Mechanism-based drug exposure classification in pharmaco-epidemiological studies

Marianne Verdel

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Mechanism-based drug exposure classification in pharmacoepidemiological studies

Mechanisme-georiënteerde classificatie van geneesmiddel-blootstelling in farmaco-epidemiologische studies
(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 29 september 2010 des middags te 2.30 uur

door

BERTHA MARIA VERDEL

geboren op 9 februari 1968 te Alphen aan den Rijn

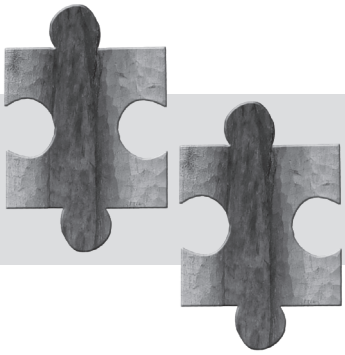
PROMOTOREN: Prof. dr. H.G.M. Leufkens
Prof. dr. A.C.G. Egberts

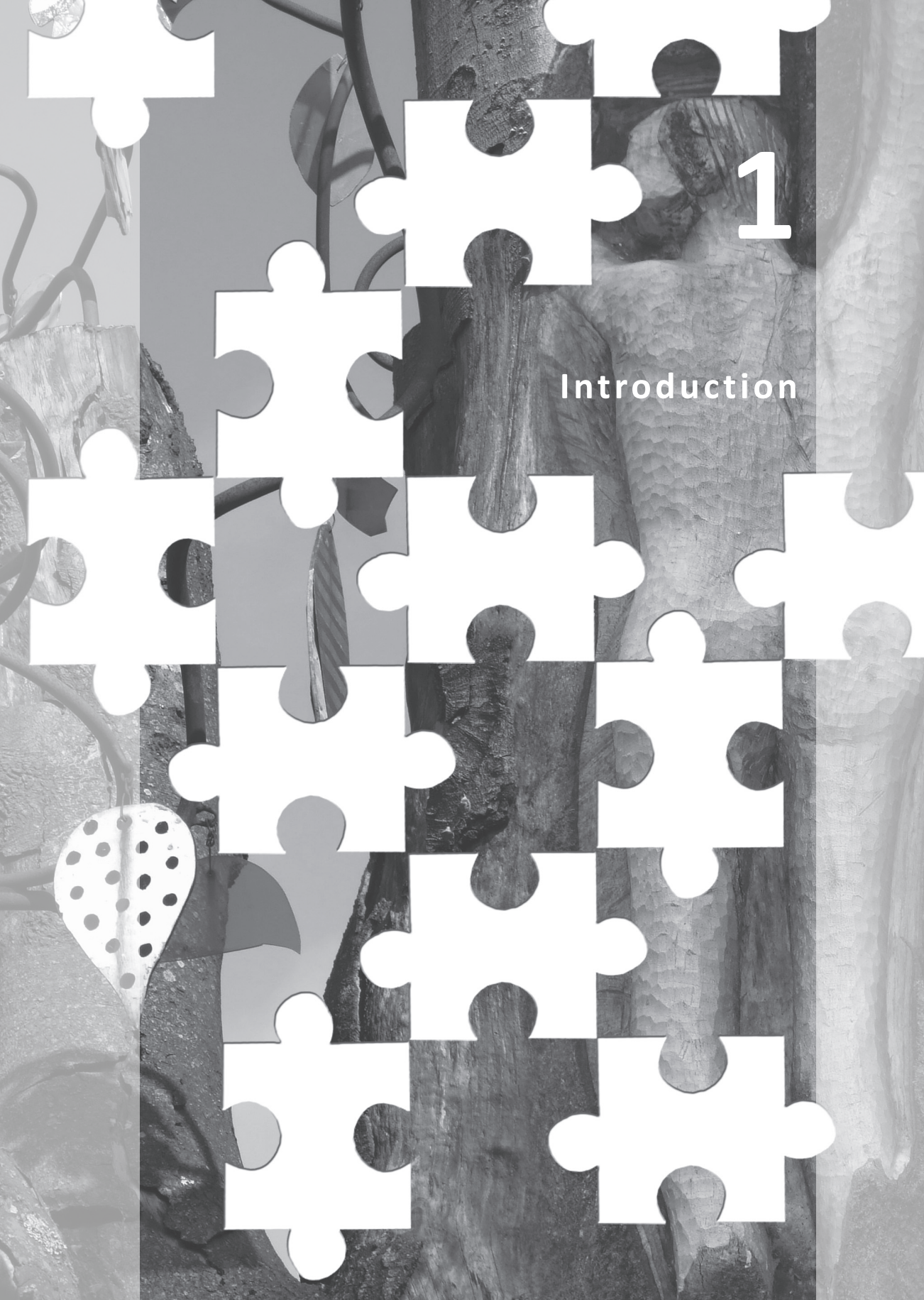
CO-PROMOTOR: Dr. P.C. Souverein

Chi guarda a ogni nuvolo,
non fai mai viaggio

He who looks at every cloud
never makes a journey
Tuscan proverb

Contents





1

Introduction

INTRODUCTION

In the year 1962, many noteworthy events took place. It was the year of the Cuban missile crisis, a confrontation between the United States and the Soviet Union. Several famous people died, for example Marilyn Monroe, American actress and singer, Her Royal Highness Princess Wilhelmina of the Netherlands, and Niels Bohr, Danish physicist and Nobel prize laureate (1922); others were born, such as king Abdullah II of Jordan and Ruud Gullit, a well-known Dutch football player. It was also the year that James Watson, Francis Crick and Maurice Wilkins received the Nobel prize of Physiology or Medicine for "their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material".[1] Furthermore, an article by Hansch *et al.*[2] was published which was an important contributor to the concept of quantitative **structure-activity relationships** (QSAR), the quantitative correlation between the physicochemical properties of molecules and biological activities.

Just before 1962, in December 1961, the Lancet published a letter of William McBride concerning the use of thalidomide and the occurrence of congenital abnormalities.[3] In response to the thalidomide tragedy, the Kefauver Harris Amendment to the Federal Food, Drug and Cosmetic Act was passed in 1962. This amendment required that drugs were to be tested for efficacy as well as safety and gave the Food and Drug Administration (FDA) the authority to require sophisticated clinical trials before approving drugs.[4] Along with the worldwide legislation regarding drug evaluation and the establishment of drug regulatory authorities – in the Netherlands, *het College ter Beoordeling van Geneesmiddelen* (Medicines Evaluation Board) was founded in 1963 – spontaneous reporting systems of suspected adverse drug reactions were initiated worldwide. The letter of McBride and the aftermath were determinative triggers for putting these (regulatory) systems in place and for a change in thinking about drugs and the weighing of their therapeutical and adverse effects.

MECHANISM-BASED CLASSIFICATION OF DRUG EXPOSURE

There are several ways to look at the concept of *drug classification*. Medicinal chemists are inclined to classify compounds according to their chemical structure, while physicians and pharmacists are more likely to consider the clinical use of drugs. In the field of biopharmaceutics, drug disposition of compounds is divided into four classes based on their solubility and permeability *in vitro* to predict oral

absorption. For pharmacoepidemiological studies, drugs are often identified and selected on the basis of the anatomical therapeutic chemical (ATC) classification system, recommended by the WHO to serve as a tool for drug utilisation studies.[5] Hence, the importance of drug classification does not extend only to the field of taxonomy. Also in the field of drug discovery and drug development, for regulatory authorities, for pricing and reimbursement of medicines, and for formulary and prescribing decision making, a meaningful classification scheme is essential.

In pharmacoepidemiology and pharmacovigilance, the relation between drug exposure and a clinical outcome is crucial. Timing of drug exposure is often organised into three levels: 1) use or no use of 'drug x'; 2) information on amount, dosage or duration of 'drug x'; and 3) multiple prescribing or dispensing moments.[6] This design can be refined even further by assessing patients' adherence to prescribed drug treatments[7] or studying timing of exposure by constructing treatment episodes.[8,9] Drugs are usually classified into classes on the basis of their therapeutical indication. Within such a group, further subdivions can be made with respect to chemical subclasses or mechanisms of action. This classification used in clinical setting has been and still is very useful. However, in the field of pharmacoepidemiology, the therapeutical classification is not always the only effective approach to categorise drug exposure. It does not take into account the fact that chemical (sub)structures or pharmacological activity may be shared by drugs which do not belong to the same class, but can be associated with the same adverse effects. This leads to the question whether there is any need for further 'refinement' in drug exposure assessment with regards to the drug itself. In other words: can we obtain useful additional information when shifting from a traditional pharmacotherapeutical classification to a more mechanistic classification of drug exposure?

Mechanism-based drug classification is not a new concept. The Martindale: the complete drug reference, first published in 1883 under the title Martindale: the extra pharmacopoeia, used and uses a drug classification based on clinical use. Although the nomenclature on a high level often has been based on indication (antihypertensive drugs, anti-Parkinson drugs), the classification of various drug classes has occurred on their mechanism of action (*e.g.* antihistaminic drugs, selective serotonin reuptake inhibitors). In addition, other drugs classes are named after their chemical (sub)structures, such as tetracyclines and fluoroquinolones. Also the Dutch therapeutic reference book *Informatorium Medicamentorum* systematises drugs according to this categorisation, whereas another Dutch manual the *Farmacotherapeutisch Kompas* uses as primary categorisation an anatomical-based classification, with a subdivision according to the drugs'

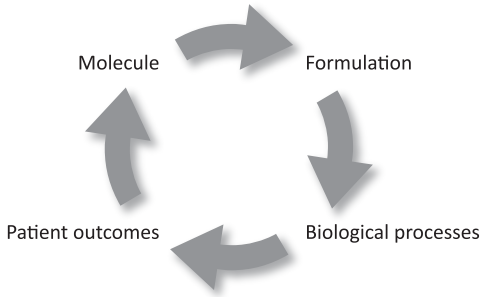
therapeutical, pharmacological and/or chemical properties. Over the last years, the attention for intrinsic molecular characteristics as a classification method in pharmacoepidemiological studies has been increased.

In this thesis, we illustrate categorisation of drug exposure – other than on the basis of therapeutical groups – in relation to ADRs from three perspectives: 1) molecular characteristic-based classification; 2) formulation-based classification; and 3) target-oriented classification.
A pharmacological active substance is not yet a drug. It has to be formulated into an efficacious and safe medicine. The molecular characteristics of a compound are important in the choice of formulation and excipients, and the type of formulation influences pharmacokinetic properties. Molecular characteristics and receptor affinity are not separate variables. The relations between molecular structure and biological activity of chemical compounds are defined as structure-activity relationships (SAR). The structural features of a drug in combination with a particular three-dimensional geometry are required for a drug in order to bind to a receptor and induce a physiological response. The idea behind SAR and quantitative SAR (QSAR) is to understand, quantify and predict the binding affinity, the acute toxicity or pharmacokinetic parameters of existing or hypothetical molecules.[10] Physicochemical properties of drugs, such as molecular weight, octanol-water partition coefficient (log P) and acid-base status (pK_a), have significant influence on their pharmacokinetic (absorption, distribution, metabolism, excretion) and pharmacodynamic (affinity for receptors, ion channels, enzymes) aspects, and therefore on their biological activity. Subsequently, patient outcomes (low efficacy, ADRs) can lead to the modification of an existing drug molecule to improve physicochemical properties (Figure 1). Although these principles apply primarily for small (chemical) molecules, there seems to be no restriction to apply these principles for biopharmaceuticals (proteins, nucleic acids) as well.

REDEFINING EXPOSURE

The WHO defined *adverse drug reaction* as "one that is noxious and unintended and occurs at doses normally used in man".[11] The most frequently used classification scheme for ADRs is the one of Rawlins and Thompson.[12] They divide ADRs in two categories, type A and type B. Later, these two categories were labelled 'augmented' and 'bizarre' for mnemonic reasons. Meyboom *et al.* described type A ADRs as *drug actions* and type B ADRs as *patient reactions*.[13] Type A reactions

Figure 1 From molecule to patient outcomes

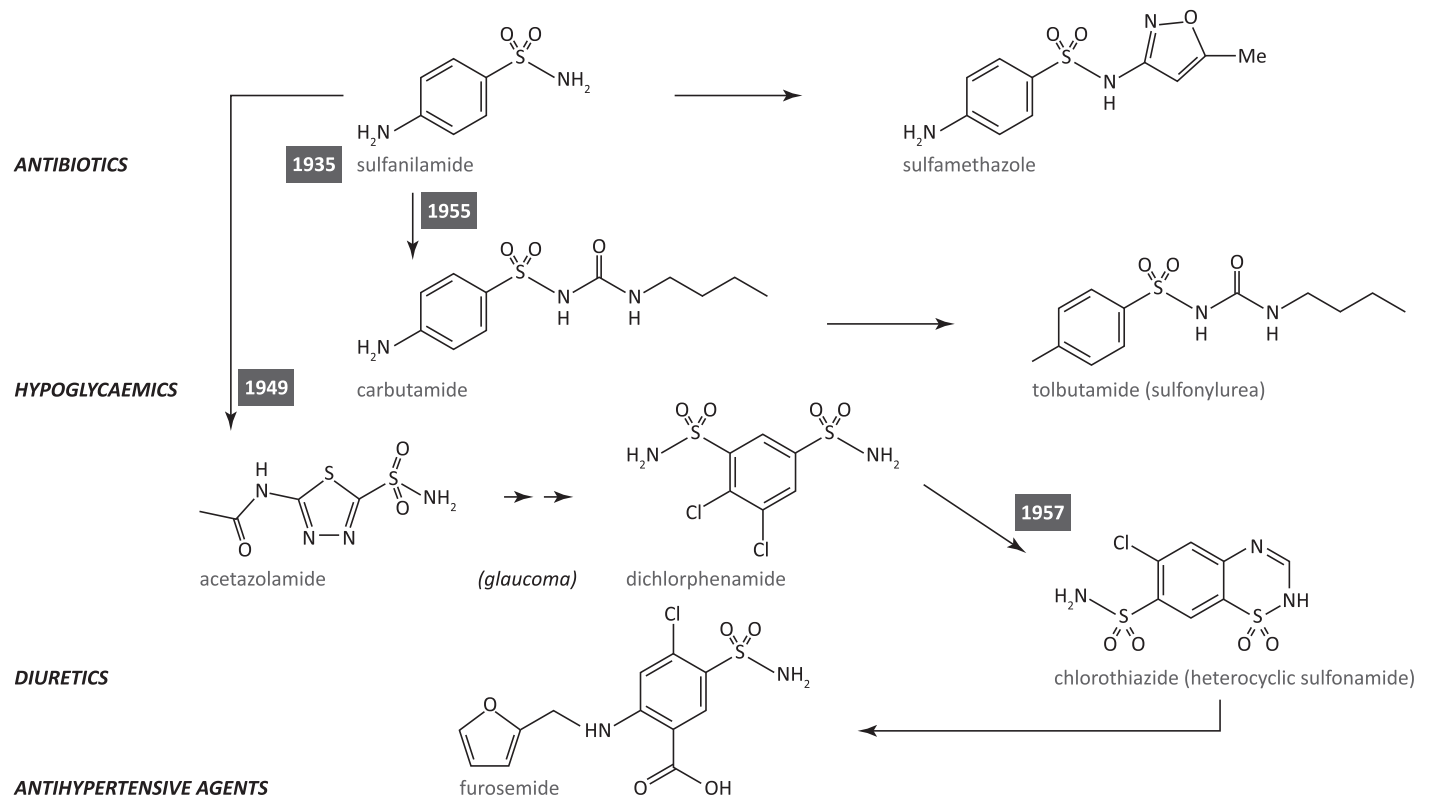


are most often common (> 1%), predictable, dose-dependent and related to the pharmacological action of a drug. Type B reactions are less common (< 1%), unexpected and usually acute, have no relationship with dosage, hypersensitive responses and not related to the pharmacological action of the drug.

Type B ADRs are usually immunoallergic reactions, such as urticaria, hepatitis and agranulocytosis. Often, these allergic reactions are caused by specific groups of atoms within a molecule. The sulfonamide ('sulfa') moiety ($R{-}SO_2N{-}R_2$) is an example of a functional group. The term 'sulfa drugs' usually refers to the group of sulfonamide antibiotics, but there are several non-antibiotic drugs which also have a sulfa moiety.[14] From sulfanilamide, other sulfonamide antibiotics, hypoglycaemic drugs and diuretics have been derived (Figure 2). When the sulfonamide antibiotics and non-antibiotic sulfonamide drugs are grouped together, or are even further regrouped on basis of substituents attached to the sulfa moiety,[15] drug exposure is then redefined from a drug (or drug class) to a molecular characteristic. Another example of **molecular characteristic-based classification** is the recategorisation of antiepileptic drugs in aromatic and non-aromatic antiepileptic drugs in relation to allergic reactions.[16]

Furthermore, it has been suggested that molecular properties are related to the inhibitory potential of the bile salt export pump (BSEP).[17] Inhibition of the BSEP is one of the proposed mechanisms of drug-induced cholestasis [18,19] and this inhibition potential might be a useful predictor for cholestasis. Drug-induced liver injury, including cholestasis, is a major clinical problem and also is the most common single adverse reaction leading to refusal of market approval.[20] Since liver injury has been associated with a wide variety of drugs,[21,22] assessment of their

Figure 2 Derivatives of sulfanilamide [23]



molecular properties could be of relevance in preclinical and clinical practice.
Examples of type A ADRs are nausea, headache, tachycardia, constipation, tinnitus and urinary retention. The occurrence of type A ADRs can be understood from their pharmacological action. When there is a biologically plausible explanation that an ADR is associated with the affinity for a certain transporter or receptor, categorisation according to the (degree of) receptor affinity would be more appropriate than according to therapeutic drug classes. For example, antidepressants drugs are classified according to their chemical structure (tricyclic antidepressants, TCAs) or on basis of their mechanism of action (selective serotonin reuptake inhibitors, SSRIs). Nevertheless, the division between these two groups is not clear-cut because among the TCAs, several have substantially affinity for the serotonin transporter.
The classification of drugs according to their anti-hERG activity in relation with drug-induced QTc-prolongation [24] is another example of **target-oriented drug classification**.
In addition to molecular characteristic-based and target-based classifications, other exposure classifications are possible. Among antidepressant drugs, several are potent inhibitors of cytochrome P-450 (CYP) 2D6, whereas others are weak inhibitors.[25] Therefore, antidepressant drugs may be classified according to the degree of inhibition of this drug-metabolising enzyme in drug-drug interaction studies. For biopharmaceuticals which have different characteristics compared to small molecule drugs, the way of synthesis, administration route and concomitant use of immune suppressants are potential classification schemes.
Categorisation of drug exposure according to chemical structure or to pharmacological activity results in **redefining exposure**. Instead of a possible association between a drug and an ADR, the focus is shifted from the drug itself towards functional groups or target-related properties (Table 1).

OBJECTIVE OF THIS THESIS

The main objective of this thesis is to evaluate the role of structure-activity relationships in the understanding and prediction of drug-induced safety problems. Although the therapeutic classification (*e.g.* diuretics, antidepressants, antidiabetic agents) can be very useful in a clinical setting, this thesis focuses on the categorisation of drugs on basis of molecular characteristics and target-related properties instead of categorisation on basis of the pharmacotherapeutical drug class. These three aspects will be assessed in several observational studies

Table 1 Redefining exposure from (A) drug to (B) molecular characteristic-based, (C) formulation-based or (D) target-oriented classifications

A	**outcome**	**no/other outcome**
drug of interest	a	b
no/other exposure	c	d

Relative risk: a/(a+b) / c/(c+d).
Odds ratio: (a/c) / (b/d) = (a×d) / (b×c).

B	**allergic reaction**	**no/other outcome**
arylamine and/or N1 substituent: yes	a	b
arylamine and/or N1 substituent: no	c	d

C	**change in blood cell counts**	**no/other outcome**
lipophilic solvents: yes	a	b
lipophilic solvents: no	c	d

D	**arrhythmias**	**no/other outcome**
HERG activity: yes (low, medium, high)	a	b
HERG activity: no	c	d

in relationship with miscellaneous drug-related adverse reactions. Such a reclassification could be a helpful tool in post-approval research.

OUTLINE OF THIS THESIS

This thesis consists of three parts. In the first part (**Chapter 2**) exposure classification according to molecular characteristics are the central point. The second part (**Chapter 3**) focuses on classification on the basis of pharmaceutical formulation. The last part (**Chapter 4**) addresses target-orientated drug exposure classification.

Chapter 2.1 describes the difference in risk of an allergic reaction to sulfonamide drugs based on the drug's chemical structure. This case-control study has been conducted using data from the General Practice Research Database (GPRD). In *Chapter 2.2 and 2.3*, we present studies which have been performed with data from the International Drug Monitoring Program of the World Health Organization (WHO). *Chapter 2.2* focuses on the association between drug-induced photosensitivity and spectroscopic and molecular characteristics; *Chapter 2.3* discusses the influence of fluorine substituents on the reporting of adverse drug reactions.

Chapter 3.1 deals with the association between lipophilic solvents and changes in circulating red blood cells. This study obtained information from the Utrecht Patient Oriented Database (UPOD).

In *Chapter 4.1*, we describe a case – non-case study using data collected by the Netherlands Pharmacovigilance Centre Lareb. The classification of the study drugs was based on the ability to affect ion transport systems in the kidneys and the inner ear. In *Chapter 4.2*, we assessed the association between transporter affinity and a first diagnosis of abnormal bleeding leading to hospitalisation, whereas in *Chapter 4.3* osteoporotic and non-osteoporotic fractures were regarded as outcome definition. According to the degree of affinity for the serotonin transporter and serotonin receptor 2A, antidepressants and serotonergic drugs are categorised in groups with high, medium and low affinity. In both studies, we used data from the PHARMO Record Linkage System.

In **Chapter 5**, the general discussion, the results of these studies are discussed in the context of past, present and future perspectives.

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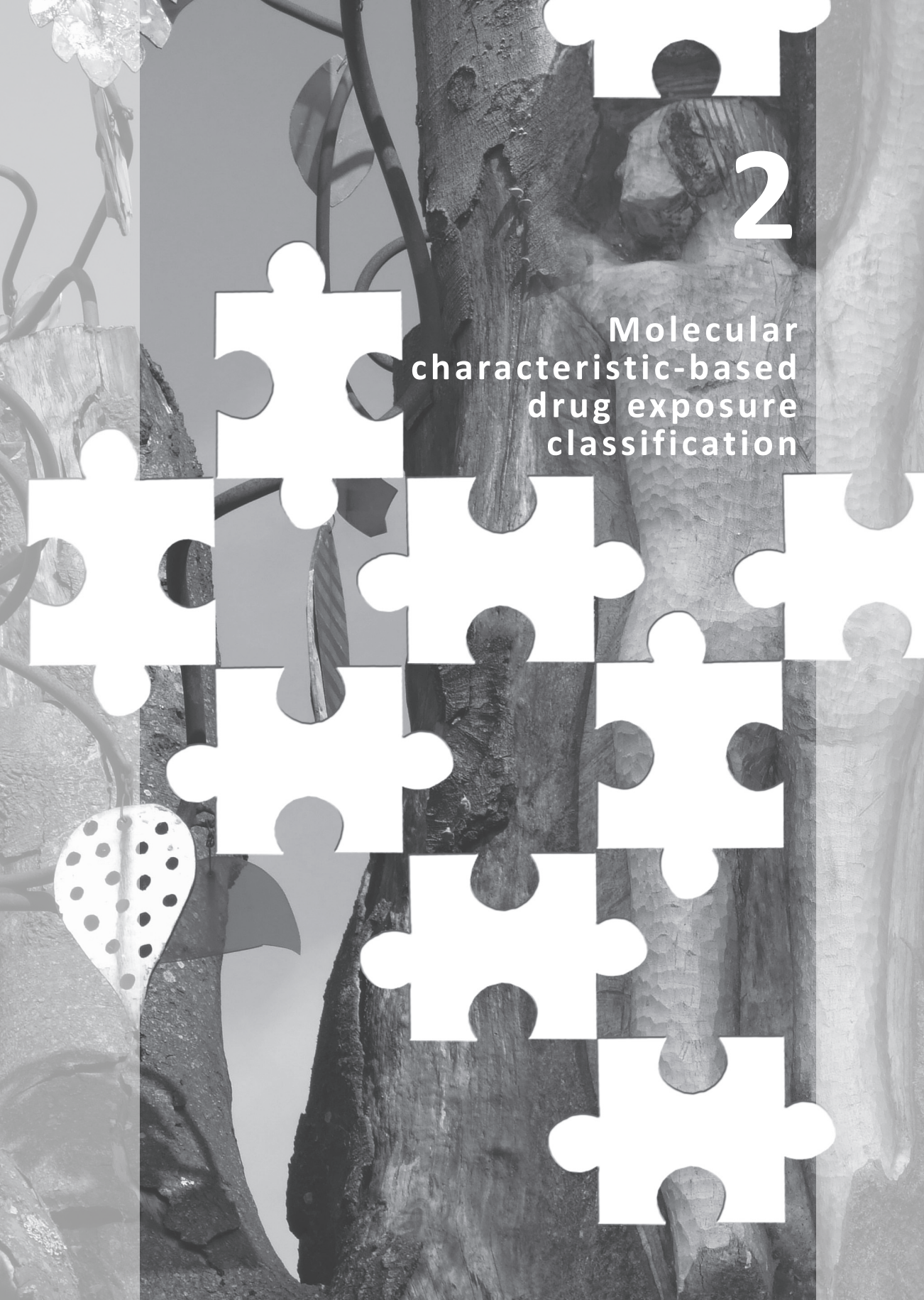


2

Molecular characteristic-based drug exposure classification

2.1

Difference in risk of allergic reaction to sulfonamide drugs based on chemical structure

Bertha Maria Verdel
Patrick C Souverein
Toine CG Egberts
Hubert GM Leufkens

Ann Pharmacother 2006;40:1040-6

ABSTRACT

Background

The chemical structure of sulfonamide antibiotics and sulfonamide non-antibiotics can affect the potential for adverse reactions.

Objective

To assess whether differences in chemical structure of the various sulfonamide drugs influence the risk of allergic events.

Methods

A case-control study was conducted among patients with diabetes mellitus (DM), using data from the General Practice Research Database. Cases were defined as patients with a diagnosis of hypersensitivity or allergic reaction. The date of the last event was the index date. Controls were matched on practice, type of DM and index date. Current use of sulfonamides was defined as use in a 14 day time window before index date. Sulfonamide drugs were classified according to the presence/absence of a N1 substituent ($N1^{+}/^{-}$) and/or an arylamine ($N4^{+}/^{-}$). Conditional logistic regression was used to estimate the strength of association and expressed as odds ratios (OR) and 95% confidence intervals (CI).

Results

Overall, current use of $N1^{+}\,N4^{+}$ sulfonamide drugs was associated with the outcome (adjusted OR 3.71; 95% CI 1.40–9.81). Current use of $N1^{+}\,N4^{-}$ and $N1^{-}\,N4^{-}$ sulfonamide drugs was also associated with the occurrence of allergic reactions, although not as strongly: adjusted OR 2.48 (95% CI 2.12–2.89) and 2.07 (95% CI 1.74–2.46), respectively. Sex and age seemed to be effect modifiers. There was no clear evidence for effect modification by immune disease state.

Conclusions

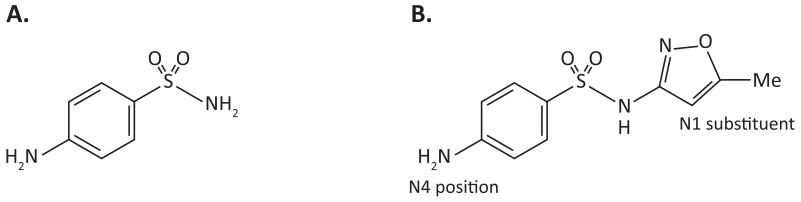
Although we did not find major differences between the groups, we believe that this approach is an innovative manner to examine adverse drug reactions by using chemical structure instead of therapeutic drug classes to classify exposure.

INTRODUCTION

Sulfonamide antibiotics and sulfonamide non-antibiotics, such as sulfonylureas and thiazide diuretics, are well known to have the capacity to cause hypersensitivity reactions.[1-3] Although in some patients possible cross-reactivity occurred between a sulfonamide antibiotic and a sulfonamide non-antibiotic,[4-8] the issue of cross-reactivity is still controversial. Available evidence suggests that sulfonamide antibiotics probably do not cross-react with sulfonamide non-antibiotics.[9-13] Both sulfonamide antibiotics and non-antibiotics are compounds with a sulfonamide moiety (SO_2NH_2). Notwithstanding this same chemical structure, the group is very heterogeneous with respect to three-dimensional structures.

Sulfonamide antibiotic-induced hypersensitivity reactions involve a complex combination of metabolic and immunological events. Sulfonamide antibiotics are derivatives of sulfanilamide (Figure 1A). They contain an aromatic amine group at the N4 position and a substituent at the N1 position. The aromatic amine moiety is considered to be the trigger for serious drug reactions, due to the formation of reactive hydroxylamine intermediates and the subsequent haptenation product.[14]

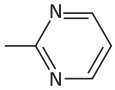
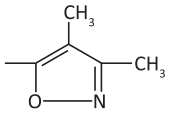
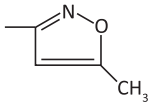
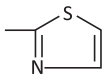
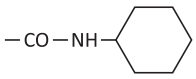
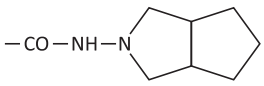
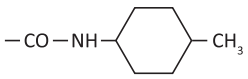
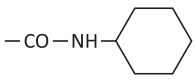
Figure 1 Sulfanilamide (A) and sulfametoxazole (B)



Type I allergic reactions to sulfonamide antibiotics appear to be directed by the substituents at the N1 position. The attachment of a 5-or 6-member aromatic heterocyclic ring with at least 1 nitrogen to the sulfonamido-N1 and the presence of a single methyl group (β-position) on the second carbon atom are important allergenic determinants (Figure 1B).[9,11,12]

Sulfonamide non-antibiotics lack both the aromatic amine moiety and an N1 substituent with a 5-or 6-member aromatic heterocyclic ring (Figure 2). On a chemical basis, it has been considered unlikely that the risk of a hypersensitivity reaction or allergic event after use of a sulfonamide antibiotic and a sulfonamide

Figure 2 Examples of sulfonamide drugs classified by N1 substituents and N4 position

N1 substituent	Arylamine (N4 position)	Sulfonamide drug
	yes	sulfadiazine
	yes	sulfafurazole
	yes	sulfametoxazole
	yes	sulfathiazole
	no	glibenclamide
	no	gliclazide
	no	glimepiride
	no	glipizide
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no	no	chlorthalidone
no	no	furosemide
no	no	hydrochlorthiazide
no	no	indapamide
no	yes	dapsone

non-antibiotic is the same. On basis of the chemical structure, Johnson *et al.* divided sulfonamides in three groups: arylamines (a sulfonamide moiety connected to a benzene ring with an unsubstituted amine moiety at the N4 position, $N4^{+}$), nonarylamines (a sulfonamide moiety connected to a benzene ring or other cyclic structure without the amine moiety at the N4 position, $N4^{-}$), and a group in which the sulfonamide moiety was not directly connected to the benzene ring.[10] However, in the group of the nonarylamines they made no distinction between sulfonamides that lacked a 5-or 6-member aromatic heterocyclic ring ($N1^{-}$) or sulfonamides, which had a different type of substituent at the N1 position ($N1^{+}$).

In this study, we classified sulfonamide drugs according to the absence or presence of N1 substituents and the absence or presence of an arylamine. The objective of this study was to determine whether the presence of N1 substituents and the aromatic amine moiety in sulfonamide drugs influence the occurrence of hypersensitivity reactions and allergic events. We chose to conduct a study with patients with diabetes mellitus (DM), as the frequency of use of sulfonamide drugs is relatively high within such patients.

METHODS

Setting

The data for this study was obtained from the General Practice Research Database (GPRD), which contains the computerised medical records of about 650 general practices, comprising over 35 million patient-years of data collected from almost nine million patients.[15] The data accrued in the GPRD include demographic information about the patient, prescription details, clinical events, preventive care provided, referrals to specialist care, hospital admissions and their major outcomes.[16] Clinical data are stored and retrieved by means of Oxford Medical Information Systems (OXMIS) and Read codes for diseases or causes of morbidity and mortality that are cross-referenced to the International Classification of Diseases (ICD-9).

Each entry into GPRD is internally validated by crosschecking within the practice and by comparisons with external statistics. Only data from practices that pass this quality control are compiled to form the GPRD database. Several independent validation studies have shown that the GPRD database has a high level of completeness and validity.[16,17] The GPRD is owned by the UK Department of Health and managed by the Medicines Control Agency.[15]

Study base

From the GPRD, we identified all patients with either a diagnosis of diabetes (ICD-9 code 250) or a prescription for a drug indicated for the treatment of diabetes mellitus (*i.e.* insulin and/or oral antidiabetic drugs) in the period from 1987–2001. Patients were followed up from their first diagnosis of DM (after practice up-to-standard) or the start of the data collection of the practice (when a patient had a first diagnosis of DM prior to the practice's up-to-standard date), up to the end of the study period.

Cases and controls

Within the study base, a nested case-control study was conducted. Cases were defined as patients with at least one diagnosis of hypersensitivity or allergic reaction (*e.g.* anaphylaxis, urticaria, angioedema, allergic rash, allergic dermatitis, and toxic epidermal necrolysis (TEN); for selected OXMIS/Read codes, see Appendix I) during the study period. The date of the last recorded event of the outcome event was the index date, because we also wanted to study the effect of prior hypersensitivity or allergic events. For each case, up to three controls were sampled and matched on type of DM, general practice and index date. Potential controls were all patients from the study base who did not have one of such outcomes during the study period. We made a distinction between patients using insulin only (type 1) and those using oral antidiabetic agents only or who had a diagnosis of DM only without medication (type 2). Both cases and controls were eligible for inclusion if they had a minimum period of 365 days of history in the GPRD prior to the index date.

Exposure assessment

Sulfonamide drugs were classified by the presence of an N1 substituent and an aromatic amine moiety (Appendix II). The abbreviations $N1^{+}$ and $N1^{-}$ indicate the presence or absence, respectively, of a substituent at the N1 position; $N4^{+}$ and $N4^{-}$ indicate the presence respectively absence of an arylamine. For each patient, we identified all prescriptions for sulfonamide drugs in the year before the index date.

Drug exposure was categorised according to the timing of use in relation to the index date. Patients were defined as current users when they had either a prescription in a 14 day time window prior to the index date or the theoretical enddate of an earlier prescription was in or after this time window. If the enddate of the last prescription was from 14 to 365 days before the index date, drug use

Table 1 Characteristics of cases and controls

Characteristics	Cases n=3 362 (100%)	Controls n=10 041 (100%)	Crude OR (95% CI)
Sex			
female	2 024 (60.2%)	4 679 (46.6%)	1.75 (1.61–1.89)
male	1 338 (39.8%)	5 362 (53.4%)	1.00 (reference)
Age (years)			
≤ 65	1 955 (58.1%)	4 663 (46.4%)	1.69 (1.55–1.83)
> 65	1 407 (41.9%)	5 378 (53.6%)	1.00 (reference)
Type of diabetes			
1	700 (20.8%)	2 090 (20.8%)	NA
2	1 817 (54.0%)	5 451 (54.3%)	NA
diagnosis only	845 (25.1%)	2 500 (24.9%)	NA
Prior hypersensitivity or allergic event	673 (20.0%)	0 (0.0%)	NA
Immune disease state [a]	868 (25.8%)	790 (7.9%)	4.15 (3.72–4.64)
Co-morbidity (in year before index date)			
amyloid disease	12 (0.4%)	22 (0.2%)	1.64 (0.81–3.31)
asthma	192 (5.7%)	234 (2.3%)	2.57 (2.11–3.13)
Behçet syndrome	6 (0.2%)	7 (0.1%)	2.57 (0.86–7.65)
colitis	10 (0.3%)	14 (0.1%)	2.14 (0.95–4.82)
eczema	208 (6.2%)	191 (1.9%)	3.38 (2.76–4.13)
erythromelalgia	1 (0.0%)	6 (0.1%)	0.50 (0.06–4.15)
rheumatoid arthritis	18 (0.5%)	25 (0.2%)	2.14 (1.17–3.92)
rhinitis	88 (2.6%)	115 (1.1%)	2.35 (1.76–3.12)
scleroderma	3 (0.1%)	1 (0.0%)	9.00 (0.94–86.5)
spondyloarthritis	43 (1.3%)	65 (0.6%)	1.97 (1.34–2.90)
vasculitis	6 (0.2%)	11 (0.1%)	1.64 (0.61–4.43)

OR = odds ratio; CI = confidence interval; NA = not applicable

a) Asthma/drugs used for asthma, rhinitis, eczema, colitis, rheumatoid arthritis, amyloid disease, Behçet syndrome, scleroderma, erythromelalgia, vasculitis, spondyloarthritis.

was defined as past. No use was defined as no prescription of a sulfonamide drug within one year before the index date.

Data analysis

The strength of the association between sulfonamide drug use and hypersensitivity and allergic reactions was ascertained by conditional logistic regression analysis and expressed as crude and adjusted odds ratios (OR) with 95% confidence

intervals (CI). The model included current and past use of sulfonamide drugs. Covariates were included in the multivariate model if they were either statistically significant ($p < 0.05$) associated with the outcome in a univariate analysis or induced a 10% change of the crude OR of the exposure variable.

It has been reported that a history of previous adverse drug reactions is an important risk factor for adverse drug reactions.[11,18] Female sex and age are also risk factors for such events.[13,18-21] Sex, age, a history of allergic events, and a history or presence of an immune disease were evaluated as effect modifiers to assess differential risk. Immune diseases included asthma, rhinitis, eczema, and rheumatoid arthritis. Patients with DM types 1 and 2 (without or without oral antidiabetic drugs) were analysed as subgroups.

RESULTS

The study base comprised 141 164 patients with either a diagnosis of DM or a prescription for a drug indicated for the treatment of DM. We identified 3362 (2.4%) cases with a record of a hypersensitivity reaction or allergic event. Urticaria was the most frequent allergic-like event (n = 1536; 45.7%), followed by allergic rash (n = 961; 28.6%) and allergic reactions (n = 432; 12.8%). Thirteen cases (0.4%) experienced anaphylactic shock; angioedema occurred in 120 patients (3.6%).

The characteristics of the study population are shown in Table 1. The mean age ± SD was 59 ± 18 years (control group 64 ± 18 years). Female gender, age 65 years or less, and a history or presence of an immune disease were associated with the occurrence of hypersensitivity reactions and allergic events.

The association between the use of sulfonamide drugs and the risk of hypersensitivity reactions and allergic events is shown in Table 2. The prevalence of current sulfonamide drug use was higher among cases (40.2%) than among controls (28.0%), yielding a crude OR of 2.16 (95% CI 1.92–2.43). This association persisted after adjusting for sex, age, and immune disease state (OR 2.36; 95% CI 2.08–2.69).

Stratification according to the presence or absence of an N1 substituent or an arylamine showed that the current use of sulfonamide drugs with $N1^+ N4^+$ was most clearly associated with the occurrence of hypersensitivity and allergic reactions (adjusted OR 3.71; 95% CI 1.40–9.81). Current use of $N1^+ N4^-$ and $N1^- N4^-$ sulfonamide drugs was also associated with the occurrence of allergic reactions, although not as strong as the association with $N1^+ N4^+$ sulfonamide drugs (see Table 2). Current use of more than one sulfonamide drug at the time

Table 2 Current use of sulfonamide drugs and risk of hypersensitivity reactions and allergic events

Exposure	Cases n=3 362 (100%)	Controls n=10 041 (100%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
None	1 166 (34.7%)	4 026 (40.1%)	1.00 (reference)	1.00 (reference)
Current use				
any	1 353 (40.2%)	2 815 (28.0%)	2.16 (1.91–2.43)	2.36 (2.08–2.69)
$N1^+ N4^+$ only	10 (0.3%)	10 (0.1%)	3.90 (1.60–9.48)	3.71 (1.40–9.81)
$N1^+ N4^-$ only	845 (25.1%)	1 856 (18.5%)	2.24 (1.94–2.59)	2.48 (2.12–2.89)
$N1^- N4^-$ only	302 (9.0%)	602 (6.0%)	1.91 (1.63–2.24)	2.07 (1.74–2.46)
$N1^- N4^+$ only	0 (0.0%)	0 (0.0%)	NA	NA
> 1 drug	196 (5.8%)	347 (3.5%)	2.74 (2.22–3.38)	3.05 (2.44–3.82)

OR = odds ratio; CI = confidence interval; $N1^+ N4^+$ = substituent at N1 position, arylamine (*e.g.* sulfonamide antibiotics); $N1^+ N4^-$ = substituent at N1 position, no arylamine (*e.g.* sulfonylureas); $N1^- N4^-$ = no substituent at N1 position, no arylamine (*e.g.* thiazide diuretics); $N1^- N4^+$ = no substituent at N1 position, arylamine (*e.g.* dapsone); NA = not applicable

a) Adjusted for sex, age and immune disease state.

yielded an OR of 3.05 (95% CI 2.44–3.82). Sex, age, and immune disease state were evaluated as effect modifiers. We also stratified on the presence or absence of N1 substituents and the presence or absence of an arylamine in sulfonamide drugs. The association between current use of a sulfonamide drug and allergic events was stronger among males compared with females, as was age greater than 65 years (Table 3). We found no difference in patients with or without an immune disease state or a history of an allergic event. Among current users of a sulfonamide drug, the risk seemed most pronounced among patients with DM using oral antidiabetic drugs (Table 3).

DISCUSSION

We found that the risk of hypersensitivity or an allergic reaction after use of a sulfonamide drug was approximately two times greater than for those who did not use any sulfonamide. Stratifying according to these substituents at the N1 and N4 positions showed that there was a differential risk between the groups of sulfonamides, although the difference was not statistically significant. The increase in risk was most pronounced for sulfonamide drugs with an N1 substituent with a 5- or 6-member aromatic heterocyclic ring and an arylamine (predominantly

Table 3 Stratified variables associated wih current use of sulfonamide drugs and risk of hypersensitivity reactions and allergic events

Characteristics	Any sulfonamide Adjusted [a] OR (95% CI)	$N1^+ N4^+$ Adjusted [a] OR (95% CI)	$N1^+ N4^-$ Adjusted [a] OR (95% CI)	$N1^- N4^-$ Adjusted [a] OR (95% CI)
Sex				
female	2.10 (1.79–2.45)[b]	4.61 (1.34–15.86)	2.26 (1.87–2.72)	1.72 (1.39–2.15)[b]
male	2.74 (2.31–3.24)	2.32 (0.42–12.96)	2.75 (2.26–3.34)	2.67 (2.05–3.49)
Age (years)				
≤ 65	1.79 (1.53–2.10)[b]	3.69 (1.18–11.57)	1.91 (1.59–2.30)[b]	1.52 (1.16–1.99)[b]
> 65	2.53 (2.12–3.02)	4.19 (0.73–24.04)	2.77 (2.26–3.39)	2.16 (1.72-2.70)
Immune disease state				
yes	2.65 (2.05–3.43)	3.37 (0.22–51.96)	2.64 (1.96–3.56)	2.73 (1.86–4.00)
no	2.31 (2.02–2.62)	3.87 (1.37–10.89)	2.44 (2.07–2.86)	1.95 (1.61–2.36)
Type of diabetes				
1	1.86 (1.29–2.67)	2.58 (0.61–10.95)	NA	1.86 (1.27–2.71)
2	2.80 (2.28–3.43)	7.25 (1.56–33.63)	2.67 (2.17–3.28)	3.04 (2.25–4.12)
diagnosis only	1.48 (1.13–1.93)	2.92 (0.26–32.85)	0.65 (0.08–5.59)	1.48 (1.13–1.93)
Previous allergic event				
yes	2.75 (2.03–3.72)	4.36 (0.26–73.92)	2.69 (1.89–3.83)	2.85 (1.91–4.25)
no	2.30 (1.99–2.65)	3.67 (1.31–10.32)	2.45 (2.06–2.90)	1.93 (1.59–2.34)

OR = odds ratio; CI = confidence interval; $N1^+ N4^+$ = substituent at N1 position, arylamine (*e.g.* sulfonamide antibiotics); $N1^+ N4^-$ = substituent at N1 position, no arylamine (*e.g.* sulfonylureas); $N1^- N4^-$ = no substituent at N1 position, no arylamine (*e.g.* thiazide diuretics); $N1^- N4^+$ = no substituent at N1 position, arylamine (*e.g.* dapsone); NA = not applicable

a) Adjusted for sex, age and immune disease state.

b) Statistical significant difference ($p < 0.05$).

sulfonamide antibiotics). This finding is in agreement with results of studies that consider the N1 and N4 substituents of the sulfonamide antibiotics powerful predictors of immunological response.[9,11,20]

Although $N1^+ N4^-$ and $N1^- N4^-$ sulfonamide drugs (primarily sulfonylureas and diuretics, respectively) lack both the N1 substituent with a 5- or 6-member aromatic heterocyclic ring and an arylamine, both groups were associated with an increased risk of hypersensitivity and allergic reactions. Both $N1^+ N4^-$ and $N1^- N4^-$ sulfonamide drugs are usually used chronically. Still, we found a twofold increase of the risk of an allergic event associated with current use of these drugs. Current use of more than one sulfonamide increased the risk of a hypersensitivity or allergic reaction three times compared to non-users.

Risk estimates were not different between patients with (a history of) immune diseases or a previous allergic event. However, stratification according to age and sex did reveal differences in risk. It has been suggested that female sex is a risk factor for the occurrence of adverse drug reactions.[18,19,21] However, our findings do not support those data. We did identify increased risk of an allergic reaction in patients older than 65 years with DM. This result is consistent with studies suggesting that adverse drugs reactions are more common in elderly patients.[22,23]
In patients with type 2 DM who are using oral antidiabetic drugs, the risk of a hypersensitivity reaction associated with current use of a sulfonamide agent was higher than in patients with type 1 DM and patients with a diagnosis DM who were not receiving drug therapy. The number of patients using $N1^{+}$ $N4^{+}$ sulfonamide drugs was small, but the association was also present in the much larger group of $N1^{-}$ $N4^{-}$ sulfonamide drug users.
There are potential methodological limitations to our study. In our case definition, we selected codes for hypersensitivity and allergic reactions and excluded codes that suggested non-unique causes. Therefore, it is possible that the lists of codes in Appendix I omitted codes for hypersensitivity reactions and allergic events. Misclassification with respect to the recording of diagnosis of hypersensitivity reactions and allergic events cannot be excluded, but it seems unlikely that misclassification was differential between cases and controls.
The timing of the outcome events was an important factor. The potential immunologic reaction related to sulfonamide antibiotics usually develops within 1–3 days of initial medication; the hypersensitivity reaction requiring the presence of an arylamine has a delayed onset, usually within 7–14 days.[9,10] Hypersensitivity reactions caused by some sulfonamide drugs ($N1^{+}$ $N4^{-}$) occur usually in the first 6–8 weeks of therapy. Because most of the outcome events are mild dermatological end points, it is possible that those events were not diagnosed or not reported directly after the onset of the events.
We did compute the OR and 95% CI for risk of a hypersensitivity reaction or allergic event within a 30 day time window prior to the index date. The ORs and 95% CIs did not differ substantial (data not shown). Furthermore, as dermatological events are known adverse effects of sulfonamide drugs, we cannot exclude the possibility that diagnostic suspicion bias accounts for part of the observed outcome events. There could also have been other variables that we could not control; therefore, residual confounding is possible.
This study was conducted among patients with DM because the use of sulfonamide drugs is usually relatively high in such patients. To our knowledge, patients with

DM are not at greater risk for hypersensitivity reactions after use of sulfonamide drugs than are patients who are not diabetic.
Previous reports which mention the risk of hypersensitivity reactions after the use of sulfonamide drugs do not categorise these agents according to their substituents. Recategorising these medications in the defined chemical structure categories could be helpful to obtain a new perspective on the problem of 'sulfa' allergy and/or cross-reactivity between drugs with a sulfonamide moiety.

CONCLUSION

In this study we used an innovative manner to examine adverse drug reactions by using chemical structures instead of a traditional pharmacology-based classification to assess exposure. Although our results did not show an obvious difference in risk of hypersensitivity or allergic reaction after using a sulfonamide drug classified according to substituents at the N1 and N4 positions, we believe that structure-activity relationships related to drug exposure will play a major role in the future.
Further research is needed to establish whether a possible association exists between the presence or absence of substituents of sulfonamide drugs and the occurrence of hypersensitivity reactions.

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Appendix I Diagnoses included in the definitions of hypersensitivity reactions and allergic events

Definition	OXMIS codes	Read codes
urticaria (nos)	7089	M28..00 – M28z.00
urticaria, allergic	7089AL	
urticaria, cholinergic	7080C	
dermatosis, allergic	7089D	
urticaria, giant	7080G	
angioneurotic oedema	7080AN	SN51.00
angio-oedema	7080AO	
oedema, allergic	7080AL	
rash, allergic	7089AR	
dermatitis allergic	6929E	
reaction allergic	6929G	
skin allergy	6929EA	
skin allergic reaction	6929ER	
erythema multiforme	6951	M151.00
Stevens-Johnsons syndrome	6951MJ	M151700
toxic epidermal necrolysis	6951NE	M151.12 – M151800
shock (circulatory)	7829	
anaphylactic shock	9994	SN50.00
hypersensitivity nos		SN53.11
hypersensitivity angiitis		G752.00
urticaria, papular	6982AR	
laryngeal spasm	508 T	H1y7400

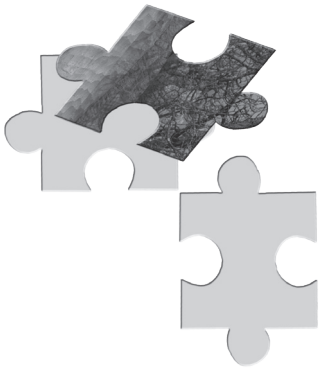
nos = not otherwise specified

Appendix II Classification of sulfonamide drugs acoording to the presence and/or absence of a N1 substituent and an arylamine (N4)

N1 substituent	Arylamine (N4)	Sulfonamide drugs
+	+	calcium sulfaloxate, sulfamoxole, sulfadiazine, sulfametoxazole, succinylsulfathiazole, sulfacetamide, sulfadimethoxine, sulfadimidine, sulfafurazole, sulfaguanidine, sulfamethizole, sulfamethoxypyridazine, sulfametopyrazine, sulfamezathine, sulfaphenazole, sulfapyridine, sulfasuxidine, sulfathiazole, amprenavir
+	–	acetohexamide, chlorpropamide, glibenclamide, glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glymidine, tolazamide, tolbutamide, almotriptan, probenacid, sotalol, torasemide
–	–	bumetanide, furosemide, piretanide, sarfrusemide, bendrofluazide, chlorothiazide, chlorthalidone, clopamide, clorexolone, cyclopenthiazide, ethiazide, hydrochlorthiazide, hydroflumethiazide, indapamide, methyclothiazide, metolazone, neo-urizide, polythiazide, quinethazone, xipamide, acetazolamide, celecoxib, diazoxide, famotidine, naratriptan, sildenafil, sulpiride, sumatriptan
–	+	dapsone

2.2

Risk of drug-induced photosensitivity: focus on spectroscopic and molecular characteristics



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ABSTRACT

Background

Drug-induced photosensitivity is difficult to predict and remains a challenge for both the dermatological clinical practice and pharmacovigilance.

Purpose

To assess the association between spectroscopic and molecular characteristics and the occurrence of photosensitivity reactions.

Methods

For 143 well-known photosensitisers (*e.g.* tetracyclines, diuretics), we retrieved information on spectroscopic and molecular parameters, including: absorption maximum λ_{max}, molar absorption coefficient ε, area under the absorption curve (AUC), molecular weight and configuration, heteroatoms and aromatic halogen atoms, lipophilicity (log P) and acid/base status (pKa). In the WHO-ADR database, all reports with suspected adverse drug reactions of the study drugs were selected. We identified all reports on photosensitivity reactions and defined them as cases. All other reports were selected as non-cases. A case – non-case approach was performed to assess the spectroscopic and molecular exposure variables as a factor for photosensitivity reactions. Logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (CI).

Results

A λ_{max} between 290 and 320 nm (OR 3.74; 95% CI 3.45–4.06), and an $\varepsilon > 20\,000$ $M^{-1}\,cm^{-1}$ (OR 5.49; 95% CI 5.10-5.92) were highly associated with the reporting of photosensitivity reactions. Risk of photosensitivity reactions was significantly increased among intermediate or high AUCs compared to low AUC. Low molecular weight and aromatic halogen atoms were associated with photosensitivity reactions (OR 2.37; 95% CI 2.07–2.71 resp. OR 3.23; 95% CI 3.02–3.47) as were log $P < 1$ and $pKa < 7$.

Conclusion

The reporting of photosensitivity reactions to established phototoxic drug classes is strongly influenced by spectroscopic and physicochemical characteristics of individual drugs.

INTRODUCTION

A specific category of cutaneous adverse drug reactions is drug-induced photosensitivity which results from a combined exposure to a drug and ultraviolet (UV) or visible light. Drug-induced photosensitivity is a general term that most commonly describes either a phototoxic or a photoallergic reaction.[1-3] Phototoxicity is an UV or visible light-induced, non-immunological response to a photoactive drug or chemical; photoallergy is an acquired immunological reactivity to a drug or chemical, initiated by formation of photoproducts after exposure to light. These reactions can be diagnosed separately, based on pathogenesis, clinical features and histology, although in clinical practice differentiation may be difficult and some drugs can induce both reaction types. After systemic drug use, phototoxicity has a higher incidence compared to photoallergy,[4,5] and it is also far more dose dependent, to the drug or its metabolite as well as to the light exposure. Skin eruptions due to the combined effect of UV/visible light and a drug are generally mild, but severe reactions may occur, and chronic interaction may be an additional factor contributing to the initiation of skin cancer.[1]

Many drugs are known to induce photosensitivity, for example quinolones, tetracyclines, diuretics and non-steroidal anti-inflammatory drugs (NSAIDs),[1,5-7] although the incidence of photosensitivity associated with individual drugs within these classes remains uncertain.[6] The majority of systemically administered drugs have not undergone controlled testing to determine their potential for photosensitivity.[8,9] Warnings of phototoxicity or photoallergy were, therefore, only included in drug leaflets after reports of photosensitivity reactions resulting from extended clinical use of these drugs. In the guidelines of the Committee for Proprietary Medicinal Products and the Food and Drug Administration, photosafety testing is currently required by regulatory authorities for new active substances that absorb UVA, UVB or visible light (290−700 nm) and are topically applied or reach the skin or eyes following systemic exposure.[8,9] For the photochemical assessment also the photoinstability of a drug *in vitro* and structure-activity relationships have to be taken into account.

Suspected photosensitive drug classes consist of drugs with different pharmacological activities and different chemical structures. However, the ability of drugs to act as photosensitisers depends on a complex of physical, chemical, pharmacokinetic and pharmacodynamic properties. Generic molecular characteristics of photosensitising agents, described in literature, include low molecular weight (200−500 Dalton), planar, tricyclic or polycyclic configurations, often with heteroatoms in their structures enabling resonance stabilisation, and aromatic halogen substituents.[1,10] A factor of potential importance in reaching

the skin at sufficient concentrations to cause photosensitivity reactions is the lipophilicity of a drug, expressed as log P (octanol/water partition coefficient). The lipid solubility is also an important characteristic with regards to the pharmacokinetics (absorption, distribution, metabolism and elimination) and pharmacodynamics (receptor affinity) of a drug. A drug's affinity for melanin (an endogenous chromophore) has been related to log P as well as to the acid/base status (pKa).[11] Spectroscopic parameters are the absorption maximum λ_{max} and the molar absorption coefficient ε, a measure of the ability of a chemical to absorb light. Although all these characteristics are considered important factors for the capability of producing photosensitive side effects, few studies have been conducted to elucidate their role in drug-induced photosensitivity in day-to-day practice.

The objective of our study was to assess the association between the drug-specific spectroscopic parameters and other molecular characteristics (as mentioned above) and the occurrence of photosensitivity reactions, using drug safety data obtained from the World Health Organization (WHO) adverse drug reactions database.

METHODS

Setting

The data have been obtained from the International Drug Monitoring Program of the WHO. The WHO adverse reaction database Vigibase is maintained by the Uppsala Monitoring Centre and contains summaries of suspected spontaneous case reports originally submitted to national pharmacovigilance centres in more than 84 countries all over the world. At the end of June 2007, this database contained more than 3.8 million individual case reports of suspected adverse drug reactions (ADRs) regarding specific, but anonymous, patients. The reports contain administrative data, patient data, ADR data, medication data and additional information. The ADRs are classified according to the WHO Adverse Reaction Terminology (ART).[12] The drugs mentioned in the reports are classified according to the Anatomical Therapeutic Chemical (ATC) classification system.[13] The information in these reports is not homogenous, at least with regard to origin, completeness of documentation or the likelihood that the suspected drugs caused the adverse events.

Vigibase has been used for data mining studies as well as to investigate drug specific ADRs.[14,15]

From the text books of Litt and Mulder *et al.*,[7,16] we selected nine drug classes and two drug substances, which are the dominant photosensitisers from the 1950s-1960s till now: sulfonamides (J01E), tetracyclines (J01A), quinolone antibiotics (J01M), non-selective monoamine reuptake inhibitors (N06AA), antimalarials (P01BA and P01BC), phenothiazine antipsychotics (N05AA, N05AB and N05AC), diuretics (C03A, C03B, C03C and C03E), non-steroidal anti-inflammatory drugs (M01A), sulfonylurea derivatives (A10BB) and the antiarrhytmic drugs quinidine (a methanolquinoline, C01BA01) and amiodarone (C01BD01). This resulted in a list of approximately 250 drugs. Subsequently, we included only those drugs for which the information on the spectroscopic parameters λ_{max} and ε were available from a single data source. The spectroscopic parameters have been measured under the same conditions accordingly,[17] as a result of which the uniformity of data collection is guaranteed. This led to 143 study drugs, expressed as the base of the pure chemical substance, for which we also gathered the physicochemical characteristics (Appendix I).

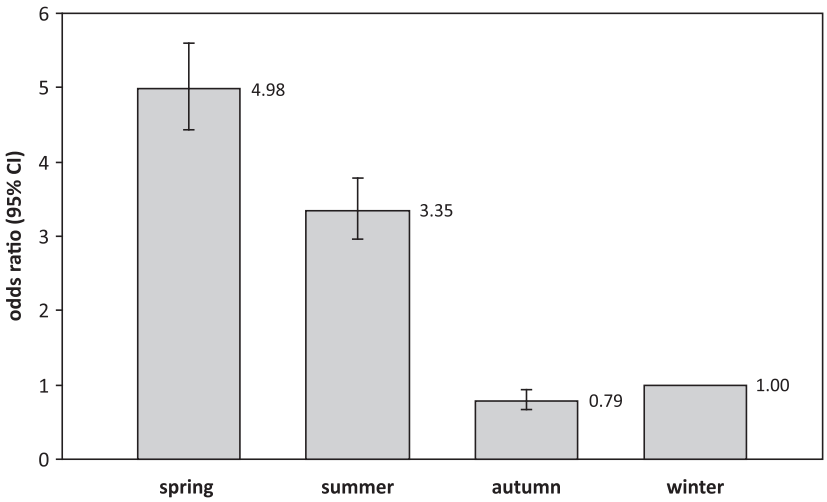
Design

In the WHO-ADR database, we selected all reports of the study drugs up to the second quarter of 2007. Only reports of drugs used systemically were included. Reports were excluded when two or more suspected drugs were reported per ADR-report. Subsequently, we identified all reports of interest by means of the WHO-ART preferred terms 'photosensitivity reaction', 'photosensitivity allergic reaction' and 'photosensitivity toxic reaction'. All suspected ADR-reports with these preferred terms were defined as cases. All other reports of the study drugs were selected as non-cases.

A case – non-case approach within the drug ADR-reports was performed to assess molecular exposure variables as a factor for photosensitivity reactions. Determinants included absorption maximum λ_{max} (< 290 nm, 290−320 nm, 320−400 nm), molar absorption coefficient ε (< 10 000, 10 000−20 000, > 20 000 M^{-1} cm^{-1}), the area under the (absorption) curve (AUC) from 290 till 360 nm (small, intermediate, large), molecular weight (200−500 Dalton, > 500 Dalton), molecular configuration (planar/tricyclic/polycyclic configuration or not), the presence of heteroatoms, the presence of aromatic halogen atoms, log P (hydrophilic compounds, lipophilic, and highly lipophilic compounds, missing data), and pKa (acid compounds, basic compounds, missing data).

The available absorption spectra have been recorded from 220 till 360 nm.[17] In case of multiple absorption maxima, the highest maximum was used with the corresponding molar absorption coefficient. We decided to estimate the AUC

Figure 1 Association between seasons and reported photosensitivity reactions



CI = confidence interval

from 290 till 360 nm because the AUC could not be determined for all 143 study drugs starting at 220 nm due to interference with the solvent (methanol). We estimated the AUC manually by counting small-area squares under the absorption curve, obtained from drugs at the same concentration (1 mg mL^{-1}). Molecular weight, chemical structure, log P and pKa were determined using electronic data sources.[18-20]

As negative control outcomes, we studied the association between spectroscopic properties and renal disorders (WHO-ART high level terms 'abnormal renal function', 'nephritis', and 'renal tubular disorder') and liver disorders (high level term 'hepatocellular damage') which do not have any direct association with the spectroscopic variables.

Data analysis

The strength of the association between the exposure variables and the occurrence of photosensitivity reactions was ascertained by logistic regression and expressed as odds ratios (OR) with 95% confidence intervals (CI). Univariate logistic regression was used for the association between spectroscopic parameters

and photosensitivity reactions. We used multivariate logistic regression to adjust for the molecular characteristics, namely molecular weight, configuration and the presence of heteroatoms and aromatic halogen atoms. Unadjusted ORs were presented for the association between the physicochemical parameters log P and pKa and photosensitivity reactions since the value of these parameters is determined by the molecular characteristics.

We analysed the association between the spectroscopic parameters in subsets of the database based on the number of reports per drug (< 5000; ≥ 5000) in order to assess the influence of drugs which are mentioned in many reports compared to other drugs in the database, because they are either frequently prescribed or have been marketed relatively early.

All statistical analyses were performed using SPSS 14.0 statistical software (SPSS Inc. Chicago, Illinois, USA).

RESULTS

At the end of June 2007, the WHO had received 210 457 case reports of suspected adverse drug reactions (ADRs) of 143 study drugs. Of these reports, 43 193 reports (20.5%) belonged to the system organ class Skin and Appendages (0100), and of which 4178 reports (9.7%) mentioned the WHO-ART preferred terms 'photosensitivity reaction' (n = 3894, 93.2%), 'photosensitivity allergic reaction' (n = 207, 5.0%) or 'photosensitivity toxic reaction' (n = 77, 1.8%). Photosensitivity reactions were statistically significantly more frequently reported in the spring or summer (Figure 1).

The association between the spectroscopic parameters λ_{max}, ε and the AUC and photosensitivity reactions is shown in Table 1. An absorption maximum within the UVB range (290–320 nm) (OR 3.74; 95% CI 3.45–4.06), and a molar absorption coefficient of more than 20 000 $M^{-1} cm^{-1}$ (OR 5.49; 95% CI 5.10–5.92) were strongly associated with the reporting of photosensitivity reactions. The risk of the occurrence of a photosensitivity reaction was also increased if the AUC was intermediate or large. The spectroscopic parameters showed no association with the negative control outcomes, except from the association between liver disorders and the molar absorption coefficient (Table 1).

In Table 2, the association between physicochemical characteristics and photosensitivity reactions is listed. A low molecular weight and the presence of aromatic halogen atoms are physicochemical variables which were significantly associated with the reporting of photosensitivity reactions (OR 2.37; 95% CI

Table 1 Association between spectroscopic exposure variables and photosensitivity reactions, and spectroscopic exposure variables and negative control outcomes

Parameter	Photosensitivity reactions	Negative controls	
		Renal disorders	Liver disorders
	Crude OR (95% CI)	Crude OR (95% CI)	Crude OR (95% CI)
λ_{max} (nm)			
< 290	1.00 (reference)	1.00 (reference)	1.00 (reference)
290–320	3.74 (3.45–4.06)	0.63 (0.56–0.71)	0.88 (0.79–0.99)
> 320	1.43 (1.30–1.56)	1.24 (1.13–1.36)	1.05 (0.95–1.15)
ε ($M^{-1}cm^{-1}$)			
< 10 000	1.00 (reference)	1.00 (reference)	1.00 (reference)
10 000–20 000	1.44 (1.33–1.57)	0.91 (0.83–1.00)	1.35 (1.22–1.48)
> 20 000	5.49 (5.10–5.92)	0.84 (0.74–0.95)	1.73 (1.55–1.94)
AUC (290–360 nm)			
small	1.00 (reference)	1.00 (reference)	1.00 (reference)
intermediate	2.54 (2.22–2.90)	0.84 (0.76–0.93)	0.92 (0.82–1.02)
large	7.89 (6.95–8.96)	0.88 (0.79–0.98)	1.07 (0.95–1.19)

OR = odds ratio; CI = confidence interval; AUC = area under curve

2.07–2.71 and OR 3.37; 95% CI 3.15–3.61, respectively). A planar, tricyclic or polycyclic configuration as such was weakly associated with photosensitivity reactions, but the presence of heteroatoms was not.

The 10 study drugs with the highest number of reports with suspected photosensitivity adverse drug reactions are listed in Table 3. For 9 out of 10 drugs, photosensitivity reactions were statistically significantly more frequently reported compared to the other drugs in our study base.

DISCUSSION

In this study, we found a statistically significant association between the spectroscopic parameters λ_{max}, ε and the AUC as well as several molecular characteristics, namely the presence of an aromatic halogen atom, hydro/lipophilicity and acid/base status, and the reporting of photosensitivity reactions.

As expected, drugs with an absorption maximum within the UVA and UVB range have a higher risk of reporting photosensitivity reactions than drugs with a maximum below 290 nm. The risk of reporting photosensitivity reactions was

Table 2 Association between physicochemical parameters and photosensitivity reactions

Parameter	Cases n=4 178 (100%)	Non-cases n=206 279 (100%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Mw (base)				
200–500	3 925 (93.9%)	194 567 (94.3%)	0.93 (0.82–1.06)	2.37 (2.07–2.71)[a]
> 500	253 (6.1%)	11 712 (5.7%)	1.00 (reference)	1.00 (reference)
Planar, tricyclic or polycyclic configuration: yes	2 385 (57.1%)	128 612 (62.3%)	0.77 (0.72–0.82)	1.13 (1.05–1.21)[b]
Heteroatoms: yes	1 661 (39.8%)	110 366 (53.4%)	0.58 (0.54–0.61)	0.43 (0.40–0.46)[c]
Aromatic halogen atoms: yes	2 723 (65.2%)	85 069 (41.2%)	2.67 (2.51–2.85)	3.37 (3.15–3.61)[d]
log P [e]				
< 1	1 548 (37.1%)	38 981 (18.9%)	2.98 (2.65–3.45)	
1–3	351 (8.4%)	26 323 (12.8%)	1.00 (reference)	
> 3	2 264 (54.2%)	137 470 (66.6%)	1.24 (1.10–1.38)	
missing	15 (0.4%)	3 505 (1.7%)	–	
pKa [f]				
< 7	2 939 (70.3%)	108 594 (52.6%)	1.91 (1.78–2.04)	
≤ 7	1 183 (28.3%)	83 319 (40.4%)	1.00 (reference)	
missing	56 (1.3%)	14 366 (7.0%)	–	

OR = odds ratio; CI = confidence interval
a) Adjusted for configuration, heteroatoms, halogen atoms.
b) Adjusted for Mw, heteroatoms, halogen atoms.
c) Adjusted for Mw, configuration, halogen atoms.
d) Adjusted for Mw, configuration, heteroatoms.
e) log P of drug or base of chemical substance.
f) pKa of drug or conjugated acid.

Table 3 Study drugs with highest number of reported cases, and their spectroscopic and molecular characteristics

Drug	λ_{max} [a] (nm)	ε (M^{-1} cm^{-1})	AUC	Mw (Dalton)	config.	hetero-atoms	aromatic halogen atoms	log P [b] (est.)	pKa [c] (est.)	number of reports	cases [d]	ROR (95% CI)
benoxaprofen	308	28 500	large	301.7		no	Cl	3.98	4.50	5 185	1 249	22.4 (20.8–24.1)
HCT	315	2 950	intermediate	297.8	polycyclic	yes	Cl	–0.10	8.45	10 093	495	2.81 (2.56–3.10)
doxycycline	352	14 290	large	444.4	tetracyclic	no	no	–1.36	3.57	5 605	331	3.35 (2.98–3.76)
azapropazone	251	43 730	large	300.4	tricyclic	yes	no	–0.03	6.45	1 483	259	11.3 (9.84–13.0)
amiodarone	240	37 700	intermediate	681.8		no	2 I	8.81	8.73	9 126	220	1.26 (1.10–1.44)
demeclocycline	373	15 330	large	464.9	tetracyclic	no	Cl	1.74	8.41	480	176	30.4 (25.2–36.7)
nalidixic acid	329	11 520	large	232.3	polycyclic	yes	no	1.64	5.40	2 899	120	2.21 (1.84–2.66)
carprofen	300	21 200	large	273.7	tricyclic	yes	Cl	4.29	4.24	297	91	22.7 (17.7–29.1)
chlorpromazine	310	4 190	intermediate	318.9	tricyclic	yes	Cl	5.20	8.87	3 236	83	1.33 (1.07–1.66)
naproxen	331	1 840	intermediate	230.3	polycyclic	no	no	3.10	4.50	9 702	77	0.39 (0.31–0.49)

AUC = area under the curve; Mw = molecular weight; config. = configuration; est. = estimated; ROR = reporting odds ratio; CI = confidence interval; HCT = hydrochlorothiazide
a) λ_{max} measured in methanol.
b) log P of drug or base of chemical substance.
c) pKa of drug or conjugated acid.
d) Number of reports with suspected photosensitivity reactions.

also increased among drugs with a high molar absorption coefficient, a measure of a drug's ability to absorb light at a given wavelength. This finding may be of importance, since in the current guidelines for photosafety testing, no information on thresholds for this coefficient is included. The AUC reflects the amount of absorption of a chemical substance over a certain wavelength range. We expected that a larger AUC would implicate a higher risk of reporting photosensitivity suspected ADRs. Indeed, the results of this study support this hypothesis.

In order to assess the influence of frequently prescribed drugs, or drugs with a relatively early year of marketing, we analysed the association between the spectroscopic parameters in subsets of the database based on the number of reports per drug (< 5000; ≥ 5000). Although the magnitude of the ORs changed in the subsets based on the number of reports, the main findings did not differ in both subsets (data not shown). When we excluded the drugs with the highest total of reported cases (benoxaprofen and hydrochlorothiazide) from the analysis, similar results were found.

Photosensitisation is a process that is additional to the normal pathological response to sunlight, *i.e.* sunburn. Wavelengths of the solar spectrum from 290 till 320 nm are considered to be most capable of inducing erythema,[1,21] but UVA penetrates more deeply into the skin because its longer wavelengths are not scattered as much as the shorter UVB wavelengths.[22] The clinical relevance of UV light below 290 nm in relation with photosensitivity reactions is relatively small, since UVC radiation (100−290 nm) is filtered by the ozone layer before reaching the earth's surface.[2,3]

Each drug has a specific absorption spectrum which is determined by the configuration of the atoms in the molecule. This molecular configuration and certain atoms are considered to be essential in inducing photosensitivity reactions. In this study, we found that, in particular, the presence of aromatic halogen atoms is associated with the reporting of photosensitivity reactions. In most aromatic halogen-containing drugs (*e.g.* chlorpromazine, hydrochlorothiazide, diclofenac, and the fluoroquinolone antibiotics), the dissociation of the bond between halogen and the aromatic compound by UV light leads to the formation of free radicals which could damage DNA and other cellular targets.[1,23-25] Although a planar, tricyclic or polycyclic configuration with heteroatoms is thought to contribute to a drug's ability to act as a photosensitiser, our results do not support this hypothesis. Some well-known photosensitising drugs are either highly lipophilic (*e.g.* amiodarone, benoxaprofen) or highly hydrophilic (*e.g.* demeclocycline, hydrochlorothiazide). Both lipophilic and hydrophilic substances are thought to interact with cell membranes, and therefore induce phototoxic damage.[4,26] Our

results correspond to this information and show that both drugs with a highly lipophilic character (log P > 3) as well as hydrophilic drugs (log P < 1) seem to give rise to the reporting of phototoxic ADRs in comparison with drugs with a moderate lipophilic character. Since the relationship between log P and the reporting of photosensitivity reactions is ambiguous, assessment of a drug's lipophilicity only is insufficient in the evaluation of a potential photosensitive capacity.

There are several limitations to this study. Firstly, we used quantitative data of a spontaneous reporting system database without personal additional qualitative verifications or causality checks. Furthermore, a spontaneous reporting system as a mean of collecting data on suspected ADRs is known to represent only a fraction of the drug-related adverse events,[27,28] dependent of the type of ADR, physician behaviour and practice settings. Selective over- and underreporting of specific ADRs may lead to misinterpretations when comparing drug classes with respect to ADRs. ADRs of relatively new drugs, severe ADRs,[29] and ADRs which are not listed in the summary of product characteristics,[30] are reported more often than others. The potential of reporting bias, which is always a concern in this type of studies, is, however, unlikely with respect to the objective of the study, since it is not to be expected that reports in daily practice are made on basis of suspicion of the molecular parameters of drugs. Secondly, the study was restricted to nine well-known phototoxic drug classes and two drug substances. Each drug class consists of drugs which are not all equally phototoxic (*e.g.* proprionic acid derivatives versus other NSAIDs, and demeclocycline and doxycycline versus other tetracycline antibiotics). From the drug classes, we included only drugs for which the UV-properties were available, resulting in 143 unique chemical substances. *A priori*, we did not know whether this inclusion criterion would result in a representative selection. Thirdly, the available UV absorption spectra covered the range from 220 till 360 nm. Therefore, our data did not cover the whole UVA (320−400 nm) or visible light range (400-800 nm). However, fortunately absorption maxima above 360 nm were listed. For some drug classes, such as tetracycline and quinolone antibiotics, with an absorption spectrum which extend to the region of visible light, potential important UV data were missing. This could have confounded the association between wavelength and AUC and the reporting of photosensitivity ADRs. Other factors in photosensitisation reactions, such as the actual energy needed to activate a molecule, excited single state and triplet states, and the forming of free radicals,[1,31] were not included in our analysis. Fourthly, it is known that some drugs, such as chlorpromazine and amiodarone, are metabolised to photoreactive products,[4,32] whereas we used the spectroscopic and molecular parameters of the parent drug. Furthermore, all values of the parameters in this

study have been assessed *in vitro*. It is to be expected that the values can differ *in vivo*. Lastly, as a consequence of our case definition, it is unknown whether the results apply for both phototoxicity reactions and photoallergic reactions.

To our knowledge, this study is the first in which molecular characteristics which are considered important parameters are assessed in relationship with the occurrence of drug-induced photosensitivity reactions in daily practice. These molecular characteristics are unlikely to be known by health care providers. The spectroscopic parameters we used seem to be objective exposure variables. The analysis in the subset of the database points in a similar direction and the negative control outcomes which should not be related to the spectroscopic parameters (renal disorders, liver disorders) are indeed unrelated to these exposure variables. This indicates that our findings represent a true connection.

The occurrence of photosensitivity reactions is not only dependent on the molecular structure or spectroscopic parameters of a drug, but also on a multifaceted biological process. Because of factors such as light exposure, drug dosage, variations in bioavailability, patient characteristics such as metabolism and skin type, and the ability of UV light to penetrate the stratum corneum,[3,6] the reported incidence of phototoxic reactions varies for individual drugs. However, in international pharmacovigilance, spectroscopic and physiochemical characteristics of individual drugs which are considered to play a role in the occurrence of photosensitivity, are strongly correlated with the reported photosensitivity reactions to established phototoxic drug classes. Whether these findings can be extrapolated to all drug classes would require further research.

In addition to the industry standard *in vitro* 3T3 neutral red phototoxicity test,[33] systemic analysis and evaluation of spectroscopic and other molecular characteristics can be a useful tool for regulatory authorities and drug development because these characteristics may attribute to the detection and prediction of agents with a potential raised risk for photoreactivity.

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Appendix I Study drugs, spectroscopic and physicochemical parameters, and the number of reports in the WHO-ADR database

Drug	λ_{max} [a] (nm)	ε (M^{-1} cm^{-1})	Mw (Dalton)	config.	hetero-atoms	aromatic halogen atoms	log P [b] (est.)	pKa [c] (est.)	number of reports	cases [d]
acemetacin	319	6 570	415.8		no	no	4.13	NA	248	2
acetohexamide	246	13 750	324.4		no	no	2.44	3.97	50	0
amiodarone	240	37 700	681.8		no	2 I	8.81	8.73	9 126	220
amitriptyline	238	14 500	329.4	tricyclic	no	no	4.95	9.47	5 210	39
amitriptyloxide	238	14 560	277.4	tricyclic	no	no	NA	6.51	43	0
amoxapine	321	6 900	313.8	tricyclic	yes	no	3.38	5.91	1 168	4
azapropazone	251	43 730	300.4	tricyclic	yes	no	−0.03	6.45	1 483	259
bemetizide	316	3 170	401.9	polycyclic	yes	Cl	NA	8.40	81	1
bendroflumethiazide	324	4 170	421.4	polycyclic	yes	no	1.82	8.27	871	20
benoxaprofen	308	28 500	301.7		no	Cl	3.98	4.50	5 185	1 249
benzthiazide	283	11 160	432.0	polycyclic	yes	Cl	NA	4.60	8	0
bufexamac	284	1 340	223.3		no	no	1.98	7.77	3	0
bumadizone	237	35 100	326.4		no	no	3.10	3.67	14	0
bumetanide	335	4 230	364.4		no	no	2.57	3.30	394	7
butaperazine	278	21 500	409.6	tricyclic	yes	no	4.81	NA	1	0
butizide	316	3 080	353.8	polycyclic	yes	Cl	NA	8.54	26	1
carbutamide	268	20 080	271.4		no	no	0.94	4.66	26	2
carprofen	300	21 200	273.7	tricyclic	yes	Cl	4.29	4.24	297	91
chloroquine	330	13 830	515.9	polycyclic	no	Cl	4.50	9.86	2 228	23
chlorothiazide	280	10 650	295.7	polycyclic	yes	Cl	−0.23	5.68	263	16
chlorpromazine	310	4 190	318.9	tricyclic	yes	Cl	5.20	8.87	3 236	83
chlorpropamide	276	430	276.7		no	Cl	2.01	3.83	1 097	19
chlortalidone	283	1 460	338.8	polycyclic	yes	Cl	1.01	8.40	858	9
cinoxacin	352	12 800	262.2	tricyclic	yes	no	1.59	4.69	382	5

(Appendix I continued)

Drug	λ_{max} [a] (nm)	ε (M^{-1} cm^{-1})	Mw (Dalton)	config.	hetero-atoms	aromatic halogen atoms	log P [b] (est.)	pKa [c] (est.)	number of reports	cases [d]
ciprofloxacin	330	11 700	331.3	polycyclic	yes	F	0.00	5.68	3 436	39
clomipramine	251	8 360	314.9	tricyclic	yes	Cl	5.65	8.93	4 593	15
clopamide	286	1 250	345.9	polycyclic	no	Cl	1.87	9.13	40	1
cyclopenthiazide	316	3 040	379.9		yes	Cl	2.07	9.13	283	15
cyclothiazide	315	2 790	357.8		yes	Cl	1.74	8.41	48	2
demeclocycline	373	15 330	464.9	tetracyclic	no	Cl	−1.14	4.00	480	176
desipramine	250	8 950	266.4	tricyclic	yes	no	4.80	9.49	2 196	5
dibenzepin	225	27 870	295.4	tricyclic	yes	no	2.61	8.90	111	0
diclofenac	282	12 030	296.2		no	2 Cl	0.57	4.12	10 568	27
dosulepin	305	2 400	295.4	tricyclic	yes	no	4.51	NA	1 052	12
doxepin	296	3 410	279.4	tricyclic	yes	no	3.99	9.49	2 236	14
doxycycline	352	14 290	444.4	tetracyclic	no	no	−1.36	3.57	5 605	331
enoxacin	344	15 500	320.3		yes	F	−0.21	5.45	551	34
etacrynic acid	270	3 120	303.1		no	2 Cl	3.41	3.44	228	0
etozolin	285	21 100	284.4		yes	no	2.36	5.63	10	0
fenbufen	282	21 170	254.3	planar	no	no	3.18	4.39	5 666	48
fenoprofen	280	3 420	242.3		no	no	3.90	4.17	1 794	11
flufenamic acid	340	7 790	281.2		no	no	5.15	4.52	96	0
fluphenazine	310	3 680	437.5	tricyclic	yes	no	4.13	7.90	1 810	4
flurbiprofen	247	19 700	244.3		no	F	3.81	4.24	1 350	11
furosemide	337	5 260	330.7		yes	Cl	2.32	3.18	3 714	34
glibenclamide	300	3 080	494.0		yes	no	4.79	4.33	4 832	43
glibornuride	263	610	366.5		yes	no	NA	4.26	36	0
gliclazide	264	635	323.4		yes	no	2.12	4.04	424	13
glimepiride	227	30 800	490.6		yes	no	3.31	4.31	913	5
glipizide	276	10 630	445.5		yes	no	3.35	4.32	2 860	10

(Appendix I continued)

Drug	λ_{max} [a] (nm)	ε (M^{-1} cm^{-1})	Mw (Dalton)	config.	hetero-atoms	aromatic halogen atoms	log P [b] (est.)	pKa [c] (est.)	number of reports	cases [d]
gliquidone	313	2 600	527.6		yes	no	4.65	4.29	15	0
glisoxepide	229	22 400	449.5		yes	no	2.50	3.98	4	0
grepafloxacin	319	13 800	359.4	polycyclic	yes	F	2.12	5.75	391	25
hydrochlorothiazide	315	2 950	297.8	polycyclic	yes	Cl	−0.10	8.45	10 093	495
hydroflumethiazide	324	3 910	331.3		yes	no	0.22	8.34	153	6
hydroxychloroquine	344	14 600	335.9	polycyclic	yes	Cl	3.03	9.14	1 357	10
ibuprofen	272	230	206.3		no	no	3.79	4.53	6 930	7
imipramine	251	8 680	280.4	tricyclic	yes	no	5.01	9.00	211	0
indometacin	316	6 150	357.8	polycyclic	yes	Cl	4.23	4.66	187	0
indoprofen	281	14 500	281.3	polycyclic	yes	no	2.32	4.41	358	1
isoxicam	347 [e]	13 200	335.3	polycyclic	yes	no	1.97	NA	309	1
kebuzone	244	14 440	322.4		no	no	1.47	NA	173	0
ketoprofen	255	17 080	254.3		no	no	3.00	4.35	373	1
ketorolac	319	20 000	376.4	polycyclic	yes	no	2.32	NA	43	0
leflunomide	261	19 800	270.2		yes	no	2.66	12.51	2 600	8
levomepromazine	304	4 450	444.6	tricyclic	yes	no	5.06	NA	556	2
lofepramine	255	24 800	419.0	tricyclic	yes	Cl	7.26	7.55	2 069	17
lonazolac	274 [f]	43 270	312.8		yes	Cl	3.67	NA	276	0
maprotiline	272	1 540	277.4		no	no	4.52	10.30	2 277	11
mefenamic acid	346	6 470	241.3		no	no	NA	4.49	3 238	13
mefloquine	316	2 760	378.3	polycyclic	yes	no	3.81	9.66	14 795	13
mefruside	284	1 190	382.9		yes	Cl	20.6	8.85	84	1
melitracen	257	14 600	291.4	tricyclic	no	no	4.81	9.50	93	1
metacycline	349	15 040	442.4	tetracyclic	no	no	−1.37	3.57	202	0
methazolamide	291	12 360	236.3		yes	no	0.33	7.57	117	0
metozalone	343	3 250	365.8	polycyclic	yes	Cl	1.84	9.44	581	3

(Appendix I continued)

Drug	λ_{max} [a] (nm)	ε (M^{-1} cm^{-1})	Mw (Dalton)	config.	hetero-atoms	aromatic halogen atoms	log P [b] (est.)	pKa [c] (est.)	number of reports	cases [d]
minocycline	348	12 630	457.5	tetracyclic	no	no	−0.42	4.07	8 229	65
mofebutazone	271	6 600	232.3		yes	no	1.90	5.98	2	0
nabumetone	333	2 000	228.3	polycyclic	no	no	3.22	NA	3 669	35
nalidixic acid	329	11 520	232.3	polycyclic	yes	no	1.64	5.40	2 899	120
naproxen	331	1 840	230.3	polycyclic	no	no	3.10	4.50	9702	77
norfloxacin	3 156	12 200	319.3	polycyclic	yes	F	−0.31	5.68	23	0
nortriptyline	239	14 160	263.4	tricyclic	no	no	4.74	10.33	2 282	4
noxiptiline	250	13 550	294.4	tricyclic	no	no	4.29	7.73	8	0
ofloxacin	298	36 000	361.4	tricyclic	yes	F	−0.20	5.76	272	0
opipramol	256	33 170	363.5	tricyclic	yes	no	3.41	7.77	333	1
oxolinic acid	336 [f]	8 600	261.3	tricyclic	yes	no	1.70	5.90	91	0
oxyphenbutazone	241	15 570	324.4		yes	no	3.04	8.99	21	0
oxytetracycline	360	14 540	460.4	tetracyclic	no	no	NA	3.57	48	0
pefloxacin	317	12 900	333.4	polycyclic	yes	F	−0.09	5.68	1 352	52
perazine	307	4 520	339.5	tricyclic	yes	no	4.15	8.04	254	10
periciazine	315	2 810	365.5	tricyclic	yes	no	3.93	8.44	174	2
perphenazine	312	4 450	404.0	tricyclic	yes	Cl	3.82	7.87	868	4
phenylbutazone	243	14 860	308.4		yes	no	3.52	4.50	843	0
pipemidic acid	325	10 000	303.3	polycyclic	yes	no	−1.70	5.34	505	9
pipotiazine	313	3 600	475.7	tricyclic	yes	no	4.42	NA	80	1
piretanide	348	3 260	362.4		yes	no	2.52	3.27	126	1
pirlindole	275	7 960	226.3	tetracyclic	yes	no	3.22	8.78	4	0
piromidic acid	350	5 270	288.3	polycyclic	yes	no	1.49	534	5	0
piroxicam	325	18 430	331.4	polycyclic	yes	no	2.58	6.01	11	0
polythiazide	314	3 000	439.9	polycyclic	yes	Cl	1.00	9.49	110	1
prochlorperazine	311	4 420	374.0	tricyclic	yes	Cl	4.79	8.68	2 961	11

(Appendix I continued)

Drug	λ_{max} [a] (nm)	ε (M^{-1} cm^{-1})	Mw (Dalton)	config.	hetero-atoms	aromatic halogen atoms	log P [b] (est.)	pKa [c] (est.)	number of reports	cases [d]
proglumetacin	318	6 140	844.4	polycyclic	yes	Cl	NA	9.79	84	0
promazine	303	4 020	382.4	tricyclic	yes	no	4.56	8.88	125	2
proquazone	356	5 750	278.4	polycyclic	yes	no	3.02	3.78	81	0
protriptyline	293	13 960	263.4	tricyclic	no	no	4.89	10.16	345	45
quinethazone	345	2 350	289.7	polycyclic	yes	Cl	0.16	9.35	14	3
quinidine	332	5 290	324.4	polycyclic	yes	no	3.29	9.11	2 485	41
quinine	333	5 060	324.4	polycyclic	yes	no	3.29	9.11	1 757	16
rolitetracycline	269	18 410	527.6	tetracyclic	no	no	−0.66	3.86	258	0
rosoxacin	317	9 700	294.3	polycyclic	yes	no	2.22	5.50	11	0
sulfacarbamide	270	19 880	215.2		no	no	−1.00	4.71	27	0
sulfadiazine	268	21 300	250.3		yes	no	−0.34	5.78	443	7
sulfadimethoxine	272	21 690	310.3		yes	no	1.17	5.13	139	1
sulfadimidine	269	22 600	278.3		yes	no	0.76	5.78	143	1
sulfadoxine	273	23 090	310.3		yes	no	−0.24	4.52	8	0
sulfafurazole	271	19 250	267.3		yes	no	1.03	5.78	66	0
sulfamerazine	270	22 730	264.3		yes	no	0.21	5.95	31	0
sulfamethoxazole	269	19 880	253.3		yes	no	0.48	5.95	371	1
sulfamethoxypyridazine	268	21 720	280.3		yes	no	0.20	6.07	70	3
sulfametopyrazine	270	20 450	280.3		yes	no	0.64	6.26	176	5
sulfametoxydiazine	270	20 710	280.3		yes	no	−0.26	6.26	304	2
sulfametrole	269	22 040	286.3		yes	no	1.88	5.67	1	0
sulfamoxole	269	22 420	267.3		yes	no	1.03	5.67	62	0
sulfathiazole	288	21 320	255.3		yes	no	−0.40	6.10	14	0
sulforidazine	315	3 250	402.6	tricyclic	yes	no	4.23	8.83	4	0
sulindac	328	13 370	356.4	polycyclic	no	F	4.28	4.22	5 244	14
tenoxicam	3 636	22 000	337.4	polycyclic	yes	no	2.40	6.70	1 495	8

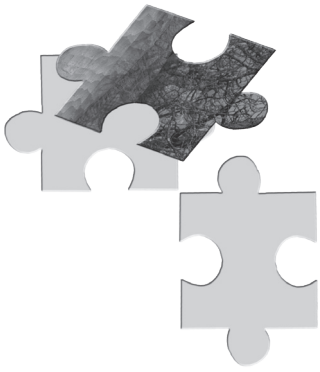
(Appendix I continued)

Drug	λ_{max} [a] (nm)	ε (M^{-1} cm^{-1})	Mw (Dalton)	config.	hetero-atoms	aromatic halogen atoms	log P [b] (est.)	pKa [c] (est.)	number of reports	cases [d]
tetracycline	363	16 300	444.4	tetracyclic	no	no	−1.33	4.00	440	19
thiopropazate	308	4 200	446.0	tricyclic	yes	Cl	4.82	7.15	12	0
thioproperazine	318	3 500	446.6	tricyclic	yes	no	2.95	NA	19	0
thioridazine	315	4 770	370.6	tricyclic	yes	no	6.45	8.90	2 531	12
tiaprofenic acid	303	14 840	260.3		yes	no	2.82	3.97	1 968	34
tolazamide	274	500	311.4		yes	no	2.23	4.04	374	2
torasemide	289	12 000	348.4		yes	no	2.40	7.10	458	5
trichlormethiazide	312	3 000	380.7	polycyclic	yes	Cl	0.23	6.28	67	3
trifluoperazine	311	3 400	480.4	tricyclic	yes	no	5.11	8.04	1 143	4
triflupromazine	308	2 680	352.4	tricyclic	yes	no	5.52	8.89	54	0
trimethoprim	288	6 730	290.3		yes	no	0.73	4.29	5 329	20
trimipramine	249	9 780	294.4	tricyclic	yes	no	5.43	8.94	782	4
trovafloxacin	342	20 300	416.4	polycyclic	yes	3 F	1.55	5.31	5 385	11
xipamide	318	3 550	354.8		no	Cl	2.29	6.48	251	3
zomepirac	324	13 600	291.7		yes	Cl	3.21	NA	8 302	4
Total									**210 457**	**4 178**

Mw = molecular weight; config. = configuration; est. = estimated; NA = not available
a) λ_{max} measured in methanol.
b) log P of drug or base of chemical substance.
c) pKa of drug or conjugated acid.
d) Number of reports with suspected photosensitivity reactions.
e) λ_{max} measured in 0.1 M sodiumhydroxid.
f) λ_{max} measured in 0.1 M hydrochloric acid.

2.3

Fluorinated drugs and the reporting of adverse drug reactions



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ABSTRACT

Background

Over the last decades, fluorine substituents have become an important drug component. Currently, 5−15% of all drugs (*e.g.* fluoroquinolones, type 2 statins and selective serotonin reuptake inhibitors) contain one or more fluorine atoms and the number of fluorinated drugs in the drug pipeline is rising. The incorporation of a fluorine atom has influence on the drug's pharmacokinetic and -dynamic properties.

Aim

To assess whether there is an association between fluorinated substituents in drug compounds and the reporting of suspected adverse drug reactions (ADRs).

Methods

All reports of suspected adverse drug reactions (ADRs) in the World Health Organization ADRs database up to 31 December 2008 were considered. We assessed whether six fluorinated study drugs (fluoxetine, dexamethasone, fluvastatin, celecoxib, mefloquine and ciprofloxacin) or their non-fluorinated counterparts (sertraline, prednisolone, pravastatin, valdecoxib, quinine and pipemidic acid) were involved as suspect agent to an ADR. For several ADRs of interest, proportional reporting ratios and 95% confidence intervals were calculated.

Results

There were 148 345 case reports in which a fluorinated or non-fluorinated study drug was identified as a suspected drug. 219 042 ADRs for a fluorinated study drug and 115 548 ADRs for a non-fluorinated study drug were reported. In these reports, psychiatric disorders were reported most frequently among the fluorinated drugs (15.2%) and non-fluorinated drugs (18.9%), followed by skin disorders. For skin and appendages disorders, the PRR was statistically significantly higher than 1 for three fluorinated/non-fluorinated pairs (fluoxetine/sertraline, dexamethasone/prednisolone, fluvastatin/pravastatin). The same applied to liver and biliary disorders. Some expected differences between fluorinated and non-fluorinated study drugs were observed: ciprofloxacin/pipemidic acid and musculo-skeletal disorders and mefloquine/quinine and psychiatric disorders.

Conclusion

Some ADRs were reported more to a fluorinated study drug than to its non-fluorinated counterpart, but it can not be concluded that there is a more general disproportional reporting of suspected ADRs for fluorinated drugs.

INTRODUCTION

In the last 50 years, fluorine substituents have become an important drug component and the role of fluorine in medicinal chemistry and drug design is well recognised.[1-4] Until 1957, when the antineoplastic agent 5-fluorouracil was synthesised, no fluorine-containing drug had been developed. Currently, approximately 5−15% of all drugs on the market contain one or more fluorine substituents[4] and 20−25% of drugs in the pharmaceutical pipeline are fluorinated.[3] Examples of well-known drug classes with fluorinated drugs are fluorine-containing glucocorticoids, fluoroquinolones, type 2 statins (atorvastatin, fluvastatin, cerivastatin, rosuvastatin) and selective serotonin reuptake inhibitors (SSRIs, except sertraline), showing a variety of both chemical structures and therapeutical indications.

The introduction of a fluorine atom changes the behaviour of a molecule by modifying its physicochemical and conformational properties (and hence absorption, distribution, metabolism and excretion). By modulating the pK_a and lipophilicity, fluorine substitution has been proven to enhance the potency/activity of a drug compared to the parent compound or prolonged the duration of action by blocking metabolic pathways.[3,5] In addition, blood brain barrier permeability increases due to changes in lipophilicity or amine pK_a.[6] Besides the influence on pharmacokinetic aspects, fluorination can also enhance hydrophobic interactions between a drug and its binding sites on receptors or enzymes.

Notwithstanding these improved drug properties, fluorine substitution can also result in unwanted consequences. Drugs with increased lipophilicity have potential toxic effect due to increased penetration through cell membranes and a prolonged half-life. Furthermore, defluorination give rise to the development of toxic compounds. In recent years, several fluorinated drugs have been withdrawn from the market due to severe adverse drug reactions (ADRs), such as trovafloxacin (2001, liver failure), cerivastatin (2001, rhabdomyolysis) and lumiracoxib (2007, hepatotoxicity), although a direct relationship between the presence of fluorine substituents and these ADRs was not established. Yet, controversy about the use of fluorinated drugs has emerged, particularly in lay media. Fluorine compounds used in dentistry and the application of water fluoridation have fuelled the debate about the possibility of fluoride poisoning, but although dental fluorosis is highly associated with the concentration of fluoride in drinking water, no scientific evidence for other adverse reactions exists.[7] Fluorinated drugs represent a different category of fluorine compounds (organofluorines) than the fluorine compounds used in dentistry and water fluoridation (inorganic fluorides). Although unlikely,

it is not impossible that organofluorines are metabolised into inorganic fluoride, and thereby increase the level of inorganic fluoride in human tissue.
In this light, we were interested to assess whether there is an association between fluorinated substituents in drug compounds and the reporting of suspected adverse drug reactions (ADRs), using the World Health Organization adverse drug reactions (WHO-ADR) database.

METHODS

Setting

The data were obtained from the International Drug Monitoring Program of the WHO. The WHO adverse reaction database Vigibase is maintained by the Uppsala Monitoring Centre and contains summaries of suspected spontaneous case reports originally submitted to national pharmacovigilance centres in more than 84 countries all over the world. At the end of December 2008, this database contained more than 3.8 million individual case reports of suspected adverse drug reactions (ADRs) regarding specific, but anonymous, patients. The reports contain administrative data, patient data, ADR data, medication data and additional information. The ADRs are classified according to the WHO Adverse Reaction Terminology (ART). The drugs mentioned in the reports are classified according to the Anatomical Therapeutic Chemical (ATC) classification system.[8] The information in these reports is not homogenous, at least with regard to origin, completeness of documentation or the likelihood that the suspected drugs caused the adverse events. Vigibase has been used for data mining studies as well as to investigate drug specific ADRs.[9-12]
From six drug classes, we selected a representative fluorinated drug and a non-fluorinated counterpart. Three drug classes were chosen on the basis of recent withdrawals from the market of a fluorinated drug. The other drug classes were considered important drug classes that are prescribed for various indications. Contrasts were made between the following six pairs of fluorinated and non-fluorinated study drugs: 1) fluoxetine versus sertraline; 2) dexamethasone versus prednisolone; 3) fluvastatin versus pravastatin; 4) celecoxib versus valdecoxib; 5) mefloquine versus quinine; and 6) ciprofloxacin versus pipemidic acid. Non-fluorinated counterparts were chosen on the basis of their similarity in molecular structure to the structure of fluorinated drug (2D) and the absence of any halogen atoms. An exception was sertraline, the only non-fluorinated SSRI, which actually has two chlorine atoms. All control drugs had the same ATC5-code as their

corresponding study drug, except pipemidic acid, as all drugs with ATC5-code J01MA (fluoroquinolones) have a fluorine substituent (Appendix I).

Study design

In the WHO-ADR database, ADRs are classified by means of the WHO-ART preferred term (PT), high level term (HLT) and categorised in system organ classes (SOC). For this study, all reports of suspected ADRs in the WHO-ADR database up to 31 December 2008 were considered. We identified all reports involving the six fluorinated study drugs or their non-fluorinated counterparts and assessed whether the fluorinated or non-fluorinated study drug was the suspected agent to an ADR. Only reports with only one suspected drug were taken into account. We included all ADRs mentioned in the reports in the analyses, except ADRs with adverse reaction record numbers 9998 (term under assessment for WHO-ART) and 9999 (term not accepted in WHO-ART). The outcomes of interest were ADRs relating to skin and appendages (SOC 0100), musculo-skeletal system disorders (SOC 0200), psychiatric disorders (SOC 0500), liver and biliary system disorders (SOC 0700) and tooth problems (adverse reaction record numbers 0333, 0334, 0335, 0336, 0704 and 1376). Musculo-skeletal system disorders and tooth problems were chosen on basis of the association with inorganic fluoride. The choice for liver and biliary system disorders and psychiatric disorders was based on the fact that, in general, the incorporation of a fluorine atom leads to altered drug metabolism and a more lipophilic molecule, effecting cell penetration and blood brain barrier passage.[1,5] Cutaneous ADRs were included because they represent a large group of mostly mild ADRs associated with several drug classes. In addition, we selected the top 50 ADRs (irrespective of SOC), classified on HLT of these six fluorinated/non-fluorinated drug pairs to assess whether the top ADRs could be linked to a mechanism depending on fluorination of the suspected drug.

Table 1 Proportional reporting ratios (PPR)[a]

	Number of reports with ADR of interest	Number of reports with all other ADRs	Total number of reports
Fluorinated study drug	a	b	a + b
Non–fluorinated study drug	c	d	c + d

ADR = adverse drug reaction
a) PRR: (a / (a+b)) / (c / (c+d)).

Data analysis

For each fluorinated study drug, we calculated the relative frequency of all outcomes of interest versus all other reported ADRs for that drug. This frequency – expressed as proportion − was divided by the corresponding frequency for its non-fluorinated counterpart. This proportional ADR reporting ratio (PRRs),[13] and 95% confidence interval (CI) was calculated for each outcome of interest (Table 1).

RESULTS

In total, there were 148 345 case reports in which one of the 16 fluorinated or one of the 16 non-fluorinated study drugs was identified as a suspected drug. These case reports comprised 219 042 ADRs for one of the six fluorinated study drugs and 115 548 ADRs for a non-fluorinated study drug. Psychiatric disorders were reported most frequently among both the fluorinated drugs (15.2%) and the non-fluorinated drugs (18.9%), followed by skin disorders (9.8% and 8.3%, respectively). The total number of reported ADRs per system organ class in fluorinated – non-fluorinated drug pairs is listed in Table 2. The ADRs reported for fluoxetine and celecoxib dominated the total number of reports for the fluorinated study drugs (74.5%), whereas the percentage of reported ADRs for sertraline among the non-fluorinated drugs was 65.6%.

Also, the PRRs for each selected SOC are presented in Table 2. For three fluorinated/non-fluorinated drug pairs, ADRs belonging to the SOC skin and appendages disorders were statistically significantly more reported for fluorinated study drugs than for their non-fluorinated counterparts (fluoxetine/sertraline, dexamethasone/prednisolone, fluvastatin/pravastatin). The same applied to liver and biliary disorders. Two out of three pairs (celecoxib/valdecoxib and ciprofloxacin/pipemidic acid) belong to drug classes, in which a drug had to be withdrawn from the market due to hepatotoxicity (lumiracoxib and trovafloxacin). Some expected differences between fluorinated and non-fluorinated study drugs were noticeable. The overall PRR of pair 6 (ciprofloxacin/pipemidic acid) for musculo-skeletal disorders was 2.21 (95% CI 1.63−3.01). Confined to tendon disorders, the PRR was 3.95 (95% CI 2.83−6.98). For psychiatric disorders, the PRR for pair 5 (mefloquine/quinine) was 10.0 (95% CI 8.63−11.6).

In Table 3, the top 50 PRRs (fluorinated study drugs versus non-fluorinated study drugs) categorised on high level terms are shown. Tendon disorders had the highest PRR (4.10; 95% CI 3.23−5.19) which is primarily driven by the known association between fluoroquinolones and tendon disorders, However,

Table 2 Number of reported ADRs for fluorinated study drugs and non-fluorinated counterparts, and the proportional reporting ratio

Pair		Number of reported ADRs									
		Total	Skin and appendages		Musculo-skeletal system disorders		Psychiatric disorders		Liver and biliary system disorders		Tooth problems	
			%	PPR (95% CI)	%	PPR (95% CI)	%	PPR (95% CI)	%	PPR (95% CI)	%	PPR (95% CI)
1	fluoxetine	99 361	9.6	1.57 (1.52–1.63)	2.2	1.28 (1.19–1.37)	25.5	0.97 (0.96–0.99)	2.2	1.34 (1.25–1.44)	0.1	0.67 (0.54–0.83)
	sertraline	75 771	6.1		1.7		25.9		1.6		0.2	
2	dexamethasone	930	24.0	2.19 (1.32–3.64)	1.9	0.18 (0.09–0.38)	6.6	0.84 (0.44–1.60)	1.9	0.83 (0.25–2.76)	0.1	NE
	prednisolone	128	10.9		15.6		7.8		2.3		0.0	
3	fluvastatin	17 775	10.5	1.52 (1.39–1.66)	15.7	1.04 (0.98–1.11)	6.2	1.03 (0.93–1.14)	7.6	0.48 (0.44–0.51)	0.1	0.49 (0.22–1.10)
	pravastatin	7 942	6.9		15.0		6.0		16.0		0.1	
4	celecoxib	63 848	11.0	0.81 (0.78–0.84)	3.3	0.90 (0.83–0.97)	5.2	0.89 (0.84–0.94)	1.8	1.87 (1.63–2.14)	0.1	1.25 (0.69–2.28)
	valdecoxib	25 426	13.5		3.7		5.9		1.0		0.1	
5	mefloquine	27 855	4.3	0.35 (0.32–0.38)	1.3	0.85 (0.67–1.09)	10.5	10.0 (8.63–11.6)	1.2	0.43 (0.36–0.52)	0.0	0.93 (0.11–7.95)
	quinine	5 174	12.3		1.5		3.3		2.9		0.0	
6	ciprofloxacin	9 273	18.6	0.52 (0.47–0.57)	8.9	2.21 (1.63–3.01)	10.3	2.49 (1.84–3.37)	5.9	1.92 (1.35–2.74)	0.1	NE
	pipemidic acid	1 107	32.8		3.7		3.8		2.8		0.0	

ADRs = adverse drug reactions; PRR = proportional reporting ratio; CI = confidence interval; NE = not estimable

Table 3 Top 50 proportional reporting ratios (fluorinated study drugs/non-fluorinated study drugs)

SOC	High level term	Number of reported ADRs		PRR (95% CI)
		fluorinated drugs [a]	non-fluorinated drugs [b]	
0200	tendon disorder	598	77	4.09 (3.23–5.19)
1210	iron metabolism disorder	22	4	2.90 (1.00–8.41)
0431	keratitis	48	9	2.81 (1.38–5.73)
0600	peptic ulcer	1 120	210	2.81 (2.43–3.26)
1810	anaphylactic reaction	473	104	2.40 (1.94–2.96)
0600	gastrointestinal haemorrhage	2 787	636	2.31 (2.12–2.52)
0100	urticaria	2 761	677	2.15 (1.98–2.34)
1500	hearing disorder congenital	4	1	2.11 (0.24–18.9)
0600	intestinal obstruction	77	20	2.03 (1.24–3.32)
0200	fasciitis	11	3	1.93 (0.54–6.93)
0800	alkalosis	18	5	1.90 (0.70–5.11)
0600	gastritis	338	100	1.78 (1.43–2.23)
0410	neuropathy	400	119	1.77 (1.44–2.17)
0600	intestinal ulceration	20	6	1.76 (0.71–4.38)
1040	vasculitis	343	104	1.74 (1.40–2.16)
0600	lip disorder	104	32	1.71 (1.15–2.55)
1210	anaemia aplastic	68	21	1.71 (1.05–2.78)
1300	oliguria	144	45	1.69 (1.21–2.36)
0100	rash	7 511	2 475	1.60 (1.53–1.67)
1210	myeloproliferative disorder	206	68	1.60 (1.21–2.10)
0600	stomatitis	645	217	1.57 (1.34–1.83)
1040	vein disorder	88	30	1.55 (1.02–2.34)
1020	pericardial effusion	35	12	1.54 (0.80–2.96)
0431	uveitis	26	9	1.52 (0.71–3.25)
1300	nephritis	126	44	1.51 (1.07–2.13)
1810	oedema	2 509	886	1.49 (1.38–1.61)
1220	granulocytopenia	408	147	1.46 (1.21–1.77)
1300	renal function abnormal	1 176	425	1.46 (1.31–1.63)
0431	cataract	203	74	1.45 (1.11–1.89)
1500	heart malformation	43	16	1.42 (0.80–2.51)
0432	vestibular disorder	64	24	1.41 (0.88–2.25)

(Table 3 continued)

SOC	High level term	Number of reported ADRs		PRR (95% CI)
		fluorinated drugs [a]	non-fluorinated drugs [b]	
0600	ileus	37	14	1.39 (0.75–2.58)
0300	lupus erythematosus syndrome	170	65	1.38 (1.04–1.83)
0100	skin discolouration	446	175	1.34 (1.13–1.60)
1040	haemorrhage intracranial	61	24	1.34 (0.84–2.15)
0100	photosensitivity reaction	536	211	1.34 (1.14–1.57)
0500	delusion	1 833	723	1.34 (1.23–1.46)
0500	schizophrenic reaction	652	258	1.33 (1.15–1.54)
0431	macular oedema	5	2	1.32 (0.26–6.79)
0300	arthritis rheumatoid	72	29	1.31 (0.85–2.01)
0800	hyperuricaemia	69	28	1.30 (0.84–2.02)
1210	anaemia macrocytic	19	8	1.25 (0.55–2.86)
1300	non–protein nitrogen increased	761	324	1.24 (1.09–1.41)
1500	death foetal	294	126	1.23 (1.00–1.52)
0200	arthropathy	1 252	537	1.23 (1.11–1.36)
0600	colitis	246	106	1.22 (0.97–1.54)
0410	dystonia	565	246	1.21 (1.04–1.41)
1040	ocular haemorrhage	136	60	1.19 (0.88–1.62)
1100	apnoea	88	39	1.19 (0.82–1.73)
0100	nail disorder	133	59	1.19 (0.87–1.61)

SOC = system organ class; ADRs = adverse drug reactions; PRR = proportional reporting ratios; CI = confidence interval

a) Fluoxetine, dexamethasone, fluvastatin, celecoxib, mefloquine, ciprofloxacin.

b) Sertraline, prednisolone, pravastatin, valdecoxib, quinine, pipemidic acid.

even without the reported ADRs for ciprofloxacin and pipemidic acid, the PRR was statistically significantly higher than 1.00 (1.35; 95% CI 1.01–1.79). Pair 4 (celecoxib/valdecoxib) was accountable for the high PRR for HLTs belonging to SOC 0600 (peptic ulcer, GI haemorrhage, intestinal obstruction and gastritis). Exclusion of the number of reports for these two drugs resulted in lower PRRs which were not statistically significant.

DISCUSSION

In this study, known differences between fluorinated study drugs and their non-fluorinated counterparts appeared from the analyses. We found a statistically significant PRR for pair 6 (ciprofloxacin/pipemidic acid) for musculo-skeletal systems disorders (tendon disorders)14,15 while the PRR for pair 5 (mefloquine/quinine) for psychiatric disorders [16,17] was statistically significantly increased as well. Pair 6 (ciprofloxacin/pipemidic acid) had also a high PRR for psychiatric disorders. We could not conclude that there was a more disproportional reporting of suspected ADRs for fluorinated drugs in general. However, we did find some interesting discrepancies in the reporting of ADRs for fluorinated and non-fluorinated study drugs, which are worthwhile to evaluate more profoundly (Appendix II).

There is a discrepancy in perception of the role of fluorine in pharmaceuticals. Medicinal chemists consider the advantages of incorporating fluorine substituents into new compounds; on the other side, there are many action groups that express their concern about fluoride in drinking water, toothpaste and pharmaceuticals. Although the association between high concentrations of fluoride in drinking water and dental fluorosis is recognised, the association between organic fluorine in pharmaceuticals and the occurrence of ADRs is uncertain. Therefore, we used the WHO-ADR database to evaluate whether fluorine substituents were associated with the reporting of suspected ADRs. We selected six fluorinated drugs and six non-fluorinated counterparts from the same ATC-group and calculated proportional reporting ratios for each pair. By evaluating the fluorinated drug with its non-fluorinated counterpart, we tried to exclude the influence of the underlying disease since in general the indication for prescribing the drug (with or without fluorine substituent) was the same.

Research to the role of organic fluorine in drugs has to be continued, especially since in the last 20 years the use of fluorine in medicinal chemistry and drug design has become commonplace. The last five years, the proportion of fluorinated drugs in the total market has increased noticeably.[4] New methodologies have become available making the incorporation of fluorine in target molecules much easier. Because of the favourable physicochemical properties, it seems likely that many new fluorinated compounds will be synthesised and eventually find their way on the pharmaceutical market.[18-20] The large – and possible growing – percentage of fluorinated drugs of the total number of drugs on the market asks for continuous evaluation of possible ADRs. A possibility will be to assess whether among the suspected ADRs reported to the WHO, the percentage of reports in which fluorinated drugs are involved is larger than the percentage of fluorinated

drugs on the market. Whether the reporting suspected ADRs is dependent of the fluorine substituent (aliphatic, aromatic) is a potential research question.
In conclusion, some ADRs were reported more to a fluorinated study drug than to its non-fluorinated counterpart. Although several fluorine-containing drugs are recently withdrawn from the market, there is at the moment no reason to presume a general association between fluorinated pharmaceuticals and the reporting of suspected ADRs. Since fluorine is likely to be of growing importance as a substituent in pharmaceuticals, regular assessment whether there is a disproportional reporting of ADRs for fluorinated drugs might be required.

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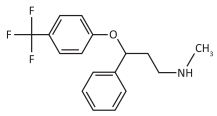
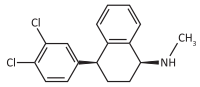
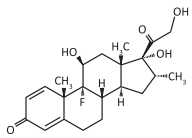
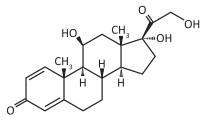
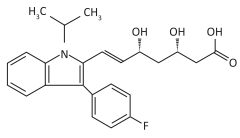
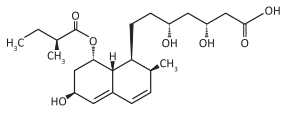
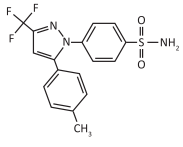
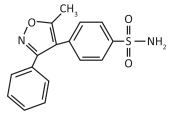
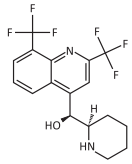
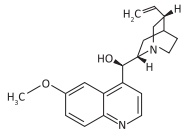
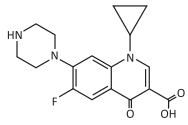
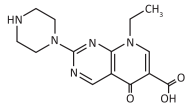
Appendix I Characteristics of the study drugs and the non-fluorinated control drugs

Pair		ATC code	Molecular structure	Mw	log P est.	pK_a est.	Year of marketing
1	fluoxetine	N06AB03		303.3	4.65	9.52	1986
	sertraline	N06AB06		306.2	5.29	8.74	1990
2	dexamethasone	H02AB02		392.5	1.72	NA	1958
	prednisolone	H02AB06		360.4	1.40	NA	1955
3	fluvastatin	C10AA04		411.5	4.85	4.07	1994
	pravastatin	C10AA03		424.5	3.10	4.23	1990
4	celecoxib	M01AH01		417.8	3.47	9.43	1998
	valdecoxib	M01AH03		314.4	2.67	10.31	2002

(Appendix I continued)

Pair		ATC code	Molecular structure	Mw	log P est.	pK_a est.	Year of marketing
5	mefloquine	P01BC02		378.3	3.85	9.65	1986
	quinine	P01BC01		324.4	3.29	9.09	?
6	ciprofloxacin	J01MA02		331.3	0.00	6.09	1987
	pipemidic acid	J01MB04		303.3	-1.70	5.20	1975

ATC = Anatomical Therapeutic Chemical; Mw = molecular weight; est. = estimated

Appendix II Proportional reporting ratios (fluorinated study drug/ non-fluorinated study drug) per system organ class

System Organ Class		Proportional reporting ratios (95% CI)					
		pair 1 [a]	pair 2 [b]	pair 3 [c]	pair 4 [d]	pair 5 [e]	pair 6 [f]
0100	Skin and Appendages disorders	1.57 (1.52–1.63)	2.19 (1.32–3.64)	1.52 (1.39–1.66)	0.81 (0.78–0.84)	0.35 (0.32–0.38)	0.52 (0.47–0.57)
0200	Musculo-skeletal system disorders	1.28 (1.19–1.37)	0.18 (0.09–0.38)	1.04 (0.98–1.11)	0.90 (0.83–0.97)	0.85 (0.67–1.09)	2.21 (1.63–3.01)
0300	Collagen disorders	1.97 (1.54–2.52)	NA	1.25 (0.84–1.85)	1.00 (0.70–1.44)	0.45 (0.19–1.09)	0.49 (0.11–2.28)
0410	Central and peripheral nervous system disorders	0.89 (0.87–0.91)	0.49 (0.30–0.82)	1.26 (1.16–1.38)	0.97 (0.92–1.02)	3.01 (2.74–3.31)	1.17 (0.96–1.43)
0420	Autonomic nervous system disorders	0.76 (0.05–12.2)		NA	1.59 (0.18–14.3)		
0431	Vision disorders	0.95 (0.88–1.02)	1.65 (0.22–12.6)	1.63 (1.33–1.99)	1.00 (0.89–1.14)	1.22 (0.98–1.51)	2.41 (1.19–4.89)
0432	Hearing and vestibular disorders	0.78 (0.69–0.88)	NE	2.04 (1.15–3.64)	1.70 (1.30–2.21)	0.11 (0.10–0.13)	3.91 (1.24–12.3)
0433	Special senses other, disorders	1.25 (1.01–1.54)		1.42 (0.93–2.17)	2.02 (1.31–3.14)	0.95 (0.45–2.03)	6.69 (1.66–27.0)
0500	Psychiatic disorders	0.97 (0.96–0.99)	0.84 (0.44–1.60)	1.03 (0.93–1.14)	0.89 (0.84–0.94)	10.0 (8.63–11.6)	2.49 (1.84–3.37)
0600	Gastro-intestinal system disorders	0.73 (0.71–0.76)	1.41 (0.82–2.41)	0.69 (0.64–0.73)	1.83 (1.76–1.91)	1.28 (1.19–1.39)	0.63 (0.55–0.73)
0700	Liver and biliary system disorders	1.34 (1.25–1.44)	0.83 (0.25–2.76)	0.48 (0.44–0.51)	1.87 (1.63–2.14)	0.43 (0.36–0.52)	1.92 (1.35–2.74)
0800	Metabolic and nutritional disorders	0.98 (0.94–1.03)	1.72 (0.41–7.18)	1.06 (0.96–1.16)	1.06 (0.97–1.15)	0.34 (0.27–0.44)	2.59 (1.22–5.49)
0900	Endocrine disorders	1.12 (0.89–1.17)	0.96 (0.12–7.77)	1.53 (0.99–2.37)	0.75 (0.53–1.07)	1.18 (0.35–3.97)	NE

(Appendix II continued)

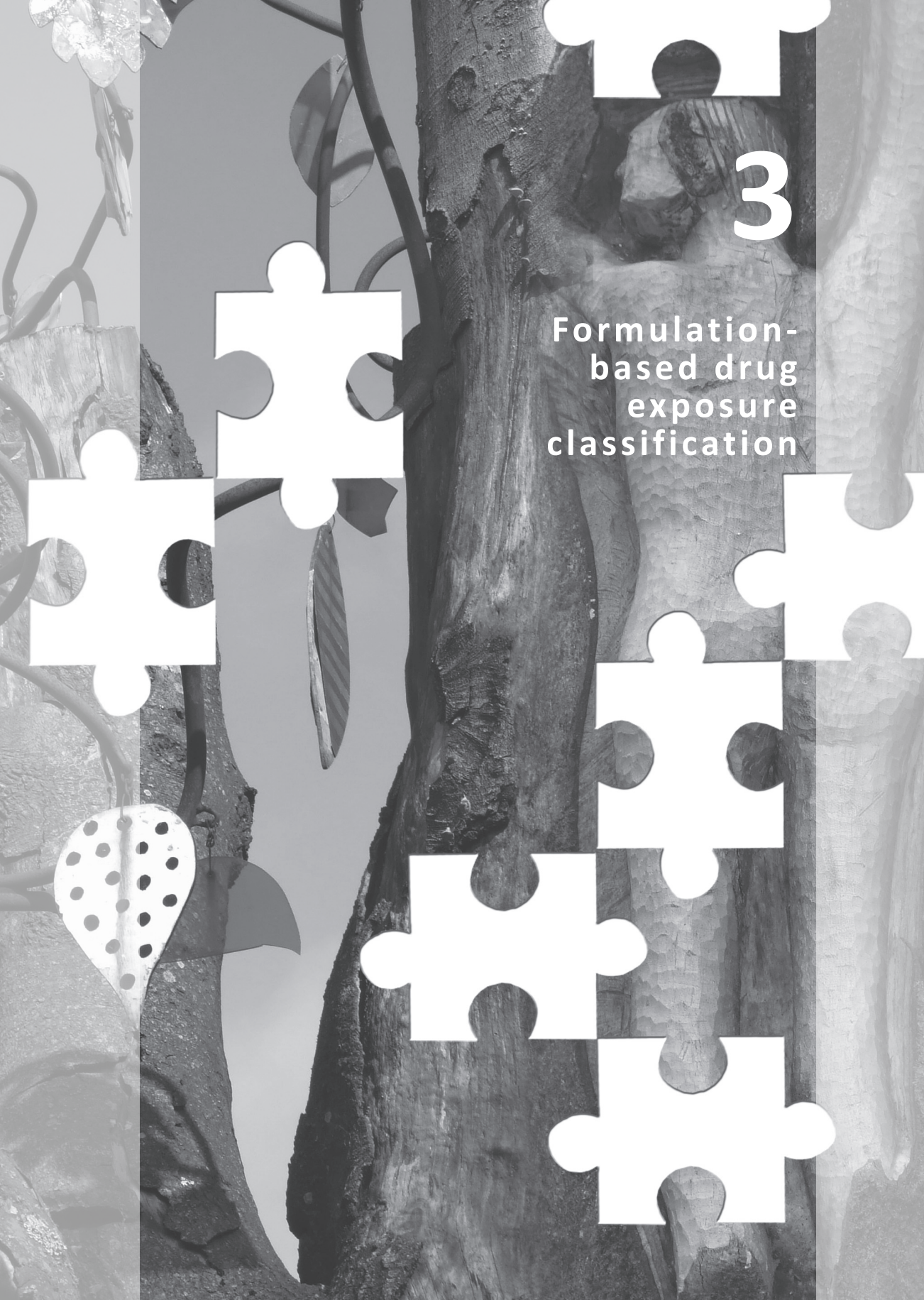
System Organ Class		Proportional reporting ratios (95% CI)					
		pair 1 [a]	pair 2 [b]	pair 3 [c]	pair 4 [d]	pair 5 [e]	pair 6 [f]
1010	Cardiovasculair disorders	0.95 (0.88–1.01)	1.01 (0.31–3.32)	1.12 (0.84–1.50)	0.75 (0.71–0.80)	0.46 (0.36–0.59)	1.02 (0.53–1.95)
1020	Myo-, endo-, percardial & valve disorders	1.02 (0.89–1.17)		1.17 (0.84–1.61)	0.68 (0.64–0.72)	0.85 (0.41–1.74)	NE
1030	Heart arte and rhythm disorders	1.10 (1.04–1.18)	NE	2.02 (1.51–2.69)	0.87 (0.79–0.95)	1.19 (1.00–1.41)	3.87 (1.43–10.4)
1040	Vascular (extracardiac) disorders	1.05 (0.95–1.16)	1.58 (0.58–4.32)	0.85 (0.65–1.11)	0.50 (0.47–0.53)	0.36 (0.25–0.52)	1.06 (0.60–1.87)
1100	Respiratory system disorders	1.13 (1.07–1.20)	0.52 (0.28–0.95)	1.47 (1.23–1.75)	1.03 (0.96–1.10)	0.34 (0.29–0.41)	1.13 (0.77–1.67)
1210	Red blood cell disorders	1.32 (1.13–1.53)	0.22 (0.07–0.66)	1.44 (0.99–2.11)	2.04 (1.72–2.44)	0.08 (0.05–0.10)	4.88 (1.20–19.8)
1220	White cell and RES disorders	1.88 (1.65–2.14)	2.89 (0.39–21.3)	1.62 (1.12–2.35)	1.85 (1.46–2.33)	0.13 (0.10–0.17)	0.62 (0.41–0.93)
1230	Platelet, bleeding & clotting disorders	1.54 (1.42–1.67)	0.41 (0.11–1.51)	0.72 (0.59–0.89)	0.93 (0.86–1.01)	0.03 (0.02–0.03)	0.60 (0.41–0.87)
1300	Urinary system disorders	1.07 (1.00–1.15)	4.13 (0.57–30.0)	1.76 (1.51–2.05)	1.20 (1.12–1.30)	0.19 (0.15–0.24)	1.75 (1.20–2.56)
1410	Reproductive disorders, male	0.86 (0.79–0.93)	NE	1.40 (0.77–2.56)	0.76 (0.49–1.18)	1.02 (0.23–4.61)	0.38 (0.08–1.85)
1420	Reporductive disorders, female	1.73 (1.62–1.84)	NE	2.00 (1.18–3.38)	1.23 (0.97–1.57)	1.11 (0.63–1.96)	2.96 (0.40–21.8)
1500	Foetal disorders	1.17 (1.03–1.32)	NE	9.38 (1.26–69.7)	0.92 (0.54–1.58)	4.21 (1.86–9.53)	0.28 (0.14–0.56)
1600	Neonatal and infancy disorders	1.43 (1.20–1.72)	0.14 (0.01–2.19)	NE	NE	1.39 (0.49–3.95)	0.11 (0.01–1.75)
1700	Neoplasms	0.97 (0.83–1.14)	NE	1.22 (0.80–1.86)	1.00 (0.82–1.23)	1.21 (0.27–5.35)	NE

(Appendix II continued)

System Organ Class		Proportional reporting ratios (95% CI)					
		pair 1 [a]	pair 2 [b]	pair 3 [c]	pair 4 [d]	pair 5 [e]	pair 6 [f]
1810	Body as a whole-general disorders	0.90 (0.88–0.93)	1.01 (0.63–1.62)	1.17 (1.09–1.26)	1.11 (1.08–1.15)	0.65 (0.59–0.71)	1.21 (0.97–1.50)
1820	Application site disorders	1.64 (1.04–2.59)	NE	1.79 (0.60–5.34)	0.65 (0.49–0.87)	0.11 (0.03–0.47)	NE
1830	Resistance mechanisms disorders	0.80 (0.68–0.95)	0.45 (0.15–1.35)	2.06 (1.13–3.75)	0.65 (0.56–0.77)	0.52 (0.28–0.95)	5.37 (0.74–38.9)
2000	Secondary terms-events	0.52 (0.48–0.57)	0.28 (0.17–1.09)	1.45 (1.02–2.07)	0.79 (0.72–0.87)	0.32 (0.23–0.43)	NE
2100	Poison specific term	0.44 (0.13–1.49)			NE	0.04 (0.01–0.13)	

CI = confidence interval; NA = not applicable (no cases); NE = not estimable (no controls); empty cells = no cases, no controls; RES = reticuloendothelial system

a) pair 1 = fluoxetine/sertraline.
b) pair 2 = dexamethasone/prednisolone.
c) pair 3 = fluvastatin/pravastatin.
d) pair 4 = celecoxib/valdecoxib.
e) pair 5 = mefloquine/quinine.
f) pair 6 = ciprofloxacin/pipemidic acid.

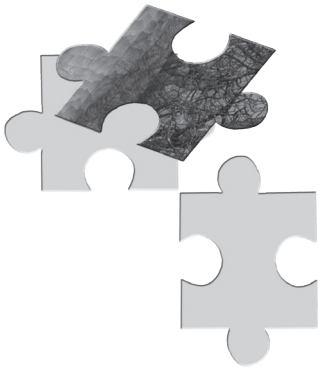


3

Formulation-based drug exposure classification

3.1

Changes in circulating red blood cells: drug-induced or excipient-induced?



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ABSTRACT

Background

The taxanes, paclitaxel and docetaxel are highly lipophilic agents and are therefore formulated with non-ionic surfactants Cremophor® EL (CrEL, polyoxyethyleneglycerol tricinoleate 35) and polysorbate 80 (Tween® 80, polyoxyethylene-sorbitan-20-monooleate). Both paclitaxel and docetaxel and the solvents can induce phosphatidylserine (PS) exposure on the cell surface of erythrocytes, which are consequently rapidly eliminated by macrophages from circulating blood. Cisplatin is also a PS exposure-inducing cytostatic agent, but is available in formulations without CrEL/polysorbate.

Aim

To assess whether the use of lipophilic solvents in cytostatic regimens affects circulating red blood cells.

Methods

Data were obtained form the Utrecht Patient Orientated Database UPOD). Within a cohort of adult oncology patients in the period 2005–2009, patients with a first chemotherapy course with paclitaxel, docetaxel, cisplatin and/or carboplatin were identified. The cytostatic cycles were classified in to the group with lipophilic solvents (taxane group) or without lipophilic solvents (platinum group). For each group, the number of erythrocytes (RBC), haemoglobin concentration (HGB), haematocrit (HT), mean corpuscular volume (MCV), number of reticulocytes (RETC), red cell distribution width (RDW), number of platelets (PLT), and mean platelet volume (MPV) were determined at baseline (T0) and during the treatment period (T1). The difference between T1 and T0 (delta) was calculated for each blood cell parameter.

Results

The study population comprised 320 patients (35.3% female, mean age of 51 years at the start of chemotherapy course) of whom 24 were treated with a taxane and 256 with a platinum compound. The deltas in RBC, HGB, HT and MPV were statistically significantly larger for the taxane group (with lipophilic solvents) and platinum group (without lipophilic solvents). There was no difference in deltas in the group of taxanes in combination with platinum compounds compared to taxanes in combinations with other cytostatic agents.

Conclusion

The results in this study suggest that there are differences between the two groups with regards to changes in red blood cell parameters. However, whether these effects can be attributed to the pharmacological compounds or the solvents has to be elucidated. The findings in this study warrant further research to unravel underlying mechanisms.

INTRODUCTION

The taxanes paclitaxel and docetaxel are anticancer drugs that are widely used in the treatment of various malignancies, such as breast cancer, ovarian cancer, non-small-cell lung cancer and prostate cancer. Just as all myelosuppressive chemotherapy, cytostatic treatment with paclitaxel and docetaxel is known to cause many adverse effects, among others thrombocytopenia and anaemia. The incidence and severity of chemotherapy-induced anaemia is dependent on the type, schedule, intensity and previous chemotherapy. Frequent symptoms of anaemia are fatigue, dyspnoea and depression, and as a result anaemia decreases functional capacity and quality of life for cancer patients.[1,2] Anaemia may result from either decreased formation of new erythrocytes (*i.e.* bone marrow suppression) or from accelerated clearance of circulating erythrocytes (eryptosis).[3] Eryptosis is characterised by cell shrinkage, membrane blebbing and phosphatidylserine (PS) exposure at the cell surface. Erythrocytes that express PS are readily recognised and phagocytosed by macrophages and thus eliminated from circulating blood. Eryptosis can be triggered by several haemoglobinopathies and sepsis or can be induced by certain drugs.[4] Examples of PS exposure inducing drugs are amiodarone, azathioprine, chlorpromazine, cisplatin and paclitaxel.[4,5]

Taxanes are highly hydrophobic agents and the development of these drugs was delayed because of problems in drug formulation.[6,7] Eventually, formulations with non-ionic surfactants Cremophor® EL (CrEL, polyoxyethyleneglycerol tricinoleate 35) and polysorbate 80 (Tween® 80, polyoxyethylene-sorbitan-20-monooleate) were used to stabilise emulsions of these hydrophobic compounds in aqueous solutions.[6] Both CrEL and polysorbate are not inert excipients; they can alter the pharmacokinetics and pharmacodynamics of the active ingredient.[6,8,9] Biological effects related to these solvents are acute hypersensitivity reactions and peripheral neuropathies. In addition, CrEL has been associated with hyperlipidaemia, abnormal lipoprotein patterns and aggregation of erythrocytes.[10]

Treatment with PS exposure-inducing agents (drugs, as well as solvents) may have an effect on circulating red blood cells. Changes could occur in red blood cell indices, such as the number of erythrocytes (red blood cells, RBC), mean corpuscular volume (MCV), and haemoglobin concentration, contributing to the anaemia and overall quality of life in patients treated with these compounds. Therefore, we conducted a preliminarily study within a cohort of oncology patients treated with paclitaxel, docetaxel, cisplatin and/or carboplatin to assess whether the use of lipophilic solvents in cytostatic regimens affects circulating red blood cells.

METHODS

Setting

For this study, data were obtained from the Utrecht Patient Oriented Database (UPOD). The structure and content of UPOD have been described in detail elsewhere.[11] In brief, UPOD is a database for (pharmaco)epidemiological research, encompassing automated data collected during clinical care on patient demographics, hospital discharge diagnoses, medication exposure, medical procedures and laboratory test for all patients treated at the University Medical Center Utrecht (UMC Utrecht), the Netherlands. In addition to these data, UPOD comprises a database with haematological data obtained with Cell-Dyn 4000 and Cell-Dyn Sapphire haematology analysers (Abbott Diagnostics, Santa Clara, CA, USA) used in routine cell analysis at the UMC Utrecht since January 2005. For each analysed blood sample, all possible blood cell parameters [12] are collected within the database, providing complete and validated automated haematological data, including absolute cell counts, cell volume indices and morphological data.[11]

Study population

The study population consisted of adult oncology patients (18 years and older) who received a first course of non-experimental chemotherapy comprising cytostatic agents paclitaxel, docetaxel, cisplatin and/or carboplatin at the in- or outpatient clinic of the UMC Utrecht in the period from 1 January 2005 to 31 December 2009 (based on information from the electronic chemotherapy order entry system). The choice for these cytostatic agents was based on a number of considerations. Since no other drug formulations of paclitaxel and docetaxel other than the ones containing CrEL and polysorbate 80 are on the market in the Netherlands, discrimination between drug-induced or excipient-induced PS expression is not possible. However, cisplatin, which is also a PS expression inducing cytostatic agent, is on the market in CrEL-free/polysorbate-free formulations. This drug is used as monotherapy or in combination with other cytostatic agents, such as paclitaxel or docetaxel. Carboplatin is also on the market in CrEL-free/polysorbate-free formulations and is considered not to induce PS expression.[3] For each patient, periods of consecutive exposure to a specific chemotherapy regiment (courses) were determined. A course was constructed from consecutive automated medications orders for cycles of chemotherapy (*i.e.* one round of chemotherapy). Cycles of chemotherapy were considered consecutive when the gap between the cycles was seven days or less. The start date of the first cycle was considered the start date of the course. The theoretical end date of the last cycle, calculated as the start date of the last cycle plus the standard length of the cycle was considered

as the end date of the course. The duration of the course was determined by subtracting the end date and the start date of the course.
Patients without baseline blood cell counts within 30 days prior to the start date of a chemotherapy course or patients having a chemotherapy course that ended within a 30-day period prior to the first relevant course (*i.e.* a course with paclitaxel, docetaxel, cisplatin and/or carboplatin) were excluded. In addition, we did not include patients without follow-up red blood cell counts during a cycle of chemotherapy or when the date of follow-up measurements was within two days after the start of the chemotherapy cycle. Furthermore, patients were excluded when they received a blood transfusion within a 120-day period prior to baseline blood cell counts or had a blood transfusion between baseline blood cell counts and the first follow-up blood cell counts.

Exposure assessment

For each cycle, the individual cytostatic agents that were part of the regimen were identified. We only took into account the first cycles in which paclitaxel, docetaxel, cisplatin and/or carboplatin were administered (either as monotherapy or in combination). We classified the cytostatic cycles into two groups according to the presence/absence of lipophilic solvents (Table 1). Furthermore, we divided the taxane group in combinations with and without platinum compounds.

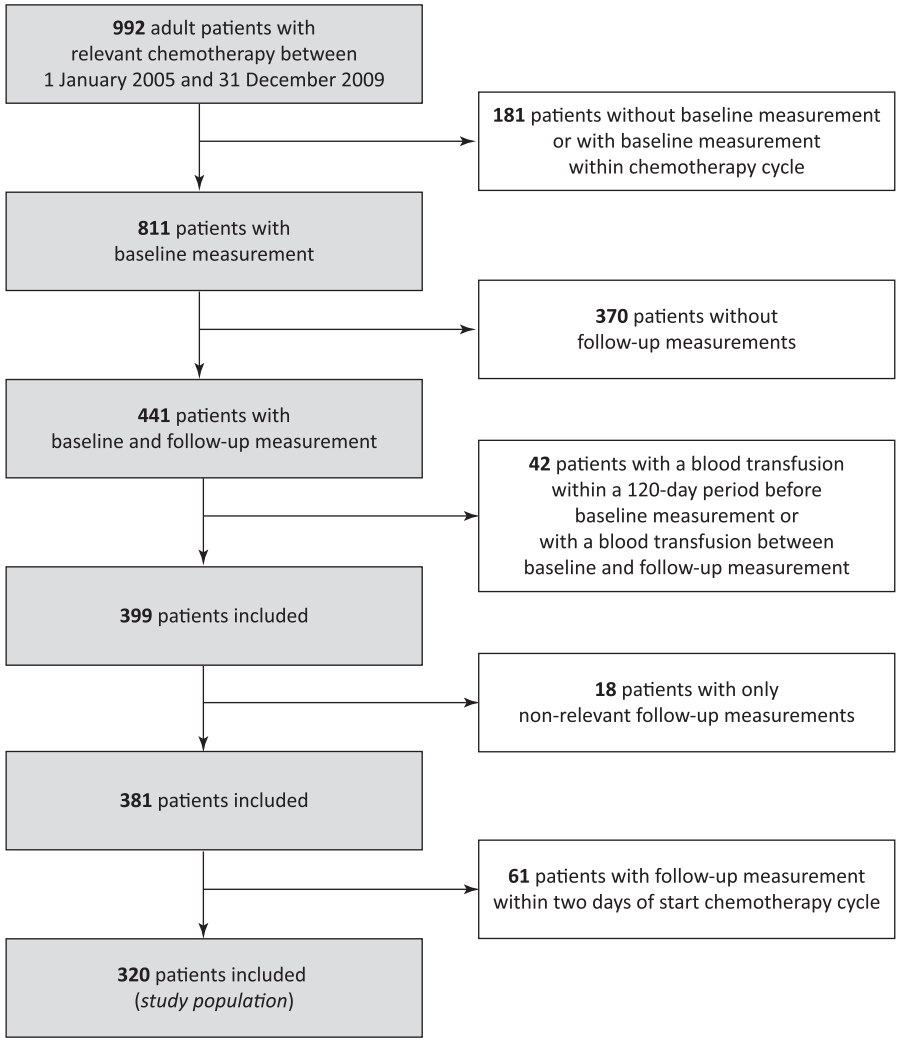
Table 1 Classification of cytostatic cycles

Cytostatic regimen		lipophilic solvents	
		yes	no
taxane group	paclitaxel, in combination docetaxel, mono or in combination	x	
platinum group	cisplatin, mono or in combination (excl. paclitaxel or docetaxel) carboplatin, mono or in combination (excl. paclitaxel or docetaxel)		x

Red blood cell indices

For each patient, blood cell counts that were performed at baseline (T0) and during the treatment period (T1) were identified. We analysed blood samples for the number of erythrocytes (RBC), mean corpuscular volume (MCV) and the number of reticulocytes (RECT). Furthermore, mean haemoglobin (HGB), haematocrit (HT), mean corpuscular haemoglobin (MCH = HGB/RBC) and mean red cell distribution

Figure 1 Schematic view of included patients



width (RDW) were assessed. Red blood cell fragments or vesicles could be falsely identified as thrombocytes. To investigate the magnitude of this phenomenon, the number of thrombocytes (PLT) and their mean volume (MPV) was determined.

Data analysis

For each patient, mean blood cell counts were assessed at baseline and during the first cycle of relevant cytostatic treatment. If more than one blood sample analysis during treatment was available, the first one was taken into account. For each relevant blood cell parameter, the difference between the value during treatment (T1) and at baseline (T0) (delta, Δ) was calculated. Differences in deltas between the taxane group (in lipophilic vehicle) and the platinum group (without lipophilic vehicle) were tested for statistical significance using students *t*-tests considering a *p*-value smaller than 0.05 statistically significant. The underlying assumption that the data follow a normal distribution was tested. Data analysis was performed using SPSS 16.0 (SPSS Inc. Chicago, Illinois, USA).

RESULTS

We initially identified 992 adult patients with a first course of non-experimental chemotherapy of paclitaxel, docetaxel, cisplatin and/or carboplatin at the UMC Utrecht in the period 2005-2009 (Figure 1). For 181 patients no baseline counts were available or these were obtained during a prior chemotherapy cycle not containing any of the cytostatics of interest. For 370 patients no follow-up measurements were available, while 42 patients received a blood transfusion within a 120-day period before baseline measurement or between baseline and follow-up measurement. For 15 patients no follow-up measurements for the relevant indices were available and 64 patients had a follow-up measurement within two days of the start of the chemotherapy cycle. This led to the inclusion of 320 patients in the study. The patient characteristics and cytostatic regimens are presented in Table 2. The study population comprised 113 (35.3 %) female patients and, on average, patients were 51 years old at the start of the course. Nineteen patients were treated with paclitaxel in combination with cisplatin, carboplatin or other agents; docetaxel was administered to five patients. A total of 24 patients were treated with a cytostatic regimen in which CrEL or polysorbate was used as a solvent. Cisplatin (monotherapy or in combination) was the cytostatic treatment for 256 (79.0 %) patients and carboplatin (monotherapy or in combination) for 40 patients.

In Table 3, the mean blood cell counts at baseline (T0) and at follow-up (T1) are listed, stratified according to the presence of lipophilic solvents. All values at T0 and T1 were within reference values, except the number of reticulocytes at T1 (reference value 25−120×10^9/L) in the platinum group. The average number of days

Table 2 Characteristics of the study population

Patient demographics		**n=320 (100%)**
Female		113 (35.3%)
Mean age at start of the first chemotherapy treatment; years (SD)		
18–39 years		50.7 (14.9)
40–59 years		92 (28.8)
40–59 years		124 (38.8)
> 60 years		104 (32.5)
Cytostatic regimen		
Lipophilic solvents (taxane group)	paclitaxel	
	– combination with cisplatin	3 (0.9%)
	– combination with carboplatin	12 (3.8%)
	– combination with other agents	4 (1.2%)
	docetaxel	
	– monotherapy	3 (0.9%)
	– combination with cisplatin	0 (0.0%)
	– combination with carboplatin	1 (0.3%)
	– combination with other agents	1 (0.3%)
No lipophilic solvents (platinum group)	cisplatin	
	– monotherapy	56 (17.5%)
	– combination with other agents (excl. paclitaxel or docetaxel)	200 (62.5%)
	carboplatin	
	– monotherapy	1 (0.3%)
	– combination with other agents (excl. paclitaxel or docetaxel)	39 (12.2%)

SD = standard deviation

between measurements at T0 and T1 was 7.1 days (SD 3.5) and ranged from 2 to 20 days. With regard to these aspects, no statistically significant differences were found between the taxane and platinum group. There was a statistically significant difference between deltas for the number of erythrocytes (RBC) (-0.20 *vs.* -0.04; $p = 0.040$), haemoglobin (-0.36 *vs.* -0.08; $p = 0.038$) and haematocrit (-2.56 *vs.* -1.00; $p = 0.025$). Also the delta in mean platelet volume was 0.40 fL larger in the taxane group compared to the platinum group, which was significantly different ($p = 0.008$).

Out of 24, 16 patients were treated with a combination of paclitaxel or docetaxel with a platinum compound. No statistically significant difference between

Table 3 Mean blood cell counts at baseline (T0) and at follow-up (T1), stratified according to the presence of lipophilic solvents

Blood cell indices	lipophilic solvents		*p*-value
	yes	no	
	taxane group (n=24)	platinum group (n=296)	
RBC ($\times 10^{12}$/L)	(SD)	(SD)	
T0	4.45 (0.45)	4.55 (0.49)	
T1	4.25 (0.56)	4.51 (0.61)	
Δ T1–T0	-0.20 (0.47)	-0.04 (0.37)	0.040
MCV (fL)			
T0	87.91 (4.60)	88.75 (5.42)	
T1	86.92 (4.62)	87.69 (5.53)	
Δ T1–T0	-0.99 (1.14)	-1.20 (1.38)	0.782
HGB (mmol/L)			
T0	8.14 (1.02)	8.40 (1.02)	
T1	7.78 (1.05)	8.32 (1.16)	
Δ T1–T0	-0.36 (0.85)	-0.08 (0.63)	0.038
HT (%)			
T0	39.09 (4.99)	40.16 (4.55)	
T1	36.53 (5.25)	39.16 (5.20)	
Δ T1–T0	-2.56 (4.41)	-1.00 (3.15)	0.025
MCH (fmol)			
T0	1.83 (0.12)	1.85 (0.14)	
T1	1.83 (0.14)	1.85 (0.15)	
Δ T1–T0	0.0083 (0.62)	0.0004 (0.07)	0.582
RETC ($\times 10^{9}$/L)			
T0 [a]	70.60 (32.15)	57.95 (20.77)	
T1 [b]	25.37 (18.83)	22.01 (20.62)	
Δ T1–T0	-46.86 (27.86)	-35.71 (26.16)	0.157
RDW (%CV)			
T0	13.04 (1.58)	12.42 (1.28)	
T1	12.89 (1.35)	12.27 (1.35)	
Δ T1–T0	-0.14 (0.58)	-0.15 (0.45)	0.941
PLT ($\times 10^{9}$/L)			
T0 [c]	340.63 (144.55)	318.24 (105.94)	
T1 [c]	272.11 (111.52)	266.52 (104.48)	
Δ T1–T0	-68.51 (102.66)	-51.72 (89.40)	0.392

(Table 3 continued)

Blood cell indices	lipophilic solvents		*p*-value
	yes	no	
	taxane group (n=24)	platinum group (n=296)	
MPV (fL)			
T0 [c]	7.36 (0.78)	7.33 (0.92)	
T1 [c]	7.92 (0.98)	7.49 (0.96)	
Δ T1−T0	0.56 (0.85)	0.16 (0.67)	0.008

RBC = red blood cells (number of erythrocytes); MCV = mean corpuscular volume; fL = femtoliter; HGB = haemoglobin; HT = haematocrit; MCH = mean corpuscular haemoglobin; RETC = number of reticulocytes; RDW = red cell distribution width; CV = corpuscular volume; PLT = mean platelet count; MPV = mean platelet volume; SD = standard deviation

a) Taxane group n = 16; platinum group n = 187.
b) Taxane group n = 13; platinum group n = 206.
c) Taxane group n = 23.

the deltas for RBC, HGB, HT and MPV were observed (Table 4). Only the delta in platelet count was statistically significantly higher in the platinum group ($p = 0.002$). In Table 5, the measurements at baseline and follow-up are listed for cisplatin chemotherapy and carboplatin chemotherapy. There was a difference between the deltas for RBC, HGB, HT, WBC and PLT. No difference was found for MPV. Although no differences in delta for MPV were found within the taxane group (taxane with/without platinum compounds) and within the platinum group (cisplatin *vs.* carboplatin), the delta for MPV was statistically significantly larger in the group with lipophilic solvents (taxane group) compared to the group without lipophilic solvents (platinum group).

DISCUSSION

We observed that, although blood cell indices changed in both groups, mean changes in blood cell parameters were statistically significantly higher in the taxane group (with lipophilic solvents) compared to the platinum group (without lipophilic solvents). The statistically significant difference in deltas was observed for three red blood cell parameters, namely the number of erythrocytes, the haemoglobin concentration and haematocrit. In addition, the delta in mean platelet volume increased significantly more in the taxane group. The decrease in number of erythrocytes as well as the lower haemoglobin concentration within days after the start of the chemotherapy cycle is unlikely to be induced by bone marrow

Table 4 Mean blood cell counts at baseline (T0) and at follow-up (T1), taxane group

Blood cell indices	taxane group (n=24)				*p*-value
	taxanes with other agents (n=8)		taxanes with platinum compounds (n=16)		
RBC ($\times 10^{12}$/L)		(SD)		(SD)	
T0	4.45	(0.40)	4.45	(0.48)	
T1	4.39	(0.45)	4.18	(0.61)	
Δ T1–T0	-0.07		-0.27		0.323
MCV (fL)					
T0	87.54	(96.25)	88.09	(3.77)	
T1	86.23	(6.19)	87.27	(3.81)	
Δ T1–T0	-1.31		-0.82		0.331
HGB (mmol/L)					
T0	8.14	(0.76)	8.14	(1.15)	
T1	8.11	(0.59)	7.61	(1.19)	
Δ T1–T0	-0.02		-0.53		0.175
HT (%)					
T0	38.58	(4.17)	39.35	(5.47)	
T1	37.04	(2.97)	36.27	(6.16)	
Δ T1–T0	-1.53		-3.08		0.431
MCH (fmoL)					
T0	1.83	(0.14)	1.82	(0.11)	
T1	1.86	(0.18)	1.82	(0.13)	
Δ T1–T0	0.03		0.00		0.245
RETC ($\times 10^{9}$/L)					
T0 [a]	74.49	(39.44)	68.26	(29.03)	
T1 [b]	43.02	(11.11)	14.34	(13.26)	
Δ T1–T0	-42.53		-49.02		0.723
RDW (%CV)					
T0	12.54	(1.14)	13.28	(1.74)	
T1	12.65	(1.13)	13.01	(1.46)	
Δ T1–T0	-0.11		-0.27		0.126
PLT ($\times 10^{9}$/L)					
T0 [c]	243.00	(54.33)	392.69	(151.69)	
T1 [c]	257.81	(96.20)	279.74	(121.40)	
Δ T1–T0	14.80		-112.95		0.002

(Table 4 continued)

Blood cell indices	taxane group (n = 24)		*p*-value
	taxanes with other agents (n=8)	taxanes with platinum compounds (n=16)	
MPV (fL)			
T0 [c]	7.65 (0.90)	7.21 (0.69)	
T1 [c]	8.00 (0.99)	7.88 (1.01)	
Δ T1–T0	0.35	0.67	0.409

RBC = red blood cells (number of erythrocytes); MCV = mean corpuscular volume; fL = femtoliter; HGB = haemoglobin; HT = haematocrit; MCH = mean corpuscular haemoglobin; RETC = number of reticulocytes; RDW = red cell distribution width; CV = corpuscular volume; PLT = mean platelet count; MPV = mean platelet volume; SD = standard deviation

a) Taxanes with other agents n = 6; taxanes with platinum compounds n = 10.
b) Taxanes with other agents n = 5; taxanes with platinum compounds n = 8.
c) Taxanes with platinum compounds n = 15.

suppression, as the life span of erythrocytes is usually about 100–120 days before they are removed from the circulation by macrophages. The difference between the two groups with regard to the decreased number of red blood cells and haemoglobin concentration suggests that there may be an alternative cause. The formation of phosphatidylserine enriched red blood cell vesicles is unlikely, since we did not observe a decline in mean corpuscular volume. Possibly, the period between the baseline and follow-up measurements was too short to observe an effect on those parameters. Alternatively, the period between measurements could have been too long. If the effects on the volume of the red blood cells (caused by vesiculation) are immediate, affected red cells will be cleared from the circulation before they can be detected by the haematocytometer. The number of reticulocytes and platelets in follow-up measurements was lower than at baseline in both groups, indicating drug-induced myelosuppression. The mean platelet volume increased statistically significantly more in the group with lipophilic solvents (taxane group) compared to the group without these solvents (platinum group). The reasons for this finding are unclear. One explanation can be that cell particles or vesicles are detected in the platelet channel of the analyser, thus adding to the measured MPV. If this is the case, the total number of platelets is too high in this patient group, since vesicles and cell fragments may be counted as platelets.

A possible complicating factor in the interpretation of the results is the fact that in the taxane group, 16 out of 24 patients were treated with a combination of paclitaxel or docetaxel with a platinum compound. Platinum

Table 5 Mean blood cell counts at baseline (T0) and at follow-up (T1), platinum group

Blood cell indices	platinum group (n=296)				p value
	cisplatin (n=256)		carboplatin (n=40)		
RBC ($\times 10^{12}$/L)		(SD)		(SD)	
T0	4.60	(0.47)	4.24	(0.51)	
T1	4.58	(0.59)	4.08	(0.54)	
Δ T1–T0	-0.02	(0.38)	-0.16	(0.28)	0.020
MCV (fL)					
T0	88.81	(5.36)	88.39	(5.80)	
T1	87.80	(5.47)	86.95	(5.90)	
Δ T1–T0	-1.01	(1.38)	-1.44	(1.34)	0.063
HGB (mmol/L)					
T0	8.51	(0.97)	7.72	(1.05)	
T1	8.46	(1.11)	7.42	(1.05)	
Δ T1–T0	-0.04	(0.65)	-0.29	(0.42)	0.018
HT (%)					
T0	40.62	(4.35)	37.23	(4.75)	
T1	39.78	(4.96)	35.20	(4.98)	
Δ T1–T0	-0.84	(3.22)	-2.03	(2.46)	0.027
MCH (fmol)					
T0	1.85	(0.13)	1.82	(0.16)	
T1	1.85	(0.15)	1.82	(0.14)	
Δ T1–T0	0.00	(0.07)	0.00	(0.06)	0.939
RETC ($\times 10^{9}$/L)					
T0 [a]	57.84	(20.71)	59.09	(22.15)	
T1 [b]	23.60	(20.75)	11.88	(16.82)	
Δ T1–T0	-35.13	(26.21)	-41.65	(25.71)	0.358
RDW (%CV)					
T0	12.28	(1.14)	13.31	(1.72)	
T1	12.13	(1.23)	13.16	(1.71)	
Δ T1–T0	-0.15	(0.44)	-0.15	(0.52)	0.987
PLT ($\times 10^{9}$/L)					
T0	309.16	(92.60)	376.34	(157.53)	
T1	271.09	(100.89)	237.25	(122.48)	
Δ T1–T0	-38.07	(77.18)	-139.09	(111.61)	0.000

(Table 5 continued)

Blood cell indices	platinum group (n=296)		p value
	cisplatin (n=256)	carboplatin (n=40)	
MPV (fL)			
T0	7.34 (0.91)	7.26 (1.00)	
T1	7.52 (0.94)	7.31 (1.06)	
Δ T1–T0	0.18 (0.64)	0.05 (0.82)	0.272

RBC = red blood cells (number of erythrocytes); MCV = mean corpuscular volume; fL = femtoliter; HGB = haemoglobin; HT = haematocrit; MCH = mean corpuscular haemoglobin; RETC = number of reticulocytes; RDW = red cell distribution width; CV = corpuscular volume; PLT = mean platelet count; MPV = mean platelet volume; SD = standard deviation

a) Cisplatin group n = 171, carboplatin group n = 16.

b) Cisplatin group n = 178, carboplatin group n = 28.

compounds, particularly carboplatin, are highly associated with anaemia and myelosuppression.[2] Therefore, we conducted an analysis in which we stratified the taxane group in combinations with (n = 16) and without platinum compounds (n = 8). The difference in delta for platelet count can be explained by toxic effects of the pharmacological compounds on circulating thrombocytes and/or bone marrow, since thrombocytes have a short half life. For the purpose of our study, the grouping of all taxane chemotherapy regimens seems to be appropriate. Whether this also applies for the platinum compound group is not certain since differences were found between the deltas for RBC, HGB, HT, WBC and PLT.

There are several limitations to this study. We only took the first chemotherapy cycle into account and we calculated differences between the first follow-up measurement and baseline measurements. We did not acquire information after multiple cycles and therefore do not know whether the effects on red blood cells will deteriorate or will reach a steady state. As stated earlier, the effect may also be instantaneously, thereby escaping detection in our study design. Furthermore, UPOD currently comprises data from only one institution. As a consequence, exposure data are limited in numbers, limiting the power of the study. We also did not study the cytostatic regimens in depth and cannot exclude the possibility that other cytostatic agents affected our findings.

We conducted this study to evaluate the effects of the lipophilic solvents CrEL or polysorbate in cytostatic regimens on circulating red blood cells. Whether the presence of the lipophilic solvents could be an explanation for the differences in decline of erythrocytes, mean haemoglobin and haematocrit and the increase of mean platelet volume in cytostatic regimens with lipophilic solvents is unclear.

However, the findings in this study warrant further research to unravel underlying mechanisms and to assess whether the differences between the groups have clinical relevance.

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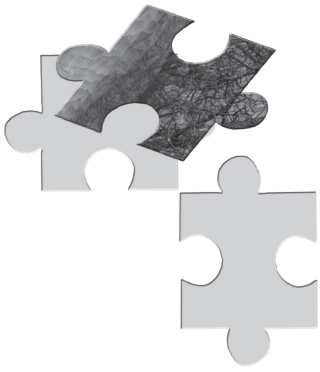
4

Target-orientated drug exposure classification

4.1

Drug-related nephrotoxic and ototoxic reactions: a link through a predictive mechanistic commonality



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ABSTRACT

Background

Drug-induced ototoxicity is a subject of interest because many diseases are treated with drugs which have potential toxic effects on the ear. There is evidence that both inner ear and kidney tissue are immunologically, biochemically and functionally related. It has been suggested that drugs which influence transport of sodium and/or potassium change ionic homeostasis in the inner ear and, hence, induce functional disturbances, such as hearing loss, tinnitus and vertigo.

Objectives

To assess whether renal suspected adverse drug reactions (sADRs) have predictive value for ear and labyrinth adverse drug reactions (ADRs) and whether drug classes involved have influence on ion transport systems.

Study design

Data were obtained from the Netherlands Pharmacovigilance Centre Lareb. The study base comprised all reports of sADRs up until 1 January 2007. Cases were all sADRs for relevant renal disorders and all sADRs for relevant ear disorders. All other reported sADRs were selected as 'non-cases'. The relationship between drug classes and renal and ear and labyrinth sADRs was evaluated by calculating reporting odds ratios (RORs). A ROR ≥ 1.50 was regarded as a cut-off value for an association. Drug classes were classified into four groups: (A) ROR kidney < 1.50 and ROR ear < 1.50 or no reports on ear ADRs (reference group); (B) ROR kidney < 1.50 and ROR ear ≥ 1.50; (C) ROR kidney ≥ 1.50 and ROR ear < 1.50 or no reports on ear ADRs; and (D) ROR kidney ≥ 1.50 and ROR ear ≥ 1.50. For each group, we calculated odds ratios (ORs) for the association between the group classification and effect on ion channels/ion transport systems in kidney and ear tissues.

Results

Of 193 drug classes with relevant ADRs for renal disorders, 120 drug classes also had reports on ototoxic reactions. Fourteen out of 120 drug classes had a ROR ≥ 1.50 for the association between the drug class and both renal and ear sADRs. Among these drug classes were several with a well-known ability to induce renal (adverse) effects and ear and labyrinth disorders, such as loop diuretics, aminoglycosides and quinine.

We found that a mechanistic commonality of the drug classes mentioned in the reports was the ability to affect ion transport systems. The percentage of drugs having this property differed between the four groups. The ORs for group D

and B were significantly higher compared to the reference group (OR 12.2; 95% confidence interval [CI] 3.0–30.5 and OR 8.7; 95% CI 2.4–18.7, respectively), whereas there was no association for group C.

Conclusion

Our data suggest that renal sADRs as such are not a marker for drug-induced ear and labyrinth disorders. However, the ability of drugs to act on ion channels or ion transport systems, and therefore have an influence on ionic homeostasis in kidney and ear might be a predictor for the possible occurrence of drug-related ototoxicity.

INTRODUCTION

In daily medical practice, drug-induced ototoxicity is a subject of interest because numerous diseases are treated with drugs which have potential toxic effects on the ear.[1] For several drugs, such as aminoglycosides, quinine, salicylates, loop diuretics and antineoplastic drugs, the association with ototoxic effects has been well documented.[1-3] However, there is little evidence for many other drugs. Usually, ototoxic effects have not been detected by the time a drug reaches the market because pharmacological effects on the ear are not routinely evaluated in pre-clinical tests and clinical trials,[4-6] and such effects may be rare. Furthermore, rates of occurrence of ototoxicity are difficult to determine because of the lack of standardised guidelines for monitoring aural function during treatment with potentially ototoxic agents and the wide differences in individual susceptibility.[7,8] The reported incidences of drug-induced ototoxicity vary widely, ranging, for example, from 10% to 63% for aminoglycosides, from 0% to 16% for macrolides and furosemide, and from 3% to 100% for platinum antitumour compounds.[7]

Ototoxicity can be defined as the tendency of certain drugs and other chemical substances to cause functional impairment and cellular degeneration of the tissues of the inner ear. The main sites of such ototoxic effects are the cochlea, vestibulum and stria vascularis.[2] Ototoxicity is clinically characterised by tinnitus, hearing loss and vestibular complaints, and has been associated with both short- and longterm exposure.[9,10] Reported risk factors for drug-induced ototoxicity are the patient's age (the young and the elderly are at a higher risk), previous use of an ototoxic agent, dose, exposure to multiple ototoxic drugs and impaired liver or renal function.[7,8] In particular, the relationship between impaired renal function and hearing loss is notable. Firstly, there are inherited renal diseases

which are accompanied by hearing disorders, such as Alport syndrome and Bartter syndrome.[11] Secondly, the incidence of hearing loss is considerably higher among patients with chronic renal failure than in the general population.[12] Lastly, renal adverse effects of some drugs (*e.g.* aminoglycosides and loop diuretics) can be accompanied by ototoxicity. It has been shown that both kidney and inner ear tissues are to some extent immunologically, biochemically and functionally related.[13] For example, the stria vascularis and the tubular epithelium in the kidney have similar ion-transport processes,[14] and chloride ion channels of the CLC-K family are expressed exclusively in the kidneys and ears.[13,15,16]

Taking these similarities into consideration, it is clinically relevant to know whether the potential adverse renal effects of drugs which are routinely monitored for, have predictive value for ear and labyrinth adverse drug effects, which are not routinely monitored for.

Thus, the first aim of this study was to assess whether there is an association between renal adverse effects and ear and labyrinth adverse effects as mentioned in spontaneous reports of adverse drug reactions (ADRs) to the Netherlands Pharmacovigilance Centre Lareb. Subsequently, we investigated whether the drug classes involved had a mechanistic commonality, namely the ability to influence ion transport systems in kidney and ear tissues which could explain a possible association.

METHODS

Setting

The Netherlands Pharmacovigilance Centre Lareb maintains the spontaneous ADR reporting system in the Netherlands on behalf of the Dutch Medicines Evaluation Board. Each year, Lareb receives approximately 6000 reports on suspected ADRs, provided by health care professionals, patients, and the marketing authorisation holders of drugs that are approved for marketing in the Netherlands. Reports contain information about the patient (*i.e.* age, sex), one or more sADRs, medication used at the time of the event (both suspected and concomitant drugs), source (physician, pharmacist or patient) and the year of reporting. Each report is evaluated by a trained physician and/or pharmacist and filed in a database. ADRs are coded according to the *Medical Dictionary for Regulatory Activities* (MedDRA)[a]

[a] MedDRA is a registered trade mark belonging to the international Federation of Pharmaceutical Manufatures Associations.

terminology; the drugs are classified according to the Anatomical Therapeutic Chemical (ATC) classification system.[17]

Selection of case reports

The study base comprised all reports of sADRS received by Lareb between 1 January 1985 and 31 December 2006. *A priori*, two of the authors (BV and EvP) selected the relevant terms for renal disorders and relevant ear and labyrinth disorders. Subsequently, we selected all sADRs for relevant renal disorders according to the MedDRA terminology among the reported sADRs in the database (Appendix I). All reported sADRs for renal disorders were defined as cases. All other ADRs (*i.e.* those not classified as relevant renal disorders) were selected as non-cases. Additionally, we selected all sADRs for relevant ear and labyrinth disorders (Appendix II) and we defined them as cases. All other ADRs (*i.e.* those not classified as relevant ear and labyrinth disorders) were selected as non-cases.

Data analysis

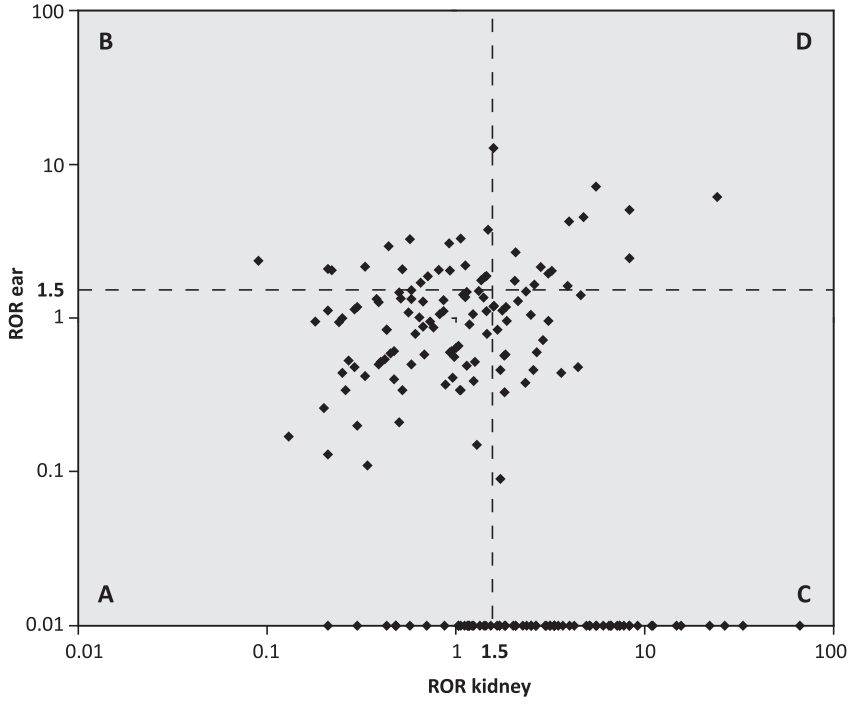
The relationship between drug classes and reports with renal sADRs and ear and labyrinth sADRs, respectively, was evaluated by calculating ADR reporting odds ratios (RORs) with a 95% confidence interval (CI). This method, using the concept of 'reaction proportion signalling', assesses whether drugs or drug classes have a disproportionate share in a certain ADR relative to all other reported ADRs.[17] A ROR significantly higher than 1 indicates a disproportionate share of a certain drug or drug class in the reporting of a certain event (*i.e.* renal or ear disorders) and is, therefore, be considered a proxy for an increased risk of the ADR of interest.[18]

An arbitrary point estimate of 1.50 was regarded as an indication for a clinically relevant association between a drug class and renal and ear disorders. As the power of this study was expected to be low because of the small numbers of reports on nephrotoxic and ototoxic reactions, we decided not use the lower limit of the 95% CI as cut-off value. Based on the RORs for renal and ear and labyrinth disorders (ROR kidney and ROR ear, respectively), drug classes were classified into four categories; *group A* ROR kidney < 1.50 and ROR ear < 1.50 or no reports on ear sADRs (reference group); *group B* ROR kidney < 1.50 and ROR ear ≥ 1.50; *group C* ROR kidney ≥ 1.50 and ROR ear < 1.50 or no reports on ear sADRs; and *group D* ROR kidney ≥ 1.50 and ROR ear ≥ 1.50.

In search for mechanistic similarities, we focused on the mechanism of actions of drugs with regards to their ability to affect ion transport processes in kidney and ear tissues. We conducted a survey in the medical literature, indexed in MEDLINE/PubMed. We used the following Medical Subject Headings (MeSH): 'ion transport'

and 'ion channel', in combination with the names of the drug classes (for drug classes see Appendices III-VI), or the name of an individual drug belonging to the related drug class, each with and without 'kidney' or 'ear' as the search limiter. For each of the four groups, we calculated the percentage of drug classes with effects on ion transport systems. We also calculated odds ratios (ORs) with 95% CIs for the association between the group classification (*i.e.* A, B, C and D) and effect on ion channels/ion transport systems.

Figure 1 Reporting odds ratio (ROR) kidney *vs.* ROR ear (n = 193), logarithmic scale



ROR ear of 0.01 indicates no reports of suspected adverse drug reactions (sADRs) involving the ear. Drug classes were classified into four categories: *group A* ROR kidney < 1.50 and ROR ear < 1.50 or no reports on ear sADRs (reference group); *group B* ROR kidney < 1.50 and ROR ear ≥ 1.50; *group C* ROR kidney ≥ 1.50 and ROR < 1.50 or no reports on ear sADRs; and *group D* ROR kidney ≥ 1.50 and ROR ear ≥ 1.50.

RESULTS

The selection of all relevant sADRs for renal disorders resulted in 1068 reports, in which 193 drug classes were involved. Renal failure was the most frequently reported renal sADR (n = 431; 36.3%), followed by urinary tract signs and symptoms (n = 203; 17.1%). Relevant sADRs for ear and labyrinth disorders were found in 727 reports, in which 160 drug classes were mentioned. Inner ear signs and symptoms were the most frequent ear and labyrinth sADRs (n = 512; 37.5 %), followed by auditory nerve disorders (n = 365; 26.8%) and hearing losses (n = 203; 14.8%).

All 193 drug classes were categorised into four groups using a ROR of 1.50 as cut-off value. Of the 193 drug classes with relevant sADRs for renal disorders, 120 drug classes (62.2%) had also reports on drug-related ototoxic reactions. Fourteen out of these 120 drug classes had a ROR ≥ 1.50 for the association between the drug class and both renal and ear and labyrinth sADRs (Figure 1, group D). The drug classes in group D with a disproportionate share in the total number of case reports of both renal and ear sADRs (ROR ≥ 1.50) are listed in Appendix III. Among these drug classes were several with a well-known ability to induce renal (adverse) effects and ear and labyrinth disorders, such as loop diuretics, aminoglycosides and quinine. For seven out of the 14 drug classes with a ROR ≥ 1.50 for the association between drug class and both renal and ear sADRs, terms which indicate ear and labyrinth disorders, such as tinnitus, hearing loss and vertigo, are listed in the summaries of product characteristics (SPCs). Specifically, these were: high-ceiling diuretics, other peripheral vasodilators, other aminoglycosides, glycopeptide antibacterials, quinine (derivatives), salicylic acid (derivatives) and carbonic anhydrase inhibitors (Appendix III, numbers 4, 5, 6, 7, 10, 12 and 13).

In search for a mechanistic commonality as an explanation for this observed association between spontaneous reports on renal and ear and labyrinth sADRs, we assessed whether the involved drugs had any mechanisms of action in kidney and ear tissues. In group D, several drug classes act on membranes influencing transport of sodium and/or potassium, chloride and calcium via direct or indirect pathways. These are locally acting corticosteroids, high-ceiling diuretics, other aminoglycosides, quinine (derivatives), salicylic acid (derivatives) and carbonic anhydrase inhibitors (Appendix III, numbers 1, 4, 6, 10, 12 and 13). Well-known ototoxic and nephrotoxic drugs, such as loop diuretics, aminoglycosides and salicylic acid (derivatives), are among those drug classes with reports on ear and renal sADRs.

The 20 drug classes in group B are listed in Appendix IV. Platelet aggregation inhibitors (Appendix IV, number 1), low-ceiling sulfonamides (numbers 3 and 4),

Table 1 Association between group classification (based on reporting odds ratio [ROR] kidney and ROR ear) and the effect on ion transport systems

Group	Group definition	Number of drug classes in group	Drug classes with effect on ion systems n (%)	OR (95% CI)
A	ROR kidney < 1.50; ROR ear < 1.50 or no reports on ear sADRs	86	5 (5.8%)	1.0 (reference)
B	ROR kidney < 1.50; ROR ear ≥ 1.50	20	7 (35.0%)	8.7 (2.4–18.7)
C	ROR kidney ≥ 1.50; ROR ear < 1.50 or no reports on ear sADRs	73	8 (11.0%)	2.0 (0.6–3.1)
D	ROR kidney ≥ 1.50; ROR ear ≥ 1.50	14	6 (42.9%)	12.2 (3.0–30.5)

OR = odds ratio; CI = confidence interval

oxicams (number 12), and quinine alkaloids (number 19) affect ion channels and/or ion transport systems. In contrast to the drug classes in Appendix III and IV, the majority of the number of drug classes with reports on renal and ear sADRs in group C and group A (reference group) (Appendix V and VI, respectively) have no effect on ion transport systems, at least not in kidney or ear tissue.

In Table 1, the association is described between the group in which the drug classes are classified and effect on ion channels/ion transport systems in kidney and ear tissues. The reference group contained those drug classes with reports on renal sADRs with a ROR < 1.50 and a ROR ear < 1.50 or no reports on ear sADRs. In group D and group B, the number of drug classes with an effect on ion systems was statistically significantly larger than in the reference group (OR 12.2; 95% CI 3.0–30.5 and OR 8.7; 95% CI 2.4–18.7, respectively) whereas there was no association for group C (OR 2.0; 95% CI 0.6–3.1).

DISCUSSION

Our main finding was that there was a high percentage of drug classes affecting ion transport systems among drug classes associated with a disproportional share of reported sADRs for both kidney and ear (group D, Appendix III). Furthermore, another interesting finding was that also in group B (Appendix IV), effects on ion

Box 1 Mechanisms of action

Loop diuretics (Appendix III, number 4) are inhibitors of the Na^+-K^+-$2Cl^-$ transport system in the loop of Henle, and **carbonic anhydrase inhibitors** (Appendix III, number 13) have a diuretic effect by reabsorption of hydrogen carbonate and the excretion of Na^+ and K^+. In vivo studies have shown that loop diuretics could induce changes in the electrolyte composition of the endolymph.[26-28] **Thiazides and related diuretics** inhibit the Na^+-Cl^- transport system in the distale tubule (Appendix IV, numbers 3, 4, and 6).

Salicylic acid derivatives and NSAIDs (Appendix III, number 12 and Appendix IV, numbers 1 and 12) affect the outer hair cells. Their main action is inhibition the prostaglandin synthesis. Prostaglandins are key regulators of ion transport in the kidney and they regulate also the Na^+-K^+-adenosine triphosphate (ATP)ase pump. Because Na^+-K^+-ATPase is present in the cochlea, it is plausible that prostaglandin synthetase inhibitors have an effect on ion transporting epithelia in the inner ear as well.

As with salicylate ototoxicity, quinine ototoxicity appears to be multifactorial in origin.[29,30] **Quinine and derivatives** (Appendix III, number 10 and Appendix IV, number 19) induce vasoconstriction and decrease cochlear blood flow. Alterations and loss of outer hair cells seem to play important roles. Antagonisms of calcium-dependent K^+ channels may have a potential role in ototoxicity. Blockage of K^+ currents may inhibit the generation of the endocochlear potential.[31]

Calcium antagonists (**benzothiazepine derivatives**; Appendix IV, number 5) block the L-type voltage-sensitive calcium channels in arterial smooth muscle, causing relaxation and vasodilatation. The L-type calcium channels are present in both cochlear and vestibular receptors.[32]

Glucocorticoids (Appendix III, number 1) are involved in the Na^+-K^+-ATPase activity in several tissues, including the stria vascularis in the inner ear.[33] It has been suggested that glucocorticoids regulate Na^+ transport in the inner ear and therefore may be beneficial in the treatment of Meniere's disease.[34,35]

Aminoglycosides (Appendix III, number 6) are antibiotics which interfere with the bacterial messenger RNA. They accumulate rapidly in the perilymph and endolymph of the inner ear, where they target the sensory hair cells. They are known to block a variety of ion channels including large-conductance Ca^{2+}-activated K^+ channels, although blockage was not thought to lead directly to ototoxic effects.[36]

systems seem to play an important role. This also applies to the group of drug classes with a ROR ear ≥ 1.50 and no reports on renal sADRs (OR 5.6; 95% CI 1.7−10.5). This outcome would imply that regardless of any (reports on) renal sADRs, the ability to act on ion transport systems in the inner ear could be an important factor in drug-induced ototoxicity.

It has been established that many drugs are capable of inducing ototoxicity and nephrotoxicity. The mechanisms by which these adverse effects are produced are not well understood, but kidney and ear tissues may relate on immunological, biochemical and functional levels.[13] In Box 1, the mechanisms of action of the drug classes (group D and B) with regards to ion transport systems are listed. Although there are some indications that other drugs can also act on ion channels,[19,20] the relevance with regards to ion systems in kidneys and/or ear is either unclear or not well established. Other drug classes do have the ability to act on ion transport systems, such as digitalis glycosides (Appendix V, number 14) and sulfonylurea

Box 2 Role of ion transport processess in the inner ear

From the literature, it is known that ion transport processes play an important role in both kidney and ear tissues.[13-15] **Changes in ionic homeostasis** in the inner ear may lead to functional disturbances, namely hearing loss, tinnitus and vertigo.[37] The major function of the inner ear is the transformation of mechanical stimuli into electrical signals. This conversion occurs in the sensory hair cells of the cochlea, and the **availability of potassium (K^+) ions** is essential to maintain normal auditory function.[16,38] K^+ is the major cation in endolymph, and the cochlear function depends on the active secretion of K^+ and absorption of sodium (Na^+).[16,39,40] In the cochlea, **Na^+-K^+-ATPase** plays an important role in maintaining ionic homeostasis and physiologic function.

derivatives (Appendix VI, number 7), but there are no indications that they influence ion systems in kidney and/or ear tissues. The importance of ion transport processes in ear tissues is discussed in Box 2.

There are several limitations to this study. Firstly, the selection of the relevant terms for renal disorders and for relevant ear and labyrinth disorders according to the MedDRA terminology was in part arbitrary. Secondly, the concept of 'reaction proportion signalling' has limitations. A spontaneous reporting system as a mean of collecting data on sADRs is known to represent only a fraction of the drug-related adverse events [21,22] and is dependent of the type of ADR. Selective over- and underreporting of specific ADRs may lead to misinterpretations when comparing drug classes with respect to ADRs. ADRs of relatively new drugs, severe ADRs [23] and ADRs which are not listed in the summary of product characteristics [24] are reported more often than others. Notwithstanding the selective underreporting, drug classes with a known ability to induce ototoxic side effects emerged from the Lareb database (see Appendices III and IV). The potential of reporting bias, which is always a concern in this type of studies, is, however, low with respect to the research question concerning mechanistic commonalities, since it is not to be expected that reports are made on basis of a suspicion that ion transport systems are involved.

As a consequence of the low numbers of reports for individual drugs, we evaluated the associations on drug class level. For the majority of drug classes, the adverse effects on kidney and ear are considered a class effect, but there are some exceptions. For example, minocycline, a tetracycline derivative, appears to produce vestibulotoxicity, whereas other tetracycline antibiotics do not. The same applies for irbesartan and telmisartan, which are able to induce tinnitus, in contrast to other angiotensin II antagonists (information from Dutch SPCs). Because of the low numbers of reports, we could not discriminate between these individual drugs and we categorised them on the basis of the overall class effect (group A).

In order to study the robustness of our results, we conducted a sensitivity analysis using RORs of 1.00 and 2.00 as cut-off values. Using a ROR of 1.00, this resulted in ORs of 6.8 (95% CI 1.7−13.4) and 2.8 (95% CI 0.6−5.7) for group D and B, respectively. When using a ROR of 2.00, the ORs were 6.5 (95% CI 1.5−20.2) and 6.1 (95% CI 1.8−15.2) for group D and B, respectively. The use of different cut-off values changed the magnitude of the ORs, but did not change our main finding.
Thirdly, all ATC codes were included, regardless of the route of administration of a drug. We did not take into account whether a drug was able to penetrate into the inner ear after administration. This could lead to overestimation of the observed association. Lastly, in this study, we did not adjust the RORs for potential confounding factors, such as patients characteristics, co-morbidity and co-medication. In the context of studying renal and ear and labyrinth sADRs, age may act as a confounder. Elderly patients are more at risk for drug-induced ototoxicity and impaired renal function, and for exposure to multiple (ototoxic) drugs. In the drug classes with both a ROR kidney and ROR ear ≥ 1.50, reports of renal and ear and labyrinth sADRs regarding elderly patients may be overrepresented.
In this study , we classified drug classes according to their mechanistic properties in relation with the calculation of RORs. In a study on anti-HERG activity in relation with drug-induced QTc-prolongation, the same concept was used.[25] However, to our knowledge, there are no other studies of this type, in which the relationship between a combination of two RORs on different organ systems and a corresponding mechanism of action is assessed.

CONCLUSION

In this study we focused on the possible association between renal events and labyrinth disorders using a spontaneous reports system and a mechanistic similarity between drug classes mentioned in those reports. Overall, our data suggest that suspected renal events as such are no marker for drug-induced ear and labyrinth disorders. However, the ability of drugs to act on ion channels or ion transport systems, and thereby influence ionic homeostasis in kidney and ear, might be a predictor for the possible occurrence of drug-related ototoxicity. Since in pre-clinical testing pharmacological effects on ear tissues are not routinely assessed, an important practical implication of our findings could be that drug classes which are able to act on ion transport systems in kidney and ear tissues might have potential ototoxic properties. However, the number of reports on

drug classes involved in the analysis was low and further research into this field is necessary to clarify the mechanistic commonality in detail.

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Appendix I Renal disorders

High level names (MedDRA)
Nephritis NEC
Nephropathies and tubular disorders NEC
Renal and urinary tract disorders congenital NEC
Renal and urinary tract injuries NEC
Renal disorders congenital
Renal disorders NEC
Renal failure and impairment
Renal haemorrhagic disorders
Renal hypertension and related conditions
Renal hypertensions
Renal infections and inflammations (excl. nephritis)
Renal lithiasis
Renal necrosis and vascular insufficiency
Renal neoplasms
Renal neoplasms benign
Renal obstructive disorders
Renal structural abnormalities and trauma
Renal vascular and ischaemic conditions
Urinary abnormalities
Urinary tract neoplasms benign
Urinary tract signs and symptoms NEC

NEC = not elsewhere classified

Appendix II Ear and labyrinth disorders

High level names (MedDRA)
Auditory nerve disorders
Ear disorders NEC
Hearing disorders NEC
Hearing losses (incl. deafness)
Hyperacusia
Inner ear disorders NEC
Inner ear infections and inflammations
Inner ear signs and symptoms
Vertigos NEC
VIIIth cranial nerve disorders

NEC = not elsewhere classified

Appendix III Drug classes in group D: ROR kidney ≥ 1.50 and ROR ear ≥ 1.50

No.	ATC5	ATC5 description	Kidney		Ear	
			n	ROR (95% CI)	n	ROR (95% CI)
1	A07EA [a]	Corticosteroids acting locally	3	3.90 (1.22–12.5)	1	1.62 (0.22–11.7)
2	A07EC	Aminosalicylic acid and similar agents	14	3.22 (1.87–5.52)	7	2.03 (0.95–4.30)
3	C02KD	Serotonin antagonists	1	2.07 (0.28–15.2)	1	2.68 (0.37–19.7)
4	C03CA [a]	Sulfonamides, plain, high-ceiling	12	3.97 (2.21–7.14)	10	4.25 (2.24–8.06)
5	C04AX	Other peripheral vasodilators	2	8.29 (1.90–36.1)	1	5.05 (0.67–38.0)
6	J01GB [a]	Other aminoglycosides	2	5.52 (1.30–23.4)	2	7.17 (1.69–30.4)
7	J01XA	Glycopeptide antibacterials	4	24.2 (7.68–76.0)	1	6.13 (0.81–46.7)
8	J06BA	Immunoglobulins, normal human	2	3.08 (0.75–12.7)	1	1.95 (0.27–14.2)
9	L03AB	Interferons	9	2.05 (1.05–3.99)	6	1.75 (0.78–3.94)
10	M09AA [a]	Quinine and derivatives	2	1.58 (0.39–6.42)	11	12.8 (6.75–24.2)
11	N01AX	Other general anesthetics	4	8.30 (2.93–23.5)	1	2.45 (0.34–17.9)
12	N02BA [a]	Salicylic acid and derivatives	5	2.81 (1.15–6.90)	3	2.15 (0.68–6.78)
13	S01EC [a]	Carbonic anhydrase inhibitors	4	4.74 (1.72–13.1)	3	4.53 (1.42–14.5)
14	V01AA	Allergen extracts	2	2.60 (0.63–10.7)	1	1.65 (0.23–11.7)

n = number of reports; ROR = reporting odds ratio; CI = confidence interval
a) Effect on ion (transport) systems in kidney and/or ear tissues.

Appendix IV Drug classes in group B: ROR kidney < 1.50 and ROR ear ≥ 1.50

No.	ATC5	ATC5 description	Kidney		Ear	
			n	ROR (95%CI)	n	ROR (95% CI)
1	B01AC [a]	Platelet aggregation inhibitors excl. heparin	15	1.48 (0.88–2.48)	28	3.75 (2.55–5.51)
2	C01BC	Antiarrhythmics, class IC	1	0.58 (0.08–4.16)	2	1.52 (0.37–6.16)
3	C03BA [a]	Sulfonamides, plain, low-ceiling	2	0.92 (0.23–3.71)	5	3.09 (1.25–7.48)
4	C03EA [a]	Low-ceiling diuretics and potassium-sparing agents	3	0.93 (0.30–2.92)	5	2.04 (0.84–4.97)
5	C08DB [a]	Benzothiazepine derivatives	4	1.45 (0.54–3.91)	4	1.88 (0.70–5.08)
6	C09BA [a]	ACE inhibitors and diuretics	4	1.36 (0.50–3.66)	4	1.76 (0.65–4.76)
7	G03CA	Natural and semisynthetic estrogens, plain	1	0.22 (0.03–1.57)	7	2.05 (0.97–4.36)
8	G03FB	Progestogens and estrogens, sequential preparations	1	0.65 (0.09–4.65)	2	1.70 (0.42–6.91)
9	J01FA	Macrolides	6	0.57 (0.25–1.28)	25	3.26 (2.17–4.90)
10	L01BA	Folic acid analogues	2	1.12 (0.28–4.54)	3	2.20 (0.70–6.95)
11	L04AX	Other immunosuppresive agents	7	1.42 (0.67–3.02)	7	1.85 (0.87–3.93)
12	M01AC [a]	Oxicams	3	0.52 (0.17–1.63)	9	2.08 (1.07–4.05)
13	N02AX	Other opioids	5	0.71 (0.29–1.71)	10	1.87 (1.00–3.52)
14	N02CC	Selective $5HT_1$-receptor agonists	8	1.32 (0.65–2.67)	7	1.50 (0.71–3.17)
15	N06AX	Other antidepressants	17	0.81 (0.50–1.32)	32	2.06 (1.44–2.95)
16	N07BA	Antismoking agents	2	0.21 (0.05–0.83)	15	2.09 (1.25–3.51)
17	P01AB	Nitroimidazole derivatives	3	1.06 (0.34–3.31)	7	3.29 (1.54–7.02)
18	P01BA	Aminoquinolines	1	0.44 (0.06–3.13)	5	2.93 (1.20–7.17)
19	P01BC [a]	Quinine alkaloids	2	0.09 (0.02–0.37)	36	2.36 (1.68–3.31)
20	R05DA	Opium alkaloids and derivatives	1	0.33 (0.05–2.32)	5	2.16 (0.89–5.27)

n = number of reports; ROR = reporting odds ratio; CI = confidence interval
a) Effect on ion (transport) systems in kidney and/or ear tissues.

Appendix V Drug classes in group C: ROR kidney ≥ 1.50 and ROR ear < 1.50 or no reports on ear ADRs

No.	ATC5	ATC5 description	Kidney		Ear	
			n	ROR (95% CI)	n	ROR (95% CI)
1	A02BD [a]	Combinations for eradication of Helicobacter pylori	1	2.07 (0.28–15.2)	0	
2	A02BX	Other drugs for treatment of peptic ulcer	2	1.81 (0.44–7.40)	0	
3	A04AA	Serotonin ($5HT_3$) antagonists	2	7.37 (1.71–31.8)	0	
4	A06AG	Enemas	1	33.1 (3.00–365.6)	0	
5	A10BA	Biguanides	9	2.26 (1.16–1.41)	0	
6	A10BF	Alpha glucosidase inhibitors	1	3.86 (0.49–27.6)	0	
7	A10BG	Thiazolidinediones	10	4.91 (2.57–9.36)	0	
8	A11CC	Vitamin D and analogues	2	6.63 (1.55–28.4)	0	
9	A11GA	Ascorbic acid (vit C), plain	2	26.5 (5.14–136.9)	0	
10	A12AA	Calcium	2	2.50 (0.61–10.3)	0	
11	B01AB	Heparin group	7	1.81 (0.85–3.84)	1	0.33 (0.05–2.32)
12	B01AD	Enzymes	4	3.32 (1.21–9.08)	0	
13	B05AA	Blood substitutes and plasma protein fractions	1	3.31 (0.44–27.7)	0	
14	C01AA	Digitalis glycosides	4	3.09 (1.13–8.43)	1	0.96 (0.13–6.93)
15	C02KX	Other antihypertensives	8	5.55 (2.69–11.5)	0	
16	C03DA	Aldosterone antagonists	9	6.46 (3.25–12.8)	0	
17	C05CA	Bioflavonoids	3	4.23 (1.32–13.6)	0	
18	C09DA [a]	Angiotension II antagonists and diuretics	4	1.76 (0.65–4.75)	2	1.12 (0.28–4.53)
19	D07XA [a]	Corticosteroids, weak, other combinations	1	5.09 (0.67–39.0)	0	
20	D10BA	Retinoids for treatment of acne	3	1.53 (0.49–4.81)	0	
21	G02CA	Ergot alkaloids and oxytocin incl. analogues, in combination	1	6.02 (0.78–46.7)	0	
22	G02CB	Prolactine inhibitors	2	2.37 (0.58–9.71)	0	
23	G03BB	5-Androstanon (3) derivatives	1	66.2 (4.41–1059.8)	0	
24	G03HA	Antiandrogens, plain	3	2.97 (0.93–9.46)	0	
25	G04BE	Drugs used in erectile dysfunction	4	1.70 (0.63–4.60)	0	
26	G04CB	Testosterone-5-alpha reductase inhibitors	5	2.89 (1.18–7.09)	1	0.72 (0.10–5.16)
27	H01BA	Vasopressin and analogues	2	2.65 (0.64–10.9)	0	
28	H02AB [a]	Glucocorticoids	22	2.68 (1.74–4.13)	4	0.60 (0.22–1.61)
29	H05AA	Parathyroid hormones and analogues	1	3.48 (0.47–26.1)	0	
30	H05BX	Other anti-parathyroid agents	1	11.0 (1.33–91.8)	0	
31	J01DA	Cephalopsporins and related substances	2	14.7 (3.18–68.3)	0	

(Appendix V continued)

No.	ATC5	ATC5 description	Kidney		Ear	
			n	ROR (95% CI)	n	ROR (95% CI)
32	J01DD	Third-generation cephalosporins	3	9.16 (3.21–26.1)	0	
33	J01DH	Carbapenems	1	6.62 (0.85–51.8)	0	
34	J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	14	1.72 (1.01–2.94)	3	0.46 (0.15–1.45)
35	J01MA	Fluoroquinolones	27	2.13 (1.44–3.14)	13	1.29 (0.74–2.25)
36	J04AB	Antibiotics	7	15.6 (6.81–35.5)	0	
37	J04BA	Drugs for treatment of lepra	1	3.15 (0.42–23.5)	0	
38	J05AE	Protease inhibitors	11	7.14 (3.82–13.4)	0	
39	J05AF	Nucleoside reverse transcriptase inhibitors	11	7.74 (4.13–14.5)	0	
40	J05AG	Non-nucleoside reverse transcriptase inhibitors	5	4.00 (1.62–9.89)	0	
41	J05AR	Antivirals for treatment of HIV infections, combinations	1	3.01 (0.41–22.4)	0	
42	L01AA	Nitrogen mustard analogues	4	1.80 (0.67–4.88)	1	0.57 (0.08–4.09)
43	L01BB	Purine analogues	1	2.01 (0.27–14.7)	0	
44	L01BC	Pyrimidine analogues	24	1.72 (1.14–2.59)	1	0.09 (0.01–0.63)
45	L01CA	Vinca alkaloids and analogues	4	4.58 (1.66–12.6)	1	1.41 (0.19–10.2)
46	L01CB	Podophyllotoxin derivatives	3	3.32 (1.04–10.6)	0	
47	L01DB	Anthracyclines and related substances	7	2.57 (1.20–5.49)	1	0.46 (0.06–3.27)
48	L01DC	Other cytotoxic antibiotics	2	2.50 (0.61–10.3)	0	
49	L01XA	Platinum compounds	30	2.33 (1.61–3.37)	4	0.38 (0.14–1.02)
50	L02AB	Progestogens	1	5.52 (0.72–42.5)	0	
51	L02AE	Gonadotropin releasing hormone analogues	4	1.83 (0.68–4.95)	1	0.58 (0.08–4.14)
52	L03AA	Colony stimulating factors	1	2.65 (0.36–19.6)	0	
53	L03AC	Interleukins	2	3.31 (0.80–13.7)	0	
54	L04AA	Selective immunosuppressive agents	74	4.43 (3.47–5.66)	7	0.48 (0.23–1.00)
55	M01AB [a]	Acetic acid derivatives and related substances	41	1.86 (1.35–2.55)	17	0.96 (0.59–1.56)
56	M01AE [a]	Propionic acid derivatives	32	1.59 (1.11–2.27)	19	1.20 (0.76–1.90)
57	M01AH [a]	Coxibs	20	1.66 (1.06–2.60)	8	0.84 (0.42–1.69)
58	M01AX [a]	Other antiinflammatory and antirheumatic agents, non-steroidal	4	1.83 (0.68–4.95)	0	
59	M01CB	Gold preparations	7	10.9 (4.87–24.2)	0	
60	M03CA	Dantrolene and derivatives	1	22.1 (2.29–212.5)	0	
61	M04AA	Preparations inhibiting uric acid production	2	1.84 (0.45–7.51)	1	1.18 (0.16–8.47)

(Appendix V continued)

No.	ATC5	ATC5 description	Kidney		Ear	
			n	ROR (95% CI)	n	ROR (95% CI)
62	M04AB	Preparations increasing uric acid excretion	2	7.37 (1.71–31.8)	0	
63	M05BB	Bisphosphonates, combinations	3	2.49 (0.78–7.89)	1	1.05 (0.15–7.53)
64	N03AX	Other antiepileptics	17	1.58 (0.97–2.57)	10	1.19 (0.64–2.23)
65	N05AC	Phenothiazines with piperidine structure	1	2.07 (0.28–15.2)	0	
66	N05AN	Lithium	10	3.61 (1.90–6.84)	1	0.44 (0.06–3.15)
67	N06AF	Monoamine oxidase inhibitors, non-selective	1	1.65 (0.23–12.1)	0	
68	N06DX	Other anti–dementia drugs	2	8.29 (1.90–36.1)	0	
69	N07BB	Drugs used in alcohol dependence	2	1.70 (0.42–6.92)	0	
70	S01EA	Symphatomimetics in glaucoma therapy	1	3.15 (0.42–23.5)	0	
71	S01EE	Prostaglandin analogues	4	2.35 (0.86–6.38)	2	1.49 (0.37–6.06)
72	V03AE	Drugs for treatment of hyperkalemia and hyperphosphatemia	1	8.28 (1.03–66.3)	0	
73	V03AF	Detoxifying agents for antineoplastic treatment	7	5.12 (2.37–11.1)	0	

n = number of reports; ROR = reporting odds ratio; CI = confidence interval
a) Effect on ion (transport) systems in kidney and/or ear tissues.

Appendix VI Drug classes in group A (reference): ROR kidney < 1.50 and ROR ear < 1.50 or no reports on auricular sADRs

No.	ATC5	ATC5 description	Kidney		Ear	
			n	ROR (95% CI)	n	ROR (95% CI)
1	A02BA	H2-receptor antagonists	2	0.27 (0.07–1.08)	3	0.53 (0.17–1.64)
2	A02BC [a]	Proton pump inhibitors	20	0.98 (0.63–1.54)	9	0.56 (0.29–1.09)
3	A03FA	Propulsives	3	0.47 (0.15–1.46)	2	0.40 (0.10–1.62)
4	A06AD	Osmotically acting laxatives	1	0.82 (0.11–5.87)	1	1.06 (0.15–7.62)
5	A08AA	Centrally acting antiobesity products	2	0.61 (0.15–2.47)	2	0.79 (0.20–3.20)
6	A10AE	Insulins and analogues, long-acting	2	1.03 (0.25–4.15)	0	
7	A10BB	Sulfonamides, urea derivatives	1	0.18 (0.03–1.29)	4	0.95 (0.35–2.56)
8	B01AA	Vitamine K antagonists	6	0.96 (0.43–2.16)	2	0.41 (0.10–1.65)
9	B01AX	Other antithrombotic agents	1	1.41 (0.19–10.2)	0	
10	B03XA	Other antianemic preparations	2	1.11 (0.27–4.51)	0	
11	C01DA	Organic nitrates	1	0.26 (0.04–1.87)	1	0.34 (0.05–2.43)
12	C03AA [a]	Thiazides, plain	2	0.56 (0.14–2.25)	3	1.09 (0.35–3.42)
13	C07AA	Beta-blocking agents, non-selective	1	0.20 (0.03–1.41)	1	0.26 (0.04–1.83)
14	C07AB	Beta-blocking agents, selective	7	0.39 (0.19–0.83)	17	1.27 (0.78–2.06)
15	C07AG	Alpha and beta-blocking agents	1	0.86 (0.12–6.18)	1	1.11 (0.15–8.02)
16	C08CA [a]	Dihydropyridine derivatives	17	1.12 (0.69–1.81)	16	1.37 (0.83–2.26)
17	C08DA [a]	Phenylalkylamine derivatives	2	0.73 (0.18–2.96)	2	0.95 (0.24–3.85)
18	C09AA	ACE inhibitors, plain	37	1.46 (1.04–2.03)	16	0.79 (0.48–1.30)
19	C09CA	Angiotensin II antagonists, plain	20	1.40 (0.90–2.20)	15	1.36 (0.81–2.27)
20	C10AA	HMG CoA reductase inhibitors	45	1.26 (0.93–1.70)	15	0.52 (0.31–0.87)
21	C10AB	Fibrates	3	1.14 (0.36–3.58)	1	0.49 (0.07–3.48)
22	C10AC	Bile acid sequestrants	1	1.44 (0.20–10.4)	0	
23	C10AX	Other lipid modifying agents	1	0.67 (0.09–4.84)	1	0.88 (0.12–6.28)
24	D01BA	Antifungals for systemic use	4	0.29 (0.11–0.79)	5	0.48 (0.20–1.15)
25	D05BB	Retinoids for treatment of psoriasis	1	1.14 (0.16–8.25)	1	1.48 (0.20–10.7)
26	G02BA	Intrauterine contraceptives	1	0.21 (0.03–1.48)	0	
27	G03AA	Progestogens and estrogens, fixed combinations	4	0.34 (0.13–0.90)	1	0.11 (0.02–0.77)
28	G03AC	Progestogens	2	0.57 (0.14–2.28)	0	
29	G03DC	Estren derivatives	1	0.30 (0.04–2.14)	3	1.18 (0.38–3.70)
30	G03HB	Antiadrogens and estrogens	1	0.42 (0.06–2.97)	1	0.54 (0.08–3.85)
31	G04BD	Urinary antispasmodics	4	0.93 (0.35–2.51)	2	0.60 (0.15–2.42)
32	G04CA	Alpha-adrenoreceptor antagonists	7	1.09 (0.52–2.31)	7	1.42 (0.67–3.01)
33	H01AC	Somatropin and somatropin agonists	2	0.95 (0.23–3.82)	1	0.61 (0.08–4.35)
34	H03AA	Thyroid hormones	1	0.40 (0.06–2.88)	1	0.52 (0.07–3.74)
35	J01AA	Tetracyclines	9	0.67 (0.35–1.30)	13	1.28 (0.74–2.22)
36	J01CA	Penicillins with extended spectrum	6	0.50 (0.22–1.12)	2	0.21 (0.05–0.86)
37	J01CE	Beta-lactamase sensitive penicillins	1	0.29 (0.04–2.07)	3	1.14 (0.37–3.58)

(Appendix VI continued)

No.	ATC5	ATC5 description	Kidney		Ear	
			n	ROR (95% CI)	n	ROR (95% CI)
38	J01CF	Beta-lactamase resistent penicillins	2	1.00 (0.25–4.06)	1	0.64 (0.09–4.62)
39	J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	6	0.68 (0.30–1.52)	4	0.58 (0.22–1.56)
40	J01EA	Trimethoprim and derivatives	4	1.06 (0.39–2.85)	1	0.34 (0.05–2.41)
41	J01XE	Nitrofuran derivatives	5	0.64 (0.27–1.55)	6	1.01 (0.45–2.26)
42	J02AC	Triazole derivatives	6	0.88 (0.39–1.97)	2	0.37 (0.09–1.50)
43	J05AB	Nucleosides and nucleotides excl. reverse transcriptase inhibitors	5	1.45 (0.60–3.52)	3	1.11 (0.36–3.49)
44	J07AH	Meningococcal vaccines	4	1.24 (0.46–3.34)	1	0.39 (0.06–2.82)
45	J07BB	Influenza vaccines	1	0.33 (0.05–2.33)	1	0.42 (0.06–3.03)
46	J07BC	Hepatitis vaccines	6	1.23 (0.55–2.78)	4	1.06 (0.39–2.85)
47	J07BD	Morbilli vaccines	4	1.22 (0.45–3.29)	0	
48	J07BL	Yellow fever vaccines	1	1.03 (0.14–7.46)	0	
49	J07CA	Bacterial and viral vaccines, combined	3	0.25 (0.08–0.79)	4	0.44 (0.16–1.18)
50	L01CD	Taxanes	12	1.16 (0.65–2.05)	0	
51	L01XC	Monoclonal antibodies	6	1.06 (0.47–2.39)	0	
52	L01XE	Protein kinase inhibitors	1	0.87 (0.12–6.27)	0	
53	L01XX	Other neoplastic agents	11	1.29 (0.71–2.35)	1	0.15 (0.02–1.05)
54	L02BA	Anti-estrogens	1	0.45 (0.06–3.24)	1	0.59 (0.08–4.20)
55	L02BB	Anti-androgens	1	0.70 (0.10–5.00)	0	
56	L02BG	Enzyme inhibitors	1	0.48 (0.07–3.43)	0	
57	L03AX	Other cytokines and immunomodulators	2	1.34 (0.33–5.43)	0	
58	M05BA	Bisphosphonates	3	0.38 (0.12–1.18)	8	1.33 (0.66–2.69)
59	N02AA	Natural opium alkaloids	2	1.03 (0.26–4.19)	1	0.66 (0.09–4.76)
60	N02BE	Anilides	4	1.05 (0.39–2.84)	1	0.34 (0.05–2.40)
61	N02CX	Other antimigraine preparations	1	0.48 (0.07–3.40)	0	
62	N03AE	Benzodiazepine derivatives	1	1.18 (0.16–8.55)	0	
63	N03AF	Carboxamide derivatives	4	0.58 (0.22–1.56)	7	1.33 (0.63–2.83)
64	N03AG	Fatty acid derivatives	6	0.86 (0.38–1.93)	7	1.31 (0.62–2.78)
65	N04BC	Dopamine agonists	3	1.17 (0.37–3.67)	0	
66	N05AD	Butyrophenone derivatives	1	0.39 (0.05–2.78)	1	0.50 (0.07–3.60)
67	N05AH	Diazepines, oxazepines and thiazepines	6	0.58 (0.26–1.29)	4	0.50 (0.19–1.33)
68	N05AX	Other antipsychotics	2	0.30 (0.08–1.22)	1	0.20 (0.03–1.39)
69	N05BA	Benzodiazepine derivatives	1	0.24 (0.03–1.71)	3	0.94 (0.30–2.95)
70	N05CD	Benzodiazepine derivatives	1	0.43 (0.06–3.11)	0	
71	N05CF	Benzodiazepine related drugs	1	0.47 (0.07–3.38)	1	0.61 (0.09–4.38)
72	N06AA	Non-selective monoamine reuptake inhibitors	8	0.76 (0.38–1.54)	7	0.87 (0.41–1.83)

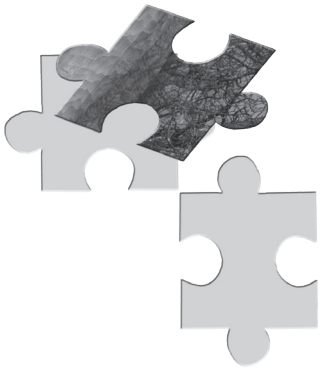
(Appendix VI continued)

No.	ATC5	ATC5 description	Kidney		Ear	
			n	ROR (95% CI)	n	ROR (95% CI)
73	N06AB	Selective serotonin reuptake inhibitors	22	0.50 (0.33–0.77)	47	1.47 (1.09–1.98)
74	N06BA	Centrally acting sympathomimetics	2	0.52 (0.13–2.11)	1	0.34 (0.05–2.41)
75	N06DA	Anticholinesterases	2	1.15 (0.28–4.67)	0	
76	N07CA	Antivertigo preparations	1	0.25 (0.04–1.82)	3	1.00 (0.32–3.13)
77	P01BB	Biguanides	2	0.43 (0.11–1.73)	3	0.84 (0.27–2.63)
78	R03AC	Selective beta-2-adrenoreceptor agonists	1	0.13 (0.02–0.96)	1	0.17 (0.02–1.25)
79	R03BA [a]	Glucocorticoids	2	0.21 (0.05–0.83)	1	0.13 (0.02–0.95)
80	R03BB	Anticholinergics	5	1.18 (0.49–2.87)	3	0.91 (0.29–2.85)
81	R03DA	Xanthines	1	0.30 (0.04–2.15)	0	
82	R03DC	Leukotriene receptor anatgonists	1	0.48 (0.07–3.45)	0	
83	R05CB	Mucolytics	4	1.24 (0.46–3.34)	0	
84	R06AE	Piperazine derivatives	1	0.21 (0.03–1.52)	4	1.12 (0.42–3.02)
85	R06AX	Adrenergics, inhalants	4	0.43 (0.16–1.15)	6	0.84 (0.38–1.89)
86	S01ED	Beta–blocking agents	1	0.51 (0.07–3.67)	2	1.34 (0.33–5.43)

n = number of reports; ROR = reporting odds ratio; CI = confidence interval
a) Effect on ion (transport) systems in kidney and/or ear tissues.

4.2

Use of serotonergic drugs and the risk of bleeding



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ABSTRACT

Background

Epidemiological studies have suggested an association between antidepressant drug use and an increased risk of bleeding. As serotonin (5-HT) seems to play a role in this adverse effect, drugs with affinity for the serotonin transporter and/or 5-HT_{2A} receptor, such as antidepressants, antipsychotics and ergoline derivatives, could interfere with haemostasis.

Objective

To assess the association between serotonergic drug use and the risk of bleeding. Serotonergic drugs were classified in the traditional therapeutically-based way as well as according to the degree of affinity for the 5-HT transporter (5-HTT) and 5-HT_{2A} receptor.

Methods

A case-control study was conducted using data from the Dutch PHARMO Record Linkage System. Cases were patients with a first hospital admission for a female genital tract, gastrointestinal or intracranial bleeding between 1997 and 2008. Up to two controls were matched to each case on gender, age, geographical area and index date. Analyses were stratified for prevalent and new users of serotonergic drugs and for patients with a low or high bleeding risk profile.

Results

The study population comprised 28 289 cases and 50 786 matched controls. Female genital tract bleedings were seen most frequently (47.4%), followed by gastrointestinal bleedings (32.7%). Current use of antidepressants was associated with an increased risk of female genital tract (odds ratio [OR] 2.37; 95% confidence interval [CI] 2.12−2.64), gastrointestinal (OR 1.36; 95% CI 1.20−1.53) and intracranial bleeding (OR 1.41; 95% CI 1.21−1.64). Use of antipsychotics was associated with gastrointestinal (OR 1.79; 95% CI 1.41−2.27) and intracranial bleeding (OR 1.44; 95% CI 1.06−1.95). In addition, current use of ergoline derivatives was only associated with female genital tract bleeding (OR 2.29; 95% CI 1.28−4.08). New users of a serotonergic drug had an increased risk for gastrointestinal and intracranial bleeding compared to prevalent users. The risk estimates for the association between serotonergic drugs and gastrointestinal and intracranial bleeding was not different in patients with a low or high bleeding risk profile, whereas the risk of female genital tract bleeding was increased in low-risk patients using a serotonergic drug compared to non-users.

Conclusions

Current use of antidepressants and antipsychotics, in contrast to use of ergoline derivatives, was associated with gastrointestinal and intracranial bleeding. The unexpected association between antipsychotic drug use and the occurrence of gastrointestinal bleeding may warrant further research. No clear association was found between the degree of affinity for the 5-HTT or 5-HT_{2A} receptor and female genital tract, gastrointestinal and intracranial bleeding.

INTRODUCTION

In the 1990s, case reports appeared on abnormal bleeding after the use of selective serotonin reuptake inhibitors (SSRIs),[1-6] followed by observational studies providing evidence concerning the risk of abnormal bleeding among patients on antidepressant therapy.[7-16] Several studies have suggested that particularly SSRI use is associated with (gastrointestinal) bleeding, although the magnitude of the risk estimate differed between studies. Mechanistically, serotonin (5-hydroxytryptamine, 5-HT) seems to play a role in this adverse effect. Peripheral 5-HT is stored in platelets and released in case of a thrombotic event, stimulating platelet aggregation.[17,18] As mature platelets are not capable to synthesise 5-HT, these are dependent on the reuptake of 5-HT from plasma. Drugs with inhibitory effects on the serotonin reuptake transporter (5-HTT) can affect the platelet serotonin content or its release from dense granules and therefore affect primary haemostasis.[19,20] This makes that serotonergic medication would not cause bleeding as such, but could influence the duration of bleeding and/or volume of blood loss in conditions with underlying diseases or with concomitant use of drugs known to cause bleedings, such as non-steroidal anti-inflammatory drugs (NSAIDs). Previous work of our group showed an association between use of serotonergic antidepressants and the need for peri-operative blood transfusion in orthopaedic surgery, whereas there was no association with non-serotonergic antidepressants.[21] Also, Meijer *et al.* showed an association between the degree of serotonin reuptake inhibition and the risk of bleeding in a cohort of new antidepressant users.[10]

Not only the function of the 5-HTT in platelet serotonin transport is well characterised, but also the role of the 5-HT_{2A} receptor, which is the only serotonergic receptor identified on the platelet membrane.[22] The 5-HT_{2A} receptor mediates 5-HT-induced platelet aggregation.[23] By interacting with the 5-HTT and the 5-HT_{2A} receptor, drugs with serotonergic properties play a major role in regulating extracellular 5-HT concentration. Although many studies have focused on the association between the use of antidepressants and risk of bleeding events, little is known with respect to the use of other serotonergic drugs. Besides antidepressants, also drugs with other therapeutic indications, such as antipsychotics and antimigraine drugs act as antagonists or agonists on the 5-HTT and 5-HT receptors.[17,24] Randomised clinical trials on the atypical antipsychotics risperidone and olanzapine (both with high affinity for the 5-HT_{2A} receptor) in elderly patients with dementia revealed an increased incidence of cerebrovascular adverse events, including cerebral haemorrhage.[25,26] Furthermore, there are some case reports describing a possible association between abnormal bleeding and

serotonergic drugs, other than antidepressants,[27,28] and observational studies in which the risk of cerebrovascular events, including intracranial haemorrhage, in elderly antipsychotic users, has been assessed.[29,30]
Therefore, the objective of this study was to assess the association between use of antidepressants, antipsychotics and ergoline derivatives (partial agonists for the 5-HT_{2A} receptor, such as ergotamine and lisuride) and the risk of three different bleeding types, namely female genital tract, gastrointestinal and intracranial bleeding. Classification of serotonergic drugs was made in the traditional therapeutically-based way as well as according to the degree of affinity for the 5-HTT and 5-HT_{2A} receptor.

METHODS

Setting

Data for this study were obtained from the PHARMO Record Linkage System (http://www.pharmo.nl). The PHARMO RLS includes the demographic details and complete medication history of more than two million community-dwelling residents of more than twenty-five population-defined areas in the Netherlands from 1985 onwards, further linked to hospital admission records as well as several other health registries, including pathology, clinical laboratory findings and general practitioner data. Since virtually all patients in the Netherlands are registered with a single community pharmacy, independent of prescriber, pharmacy records are virtually complete with regard to prescription drugs.[31]
For this study, drug dispensing data and hospitalisation data were used. The computerised drug dispensing histories contain information concerning the dispensed drug, dispensing date, the prescriber, amount dispensed, prescribed dosage regimen, and the estimated duration of use. The duration of use of each dispensed drug is estimated by dividing the number of dispensed units by the prescribed number of units to be used per day. Drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification.[32] The Hospital Admission Register comprises all hospital admissions in the Netherlands, including detailed information concerning the primary and secondary discharge diagnoses, diagnostic, surgical and treatment procedures, type and frequency of consultations with medical specialists and dates of hospital admission and discharge. All diagnoses are coded according to the International Classification of Diseases, 9th edition (ICD-9-CM).

Study population

Within the PHARMO RLS, a case-control study was conducted. Cases were defined as patients 18 years and older with a first hospital admission for gastrointestinal, intracranial and female genital tract bleeding (for ICD-9 codes see Appendix I) in the period January 1998 to December 2007. The date of hospital admission was the index date. For each case, up to two control patients without a history of a bleeding during the study period were matched on gender, year of birth, geographical area, index date and duration of exposure history in the PHARMO RLS prior to the index date. Both cases and controls were eligible for inclusion if they had a minimum period of 365 days of history in the PHARMO RLS prior to the index date.

Exposure definition and assessment

For each case and control, all prescriptions for antidepressants, antipsychotics and ergoline derivatives (Appendix II) before the index date were identified.[33,34] Serotonergic drugs were classified according to their pharmacotherapeutical group, as well as categorised on basis of their affinity for the 5-HTT and the 5-HT_{2A} receptor (high: $K_i < 10$ nM; medium: K_i 10−1000 nM; low: $K_i > 1000$ nM; or no data). Lower affinity constants (K_i) reflect a higher affinity and therefore a higher agonism or antagonism of the serotonin-induced effects. K_i data were obtained from the Psychoactive Drug Screening Program (PDSP) funded by the National Institute of Mental Health (NIMH).[35] This programme provides screening of psychoactive compounds for pharmacological and functional activity at cloned human or rodent CNS receptors, channels, and transporters. The database contains more than 47 000 K_i values and accrues new information on a regular basis. The K_i values were taken from experiments with human receptor cell lines. When there was more than one K_i value for a specific serotonergic drug – receptor interaction, an average value was calculated. When no K_i value from a human receptor cell line was available, a K_i for a drug-animal receptor interaction was taken.

Exposure to serotonergic drugs was classified according to the timing of use in relation to the index date. Current users were defined as patients with a prescription for a serotonergic drug within 90 days before the index date. Recent users had a last prescription between 91 and 180 days before the index date. A prescription for a serotonergic drug between 181 and 365 was considered as past use and distant past users had a prescription for a serotonergic drug more than 365 days before the index date. Exposure to serotonergic drugs was categorised as no use when there was no recorded use of serotonergic medication from the first entry in the PHARMO RLS until the index date. Among current users, we

assessed whether patients were new users of serotonergic medication, defined as not having a prescription in a 6-month time window before the dispensing date of the previous prescription.

Data analysis

The strength of the association between use of serotonergic drugs and the occurrence of female genital tract, gastrointestinal and intracranial bleeding was estimated using conditional logistic regression and was expressed as crude and adjusted odds ratios (OR) with 95% confidence intervals (CI). Potential confounders were prescription drugs that have been associated with bleeding and drugs used in the treatment for medical conditions associated with bleeding, such as NSAIDs, oral glucocorticoids, proton pump inhibitors and platelet aggregation inhibitors. Use of concomitant drugs was recorded within a 6-month period prior to the index date, as well as hospitalisations (ever) for several comorbidities. For each bleeding type, a separate model was fitted. Covariates were included in the regression model if they induced a 5% change or more in the crude matched OR for current use of serotonergic drugs. We stratified our analyses for patients with a low or high bleeding risk profile, defined as use of NSAIDs, vitamin K antagonists, heparin, platelet aggregation inhibitors, direct and other thrombin inhibitors, antifibrinolytics or vitamin K and other haemostatics in a 6-month time window before the index date. All statistical analyses were performed using SPPS for Windows (version 16.0.1; SPSS Inc. Chicago, Illinois).

RESULTS

The study population comprised 28 289 patients with a hospital admission for a female genital tract, gastrointestinal or intracranial bleeding during the study period and 50 786 matched controls. The characteristics of cases and controls are shown in Table 1. Female genital tract bleedings were the most frequent type of bleeding (n = 13 399; 47.4%), followed by gastrointestinal (n = 9239; 32.7%) and intracranial bleedings (n = 5651; 20.0%). Frequently prescribed drugs among patients with a gastrointestinal and intracranial bleeding and their matched controls were NSAIDs, platelet aggregation inhibitors, diuretics and statins. Patients hospitalised for a female genital tract bleeding differed from patients with gastrointestinal and intracranial bleeding with respect to age (81% were younger than 60), fewer prior hospital admissions for other causes and less comedication (except use of NSAIDs and iron preparations).

Table 1 Characteristics of cases and control patients

	Female genital tract bleeding			Gastrointestinal bleeding			Intracranial bleeding		
	Cases n=13 399 (100%)	Controls n=25 683 (100%)		Cases n=9 239 (100%)	Controls n=15 605 (100%)		Cases n=5 651 (100%)	Controls n=9 498 (100%)	
Age (years)									
18–39	2 932 (21.9%)	5 748 (22.4%)	NA	782 (8.5%)	1 514 (9.7%)	NA	242 (4.3%)	473 (5.0%)	NA
40–59	7 991 (59.6%)	15 100 (58.8%)	NA	2 115 (22.9%)	3 867 (24.8%)	NA	1 403 (24.8%)	2 572 (27.1%)	NA
60–79	2 120 (15.8%)	4 126 (16.1%)	NA	4 135 (44.8%)	6 806 (43.6%)	NA	2 781 (49.2%)	4 594 (48.4%)	NA
≥ 80	356 (2.7%)	709 (2.8%)	NA	2 207 (23.9%)	3 418 (21.9%)	NA	1 225 (21.7%)	1 859 (19.6%)	NA
Gender (female)	13 399 (100%)	25 683 (100%)	NA	4 362 (47.2%)	7 421 (47.6%)	NA	2 978 (52.7%)	5 063 (53.3%)	NA
Hospital admissions (ever) before index date									
angina pectoris	75 (0.6%)	106 (0.4%)		314 (3.4%)	276 (1.8%)		127 (2.2%)	193 (2.0%)	NS
cancer	419 (3.1%)	1 237 (4.8%)		920 (10.0%)	667 (4.3%)		343 (6.1%)	415 (4.4%)	
cardiac dysrhythmia	117 (0.9%)	165 (0.6%)		472 (5.1%)	418 (2.7%)		289 (5.1%)	246 (2.6%)	
cataract	273 (2.0%)	472 (1.8%)	NS	943 (10.2%)	1 268 (8.1%)		578 (10.2%)	720 (7.6%)	
heart faliure	47 (0.4%)	129 (0.5%)		411 (4.4%)	242 (1.6%)		144 (2.5%)	127 (1.3%)	
myocardial infarction	60 (0.4%)	101 (0.4%)	NS	322 (3.5%)	296 (1.9%)		131 (2.3%)	169 (1.8%)	
peptic ulcer	6 (0.0%)	19 (0.1%)	NS	154 (1.7%)	24 (0.2%)		20 (0.4%)	14 (0.1%)	
stroke	25 (0.2%)	66 (0.3%)	NS	125 (1.4%)	98 (0.6%)		148 (2.6%)	65 (0.7%)	

(Table 1 continued)

	Female genital tract bleeding			Gastrointestinal bleeding			Intracranial bleeding		
	Cases n=13 399 (100%)	**Controls n=25 683 (100%)**		**Cases n=9 239 (100%)**	**Controls n=15 605 (100%)**		**Cases n=5 651 (100%)**	**Controls n=9 498 (100%)**	
Comedication within 6 months before index date									
NSAIDs	4 340 (32.4%)	4 365 (17.0%)		2 454 (26.6%)	2 686 (17.2%)		1 201 (21.3%)	1 712 (18.0%)	
acetylsalicylic acid, low dose	391 (2.9%)	555 (2.2%)		1 427 (15.4%)	1 663 (10.7%)		771 (13.6%)	982 (10.3%)	
platelet aggregation inhibitors	692 (5.2%)	1 084 (4.2%)		2 539 (27.5%)	2 959 (19.0%)		1 438 (25.4%)	1 788 (18.8%)	
diuretics	1 141 (8.5%)	1 682 (6.5%)		2 421 (26.2%)	2 570 (16.5%)		1 055 (18.7%)	1 594 (16.8%)	NS
proton pump inhibitors	1 268 (9.5%)	1 720 (6.7%)		2 227 (24.1%)	1 797 (11.5%)		729 (12.9%)	1 094 (11.5%)	
oral antidiabetic drugs	589 (4.4%)	863 (3.4%)		1 141 (12.3%)	1 373 (8.8%)		513 (9.1%)	860 (9.1%)	NS
statins	778 (5.8%)	955 (3.7%)		1 615 (17.5%)	2 136 (13.7%)		880 (15.6%)	1 328 (14.0%)	
vitamin K antagonists	282 (2.1%)	406 (1.6%)		1 465 (15.9%)	989 (6.3%)		950 (16.8%)	591 (6.2%)	
oral glucocorticoids	972 (7.3%)	1 480 (5.8%)		1 208 (13.1%)	1 209 (7.7%)		524 (9.3%)	817 (8.6%)	NS
iron preparations	1 516 (11.3%)	517 (2.0%)		698 (7.6%)	303 (1.9%)		169 (3.0%)	157 (1.7%)	
paracetamol	504 (3.8%)	939 (3.7%)	NS	1 111 (12.0%)	893 (5.7%)		462 (8.2%)	535 (5.6%)	

NA = not applicable; NS = not significant ($p > 0.05$); NSAIDs = non-steroidal anti-inflammatory drugs

Table 2 Association between current use of one serotonergic drug and different bleeding types

Female genital tract bleeding			
Drug classes	**Cases n=13 399 (100%)**	**Controls n=25 683 (100%)**	**Adjusted [a] OR (95% CI)**
Antidepressant drugs	929 (6.9%)	703 (2.7%)	2.37 (2.12–2.64)
SSRIs	561 (4.2%)	427 (1.7%)	2.30 (2.01–2.64)
TCAs	190 (1.4%)	154 (0.6%)	2.12 (1.69–2.66)
other antidepressants	178 (1.3%)	122 (0.5%)	2.50 (1.95–3.20)
Antipsychotic drugs	72 (0.5%)	139 (0.5%)	0.89 (0.66–1.21)
phenothiazines	13 (0.1%)	19 (0.1%)	1.07 (0.51–2.26)
butyrophenones	11 (0.1%)	60 (0.2%)	0.30 (0.15–0.58)
other antipsychotics	48 (0.4%)	60 (0.2%)	1.48 (0.99–2.22)
Ergoline derivatives	33 (0.2%)	21 (0.1%)	2.29 (1.28–4.08)
Gastrointestinal bleeding			
Drug classes	**Cases n=9 239 (100%)**	**Controls n=15 605 (100%)**	**Adjusted [b] OR (95% CI)**
Antidepressant drugs	622 (6.7%)	701 (4.5%)	1.36 (1.20–1.53)
SSRIs	338 (3.7%)	386 (2.5%)	1.39 (1.19–1.63)
TCAs	174 (1.9%)	190 (1.2%)	1.29 (1.03–1.61)
other antidepressants	110 (1.2%)	125 (0.8%)	1.25 (0.95–1.65)
Antipsychotic drugs	171 (1.9%)	147 (0.9%)	1.79 (1.41–2.27)
phenothiazines	22 (0.2%)	13 (0.1%)	2.99 (1.47–6.09)
butyrophenones	94 (1.0%)	74 (0.5%)	1.79 (1.29–2.47)
other antipsychotics	55 (0.6%)	60 (0.4%)	1.49 (1.01–2.19)
Ergoline derivatives	22 (0.2%)	18 (0.1%)	1.80 (0.94–3.45)
Intracranial bleeding			
Drug classes	**Cases n=5 651 (100%)**	**Controls n=9 498 (100%)**	**Adjusted [c] OR 95% CI**
Antidepressant drugs	372 (6.6%)	429 (4.5%)	1.41 (1.21–1.64)
SSRIs	196 (3.5%)	223 (2.3%)	1.39 (1.13–1.70)
TCAs	108 (1.9%)	126 (1.3%)	1.35 (1.03–1.78)
other antidepressants	68 (1.2%)	80 (0.8%)	1.46 (1.03–2.05)
Antipsychotic drugs	90 (1.6%)	97 (1.0%)	1.44 (1.06–1.95)
phenothiazines	16 (0.3%)	12 (0.1%)	1.48 (0.92–2.38)
butyrophenones	41 (0.7%)	38 (0.4%)	1.29 (0.81–2.06)
other antipsychotics	33 (0.6%)	47 (0.5%)	1.26 (0.76–2.09)
Ergoline derivatives	14 (0.2%)	24 (0.3%)	0.93 (0.47–1.85)

(legend Table 2)

OR = odds ratio; CI = confidence interval; SSRIs = selective serotonin reuptake inhibitors; TCAs = tricyclic antidepressants

a) Adjusted for use of non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors and iron preparations (6 months before index date).
b) Adjusted for use of NSAIDs, proton pump inhibitors and paracetamol (6 months before index date).
c) Adjusted for use of NSAIDs, platelet aggregation inhibitors and vitamin K antagonists (6 months before index date).

After adjustment for confounders, current use of serotonergic medication was associated with an increased risk of hospitalisation for female genital tract (OR 2.08; 95% CI 1.89−2.28), gastrointestinal (OR 1.49; 95% CI 1.35−1.65) and intracranial bleeding (OR 1.42; 95% CI 1.16−1.49). Current use of two or more serotonergic drugs increased the risk of a female genital tract and gastrointestinal bleeding approximately 2.5-fold compared to non-use (data not shown). The association between current use of a serotonergic drug, classified into drug classes, and the risk of female genital tract, gastrointestinal and intracranial bleeding is shown in Table 2. Current use of antidepressants was associated with an increased risk of all three bleeding types, whereas current use of an antipsychotic drug was associated with the occurrence of gastrointestinal and intracranial bleeding. In addition, current use of ergoline derivatives, which was not associated with gastrointestinal (OR 1.80; 95% CI 0.94−3.45) and intracranial bleeding (OR 0.93; 95% CI 0.47−1.85), increased the risk of female genital tract bleeding (OR 2.29; 95% CI 1.28−4.08). No differences were found between the different antidepressant and antipsychotic drug classes and the occurrence of bleedings.

The risk of gastrointestinal and intracranial bleeding was higher in new users of antidepressants and antipsychotics compared to prevalent users. Antidepressant drug use was not associated with female genital tract bleeding in new users in contrast to prevalent users (Table 3). In Table 4, the association is shown between the degree of affinity for the 5-HTT and 5-HT_{2A} receptor and female genital tract, gastrointestinal and intracranial bleeding. No association between the degree of affinity for the 5-HTT and 5-HT_{2A} receptor and female genital tract, gastrointestinal or intracranial bleeding was found. Table 5 shows the results of stratification according to high or low bleeding risk profiles. The risk of female genital tract and gastrointestinal bleeding was statistically significantly increased in both low bleeding risk and high bleeding risk patients using a serotonergic drug compared to non-users. The risk estimates for gastrointestinal bleeding did not differ between the two groups. In the low risk group, the risk of female genital tract bleeding was increased in patients using a serotonergic drug compared to non-users (OR 2.50; 95% CI 2.21−2.83), whereas the risk estimate for patients in

Table 3 Association between serotonergic drugs and bleeding for prevalent and new users

Female genital tract bleeding			
Drug classes	**Cases n=13 399 (100%)**	**Controls n=25 683 (100%)**	**Adjusted [a] OR (95% CI)**
Antidepressants			
prevalent users	909 (6.8%)	671 (2.6%)	2.43 (2.18–2.72)
new users	20 (0.1%)	32 (0.1%)	1.20 (0.67–2.18)
Antipsychotics			
prevalent users	70 (0.5%)	113 (0.4%)	1.14 (0.83–1.57)
new users	2 (0.0%)	26 (0.1%)	0.14 (0.03–0.61)
Ergoline derivatives			
prevalent users	28 (0.2%)	18 (0.1%)	2.30 (1.23–4.28)
new users	5 (0.0%)	3 (0.0%)	3.83 (0.80–18.4)
Gastrointestinal bleeding			
Drug classes	**Cases n=9 239 (100%)**	**Controls n=15 605 (100%)**	**Adjusted [b] OR (95% CI)**
Antidepressants			
prevalent users	587 (6.4%)	685 (4.4%)	1.34 (1.19–1.51)
new users	35 (0.4%)	16 (0.1%)	3.06 (1.63–5.74)
Antipsychotics			
prevalent users	143 (1.5%)	137 (0.9%)	1.67 (1.30–2.15)
new users	28 (0.3%)	10 (0.1%)	4.22 (1.98–9.00)
Ergoline derivatives			
prevalent users	22 (0.2%)	17 (0.1%)	1.94 (1.00–3.75)
new users	0 (0.0%)	1 (0.0%)	NE
Intracranial bleeding			
Drug classes	**Cases n=5 651 (100%)**	**Controls n=9 498 (100%)**	**Adjusted [c] OR (95% CI)**
Antidepressants			
prevalent users	359 (6.4%)	421 (4.4%)	1.40 (1.20–1.63)
new users	13 (0.2%)	8 (0.1%)	2.30 (0.90–5.88)
Antipsychotics			
prevalent users	71 (1.3%)	93 (1.0%)	1.24 (0.89–1.72)
new users	19 (0.3%)	4 (0.0%)	6.43 (2.09–19.8)
Ergoline derivatives			
prevalent users	10 (0.2%)	24 (0.3%)	0.70 (0.33–1.51)
new users	4 (0.1%)	0 (0.0%)	NE

(*legend Table 3*)

OR = odds ratio; CI = confidence interval; NE = not estimable

a) Adjusted for use of non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors and iron preparations (6 months before index date).
b) Adjusted for use of NSAIDs, proton pump inhibitors and paracetamol (6 months before index date).
c) Adjusted for use of NSAIDs, platelet aggregation inhibitors and vitamin K antagonists (6 months before index date).

the high risk group was considerably lower (OR 1.58; 95% CI 1.37−1.83). The risk for intracranial bleeding was not increased in the group of patients with a low risk group in contrast to the high risk group.

DISCUSSION

In this study, we found that not only use of antidepressants, but also use of antipsychotic drugs was associated with an increased risk of hospital admission for gastrointestinal bleeding. Furthermore, antidepressant drugs were also associated with an increased risk of female genital tract and intracranial bleeding. Ergoline derivatives were only associated with female genital tract bleeding. After stratification on high and low bleeding risk profile, the association between current use of a serotonergic drug and female genital tract bleeding was statistically significantly increased among patients with a low bleeding risk.

We performed stratified analyses on bleeding types because of their different aetiology. Gastrointestinal and intracranial bleedings are often caused by trauma (*e.g.* vascular rupture or peptic ulcers and erosions) in contrast to female genital tract bleedings which usually result from hormonally related or structural gynaecologic disorders. We assessed the association between current use of antidepressants, antipsychotics and ergoline derivatives and female genital tract, gastrointestinal and intracranial bleeding. Although other observational studies did not find an association between use of SSRIs and risk of haemorrhagic stroke,[11,36,37] we found a statistically significantly increased risk of intracranial bleeding associated with the use of SSRIs (OR 1.39; 95% CI 1.19−1.63). Antipsychotic drugs were associated with the risk of gastrointestinal (OR 1.79; 95% CI 1.41−2.27) and intracranial bleeding (OR 1.44; 95% CI 1.06−1.95). Whether the observed association between antipsychotics with affinity for the 5-HT_{2A} receptor, but not for the 5-HTT and the risk of gastrointestinal and intracranial bleeding is a result of decreased platelet aggregation is not clear. Serotonin, 5-HTT and the 5-HT_{2A} receptor play a role in platelet aggregation. Drugs with affinity for this transporter or receptor could therefore affect haemostasis. Ergoline derivatives, a group with (partial) agonistic

Table 4 Association between the affinity for the 5-HTT and the 5-HT2A receptor and different bleeding types

Female genital tract bleeding			
Drug classes	**Cases n=13 399 (100%)**	**Controls n=25 683 (100%)**	**Adjusted [a] OR (95% CI)**
Antidepressants			
5-HTT affinity			
high	619 (4.6%)	461 (1.8%)	2.43 (2.12–2.77)
medium	240 (1.8%)	176 (0.7%)	2.39 (1.94–2.94)
low	64 (0.5%)	61 (0.2%)	1.91 (1.31–2.79)
Antipsychotics			
5-HT_{2A} receptor affinity			
high	20 (0.1%)	43 (0.2%)	0.87 (0.50–1.51)
medium	28 (0.2%)	61 (0.2%)	0.89 (0.55–1.42)
low	0 (0.0%)	2 (0.0%)	NE
Ergoline derivatives			
5-HT_{2A} receptor affinity			
high	28 (0.2%)	13 (0.1%)	3.49 (1.72–7.07)
medium	5 (0.0%)	8 (0.0%)	0.98 (0.31–3.11)
Gastrointestinal bleeding			
	Cases n=9 239 (100%)	**Controls n=15 605 (100%)**	**Adjusted [b] OR (95% CI)**
Antidepressants			
5-HTT affinity			
high	360 (3.9%)	429 (2.7%)	1.37 (1.17–1.59)
medium	200 (2.2%)	199 (1.3%)	1.45 (1.17–1.78)
low	56 (0.6%)	69 (0.4%)	1.19 (0.82–1.73)
Antipsychotics			
5-HT_{2A} receptor affinity			
high	70 (0.8%)	55 (0.4%)	2.01 (1.38–2.93)
medium	79 (0.9%)	65 (0.4%)	1.88 (1.32–2.67)
low	2 (0.0%)	0 (0.0%)	NE
Ergoline derivatives			
5-HT_{2A} receptor affinity			
high	5 (0.1%)	6 (0.0%)	1.38 (0.40–4.73)
medium	17 (0.2%)	12 (0.1%)	2.06 (0.95–4.46)

(Table 4 continued)

Intracranial bleeding			
	Cases n=5 651 (100%)	**Controls n=9 498 (100%)**	**Adjusted [c] OR (95% CI)**
Antidepressants			
5-HTT affinity			
high	217 (3.8%)	247 (2.6%)	1.45 (1.19–1.76)
medium	110 (1.9%)	135 (1.4%)	1.27 (0.97–1.66)
low	42 (0.7%)	45 (0.5%)	1.63 (1.04–2.54)
Antipsychotics			
5-HT_{2A} receptor affinity			
high	26 (0.5%)	38 (0.4%)	1.20 (0.71–2.02)
medium	48 (0.8%)	32 (0.3%)	2.16 (1.35–3.46)
low	0 (0.0%)	1 (0.0%)	NE
Ergoline derivatives			
5-HT_{2A} receptor affinity			
high	6 (0.1%)	8 (0.0%)	1.44 (0.48–4.30)
medium	8 (0.1%)	16 (0.2%)	0.74 (0.30–1.78)

OR = odds ratio; CI = confidence interval; 5-HTT = serotonin reuptake transporter; 5-HT = serotonin; NE = not estimable

a) Adjusted for use of non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors and iron preparations (6 months before index date).
b) Adjusted for use of NSAIDs, proton pump inhibitors and paracetamol (6 months before index date).
c) Adjusted for use of NSAIDs, platelet aggregation inhibitors and vitamin K antagonists (6 months before index date).

effect on the 5-HT_{2A} receptor, increased the risk of female genital tract bleeding. Among the ergoline derivatives, ergotamine preparations comprised 50% of all dispensings. Ergotamine is used in the treatment of migraine. The occurrence of migraine is related to the female menstrual cycle, probable due to a fall in oestrogen level. The association between current use of ergoline derivatives and female genital tract bleeding is therefore unsurprising. No association was found between the use of ergoline derivatives and gastrointestinal and intracranial bleeding. In theory, antagonists of the 5-HT_{2A} receptor, such as ketanserin, should be associated with bleeding complications. Unfortunately, the number of patients using 5-HT_{2A} receptor antagonists was too low to be able to evaluate an association with an increased risk of bleeding. In new users, the dissimilarity in results between gastrointestinal and intracranial bleeding on the one side and to female genital tract bleedings on the other side is obvious. No association was observed between use of an antidepressant or antipsychotic drug and female genital tract bleeding in new users. The association between serotonergic drugs

Table 5 Association between current use of serotonergic drugs and bleeding, stratified according to risk profile [a]

	Female genital tract bleeding	Gastrointestinal bleeding	Intracranial bleeding
	Adjusted [b] OR (95% CI)	Adjusted [c] OR (95% CI)	OR (95% CI)
Low risk profile			
no use	1.00 (reference)	1.00 (reference)	1.00 (reference)
current use	2.50 (2.21–2.83)	1.45 (1.24–1.70)	1.20 (0.99–1.45)
High risk profile			
no use	1.00 (reference)	1.00 (reference)	1.00 (reference)
current use	1.58 (1.37–1.83)	1.44 (1.26–1.65)	1.63 (1.38–1.92)

OR = odds ratio; CI = confidence interval

a) High risk profile: use of non-steroidal anti-inflammatory drugs (NSAIDs), vitamin K antagonists, heparin, platelet aggregation inhibitors, direct and other thrombin inhibitors, antifibrinolytics and vitamin K and other haemostatics (6 months before index date).

b) Adjusted for use of proton pump inhibitors and iron preparations (6 months before index date).

c) Adjusted for use of proton pump inhibitors and paracetamol (6 months before index date).

and increased bleeding risk is probably not a consequence of a toxic reaction to the drug itself, but the result of decreased platelet aggregation. This effect has been demonstrated for SSRIs in patients with depression and healthy controls.[38] Paroxetine decreases serotonin storage in the platelets and platelet function by more than 80% after 14–21 days.[39] The difference between prevalent users and new users can be explained by the fact that relative shortage of serotonin induced by starting using a serotonergic drug may correct itself after a few weeks resulting in a new balance.

The strength of this study was that we used a large population-based database which enables us to evaluate several types of bleeding. All drug dispensings are routinely recorded and information bias of drug exposure is therefore unlikely. Our study had several potential limitations. In the PHARMO RLS database, no information on smoking status, alcohol intake and over-the-counter medicines is recorded. These factors are considered important confounders of the association between drug use and the risk of bleeding. Due to the use of hospital admission data, only bleeding events leading to hospitalisation were included in our analyses. The possibility that serotonergic drug use may increase the risk of mild bleeding events cannot be excluded. We included only antidepressants, antipsychotics and ergoline derivatives in our analyses, but it can not be ruled of that patients used other medications with affinity for the 5-HTT and 5-HT_{2A} receptor, such as

tramadol or ketanserin. However, it seems unlikely that there will be differential use of these drugs between cases and controls. Furthermore, confounding by indication may not be excluded. Other risk factors, such as depression, were not taken into account. In patients with depression, abnormalities in pathways involved in platelet activation have been shown.

In summary, current use of antidepressants and antipsychotic drugs may increase the risk of gastrointestinal and intracranial bleeding. New users of a serotonergic drug have an increased risk of gastrointestinal and intracranial bleeding in contrast to prevalent users. The association between antipsychotics and gastrointestinal bleeding may warrant further research, since this association was rather unexpected. Risk of female genital tract, gastrointestinal of intracranial bleeding may not be associated with the degree of affinity for the 5-HTT and 5-HT_{2A} receptor.

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Appendix I Outcomes

Diagnosis	ICD-9 code
Subarachnoid hemorrhage	430
Intracerebral hemorrhage	431
Nontraumatic extradural hemorrhage	432.0
Subdural hemorrhage	432.1
Unspecifed intracranial hemorrhage	432.9
Other and unspecified capillary diseases	448.9
Gastric ulcer, acute with hemorrhage	531.00
Gastric ulcer, acute with hemorrhage and perforation	531.2
Gastric ulcer, chronic or unspecified with hemorrhage	531.4
Gastric ulcer, chronic or unspecified with hemorrhage and perforation	531.6
Duodenal ulcer, acute with hemorrhage	532.0
Duodenal ulcer, acute with hemorrhage and perforation	532.2
Duodenal ulcer, chronic or unspecified with hemorrhage	532.4
Duodenal ulcer, chronic or unspecified with hemorrhage and perforation	532.6
Peptic ulcer, acute with hemorrhage	533.0
Peptic ulcer, acute with hemorrhage and perforation	533.2
Peptic ulcer, chronic or unspecified with hemorrhage	533.4
Peptic ulcer, chronic or unspecified with hemorrhage and perforation	533.6
Gastrojejunal ulcer, acute with hemorrhage	534.0
Gastrojejunal ulcer, acute with hemorrhage and perforation	534.2
Gastrojejunal ulcer, chronic with hemorrhage	534.4
Gastrojejunal ulcer, chronic with hemorrhage and perforation	534.6
Gastritis and duodenitis, with hemorrhage	535.01
Hemoperitoneum (nontraumatic)	568.81
Hemorrhage of rectum and anus	569.3
Gastrointestinal hemorrhage	578.0
Blood in stool	578.1
Hemorrhage of gastrointestinal tract unspecified	578.9
Excessive or frequent menstruation	626.2
Puberty bleeding	626.3
Ovulation bleeding	626.5
Metrorrhagia	626.6
Postcoital bleeding	626.7
Other uterine haemorrhage	626.8
Unspecified uterine haemorrhage	626.9
Premenopausal menorrhagia	627.0
Postmenopausal bleeding	627.1

Appendix II Serotonergic study drugs and their affinity contants for the 5-HTT and the 5-HT_{2A}-receptor [35]

Drug class	Drug	Affinity for 5-HTT (in nM)	Affinity for 5-HT_{2A} (in nM)
Ergoline derivatives [a]			
	lisuride	-	2.15
	cabergoline	-	6.17
	dihydroergotamine	-	39 (rat)
	ergotamine	-	0.81
	methysergide	> 10,000	21.21
	bromocriptine	-	107.2
	pergolide	-	38.1
Antipsychotic drugs			
phenothiazines	chlorpromazine	1296	42.4
	levomepromazine	-	-
	fluphenazine	5950	29.4
	perphenazine	-	5.6
	prochlorperazine	-	15
	perazine	-	-
	periciazine	-	-
	thiordiazine	1259	17.9
	pipotiazine	-	-
butyrophenones	haloperidol	> 1000	96.7
	pipamperone	> 1000	6.3
	bromperidol	-	26 (rat)
	benperidol	-	2.5 (rat)
	droperidol	-	3.5 (rat)
other antipsychotics	sertindole	> 1000	0.43
	flupentixol	-	87.5
	chlorprothixene	-	0.43
	zuclopenthixol	-	-
	pimozide	-	13.7
	penfluridol	-	104.5 (rat)
	clozapine	> 1000	7.9
	olanzapine	> 1000	5.2
	quetiapine	> 1000	344
	tetrabenazine	-	-
	sulpiride	-	10 000 (rat)
	tiapride	-	-
	lithium	-	-
	risperidone	> 1000	1.21
	aripiprazole	> 1000	21.9

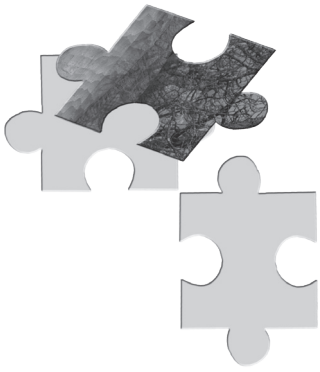
(Appendix II continued)

Drug class	Drug	Affinity for 5-HTT (in nM)	Affinity for 5-HT_{2A} (in nM)
Antidepressants			
TCAs	desipramine	95.4	105 (rat)
	imipramine	8.37	77.8 (rat)
	clomipramine	0.21	35.5
	opipramol	-	-
	trimipramine	149	-
	amitriptyline	27.6	23
	nortriptyline	207	5.0 (rat)
	doxepin	68	26.0 (rat)
	dosulepin	-	-
	maprotiline	5800	51 (rat)
SSRIs	fluoxetine	5.42	196.7
	citalopram	6.09	> 10 000
	paroxetine	0.26	> 10 000
	sertraline	1.11	> 1000 (rat)
	fluvoxamine	5.55	> 10 000 (rat)
	escitalopram	1.80	-
other antidepressants	phenelzine	> 10,000	-
	tranylcypromine	39,000	> 10 000
	moclobemide	-	-
	mianserin	4000	19.4
	trazodone	367	35.8
	nefazodone	403	8.55 (rat)
	mirtazapine	> 10,000	69
	venlafaxine	68.7	> 1000 (rat)
	duloxetine	1.73	504 (rat)

5-HTT = serotonin reuptake transporter; 5-HT = serotonin; TCAs = tricyclic antidepressants; SSRIs = selective serotonin reuptake inhibitors
a) (partial) agonist for the 5-HT_{2A} receptor.

4.3

Use of antidepressant drugs and risk of osteoporotic and non-osteoporotic fractures



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Bone (in press)

ABSTRACT

Aim

Both tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) have been associated with an increased risk of fractures. The serotonin transporter (5-HTT) has been located in the bone and may play a role in bone physiology. We assessed the association between antidepressant drug use, categorised in a therapeutically-based way and on basis of their affinity for the 5-HTT, and the risk of both osteoporotic and non-osteoporotic fractures.

Methods

A case-control study was conducted using the PHARMO RLS. Cases were patients with a first hospital admission for a fracture during the study period. Up to four controls were matched to each case on gender, age, geographical area, and index date.

Results

We identified 16 717 cases, of whom 59.5% had an osteoporotic fracture, and 61 517 controls. Compared to no use, current use of SSRIs was associated with a statistically significant increased risk of osteoporotic fractures (OR 1.95; 95% CI 1.69–2.26), as was current use of TCAs and non-SSRI/non-TCA antidepressant drugs (OR 1.37; 95% CI 1.16–1.63 and OR 1.40; 95% CI 1.06–1.85, respectively).
The risk of an osteoporotic fracture was statistically significantly higher for antidepressants with a high affinity for the 5-HTT (OR 1.86; 95% CI 1.63–2.13) compared to antidepressants with a medium or low affinity (OR 1.43; 95% CI 1.19–1.72 (medium) and OR 1.32; 95% CI 0.98–1.79 (low) ($p < 0.05$ for trend). The risk of non-osteoporotic fractures did not show the same trend.

Conclusions

The extent of affinity for the 5-HTT may contribute to the increased risk of osteoporotic fractures related to antidepressant drug use. The pharmacological mechanism-based classification could to be an appropriate alternative for traditional classification to study the association between the use of antidepressants and the risk of fractures.

INTRODUCTION

Osteoporosis is a skeletal disease which is characterised by low bone mass and disruption of the micro-architecture of bone, resulting in increased bone fragility and increased risk of fractures.[1-3] Osteoporotic fractures (especially fractures of the femur, vertebrae and distal forearm) are a major health problem in the elderly, and the annual costs for this type of fractures are high. Several drug classes have been associated with an increased risk of falls and fractures, including antidepressants.[4-12] Several pharmacological mechanisms have been proposed to explain this adverse effect of antidepressant drug treatment. Tricyclic antidepressants (TCAs) may cause orthostatic hypotension, sedation and confusion/dizziness by blocking the alpha adrenergic, histaminic H_1, and cholinergic (muscarinic) M_3 receptors, and thereby increase the risk of falls and subsequent fractures. Although it was expected that selective serotonin reuptake inhibitors (SSRIs) would cause fewer problems in this respect, given their weak affinity for the α-, H_1- and M_3-receptors, they also have been associated with an increased risk of falls and fractures.[5,13] Long-term use of SSRIs has been linked with a reduction of bone mass and may affect bone micro architecture.[14-16] The underlying mechanism has been related to the selective blockade of the serotonin (5-hydroxytryptamine, 5-HT) reuptake transporter (5-HTT). The 5-HTT and several functional 5-HT-receptors have been found in osteoblastic cell lines,[17,18] as well as in osteoclasts [19] and may therefore be important in bone physiology. However, among antidepressants, not only SSRIs block the 5-HTT. For instance, within the group of TCAs, clomipramine has more pharmacological similarities with SSRIs than with other TCAs. Imipramine has a high affinity for the 5-HTT as well. This raises the question whether the therapeutical classification of antidepressants (TCAs versus SSRIs), which is mainly based on molecular structure (tricyclic) and mechanism of action (interference with the serotonergic neurotransmitter system), is an appropriate way to evaluate the association between antidepressant use and the occurrence of fractures. Recently, two studies evaluated the effect of antidepressants' affinity for the 5-HTT on hip/femur fractures and any fracture.[10,12] In both studies, fracture risk increased with an increasing degree of affinity for the 5-HTT. Currently, there is no information available whether this association applies also for other types of fractures.

Therefore, the aim of this study was to assess the association between the use of antidepressant drugs, categorised both in a therapeutically-based way (SSRIs, TCAs) as well as on their affinity for the 5-HTT, and the risk of both osteoporotic and non-osteoporotic fractures.

METHODS

Setting

Data for this study were obtained from the PHARMO record linkage system (http://www.pharmo.nl). The PHARMO RLS includes the demographic details and complete medication history of more than two million community-dwelling residents of more than twenty-five population-defined areas in the Netherlands from 1985 onwards, further linked to hospital admission records as well as several other health registries, including pathology, clinical laboratory findings and general practitioner data. Since virtually all patients in the Netherlands are registered with a single community pharmacy, independent of prescriber, pharmacy records are virtually complete with regard to prescription drugs.[20]

For this study, drug dispensing data and hospitalisation data were used. The computerised drug dispensing histories contain information concerning the dispensed drug, dispensing date, the prescriber, amount dispensed, prescribed dosage regimen, and the estimated duration of use. The duration of use of each dispensed drug is estimated by dividing the number of dispensed units by the prescribed number of units to be used per day. Drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification.[21] The Hospital Admission Register comprises all hospital admissions in the Netherlands, including detailed information concerning the primary and secondary discharge diagnoses, diagnostic, surgical and treatment procedures, type and frequency of consultations with medical specialists and dates of hospital admission and discharge. All diagnoses are coded according to the International Classification of Diseases, 9th edition (ICD-9-CM).

Validation studies on PHARMO RLS have confirmed a high level of data completeness and validity with regards to fractures [22] and PHARMO RLS has been used more often to address risk factors of hip/femur fracture.[12,23]

Study population

Within PHARMO RLS, a population-based case-control study was conducted. Cases were defined as patients 18 years and older with a first record for a fracture leading to hospitalisation, identified through the ICD-9 codes (ICD-9 800–829) in the period from 1 January 1991 until 31 December 2002. Fractures were classified as osteoporotic fractures, defined as hip/femur (ICD-9 819–821), radius/ulna (ICD-9 813), humerus (ICD-9 812), vertebral (ICD-9 805–806), rib (ICD-9 807) and clavicle fractures (ICD-9 810), and non-osteoporotic fractures (ICD-9 codes 800–804, 808, 809, 811, 814–818, 822–829). The index date was the date of hospital admission. For each case, up to four controls without a history of fractures during the study

period were matched on year of birth, gender, geographical area, and calendar time (the index date of the corresponding case). Both cases and controls were eligible for inclusion if they had a minimum period of 365 days of history in the PHARMO RLS prior to the index date.

Exposure assessment

For each case and control, all prescriptions before the index date for antidepressant drugs were identified. Antidepressant drugs were classified in accordance with the ATC-code (N06AA Non-selective monoamine ruptake inhibitors = TCAs and related substances; N06AB Selective serotonin reuptake inhibitors; N06AF/G/X Other antidepressant drugs), as well as grouped on basis of their affinity for the 5-HTT (Table 1). Lower affinity constants (K_i) reflect a higher affinity for the transporter and therefore a higher antagonism of the 5-HTT. K_i data were obtained from the Psychoactive Drug Screening Program (PDSP) funded by the National Institute of Mental Health (NIHM). This program provides screening of psychoactive compounds for pharmacological and functional activity at cloned human or rodent CNS receptors, channels, and transporters. The database contains more than 47 000 K_i values and accrues new information on a regular basis. We classified the antidepressants into three groups: high: $K_i < 10$ nM; medium: K_i 10–1000 nM; low: $K_i > 1000$ nM. The K_i values were taken from experiments with human receptor cell lines. When there was more than one K_i value for a specific antidepressant drug-receptor interaction, an average value was calculated.

Exposure to antidepressant drugs was classified according to the timing of use in relation to the index date. Current use was defined as a patient using an antidepressant on the index date, based on the dispensing date and the calculated enddate of the prescription. Current users of more than one antidepressant drug concurrently were categorised as a separate group. Recent users were patients who were not current users, but used antidepressants within three months before the index date. Past use was defined as use of an antidepressant in the year before the index date, not being current or recent use. Distant past users were patients who were no past user, but used antidepressants more than 365 days prior to the index date. Exposure to antidepressants was categorised as no use when there was no recorded use of antidepressant medication from the first entry in the PHARMO RLS till the index date.

Assessment of potential confounders

Potential confounders in this study were prior medication use and/or medical conditions known to be associated with falls, fractures or known to be associated

Table 1 Therapeutically-based versus mechanism-based classification (affinity for the 5-HTT) of antidepressant drugs

Therapeutical classification	Affinity for the 5-HTT [26]			
	high (K_i < 10 nM)	medium (K_i 10–1000 nM)	low (K_i > 1000 nM)	no data available
SSRIs				
citalopram	+			
fluoxetine	+			
fluvoxamine	+			
paroxetine	+			
sertraline	+			
TCAs and related drugs				
amitriptyline		+		
clomipramine	+			
desipramine		+		
dibenzepin				+
dosulepin				+
doxepin		+		
imipramine	+			
maprotiline			+	
nortriptyline		+		
opipramol				+
trimipramine		+		
Non-SSRI/non-TCA				
mianserin			+	
mirtazapine			+	
moclobemide				+
nefazodone		+		
tranylcypromine			+	
trazodone		+		
venlafaxine		+		

5-HTT = serotonin transporter; K_i = affinity constant; SSRIs = selective serotonin reuptake inhibitors; TCAs = tricyclic antidepressants

with either bone anabolic or catabolic effects. Medication use within a six-month period prior to the index date was evaluated and included antiarrhytmic drugs, antidiabetic drugs, antiepileptic drugs, anti-Parkinson drugs, antipsychotics, benzodiazepines (within a three-month period), beta-blocking agents, disease-modifying antirheumatic drugs (DMARDs), hormone replacement therapy (HRT), lithium, non steroidal anti-inflammatory drugs (NSAIDs), opioids, oral and

inhaled glucocorticoids, thiazide diuretics and thyroid hormones. The different time window for benzodiazepines has been chosen because in the Netherlands, benzodiazepines are dispensed for a maximum of 30 days in contrast with other drugs which are dispensed for a maximum of 90 days.
Hospitalisation records were assessed for a history of hospitalisation before the index date for cancer, cardiovascular diseases, cerebrovascular accidents, chronic obstructive airway disease (COPD), impaired renal function, inflammatory bowel diseases, mental disorders, musculoskeletal diseases, and rheumatoid arthritis.

Data analysis

The strength of the association between use of antidepressant drugs and the occurrence of fractures was evaluated using conditional logistic regression analysis and was expressed as odds ratios (ORs) with 95% confidence intervals (CIs). The model included current, recent, past and distant past use of antidepressant drugs. Data were stratified according to fracture type. We fitted separate models for osteoporotic and non-osteoporotic fractures. We used a stepwise backward selection procedure to determine which of the covariates had to be included in the final regression model using a significance level of 0.05.
Data were analysed using SPPS 14.01.1 (SPSS Inc. Chicago, Illinois, USA).

RESULTS

The study population consisted of 16 717 patients with a hospitalisation for any type of fracture and 61 517 matched controls. Osteoporotic fractures were most frequent (n = 9943; 59.5%). The characteristics of the patients with osteoporotic and non-osteoporotic fractures are shown in Table 2. The majority of the patients with an osteoporotic fracture were older than 60 years (71.6%) (mean age 67.8 ± 19.3 years) and were females (66.0%) in contrast to patients with a non-osteoporotic fracture (29.2% 60 years or older; 48.6% female; mean age 47.9 ± 20.0 years).
Compared to control patients, both patients with an osteoporotic or a non-osteoporotic fracture had a higher prevalence of medical conditions and used more medication. The most frequently prescribed drugs among cases and controls were benzodiazepines and NSAIDs. Patients with osteoporotic fractures had more hospitalisation records for cancer, cardiovascular diseases and cerebrovascular diseases compared to patients with non-osteoporotic fractures.

Table 2 Characteristics of case and control patients

	Osteoporotic fractures		Non-osteoporotic fractures	
	Cases n=9 943 (100%)	Controls n=36 359 (100%)	Cases n=6 774 (100%)	Controls n=25 158 (100%)
Age (years)				
18–39	1 182 (11.9%)	4 299 (11.8%)	2 659 (39.3%)	9 712 (38.6%)
40–59	1 644 (16.5%)	6 221 (17.1%)	2 137 (31.5%)	8 092 (32.2%)
60–79	3 808 (38.3%)	14 259 (39.2%)	1 454 (21.5%)	5 522 (21.9%)
≥ 80	3 309 (33.3%)	11 580 (31.8%)	524 (7.7%)	1 832 (7.3%)
Gender (female)	6 561 (66.0%)	23 818 (65.5%)	3 291 (48.6%)	12 224 (48.6%)
Fracture site				
hip/femur	5 839 (58.7%)			
radius/ulna	1 392 (14.0%)			
vertebral	1 012 (10.2%)			
humerus	765 (7.7%)			
rib	715 (7.2%)			
clavicle	220 (2.2%)			
ankle			2 163 (31.9%)	
tibia/fibula			1 356 (20.0%)	
face bones			1 023 (15.1%)	
pelvis			658 (9.7%)	
Hospitalisation data				
cancer	490 (4.9%)	1 343 (3.7%)[a]	126 (1.9%)	469 (1.9%)
cardiovascular diseases	1 630 (16.4%)	4 505 (12.4%)[a]	742 (11.0%)	1 772 (7.0%)[a]
cerebrovascular disease	360 (3.6%)	699 (1.9%)[a]	61 (0.9%)	199 (0.8%)
inflammatory bowel disease	305 (3.1%)	705 (1.9%)[a]	119 (1.8%)	353 (1.4%)[b]
obstructive airway disease	3 231 (32.5%)	8 751 (24.1%)[a]	1 794 (26.5%)	5 117 (20.3%)[a]
mental disorders	35 (0.4%)	56 (0.2%)[a]	27 (0.4%)	34 (0.1%)[a]
musculoskeletal diseases	956 (9.6%)	2 704 (7.4%)[a]	696 (10.3%)	1 653 (6.6%)[a]
Co-medication				
antiarrhytmic drugs	139 (1.4%)	410 (1.1%)[b]	62 (0.9%)	119 (0.5%)[a]
antidiabetic drugs	963 (9.7%)	2 640 (7.3%)[a]	305 (4.5%)	942 (3.7%)[c]
antiepileptic drugs	304 (3.1%)	514 (1.4%)[a]	121 (1.8%)	270 (1.1%)[a]
anti-Parkinson drugs	251 (2.5%)	402 (1.1%)[a]	45 (0.7%)	100 (0.4%)[c]
antipsychotic drugs	460 (4.6%)	898 (2.5%)[a]	127 (1.9%)	283 (1.1%)[a]
benzodiazepines	2 472 (24.9%)	6 742 (18.5%)[a]	900 (13.3%)	2 450 (9.7%)[a]

(Table 2 continued)

	Osteoporotic fractures		Non-osteoporotic fractures	
	Cases n=9 943 (100%)	**Controls n=36 359 (100%)**	**Cases n=6 774 (100%)**	**Controls n=25 158 (100%)**
beta-blocking agents	1 281 (12.9%)	5 107 (14.0%)[c]	562 (8.3%)	1 940 (7.7%)[b]
DMARDs	158 (1.6%)	284 (0.8%)[a]	62 (0.9%)	152 (0.6%)[c]
HRT	236 (2.4%)	937 (2.6%)	158 (2.3%)	533 (2.1%)
inhaled corticosteroids	844 (8.5%)	2 548 (7.0%)[a]	453 (6.7%)	1 411 (5.6%)[a]
lithium	22 (0.2%)	51 (0.1%)[b]	18 (0.3%)	47 (0.2%)
NSAIDS	2 513 (25.3%)	6 532 (18.0%)[a]	1 537 (22.7%)	3 696 (14.7%)[a]
opioids	364 (3.7%)	560 (1.5%)[a]	112 (1.7%)	210 (0.8%)[a]
oral glucocorticoids	616 (6.2%	1 496 (4.1%)[a]	216 (3.2%)	592 (2.4%)[a]
thiazide diuretics	907 (9.1%)	3 251 (8.9%)	290 (4.3%)	985 (3.9%)

DMARDs = disease modifying antirheumatic drugs; HRT = hormone replacement therapy; NSAIDs = nonsteroidal anti-inflammatory drugs

a) $p < 0.005$.
b) $p < 0.05$.
c) $p < 0.01$.

The association between the use of antidepressant drugs and the risk of osteoporotic and non-osteoporotic fractures is shown in Table 3. After adjustment for potential confounders, current use was associated with an equally increased risk of fractures (OR 1.64; 95% CI 1.48−1.83, and OR 1.60; 95% CI 1.37−1.86, respectively).

We stratified current antidepressant drug use according to both the therapeutically-based categorisation and affinity for the 5-HTT. Current use of SSRIs yielded higher risk estimates compared to TCAs (and related substances) and other non-SSRI/non-TCA antidepressant drugs (OR 1.95; 95% CI 1.69−2.26, OR 1.37; 95% CI 1.16−1.63, and OR 1.40; 95% CI 1.06−1.85, respectively) for osteoporotic fractures. For non-osteoporotic fractures the ORs were 1.59 (95% CI 1.30−1.93; SSRIs), 1.50 (95% CI 1.14−1.96; TCAs) and 2.00 (95% CI 1.33−3.01; non-SSRI/non-TCA antidepressants). When using the classification based on affinity for the 5-HTT, we found that the risk of an osteoporotic fracture was statistically significantly higher for current use of antidepressants with a high affinity for this transporter compared to antidepressants with a medium or low affinity: OR 1.86; 95% CI 1.63−2.13 (high affinity), OR 1.43; 95% CI 1.19−1.72 (medium affinity) and OR 1.32; 95% CI 0.98−1.79 (low affinity) (Figure 1). Although current use of

Table 3 Use of antidepressant drugs and the risk of osteoporotic and non-osteoporotic fractures

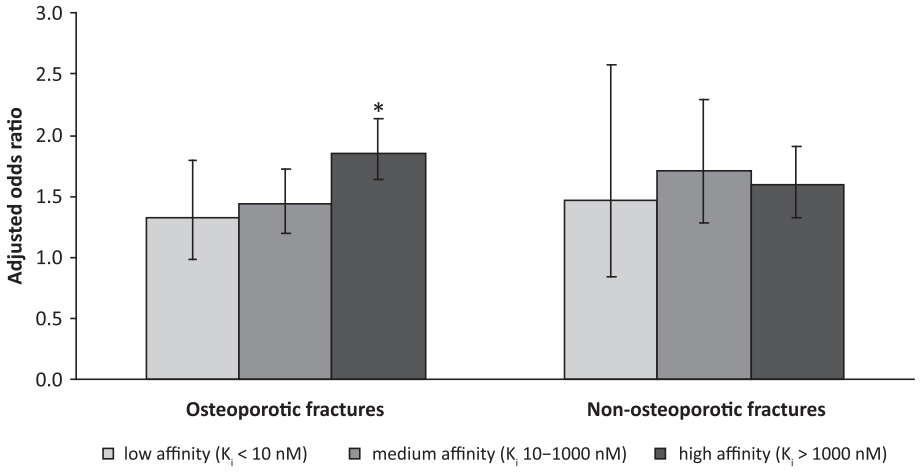
	Osteoporotic fractures			Non-osteoporotic fractures		
	Cases n=9 943 (100%)	Controls n=36 359 (100%)	Adjusted [a] OR (95% CI)	Cases n=6 774 (100%)	Controls n=25 158 (100%)	Adjusted [b] OR (95% CI)
Current use	665 (6.7%)	1 233 (3.4%)	1.64 (1.48–1.83)	290 (4.3%)	606 (2.4%)	1.60 (1.37–1.86)
Recent use	168 (1.7%)	397 (1.1%)	1.25 (1.03–1.51)	82 (1.2%)	237 (0.9%)	1.12 (0.86–1.45)
Past use	190 (1.9%)	589 (1.6%)	1.03 (0.86–1.22)	121 (1.8%)	318 (1.3%)	1.29 (1.04–1.61)
Distant past use	547 (5.5%)	1 646 (4.5%)	1.13 (1.02–1.25)	295 (4.4%)	955 (3.8%)	1.07 (0.93–1.23)
No use	8 373 (84.2%)	32 494 (89.4%)	1.00 (reference)	5 986 (88.4%)	23 042 (91.6%)	1.00 (reference)

OR = odds ratio; CI = confidence interval; SSRIs = selective serotonin reuptake inhibitors; TCAs = tricyclic antidepressants; 5-HTT = serotonin transporter; AD = antidepressant drug

a) Adjusted for: cancer, cardiovascular disease, cerebrovascular disease, inflammatory bowel disease, mental disorders, obstructive airway disease, and use of antidiabetics, antiepileptics, anti-Parkinson drugs, antipsychotics, benzodiazepines, beta-blocking agents, DMARDs, hormone replacement therapy, NSAIDs, oral glucocorticoids and opioids.

b) Adjusted for: cardiovascular disease, mental disorders, musculoskeletal disease, obstructive airway disease, rheumatic diseases and use of agents acting on the renin-angiotensin system, antiarrhytmics, antidiabetics, antiepileptics, benzodiazepines, NSAIDs and opioids.

Figure 1 Association between current use of antidepressants, categorised according to the affinity for the 5-HTT, and (non-)osteoporotic fractures



5-HTT = serotonin transporter; K_i = affinity constant
For adjustments: see Table 3.
* $p < 0.05$ high vs. medium affinity and high *vs.* low affinity.

antidepressants increased the risk of non-osteoporotic fractures by approximately 50%, there was no trend with increasing affinity for the 5-HTT (high: OR 1.59; 95% CI 1.33–1.91, medium: OR 1.71; 95% CI 1.28–2.29, low: OR 1.47; 95% CI 0.83–2.58). In separate analyses for various types of fracture (hip/femur, radius/ulna, facial bones, vertebral, tibia/fibula and ankle), we did not see the same trend with the exception of hip/femur fractures (58.7% of all osteoporotic fractures).

Among patients with an osteoporotic fracture, users of antidepressants with a medium affinity for the 5-HTT were older compared to users of an antidepressant with a high or low affinity (87% 60 years and older, versus 77% and 57%, respectively). Users of antidepressants with a medium or low affinity use took more benzodiazepines, antiepileptic drugs, opioids and anti-Parkinson drugs in comparison with users of an antidepressant drug with high affinity for the 5-HTT (data not shown).

DISCUSSION

The main finding of this study was the association between the degree of 5-HTT inhibition of antidepressants and the risk of osteoporotic fracture. This association was absent when the risk of non-osteoporotic fracture was evaluated. Current use of antidepressant drugs with a high affinity for the 5-HTT was associated with a higher risk of osteoporotic fractures compared to use of antidepressants with a medium or low affinity. The 5-HTT has been located in osteoclasts, osteoblasts and osteocytes and the effect of the inhibition of 5-HTT in bone has been assessed.[24,25] *In vivo* studies have found that 5-HT could alter bone architecture and could reduce bone mass and density.[24] The finding that especially the risk of osteoporotic fractures was related with the use of antidepressant drugs with a high degree of inhibition of the 5-HTT in contrast to the risk of non-osteoporotic fractures, is, in this perspective, noteworthy. Osteoporotic fractures are, unlike non-osteoporotic fractures, related to low bone mass and micro architectural deterioration. Non-osteoporotic fractures, which are more often high-energy fractures, are probably less dependent on low bone mineral density. The influence of drugs with the ability to interact with bone physiology, therefore, may be greater on osteoporotic fracture risk than on non-osteoporotic fracture risk. Fractures and their sequelae can have important personal as well as (economic) implications for society. Therefore, attention on the issue of bone health among patients using antidepressants is warranted.

In Table 1, it is shown that all SSRIs belong to the group of antidepressants with a high affinity for the 5-HTT. However, TCAs and non-SSRI/non-TCA antidepressants are heterogeneous groups which cannot be divided into a specific group on basis of their affinity for the 5-HTT. Clomipramine and imipramine both have a high affinity for the 5-HTT. However, current use of clomipramine was not associated with osteoporotic fractures whereas current use of imipramine increased the risk of an osteoporotic fracture more than 3-fold compared to non-use. This finding suggests that not only affinity for the 5-HTT but also other pharmacological mechanisms are accountable for the increased risk of osteoporotic fractures.

Among current users with an osteoporotic fracture, paroxetine and amitriptyline were the most used antidepressant drugs within the different groups (therapeutically-based classification: 62% (SSRI) and 56% (TCA) respectively; based on 5-HTT affinity: 54% (high) and 67% (medium) respectively), and were assumed to be mainly responsible for the observed effects. We did not have data on affinity for the 5-HTT for all antidepressant drugs. However, the proportion of cases and controls who were current users of an antidepressant for which no data on affinity were available was very low (0.9% and 1.7%, respectively).

We used both a therapeutically-based way as well as a mechanism-based classification to define exposure to antidepressant drugs. Affinity constants for the 5-HTT were obtained from the Psychoactive Drug Screening Program (PDSP).[26] In other studies which evaluated the association between inhibition of 5-HTT by antidepressant drugs and the risk of fractures,[10,12] other classifications were used.[27,28] Also, the cut off points for the classification in the *low-medium-high* affinity groups were not identical between the different studies. We did a sensitivity analysis by also classifying the degree of inhibition of the 5-HTT according to Goodman and Gilman's The Pharmacological Basis of Therapeutics,[27] and found the same trend: OR 1.37; 95% CI 1.07−1.75 (low affinity), OR 1.56; 95% CI 1.31−1.86 (medium affinity), and OR 1.83; 95% CI 1.58−2.12 (high affinity). Classifying the degree of inhibition according to both methods did result in the same trend. The advantages of using the PDSP are the large number of available affinity constants for many receptors, originating from many studies, and the constant screening for new pharmacological data. The mechanism-based classification seems to be adequate and a useful addition to the traditional classification of antidepressant drugs.

There are several limitations of this study. Firstly, as with each observational retrospective study, the findings were prone to unmeasured confounding. Unmeasured potential confounders include body mass index, smoking status, alcohol intake, postmenopausal status and cognitive and physical impairment. Control patients did not have a history of fractures, and were not selected using the incidence density sampling technique. However, fractures are a relatively rare disease and we think that this will not have substantially influenced our results. PHARMO did not contain information on the aetiology of fractures, such as falls. In addition, there was no information on the severity of depression in our patients. Depression as such is an important risk factor for falls [29] and a major risk factor for reduced lower spine bone mineral density and fractures. Physiological effects of major depression disorder (*e.g.* increased cortisol levels, cytokines) and changes in life-style could also lead to poorer bone health.[30,31] Also, there might have been selective prescribing of SSRIs instead of TCAs to patients with a history of falls or a high risk of falling. In addition, there is always the concern of residual confounding even after adjustment for many confounding variables.

Secondly, antidepressant drugs interact with numerous neurotransmitter receptors. The categorisation of antidepressant drugs has been made on basis of affinity for one neurotransmitter transporter, namely the 5-HTT. Most antidepressant drugs have additional effects on other receptors or transporters which have not been assessed in this study.[32] Furthermore, it is known that some drugs, such as

amitriptyline, imipramine and fluoxetine, are metabolised into active compounds, whereas we used the affinity constants of the parent drug.

Lastly, in contrast to the Danish' national Hospital Discharge Register and the General Practice Research Database in the United Kingdom [10,33] only hospitalisation admissions are recorded in the PHARMO RLS, leading to an underestimation of the actual number of fractures. The underrepresentation of fractures in this study applies for both osteoporotic and non-osteoporotic fractures and is expected to be independent of the exposure to antidepressant drugs, making differential misclassification unlikely.

In conclusion, our findings suggest that the extent of affinity for the 5-HTT of antidepressant drugs is associated with the magnitude of the risk of osteoporotic fractures, although we could not exclude other alternative explanations, such as underlying disease, smoking and fall-related effects. High affinity for the 5-HTT seems to a prominent factor in the increase of risk of fractures related to antidepressant drug use. The classification based on this pharmacological mechanism, could to be an appropriate alternative to study the association between the use of antidepressants and the risk of fractures.

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Appendix I Association between current use of antidepressant drugs and the risk of osteoporotic and non–osteoporotic fractures

Drug	ATC [b]	Affinity for	Osteoporotic fractures			Non–osteoporotic fractures		
		5-HTT [b] (K_i in nM)	Cases n=9 943 (100%)	Controls n=36 359 (100%)	Adjusted [c] OR (95% CI)	Cases n=6 774 (100%)	Controls n=25 158 (100%)	Adjusted [d] OR (95% CI)
amitriptyline	TCA	27.61	130 (1.3%)	277 (0.8%)	1.28 (1.02–1.60)	43 (0.6%)	95 (0.4%)	1.33 (0.92–1.94)
citalopram	SSRI	6.09	26 (0.3%)	37 (0.1%)	2.11 (1.26–3.55)	11 (0.2%)	27 (0.1%)	1.20 (0.58–2.46)
clomipramine	TCA	0.21	37 (0.4%)	95 (0.3%)	1.11 (0.75–1.65)	24 (0.4%)	49 (0.2%)	1.48 (0.90–2.45)
desipramine	TCA	95.40	1 (0.0%)	0 (0.0%)	NA	0 (0.0%)	2 (0.0%)	NA
dibenzepin	TCA	no data	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
dosulepin	TCA	no data	3 (0.0%)	14 (0.0%)	0.64 (0.18–2.26)	0 (0.0%)	4 (0.0%)	NA
doxepin	TCA	68.00	12 (0.1%)	20 (0.1%)	1.44 (0.68–3.05)	2 (0.0%)	4 (0.0%)	1.88 (0.33–10.5)
fluoxetine	SSRI	5.42	52 (0.5%)	91 (0.3%)	1.75 (1.23–2.49)	28 (0.4%)	58 (0.2%)	1.48 (0.93–2.36)
fluvoxamine	SSRI	5.55	39 (0.4%)	71 (0.2%)	1.61 (1.07–2.42)	26 (0.4%)	59 (0.2%)	1.42 (0.88–2.29)
imipramine	TCA	5.46	12 (0.1%)	11 (0.0%)	3.61 (1.50–8.66)	5 (0.1%)	8 (0.0%)	1.81 (0.58–5.67)
maprotiline	TCA	5 800	25 (0.3%)	47 (0.1%)	1.51 (0.91–2.50)	6 (0.1%)	15 (0.1%)	1.36 (0.51–3.59)
mianserin	Other	4 000	8 (0.1%)	29 (0.1%)	0.74 (0.33–1.66)	4 (0.1%)	5 (0.0%)	2.61 (0.70–10.1)
mirtazapine	Other	> 100 000	31 (0.3%)	76 (0.2%)	1.08 (0.70–1.69)	8 (0.1%)	25 (0.1%)	1.02 (0.45–2.31)
moclobemide	Other	no data	4 (0.0%)	7 (0.0%)	1.65 (0.47–5.81)	3 (0.0%)	7 (0.0%)	1.62 (0.42–6.34)
nefazodone	Other	402.67	1 (0.0%)	2 (0.0%)	1.27 (0.11–14.9)	0 (0.0%)	3 (0.0%)	NA
nortriptyline	TCA	204.17	19 (0.2%)	41 (0.1%)	1.13 (0.64–2.01)	8 (0.1%)	13 (0.1%)	2.20 (0.89–5.44)
opipramol	TCA	no data	0 (0.0%)	1 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
paroxetine	SSRI	0.26	210 (2.1%)	342 (0.9%)	1.87 (1.56–2.24)	98 (1.4%)	200 (0.8%)	1.61 (1.25–2.07)

(Appendix I continued)

Drug	ATC [b]	Affinity for	Osteoporotic fractures			Non–osteoporotic fractures		
		5-HTT [b] (K_i in nM)	Cases n=9 943 (100%)	Controls n=36 359 (100%)	Adjusted [c] OR (95% CI)	Cases n=6 774 (100%)	Controls n=25 158 (100%)	Adjusted [d] OR (95% CI)
sertraline	SSRI	1.11	23 (0.2%)	22 (0.1%)	3.06 (1.68–5.58)	4 (0.1%)	9 (0.0%)	1.37 (0.41–4.58)
tranylcypromine	Other	39 000	5 (0.1%)	3 (0.1%)	5.39 (1.23–23.7)	0 (0.0%)	0 (0.0%)	NA
trazodone	Other	367.33	15 (0.2%)	21 (0.1%)	1.59 (0.77–3.28)	3 (0.0%)	6 (0.0%)	1.89 (0.47–7.61)
trimipramine	TCA	149.00	0 (0.0%)	1 (0.0%)	NA	0 (0.0%)	0 (0.0%)	NA
venlafaxine	Other	68.70	23 (0.2%)	39 (0.1%)	1.59 (0.92–2.73)	21 (0.3%)	24 (0.1%)	3.02 (1.65–5.50)

OR = odds ratio; CI = confidence interval; NA = not applicable; TCAs = tricyclic antidepressants; SSRIs = selective serotonin reuptake inhibitors; 5–HTT = serotonin transporter

a) TCA and related drugs (N06AA); SSRI (N06AB); other (non–SSRI/non–TCA) antidepressant drugs (N06AF/G/X).

b) http://pdsp.med.unc.edu [Accessed on 23 January 2009].

c) Adjusted for: cancer, cardiovascular disease, cerebrovascular disease, inflammatory bowel disease, mental disorders, obstructive airway disease, and use of antidiabetics, antiepileptics, anti–Parkinson drugs, antipsychotics, benzodiazepines, beta–blocking agents, DMARDs, hormone replacement therapy, NSAIDs, oral glucocorticoids and opioids.

d) Adjusted for: cardiovascular disease, mental disorders, musculoskeletal disease, obstructive airway disease, rheumatic diseases and use of agents acting on the renin–angiotensin system, antiarrhytmics, antidiabetics, antiepileptics, benzodiazepines, NSAIDs and opioids.

5

General discussion

"Crude classifications and false generalisations are the curse of organised life"

George Bernard Shaw
(Irish playwright and essayist, 1856-1950)

In this thesis, the concept of mechanism-based drug exposure classification was used to study associations between drug exposure and adverse effects of drugs within the domain of pharmacoepidemiology and pharmacovigilance. In seven pharmacoepidemiological studies, using five different datasets, we assessed whether it was feasible to classify drugs on the basis of their molecular and pharmacological properties. Instead of the most commonly applied pharmacotherapeutical classification, molecular characteristic-based, formulation-based and target-oriented drug classification schemes were used. In the studies described in *Chapters 2.1*, *2.2*, *4.2* and *4.3*, we tested molecular characteristic-based and target-oriented drug exposure classifications with known associations between drug classes and the risk of certain outcomes (allergic reactions, photosensitivity reactions, bleeding and fractures, respectively). In these studies, there was *a priori* a rationale to classify drugs on the basis of their substituents/physicochemical parameters or the degree of the affinity for certain receptors. In addition, we conducted three studies in which we evaluated whether a different approach in classifying drugs would give insight in the role of certain drug characteristics in the occurrence of ADRs. In *Chapter 2.3*, we assessed whether fluorine substituents were involved in the reporting of ADRs, whereas in *Chapter 3.1* and *Chapter 4.1*, a formulation-based and a target-orientated drug exposure classification was used to study changes in blood cell counts and drug-induced nephrotoxicity and ototoxicity. In the field of medicinal chemistry, the relationship between structure or binding affinities and drug-induced events are routinely evaluated *in vitro*, as well as *in vivo* and nowadays also *in silico* (via computer simulation). Several databases [1,2] have emerged in which the relationships between structure and biological properties of chemicals can be programmed to predict drug-target relationships in preclinical development programmes and consequently the therapeutic effects and adverse reactions. The predictive value varies depending on the methods for prediction (neural networks, functional group filters, quantitative structure-activity relationships, decision trees), but chemoinformatics is an effective tool in drug discovery and drug development.[3] However, in pharmacoepidemiological studies the chemoinformatics approach is relatively new. Pharmacoepidemiological databases,

such as spontaneous ADR reporting systems, general practitioner databases and drug dispensing databases certainly offer possibilities for evaluating molecular and/or pharmacological drug characteristics. The purpose of this chapter is to put the previous chapters in broader perspective and to describe the importance of molecular and pharmacological drug characteristics in classifying drug exposure and the consequences for pharmacoepidemiological studies and data mining in pharmacovigilance.

Three themes will be discussed:

- molecular characteristic-based drug exposure classification, including aspects of pharmaceutical formulation;
- target-oriented drug exposure classification systems;
- implications for drug safety.

These themes will be preceded by an overview of general classification schemes of medicines.

CLASSIFICATION SCHEMES OF MEDICINES

The science of classification is called taxonomy. Taxonomy derived from the Greek words *τάξις*, *taxis* (meaning 'order', 'arrangement') and *νόμος*, *nomos*, meaning 'law' or 'science'. Taxonomy and classification systems enable us to identify similarities and differences among a large number of organisms, objects, concepts, relationships and medications. However, it is sometimes not easy to choose the appropriate classification system. Exemplary are the group of antibacterials; for this group many classification schemes are possible based on bacterial spectrum (*e.g.* small versus broad spectrum activity), route of administration (*e.g.* oral, topical or parenteral) or chemical structure (beta-lactam antibacterials, sulfonamides, tetracyclines). Drug classification systems are generally based on chemical or biological properties. Examples are the biopharmaceutics classification system (BCS) and target-based classifications. In 1995, Amidon *et al.* defined the BCS to categorise drugs into one of four biopharmaceutical classes according to their water solubility and membrane permeability characteristics.[4] The BCS is used as an instrument to facilitate oral drug development and serves as a regulatory tool for setting bioavailability/bioequivalence standards for the USA Food and Drug Administration, the European Medicines Agency and the World Health Organization (WHO).[5,6] When looking at the nature of drug targets, Imming *et al.* proposed a classification based on this consideration to estimate the number of

known drug targets.[7] This mechanistic classification is generally used for better understanding of the underlying mechanisms of action. This classification has been used to classify the group of addictive drugs, a chemically heterogeneous group with different molecular targets. Yet, three classes can be distinguished: drugs that activate G protein-coupled receptors (*e.g.* opioids), drugs that bind to ionotropic receptors and ion channels (*e.g.* nicotine, alcohol, benzodiazepines) and drugs that bind to transporters of biogenic amines (*e.g.* amphetamines, ecstasy).[8] This concept can also be applied within a drug class. Non-steroidal anti-inflammatory drugs (NSAIDs) are a structurally diverse group and are usually classified accordingly. However, classification of NSAIDS by their selectivity of inhibition of cyclooxygenase (COX) 1 and/or 2 results in a useful classification system.[9]

The anatomical therapeutic chemical (ATC) classification system, recommended by the WHO for drug utilisation studies is a well-known drug classification system often used in pharmacoepidemiology. The ATC code connects chemical classification and therapeutic approach.[10] Drugs are classified at five different levels, ranging from the area of action, therapeutic indication, pharmacological and chemical properties, and finally the specific drug substance. Drug classes on ATC4 level are sometimes referred to by therapeutic use (*e.g.* N05A antipsychotics), pharmacological mode of action (*e.g.* C07A beta-blocking agents) and chemical structure (*e.g.* J01A tetracyclines). Moreover, the ATC classification is rather arbitrary. For example, selective serotonin reuptake inhibitors (SSRIs) are considered to be antidepressants, but have also efficacy in anxiety disorders. However, if the pharmaceutical company had decided to test the SSRIs in anxiety disorders first, they would have be considered anxiolytics with efficacy in depressive disorders and classified accordingly. The same applies to antiepileptic drugs that are also prescribed for mood disorders rather than epilepsy. The activity of a drug is not the result of its therapeutic label but instead of its mechanism of action, which may have an impact on many different physiological systems within the body.

A new challenge is the classification of biopharmaceuticals. Biopharmaceuticals have specific characteristics and differ from small molecule medicines with respect to for example molecular weight, stability, manufacturing and route of administration.[11] On the basis of production (originated from microbial cells, mammalian cell lines, plant cell cultures), clinical use (*e.g.* treatment of anaemia, leukaemia, multiple sclerosis, rheumatoid arthritis) or their biological action (blood factors, thrombolytic agents, hormones, haematopoietic growth factors,

interferons, vaccines, monoclonal antibodies), biopharmaceuticals can be classified in different categories.

In summary, drug exposure classification can be looked at from different angles. The three major ways to classify small molecule drugs are on the basis of 1) therapeutic use; 2) chemical structure/molecular properties; and 3) pharmacology (pharmacokinetics, pharmacodynamics). This thesis has focused on the mechanism-based drug exposure classification instead of categorisation on the basis of therapeutic drug classes. Relevant molecular and pharmacological properties in classification schemes of medicines are discussed.

MOLECULAR CHARACTERISTIC-BASED DRUG EXPOSURE CLASSIFICATION, INCLUDING ASPECTS OF PHARMACEUTICAL FORMULATION

What makes a 'class' a 'class'?

The sulfonamide moiety is the chemical basis of several drugs. Usually, the term 'sulfa' drugs refers to sulfonamide antibiotics, but also sulfonylureas, thiazide diuretics and some COX-2 inhibitors (*e.g.* celecoxib) possess such a functional group. In 2003, an article was published in the New England Journal of Medicine regarding the absence of cross-reactivity between sulfonamide antibiotics and sulfonamide non-antibiotics.[12] All drugs with a sulfonamide moiety and not being an antibiotic were assigned to the group 'sulfonamide non-antibiotic drugs', creating a heterogeneous drug class.

"Showing the structure of a drug in a lecture is usually the best way to turn off a clinical audience" wrote Sheldon H. Preskorn in 2003.[13] Although unjustly, it is understandable that prescribers think about drugs in terms of their therapeutic classification. However, a drug's molecular properties (chemical, physical and structural) are important factors for the pharmacokinetics and pharmacodynamics in the human body. The physicochemical properties of a drug on the one hand and the physiological processes in the organism on the other hand determine its efficacy and toxicological effects.

Based on the observation that most drug candidates are relatively small and moderately lipophilic molecules, Lipinski *et al.* formulated guidelines to predict good oral absorption and permeability of potential drug candidates.[14] In general, an orally administered compound must comply with at least three of the following criteria:

- not more than 5 hydrogen bond donors (nitrogen (N) or oxygen (O) atoms with one or more H atoms);

- not more than 10 H-bond acceptors (N or O atoms);
- a molecular weight under 500 Daltons;
- an octanol-water partition coefficient log P of less than 5.

These guidelines are better known as *Lipinski's rule of five*, because all numbers are multiples of five. Since the formulation of the *rule of five*, many analyses have been done to identify changes in physicochemical properties of marketed oral drugs over time and in different stages of development.[15-19] Overall, in the last 70 years the mean lipophilicity, the number of H-bonds and the mean polar surface area have hardly changed. There is, however, a steady increase in mean and median molecular weight and number of rings of newer drugs.[17,18] However, the *rule of five* does not predict whether a compound is pharmacologically active. The recognition of certain chemical structures may lead to an estimation of a chemical's reactivity in a biological system.[20] In addition, physicochemical parameters, such as lipo- and hydrophilicity, molecular weight, may add to the insight in the relation between chemical structure and toxicity. This type of thinking has led to the development of quantitative structure-activity relationships (QSARs). QSARs are mathematical equations used as a predictive tool to estimate efficacy or toxicity of new chemicals based upon a training set of chemicals with known activity and a defined chemical space.[21] However, these QSARs are often limited to certain chemical groups and to well-defined biological processes. QSARs are used at the beginning of the *drug pipeline* to predict a chemical's activity and/or toxicity. Although significant advancements have been made to handle drug safety during drug discovery and development processes, some of the safety issues of medications only appear when a drug is approved to the market. In **Chapter 2**, we have given examples of the application of the concept of structure-activity relationships at the end of the pipeline, namely at the level of drug use in populations. In *Chapter 2.1*, we classified sulfonamide antibiotics and sulfonamide non-antibiotics according to the substituents of the 'sulfa' moiety (SO2NR). An aromatic amine group at the N4 position and a substituent at the N1 position are considered to be triggers for serious allergic drug reactions. These substituents are present in sulfonamide antibiotics, but sulfonamide non-antibiotics (*e.g.* sulfonylureas, thiazide diuretics) lack one or both substituents. In the study presented in *Chapter 2.2*, we classified photosensitive drugs according to their spectroscopic parameters (absorption maximum, molar absorption coefficient) and molecular characteristics (low MW, planar or tricyclic configuration, aromatic halogen substituents, log P). Although in literature generic molecular and spectroscopic characteristics are often mentioned as important factors for the ability to induce photosensitive side effects, little evidence is available whether this is of importance in daily clinical practice.

In the last ten years, some well-known fluorinated drugs, such as cerivastatin and trovafloxacin have been withdrawn for the market. Since the number of fluorinated drugs tends to increase, the role of fluorine in the reporting of adverse drug reactions was studied in *Chapter 2.3*. There are more examples of studies in which drug exposure classification is made on the basis of the chemical structure. It appeared that antiepileptic hypersensitivity syndrome was associated with the use of aromatic antiepileptic drugs (AEDs).[22] In a study of our group, the chemical structure of AEDs was the determinant of interest. It was found that AEDs with an aromatic ring (carbamazepine, phenytoin, lamotrigine) had a two-fold increased risk of hypersensitivity reactions compared to AEDs without an aromatic ring (valproate, pregabalin).[23] Compound lipophilicity is an important factor in drug metabolism, particularly to cytochrome P450 binding.[24] Also the three dimensional conformation plays a part in drug metabolism and drug-target (*e.g.* enzyme, receptor) interaction. It has been long acknowledged that stereochemistry in drug-receptor interaction is an important factor and as early as in 1860, Louis Pasteur recognised that different stereoisomers could have very different physiological properties.[25] Pharmacological active substances such as thalidomide, methadone and ibuprofen have two enantiomers with different activities. The thalidomide molecule is a racemic mixture of (*S*)(−) and (*R*)(+) thalidomide. It was marketed as a sedative and antiemetic for morning sickness. Only the (*R*)(+) form possess these actions, whereas the (*S*)(−) form has antivascular effects, which has led to the development of birth defects.[26] In the case of methadone, the (*S*)(−) form can block the human ether-à-go-go-related gene (hERG) voltage gated potassium channel that can result in prolongation of the QT interval. A reduction of the QT interval has been shown by replacing (*RS*)methadone with (*R*)methadone. Also the metabolism of methadone mediated by CYP2B6 showed stereoselectivety.[27] Ibuprofen is also a racemate with the (*S*)(+) form as active enantiomer whereas (*R*)(−)ibuprofen is pharmacological inactive.

Aspects of pharmaceutical formulation

The experiences with inferior sulfanilamide and paracetamol elixirs show that ADRs are not exclusively induced by the active ingredient of a medication. In the 'elixir' incidents, the cause of the adverse events was the highly toxic solvent diethylene glycol. Also in other medicines, excipients may cause unwanted effects. The main potential adverse effect to an excipient is usually an intolerance or allergy to a specific additive. Sorbitol solutions, used as a sugar free solvent, may cause gastrointestinal side effects and for patients with coeliac disease, tablets with gluten are undesirable. However, sorbitol and gluten are considered 'inert';

only in large quantities or in a limited patient population their adverse effects can occur. In the Netherlands, the Medicine Evaluation Board provides lists with medical products containing gluten, benzyl alcohol and propylene glycol[28] and the European Medicines Agency has issued a guideline for the information on label and packet leaflet with regards to excipients.[29] On the other hand, there are excipients that are not inert and are pharmacological active. An example is the surfactant Cremophor EL® (polyoxyethyleneglycerol tricinoleate 35) used in formulations with hydrophobic compounds. Cremophor EL is known to cause hypersensitivity reactions and peripheral neuropathies, as well as aggregation of erythrocytes. In *Chapter 3.1*, we studied the effects of lipophilic solvents on circulating red blood cells. Cycles of chemotherapy were classified according to the presence or absence of these lipophilic solvents.

In November 1937 and September 1938, several reports appeared in the *Journal of the American Medical Association* dealing with the 'pathologic effects of elixir of sulfanilamide (diethylene glycol) poisoning'.[30-32] At least seventy-six people died as a result of the use of this elixir. Not sulfanilamide, but the solvent diethylene glycol (DEG) was responsible for the pathologic effects, such as tubular necrosis and hepatic damage. Information about toxic effects of DEG was available before market introduction of this elixir. However, the sulfanilamide elixir had not been submitted to the Council on Pharmacy and Chemistry of the American Medical Association nor did the Food and Drug Administration know of its composition. Years later, in the 1990s, the same tragedy took place in Bangladesh and Haiti. The presence of the highly toxic agent DEG (used as a less expensive substitute for propylene glycol and as a contamination of the solvent glycerine, respectively) in paracetamol elixir was responsible for an outbreak of fatal renal failure among children.[33,34] In 2007, the Food and Drug Administration issued a *Guidance for Industry* for the testing of glycerine for DEG after an outbreak of DEG poisoning occurred in Panama.[35]

There is some debate whether substitution of trade name drugs with generic drugs is always appropriate, particularly in case of a narrow therapeutic window or with delivery systems having specific properties. In these cases, switching from a trade name drug to a generic drug is not advisable in patients who are already using them, even when the generic drug is bioequivalent to the trade name drug. Antiepileptic drugs are under continuous scrutiny[36,37] and also substitution of immunosuppressants fuels debate.[38,39] The effect of excipients on the rate and availability of the active ingredient in bioequivalence studies is, however, in general marginal. Unlike small molecule drugs, substitution of a biopharmaceutical with a so-called 'biosimilar' is currently not straightforward because the manufacturing process of biopharmaceuticals is sensitive for changes. A decade ago, an increase in the incidence of pure red cell aplasia (PRCA) was observed that was associated with the use of Eprex® (epoetin alpha). It is still not entirely clear whether the change in formulation (in 1998 human serum albumin had been replaced by

polysorbate 80 as a stabiliser) is the cause of the erythropoietin-associated PRCA incidents.[40] Biosimilars may differ from the original biopharmaceutical product with respect to biological and clinical properties (immunogenicity). The European Medicines Agency has issued guidelines in which biosimilars are set apart from generic drugs since regular bioequivalence studies are not applicable for biopharmaceuticals.[41]

TARGET-ORIENTED DRUG EXPOSURE CLASSIFICATION SYSTEMS

"Corpora non agunt nisi fixata"
Paul Ehrlich (German medical scientist, 1854-1915)

At the end of the 19th century, John Langley and Paul Ehrlich independently formulated the theory of 'receptive substance' or 'receptor', although only in 1907 the concepts also included the binding of drugs to receptors.[42] In 1948, Raymond Ahlquist postulated that there were two types of adrenergic receptors, which he called α and β. Since then there has been a growing interest in the classification of receptors and the challenge is to link together drug-related characteristics with functional activity and the characterisation of a receptor (amino acid sequence).[43]

As stated earlier, drugs can be classified according to their pharmacological targets. In Table 1, an overview of target-based classifications is presented.[7] Names of many drug classes are derived from their pharmacological actions, for example HMG-CoA reductase inhibitors, beta-blocking agents, SSRIs and proton pump inhibitors. Yet, affinity for a specific receptor or (ion) transporter is not always exclusively reserved to one drug class. Therefore, in **Chapter 4**, we put the target-orientated classification into practice. In *Chapter 4.1*, we assessed whether drugs with nephrotoxic and ototoxic ADRs were linked together by a mechanistic commonality, in this case the ability to affect ion channels/transporters in the kidney and inner ear. In addition, we conducted two studies (described in *Chapters 4.2* and *4.3*) in which the degree of affinity for the serotonin (5-HT) transporter (5-HTT) and 5-HT_{2A} receptor was the main exposure determinant in relation with the risk of fractures and the risk of bleeding. Drugs were classified on the basis of their degree of affinity for the transporter or receptor. In both studies, there were previous indications that the neurotransmitter 5-HT was involved in biological processes, which could explain the various adverse events. Particularly, the 5-HTT has gained lots of interest and is subject of many studies. The occurrence of hip fractures and bleeding complications seems to be associated with the degree of

Table 1 Target-based classification of drugs [7]

Target	Example	Activity of drug	Drug examples
Enzymes	cyclooxygenase DNA gyrases (bacterial)	COX2 inhibitor inhibitor	celecoxib fluoroquinolones
Substrates, metabolites and proteins	asparagine		asparaginase
Receptors	GABA receptors	benzodiazepine binding site agonists	benzodiazepines
	adrenoreceptors	β_2-receptor agonists	salbutamol
Ion channels	voltage-gated K^+ channels	inhibitor	amiodarone
Transport proteins	proton pump	H^+/K^+ ATPase	omeprazole
DNA/RNA and the ribosome	DNA and RNA	complexation	cisplatin
Targets of monoclonal antibodies	tumour necrosis factor α		infliximab
Various physicochemical mechanisms		acid binding	magnesium hydroxide
Unknown mechanism of action			4-aminosalicylic acidalendronate

affinity for this transporter.[44-46] There are other studies that used the concept of drug exposure classification by receptor affinity. The α_1-blocking and histamine H_1-blocking properties of antipsychotics have been studied in relation with various outcomes: priapism, fractures and pneumonia.[47-49] In 2000, Sawada *et al.* observed that drugs containing a diethyl amino methyl moiety ($RCH_2{-}NH{-}(CH_2R)_2$), such as haloperidol and cinnarizine seem to play a part in inducing parkinsonism by binding to dopamine D_1 and D_2 receptors.[50] It has been suggested that aripiprazole, an atypical antipsychotic, would be less likely to cause movement disorders because of its partial D_2 agonism. Yet, there are case reports in which aripiprazole – with a diethyl amino methyl moiety – is associated with parkinsonism.[51,52] Since many drugs do not target only one receptor but have affinities for several receptors and/or transporters, a suitable method of approach is to classify drugs on the basis of a combination of binding properties for transporter- and receptor sites. Derijks *et al.* calculated receptor occupancy of antidepressant drugs for the 5-HTT, norepinephrine transporter, muscarine 3 receptor, H_1 receptor, α_1 receptor and the 5-HT_{2C} receptor. Hierarchical cluster analysis was used to identify clusters of

antidepressants with similar binding profile.[53] This classification may give insight whether similar binding profiles are associated with similar therapeutic and adverse effects.

There has always been much interest in the link between molecular structure and biological effect. This has evolved in further attention for the relationship between diseases and underlying physiological processes, drugs and their targets, and drug targets and the occurrence of ADRs. Technological advancements have made it possible to explore possible interactions of investigational and approved drugs with hundreds of targets. Nevertheless, the classification of drugs in accordance with their molecular targets has been complicated by the fact that in many cases the exact biological pathways in which drugs may intervene are not fully known. In some fields, such as in psychiatry, our understanding of pathophysiological processes is limited and therefore the assessment of the association with certain ADRs can be problematic. On the other hand, the analysis of ADRs can be an instrument to examine a disease mechanism. In case of loss of impulse control disorders (ICDs) in Parkinson disease, the finding that particularly the use of dopamine agonists was associated with ICDs was an indication that dopaminergic pathways were involved in the development of these ADRs.[54,55]

IMPLICATIONS FOR DRUG SAFETY

In the studies described in the previous chapters, we used data originating from several data sources, containing heterogeneous information, and used different study designs. We showed that it is feasible to conduct pharmacoepidemiological studies in which drug exposure has been classified using a mechanism-based approach. In this chapter, other examples of classification schemes are discussed. Two issues remain: 1) what are the implications of mechanism-based drug exposure classification for post-approval research, in particular for testing of associations between drugs and ADRs as well as for the detection of such associations? and 2) what is the consequence of mechanism-based drug exposure classification for the 'class' discussion in drug development, drug regulatory affairs and clinical practice?

The major aim of pharmacovigilance is the early detection and assessment of previously unknown ADRs. A valuable tool for the detection of these ADRs is the analysis of spontaneous reports of suspected ADRs, which are reported by physicians, pharmacists, pharmaceutical manufactures and patients to national

and international pharmacovigilance centres. These individual case reports are organised in databases and subsequently analysed and interpreted, generating hypotheses of new potential drug-ADR associations (*i.e.* a 'signal'), which can encourage further research.[56] Automated quantitative signal detection is indispensable, since large numbers of reports are stored in the databases and it is impossible to assess all individual reports. Methods for quantitative signal detection aim to detect combinations of a drug and a clinical event that are disproportionally highly represented in the database.[56,57] Several measures of determining disproportionality are applied, such as the reporting odds ratio (ROR), proportional ADR reporting ratio (PRR), the Bayesian Confidence Propagation Neural Network analysis and in the field of pharmacogenetics, techniques for 'large-scale multiple testing' have been developed. Currently, data mining approaches in spontaneous reporting drug safety data are focused on a drug-ADR combination and do not take into consideration the possibility of categorising drug exposure according to similarities in molecular structure or biological activity. This raises the question whether a combination of a molecular characteristic or an underlying mechanism and an ADR will be observed and hence whether we are not missing valuable information for generating alerts in post marketing drug surveillance and pharmacovigilance. Mechanism-based drug exposure classification in drug safety can be very useful as the study of anti-hERG activity and drug-induced arrhythmias has proven.[58] Therefore, it is a challenge to include mechanism-based determinants in data mining approaches. Subsequently, hypotheses of potential signals could be extended to target-ADR and molecular (sub)structure-ADR associations. There are four possible situations in which mechanism-based drug exposure classification instead of or along with pharmacotherapeutical classification can be put into practice (Table 2). When there is 'evidence' for an association between a drug and a certain ADR, and there is a biological rationale (situation I), mechanism-based drug exposure classification is the appropriate method to support or confirm previous results, as done in *Chapters 2.1*, *2.2* and *4.3*. In situation II, mechanism-based drug exposure classification can provide or enhance knowledge about underlying mechanisms in a drug (class) – ADR combination (*Chapters 4.1* and *4.2*). In situations III and IV, mechanism-based drug exposure classification in data mining might generate new hypotheses (*Chapters 2.3* and *3.1*). An open mind for expected and unexpected target/molecule – ADR associations and the application of structure-activity-ADR relationships in pharmacoepidemiological studies are indispensable tools in post marketing drug surveillance.

Table 2 Four situations in mechanism-based drug exposure classification

		suspected mechanism/biological rationale	
		yes	no
'evidence'/hypothesis for signal (drug – ADR)	yes	I	II
	no	III	IV

Is the discussion *what makes a 'class' a 'class'*? not already outdated with the introduction of biological drugs as their share of all launched new molecular entities is increasing? Probably not, since also the group of biopharmaceuticals is a heterogeneous one and different classes are recognisable within this group. Small chemical molecules will always remain a part of the pharmacotherapeutical arsenal and consequently, in drug development, in regulatory affairs and in clinical practice, practical classification schemes of medicines will be necessary. Drug regulatory authorities are confronted with a variety of drugs, with various molecular properties and mechanisms of action. With each registration of a new chemical entity or with a new safety issue, regulatory authorities have to go into the subject of drug classification. In drug discovery and development, mechanism-based classification is routinely executed. Many databases are freely available for research on drug – target relationships,[1] new molecular targets for known drugs,[59] links between drug classes and metabolism,[60] and the use of side effects to identify drug targets.[61] All this information may generate many novel drug – target relationships, but this information has to be disseminated from the field of drug discovery to the level of clinical use of new and older drugs. The challenge will be to link the type of databases used in drug innovation and drug development with databases used in post-approval studies. Information on the effect of drugs in population-based settings and in real-life circumstances and the use of spontaneous reported adverse drug reactions databases may enhance further understanding in the complex effects of existing drugs.

In clinical practice, the most important aspect of a drug is that it lives up to its expectations; it has to be effective without serious side effects. However, the understanding of underlying biological mechanisms is equally important in order to explain, for example, differences in drug-drug interactions for drug classes that are thought to be homogenous with regards to their actions (*e.g.* SSRIs and tamoxifen). Because physicochemical properties such as specific

molecular substructures, lipophilicity and molecular weight are of influence of physiological properties (metabolic inactivation, transport to gastrointestinal tract and kidneys, membrane permeability and binding to plasma albumin and tissues), knowledge of these intrinsic drug characteristics is essential. Health care professionals should be aware of structure-activity-relationships (SAR) in drug monitoring. Understanding of SAR may contribute information in the analysis of adverse drug effects and subsequently, the reporting of suspected adverse drug reactions to pharmacovigilance centres. Pharmacists are the dedicated persons to play an important role in bringing together the knowledge about chemical and pharmacological properties with clinical issues.

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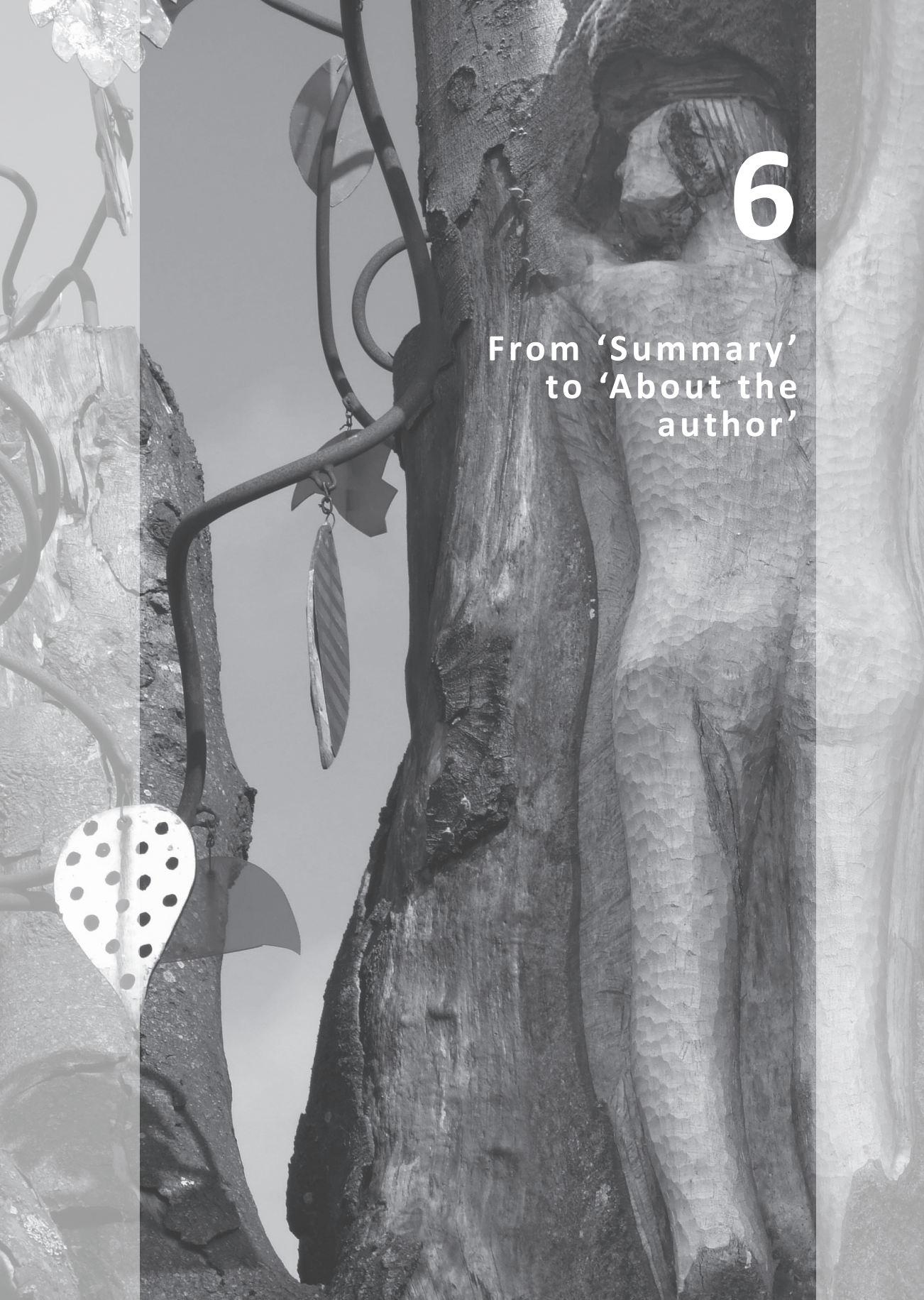
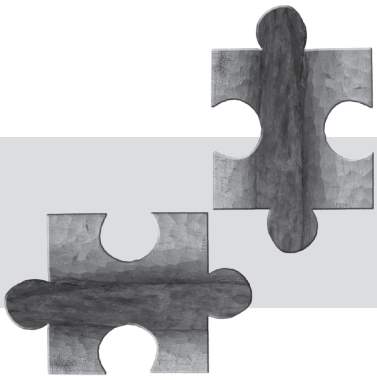


6

From ‘Summary’ to ‘About the author’

Summary



CHAPTER 1

In pharmacoepidemiology and pharmacovigilance, the relation between drug exposure and clinical outcomes is crucial. Exposure classification in pharmacoepidemiological studies is traditionally based on pharmacotherapeutical reasoning, but other classification schemes on the basis of molecular characteristics, pharmaceutical formulation or pharmacological mechanisms are also possible. The concept of quantitative structure-activity relationships (QSAR), the recognition of James Watson, Francis Crick and Maurice Wilkins for their work on the molecular structure of nucleic acids and the McBride-letter in the Lancet on his observations of congenital malformations among women using thalidomide − all milestones of the 1960s − build the landscape for the central theme described in this thesis, the mechanism-based classification of drugs, a merger of the fields of chemistry, biology and drug safety. **Chapter 1** describes the concept of the mechanism-based classification of drugs and the objective and outline of this thesis. Although the concept of mechanism-based drug classification is not a new concept, there is increasing attention for this subject in pharmacoepidemiological studies. The objective of this thesis was to evaluate the role of structure-activity relationships in the understanding and prediction of drug-induced safety problems.

CHAPTER 2

The three studies presented in **Chapter 2** focused on drug exposure classification according to molecular characteristics. In *Chapter 2.1*, we assessed whether differences in chemical structure of various sulfonamide drugs influenced the risk of allergic reactions. The substituents at the N1 (5- or 6-member aromatic heterocyclic ring) and N4 position (arylamine) of the 'sulfa' group are considered to be important allergenic determinants. Sulfonamide antibiotics contain both substituents, in contrast to sulfonamide non-antibiotics. Data were obtained

from the General Practice Research Database (GPRD). We identified all patients with a diagnosis of diabetes mellitus or a prescription for a drug indicated for its treatment in the period form 1987−2001. We performed a nested case-control study within this study base. Cases were patients with at least one diagnosis of hypersensitivity or allergic reaction and they were matched up to three controls. For each patient, we identified all prescriptions for sulfonamide drugs. Sulfonamide drugs were classified according to their presence/absence of the N1 substituent or an arylamine at the N4 position. The study population consisted of 3 362 case patients and 10 041 control patients. Overall, current use of sulfonamide drugs was associated with an increased risk of hypersensitivity reactions and allergic outcomes (adjusted odds ratio [OR] 2.36; 95% confidence interval [CI] 2.08−2.69). Stratified results did not show statistically significant differences between the groups of sulfonamides, although a more pronounced increase in risk for $N1^+$ $N4^+$ sulfonamide drugs (predominantly sulfonamide antibiotics) was found (adjusted OR 3.71; 95% CI 1.40−9.81).

In *Chapters 2.2* and *2.3*, we used data from the International Drug Monitoring Program of the World Health Organization (WHO). The WHO-ADR database, Vigibase, contains summaries of individual case safety reports originally submitted to national pharmacovigilance centres all over the world. The study, in which the association between spectroscopic and other molecular drug characteristics and the occurrence of photosensitivity reactions was assessed, is described in *Chapter 2.2*. Molecular exposure variables, such as absorption maximum λ_{max}, molar absorption coefficient ε, area under the (absorption) curve (AUC), presence of aromatic halogen atoms and lipophilicity, were determined for 143 study drugs, derived from drug classes that are known to be dominant photosensitisers (*e.g.* tetracyclines, quinolone antibiotics, phenothiazine antipsychotics and diuretics). Photosensitivity reactions were strongly associated with a λ_{max} between 290 and 320 nm (OR 3.74; 95% CI 3.45−4.06), an $\varepsilon > 20\,000$ (OR 5.49; 95% CI 5.10−5.92), a large AUC (OR 7.89; 95% CI 6.95−8.96) and aromatic halogen atoms (OR 3.37; 95% CI 3.15−3.61). Analysis and evaluation of spectroscopic and molecular characteristics can be useful for regulatory authorities and drug development because they may contribute to the detection and prediction of agents with photoreactive potential.

Particularly in the lay media, concern about the use of fluorinated drugs is expressed. Fluorine compounds used in dentistry and water fluoridation have fuelled the debate about fluoride poisoning. Although these fluorine compounds

(inorganic fluorides) are different from fluorinated drugs (organofluorines), the introduction of a fluorine atom can change the behaviour of a drug molecule by modifying its physicochemical properties. In this light, in *Chapter 2.3* we present a study in which we assessed whether there was an association between the presence of fluorine atoms in drug compounds and the reporting of ADRs. The outcomes of interest were ADRs relating to skin and appendages, musculo-skeletal system disorders, psychiatric disorders and liver and biliary system disorders. From six drug classes, we selected a representative fluorinated drug and a non-fluorinated counterpart and calculated the proportional ADR reporting ratio (PRR) for each outcome of interest. The six pairs were: 1) fluoxetine *vs.* sertraline, 2) dexamethasone *vs.* prednisolone, 3) fluvastatin *vs.* pravastatin, 4) celecoxib *vs.* valdecoxib, 5) mefloquine *vs.* quinine and 6) ciprofloxacin *vs.* pipemidic acid. For three out of six pairs, ADRs for skin and appendage disorders and liver and biliary disorders were statistically significantly more frequently reported for fluorinated study drugs than for their non-fluorinated counterparts (pair 1−3 and pair 1, 4 and 6, respectively). Known differences (*e.g.* tendon disorders associated with fluoroquinolones) between fluorinated and non-fluorinated study drugs appeared from the analyses. There was a statistically significant PRR for ciprofloxacin/pipemidic acid and musculo-skeletal system disorders (tendon disorders) and also for mefloquine/quinine for psychiatric disorders. No conclusion could be drawn with regards to a more disproportional reporting of ADRs for fluorinated drugs in general.

CHAPTER 3

Chapter 3.1 addresses the classification on the basis of pharmaceutical formulation. We studied whether the presence of lipophilic solvents in cytostatic drug regimens affected circulating red blood cells. Data were obtained from the Utrecht Patient Oriented Database, a database encompassing automatic data collected during clinical care on patient demographics, hospital discharge diagnoses, medication exposure, medical procedures and laboratory tests for all patients treated at the University Medical Center Utrecht. The study population consisted of adult oncology patients who received a first course of chemotherapy comprising taxanes (paclitaxel and docetaxel) and/or platinum compounds (cisplatin and carboplatin). Paclitaxel and docetaxel formulations contain the lipophilic solvents Cremophor EL® and polysorbate 80, respectively, in contrast to platinum compounds, which are not formulated with lipophilic solvents. These solvents, as well as taxanes

and cisplatin, have the ability to induce phosphatidylserine (PS) exposure at the cell surface of erythrocytes. Erythrocytes that express PS are readily eliminated from circulating blood and accelerated clearance can result in anaemia. Cytostatic regimens were classified according to the presence of lipophilic solvents (taxane group versus platinum group). For each patient, blood cell counts had been performed at baseline (T0) and during the first treatment period (T1). A number of routinely measured blood cell parameters were evaluated. The difference (delta) between both measurements for each parameter was calculated. The study included 320 patients, of whom 24 patients were treated with a cytostatic regimen with lipophilic solvents. There was a statistically significant difference in deltas for the number of erythrocytes, mean haemoglobin, haematocrit and mean platelet volume between the taxane and the platinum group. Although we could not conclude whether the presence of lipophilic solvents was responsible for the observed differences in blood cell indices, the findings in this study warrant further research to unravel underlying mechanisms and to assess to possible clinical relevance.

CHAPTER 4

Chapter 4 focused on target-orientated drug exposure classification. In *Chapters 4.1*, *4.2* and *4.3*, affinity for pharmacological targets such as ion channels, transporters and receptors was regarded as exposure variable.

Several drugs are associated with both renal adverse effects and ototoxicity, such as aminoglycosides and loop diuretics. Ototoxic effects are usually registered when drugs already are on the market, as in pre-clinical and clinical studies effects on the ear are − in contrast to the effects of drugs on the kidneys − not routinely evaluated. For the study described in *Chapter 4.1*, data from the Netherlands Pharmacovigilance Centre Lareb were used to assess whether renal ADRs had predictive value for ear and labyrinth ADRs and whether the involved drug classes influenced ion transport systems. We selected all reports with suspected ADRs for relevant renal disorders and defined them as cases. In addition, all reports with suspected ADRs of ear and labyrinth disorders were defined as cases. All other ADRs not being renal or ear disorders were defined as non-cases. Te estimate the association between drug classes and reports with renal ADRs and ear and labyrinth disorders, respectively, reporting odds ratios (ROR) were calculated. Drug classes were classified into four groups on the basis of the ROR for renal (ROR kidney) and ear and labyrinth (ROR ear) disorders: *group A* ROR kidney

< 1.50 and ROR ear < 1.50 or no reports on ear ADRs (reference group); *group B* ROR kidney < 1.50 and ROR ear ≥ 1.50; *group C* ROR kidney ≥ 1.50 and ROR ear < 1.50 or no reports on ear ADRs; and *group D* ROR kidney ≥ 1.50 and ROR ear ≥ 1.50. In addition, we evaluated whether the drug classes had the ability to affect ion transport processes in kidney and inner ear tissues. There were 1068 reports with relevant renal ADRs in which 193 drug classes were involved. Fourteen drug classes had a ROR ≥ 1.50 for both kidney and ear ADRs (*group D*). In this group, there were drug classes with well-known effects on kidneys and ears (aminoglycosides, loop diuretics, quinine). Compared to the reference group, drug classes in *group D* were more likely to be involved with ion transport processes (OR 12.2; 95% CI 3.0−30.5). Also drug classes in *group B* were associated with the ability to affect ion channels (OR 8.6; 95% CI 2.4−18.7). Renal ADRs were not a marker for ear and labyrinth disorders as such, but the ability of drugs to act on ion transporters or ion channels might be a predictor for the possible occurrence of drug-related ototoxicity.

Two case-control studies were conducted that used data from the Dutch PHARMO Record Linkage System (RLS), in which demographic details and complete medication history of more than two million community-dwelling residents are linked with hospital admission records (*Chapter 4.2* and *4.3*). Previous research has suggested that serotonin (5-HT) plays a role in the increased risk of bleeding complications after intake of antidepressants drugs, particularly selective serotonin reuptake inhibitors (SSRIs). The 5-HT_{2A} receptor is the only 5-HT receptor located on the membrane of platelets. Since not only antidepressants have affinity for the 5-HT transporter (5-HTT) or 5-HT_{2A} receptor, other serotonergic drugs, such as antipsychotics, may be associated with the risk of bleeding. Therefore, we assessed whether there was an association between serotonergic drug use and the risk of female tract, gastrointestinal or intracranial bleeding (*Chapter 4.2*). Adult patients with a first hospital admission for one of the bleeding types were regarded as cases, matched with up to two control patients. For all patients, all dispensings for antidepressants, antipsychotics and ergoline derivatives (agonists for the 5-HT_{2A} receptor) were identified. Classification of these drugs was made in a therapeutically-based way, as well as on the basis of affinity (high-medium-low) for the 5-HTT and 5-HT_{2A} receptor. The study population comprised 28 286 cases. Female genital tract bleedings were the most frequent type of bleeding (47.4%), followed by gastrointestinal bleeding (32.7%). Current use of antidepressant drugs was associated with an increased risk of female genital tract, gastrointestinal and intracranial bleeding (OR 2.37; 95% CI 2.12−2.64, OR 1.36,

95% CI 1.20−1.53, OR 1.41; 95% CI 1.21−1.64, respectively). Use of antipsychotic drugs increased the risk of gastrointestinal and intracranial bleeding (OR 1.79; 95% CI 1.41−2.27, OR 1.44; 95% CI 1.06−1.95), whereas ergoline derivatives were only associated with female genital tract bleedings (OR 2.29, 95% CI 1.28−4.08). The risk estimates for gastrointestinal and intracranial bleeding were higher for new users of antidepressants and antipsychotic drugs compared to prevalent users. An explanation for this finding may be that the decrease in serotonin levels is neutralised after a few weeks. No association was found between the degree of affinity for the 5-HTT and 5- HT2A receptor and any type of bleeding.

In *Chapter 4.3*, the association between the degree of inhibition of the 5-HTT and the occurrence of osteoporotic and non-osteoporotic fractures was assessed. In literature, several drug classes have been associated with risk of falls and fractures, including antidepressant drugs. The reduction of bone mass and the effect on bone micro architecture has been related to the inhibition of the 5-HTT. From the PHARMO RLS, we identified all adult patients with a first record for a hospital admission for a fracture (cases, n = 16 717). They were matched with up to four control patients on age, gender, geographical area and calendar time (n = 61 517). Antidepressants were categorised into three groups according to their affinity for the 5-HTT (high-medium-low). Fractures were classified as osteoporotic (hip/femur, radius/ulna, humerus, vertebral, rib and clavicle fractures) or non-osteoporotic. Osteoporotic fractures were the most frequent fracture type (59.5%). Current use of SSRIs, tricyclic antidepressants (TCA) and non-SSRI/non-TCA antidepressants was associated with an increased risk of both osteoporotic and non-osteoporotic fractures. When classifying antidepressants according to their affinity for the 5-HTT, we found that that the risk of an osteoporotic fracture was higher for antidepressants with a high affinity for the 5-HTT (OR 1.86; 95% CI 1.63−2.13), compared to antidepressants with a medium (OR 1.43; 95% CI 1.19−1.72) or low affinity (OR 1.32; 95% CI 0.98−1.79). Any association between the degree of 5-HTT inhibition and non-osteoporotic fractures was not observed.

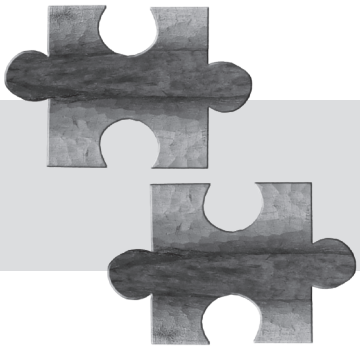
In conclusion, the findings in *Chapters 4.2* and *4.3* showed that classification on the basis of pharmacological mechanisms could be an appropriate method to study the associations between the use of drugs and the risk of adverse drug reactions.

CHAPTER 5

In **Chapter 5**, the general discussion, the concept of mechanism-based drug exposure classification was put into broader perspective and the importance of

molecular and pharmacological drug characteristics in classifying drug exposure was described. In post-approval research, mechanism-based drug exposure classification can be used to support or confirm previous results (*Chapters 2.1*, *2.2* and *4.3*), provide or enhance knowledge about underlying mechanisms in drug – ADR combinations (*Chapters 4.1* and *4.2*) or might generate new hypotheses (*Chapters 2.3* and *3.1*). It will be a challenge to link databases used in drug development with drug – target relationships and databases used in post-approval studies with information on drug – ADR combinations. These combined databases may be profitable for drug development, regulatory affairs and clinical practice. Where chemistry, biology and pharmacotherapy come together, pharmacists are indispensable in the evaluation of adverse drug effects and drug monitoring.

Samenvatting



HOOFDSTUK 1

In het begin van de jaren zestig van de vorige eeuw was het een roerige tijd. De Vietnamoorlog was op zijn hoogtepunt en de Verenigde Staten en de Sovjetunie hielden de wereld in de greep tijdens de Cubacrisis. Het was het jaar waarin Marilyn Monroe stierf en Ruud Gullit werd geboren. Op wetenschappelijk gebied waren er enkele opmerkelijke gebeurtenissen. In 1962 verscheen er een artikel van Corwin Hansch en collega's waarin het concept van kwantitatieve structuur-activiteitsrelaties werd besproken. In datzelfde jaar ontvingen James Watson, Francis Crick en Maurice Wilkins de Nobelprijs voor Fysiologie of Geneeskunde voor "hun ontdekkingen met betrekking tot de moleculaire structuur van nucleïnezuren". Eind 1961 publiceerde de Lancet een ingezonden brief van William McBride, een Australische arts, over het gebruik van Softenon® (thalidomide, een middel tegen ochtendmisselijkheid tijdens de zwangerschap) en het optreden van aangeboren afwijkingen. Deze feiten op het gebied van chemie, biologie en geneesmiddelveiligheid vormen de basis voor het thema dat in dit proefschrift is beschreven, het concept van mechanisme-georiënteerde classificatie van geneesmiddelen.

Hoofdstuk 1 is een algemene inleiding waarin wordt ingegaan op mechanisme-georiënteerde classificatie van geneesmiddelen. In de farmaco-epidemiologie en farmacovigilantie is de relatie tussen blootstelling aan geneesmiddelen en klinische uitkomsten een belangrijk onderzoeksgebied. Geneesmiddelclassificatie in farmaco-epidemiologische studies is van oudsher gebaseerd op farmaco-therapeutische gronden, maar ook andere classificaties op basis van moleculaire eigenschappen, formulering of farmacologische mechanismen zijn mogelijk. Hoewel mechanisme-georiënteerde classificatie van geneesmiddelblootstelling geen nieuw concept is, wordt hieraan in farmaco-epidemiologische studies wel steeds meer aandacht geschonken. Het doel van dit proefschrift is de rol van

structuur-activiteitsrelaties te evalueren teneinde problemen op het gebied van geneesmiddelveiligheid beter te begrijpen en te voorspellen.

HOOFDSTUK 2

In de drie studies die beschreven staan in **Hoofdstuk 2**, wordt het concept van classificatie van geneesmiddelenblootstelling op basis van moleculaire eigenschappen toegepast. In *Hoofdstuk 2.1* is onderzocht of verschillen in chemische structuur van diverse sulfonamidegeneesmiddelen het risico van allergische reacties beïnvloedden. Voornamelijk de substituenten aan de N1-(aromatisch heterocyclische 5- of 6-ring) en N4-positie (arylamine) van de 'sulfa'groep zouden verantwoordelijk zijn voor het optreden van deze reacties. Geneesmiddelen die deze beide groepen bevatten zijn de sulfonamide-antibiotica, dit in tegenstelling tot sulfonamide-nietantibiotica, zoals sulfonylureaderivaten en thiazidediuretica. Gegevens voor de studie waren afkomstig van de General Practice Research Database (GPRD), een Britse databank met gegevens van ongeveer 650 huisartsenpraktijken. De GPRD bevat informatie over patiëntenkarakteristieken, voorgeschreven geneesmiddelen, verwijzingen naar specialisten en ziekenhuisopnames. Patiënten met een diagnose diabetes mellitus of een voorschrift voor de behandeling van diabetes in de studieperiode van 1987–2001 werden geïdentificeerd. Het onderzoek was een patiënt-controleonderzoek. Er werden 3362 patiënten geïdentificeerd met een diagnose voor een allergische reactie en 10.041 patiënten zonder allergische reactie werden geselecteerd als controles. Voor zowel patiënten als controlepatiënten werden alle voorschriften voor sulfonamidegeneesmiddelen verzameld. De sulfonamidegeneesmiddelen werden ingedeeld in vier groepen, al naar gelang de aanwezigheid van N1- of N4-substituenten ($N1^+N4^+$, $N1^+N4^-$, $N1^-N4^+$ en $N1^-N4^-$). Het gebruik van sulfonamidegeneesmiddelen was geassocieerd met een verhoogd risico van allergische reacties (odds ratio [OR] 2,36; 95% betrouwbaarheidsinterval [BI] 2,08–2,69). Er werden geen statistisch significante verschillen gevonden tussen de verschillende groepen sulfonamidegeneesmiddelen, hoewel voor $N1^+N4^+$ sulfageneesmiddelen (voornamelijk sulfonamide-antibiotica) een duidelijk verhoogd risico werd gevonden (OR 3,71; 95% BI 1,40–9,81).

In de *Hoofdstukken 2.2* en *2.3* werden gegevens gebruikt van het International Drug Monitoring Program van de Wereld Gezondheidsorganisatie (World Health Organization, WHO). Vigibase, de databank van de WHO, bevat gegevens over

vermoedelijke bijwerkingen van geneesmiddelen die oorspronkelijk zijn gemeld aan nationale farmacovigilantiecentra. De studie waarin de associatie tussen spectroscopische en andere moleculaire geneesmiddelkarakteristieken en het optreden van fotosensitiviteitsreacties werd geëvalueerd, wordt beschreven in *Hoofdstuk 2.2*. Voor 143 geneesmiddelen, afkomstig uit geneesmiddelklassen die bekend staan als fototoxisch (bijv. tetracyclines, chinolonantibiotica, fenothiazine antipsychotica en diuretica) werden moleculaire variabelen bepaald, zoals het absorptiemaximum λ_{max}, de molaire absorptiecoëfficiënt ε, oppervlakte onder de absorptiecurve (AUC), aanwezigheid van aromatische halogeenatomen en lipofiliteit. Fotosensitiviteitsreacties waren sterk geassocieerd met een λ_{max} tussen 290 en 320 nm (OR 3,74; 95% BI 3,45−4,06), een ε > 20.000 (OR 5,49; 95% BI 5,10−5,92), een grote AUC (OR 7,89; 95% BI 6,95−8,96) en aromatische halogeenatomen (OR 3,37; 95% BI 3,15−3,61). Analyse en evaluatie van spectroscopische en moleculaire parameters kunnen nuttig zijn voor toelatingsautoriteiten en in geneesmiddelontwikkeling, omdat zij kunnen bijdragen in de detectie en voorspelling van chemische verbindingen met fotoreactieve eigenschappen.

Het gebruik van gefluorideerde geneesmiddelen staat − voornamelijk in Amerikaanse media − in een kwaad daglicht, omdat fluorideverbindingen die gebruikt worden in de tandheelkunde en voor waterfluoridering aanleiding hebben gegeven tot discussie over mogelijke fluoridevergiftiging. Hoewel deze fluorideverbindingen (anorganische fluoriden) verschillen van gefluorideerde geneesmiddelen (organische verbindingen), kan de aanwezigheid van een fluoratoom het gedrag van een geneesmiddel veranderen door de fysisch-chemische kenmerken te modificeren. In *Hoofdstuk 2.3* presenteren we een studie waarin werd gekeken naar de associatie tussen de aanwezigheid van fluoratomen in geneesmiddelen en de rapportage van verschillende typen bijwerkingen, namelijk huidreacties, spier- en gewrichtsaandoeningen, psychiatrische aandoeningen en leveraandoeningen. Uit zes geneesmiddelgroepen werd een representatief geneesmiddel met een fluorgroep en een niet-gefluorideerd controlegeneesmiddel geselecteerd en werd de proportional reporting ratio (PRR) berekend. De zes geneesmiddelparen waren: 1) fluoxetine *vs.* sertraline, 2) dexamethason *vs.* prednisolon, 3) fluvastatine *vs.* pravastatine, 4) celecoxib *vs.* valdecoxib, 5) mefloquine *vs.* kinine en 6) ciprofloxacine *vs.* pipemidinezuur. Voor drie van de zes paren werden huidreacties en leveraandoeningen statistisch significant vaker gerapporteerd voor de fluorgeneesmiddelen dan voor hun controlegeneesmiddel (respectievelijk paar 1 t/m 3 en paar 1, 4 en 6). Bekende

verschillen (zoals achillespeesaandoeningen bij fluorchinolonen) tussen gefluorideerde en niet-gefluorideerde geneesmiddelen werden in de analyses duidelijk. De PRR was statistisch significant verhoogd voor ciprofloxacine/pipemidinezuur en spier- en gewrichtsaandoeningen (achillespeesblessures) en ook voor mefloquine/kinine en psychiatrische aandoeningen. Op basis van deze studie kan echter niet de conclusie worden getrokken dat er in het algemeen een disproportionele rapportage van bijwerkingen is voor fluorgeneesmiddelen.

HOOFDSTUK 3

Hoofdstuk 3.1 richt zich op de classificatie van geneesmiddelen op basis van farmaceutische formulering. Er werd bestudeerd of de aanwezigheid van lipofiele oplosmiddelen in cytostaticakuren rode bloedcellen kon aantasten. De gegevens werden verkregen van de Utrecht Patient Oriented Database (UPOD), een databank waarin gegevens zijn verzameld van patiënten tijdens hun behandeling in het Universitair Medisch Centrum Utrecht (UMC Utrecht). Naast demografische gegevens, gegevens over geneesmiddelblootstelling, medische verrichtingen en ontslagdiagnoses, bevat UPOD elektronisch vastgelegde laboratoriumuitslagen en unieke gegevens over het bloedbeeld van patiënten die routinematig zijn bepaald in het diagnostisch laboratorium van het UMC Utrecht. De studiepopulatie bestond uit volwassen patiënten met kanker die een eerste chemokuur met een taxaan (paclitaxel en docetaxel) en/of een platinaverbinding (cisplatine en carboplatine) kregen in de periode 2005−2009. Paclitaxel en docetaxel zijn alleen op de markt in een formulering met lipofiele oplosmiddelen, namelijk Cremophor EL® (CrEL) and polysorbaat 80, terwijl platinaverbindingen zonder deze oplosmiddelen in de handel zijn. Net als de taxanen en cisplatine, kunnen CrEL en polysorbaat zorgen voor expressie van fosfatidylserine (FS) aan de oppervlakte van erytrocyten. Erytrocyten met FS-expressie kunnen gemakkelijk uit de bloedsomloop worden verwijderd en deze versnelde uitscheiding kan resulteren in bloedarmoede. Cytostaticakuren werden geclassificeerd op basis van de aanwezigheid van lipofiele oplosmiddelen (taxaangroep *vs.* platinagroep). Voor elke patiënt werd het bloedbeeld bepaald voordat aan een cytostaticakuur werd begonnen (T0) en gedurende de eerste behandeling (T1). Het verschil (delta) tussen de waarden op T1 en T0 werd berekend voor verschillende hematologische parameters. Van de 320 patiënten in deze studie werden er 24 behandeld met een cytostaticakuur met lipofiele oplosmiddelen. De delta bij het aantal erytrocyten, het hemoglobine, hematocriet en gemiddeld plaatjesvolume was groter bij de taxaan- dan bij de

platinagroep. Of de aanwezigheid van lipofiele oplosmiddelen verantwoordelijk was voor de verschillen in hematologische parameters werd niet duidelijk, maar de bevindingen in deze studie geven wel aanleiding tot het doen van verder onderzoek naar de onderliggende mechanismen en naar de mogelijke klinische relevantie.

HOOFDSTUK 4

In **Hoofdstuk 4** staat de target-georiënteerde classificatie van geneesmiddel-blootstelling centraal. In de *Hoofdstukken 4.1*, *4.2* en *4.3* werd de blootstelling aan geneesmiddelen ingedeeld aan de hand van affiniteit voor farmacologische targets zoals ionkanalen, transporters en receptoren.
Er zijn diverse geneesmiddelen die zowel bijwerkingen kunnen veroorzaken op de nier als op het oor, zoals aminoglycosiden en lisdiuretica. Ototoxische bijwerkingen worden vaak pas opgemerkt nadat de geneesmiddelen al op de markt zijn verschenen, omdat in pre-klinische studies en klinische trials effecten op het oor niet routinematig worden onderzocht in tegenstelling tot effecten op de nieren. Voor de studie die wordt beschreven in *Hoofdstuk 4.1* werd gebruik gemaakt van gegevens afkomstig van het Nederlands Bijwerkingencentrum Lareb. Er werd onderzocht of bijwerkingen op de nier voorspellend waren voor bijwerkingen op het oor en of de betrokken geneesmiddelklassen iontransportsystemen konden beïnvloeden. Alle rapporten met meldingen betreffende vermoedelijke bijwerkingen op de nieren werden geselecteerd en als 'cases' aangemerkt. Verder werden ook alle rapporten met vermoedelijke bijwerkingen op het oor geselecteerd en tevens aangemerkt als 'cases'. Alle andere rapporten met de overige bijwerkingen waren de 'non-cases'. Reporting odds ratios (ROR) werden berekend voor de associatie tussen geneesmiddelklassen en rapporten met gemelde bijwerkingen op de nier en op het oor. De ROR is een maat voor de disproportionaliteit in het aandeel van een bepaald geneesmiddel of een bepaalde geneesmiddelgroep bij het rapporteren van een bijwerking ten opzichte van alle andere middelen. Geneesmiddelklassen werden in vier groepen ingedeeld op basis van de ROR voor nieraandoeningen (ROR nier) en gehoorproblemen (ROR oor): *groep A* ROR nier < 1,50 en ROR oor < 1,50 of geen rapporten voor oorbijwerkingen (referentiegroep); *groep B* ROR nier < 1,50 and ROR oor ≥ 1,50; *groep C* ROR nier ≥ 1,50 and ROR oor < 1,50 of geen rapporten voor oorbijwerkingen; and *groep D* ROR nier ≥ 1,50 and ROR oor ≥ 1,50. Er waren 1068 rapporten waarin relevante bijwerkingen op de nier werden vermeld; hierbij waren 193 geneesmiddelgroepen betrokken. Veertien

geneesmiddelgroepen hadden zowel een ROR nier als een ROR oor ≥ 1,50 (*groep D*). Onder deze 14 groepen waren verschillende geneesmiddelklassen die bekend staan om hun bijwerkingen op nier en oor (aminoglycosiden, lisdiuretica, kinine). Vergeleken met de referentiegroep was de kans hoger dat geneesmiddelklassen in *groep D* betrokken waren bij iontransportprocessen (OR 12,2; 95% BI 3,0−30,5). Ook geneesmiddelklassen in *groep B* waren geassocieerd met een effect op ionkanalen (OR 8,6; 95% BI 2,4−18,7). Renale bijwerkingen kunnen niet als zodanig gebruikt worden als 'marker' voor bijwerkingen op het oor, maar de invloed van geneesmiddelen op iontransporters of ionkanalen kan een maatstaf zijn voor het optreden van geneesmiddelgerelateerde ototoxiciteit.

Twee patiënt-controleonderzoeken werden uitgevoerd met gegevens uit het PHARMO Record Linkage System. Deze Nederlandse onderzoeksdatabank wordt gebruikt voor farmaco-epidemiologisch onderzoek en bevat demografische gegevens en complete medicatiehistories van meer dan twee miljoen personen, gekoppeld aan ziekenhuisopnamen (*Hoofdstukken 4.2* en *4.3*). Uit eerder onderzoek is gebleken dat serotonine (5-HT) een rol speelt bij de verhoogde kans op bloedingrisico bij gebruik van antidepressiva, met name serotonine heropnameremmers (SSRIs). De 5-HT_{2A} receptor is de enige 5-HT receptor die zich op de membraan van bloedplaatjes bevindt. Niet alleen antidepressiva hebben een affiniteit voor de 5-HT transporter (5-HTT) of 5-HT_{2A} receptor, maar ook andere geneesmiddelgroepen zoals antipsychotica. Deze geneesmiddelen zouden derhalve ook een verhoogde kans op bloedingen kunnen geven. Daarom onderzochten we, in de studie beschreven in *Hoofdstuk 4.2*, of er een associatie was tussen het gebruik van serotonerge geneesmiddelen en het risico van bloedingen. In dit patiënt-controleonderzoek werden patiënten met een eerste ziekenhuisopname voor een menstruele, gastrointestinale of intracraniële bloeding gedefinieerd als cases, die werden gematcht met maximaal twee controlepatiënten. Voor alle patiënten werden alle voorschriften voor antidepressiva, antipsychotica en ergolinederivaten (agonisten voor de 5-HT_{2A} receptor) geïdentificeerd. Deze geneesmiddelen werden ingedeeld in geneesmiddelklassen en tevens geclassificeerd al naar gelang hun affiniteit (hoog-midden-laag) voor de 5-HTT en 5-HT_{2A} receptor. In de periode van 1998−2007 werden 28.286 patiënten met een ziekenhuisopname voor een bloeding geïdentificeerd. Menstruele bloedingen kwamen het meeste voor (47.4%), gevolgd door gastrointestinale bloedingen (32.7%). Gebruik van antidepressiva was geassocieerd met een verhoogd risico op menstruele, gastrointestinale en intracraniële bloedingen (respectivelijk OR 2,37; 95% BI 2,12−2,64, OR 1,36; 95% BI 1,20−1,53, OR 1,41; 95% BI 1,21−1,64). Het

gebruik van antipsychotica verhoogde de kans op gastrointestinale en intracraniële bloedingen (OR 1,79; 95% BI 1,41−2,27, OR 1,44; 95% BI 1,06−1,95), terwijl het gebruik van ergolinederivaten alleen was geassocieerd met menstruele bloedingen (OR 2,29; 95% BI 1,28−4,08). In deze studie vonden we verschillen tussen patiënten die al geruime tijd antidepressiva of antipsychotica gebruikten en patiënten die daarmee recentelijk gestart waren. Bij nieuwe gebruikers was het risico van een gastrointestinale of intracraniële bloeding verhoogd ten opzichte van prevalente gebruikers. Dit verschil zou kunnen komen doordat de serotoninespiegel, die aanvankelijk was gedaald door deze serotonerge geneesmiddelen, na een aantal weken weer op het oude niveau terugkeerde. Er werd geen associatie gevonden tussen de mate van remming van de 5-HTT en 5-HT_{2A} receptor en het optreden van een van de drie bloedingen.

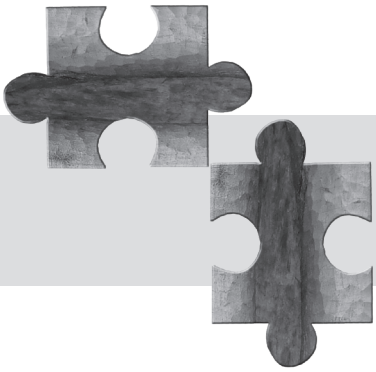
In *Hoofdstuk 4.3* bestudeerden we de associatie tussen de mate van remming van de 5-HTT en het optreden van osteoporotische en niet-osteoporotische fracturen in een patiënt-controleonderzoek. Het is bekend dat verschillende geneesmiddelgroepen, waaronder antidepressiva, zijn geassocieerd met het risico van vallen en botbreuken. De afname van botmassa en het effect op de botmatrix is gerelateerd aan de remming van de 5-HTT. In de PHARMO databank identificeerden wij alle volwassen patiënten met een eerste ziekenhuisopname voor een botbreuk (n = 16.717). Zij werden gematcht met maximaal vier controlepatiënten op leeftijd, geslacht, geografisch gebied en indexdatum (n = 61.517). Antidepressiva werden ingedeeld in drie groepen op basis van hun affiniteit voor de 5-HTT (hoog-midden-laag). Fracturen werden geclassificeerd als osteoporotisch (breuk van heup/dijbeen, spaakbeen/ellepijp, bovenarm, ruggenwervels, rib en sleutelbeen) of niet-osteoporotisch. Zestig procent van de patiënten die werden opgenomen in het ziekenhuis voor een botbreuk, had een osteoporotische fractuur. Gebruik van SSRIs, tricyclische antidepressiva en overige antidepressiva was geassocieerd met een verhoogd risico op zowel osteoporotische als niet-osteoporotische fracturen. Het risico van een osteoporotische fractuur was hoger bij gebruik van een antidepressivum met een hoge affiniteit voor de 5-HTT (OR 1,86; 95% BI 1,63−2,13), vergeleken met een antidepressivum met een gemiddeld of lage affiniteit (respectievelijk OR 1,43; 95% BI 1,19−1,72 en OR 1,32; 95% BI 0,98−1,79). In deze studie vonden we geen associatie tussen de mate van remming van de 5-HTT en het optreden van niet-osteoporotische fracturen.

Concluderend kan gesteld worden dat de resultaten in *Hoofdstukken 4.2* en *4.3* laten zien dat classificatie op basis van farmacologische mechanismen een geschikte methode kan zijn om de relatie tussen het gebruik van geneesmiddelen en verschillende bijwerkingen te bestuderen.

HOOFDSTUK 5

In **Hoofdstuk 5**, de algemene discussie, wordt het concept van mechanisme-georiënteerde classificatie van geneesmiddelblootstelling in breder perspectief geplaatst en werd het belang van moleculaire en farmacologische geneesmiddelkarakteristieken bij het classificeren van geneesmiddelblootstelling beschreven. Bij het bestuderen van geneesmiddelen nadat ze op de markt zijn verschenen, kan de mechanisme-georiënteerde classificatie van geneesmiddel-blootstelling worden gebruikt om eerdere associaties tussen het gebruik van geneesmiddelen en het optreden van bijwerkingen (*Hoofdstukken 2.1*, *2.2* en *4.3*) te ondersteunen of te bevestigen. Ook kan de mechanisme-georiënteerde classificatie reeds bestaande kennis versterken op het gebied van onderliggende mechanismen in geneesmiddel–bijwerkingcombinaties (*Hoofdstukken 4.1* en *4.2*) of nieuwe hypothesen genereren (*Hoofdstukken 2.3* en *3.1*). Het is een grote uitdaging databases die gebruikt worden in de farmaceutische industrie bij geneesmiddelontwikkeling met geneesmiddel–targetrelaties, te combineren met databases die gebruikt worden in farmacovigilantie met informatie over geneesmiddel–bijwerking combinaties. Deze gekoppelde databanken kunnen veel nuttige informatie opleveren bij het ontwikkelen van nieuwe geneesmiddelen, regelgeving en de klinische praktijk. Bij de medicatiebewaking, waar chemie, biologie en farmacotherapie samenkomen, zijn apothekers bij uitstek geschikt om bijwerkingen van geneesmiddelen te beoordelen en evalueren.

Dankwoord



In de vele jaren die ik nu bij het departement Farmaceutische Wetenschappen werkzaam ben, heb ik mij geen moment verveeld. Ik heb mij mogen bezighouden met veel uiteenlopende taken op het gebied van onderwijs, management en onderzoek. Al deze verschillende onderdelen zijn als stukjes die bij elkaar een mooie puzzel vormen. Tussen de mededeling “Schrijf je eigen onderzoeksvoorstel maar” en dit stukje/proefschrift ligt ongeveer zes jaar. Het raamwerk (de zogenaamde ‘rechte’ stukjes) was mij in het begin nog niet helemaal duidelijk. Wat wel vaststond was de kern, het kleurrijke gedeelte van de puzzel, waarmee iedereen een legpuzzel begint. Zonder deze basis zou de puzzel nooit zijn afgekomen. Prof. H.G.M. Leufkens, prof. dr. A.C.G. Egberts en dr. P.C. Souverein, jullie hebben de afgelopen jaren een belangrijke rol gespeeld bij de totstandkoming van mijn ‘farmaciepuzzel’. Het was een voorrecht om twee promotoren en een co-promotor te hebben met verschillende kwaliteiten, waardoor ik op diverse vlakken veel heb geleerd.

Beste Bert, allereerst veel dank dat jij mij zes jaar geleden samen met prof. dr. Ton de Boer zo hartelijk welkom heette op de afdeling Farmaco-epidemiologie & Farmacotherapie (F&F) na mijn overstap van de afdeling Analyse & Toxicologie (A&T). Jij had vanaf het begin een duidelijk beeld van het kader van dit proefschrift. Uiteindelijk vielen ook voor mij alle puzzelstukjes op hun plaats, omdat je mij hebt geleerd mijn onderzoek in een bredere context te plaatsen.

Beste Toine, vaak als ik met een probleem zat hoe ik iets moest aanpakken, wist jij met een schets van een tabel of figuur het probleem op eenvoudige wijze in kaart te brengen en lag een concrete oplossing voor de hand. En het is op deze plaats in proefschriften al vaker gememoreerd hoe fijn het is dat op vragen en stukken altijd een razendsnelle reactie volgde die de vaart in het proces hield.

Beste Patrick, ik had het niet beter kunnen treffen dan met jou als co-promotor. Jouw kennis op het gebied van databases en het koppelen daarvan is ongekend en ik heb dankbaar gebruik gemaakt van deze expertise als ‘jouw AIO’. Verder kon ik altijd bij je binnenlopen voor kleine en grotere problemen (waar zijn nu

die 800 patiënten gebleven?) en is mijn Engels door jouw correcties aanmerkelijk verbeterd.
Bert, Toine en Patrick, dit paar zinnen doen jullie geen recht, maar weet dat ik jullie uitstekende begeleiding heel erg heb gewaardeerd.

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Mrs dr. J.K. Jones, dr. E.P. van Puijenbroek, prof. dr. A.F.A.M. Schobben, prof. dr. B.H.C. Stricker and prof. dr. N.P.E. Verhoeven, members of the thesis committee, are gratefully acknowledged for their assessment of this thesis.

In 2007 kreeg ik de gelegenheid om onderwijsmanager te worden bij het bureau van het departement Farmaceutische Wetenschappen en die mogelijkheid heb ik met beide handen aangegrepen. Dat betekende dat het groene gedeelte werd uitgebreid en ik er nog meer collega's bij kreeg. Hoewel ik officieel niet bij de afdeling Onderwijs- & Studentenzaken hoor, brengen mijn werkzaamheden mij regelmatig in contact met de medewerkers van het studiepunt farmacie. Ik wil Dicky van Heuven, Nel Annen, Edith van den Ham, Manon Thijssen, Heleen Gerwig en Anita van Oyen (ook iemand die eigenlijk bij het 'bureau' hoort) bedanken voor de prettige samenwerking. Verder kwam ik als onderwijsmanager in verschillende overlegorganen terecht. Wat mij betreft is het belangrijkste daarvan het onderwijsmanagementteam (MT). Samen met Fred Schobben, Andries Koster, Ed Moret, Tom Schalekamp en Marcellina Vermeesch vergader ik (bijna) wekelijks over vrijwel alles wat met het onderwijs in de bachelor en master farmacie en

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De luchtpartij van een puzzel bestaat uit vele stukjes wit en diverse kleuren blauw. Soms bestaat de neiging om dit gedeelte van de puzzel over te slaan, maar juist deze stukjes zijn belangrijk voor het totaalplaatje.
Prof. dr. Auke Bult, in mijn tijd bij A&T 'dwong' jij mij om lid te worden van de curriculumcommissie (CURC), die een nieuw bachelor-masteronderwijsprogramma binnen de opleiding farmacie moest vormgeven. Ik had toen niet kunnen vermoeden dat mijn deelname aan de CURC een belangrijke stap is geweest in mijn carrière bij farmacie. Het heeft mijn visie op het gebied van onderwijs verruimd, mij in contact gebracht met collega's buiten de eigen afdeling en de deur geopend naar mijn managementfunctie. Jantien Kettenes dank ik voor haar belangstelling en begeleiding in de tijd dat duidelijk werd dat ik 'moest' promoveren. Collega's bij de postdoctorale cursussen Geneesmiddelonderzoek (GO), Analyse van Bereidingen (AB) en Kwaliteitsbeheer zorgden ervoor dat ik mij binnen een dag weer helemaal thuis voelde bij farmacie, toen ik na drie jaar apotheek de overstap maakte naar het universitaire onderwijs. Ik denk met genegenheid terug aan ons clubje in OC026.
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kunnen doen, heb jij hierbij het tegendeel bewezen. Ik vind dat het een prachtig geheel is geworden.

Ik wist al vrij snel wie ik als paranimfen zou vragen en ik ben blij dat zij deze 'taak' op zich hebben willen nemen. Lieve Marcel, we kennen elkaar vanaf de tweede klas van het gymnasium. Toentertijd gingen we niet vaak met elkaar om, maar dat veranderde toen we in 1986 farmacie in Utrecht gingen studeren en daarna vlak bij elkaar op het IBB gingen wonen. Ook na onze studententijd is onze vriendschap gebleven en ik vind het heel fijn dat jij bij deze gelegenheid mij terzijde wilt staan. Lieve Diane, toen ik jou voor het eerst zag was jij student en ik net begonnen als docent bij de postdoctorale cursussen GO en AB. Onze relatie veranderde toen ook jij als docent bij A&T kwam werken. Na jouw overstap naar de afdeling F&F duurde het niet lang of ook ik stapte over en het was heel fijn om vanaf die tijd een werkkamer te delen. Leuk dat jij, na de afronding van jouw eigen promotietraject waarbij ik als paranimf mocht optreden, nu mijn paranimf wilt zijn.

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Dertien jaar na mijn eerste werkdag bij farmacie gaat er weer een nieuwe fase in. Wat zullen de komende jaren voor mij in petto hebben? Ik ben benieuwd, op naar de volgende puzzel.

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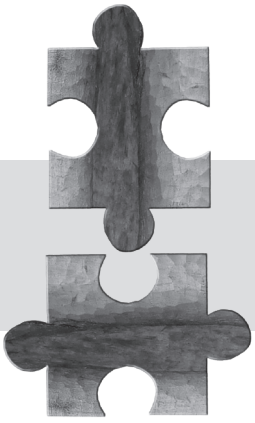

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Marianne Verdel was born in Alphen aan den Rijn, The Netherlands, on 9 February 1968. In 1986, she completed secondary school at the Christelijk Lyceum (Gymnasium) in Alphen aan den Rijn. Subsequently, she studied pharmacy at the Utrecht University. In 1992, she obtained her Master's degree in pharmacy, followed by her pharmacist degree in January 1994. After working for three and a half years as a pharmacist in several community pharmacies, she changed careers and accepted a position as university teacher at the division of Biomedical Analysis at the (then-called) Faculty of Pharmacy, Utrecht University. In January 2004, she was appointed as an Assistant Professor at the division of Pharmacoepidemiology and Pharmacotherapy. In addition, she worked at this division on the studies described in this thesis.

In May 2007, she became the Education Manager of the department of Pharmaceutical Sciences, Faculty of Science, Utrecht University, temporarily leaving her teaching job. Since May 2010, she combines this position with an appointment as Assistant Professor at the division of Pharmacoepidemiology and Clinical Pharmacology at the same department.