the parent compound or its metabolites. This raises the question whether the guidelines on safe-handling of antineoplastic drugs, treated animals and associated wastes developed by e.g. the European College of Veterinary Internal Medicine- Companion Animals (ECVIM-CA)[18] is adequate to prevent exposure of care-takers to the antineoplastic drug. Hence, the exposure of care-takers to this type of formulations should be evaluated.

Table 1 Characteristics of pet dogs treated with carboplatin sDDS

Dog	n	Age[a,b]	Breed	Gender	Tumor type	Period[a,c]	Administered dose[a]	Cumulative dose[a, b]
1	27	8	American Bulldog	M	Osteosarcoma	2 – 13	300	500
2	37	9	Jack Russell Terrier	M	Squamous – cell carcinoma	6 – 32	250	100
3	13	8	Riesenschnauzer	M	Osteosarcoma	27 – 54	250	650
4	15	8	German Longhaired Pointer	M	Thyroid carcinoma	2 – 27	250	250

[a]: age in years, administered dose in mg m^{-2}, cumulative dose in mg

[b]: at time of treatment and participation in the study

[c]: Start – end of sample collection period, after administration of carboplatin sDDS, expressed as day x after dosing to day y. M: male; n: number of samples

In this article, we describe excretion of platinum (Pt) in various excretion products after administration of an experimental carboplatin-containing polymer sDDS (carboplatin sDDS) to pet-dogs. We have measured Pt levels in canine urine, feces, saliva, sebum and cerumen, in order to assess potential exposure of care-takers via these excreta. In addition, we monitored Pt in surface samples, collected at the homes of treated pet-dogs.

Materials and Methods

Patients and chemotherapy regimes

Pet-dogs treated with an intramuscular (IM) single agent regimen with carboplatin sDDS (every four weeks) were eligible for this study. Great heterogeneity exists in the patient population with regard to administered dose. Dogs were enrolled between February 2011 and May 2012. The characteristics of the dogs are presented in Table 1. All dogs included in the study were client-owned patients, and written informed consent was obtained from the owners. Monitoring of environmental contamination with Pt was performed in three homes of treated pet-dogs. The characteristics of the families are presented in Table 2.

Table 2 Characteristics of the families with treated pet-dogs

Home	Dog[a]	Location	Number of family members	Children still at home	Main living area dog	Days[a]	Number of samples
1	2	Town	2	No	Family room	60	8
2	3	Town	2	No	Entire home	26	10
3	4	Town	3	Yes (adult)	Family room	42	10

[a]: Pet-dog of the family
Time period in days, between end of treatment and sample collection

Sample collection

All excreta were collected by the pet owners after administration of carboplatin sDDS up until day 27, using nitrile gloves.[a] Urine samples were collected in 30 mL polypropylene tubes[b] using voided urine. Canine fecal samples were collected in 50 mL polypropylene tubes.[c] Saliva samples of pet-dogs were collected by letting the dog chew on a Salivette® cotton swab[d] and by inserting the swab between the teeth and the cheek of the dog, while holding the swab. Saliva production was stimulated beforehand by the presentation of food or by exercise. Sebum sampling was performed by wiping a body surface area of 10x10 cm on the skin (of the lower abdomen) and the coat (of the thorax) of the pet-dog. Each predefined surface area was wiped three times. Cerumen samples were collected by wiping the accessible part of the ear canal. Sebum and cerumen wipe[e] samples were stored in 50mL disposable polypropylene flasks.[f] The owners stored the samples at –20 °C, until the samples were transported to the lab. All samples were then stored at –20 °C until analysis.

The environmental monitoring was announced in advance. The same person collected the samples. A surface area of 10x10 cm was wiped, unless this was not possible. In that case, the complete top of the device (e.g. computer mouse) was sampled, while the surface area was estimated. All wipe[g] samples were collected while using a uniform sampling procedure by wiping the predefined surface three times. A new pair of gloves[h] was used for each wipe sample. Each wipe was moistened with 500 µL with pure water.[i] During each visit two procedural blanks were prepared by moistening the tissues with 500 µL water. Wipe samples were stored in 30 mL polypropylene tubes[j] at -20 °C until further processing.

Table 3 Excretion of Pt in urine of pet-dogs treated with carboplatin sDDS

		Pt in urine (in mg L^{-1})				
		Dog 1	Dog 2	Dog 3	Dog 4	All dogs
Dose [a]		500	100	300	250	
Overall	Median	9.1	0.7	0.1	0.2	0.8
	Range [b]	1.6 – 40.3	0.4 – 2.7	0.07 – 0.1	0.1 – 2.4	0.1 – 40.3
	n samples	8	8	5	6	27
Day 1-7	Median	15.2	0.6	[c]	1.6	6.8
	Range [b]	3.6 – 40.3	[c]	[c]	0.8 – 2.4	0.6 – 40.3
	n samples	6	1	[c]	2	9
Day 8-28	Median	1.7	0.8	0.1	0.2	0.7
	Range [b]	1.6 – 1.9	0.4 – 2.7	[c]	0.1 – 0.2	0.1 – 2.7
	n samples	2	7	1	4	15
Day 29-47	Median	[c]	[c]	0.1	[c]	0.1
	Range [b]	[c]	[c]	0.07 – 0.1	[c]	0.1 – 0.6
	n samples	[c]	[c]	4	[c]	5

[a]: dose administered at treatment prior to sampling, in mg
[b]: minimum – maximum concentrations
[c]: not available for measurement
n samples: number of samples

Analytical procedures

Pt analyses were performed as previously reported.[19-21] The methods are based on the quantification of Pt by inductively coupled plasma mass spectrometry (ICPMS),[k] with lower limits of quantification (LLOQ) of 7.50 ng L^{-1} Pt in human and canine urine, 15.0 ng L^{-1} Pt in canine oral fluid, and 0.105 ng g^{-1} Pt in canine feces, 0.15 pg cm^{-2} Pt in canine sebum, 7.50 pg per sampled external ear canal, and 0.05 pg cm^{-2} in wipe samples. The used methods have been fully validated according to the FDA guidelines.[22]

Sample pretreatment involved mainly dilution with appropriate diluents. In short, urine samples were thawed, whirl mixed and subsequently diluted 100-fold with 1% HNO_3[l] (*v*/*v*) solution. Canine fecal samples were thawed, weighed, homogenised (1:3 feces:water[m]) and whirl mixed. The samples were then centrifuged at 2,100 *g* for five min. The supernatant was centrifuged at 10,300 *g* for five min and afterwards diluted 100-fold with 1% HNO_3 solution. The resulting solution was then filtered with a 0.2 µm filter.[n] A procedure similar

to the pretreatment of urine samples was followed for oral fluid samples. These were diluted with a 0.01% (*g*/*v*) ammonium EDTA[o] and Triton X-100[p] solution (in water) and if necessary successively with an oral fluid: 0.01% EDTA/Triton X solution (1:100, *v*/*v*). To each mL sample, 10 µL internal standard solution (iridium,[q] 100 ng L^{-1}) is added. Afterwards, diluted samples were transferred to autosampler tubes. Sebum, cerumen and surface wipes were extracted with 10 mL 1% HCl[r] (*v*/*v*). The vessels were subsequently kept in an ultrasonic bath at 40 °C for 60 min. Then, samples were filtered using a 0.2 µm filter[s]. If necessary the filtrated solution was diluted further with 1% HCl. Afterwards, internal standard was added (iridium, 100 ng L-1, 10 µL per mL sample). Two mL of sample were introduced directly into the ICPMS.

Table 4 Excretion of Pt in feces of pet-dogs treated with carboplatin sDDS

		Pt in feces (in ng g^{-1})				
		Dog 1	Dog 2	Dog 3	Dog 4	All dogs
Dose [a]		500	100	300	250	
Overall	Median	2618	61.5	6.1	22.8	29.7
	Range [b]	1254 – 4245	8.6 – 200.4	5.0 – 9.8	4.0 – 437	4.0 – 4245
	n samples	3	8	6	8	25
Day 1-7	Median	2618	114	[c]	223	437
	Range [b]	1254 – 4245	[c]	[c]	220 – 437	114 – 4245
	n samples	3	1	[c]	3	7
Day 8-28	Median	[c]	57.8	5.0	14.9	24.5
	Range [b]	[c]	8.6 – 200.4	[c]	4.0 – 29.7	4.0 – 200.4
	n samples	[c]	7	1	5	13
Day 29-54	Median	[c]	[c]	6.2	[c]	6.2
	Range [b]	[c]	[c]	5.5 – 9.8	[c]	5.5 – 9.8
	n samples	[c]	[c]	5	[c]	5

[a]: dose administered at treatment prior to sampling, in mg
[b]: minimum – maximum concentrations
[c]: not available for measurement
n samples: number of samples

Results

Excretion products of pet-dogs treated with carboplatin sDDS

A total of 92 samples were collected in four pet-dogs treated with carboplatin sDDS, on several time points after administration (see Tables 1, 3, 4 and 5). Periods of sample collection varied between the pet-dogs. Furthermore, the exact days of sample collection within each sampling period varied between pet-dogs and within pet-dogs regarding different matrices. For example, in dog 2 one cerumen sample was collected on day 32 after the last administration of carboplatin sDDS, while all other matrices (urine, feces, saliva, sebum of the coat, sebum of the skin) were collected up to day 27. Not all samples were collected in each pet-dog. Dog 1 provided urine, feces, saliva, sebum (of the coat), and

cerumen samples. From dog 3 urine, feces, saliva and cerumen were collected. In dog 4, urine, feces and saliva were sampled. None of the collected salivary samples contained sufficient amounts for measurement. By measuring Pt background in excreta of pet-dogs prior to first treatment with carboplatin, we were able to determine normal Pt background variation in veterinary oncology patients.

Table 5 Excretion of Pt in sebum of the coat and in cerumen of pet-dogs treated with carboplatin sDDS

		Pt in sebum of the coat (in pg per 100 cm^2)			
		Dog 1	Dog 2	Dog 3	All dogs
Dose [a]		500	100	300	
Overall	Median	602	146	[c]	216
	Range [b]	150 – 803	105 – 237	[c]	105 – 803
	n samples	8	8	[c]	16
Day 1-7	Median	630	105	[c]	603
	Range [b]	571 – 803	[c]	[c]	105 – 803
	n samples	6	1	[c]	7
Day 8-28	Median	259	149	[c]	150
	Range [b]	150 – 369	130 – 237	[c]	130 – 369
	n samples	2	7	[c]	9
		Pt in cerumen (in pg per sample)			
		Dog 1	**Dog 2**	**Dog 3**	**All dogs**
Overall	Median	1290	143	1141	1023
	Range [b]	746 – 2184	126 – 673	1030 – 1252	126 – 2184
	n samples	8	7	2	17
Day 1-7	Median	1290	126	[c]	1123
	Range [b]	746 – 2184	[c]	[c]	126 – 2184
	n samples	6	1		7
Day 8-28	Median	1317	147	1252	420
	Range [b]	1023 – 1611	129 – 673	[c]	129 – 1611
	n samples	2	5	1	8
Day 29-54	Median	[c]	143	1030	587
	Range [b]	[c]	[c]	[c]	143 – 1030
	n samples	[c]	[c]	[c]	2

[a]: dose administered at treatment prior to sampling, in mg
[b]: minimum – maximum concentrations
[c]: not available for measurement
n samples: number of samples

Mean Pt level in urine samples on day 2 is 4.6 mg L^{-1} (see Figure 1). It rises to 40.3 mg L^{-1} on day 3. The mean urinary Pt concentration then declines gradually over the course of the following 4 weeks to 0.4 mg L^{-1} on day 27. The urine sample collected on day 47 contained a Pt amount at 0.1 mg L^{-1}. After the administration of carboplatin sDDS, mean fecal Pt concentration on day 2 is 1421 ng g^{-1}. The mean Pt level in feces declines to 31.9 ng g^{-1} by day 9. Afterwards, the fecal Pt levels decrease slowly in two dogs, while the fecal Pt concentrations in dog 2 vary considerably up to day 27, ranging between 8.6 ng g^{-1} (on day 15) and 200 ng g^{-1} (on day 21). One fecal sample, collected on day 54 in dog 3, contained a Pt amount of 5.5 ng g^{-1}. After dosing of carboplatin sDDS the Pt level in sebum of the coat of

dog 1 is 803 pg per 100 cm^2 body surface (day 2). The Pt concentration in sebum of the coat of that dog slowly falls to 150 pg per 100 cm^2 (day 13, last time point). In sebum samples of the coat of dog 2, Pt levels vary between sampling time points without a distinguishable pattern. Sebum samples of the skin were also collected in dog 2, and again, the Pt concentrations varied between sampling time points. Pt levels in cerumen samples showed no distinguishable decline in Pt concentrations throughout the sampling period, but varied between sampling time points within each dog.

Environmental contamination at the homes of pet-dogs treated with carboplatin sDDS

None of the families with pet-dogs treated with carboplatin sDDS were able to allow environmental monitoring before the first course. Results of Pt surface contamination are presented in Table 5. None of the procedural blanks prepared at the visits contained quantifiable levels of Pt exceeding the LLOQ.

After the administration of carboplatin sDDS, 35.7 % of the samples contained levels at or above the LLOQ. The number of contaminated wipe samples and location of the contamination varied between families. Samples showing high contamination were wipe samples of the floor and of the pet-dogs favorite resting place.

Table 6 Pt levels at the homes of pet-dogs after treatment with carboplatin sDDS

Sampled surface	Pt levels (in pg cm^{-2}) Homes		
	1	**2**	**3**
Cumulative dose [a]	100	650	250
Days [b]	60	26	42
Resting place	[d]	3.6	1.7
drinking	0.6	6.8	[d]
Kitchen			
Door handle	[c]	[c]	[c]
Work surface	[c]	[c]	[c]
Floor	[d]	[d]	0.3
refrigerator	[c]	[c]	[c]
Family room			
Door handle	[d]	[d]	[c]
Small table	[d]	[d]	[c]
Floor	1.7	10.8	[d]
Remote control	[c]	0.2	[c]
Handle telephone	[c]	[c]	[c]
Computer mouse	[d]	0.2	[d]
Waste bin top	[c]	[c]	0.3

[a]: cumulative dose administered to pet-dog, at time of environmental monitoring, in mg
[b]: time period between environmental monitoring and last treatment with carboplatin sDDS, in days
[c]: Below LLOQ (0.05 pg cm^{-2})
[d]: not available for measurement

Discussion

In this article, we describe the excretion of Pt after IM administration of a newly developed one-month sustained drug release formulation of carboplatin to pet-dogs. The carboplatin sDDS was effective at achieving and maintaining increased systemic Pt levels in pet-dogs throughout the first four weeks after administration, as evidenced by the excretion profile of Pt in canine urine, feces, sebum and cerumen. In one dog urine and feces was collected from day 27 up to day 54 after the last course with carboplatin sDDS. Relatively high Pt levels could be detected in these samples up until day 54. The detection of increased amounts of Pt in sebum samples might be the result of external contamination of the coat and skin of the dog. Normal dog behavior (e.g. licking), droplet formation when urinating, or transfer from contaminated surfaces may have caused these increased Pt levels. However, the presence of increased Pt concentrations in cerumen indicates that Pt is also transported transdermally, since the ear canal is more shielded from external influences. These increased levels have been reported in sebum and cerumen of pet-dogs treated with carboplatin, as well.[23]

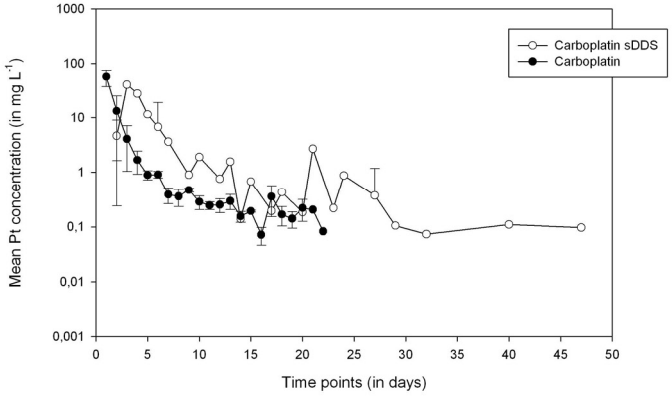

Figure 1 Mean urinary Pt concentrations in pet-dogs treated with carboplatin[23] or carboplatin sDDS, monitored up to 21 and 47 days after treatment, respectively. Mean concentrations are based on 90 samples collected in 10 pet-dogs or 27 samples collected in four dogs treated with carboplatin or carboplatin sDDS, respectively. Error bars are calculated using standard errors*2.

When carboplatin is administered to dogs the main route of excretion of Pt is via the kidneys.[23-25] Our findings indicate that this applies to the carboplatin sDDS used in this study as well. Urinary Pt concentrations rose between day 2 and day 3 when carboplatin sDDS was administered to pet-dogs, while maximal Pt levels were detected on day 1 if carboplatin was administered[23] (see Figure 1). The mean Pt concentration in canine urine and feces decreases at a slower rate in pet-dogs treated with carboplatin sDDS. As a result, mean Pt levels are, respectively, up to 12 and up to 27 times higher than the Pt concentrations in urine and fecal samples collected on the same day in pet-dogs treated with

carboplatin.[23] Furthermore, the Pt levels in urine and feces depend on the amount of administered carboplatin sDDS. Shimakura et al. (2002) [26] and Kitamura et al. (2011) [27] describe the selective delivering of a lipophilic Pt-containing compound in combination with an oily drug carrier, to the liver of dogs. The authors also reported that urine was the main route of excretion of Pt after release of the active drug from the carrier. Furthermore, Shimakura et al. (2002) [26] reported Pt excretion in urine and feces of dogs up to at least 13 weeks after dosing.

Table 7 Summary of normal Pt background and Pt levels after treatment with carboplatin sDDS

Surface samples	n subjects	n samples[a]	Mean[b]	Median[b]	Range[b,c]
Normal Pt[d]	6	14/85	0.3	0.1	0.1 – 1.7
After treatment	3	10/28	2.6	1.1	0.2 – 10.8

[a]: number of samples > LLOQ per total number of samples

[b]: expressed in mg L^{-1} (urine), pg cm^{-2} (surface samples)

[c]: minimum – maximum

[d]: Pt background in surface samples collected at the homes of pet-dogs, prior to treatment with carboplatin[23]

Increased Pt levels are evident in surface samples collected in homes of pet-dogs treated with carboplatin sDDS, compared to normal Pt background variation measured in homes of pet-dogs before the first course with carboplatin[23] (see Table 6). An increase in Pt surface contamination was also observed in homes of pet-dogs treated with carboplatin.[23] The Pt levels in surface samples were generally higher after administration of carboplatin. Factors that could affect the environmental contamination with Pt after administration of carboplatin sDDS are: the administered dose, individual differences in Pt elimination, frequency of vomiting, diarrhea and in-house urination, cleaning procedures, and implementation of and compliance to the veterinary safe handling guidelines. Environmental monitoring in the homes of pet-dogs treated with carboplatin was performed three weeks after dosing. In contrast, collection of surface samples in the homes of pet-dogs treated with carboplatin sDDS was performed 26 to 60 days after the last course of carboplatin sDDS. Furthermore, the environmental contamination by Pt measured at home 2, 26 days after administration of carboplatin sDDS, was considerably higher than the levels found at the other homes of pet-dogs treated with carboplatin sDDS. On the other hand, the cumulative dose administered to the pet-dog belonging to home 2 was higher compared to that of the other pet-dogs treated with carboplatin sDDS. Consequently, the lower Pt surface contamination at homes 1 and 3 described in the current study could be the result of lower administered doses, cleaning procedures, decreases in excretion of Pt by the treated pet-dog, or a combination of these factors.

Although Pt levels in surface samples were relatively low, some surfaces that were not accessible to the pet-dog, such as the computer mouse and the waste bin top, were also contaminated with Pt. This indicates that Pt may be dispersed by family members. This is further substantiated by the findings of the environmental monitoring of Pt at the homes of

pet-dogs treated with carboplatin, which also indicated that transference of Pt by owners occurred.[23] Furthermore, the dispersion of antineoplastic drugs to surfaces that are not expected to be contaminated (e.g. computer mouse) has been reported in veterinary oncology centers,[28,29] and human hospitals and pharmacies as well.[19,30,31] As gloves were only worn when handling the dog or its wastes during the first five days after administration of carboplatin, we can conclude that some skin contact with Pt may have occurred.

It is difficult to establish whether the Pt concentrations described in this article represent true health risks. Because we only measured Pt, we are unable to distinguish between carboplatin and its metabolites. Consequently, we cannot provide information on the biological activity of the measured Pt. As a result, we are presently investigating the DNA binding activity of the Pt measured in canine urine of pet-dogs treated with carboplatin sDDS.

Conclusion

The guidelines regarding the handling of antineoplastic drugs, treated animals and associated wastes developed by the ECVIM-CA,[18] list five days as being an expected period of risk for carboplatin. It is obvious from our study that five days is not sufficient when a sustained drug delivery formulation of carboplatin is administered. Furthermore, our findings indicate that sebum may be another source of exposure by pet-dogs treated with carboplatin sDDS. It is also evident that some Pt surface contamination occurs in homes of treated pet-dogs. Moreover, Pt can be dispersed by owners. Hence, skin contact with contaminated surfaces may occur. It is therefore advisable to reconsider the veterinary guidelines and adjust accordingly, when carrier-mediated antineoplastic drugs are used.

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Footnotes

[a] Klinion, Medeco, Oud-Beijerland, the Netherlands
[b] Sarstedt AG&Co, Nümbrecht, Germany
[c] Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA
[d] Salivette, Sarstedt AG&Co, Nümbrecht, Germany
[e] Kimtech Science precision wipes, Kimberley- Clark Professional, Irving, TX, USA
[f] Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA
[g] Kimtech Science precision wipes, Kimberley-Clark Professional, Irving, Tx, USA
[h] Klinion, Medeco, Oud-Beijerland, the Netherlands
[i] Aqua B. Braun Medical, Melsungen, Germany
[j] Sarstedt AG&Co, Nümbrecht, Germany
[k] Varian 810-MS, Mulgrave, Victoria, Australia
[l] Mallinckrodt Baker, Philipsburg, NJ, USA
[m] Aqua B. Braun Medical, Melsungen, Germany
[n] Sartorius minisart, Sarstedt AG&Co, Nümbrecht, Germany
[o] Sigma-Aldrich, St. Louis, MO, USA
[p] Sigma-Aldrich, St. Louis, MO, USA
[q] Merck, Darmstadt, Germany
[r] Mallinckrodt Baker, Philipsburg, NJ, USA
[s] Sartorius minisart, Sarstedt AG&Co, Nümbrecht, Germany

Chapter 4

Doxorubicin and epirubicin: excretion in dogs and environmental contamination

Chapter 4

The determination of doxorubicin, epirubicin, and their C-13 alcohols in canine excretion products and environmental samples

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Abstract

Objective: Monitoring of the excretion of doxorubicin, epirubicin and their C-13 metabolites in pet-dogs and evaluation of environmental contamination.

Methods: Liquid chromatography-tandem mass spectrometry was used to measure doxorubicin, epirubicin and their C-13 metabolites in canine urine, feces and saliva, and in surface samples taken in homes of treated pet-dogs and in veterinary oncology centers.

Results: Doxorubicin, epirubicin and their C-13 metabolites were detected in all excretion products, up to the last day of sampling. Contamination was demonstrated in 10 out of 93 and 3 out of 28 surface samples of the homes and the oncology centers respectively.

Conclusion: Administration of doxorubicin or epirubicin to pet-dogs results in excretion via all sampled routes, up to at least 21 days after dosing. Contamination was detected on 11% of the surfaces at the homes and the oncology centers, and the pattern of contamination indicates that skin contact with contaminated surfaces may occur.

Introduction

Exposure to antineoplastic drugs may induce adverse health effects.[1] As a result, guidelines have been developed in veterinary oncology, regarding safe handling of antineoplastic drugs, treated animals and related wastes.[2] In general, the treated animals return home after administration of the compound. Consequently, owners may be exposed to the administered antineoplastic drug, via their dog's excreta. Therefore, the guidelines also instruct the owners on the safe handling of their dog and its wastes. However, since exposure to antineoplastic drugs has rarely been monitored in veterinary medicine,[3,4] these guidelines are mainly based on knowledge transferred from human oncology. Thus, the guidelines and protective measures might not be entirely adequate for the veterinary field. Chemotherapy is increasingly used in veterinary oncology. The tailoring of the implemented guidelines to the reality and needs of veterinary oncology has therefore become mandatory.

Doxorubicin is a standard component in many chemotherapy protocols in veterinary oncology. It is used for the treatment of several tumor types, including lymphoma, osteosarcoma and hemangiosarcoma.[5-7] Anorexia, nausea, vomiting and diarrhea are common side-effects, apart from the cumulative cardiac toxicity.[8] Epirubicin is a stereoisomer of doxorubicin. Compared to doxorubicin, epirubicin has nearly the same spectrum of activity and mode of action. On the other hand, it has different pharmacokinetic and metabolic properties,[9] e.g. shorter elimination half-life and differences in the levels of metabolites. As a result epirubicin shows reduced cardiotoxicity in animal models and some in-vivo studies.[10] The use of epirubicin in veterinary oncology, however, has only sporadically been described.[11,12] Gastrointestinal side-effects are frequently reported in dogs.[11,12] Cardiotoxicity has, so far, not been described in dogs treated with epirubicin, though the number of investigated dogs was limited.[11,12] Doxorubicinol and epirubicinol are C-13 secondary alcohols of doxorubicin and epirubicin.[13] These metabolites have also been correlated with cardiotoxicity, but they are less cytotoxic than their parent compound.[9,14,15] Few studies monitored potential exposure of people to doxorubicin in veterinary medicine.[3,16] To our knowledge, no research investigating the exposure of people to epirubicin in veterinary oncology, has been reported in the literature. To evaluate potential exposure of owners, we determined the excretion of doxorubicin, doxorubicinol, epirubicin and epirubicinol in urine, feces and saliva of treated pet-dogs. Furthermore, epirubicin and epirubicinol were monitored in sebum and cerumen samples obtained from one dog. In addition, we also monitored environmental contamination with doxorubicin, doxorubicinol, epirubicin and epirubicinol at the homes of treated pet-dogs and at two veterinary oncology centers.

Materials and Methods

Patients and chemotherapy regimes

Client-owned pet dogs treated with doxorubicin[ab] or epirubicin,[c] as a single agent protocol (every three weeks), in combination with carboplatin (every six weeks), or as part of the CHOP-protocol, were eligible for this study. Dogs were enrolled in the study between May 2010 and May 2012. The characteristics of the dogs are presented in Table 1. Great heterogeneity exists in the patient population with regard to body weight and body surface area, due to the limited number of eligible dogs. A signed informed consent form was obtained from the owners.

Monitoring of surface contamination at the homes of pet-dogs treated with epirubicin

Wipe samples were collected at the homes of four families that had their pet-dog treated with epirubicin. The homes were situated in the Netherlands and Flanders. All the dogs lived in the house, though access was restricted to certain areas (e.g. family room). Most households consisted of two members, apart from one which consisted of three family members. No children younger than 18 years old were present. Several veterinary oncology centers in the Netherlands and Flanders participated in the study by informing owners of eligible dogs about this investigation. Surface contamination was monitored three weeks after the first administration of epirubicin.

Table 1 Characteristics of pet-dogs treated with doxorubicin or epirubicin

Dog	n[a]	Age[b]	Breed	Gender	Tumor type	AD	Administered dose[b]	Cumulative dose[b,c]
1	61 (13)	9	Bobtail	F	Lymphoma	Epi	33 (30)	33
2	0 (50)	13	German pointer	M	Lymphoma	Epi	25 (25)	25
3	0 (22)	8	Irish setter	F	Thyroid carcinoma	Epi	50 (30)	50
4	19 (0)	10	Airedale terrier	M	Lymphoma	Epi	30 (30)	150
5	29 (8)	6	Rhodesian ridgeback	F	Lymphoma	Epi	30.6 (30)	30.6
6	9 (0)	3	Bordeaux dog	M	Lymphoma	Epi	42 (30)	84
7	33 (0)	11	French bulldog	F	Mammary carcinoma	Epi	14.75 (25)	29.5
8	11 (0)	7	Bernese mountain dog	F	Lymphoma	Dox	32.7 (30)	96.7
9	7 (0)	12	Belgian sheepdog	F	Thyroid carcinoma	Dox	24 (30)	126.4
10	18(0)	5	Rottweiler	M	Lymphoma	Dox	40 (30)	40
11	33 (0)	5	Bernese mountain dog	M	Lymphoma	Dox	43.5 (30)	174
12	24 (0)	13	Afghan hound	M	Lymphoma	Dox	20 (22.5)	20
13	27 (0)	11	Golden retriever	F	Mammary carcinoma	Dox	12.5 (25)	12.5

[a]: number of biological samples (number of environmental samples)
[b]: units of measurement: age at treatment in years, administered dose at sampled course in mg (in mg m-2), cumulative dose at sampled course in mg
[c]: cumulative dose: the total amount of doxorubicin (doxo) or epirubicin (epi) received by the dog, at the time of sample collection
AD: antineoplastic drug, F: female, M: male

Table 2 Excretion of doxorubicin and doxorubicinol in urine, feces and saliva of treated pet-dogs

	Urine[a]				Feces[a]				Saliva[a]			
Day	Doxorubicin Median (range) [b]	n	Doxorubicinol Median (range) [b]	n	Doxorubicin Median (range) [b]	n	Doxorubicinol Median (range) [b]	n	Doxorubicin Median (range) [b]	n	Doxorubicinol Median (range) [b]	n
1	1895 (1060 – 2730)	2/2	83.8 (62.2 – 105)	2/2	3.7	1/1	[d]	0/1	2.5	1/1	0.09	1/1
2	1020 (1.6 – 1270)	3/3	105 (57.3 – 130)	3/3	38.8 (22.1 – 43.0)	4/4	37.0 (24.3 – 74.5)	4/4	2.7 (0.37 – 5.0)	2/2	0.22	1/2
3	250 (68.9 – 510)	3/4	57.6 (27.8 – 112)	3/4	6.7 (5.9 – 12.7)	3/3	9.5 (5.4 – 13.9)	3/3	1.4 (0.19 – 1.5)	3/3	0.16 (0.08 – 0.24)	2/3
4	332 (73.3 – 479)	4/5	100 (36.9 – 142)	4/5	23.3 (1.7 – 112)	4/4	141 (112 – 170) [c]	2/4	0.91 (0.27 – 7.7)	3/3	0.18 (0.16 – 0.20)	2/3
5	192 (49.8 – 405)	3/3	109 (29.4 – 128)	3/3	10.6 [c]	1/3	20.4 (3.0 – 37.8)	2/3	1.3 (0.27 – 1.6)	3/3	0.23 (0.16 – 0.30)	2/3
6	59.4 (34.9 – 251)	3/3	39.0 (24.9 – 99.9)	3/3	37.8 [c]	1/2	37.1 (4.2 – 69.9)	2/2	0.29 [c]	1/2	0.08	1/2
7	162 (25.5 – 217) [c]	3/3	59.7 (25.5 – 85.3)	3/3	12.1 (2.8 – 21.5) [c]	2/4	5.1 (3.0 – 73.4) [c]	3/4	0.43 (0.42 – 0.44)	2/2	0.09	2/2
9	12.2	1/2	14.0	1/2	-	0	-	0	[d]	0/1	[d]	0/1
10	26.2 (11.8 – 98.7)	4/4	24.1 (12.3 – 52.8)	4/4	111 (3.6 – 218) [c]	2/4	7.0 (2.2 – 497)	4/4	0.08	1/1	[c]	0/1
11	63.4	1/1	41.6	1/1	[d]	0/1	[d]	0/1	-	0	-	0
12	28.1	1/2	35.8	1/2	[d]	0/1	1.6	1/1	[d]	0/1	[d]	0/1
13	4.3 (3.9 – 4.6)	2/2	5.7 (4.5 – 7.0)	2/2	1.1 (1.3 – 1.2)	2/3	7.6	1/3	[c]	0/2	0.05 [c]	1/2
14	45.4	1/1	35.8	1/1	[d]	0/1	[d]	0/1	[d]	0/1	[d]	0/1
15	-	0	-	0	-	0	-	0	[d]	0/1	[d]	0/1
16	11.1 (7.0 – 15.1)	2/2	9.6 (5.4 – 13.9)	2/2	[c]	0/2	7.1	1/2	[d]	0/2	[d]	0/2
17	17.8	1/1	16.6	1/1	[d]	0/1	[d]	0/1	[d]	0/1	[d]	0/1
18	16.2	1/2	10.7 (1.6 – 19.8)	2/2	[d]	0/1	[d]	0/1	[d]	0/1	[d]	0/1
19	2.7	1/1	3.3	1/1	[c]	0/2	6.3	1/2	[d]	0/1	[c]	0/1
20	-	0	-	0	-	0	-	0	3.3	1/1	3.2	1/1
21	0.94	1/1	1.2	1/1	[d]	0/1	[d]	0/1	[d]	0/1	[d]	0/1
22	-	0	-	0	-	0	-	0	[d]	0/1	[d]	0/1

[a]: lower limit of quantification (LLOQs): 0.10 or 1.0 ng mL^{-1} (in urine), 0.35 or 0.88 ng g^{-1} (in feces), and 0.05 or 0.10 ng mL^{-1} (in saliva)

[b]: : median (minimum – maximum), in ng mL^{-1} (urine, corrected for matrix effect), ng g^{-1} (faeces) or ng mL^{-1} (saliva)

[c]: one or more samples below LLOQ, though a peak could be observed

[d]: all samples below LLOQ

n: number of samples above LLOQ/total number of samples, -: not available for measurement

Monitoring of surface contamination at veterinary oncology centers

The wipe samples were taken in two veterinary oncology centers in the Netherlands (site 1) and Flanders (site 2). Each center had a preparation room, while administration occurred in

a general consultation room. Site 1 used a biological safety cabinet (BSC), while site 2 used a vertical laminar air flow (LAF) hood. Site 1 used the Tevadaptor® drug-handling device (DHD),[d] while site 2 used the PhaSeal® closed system drug transfer device (CSTD).[e] Samples were collected at surfaces that were prone to contamination, such as surfaces in the preparation rooms, but also at other locations, such as surfaces in the canteen. Samples from site 1 were monitored for doxorubicin and doxorubicinol, while samples from site 2 were monitored for epirubicin and epirubicinol.

Table 3 Excretion of epirubicin and epirubicinol in urine, feces and saliva of treated pet-dogs

	Urine[a]				Feces[a]				Saliva[a]			
	Epirubicin		Epirubicinol		Epirubicin		Epirubicinol		Epirubicin		Epirubicinol	
Day	Median (range) [b]	n	Median (range) [b]	n	Median (range) [b]	n	Median (range) [b]	n	Median (range) [b]	n	Median (range) [b]	n
1	4052 (320 – 11836)	3/3	191 (39.6 – 341)	3/3	5.4 (2.4 – 8.3)	2/2	[d]	0/2	0.22		[d]	0/2
2	361 (312 – 518)	5/5	39.3 (12.3 – 181)	4/5	15.9 (1.9 – 67.2)	4/5	4.3 [c]	1/5	0.40 [c]		[d]	0/3
3	139 (85.8 – 392)	4/4	71.0 (25.9 – 247)	4/4	4.9 (2.7 – 39.6)	4/4	4.1 (3.4 – 4.8)	2/4	0.68		[d]	0/2
4	87.7 (25.9 – 143)	4/4	52.7 (11.0 – 59.4)	4/4	5.8 (2.7 – 8.9)	2/2	3.4 (2.1 – 4.8)	2/2	0.63 (0.44 – 0.81)		0.11	
5	29.7 (9.2 –189)	4/4	23.2 (9.7 – 166)	4/4	4.2 (2.0 – 325)	5/5	4.2 (1.8 – 322)	5/5	[d]	0/3	0.07	
6	20.0 (6.0 – 48.0)	3/3	8.7 (2.4 – 39.2)	3/3	42.0 (7.7– 93.5)	3/3	76.3 (7.9 – 97.7)	3/3	[d]	0/1	0.09	
7	43.0 (18.9 – 114) [c]	3/3	41.9 (11.7 – 97.3)	3/3	6.3 [c]	1/2	16.1	1/2	[d]	0/3	[d]	0/3
8	30.1 (13.6 – 46.6)	2/2	41.5 (8.5 – 74.5)	2/2	15.0 (7.4 – 22.6)	2/2	14.5 (12.3 – 16.8)	2/2	[d]	0/1	[d]	0/1
9	13.1	1/1	21.0	0/1	[c]	0/1	[c]	0/1	[d]	0/1	[d]	0/1
10	7.4 (2.6 – 12.3)[c]	2/3	9.5 (4.4 – 17.7) [c]	2/3	12.4 (6.3 – 18.4) [c]	2/3	21.6 (11.2 – 32.1)	2/3	0.14		[d]	0/3
11	4.5 (3.9 – 5.1)	2/2	9.3 (2.9 – 15.7)	1/2	34.7	1/2	53.9	1/2	-	0	-	0
12	1.6	1/1	3.3	1/1	-	0	-	0	-	0	-	0
13	7.7 [c]	1/2	8.5 [c]	1/2	8.3	1/2	8.3	1/2	[d]	0/2	[d]	0/2
15	4.0	1/2	5.0	1/2	-	0	-	0	-	0	-	0
16	2.8 [c]	1/2	4.2 (13.8 – 20.2) [c]	1/2	[d]	0/2	[d]	0/2	0.20		[d]	0/2
18	[d]	0/1	[d]	0/1	[d]	0/1	[d]	0/1	-	0	-	0
19	[c]	0/2	2.0 [c]	1/2	[c, d]	0/2	1.7	1/2	[d]	0/2	[d]	0/2
21	[d]	0/3	1.6	1/3	[d]	0/3	[d]	0/3	0.06		-	0
23	[d]	0/1	[d]	0/1	[d]	0/1	[d]	0/1	-	0	-	0

[a]: lower limit of quantification (LLOQs): 0.10 or 1.0 ng mL^{-1} (in urine), 0.35 or 0.88 ng g^{-1} (in feces), and 0.05 or 0.10 ng mL^{-1} (in saliva)

[b]: : median (minimum – maximum), in ng mL^{-1} (urine), ng g^{-1} (faeces) or ng mL^{-1} (saliva)

[c]: one or more samples below LLOQ, though a peak could be observed

[d]: all samples below LLOQ

n: number of samples above LLOQ/total number of samples, -: not available for measurement

Sample collection

Canine excretion products of pet-dogs treated with doxorubicin or epirubicin

All samples were collected by the pet owners on the day of drug administration (after dosing) up until day 21 post-infusion. A new pair of disposable gloves[f] was used for each sample. Canine urine samples were collected in 30 mL polypropylene tubes[g] using voided urine. Canine fecal samples were collected in 50 mL polypropylene tubes.[h] Canine saliva samples were collected by letting the dog chew on Salivette® cotton swabs[i] and by inserting the swab between the teeth and the cheek of the dog, both while holding the swab, until the swab was soaked with saliva or the dog consistently resisted sampling. Saliva production

was stimulated by exercise or the presentation of food. In one dog, treated with epirubicin, the owners also collected sebum and cerumen samples. Sebum sampling was performed by wiping a body surface area on the coat of the thorax and the skin of the lower abdomen of the dog. All wipe samples were collected using a uniform sampling procedure by wiping three times a predefined body surface of 10x10 cm. Cerumen sampling was performed by wiping the accessible part of the ear canal. Kimtech Science precision wipes were used.[j] Wipe samples were stored in 50mL disposable polypropylene flasks.[k] The owners stored the samples at –20 °C, until the samples were transported to the lab. All samples were then stored at –20 °C until analysis.

Surface wipe samples

Several wiping procedures were used for the collection of the wipe samples, based on information in the literature.[17-19] In **procedure 1** Kimtech Science precision wipes[l] were moistened with 500 µL pure water,[m] while one sample per surface area was collected. **Procedure 2** used 1/4 Kimtech Science precision wipes moistened with 500 µL 50% acetonitrile (ACN) in water, while one sample per surface area was collected. For **procedure 3** 1/4 Kimtech Science precision wipes were moistened with 500 µL 70% 2-propanol in water, while one sample per surface area was collected. **Procedure 4** used 1/8 grade 42 Whatman ashless cellulose filters[n] moistened with 100 µL 80% ethanol (EtOH)[o] in water, while three samples were collected per surface area. The environmental monitoring was announced in advance. Sampling was performed by the same person by wiping a surface area of 10x10 cm. For surfaces for which it was not possible to take a 10x10 cm sample, the complete top of the device was sampled and the size of the area was estimated. All wipe samples were collected using a uniform sampling procedure by wiping the predefined in three different directions. A new pair of nitrile gloves was used for each wipe sample. For each visit 2 procedural blank samples were prepared by moistening the wiping tissues with 100 or 500 µL wiping solution, without further use. After wiping, samples were stored in 30 mL polypropylene tubes[p] at -20 °C until further processing.

Sample preparation

All samples were protected from the light during sample preparation, using amber-colored eppendorf cups, amber-colored vials or aluminum foil. Urine samples were thawed, vortex mixed, centrifuged for three min at 23,100 *g* and diluted 10-fold with ACN:0.1% formic acid (HCOOH) in water (1:3.5, *v*/*v*). Per gram feces 3.5 mL ACN:0.1% HCOOH in water (1:2, *v*/*v*) was added. The samples were vortex mixed and then ultrasonicated for 20 min. The fecal homogenate was subsequently centrifuged for 10 min at 23,100 *g*. The supernatant was then diluted twofold with 0.1% HCOOH in water. Saliva samples were centrifuged at 5000 rpm for 10 min. When saliva could be not be collected from the salivette® cotton swabs, 200 µL ACN:0.1% HCOOH in water (1:4, *v*/*v*) was added to the swab and the centrifugation step

was repeated. To sebum, cerumen and surface wipe samples 5.0 mL ethanol[q] (EtOH) pH3 (using HCl)[r] was added. The samples were subsequently mixed for 30 min at 1,250 rpm. The solution was then transferred to a clean tube, and evaporated to dryness using a nitrogen gas flow at 40 °C. Afterwards, the samples were reconstituted using 100 µL ACN:0.1% HCOOH in water (1:4, *v*/*v*). The subsequent solution was then vortex mixed and centrifuged at 5,000 rpm for two min at 4.0 °C.

Liquid chromatography coupled with tandem mass spectrometric detection

An API 4000 triple quadrupole MS with electrospray ionisation[s] was coupled to an Agilent HP1100 liquid chromatographic system.[t] The Agilent 1100 system consisted of a binary pump, an autosampler, a mobile phase degasser and a column oven. Chromatographic separation was obtained using a Kinetex C18 column[u] (specifics: 50x2.1 mm internal diameter, particle size 2.6 µm) protected with an inline filter (0.5 µm). The mobile phase was as follows: isocratic elution with 77% phase A (0.1% HCOOH in water (*v*/*v*)) and 23% phase B (ACN), at a flow of 0.2 mL min^{-1} for 5 min. The column oven was set at 40 °C, while the autosampler was thermostatted at 4 °C. A sample volume of 10 µL was injected and the injection needle was washed for 5 seconds with ACN after each injection. Mass transitions and ion specific parameters were optimized for each compound in positive ion mode. The precursor ions for doxorubicin, doxorubicinol, epirubicin and epirubicinol were *m*/*z* 544, 546, 544 and 546 respectively, while the product ions were *m*/*z* 397, 399, 397 and 363 respectively. The total run time was 5 minutes. For quantification, the multiple reaction monitoring (MRM) chromatograms were acquired with Analyst software version 1.5.[v]

Urine, feces and saliva of pet-dogs treated with epirubicin or doxorubicin were measured using calibration standards containing epirubicin and epirubicinol, prepared in reconstitution solution (ACN:0.1% HCOOH in water 1:4 *v*/*v*). The lower limits of quantification (LLOQs) of epirubicin and epirubicinol ranged from: 0.10 to 1.0 ng mL^{-1} for urine, 0.10 to 0.25 ng mL^{-1} corresponding to 0.35 to 0.88 ng g^{-1} for feces, and 0.05 to 0.10 ng mL^{-1} for saliva. The matrix effect factor for epirubicin and epirubicinol in urine is 0.66. Doxorubicin, doxorubicinol, epirubicin or epirubicinol in surface wipe samples, sebum and cerumen samples were measured using calibration standards containing doxorubicin and doxorubicinol, or epirubicin and epirubicinol prepared in reconstitution solution. The LLOQs for wipe samples for doxorubicin, doxorubicinol, epirubicin, and epirubicinol varied between 0.005 and 0.50 ng mL^{-1}, corresponding to 0.05 and 5.0 pg cm^{-2}. The LLOQs for sebum and cerumen were: 0.05 ng mL^{-1} for epirubicin and 0.25 ng mL^{-1} for epirubicinol, corresponding to 0.05 and 0.25 ng per sample, respectively.

Results

Canine excretion products of pet-dogs treated with doxorubicin

Doxorubicin and doxorubicinol were measured in 111 samples, collected on several time

points after administration of doxorubicin, in six dogs (see Table 2). Mean urinary level on day 1 (day of administration, after dosing) is 1895 ng mL^{-1} for doxorubicin and 83.8 ng mL^{-1} for doxorubicinol. The mean urinary doxorubicin concentration declines to 135 ng mL^{-1} on day 7. Afterwards, the doxorubicin levels in urine decrease more gradually to 11.1 ng mL^{-1} on day 16 (mean). Doxorubicinol levels in urine decreased gradually up to day 18 (mean: 10.7 ng mL^{-1}). Mean doxorubicin and doxorubicinol level on day 2 in canine feces is 35.7 ng g^{-1} and 43.2 ng g^{-1}, respectively. This gradually declines to 1.7 ng g^{-1} (mean concentration on day 13) for doxorubicin and to 6.3 ng g^{-1} on day 21 for doxorubicinol. Mean salivary concentrations of doxorubicin decreased from 2.5 ng mL^{-1} on day 1 to 0.43 ng mL^{-1} on day 7. Mean doxorubicinol level increased from 0.09 ng mL^{-1} on day 1 to 0.22 ng mL^{-1} on day 2. Afterwards the doxorubicinol levels declined slowly to 0.09 ng mL^{-1} on day 7.

Table 4 Excretion of epirubicin and epirubicinol in sebum and cerumen of a pet-dog treated with epirubicin

	Sebum of the coat[a, b]		Sebum of the skin[a, b]		Cerumen[a, b]	
Day	Epirubicin	Epirubicinol	Epirubicin	Epirubicinol	Epirubicin	Epirubicinol
1	[c]	[c]	0.29	[c]	[c]	[c]
2	[c]	[c]	2.6	[c]	[c]	[c]
3	[c]	[c]	0.69	[c]	[c]	[c]
4	[c]	[c]	[c]	[c]	[c]	[c]
5	[c]	[c]	0.28	[c]	[c]	[c]
6	[c]	[c]	[c]	[c]	0.05	[c]
7	[c]	[c]	0.13	[c]	0.12	[c]
8	[c]	0.30	0.16	[c]	0.08	[c]
9	[c]	[c]	0.07	[c]	[c]	[c]
10	[c]	0.11	[c]	[c]	0.33	[c]
11	[c]	[c]	0.26	[c]	0.25	[c]

[a]: lower limit of quantification (LLOQ): 0.05 ng per sample (epirubicin), or 0.25 ng per sample (epirubicinol) for sebum and cerumen samples

[b]: in ng per sample (e.g. 100 cm^2 body surface area or per sampled ear)

[c]: below LLOQ

Canine excretion products of pet-dogs treated with epirubicin

Epirubicin and epirubicinol were monitored in 123 samples, collected in five dogs, on several time points after administration of epirubicin (see Table 3 and 4). Mean epirubicin and epirubicinol concentrations on day 1 in urine are 5402 ng mL^{-1} and 191 ng mL^{-1}, respectively. Mean urinary level of epirubicin declines rapidly to 189 ng mL^{-1} (mean) on day 3. Afterwards, the urinary epirubicin levels decrease more slowly up to day 16 (mean: 2.8 ng mL^{-1}). Urine levels of epirubicinol decreased to 1.6 ng mL^{-1} on day 21. Mean fecal concentration on day 1 is 5.4 ng g^{-1} for epirubicin. Mean epirubicin level increases until day 5 to 81.6 ng g^{-1}. Afterwards it gradually declines to 8.3 ng g^{-1} on day 13. Epirubicinol cannot be detected in feces until day 2. The mean fecal level of epirubicinol then rises to 74.6 ng g^{-1} on day 5. This decreases gradually to 1.7 ng g^{-1} on day 19. Eleven saliva samples contained

levels of epirubicin and epirubicinol at or above the LLOQ. Some of the sebum and cerumen samples collected in one dog contained measurable levels of epirubicin or epirubicinol (see Table 4).

Environmental monitoring

Retrospectively, we have investigated the influence of the type and size of the wipe material, the wiping solution, the number of samples per surface area and manual pressure during the wiping procedure on the recovery from the surface. Our findings indicate that wiping with Whatman filters (size: 1/4), using three wipes per surface area, with 80% EtOH as wiping solution, while applying maximal pressure, provided the best results in our hands, with up to 62% recovery from a linoleum surface (data not shown).

Environmental contamination at the homes of pet-dogs treated with epirubicin

Surface contamination of all standard locations is presented in Table 5. At four homes of treated pet-dogs a total of 93 surface samples were collected. Only 4.3% of the wipe samples contained quantifiable levels of epirubicin and 6.5% of the wipes contained epirubicinol at or above the LLOQ. None of the procedural blanks contained epirubicin or epirubicinol at or above the LLOQ.

Environmental contamination at veterinary oncology centers

Surface contamination of all standard locations is presented in Table 5. A total of 28 surface wipe samples were collected at two veterinary oncology centers. At site 1 only one sample contained a quantifiable level of epirubicinol. At site 2 one sample contained both doxorubicin and doxorubicinol at levels at or above the LLOQ, while another sample demonstrated contamination with only doxorubicinol. None of the procedural blanks contained doxorubicin, doxorubicinol, epirubicin or epirubicinol at or above the LLOQ.

Discussion

This study demonstrates doxorubicin, doxorubicinol, epirubicin, and epirubicinol excretion by pet-dogs up to 21 days after treatment. Furthermore, environmental contamination with doxorubicin, epirubicin and their C-13 alcohol metabolites was detected at some surfaces in the homes of treated pet-dogs and in veterinary oncology centers.

Table 5 Surface contamination in homes of treated pet-dogs and in veterinary oncology centers, in the Netherlands and Flanders

Location	Room	Sampled surface	Area[a]	n	Epirubicin[a, b]	n	Epirubicinol[a, b]
Homes	Miscellaneous	Resting place	100	1/8	0.47	0/8	[c]
		Area for feeding and drinking	100	0/6	[c]	1/6	0.84
	Kitchen	Door handle	15	1/8	1.80	0/8	[c]
		Work surface	100	0/8	[c]	0/8	[c]
		Floor	100	0/6	[c]	1/6	0.30
		Door handle refrigerator	15	0/6	[c]	0/8	[c]
	Family room	Table	100	1/8	0.06	0/8	[c]
		Door handle	15	1/8	0.53	0/8	[c]
		Small table	100	0/5	[c]	0/5	[c]
		Floor	100	0/7	[c]	2/7	(0.16 – 0.91)
		Remote control	50	0/7	[c]	0/7	[c]
		Handle phone	50	0/7	[c]	0/7	[c]
		Computer mouse	50	0/3	[c]	0/3	[c]
		Waste bin top	100	0/4	[c]	2/4	(0.71 – 2.13)
Location	**Room**	**Sampled surface**	**Area[a]**	**n**	**Epirubicin (Doxorubicin)[a, b]**	**n**	**Epirubicinol (Doxorubicinol)[a, b]**
Oncology centers	Waiting room	Floor	100	0/2	[c] ([c])	0/2	[c] ([c])
	Reception	Floor	100	0/1	[c] ([c])	0/1	[c] ([c])
	Consultation room	Floor	100	0/2	[c] ([c])	0/2	[c] ([c])
		Table	100	0/2	[c] ([c])	0/2	[c] ([c])
		Door handle	15	0/2	[c] ([c])	0/2	[c] ([c])
		Handle phone	50	0/2	[c] ([c])	0/2	[c] ([c])
		Mouse computer	50	0/2	[c] ([c])	0/2	[c] ([c])
		Door handle refrigerator	15	0/1	[c] ([c])	0/1	[c] ([c])
		Waste bin top	100	1/1	(0.15)	1/1	(2.5)
	Preparation room	Floor	100	0/1	[c] ([c])	0/1	[c] ([c])
		Work bench	100	0/2	[c] ([c])	0/2	[c] ([c])
		Door handle	15	0/1	[c] ([c])	0/1	[c] ([c])
		Waste bin top	100	0/1	[c] ([c])	0/1	[c] ([c])
		Transport box	100	0/1	[c] ([c])	0/1	[c] ([c])
	Canteen	Floor	100	0/2	[c] ([c])	1/2	[c] (0.59)
		Table	100	0/2	[c] ([c])	1/2	3.3 ([c])
		Kitchen sur	100	0/1	[c] ([c])	0/1	[c] ([c])

[a]: surface area in cm^2; epirubicin, doxorubicin, epirubicinol and doxorubicinol in pg cm^{-2}

[b]: lower limit of quantification (LLOQ): between 0.05 and 5.0 pg cm^{-2} (epirubicin, doxorubicin, epirubicinol and doxorubicinol)

[c]: all samples below LLOQ

n: number of samples above LLOQ /total number of samples

The sampling period, during which canine excretion products were sampled, lasted up to 21 days after treatment of the pet with doxorubicin or epirubicin. Doxorubicin, doxorubicinol, epirubicin and epirubicinol were found predominantly in urine and fecal samples from treated pet-dogs. In addition, some saliva samples contained measurable amounts of these

compounds as well. The levels varied depending on the compound, excretion product, time interval between treatment and sample collection, and pet-dog. There was not enough data available to evaluate whether the cumulative dose at time of sampling may have influenced the level of excretion. Residues of doxorubicin, doxorubicinol, epirubicin and epirubicinol could be measured up to day 21 after dosing in urine, up to day 19 in feces, and up to day 20 or day 21 (after administration of doxorubicin or epirubicin, respectively) in saliva.

The prolonged excretion of doxorubicin, doxorubicinol, epirubicin and epirubicinol was not unexpected. Knobloch et al. (2010) [3] have also monitored doxorubicin in urine of treated pet-dogs and they measured traces up to 21 days after dosing (range: 0.0 – 2.5 ng mL^{-1}, dose: 30 mg m^{-2}). The persistence of the anthracyclines and their C-13 alcohols may be explained by their moderate protein-binding, e.g. 50-80% and 45% for doxorubicin and epirubicin, respectively.[20,21] Furthermore, doxorubicin and epirubicin are associated with high tissue-binding, mainly at the nucleus and lysosomes.[22,23]

The presence of epirubicin and epirubicinol in sebum and cerumen samples of a treated pet-dog could indicate that these compounds can be excreted via the skin. However, this should be confirmed in other dogs treated with epirubicin. Moreover, this should also be investigated in pet-dogs treated with doxorubicin. It is unlikely that epirubicin and epirubicinol are secreted into sebum or cerumen, since the time lapse between sebum production and its excretion onto the skin surface averages eight days in humans,[24] but is unknown in dogs. Because the intracellular matrix of the keratinocytes is virtually impenetrable,[25] intercellular transdermal transport of epirubicin and epirubicinol would be the most likely route of excretion, if skin penetration truly transpires. The presence of epirubicin and epirubicinol in sebum samples might also be caused by transference from contaminated surroundings, by normal dog behavior (e.g. licking), or by droplet formation when urinating. However, we hypothesize that the external ear canal forms an enclosed environment, thus reducing the contribution of potential external contamination. Additionally, we have recently reported increased levels of platinum (Pt) in sebum and cerumen samples of pet-dogs after administration of carboplatin,[26] which, consequently, further substantiates the findings described here.

The safe handling guidelines of the European College of Veterinary Internal Medicine – Companion Animals (ECVIM-CA) consider the first seven days after administration of doxorubicin as the period of risk. The results reported in this article, however, indicate that this period may be too short. This confirms the results reported by Knobloch et al. (2010),[3] who also detected traces of doxorubicin in canine urine up to 21 days after administration.

Poor recovery of doxorubicin from surfaces[27,28] and the photodegradation[29] of doxorubicin and epirubicin are well known in the literature. Nonetheless, these drugs were present in surface samples. This demonstrates that actual exposure to doxorubicin, epirubicin and their C-13 alcohols can occur. To our knowledge, no previous studies describe environmental contamination with doxorubicin or epirubicin at veterinary oncology centers or at homes of

pet-dogs treated with these anthracyclines. Furthermore, few studies reporting on surface contamination with doxorubicin and epirubicin in human oncology could be traced. Turci et al. (2011) [30] were not able to detect measurable levels in surface samples, though the developed assay was probably not as sensitive as the method reported here (the LLOQ was not disclosed). Nussbaumer et al. (2012) [19] were also unable to measure doxorubicin or epirubicin in surface samples. They report a LLOQ of 100 pg cm^{-2} for doxorubicin and epirubicin. This is markedly higher than the LLOQs for wipe samples reported in our article, which ranged between 0.05 and 5.0 pg cm^{-2}. To the authors' knowledge, the current article is also the first environmental monitoring of doxorubicinol and epirubicinol. No reports could be traced regarding surface monitoring of these compounds in human and veterinary oncology centers, or in homes of human and canine oncology patients. Three surface samples collected at the veterinary oncology units contained traces of doxorubicinol or epirubicinol, while only one contained doxorubicin. This indicates that some of the environmental contamination in the oncology units is the result of excretion by treated pet-animals. However, none of the veterinary oncology units reported hospitalization of pet-dogs treated with doxorubicin or epirubicin. Consequently, it seems that during the relative short period of the duration of consults with pet-dogs treated with these compounds, excretion occured. This may have taken place during and shortly after treatment, or when consultations took place during the period of excretion of doxorubicinol or epirubicinol. This period may last up until at least 21 days after administration of the corresponding anthracycline. At the homes of treated pet-dogs, surface contamination with epirubicin and epirubicinol was detected. Although only few samples exceeded the LLOQ, the detection of doxorubicin, doxorubicinol, epirubicin and epirubicinol at waste bin tops, door handles and tables at the homes and the oncology centers indicate that transference by people occurred. As a result, we may assume that skin contact with these compounds was possible. This confirms our earlier findings,[26] which also demonstrated dispersion of Pt by people at veterinary oncology centers and at the homes of pet-dogs treated with carboplatin.

We cannot establish whether the levels of doxorubicin, epirubicin and their C-13 metabolites, reported in this article, represent true health risks. However, these compounds have cytotoxic and cardiotoxic potency.[9,14,15] Furthermore, topoisomerase II inhibitors have been associated with therapy-related carcinogenicity,[31] and occupational exposure to antineoplastic drugs has been correlated with genotoxic effects.[32] In theory, any exposure to carcinogenic compounds could result in adverse health effects.[33] It is therefore advisable to continue to assess actual exposure of veterinary personnel, pet owners and other care-takers to antineoplastic drugs, and to continue to improve safe handling guidelines to keep exposure as low as possible.

Conclusion

Administration of doxorubicin or epirubicin to pet-dogs resulted in excretion of these compounds and their C-13 metabolites in urine, feces and saliva of treated pet-dogs. Quantifiable levels could be measured up to 21 days after dosing, depending on the compound and the pet-dog. Hence, the period of risk used in the veterinary safe handling guidelines may not be adequate to prevent exposure. Furthermore, sebum and cerumen samples collected from one dog indicated that transdermal excretion may occur. Consequently, sebum may be a source of exposure when handling treated pet-dogs. In addition, some surface contamination was observed at veterinary oncology centers and at the homes of treated pet-dogs. The pattern of contamination indicates that veterinary personnel, owners and other care-takers may have skin contact with contaminated surfaces.

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Footnotes

[a] Doxorubin, Teva Pharmachemie, Haarlem, Nederland
[b] Doxorubicin, Accord Healthcare, Middlesex, UK
[c] Epirubicin, Hospira Benelux, Brussels, Belgium
[d] Teva Medical, Netanya, Israel
[e] Carmel Pharma AB, Göteborg, Sweden
[f] Klinion, Medeco, Oud-Beijerland, the Netherlands
[g] Sarstedt AG&Co, Nümbrecht, Germany
[h] Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA
[i] Sarstedt AG&Co, Nümbrecht, Germany
[j] Kimberley- Clark Professional, Irving, TX, USA
[k] Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA
[l] Kimberley-Clark Professional, Irving, Tx, USA
[m] Aqua B. Braun Medical, Melsungen, Germany
[n] Whatman Nederland, 's Hertogenbosch, the Netherlands
[o] Biosolve, Valkenswaard, the Netherlands
[p] Sarstedt AG&Co, Nümbrecht, Germany
[q] Biosolve, Valkenswaard, the Netherlands
[r] Merck, Schiphol-Rijk, the Netherlands
[s] AB Sciex, Nieuwerkerk aan de Ijssel, the Netherlands
[t] Agilent Technologies, Palo Alto, CA, USA
[u] Phenomenex, Utrecht, the Netherlands
[v] AB Sciex, Nieuwerkerk aan de Ijssel, the Netherlands

Chapter 5

Exposure assessment

Chapter 5

Antineoplastic drugs in veterinary oncology: assessing exposure of veterinary personnel and owners to carboplatin, doxorubicin and epirubicin

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Abstract

Objective: Evaluating exposure to carboplatin, doxorubicin and epirubicin, in veterinary oncology.

Methods: Platinum, doxorubicin, doxorubicinol, epirubicin and epirubicinol were monitored in urine samples from veterinary personnel and owners of treated pet-dogs. Furthermore, *ex vivo* DNA-binding activity of platinum in canine urine was evaluated.

Results: One out of 181 urine samples collected from owners demonstrated that exposure to platinum had occurred. One out of 63 samples collected from owners contained doxorubicinol at a quantifiable level, while in three other samples detectable - though not quantifiable - amounts of doxorubicinol or epirubicin were present. None of the 49 samples from personnel showed detectable levels of the investigated compounds. Pt in urine of treated pet-dogs had retained up to 5.3% and 60% DNA binding activity, respectively.

Conclusion: The results of this study indicate that uptake of carboplatin, doxorubicin, epirubicin or their metabolites may occur in owners of treated pet-dogs. This was not observed in veterinary personnel. In addition, it was also shown that Pt species in canine urine were present in a reactive form.

Introduction

The first indication that antineoplastic drugs could induce cancer was found in 1948 (Haddow, 1948).[1] Since then, many studies have confirmed the carcinogenic properties of several antineoplastic drugs.[2,3] It took until the 1970s for people to realize that occupational exposure to these drugs might induce harmful health effects as well.[4] This realization resulted in an extensive body of literature describing environmental contamination with antineoplastic drugs,[5,6] uptake of these drugs by workers[7,8] and induction of genotoxic responses as a result of occupational exposure.[9,10] As a consequence, guidelines on safe handling of antineoplastic drugs were developed in the 1980s.[11] Training of staff and implementation of protective measures resulted quickly in reduction of the exposure. By now, however, it has become apparent that occupational exposure cannot be excluded completely.[5,11]

Cancer chemotherapy is increasingly used in veterinary oncology. As a result, exposure to these drugs is an inevitable risk for an increasing number of people. Moreover, the treated animal is sent home with its owners after administration of the antineoplastic drugs, unless side-effects make a hospital stay necessary. This reduces the risk of exposure of veterinary personnel on the one hand, but could result in exposure of the owners or other care-takers, on the other hand. Monitoring of occupational exposure to antineoplastic drugs in veterinary medicine has been rare. However, research does confirm that occupational exposure is an issue in veterinary oncology.[12-17] These studies have focused on environmental contamination and sources of exposure (e.g. serum and urine of dogs). In the current study we focus on the potential uptake of antineoplastic drugs by veterinary personnel and owners exposed to antineoplastic drugs by measuring the urinary excretion of platinum (Pt), doxorubicin, doxorubicinol, epirubicin and epirubicinol by veterinary personnel and owners. Doxorubicin, epirubicin, and their C-13 alcohols (doxorubicinol and epirubicinol) are measured by liquid chromatography-tandem mass spectrometry. The analysis of Pt originating from carboplatin or metabolites was done by inductively coupled plasma-mass spectrometry (ICPMS). To address the question whether the Pt, which personnel and owners were exposed to, was still present in a biologically active form, we evaluated its DNA binding activity in urine of treated pet-dogs.

Materials and Methods

Study design

Workers were selected from three veterinary oncology centers in the Netherlands and Flanders. These centers comprise a small, medium-sized and relatively large veterinary oncology unit. The larger unit processed up to 1 mg Pt, 0.6 g doxorubicin and 1.5 g epirubicin each year. Each center followed current guidelines for safe handling of antineoplastic drugs as recommended by the European College of Veterinary Internal

Medicine Companion Animals (ECVIM-CA). All centers had biological safety cabinets, but the type and venting characteristics varied. Each center prepared the antineoplastic drugs in a separate room, though in one center that room was also used for storage. Only one site used a separate room for administration of the drugs. All centers used a closed-system transfer device for the preparation of antineoplastic drugs. When preparing the drugs, centers used disposable gloves designed for handling antineoplastic drugs, protective clothing (either sleeves or overalls) and face masks. However, in some centers, the protective clothing and masks were not always used. Preparation of the drug applications was performed by the veterinarians. Veterinary technicians handled the wastes and assisted the veterinarian when handling the treated animals.

Owners of pet-dogs treated with doxorubicin,[ab] epirubicin,[c] carboplatin[de] or a sustained drug delivery system of carboplatin (sDDS), older than 18 years, were eligible for inclusion. Eligible owners were informed about the study via their veterinary oncologist. All owners were informed about potential health risks associated with exposure to antineoplastic drugs. They were instructed about the handling of their pet-dogs and its excretion products, as recommended by the guidelines of the ECVIM-CA. During the period of risk after administration of carboplatin and doxorubicin, these include (but are not restricted to): use of disposable gloves when handling the dog, its excretion products and utensils necessary for care-taking (such as water bowl and feeder), avoiding skin contact with the treated pet and more frequently cleaning of the home. The duration of this period of risk is five days for carboplatin and seven days for doxorubicin. When epirubicin was administered, owners followed the guidelines of doxorubicin.

Due to pollution by car exhaust catalysts, Pt is ubiquitous in the environment.[18] For comparison of Pt background, urine samples of veterinary personnel were collected after a holiday of at least 1 week, urine samples of owners were collected prior to treatment of the pet-dogs, and urine samples of a non-exposed population were collected on three non-consecutive days. The non-exposed population consisted of healthy volunteers, without previous contact with Pt-containing compounds, with age ranging between 25 and 54 years old, who worked in laboratories or offices.

The research involving human subjects has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki.[19] The Netherlands Cancer Institute Independent Ethics Committee approved the protocol involving human subjects.

Sample collection

Human urine samples were collected from veterinary personnel at several time points. Personnel of medium and large oncology units collected their samples during a normal working week, on the following days: Monday before the shift (after a weekend off), Monday (after the shift), Wednesday (after the shift), Friday (after the shift), and 3 weeks

after last sample collection (after the shift). Whenever possible, samples were also collected after a holiday. Personnel of the small oncology unit collected their samples dependent on the day of administration of carboplatin: on the day of administration (before the shift and after the shift), 2 days after administration (after the shift), 4 days after administration (after the shift), and 26 days after administration (after the shift). Urine samples of the owners of treated pet-dogs were collected prior to treatment (for carboplatin), during three weeks after administration of carboplatin, doxorubicin or epirubicin to the pet-dog, or during four weeks after administration of carboplatin sDDS to the pet-dog. Canine urine samples were collected by the pet owners prior to treatment (when carboplatin was administered), and on the day of administration of carboplatin or the anthracycline after dosing, up until day 21 post-infusion. Owners were advised to collect their own and their pets' urine samples on 1 to 7 days after treatment, and on 10, 13, 16, 19 and 21 days after infusion. During the sampling period, samples were collected on day 1 to 5, day 7 and from that point onwards, every three days up to day 19. If possible, owners also collected samples on day 21. Deviations from this schema were allowed. Human and canine urine samples were collected in 30 mL polypropylene tubes[f] using voided urine. The oncology centers and owners stored the samples at –20 °C, until the samples were transported to the laboratory, where the samples were then stored at –20 °C until analysis.

Ex vivo assessment of DNA binding activity of Pt in canine urine

Urine samples (250 μL) from five pet-dogs treated with carboplatin and four pet-dogs treated with carboplatin sDDS were incubated in duplo with an excess of calf thymus DNA (250 μg) to achieve maximal binding. Furthermore, urine from a healthy, untreated human volunteer (control sample) was incubated with 250 μg of calf thymus DNA and carboplatin, with Pt concentrations (originating from carboplatin) equivalent to the investigated samples collected on day 1. Five control samples using this urine were thus prepared. This was done in order to assess the binding capacity of carboplatin at concentrations in the same range as the Pt levels in the canine urine samples collected on day 1. Lastly, urine from the volunteer was also incubated with calf thymus DNA without addition of carboplatin, in duplo (procedural blanks). The samples were incubated for 48 h to allow maximal Pt-DNA binding. Afterwards, the DNA was precipitated using 100% ethanol, washed twice with 75% ethanol, and dissolved in water, as previously described by Brouwers et al. (2008).[20]

Sample preparation

For platinum analysis, urine samples were thawed, whirl mixed and subsequently diluted 100-fold with 1% HNO_3 (*v*/*v*) solution.[g] To each 2 mL diluted sample, 20 μL of internal standard solution was added. Subsequently, diluted samples were transferred to autosampler tubes. The DNA samples were hydrolysed in 1% HNO_3 (*v*/*v*) solution (1:1) at 70 °C for 24 h and injected directly into the ICPMS, after addition of internal standard solution

(100 ng L^{-1}, 10 µL per mL sample). For doxorubicin, doxorubicinol, epirubicin and epirubicinol detection, urine samples were thawed, vortex mixed, centrifuged for three min at 23,100 *g* and diluted 10-fold with ACN:0.1% HCOOH in water (1:3.5, *v*/*v*).

Inductively coupled plasma mass spectrometric detection

Pt analyses were performed on an inductively coupled plasma-quadrupole-mass spectrometer[h] (ICPMS) equipped with a 90° reflecting ion mirror. The sample introduction system consisted of a Micromist glass low flow nebulizer (sample uptake 0.12 mL min^{-1}), a peltier-cooled (4°C) double pass glass spray chamber, a quartz torch, and a nickel sampler and skimmer cone.[i] Sample transport from the SPS-3 autosampler[j] to the nebulizer was perfomed using a peristaltic pump. Data were acquired and processed using the ICPMS Expert Software version 1.1 b49.[k] Further data handling was performed using Excel 2003.[l] All solutions were prepared using plastic pipettes[m] and 10 mL[n] and 30 mL[o] polypropylene tubes. For the detection of Pt, the isotope used was ^{194}Pt. The detection mode was based on peak jumping with peak dwell times of 968 ms, 25 scans per replicate and 3 replicates per sample. Quantification was based on the mean concentration of 3 replicates analyzed against a calibration curve using weighted linear regression analysis. Pt analyses were performed using validated methods, as described elsewhere.[21] The lower limit of quantification (LLOQ) of the method was 7.50 ng L^{-1} Pt in undiluted urine, and the absolute sensitivity for Pt bound to DNA was 7.50 fg μg^{-1} Pt in DNA (when using 100 µg DNA).

Liquid chromatography coupled with tandem mass spectrometric detection

An API 4000 triple quadrupole MS with electrospray ionisation[p] was coupled to an Agilent HP1100 liquid chromatographic system.[q] The Agilent 1100 system consisted of a binary pump, an autosampler, a mobile phase degasser and a column oven. Chromatographic separation was obtained using a Kinetex C18 column[r] (specifics: 50x2.1 mm internal diameter, particle size 2.6 µm) protected with an inline filter (0.5 µm). As mobile phase isocratic elution with 77% phase A (0.1% HCOOH in water (*v*/*v*)) and 23% phase B (ACN), at a flow of 0.2 mL min^{-1} for 5 min, was used. The column oven was set at 40 °C, while the autosampler was thermostatted at 4 °C. A sample volume of 10 µL was injected and the injection needle was washed for five seconds with ACN after each injection. Mass transitions and ion specific parameters were optimized for each compound in positive ion mode. The precursor ions for doxorubicin, doxorubicinol, epirubicin and epirubicinol were *m*/*z* 544, 546, 544 and 546 respectively, while the product ions were *m*/*z* 397, 399, 397 and 363 respectively. The total run time was five minutes. For quantification, the multiple reaction monitoring (MRM) chromatograms were acquired with Analyst software version 1.5.[s]

Urine was measured using calibration standards containing epirubicin and epirubicinol, prepared in reconstitution solution (ACN:0.1% HCOOH in water 1:4 *v*/*v*). The lower limits of quantification (LLOQs) ranged from 0.1 to 1.0 ng mL^{-1} (epirubicin, epirubicinol) for urine.

Results

Pt levels in urine from veterinary personnel and owners of pet-dogs treated with carboplatin

A total of 260 urine samples were collected from veterinary personnel, owners of treated pet-dogs and healthy volunteers. Pt levels were determined in 49 urine samples collected from 10 veterinary personnel. Pt was also monitored in 148 urine samples, collected from 13 owners of pet-dogs throughout the sampling period of 21 days after administration of carboplatin to the dog. Furthermore, Pt levels were determined in 33 samples collected from four owners of pet-dogs treated with carboplatin sDDS, up to 27 days after dosing. In addition, Pt concentrations were measured in 30 urine samples from 10 normal, healthy volunteers. Of the 260 samples collected from potentially exposed people, 13 samples were collected by owners before the first course of their pet-dog with carboplatin, and six samples were collected by veterinary personnel after a holiday. One sample, collected at day 21 from an owner of a pet-dog treated with carboplatin sDDS, contained Pt concentrations at 250 ng L^{-1}. None of the other 259 urine samples contained Pt levels at or above the LLOQ.

Doxorubicin, epirubicin and the C-13 alcohols in urine from veterinary personnel and owners of pet-dogs treated with doxorubicin or epirubicin

A total of 78 urine samples were collected from veterinary personnel, and owners of treated pet-dogs. Doxorubicin, doxorubicinol, epirubicin and epirubicinol were monitored in 15 samples collected from three veterinary personnel. Additionally, doxorubicin and doxorubicinol were monitored in 27 urine samples of four owners of pet-dogs treated with doxorubicin, collected up to 19 days after administration. Epirubicin and epirubicinol were monitored in 36 urine samples collected up to 21 days after dosing by seven owners of pet-dogs treated with epirubicin. One sample collected on day 6 from an owner of a pet-dog treated with doxorubicin contained doxorubicinol at 0.8 ng mL^{-1} (quantified using epirubicinol calibration standards). None of the other 77 urine samples contained quantifiable levels of doxorubicin, epirubicin or their C-13 alcohols. However, in two samples, one collected on day 3 in the previously mentioned owner and one collected on day 1 from another owner of a pet-dog treated with doxorubicin, a peak could be detected in the LC-MS/MS chromatogram above the background noise, but below the LLOQ at the retention time of doxorubicinol. In one sample collected on day 4 from an owner of a pet-dog treated with epirubicin, a peak could be detected below the LLOQ at the retention time of epirubicin.

Ex vivo assessment of DNA binding activity of Pt in urine of pet-dogs treated with carboplatin

Addition of carboplatin to urine, and *ex vivo* incubation of the resulting control samples with DNA for two days, revealed that 0.7% of the added Pt was bound to DNA (see Figure 1). The urine samples of pet-dogs treated with carboplatin demonstrated a 1.2 to 7.4 times higher DNA binding capacity compared with the control samples, though Pt in one sample of a treated pet-dog did not show DNA binding capacity (see Table 1). Urine samples of pet-dogs treated with carboplatin sDDS, showed a 0.9 to 82.9 times higher DNA binding capacity compared with the control samples (see Table 1). The procedural blanks did not contain levels of Pt at or above the LLOQ.

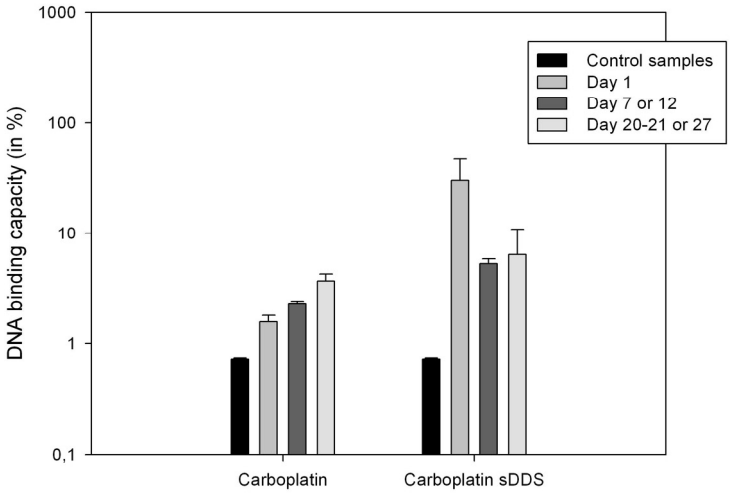

Figure 1 Mean *ex vivo* DNA binding capacity of Pt in urine samples of five pet-dogs treated with carboplatin (number of samples (n): 9) and of four pet-dogs treated with a carboplatin sustained drug delivery system (sDDS) (n: 6), compared with Pt in control samples (n: 5). Urine samples were collected on day 1, 7 or 12, and 20-21 or 27, after administration of carboplatin or carboplatin sDDS, respectively. Graph in logarithmic scaling (common log). DNA binding capacity is calculated by the following formula: (mean amount recovered Pt (in ng Pt/µg DNA)/mean amount urinary Pt (in ng Pt/µg DNA))*100.

Discussion

In this article, we report the monitoring of urinary lelvels of Pt, doxorubicin, doxorubicinol, epirubicin and epirubicinol in veterinary personnel and owners of pet-dogs treated with carboplatin, doxorubicin and epirubicin. Furthermore, we evaluated DNA binding activity of the Pt excreted in urine by pet-dogs treated with carboplatin.

Table 1 Pt-DNA adduct determination in urine of pet-dogs treated with carboplatin or carboplatin sDDS

Carboplatin standard solution					
Sample[a]	Day[b]	Administered dose[c]	Urinary Pt level[c]	Mean amount of recovered Pt-DNA adduct[c]	DNA binding capacity[c,d]
A	1	350	65.4	347	1.8
B	1	210	138	725	2.1
C	1	390	10.7	273	0.9
D	7	350	0.371	23	2.2
E	7	390	0.427	30	2.4
F	7	460	0.795	<LLOQ	n.a.
G	20	350	0.153	12	2.7
H	20	72	0.121	18	5.3
I	21	350	0.131	11	2.9
Carboplatin sustained drug delivery system					
Sample[e]	Day[b]	Administered dose[c]	Urinary Pt level[c]	Mean amount of recovered Pt-DNA adduct[c]	DNA binding capacity[c,d]
J	1	500	6.81	10339	60
K	1	250	2.43	96	0.7
L	12	500	1.56	216	5.9
M	12	250	0.185	25	4.7
N	27	100	0.630	21	1.3
O	27	300	0.116	43	12.3

[a]: Dog 1 provided samples A, D, G and I; Dog 2 provided sample B; dog 3 provided samples C and E; dog 4 provided sample F; dog 5 provided sample H

[b]: Day: time point of sample collection, with day 1 corresponding to day of administration, after administration

[c]: dose in mg, urinary Pt level in mg L^{-1}, amount of recovered Pt-DNA adduct in ng Pt per µg DNA, DNA binding capacity in %

[d]: DNA binding capacity is calculated by the following formula: (mean amount recovered Pt (in ng Pt/µg DNA)/mean amount urinary Pt (in ng Pt/µg DNA))*100

[e]: Dog 6 provided samples J and L; Dog 7 provided samples K and M; dog 8 provided sample N and dog 9 provided sample O

LLOQ: lower limit of quantification, n.a.: not applicable

Owners may be exposed to antineoplastic drugs via contaminated surfaces, and when handling their treated pet or its wastes. Consequently, the most likely routes of exposure in these cases are via skin absorption or ingestion. However, the oral bioavailability of carboplatin, doxorubicin and epirubicin is rather poor.[22-24] Furthermore, skin penetration has been investigated for doxorubicin[25] and cisplatin,[26] and these compounds showed low transdermal permeation.

As far as we know, this is the first report on biological monitoring of veterinary personnel and owners of pet-dogs, exposed to antineoplastic drugs. The detection of a quantifiable

level of doxorubicinol in a sample of an exposed owner, demonstrates that uptake of doxorubicin or doxorubicinol occurred. In addition, in three samples we detected doxorubicinol or epirubicin, though below the LLOQ of 0.1 or 1.0 ng mL^{-1}. Of course, the absence of detectable levels of doxorubicin, epirubicin and their C-13 alcohols in the other samples does not imply that no uptake occurred. Moreover, doxorubicin, doxorubicinol, epirubicin and epirubicinol are mainly excreted via bile in humans.[27,28] The measurement of doxorubicin, epirubicin and their C-13 alcohols in feces might, as a result, have yielded higher quantifiable levels of these compounds, in a larger number of samples. However, urine is much easier to collect and will likely result in higher enrolment of participants. Due to the anticipated limited number of study participants, we therefore opted for the use of urine as the matrix for exposure assessment. The majority of the authors evaluating urinary concentrations of doxorubicin or epirubicin in human oncology, found levels of these anthracyclines in some of the samples of exposed pharmacy and hospital personnel.[29-32] The quantifiable amounts varied in general between 0.1 to 1 ng mL^{-1} for doxorubicin and epirubicin, which is comparable to the results reported by us.

Since Pt is ubiquitous in the environment due to car exhaust catalysts,[18] it is important to measure normal Pt background variation in biological samples. The Pt levels of urine samples collected by veterinary personnel after a holiday, by owners before first treatment with carboplatin, and by normal volunteers, did not exceed the LLOQ of the method. Furthermore, the Pt background was comparable to levels reported in the literature.[33,34] Consequently, the Pt level exceeding the LLOQ in a sample of an owner collected after treatment of the pet-dog with carboplatin sDDS, indicates that uptake of Pt occurred, either as carboplatin or as a metabolite. In human oncology, increased Pt levels in urine samples of exposed pharmacy and hospital personnel have been described by some authors,[29,35] while others were not able to detect a significant difference with normal Pt background.[31,36] Urinary Pt concentrations up to 140 and 1300 ng L^{-1} have been reported, by Pethran et al. (2003) [29] and Turci et al. (2002),[35] respectively. The urine sample of the owner of a pet-dog treated with carboplatin sDDS contained Pt at a concentration of 250 ng L^{-1}.

Even though only five out of the 293 investigated samples contained detectable levels of doxorubicinol, epirubicin and Pt (compared to normal Pt background), these levels indicate that uptake occurred. Moreover, the five samples had all been collected from owners of treated pet-dogs. Though the number of urine samples collected from the owners is larger than the number of samples collected from veterinary personnel, the absence of detectable amounts in urine from veterinary personnel indicates that the employed working procedures and protective measures were successful in reducing exposure to carboplatin, doxorubicin, epirubicin or metabolites. On the other hand, the veterinary safe handling guidelines were not able to prevent uptake of these compounds by some owners of treated pet-dogs. Furthermore, the findings of the current study also indicate that failure of safe handling guidelines may occur several times throughout the treatment of the pet-dog.

The clinical consequences of the urinary concentrations of Pt, doxorubicinol and epirubicin, reported in the current study, remain to be established. However, doxorubicinol and epirubicin are cytotoxic and cardiotoxic agents. Consequently, uptake of these compounds may induce these harmful effects.[37-39] The ICPMS is not able to distinguish between carboplatin and its metabolites. As a result, we cannot provide information on the biological activity of the Pt found in the urine samples of pet-dogs treated with carboplatin or carboplatin sDDS. To establish whether the excreted Pt still has DNA binding potency, we attempted to assess the DNA binding activity *ex vivo*. Thus, we were able to establish that up to 5.3% of the Pt present in urine samples from pet-dogs treated with carboplatin, had retained the DNA binding capacity of carboplatin. In addition, 0.9 to 60% of the Pt found in urine samples from pet-dogs treated with carboplatin sDDS still had DNA binding activity. The overall higher biological reactivity of the Pt in urine samples of pet-dogs treated with carboplatin sDDS may be explained by the prolonged release of carboplatin from the sustained drug release formulation. Sustained DDSs allow long-term administration of drugs at therapeutic doses, while conventional methods of drug administration (such as intravenous injection), result in a 'peak and trough' pattern of plasma drug concentrations between doses.[40,41] Thusly, administration of carboplatin sDDS may result in relatively higher plasma levels of carboplatin, compared to standard formulations, due to the long-term release of the active parent compound from the formulation, opposed to the release from Pt bound to tissues[42-45] and blood proteins.[46] Pt is mainly excreted via the kidneys when released into the bloodstream.[47] Hence, the amount of active carboplatin relative to the total amount of Pt in urine could be higher when carboplatin sDDS is administered.

The markedly higher DNA binding activity of urinary Pt in one pet-dog, treated with carboplatin sDDS, is difficult to explain. However, this dog was the first dog treated with the sDDS formulation of carboplatin, at a dose of 300 mg m^{-2}. After 14 days, severe myelosuppression necessitated a hospital stay. Consequently, the veterinary oncology center adjusted the protocol. Since then, all pet-dogs treated with carboplatin sDDS received doses of maximally 250 mg m^{-2}. The severe side-effects were thus avoided and none of the urine samples collected in these dogs demonstrated that substantially higher biological reactivity.

The overall higher DNA binding of the Pt in canine urine samples compared to control samples may be explained by the presence of metabolites, with higher biological activity than carboplatin, in canine urine. Reports indicate that carboplatin is the main Pt species present in urine.[48,49] However, one of the few metabolites found in urine after administration of carboplatin, is cisplatin.[49] Various studies have demonstrated that cisplatin has a 10 to 100-fold higher DNA binding capacity compared to similar concentrations of carboplatin.[50,51] The presence of low amounts of cisplatin in the urine of treated pet-dogs would, therefore, explain the observed higher Pt DNA binding.

Conclusion

Even though only five out of 293 urine samples contained an increased Pt level or detectable amounts of doxorubicinol or epirubicin, the results of this study demonstrate that uptake of antineoplastic drugs may occur in owners and other care-takers of treated pet-dogs. Moreover, the findings imply that uptake of these compounds by owners may occur several times throughout the treatment of their pet-dog. On the other hand, uptake was not observed in veterinary personnel. Hence, the working procedures were able to reduce exposure to carboplatin, doxorubicin and epirubicin in this population at risk. This implies that appropriate protective measures and guidelines can reduce exposure in veterinary oncology. As a result, this may also be achieved in owners.

Furthermore, it was demonstrated that Pt species in canine urine had retained *ex vivo* DNA binding capacity. Uptake of Pt may, therefore, result in adverse health effects. As doxorubicinol and epirubicin have cytotoxic and cardiotoxic potency,[37-39] their presence in urine samples implies that at least some owners were potentially exposed to these effects. However, whether the measured levels of Pt, doxorubicinol and epirubicin present true health risks remains to be established.

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Footnotes

[a] Doxorubin, Teva Pharmachemie, Haarlem, the Netherlands
[b] Doxorubicin, Accord Healthcare, Middlesex, UK
[c] Epirubicin, Hospira Benelux, Brussels, Belgium
[d] Carboplatin, Hospira Benelux, Brussels, Belgium
[e] Carbosin, Teva Pharmachemie, Haarlem, the Netherlands
[f] Sarstedt AG&Co, Nümbrecht, Germany
[g] Mallinckrodt Baker, Philipsburg, NJ, USA
[h] Varian 810-MS, Mulgrave, Victoria, Australia
[i] Varian, Mulgrave, Victoria, Australia
[j] Varian, Mulgrave, Victoria, Australia
[k] Varian, Mulgrave, Victoria, Australia
[l] Microsoft, Redmond, WA, USA
[m] VWR International B.V., Amsterdam, The Netherlands
[n] Plastiques-Gosselin, Hazebrouck Cedex, France
[o] Sarstedt AG&Co, Nümbrecht, Germany
[p] AB Sciex, Nieuwerkerk aan de Ijssel, the Netherlands
[q] Agilent Technologies, Palo Alto, CA, USA
[r] Phenomenex, Utrecht, the Netherlands
[s] AB Sciex, Nieuwerkerk aan de Ijssel, the Netherlands

Chapter 6

Conclusions and perspectives

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Chemotherapy has been gaining popularity in veterinary oncology for the past decades, and it is unlikely that this trend will stop. Consequently, exposure to antineoplastic drugs is an inevitable risk for an increasing number of people. Veterinary personnel and owners may be exposed to antineoplastic drugs via contaminated surfaces, or when handling treated pet-animals and its wastes. Furthermore, handling and preparing of antineoplastic drugs and handling of associated wastes by veterinary personnel may result in exposure as well. Research demonstrates that the implementation of appropriate staff training, implementation of guidelines on safe handling of antineoplastic drugs, and use of protective measures reduces the exposure to antineoplastic drugs. However, the literature also reports that a certain amount of exposure cannot be avoided. Hence, adjusting the protective measures and guidelines to the needs of the populations at risk is imperative to keep the exposure to antineoplastic drugs as low as achievable. Yet, few studies have investigated occupational exposure to anticancer drugs in veterinary medicine. Thus, in this thesis, we aimed to expand current knowledge on low-dose exposure to antineoplastic drugs in veterinary oncology.

Excretion of antineoplastic drugs in treated pet-dogs

In the Netherlands and Flanders, personnel in veterinary oncology and owners of pet-dogs treated with antineoplastic drugs comply with the guidelines on safe handling of antineoplastic drugs, treated animals and associated wastes, developed by the European College of Veterinary Internal Medicine Companion Animals (ECVIM-CA). The ECVIM-CA guidelines list five days and seven days as a period of risk for carboplatin and doxorubicin, respectively. This is mainly based on knowledge transferred from human oncology. The studies described in the current thesis, however, demonstrate that Pt, doxorubicin, doxorubicinol, epirubicin, and epirubicinol may be excreted by the pet-dogs up to three weeks after administration of carboplatin, doxorubicin or epirubicin. In addition, Pt was detected in excretion products of pet-dogs treated with a sustained drug delivery formulation of carboplatin (carboplatin sDDS). In these dogs, excretion via all sampled excretion products could be measured up to at least four weeks after dosing.

Excretion was monitored and detected in urine, feces and saliva of pet-dogs treated with carboplatin, carboplatin sDDS, doxorubicin or epirubicin. Moreover, this thesis demonstrates that sebum and cerumen may also contain Pt, in pet-dogs treated with carboplatin or carboplatin sDDS. Additionally, results indicated that this may apply to pet-dogs treated with epirubicin as well. Thus caressing of pet-dogs, may form a potential source of exposure.

Environmental contamination with antineoplastic drugs in veterinary oncology centers

We monitored environmental contamination with Pt, doxorubicin, doxorubicinol, epirubicin, and epirubicinol in veterinary oncology centers. The results of these investigations demonstrated that low levels of these compounds could be measured in surface samples collected at the centers. In addition, dispersion of the investigated compounds by personnel was observed, with contamination found outside areas where antineoplastic drugs were handled. As a result, people could be at risk of exposure not only when actively handling these drugs or associated wastes. As surfaces deemed 'safe' could be contaminated with Pt, doxorubicin, doxorubicinol, epirubicin, or epirubicinol, skin contact with these compounds may occur. Future research investigating surface contamination with antineoplastic drugs should, therefore, also include surfaces outside areas where antineoplastic drugs were handled.

Environmental contamination with antineoplastic drugs in homes of cancer patients

Surface contamination with Pt, epirubicin and epirubicinol was monitored, and detected, in homes of veterinary and human cancer patients. The environmental contamination with Pt was, in general, lower in the homes of human cancer patients. It was hypothesized that the inability of the pet-dogs to access a garden at will, resulted in contamination of the home whenever vomiting, diarrhea or urination occurred unexpectedly. Still, the levels of Pt, epirubicin and epirubicinol found in surface samples collected in the homes were relatively low. On the other hand, transference of these compounds by owners was observed. Consequently, skin contact with these compounds may have occurred. Moreover, not only family members may be exposed to Pt, epirubicin and epirubicinol via contaminated surfaces, but other care-takers and visitors as well.

Exposure assessment of veterinary personnel and owners of treated pet-dogs

To assess actual exposure in veterinary oncology, uptake of antineoplastic drugs should be monitored. Thus, the presence of Pt, doxorubicin, doxorubicinol, epirubicin, and epirubicinol in urine samples of veterinary personnel and owners of treated pet-dogs was evaluated. The absence of measurable amounts of Pt, doxorubicin, doxorubicinol, epirubicin, and epirubicinol in urine of veterinary personnel indicates that the veterinary safe handling guidelines are sufficient to reduce uptake of these compounds to extremely low levels, if uptake occurred at all. On the other hand, a few samples from owners demonstrated the uptake of carboplatin, doxorubicin, epirubicin, or metabolites. Hence, uptake may occur in owners, and it may occur several times throughout the treatment of their pet-dog. These findings may be used to further improve the current veterinary guidelines on safe handling of antineoplastic drugs, treated animals and associated wastes.

Potential harmful effects of exposure to antineoplastic drugs

The investigations described in the current thesis, show that Pt species in urine of pet-dogs treated with carboplatin or carboplatin sDDS had retained DNA binding capacity. Uptake of Pt may, therefore, induce harmful effects. As doxorubicin, epirubicin and their C-13 metabolites have cytotoxic and cardiotoxic potency, uptake of these compounds may also result in adverse health effects. However, the clinical consequences of the observed levels remain to be established. Presently, there is a lack of statistical power in quantifying effects at low levels. In addition, most causal factors of complex diseases such as cancer have relatively weak effects. However, our methodology was developed to detect strong risk factors. Moreover, the investigated occupational risk factor is only one of many possible genotoxic agents. Thus, to be able to address the question whether low dose exposure to antineoplastic drugs may cause adverse health effects, conceptual and methodological rethinking of (exposure) science is needed. Meanwhile, as long as antineoplastic drugs with potentially harmful effects are used while the risks involved with low dose exposure remain unknown, the environmental contamination with and uptake of these compounds should be kept at as low as reasonable achievable levels.

We may conclude that exposure to antineoplastic drugs in veterinary oncology is an inevitable possibility. Consequently, occupational exposure assessment is crucial to evaluate the actual exposure in veterinary oncology. This assessment may monitor potential sources of exposure to anticancer drugs and evaluate uptake by exposed people. Occupational exposure assessment should include owners and other care-takers of pet-animals treated with antineoplastic drugs, since they are a population at risk as well. As the pharmacokinetics of drugs is species-dependent, and the behavior of the animal may influence the exposure of the owner, future research should also focus on other animals, such as cats. When assessing environmental contamination with antineoplastic drugs, it is important to include surfaces expected to be ‘safe’. For these surfaces, more sensitive methods, like the ICPMS assay described in the current thesis, are advisable. These assays depict actual exposure more accurately and are thus of greater value when ascertaining whether the employed guidelines are successful at keeping exposure as low as achievable. Furthermore, the limitations of (exposure) science need to be addressed before it will be able to answer the question: 'what are the risks involved with occupational exposure to anticancer drugs'.

The findings reported in this thesis may be used to further improve veterinary guidelines on safe handling of antineoplastic drugs, treated animals and associated wastes, and may thereby contribute to the reduction of exposure in all populations at risk in veterinary oncology.

Summary

Since their discovery in 1940s, it has become apparent that anticancer drugs can cause adverse health effects The realization that occupational exposure to antineoplastic drugs might pose health risks as well first dawned in the 1970s. By now, numerous studies have demonstrated surface contamination in oncology centers. Moreover, in human oncology, uptake of antineoplastic drugs by occupationally exposed personnel has been demonstrated and the induction of genotoxic effects has been associated with occupational exposure to antineoplastic drugs.

The past decades, the use of chemotherapy in veterinary medicine has been gaining popularity. Thus, the number of exposed people is on the rise in these settings. So far, few studies have monitored occupational exposure in veterinary oncology. This thesis attempts to contribute to current knowledge on this issue. Potential sources of exposure were investigated, such as excretion products of treated pet-dogs and contaminated surfaces. In addition, uptake of anticancer drugs by people at risk of exposure was assessed.

In **chapter 1.1** an overview of research investigating occupational exposure of personnel in veterinary and human oncology is presented. This demonstrated that environmental contamination of workplaces continues to occur, despite development and implementation of safe handling guidelines in human and veterinary oncology. Furthermore, uptake of anticancer drugs by personnel and induction of biological effects at DNA-level in exposed people was investigated in human oncology and could be observed as well.

Chapter 1.2 describes an overview of the potential harmful effects in human patients, associated with administration of chemotherapy. These may be: adverse effects on DNA-level, induction of cancer, toxic effects for the embryo or unborn child and fertility problems in men and women. Additionally, studies investigating the harmful effects of exposure to anticancer drugs in personnel in human oncology are reviewed. These studies are, however, rarely reported in the literature. As a result, only miscarriages have been relatively consistently related with occupational exposure of women to chemotherapy, so far. Furthermore, this chapter discusses inherent limitations in the methodology of epidemiology. To be able to give a straightforward answer to the question: 'what are the risks involved with occupational exposure to anticancer drugs', (exposure) science needs conceptual and methodological rethinking. The true health risks associated with exposure to anticancer drugs remain, meanwhile, unknown. Consequently, exposure should be kept at as low as reasonable achievable levels.

To allow monitoring of human urine and canine excretion products at ultrasensitive levels, we have developed and validated bioanalytical assays using inductively coupled plasma mass spectrometry (ICPMS) (**Chapter 2**). These methods may be used to measure platinum

(Pt) attributable to carboplatin in human urine, and in urine, feces, and saliva of pet-dogs treated with carboplatin (**Chapter 2.1**). Furthermore, the assays may also be used to monitor Pt in earwax, sebum of the coat and sebum of the skin of pet-dogs treated with carboplatin (**Chapter 2.2**).

Exposure to anticancer drugs may occur when handling treated pets or their excretes. Consequently, we have monitored several excretion products of pet-dogs treated with carboplatin, a carboplatin sustained drug delivery system, doxorubicin and epirubicin (**Chapter 3 and 4**).

Pt attributable to carboplatin could be measured up to at least three weeks after administration in urine, feces, saliva, earwax, sebum of the coat and sebum of the skin of treated pet-dogs (**Chapter 3.1**). The bulk of excretion occurred during the first five days. Urine was the most important route of excretion. Further research is needed to investigate whether Pt excretion in pet-dogs occurs over longer periods of time.

To improve the cure rates of cancer, research focuses on the development of new anticancer drugs, but also on new ways of administering 'old' anticancer drugs. One such way is by administering carboplatin using a so-called sustained drug delivery system (sDDS). Carboplatin is then administered within a drug carrier, which slowly releases the carboplatin into the body of the patient after administration. This may result in overall lower doses of carboplatin in the body and bloodstream (and thus may induce fewer side-effects), and in a prolonged exposure of the tumor to the anticancer drug. Since slow release of carboplatin into the body, may also lead to differences in Pt excretion in the treated pet-dogs, we monitored urine, feces, earwax, sebum of the coat and sebum of the skin in pet-dogs treated with an experimental carboplatin sDDS (**Chapter 3.4**). As it turned out, Pt attributable to carboplatin could be detected throughout the sampling period of four weeks. Moreover, in one dog, samples were obtained up until day 54 after administration. All samples contained traces of Pt. Thus, excretion may take place over even longer periods of time. Excretion occurred mainly via urine. Moreover, the amounts of Pt detected in urine and feces of pet-dogs treated with carboplatin sDDS were higher than the Pt levels found in canine urine and feces after treatment with carboplatin (the 'old' anticancer drug).

In a next step, potential exposure to other anticancer drugs in veterinary oncology was explored. Thus, biological samples of pet-dogs treated with doxorubicin and epirubicin were monitored. Doxorubicinol and epirubicinol are important metabolites (C-13 alcohols), formed by the dog after administration of doxorubicin and epirubicin, respectively. These metabolites, like their parent compounds, may induce harmful effects. As a consequence, we have also monitored doxorubicinol and epirubicinol in biological samples. Depending on the compound under analysis, the investigated pet-dog and the specific excretion product (urine, feces or saliva), doxorubicin, doxorubicinol, epirubicin and epirubicinol could be measured up to three weeks after treatment, though excretion may occur over longer

periods of time (**Chapter 4**). The bulk of excretion occurred via urine and feces, mainly during the first ten and seven days after administration of doxorubicin or epirubicin respectively. Results indicate that sebum and earwax may contain traces of these compounds as well.

The handling of antineoplastic drugs, treated pets and wastes, may result in contamination of the environment. Consequently, surfaces were monitored and traces of Pt attributable to Pt-containing compounds could be detected in veterinary oncology centers (**Chapter 3.2**) and at the homes of veterinary cancer patients (**Chapter 3.3 and chapter 3.4**). To place data in a broader perspective, Pt surface contamination was also monitored, and detected, in human oncology centers (**Chapter 3.2**) and homes of human cancer patients (**Chapter 3.3**). Environmental contamination with Pt attributable to Pt-containing compounds was lower in veterinary oncology centers, compared with human oncology centers. The Pt surface contamination in the homes of pet-dogs treated with carboplatin was, on the other hand, usually slightly higher than the contamination in homes of human oncology patients treated with carboplatin. Additionally, dispersion of Pt by people was observed in the human and veterinary oncology centers and the homes of human cancer patients and treated pet-dogs. Furthermore, environmental contamination with traces of doxorubicin, epirubicin, doxorubicinol and epirubicinol could be detected in veterinary oncology centers and in homes of treated pet-dogs as well (**Chapter 4**). Transference of doxorubicin, epirubicin and their C-13 alcohols by people was also observed in the veterinary oncology centers and homes of treated pet-dogs. Hence, skin contact with surfaces contaminated with residues of carboplatin, doxorubicin or epirubicin may have occurred. Moreover, not only personnel working in oncology centers, human cancer patients and owners of treated pet-dogs may be exposed via contaminated surfaces, but others as well.

In order to evaluate actual exposure in veterinary oncology, biological monitoring of the people at risk is needed. In a first step, uptake of antineoplastic drugs should be monitored. Therefore, the presence of Pt, doxorubicin, doxorubicinol, epirubicin, and epirubicinol in urine samples of veterinary personnel and owners of treated pet-dogs was evaluated (**Chapter 5**). A few samples of owners evidenced the uptake of carboplatin, doxorubicin, epirubicin, or metabolites. Doxorubicin, epirubicin and their C-13 metabolites can induce harmful effects. However, as the ICPMS only measures Pt atoms, no information on the biological activity of the Pt can by provided. We have therefore evaluated the capacity of Pt in urine samples of treated pet-dogs to bind to naked DNA (**Chapter 5**). It was obvious that the urinary Pt still had DNA-binding activity, which thus may result in DNA damage. Moreover, the findings indicated that urinary Pt of pet-dogs treated with carboplatin sDDS is generally more able to bind the DNA. In addition, the results seem to demonstrate the presence of metabolites with higher biological activity than that of carboplatin, in urine of pet-dogs treated with carboplatin or carboplatin sDDS. All in all, uptake of Pt by exposed

people in veterinary oncology may induce harmful effects as well.

The veterinary guidelines on safe handling of antineoplastic drugs, treated pets and wastes consider a period of risk of five days for carboplatin and seven days for doxorubicin, after administration to the pet. The results of the current thesis indicate that this may be inadequate to reduce potential exposure sufficiently. In addition, our findings also indicate that these periods of risk may not be sufficient to keep exposure as low as possible when sDDS formulations of anticancer drugs are used.

The guidelines were, however, able to keep environmental contamination at low levels. On the other hand, dispersion of Pt, doxorubicin, doxorubicinol, epirubicin, and epirubicinol by people could not be avoided. In this thesis, very sensitive to extremely sensitive assays were used to monitor environmental contamination. These assays are more sensitive than most methods used to monitor surface contamination with anticancer drugs and were therefore able to detect the dispersion. Contamination of surfaces outside areas where contamination can be expected is usually regarded as a failure of working procedures and guidelines. Thus, the use of ultrasensitive assays to measure contamination more accurately on these surfaces would be of greater value.

The absence of measurable residues of Pt, doxorubicin, doxorubicinol, epirubicin, and epirubicinol in urine of veterinary personnel indicates that the applied guidelines are sufficient to reduce uptake of these compounds to extremely low levels, if uptake occurred at all. In contrast, the uptake evidenced in a few samples of owners indicated that uptake may occur. Moreover, uptake may occur several times throughout the treatment of the pet-dog. These findings may be used to further improve the current veterinary guidelines and thus, reduce the exposure of owners as much as possible. The clinical consequences of the observed levels remain to be established. Presently, (exposure) science is not able to determine the risks associated with exposure, due to several inherent, methodological limitations.

In conclusion, monitoring of canine excretion products, environmental contamination and uptake of antineoplastic drugs by exposed people, is imperative to adapt the veterinary safe handling guidelines to the realities of exposure to anticancer drugs in all populations at risk. This thesis is hopefully an important step towards a better understanding and improvement of the prevention of exposure to antineoplastic drugs in veterinary oncology.

Samenvatting

Sinds hun ontdekking in de jaren '40 is het duidelijk geworden dat chemotherapeutica zelf schadelijk kunnen zijn voor de gezondheid. Pas in de jaren '70 werd men er zich voor het eerst van bewust dat ook beroepsmatige blootstelling aan chemotherapie schadelijk zou kunnen zijn. Ondertussen hebben vele studies aangetoond dat er omgevingsbesmetting met chemotherapie optreedt. Gegevens uit de humane oncologie tonen aan dat er opname van chemotherapeutica door beroepsmatig blootgesteld personeel mogelijk is, en dat deze beroepsmatige blootstelling is gecorreleerd met de inductie van genotoxische effecten.

Het gebruik van chemotherapie in de diergeneeskunde neemt de laatste jaren toe. Dit heeft als gevolg dat het aantal mensen dat blootgesteld kan worden aan chemotherapie ook toeneemt. Tot nu toe hebben slechts weinig studies de beroepsmatige blootstelling aan chemotherapeutica in de diergeneeskunde onderzocht. Deze thesis tracht bij te dragen aan de huidige kennis over dit onderwerp. Daarom zijn mogelijke bronnen van blootstelling, zoals uitscheidingsproducten van behandelde honden en besmette oppervlaktes, onderzocht. Daarnaast werd er ook nagegaan of er opname was van chemotherapeutica door mogelijk blootgestelde personen.

In **hoofdstuk 1.1** wordt een overzicht gegeven van de studies die de beroepsmatige blootstelling aan chemotherapie in de humane en diergeneeskundige oncologie onderzocht hebben. Hieruit blijkt dat, zowel in de humane en diergeneeskundige oncologie, omgevingsbesmetting van de werkomgeving blijft plaatsvinden, ondanks de ontwikkeling en in gebruik name van veiligheidsrichtlijnen. Verder werd in humane oncologie de opname van chemotherapeutica door beroepsmatig blootgesteld personeel en de inductie van biologische effecten op DNA-niveau in blootgestelde personen onderzocht en aangetoond,.

Hoofdstuk 1.2 beschrijft de schadelijke effecten die geassocieerd worden met de toediening van chemotherapie in humane patiënten. Deze kunnen zijn: schadelijke effecten op DNA-niveau, inductie van kanker, toxiciteit in het embryo of het ongeboren kind en vruchtbaarheidsproblemen bij mannen en vrouwen. Daarnaast wordt er een overzicht gegeven van het onderzoek naar de schadelijke gevolgen van beroepsmatige blootstelling aan chemotherapeutica in de humane oncologie. Deze studies zijn slechts zelden terug te vinden in de literatuur. Tot nu toe zijn alleen miskramen relatief consequent gerelateerd met beroepsmatige blootstelling van vrouwen aan chemotherapie. Voorts bespreekt dit hoofdstuk inherente beperkingen van de epidemiologische methodologie. Om een duidelijk antwoord te kunnen geven op de vraag wat de risico's van beroepsmatige blootstelling aan chemotherapeutica zijn, moeten de concepten en de methodologie van wetenschap (en in het bijzonder: van blootstellingsonderzoek) heroverwogen worden. De werkelijke gezondheidsrisico's van beroepsmatige blootstelling aan chemotherapie blijven in de tussentijd onbekend. Daarom zou de blootstelling zo laag mogelijk gehouden moeten worden.

Om humane urine en caniene uitscheidingsproducten op ultragevoelig niveau te kunnen monitoren, hebben we bioanalytische methoden ontwikkeld die gebruik maken van de inductief-gekoppelde plasma-massa-spectrometer (ICPMS) (**hoofdstuk 2**). Deze methoden kunnen gebruikt worden om platina (Pt), dat toegeschreven kan worden aan carboplatine, te meten in humane urine en urine, feces en speeksel van honden behandeld met carboplatine (hoofdstuk 2.1). Verder kunnen de methoden gebruikt worden om Pt te monitoren in oorsmeer, talg van de vacht en talg van de huid van honden behandeld met carboplatine (**hoofdstuk 2.2**).

Blootstelling aan chemotherapie kan optreden in de omgang met behandelde honden en hun excreta. We hebben daarom verschillende uitscheidingsproducten van honden, behandeld met carboplatine, een carboplatine preparaat met vertraagde afgifte, doxorubicine of epirubicine, gemonitord (**hoofdstukken 3 en 4**).

Pt kon tot en met minimaal drie weken na toediening gemeten worden in urine, feces, speeksel, talg van de vacht, talg van de huid en oorsmeer van honden behandeld met carboplatin (**hoofdstuk 3.1**). Het overgrote deel van de uitscheiding vond de eerste vijf dagen na toediening plaats. Urine was de belangrijkste uitscheidingsroute. Verder onderzoek is nodig om na te gaan of de uitscheiding in honden ook gedurende langere periodes na toediening plaats heeft.

Om de overlevingskansen van patiënten met kanker te verbeteren, richt onderzoek zich niet alleen op de ontwikkeling van nieuwe soorten chemotherapie, maar ook op het vinden van andere manieren om 'vertrouwde' chemotherapeutica toe te dienen. Een manier om dit te bereiken is door carboplatine toe te dienen in de vorm van een preparaat met vertraagde afgifte (PVA). Carboplatine wordt dan toegediend in een drager, dat het carboplatine langzaam vrij geeft aan het lichaam van de patiënt. Over het algemeen kan dit leiden tot lagere doses (wat kan resulteren in minder bijwerkingen), en een verlengde blootstelling van de tumor aan het chemotherapeuticum. Omdat deze vertraagde afgifte van carboplatine ook kan leiden tot verschillen in Pt excretie in de behandelde honden, hebben we de urine, feces, talg van de vacht, talg van de huid en oorsmeer van honden, behandeld met een experimenteel carboplatine PVA, gemonitord (**hoofdstuk 3.4**). Hieruit bleek dat het Pt gemeten kon worden gedurende minimaal vier weken na toediening van het carboplatine PVA. De uitscheiding gebeurde voornamelijk via urine. Meer nog, de hoeveelheid Pt in urine en feces van honden behandeld met carboplatine PVA was hoger dan het gehalte aan Pt in urine en feces van honden behandeld met carboplatine (het 'originele' chemotherapeuticum).

In een volgende stap werd in de diergeneeskundige oncologie de blootstelling aan andere chemotherapeutica onderzocht. Daartoe werd de aanwezigheid van doxorubicine en epirubicine in biologische excreta werd gemonitord. Doxorubicinol en epirubicinol zijn belangrijke metabolieten (C-13 alcoholen), die gevormd worden door de hond na toediening

van respectievelijk doxorubicine en epirubicine. Deze metabolieten kunnen, net als hun moederverbindingen, schadelijk zijn voor de gezondheid. Om die reden hebben we ook de aanwezigheid van doxorubicinol en epirubicinol in biologische monsters geëvalueerd. Afhankelijk van de geanalyseerde stof, de hond en het specifieke uitscheidingsproduct (urine, feces of speeksel), kon doxorubicine, doxorubicinol, epirubicine en epirubicinol teruggevonden worden tot drie weken na toediening, hoewel uitscheiding over een langere periode niet uitgesloten is (**hoofdstuk 4**). De uitscheiding heeft voornamelijk gedurende de eerste tien en zeven dagen na toediening van respectievelijk doxorubicine en epirubicine plaats. Er zijn aanwijzingen dat ook talg en oorsmeer sporen van deze stoffen kunnen bevatten.

Het omgaan met chemotherapeutica, behandelde dieren en daaraan gerelateerd afval, kan aanleiding geven tot besmetting van de omgeving. Daarom zijn er oppervlaktes gemonitord en konden er sporen van Pt, dat toegeschreven kan worden aan Pt-houdende chemotherapeutica, teruggevonden worden in diergeneeskundige oncologische centra (**hoofdstuk 3.2**) en in de huiselijke omgeving van caniene patiënten (**hoofdstuk 3.3 en 3.4**). Om de resultaten in een breder kader te kunnen plaatsen, werd ook de omgevingsbesmetting met Pt in humane oncologische centra geëvalueerd en aangetoond (**hoofdstuk 3.2**). Tevens werd de huiselijke omgeving van humane kankerpatiënten onderzocht (**hoofdstuk 3.3**). De besmetting van oppervlaktes met Pt was hoger in de humane oncologische centra, vergeleken met de diergeneeskundige. De Pt omgevingsbesmetting in de huiselijke omgeving van de caniene patiënten was licht hoger, vergeleken met de huiselijke omgeving van de humane patiënten. De resultaten toonden ook aan dat het Pt door mensen, zowel in de humane en diergeneeskundige oncologische centra als in de huiselijke omgeving van humane en caniene patiënten, verspreid werd. Verder werden er sporen van doxorubicine, doxorubicinol, epirubicine en epirubicinol teruggevonden op oppervlaktes in diergeneeskundige oncologische centra en in de huiselijke omgeving van behandelde honden (**hoofdstuk 4**). De verspreiding van doxorubicine, epirubicine en hun C-13 alcoholen door mensen in de veterinaire oncologische centra en in de huiselijke omgeving van behandelde honden werd ook waargenomen. Deze verspreiding impliceert dat er huidcontact met besmette oppervlaktes kan zijn opgetreden. Bovendien kunnen naast personeel van oncologische centra, humane patiënten en eigenaars van behandelde honden ook anderen aan chemotherapie worden blootgesteld via een besmette omgeving.

Om na te gaan wat de daadwerkelijke blootstelling aan chemotherapeutica in de diergeneeskundige oncologie is, is biologische monitoring van mensen met een blootstellingsrisico nodig. In een eerste stap moet de opname van chemotherapeutica door deze personen, geëvalueerd worden. Om deze reden werd de aanwezigheid van Pt, doxorubicine, doxorubicinol, epirubicine en epirubicinol in urinemonsters van veterinair

personeel en eigenaars van behandelde honden nagegaan (**hoofdstuk 5**). Enkele monsters van eigenaars toonden de opname van carboplatine, doxorubicine, epirubicine of hun metabolieten aan. Doxorubicine, epirubicine en hun C-13 alcoholen kunnen schadelijk zijn voor de gezondheid. Echter, doordat alleen Pt atomen worden gemeten door de ICPMS, kan er geen informatie gegeven worden over de biologische activiteit van het Pt. We hebben daarom de DNA-bindingscapaciteit van het urinaire Pt van honden behandeld met carboplatine geëvalueerd (**hoofdstuk 5**). Het was duidelijk dat het Pt in de urine nog steeds met DNA kon binden, wat dus aanleiding kan geven tot DNA-schade. Bovendien toonde het onderzoek aan dat het Pt in urinemonsters van honden behandeld met carboplatine PVA, over het algemeen, beter in staat was om met DNA te binden. Daarnaast lijken de resultaten aan te tonen dat er in urine van met carboplatine of carboplatine PVA behandelde honden, metabolieten aanwezig zijn die een hogere biologische activiteit bezitten dan carboplatine zelf. Hieruit zou geconcludeerd kunnen worden dat de opname van Pt door blootgestelde mensen in de diergeneeskundige oncologie ook schadelijke gevolgen zou kunnen hebben voor de gezondheid.

De diergeneeskundige richtlijnen met betrekking tot het veilig omgaan met chemotherapeutica, behandelde dieren en daaraan gerelateerd afval, houden een risicovolle periode aan van vijf dagen voor carboplatine en zeven dagen voor doxorubicine, na toediening aan het dier. De resultaten van de huidige thesis geven aan dat deze periode ontoereikend zou kunnen zijn om mogelijke blootstelling voldoende te beperken. Daarnaast geven onze bevindingen aan dat deze risicovolle periodes ook onvoldoende zouden kunnen zijn voor het verminderen van blootstelling, wanneer chemotherapeutica in de vorm van PVA worden toegediend.

De veiligheidsrichtlijnen blijken echter wel in staat om de omgevingsbesmetting laag te houden. Aan de andere kant kon de verspreiding van Pt, doxorubicine, doxorubicinol, epirubicine en epirubicinol door mensen, niet voorkomen worden. In dit proefschrift werd er gebruik gemaakt van zeer gevoelige tot extreem gevoelige methoden om omgevingsbesmetting te monitoren. Deze methoden zijn gevoeliger dan de meeste methoden die gebruikt worden voor het evalueren van de besmetting van oppervlaktes met chemotherapeutica en waren daardoor in staat om de verspreiding te detecteren. De besmetting van oppervlaktes buiten zones waar deze besmetting verwacht kan worden, wordt doorgaans beschouwd als het falen van de werkprocedures en veiligheidsrichtlijnen. Daarom is het gebruik van ultragevoelige methoden om besmetting op dit soort oppervlaktes te monitoren, van meerwaarde.

Het niet kunnen meten van sporen van Pt, doxorubicine, doxorubicinol, epirubicine en epirubicinol in urinemonsters van personeel van diergeneeskundige, oncologische centra, wijst erop dat de gebruikte richtlijnen toereikend zijn om opname van deze stoffen te verminderen tot extreem lage hoeveelheden, als er al opname plaats heeft gevonden.

Hiertegenover staat dat de opname, vastgesteld in een aantal urinemonsters van eigenaars, er op duidt dat opname van chemotherapie kan optreden. Bovendien kan deze opname meerdere malen tijdens de behandeling van de hond voorvallen. Deze bevindingen kunnen gebruikt worden om de huidige, diergeneeskundige veiligheidsrichtlijnen te verbeteren en zo de blootstelling van eigenaars zoveel mogelijk te reduceren. Wat de werkelijke gezondheidsrisico's zijn van de gemeten hoeveelheden chemotherapie, moet nog vastgesteld worden. Op dit moment, echter, is (blootstellings)onderzoek niet in staat om deze risico's te bepalen doordat het een aantal inherente, methodologische beperkingen heeft.

Samenvattend kunnen we stellen dat het monitoren van uitscheidingsproducten van de hond, mogelijke omgevingsbesmetting en potentiële opname van chemotherapeutica door mensen met blootstellingsrisico, noodzakelijk is. Op grond van de resultaten kunnen de veterinaire veiligheidsrichtlijnen aangepast worden aan de werkelijke blootstelling aan chemotherapie, zoals die optreedt in alle blootgestelde populaties. Deze thesis is hopelijk een belangrijke stap in de richting van een beter begrip en verbetering van de preventie van blootstelling aan chemotherapeutica in de diergeneeskundige oncologie.

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Tine

Haarlem, 2012

Curriculum Vitae

Tine Janssens was born on October 9, 1984 in Jette, Belgium. She gained her gymnasium diploma in 2002 at the Koninklijk Atheneum in Etterbeek, Belgium. In the same year she started the study Veterinary Medicine at the Ghent University, in Belgium. She obtained her bachelor's degree "Kandidaat Dierenarts" in 2005. Her graduation project, entitled "An in vitro model for investigation of the interactions of viruses with feline oocytes and embryo's.", was executed at the department of Obstetrics, Reproduction and Herd health, faculty of Veterinary Medicine (Ghent University), whereby she was supervised by Prof. Dr A Van Soom and Prof. Dr H Nauwynck. In 2008 she obtained her Doctor of Veterinary Medicine degree, specialized in Research and Industry in companion animals. In the same year she started the investigations described in this thesis, supervised by Prof. Dr JH Beijnen, Prof. Dr JHM Schellens and Dr EEM Brouwers. In 2010 she started the Hbo Lifecoach schooling at the Atma Institute, Amsterdam, which she successfully accomplished in 2012.

Curriculum Vitae

Tine Janssens werd geboren op 9 oktober 1984 te Jette, België. In 2002 behaalde ze haar gymnasium diploma aan het Koninklijk Atheneum in Etterbeek, België. In hetzelfde jaar begon ze met de studie diergeneeskunde aan de universiteit Gent, in België. Ze behaalde haar diploma Kandidaat Dierenarts in 2005. Haar afstudeeronderzoek, getiteld "Een in vitro model voor het bestuderen van virusinteracties met eicellen en embryo's van de kat", voerde ze uit aan de vakgroep Verloskunde, Voortplanting en Bedrijfsdiergeneeskunde van de faculteit diergeneeskunde (Universiteit Gent) onder leiding van Prof. Dr A Van Soom en Prof. Dr H Nauwynck. In 2008 studeerde ze af als algemeen dierenarts, met specialisatie in Industrie en Onderzoek bij gezelschapsdieren. In datzelfde jaar begon zij aan het promotieonderzoek dat beschreven is in dit proefschrift, onder leiding van Prof. Dr JH Beijnen, Prof. Dr JHM Schellens en Dr EEM Brouwers. In 2010 startte zij met de Hbo-opleiding tot Lifecoach aan het Atma Instituut, Amsterdam, welke ze afrondde in 2012.

List of publications

Articles related to this thesis

1. Janssens T, Brouwers EE, de Vos JP, Schellens JH, Beijnen JH. Antineoplastic drugs in veterinary oncology – Part 1: Occupational exposure. *Submitted for publication*.
2. Janssens T, Brouwers EE, de Vos JP, Schellens JH, Beijnen JH. Antineoplastic drugs in veterinary oncology – Part 2: Adverse health effects and risk assessment. *Submitted for publication*.
3. Janssens T, Brouwers EE, de Vos JP, Schellens JH, Beijnen JH. Determination of platinum originating from carboplatin in human urine and canine excretion products by inductively coupled plasma-mass spectrometry. *Journal of Analytical Toxicology.* 2011;**35**(3):153-161.
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Abstract

Janssens T, Brouwers EE, de Vos JP, Schellens JH, Beijnen JH. Treatment of dogs with carboplatin: excretion, contamination of the environment and uptake by owners. Presentation at the World Veterinary Cancer Congress, 1-3 march 2012, Paris, France.

The Road goes ever on and on
Down from the door where it began.
Now far ahead the Road has gone,
And I must follow, if I can,
Pursuing it with eager feet,
Until it joins some larger way
Where many paths and errands meet.
And whither then? I cannot say.

J.R.R. Tolkien ~The lord of the Rings, 1965