Regional Chemotherapy of the Lung: Investigations of Isolated Lung Perfusion and Selective Pulmonary Artery Perfusion

Marco Grootenboers

Regional Chemotherapy of the Lung: Investigations of Isolated Lung Perfusion and Selective Pulmonary Artery Perfusion

Regionale Chemotherapie van de Long:
Onderzoeken naar Geïsoleerde Long Perfusie en
Selektieve Arteria Pulmonalis Perfusie
(met een samenvatting in het Nederlands)

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Promotores:
Prof. dr. J.M.M. van den Bosch
Prof. dr. A. Brutel de La Rivière

Co-promotor:
Dr. F.M.N.H. Schramel

Promotiecommissie:
Prof. dr. L.A. van Herwerden
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Chapter 1

General introduction

General introduction

This thesis focuses on alternative ways of administering, selectively, cytostatic agents to patients with malignant tumors in the lungs. The two modalities are: Isolated Lung Perfusion (ILuP) and Selective Pulmonary Artery Perfusion (SPAP). These two modes of regional cytostatic treatment of tumors within the lung are investigated in a human and in an animal model, respectively. In this chapter, we introduce the study populations with pulmonary malignancies eligible for these two different techniques, that is, patients with pulmonary metastases of non-primary lung cancer for ILuP, and patients with primary lung cancer for SPAP. Both techniques are introduced and described in a summary. Finally, a short outline of the thesis is given.

Isolated Lung Perfusion (ILuP)

Isolated lung perfusion is an effective method of delivering high-doses of local chemotherapy with minimal systemic concentrations, avoiding toxic drug metabolism through the liver and kidneys [1]. The therapeutic agent is delivered into the pulmonary artery, whereas the pulmonary venous effluent is drained off, minimizing systemic exposure. Theoretically, the lung is an ideal organ for isolated organ perfusion, because it is symmetric, has a readibly accessible pulmonary vascular bed for the surgeon, and is supplied arterially almost exclusively by the pulmonary artery with complete venous drainage via the two pulmonary veins.

Creech was the first to describe ILuP in 1958, with pioneer investigations by Johnston since the 1980's, further addressing feasibility, pharmacokinetics and toxicity in ILuP (with doxorubicine) [2,3]. Description of the different ILuP circuits, explored since the 1980's, is beyond the scope of this dissertation. In our human experiments, we did not adopt a single pass system discarding the effluent after one passage. Instead, we used a recirculating system redelivering the pulmonary venous effluent back into the lung (Figure 1). Furthermore, in our current design, we did not perform bilateral thoracotomies in case of bilateral disease. We chose not to perfuse both lungs simultaneously, as we anticipated increased morbidity related to more substantial surgical trauma and combined pulmonary metastasectomy.

Ideally, the cytotoxic agent administered by ILuP should be taken up rapidly from the circulation by the lung tissue without pulmonary toxicity at significantly higher doses, when compared to intravenous (systemic) therapy. Melphalan was chosen as the chemotherapeutic agent, because of successful previous experimental work of ILuP in rodent models [4-6], and investigations in other isolated organ perfusion, such as isolated limb perfusion [7]. Hyperthermia was applied as an additional treatment modality, because of preclinical and clinical studies revealing enhanced cytostatic drug uptake with increased tumor delay [8]. Furthermore, the normal lung appears to be tolerant to fairly severe hyperthermia in isolated perfusion [9].

Originally, ILuP was developed for patients with unresectable pulmonary metastastic disease in a palliative setting. Alternatively, the objective was to convert unresectable to resectable disease. In our research, we have focused on patients with resectable lungmetastases, aiming to treat undetected, residual pulmonary micrometastatic disease, most probably responsible for high, local recurrences rates. Thus, besides avoiding potentially systemic dose-limiting toxicity, the rationale of concomitant adjuvant chemotherapy via ILuP after pulmonary metastasectomy is eliminating occult disease at the treated site, thereby hopefully improving prognosis.

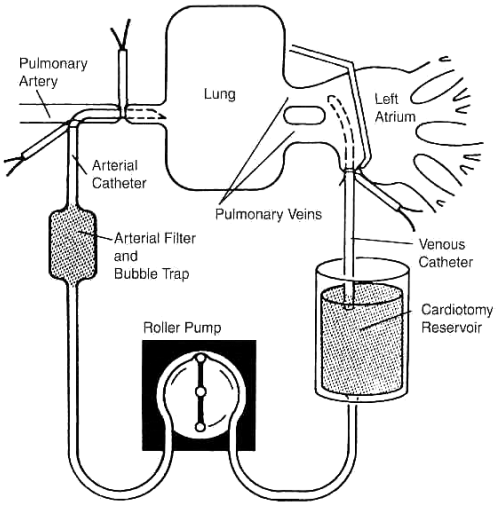

Figure 1. Isolated lung perfusion circuit. A roller pump perfuses the left lung via the pulmonary artery. Entrance of clots and air are prevented by the filter and bubble trap. (*From* Johnston MR, Minchin R, Shull JH, et al. Isolated lung perfusion with adriamycin. A preclinical study. Cancer 1983:52:404-9; with permission.)

Selective Pulmonary Artery Perfusion (SPAP)

As indicated above, ILuP with cytostatics is a surgical technique requiring cannulation of the pulmonary vessels via thoracotomy, and is therefore characterized by its invasive nature limiting repetitive application. In contrast, SPAP requires only pulmonary artery catheterization - like introducing a Swann-Ganz catheter – and does not include collection of the venous effluent. In fact, physiologic venous return into the left atrium is allowed. This non-invasive experimental modality of regional chemotherapy was first described in 1958 by Blades and colleagues [10]. The endovascular technique of blood flow occlusion (BFO) with an inflated balloon catheter to delay pulmonary artery washout (Figure 2), proved to be a means of achieving higher locoregional drug concentrations with minimal systemic concentrations, and thus minimal systemic side-effects compared to intravenous (systemic) administration [11-15]. Pharmacokinetically, however, SPAP with BFO is still inferior to ILuP [16].

In search for a less invasive procedure of regional chemotherapy of the lung, we developed a catheterization model of SPAP and investigated drug delivery parameters to optimize its pharmacokinetic profile in healthy pigs (as, unfortunately, there is no pulmonary tumor model in pigs to date). We have elected to apply BFO after the injection of the cytotoxic agent, while all previously reported studies occluded the pulmonary artery before the injection. Gemcitabine was our choice of chemotherapeutic agent based on previous SPAP experiments with gemcitabine, and its potential in the future treatment of non-small cell lung cancer (NSCLC) [11].

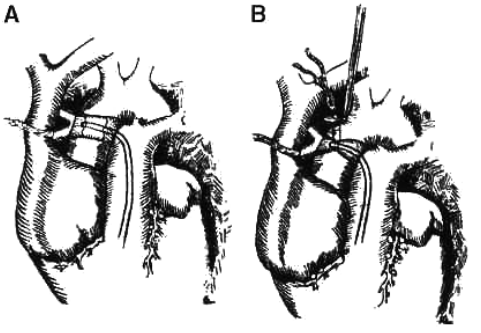

Figure 2. Endovascular occlusion of the pulmonary artery with a balloon (A) or extravascular occlusion with snare, necessitating an open –thoracotomy- approach (B). (From Smyth NP, Blades B. Selective chemotherapy of the lung during unilateral pulmonary arterial occlusion with a balloon-tipped catheter. J Thorac Cardiovasc Surg 1960; 40(5):653-66; with permission)

Pulmonary metastases of non-primary lung cancer

The lung is the most common site of metastases for all malignancies, with the possible exception of those that develop in the area of the portal venous drainage. Twenty to 30% of all patients with metastatic cancer have pulmonary metastases at the time of autopsy [17,18]. Systemic intravenous chemotherapy has not resulted in significant survival prolongation with, unfortunately, most patients dying within one year after detection of the dissemination. Moreover, efficacy in systemic chemotherapy is limited by systemic toxicity. Pulmonary metastasectomy, i.e. surgical extirpation from the lung of metastatic disease, was first performed in 1882 and has been adopted for the treatment of malignant tumors with a variety of histologies. The evidence of the exact role of pulmonary metastasectomy, however, is incomplete, as are the data on the efficacy of induction or adjuvant chemotherapy in the treatment of pulmonary metastases. In the work-up of patients presenting with pulmonary metastases who could benefit from surgical resection, various issues should be addressed, such as: is the primary tumor controlled or controllable, is extrathoracic disease present, can the patient tolerate surgery, and is complete resection possible? Given these restrictions, most patients with lung metastases will not benefit from pulmonary metastasectomy. For instance,

in metastatic colorectal disease, less than 3% of patients presenting with pulmonary metastases proved to be candidate for surgical resection [19]. The current 5-year survival, even after complete surgical resection of pulmonary metastases, remains low at 20-40% [20]. High recurrence rates of pulmonary metastases after resection are probably related to the presence of undetected micrometastases at the time of surgery and not to an inadequate resection.

Potential for primary lung cancer

The rationale of increasing the concentration of cytostatics in the lung harbouring either a primary bronchus carcinoma or metastases from an extrathoracic malignancy is appealing, as it may reduce the tumor itself and/or the accompanying pulmonary metastases. As pulmonary metastases receive most of their blood from the pulmonary circulation [21], most studies in ILuP have been performed in subjects with pulmonary metastases and demonstrate excellent separation between pulmonary and systemic circulation. In primary lung cancer, however, a dual circulation is present from both the pulmonary and bronchial circulation, making pulmonary artery perfusion (with or without isolation of the venous effluent) a potentially less efficient treatment modality. The pathophysiologic rationale for SPAP in NSCLC is supported by documented physiological anastomoses and shunts between the bronchial and pulmonary circulations [22], enlarged in case of pathologic conditions [23].

Approximately 70% of patients with NSCLC present with unresectable disease and therefore are candidates for systemic chemotherapy. Selective pulmonary artery perfusion could be a promising technique combining properties of both regional and systemic chemotherapy, potentially offering the best of both worlds: equivalent systemic cytostatic drug exposure comparable to the currently used intravenous administration, and high-dose pulmonary concentrations, possibly enhancing anti-tumor efficacy and improving locoregional control. Inadequate control of locoregional disease in lung cancer is correlated with a poor survival. Furthermore, this relatively simple procedure could be repeated indefinitely, in contrast to ILuP which requires a thoracotomy. In case of NSCLC, SPAP with gemcitabine could potentially convert unresectable disease to resectable disease and serve as a neoadjuvant chemotherapeutic modality, or could be of use in a palliative setting.

Outline of this thesis

The aim of this thesis was two-fold. First, - part I of the dissertation -, to explore ILuP with melphalan and hyperthermia followed by pulmonary metastasectomy in patients with resectable pulmonary metastases. Second, - part II -, to investigate the feasibility and pharmacokinetics of SPAP with gemcitabine, studied in a tumor-free porcine model.

Chapter 1 briefly introduces the two techniques of pulmonary artery perfusion and identifies (future) study populations.

Part I. Isolated Lung Perfusion:

Chapter 2 is a review of all human and animal studies in ILuP with melphalan.
The development of the isolated perfusion technique used in our institute is described.
Chapter 3 reports the protocol of a dose-finding phase I trial in patients with resectable pulmonary metastases treated with melphalan through ILuP followed by pulmonary metastasectomy.
Chapter 4 offers the results of this phase I trial of ILuP with melphalan and defines dose limiting toxicity and maximum tolerated dose.
Chapter 5 describes the pharmacokinetic analysis, justifying the concept of ILuP with melphalan performed in the phase I trial and the extension trial.
Chapter 6 discusses toxicity and clinical-follow up, resulting in an amendment of the previously reported maximum tolerated dose.

Part II. Selected Pulmonary Artery Perfusion:

Chapter 7 reviews investigations of SPAP in animal and human studies with emphasis on BFO, anti-tumor efficacy, feasibility and safety.
Chapter 8 outlines the development of a catheterization model of SPAP in pigs, and investigates pharmacokinetics of gemcitabine with variations in blood flow and perfusion time.
Chapter 9 was designed to evaluate SPAP of gemcitabine with dose escalation and variations in BFO times in a porcine model.
Chapter 10 discusses all previous results and suggests some future directions.
Chapter 11 gives a summary of the main results obtained in this thesis.

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PART 1 - ISOLATED LUNG PERFUSION

Chapter 2

Isolated lung perfusion for pulmonary metastases, a review and work in progress

Grootenboers MJ, Heeren J, Hendriks JM, Van Putte B, Van Boven WJ, Van Schil PE, Schramel, FM. Isolated lung perfusion for pulmonary metastases, a review and work in progress. Perfusion 2006;21(5):267-76.

Abstract

Pulmonary metastasectomy is a widely accepted treatment for many patients with pulmonary metastases from various solid tumors. Nevertheless, 5-year survival is disappointing with rates of 25-40%, and many patients develop recurrences. Isolated lung perfusion (ILuP) is a promising new technique to deliver high-dose chemotherapy to the lungs, while minimizing systemic toxicities. This procedure is technically safe and feasible. However, clinical value and efficacy remain unclear. The aim of this paper is to review literature on ILuP in humans and to describe the development of the perfusion procedure in our institute.

Introduction

The lung is the most common site of metastatic disease due to its filtering capacity for the entire circulation. Pulmonary metastases occur in approximately 30% of patients dying of cancer [1]. Most frequent, resected metastatic pulmonary diseases originate from soft tissue sarcoma and colorectal carcinoma. Although surgical resection is presently a widely accepted treatment for pulmonary metastases, 5-year survival rates of 20-40% after complete resection of pulmonary metastases remain poor [2]. Many patients develop recurrent disease, even when complete resection was achieved. Isolated lung perfusion (ILuP) is an effective method of delivering high-dose local chemotherapy with minimal systemic concentrations, avoiding toxic drug metabolism through the liver and kidneys [3]. Safety and pharmacokinetic superiority have been demonstrated in numerous experimental dog and rat models [4-6]. A number of phase I studies of ILuP in humans have been performed and proved to be feasible and safe procedures [7-13]. A mini-review of all human and animal studies in isolated perfusion with melphalan will be described in this paper, along with comments on the development of a recently performed phase I study of ILuP with melphalan in humans in our institution in general, and the used perfusion procedure more specifically.

Isolated lung perfusion

Isolated lung perfusion in humans

Reports of ILuP in humans are relatively scarce, and the study population and its treatment are heterogeneous (Table 1). Most studies are performed in patients with unresectable disease, in contrast to our study in which patients with resectable disease were included. Furthermore, all investigations were made in patients with metastatic disease of different histopathological origin, e.g., colon carcinoma, sarcoma, renal carcinoma, etc. Also, a variety of chemotherapeutics has been used, making pharmacokinetic comparison a hazardous task. The usage of hyperthermia in comparison to normothermia introduces, again, another variable in ILuP procedures. We will now, briefly, describe all published investigations of ILuP in humans in chronological order.

Year	Author	Ref.	No. of patients	Drug	Histology
1958	Creech *et al.*	14	1	Nitrogen mustard	Primary lung carcinoma
1984	Minchin *et al.*	15	3	Doxorubicin	Sarcoma
1995	Johnston *et al.*	7	8	Doxorubicin/cisplatin	Sarcoma, alveolar cell carcinoma
1996	Pass *et al.*	8	15	TNF-α	Ewing sarcoma, melanoma, other
1996	Ratto *et al.*	9	6	Cisplatin	Sarcoma
2000	Burt *et al.*	10	8	Doxorubicin	Sarcoma
2002	Putnam	11	16	Doxorubicin	Sarcoma
2002	Schröder *et al.*	12	4	Cisplatin	Primitive neuroectodermal tumors, osteosarcoma and rhabdomyosarcoma
2004	Hendriks *et al.*	13	16	Melphalan	Colorectal carcinoma, renal cell carcinoma, sarcoma and salivary gland carcinoma
2007	Grootenboers *et al.* (to be published)	NA	23	Melphalan	Colorectal carcinoma, renal cell carcinoma and sarcoma

Table 1. Human ILuP studies

Creech et al. were the first to perform a human ILuP procedure in 1958, notably performed prior to any animal studies [14]. Their reported patient had an unresectable bronchogenic carcinoma and was treated with nitrogen mustard via ILuP for 30 minutes. Perfusion temperature was not documented in the original article. The patient's condition after the operation was uneventful except for bronchitis. After this procedure, different animal studies with ILuP were performed until the next human model in 1984 by Minchin.

Minchin et al. studied ILuP with 0.56-0.98 µg/mL of doxorubicin in three patients with multiple, unresectable lung sarcoma metastases [15]. Perfusion temperature was set at 25°C. Results showed considerably less uptake of doxorubicin in the lung tissue in comparison to tumor tissue. All patients recovered without surgical complications and were able to leave the hospital within 7 days. No clinical tumor response could be reported.

A pilot study of ILuP was performed by Johnston et al. in four patients with metastatic sarcoma to the lungs, and four patients with diffuse bronchioloalveolar carcinoma of the lung [7]. Six patients were treated with an escalating doses of doxorubicin (1-10 µg/mL perfusate), and two patients with cisplatin (14 and 20 µg/mL perfusate). Systemic drug levels varied from 0 to <15% of the measured peak drug concentration of the pulmonary perfusate. Drug concentrations in the lung and tumor generally increased with higher drug dosages. There were no objective responses. Major postoperative complications occurred in two patients with bronchioloalveolar carcinoma. One patient developed a pneumonia with good response to antibiotic treatment, and another patient developed progressive respiratory failure with

pneumonia and empyema and, consequently, died with massive tumor involvement at postmortem examination.

Isolated lung perfusion with tumor necrosis factor-alpha (TNF-α) was tested by Pass et al. [8]. Fifteen patients with unresectable disease were perfused with 0.3-6.0 mg of TNF-α combined with 0.2 mg of γ-interferon under moderate hyperthermic conditions (38-39.5°C). In 10 patients, complete isolation was possible without any leakage. Maximum systemic TNF-α level was 8 ng/mL. No systemic changes in cardiac output or systemic blood pressure could be observed. There were no deaths, and mean hospitalization period was 9 days.

A multimodality approach, including operation and ILuP with cisplatinum (200 mg/m^2) was studied by Ratto et al. in six patients with lung metastases from soft tissue sarcomas [9]. Perfusion was performed for 60 minutes under normothermic conditions (37-37.5°C). Pulmonary serum levels were 43 times higher than systemic levels. Area under the curve values for total platinum concentrations were 12.8 and 0.30 mg/mL x minute for pulmonary and serum serum, respectively. No differences in platinum concentrations were found between lung and tumor tissue. All procedures were performed without any deaths, operative complications or systemic toxicity. Two patients developed interstitial and alveolar edema, necessitating respiratory support in one patient.

Isolated lung perfusion with doxorubicin, according to a dose-escalating schedule, was investigated by Burt et al. in eight patients with inoperable pulmonary metastases from sarcoma [10]. Seven patients were treated with a dose of 40 mg/m^2 or less, and one patient received 80 mg/m^2. Minimal systemic concentrations were detected on conclusion of the procedure. Lung concentrations showed correlation with the given dose, and intrapulmonary levels were higher in comparison to tumor levels in three out of four patients. In seven patients, significant decline in lung function was established and the patient treated with 80 mg of doxorubicin showed no ventilation or perfusion in the perfused lung. There were no peri-operative deaths.

A phase I study of isolated single-lung perfusion with doxorubicin in 16 patients with unresectable pulmonary metastatic disease was presented by Putnam [11]. Dose and concentrations of doxorubicin were: level 1 (60 mg/m^2, 200 mg/L, n=7), level 2 (75 mg/m^2, 250 mg/L, n=4), level 0.5 (60 mg/m^2, 100 mg/L, n=4). Minimal or undetectable systemic levels were documented. Two patients (level 2) experienced grade 4 pulmonary toxicity and overall operative mortality was 3 out of 16 (18.8%). Only one major response occurred (51% reduction) and median survival was 19.1 months.

More recently, Schröder et al. performed a feasibility study in four patients with unilateral and bilateral sarcoma metastases [12]. Metastasectomy was followed by ILuP with high-doses of cisplatin (70 mg/m^2) at hyperthermic conditions (41°C). Systemic serum levels of cisplatin were low and no systemic drug toxicity could be observed. At the end of the procedure, pulmonary uptake up to 98.3 ng/mg tissue was found. All patients developed transient pulmonary toxicity,

occurring as non-cardiogenic oedema and ischemic bronchial mucosal changes, improving in the next 12 weeks. Three patients were alive and disease-free after a median follow-up of 12 months. One patient died from cerebral metastases without autopsy evidence of local recurrence.

In conclusion, based on the published human studies in ILuP to date, one may conclude that the procedure itself is a feasible and safe technique for delivering high-dose cytostatic agent to the lung, while minimizing systemic toxicity. Efficacy, however, remains unclear.

Isolated lung perfusion and melphalan

Alkalyting agents have played an important historical role in cancer chemotherapy. Nitrogen mustard was actually used in the first human perfusion procedure ever, performed by Creech et al. in 1958 [14]. Melphalan is an alkylating agent used in the treatment of a variety of malignancies, such as ovarian cancer, rhabdomyosarcoma, pancreatic carcinoma, osteogenic sarcoma and multiple myeloma. With regards to selected perfusion of a target organ, this agent has the advantage of being investigated quite extensively, e.g., in isolated limb perfusion for melanoma in combination with TNF-α [16,17] and in isolated hepatic perfusion for colon carcinoma [18,19].

Several studies in rodent models have shown that ILuP with melphalan results in higher lung concentrations and lower systemic concentrations in comparison to intravenous (IV) treatment. Hendriks et al. investigated the pharmacokinetics of ILuP with melphalan and TNF-α using a rat model of metastatic pulmonary adenocarcinoma of the colon [20]. Ten rats were treated with different concentrations of melphalan IV and through ILuP. Lung melphalan levels were significantly higher in the ILuP groups (40.9 and 50.5 μg/g) compared to the IV-treated groups (0.8 and 1.7 μg/g). Pulmonary effluent levels of melphalan were high after 5 minutes, remained almost constant during the perfusion, and dropped to zero during the wash-out. In contrast to the rats treated with IV injection, no melphalan was detected in the serum of rats treated with ILuP. In conclusion, this study reports significantly higher lung levels of melphalan after ILuP in comparison to IV administration. No systemic levels of melphalan could be observed in contrast to the IV-treated groups.

Nawata et al. investigated ILuP with melphalan in rats with induced pulmonary sarcoma metastases [4]. Nineteen rats were randomized and treated with different concentrations of melphalan via IV administration and ILuP. Lung melphalan levels were significantly higher in the ILuP-group (62.2 μg/g) in comparison to the IV-treated groups (6.9 and 3.3 μg/g, respectively). Again, melphalan levels in the ILuP-group elevated within minutes and remained so for the time of perfusion, followed by a drop to zero in the wash-out period. In contrast to the IV-treated group, no increase of the serum melphalan levels was documented in the ILuP-treated group. In summary, this study, again, confirmed the ability of ILuP to deliver high-dose chemotherapy to the perfused organ with 20 times higher concentrations in comparison to the IV-treated group.

Eradication of pulmonary metastases of sarcoma and carcinoma has been documented after treatment with ILuP with melphalan. Hendriks et al., as previously reported, investigated ILuP with melphalan and TNF-α for metastatic pulmonary adenocarcinoma in the rat and conducted an efficacy study in 23 male rats [21]. The authors reported an evident anti-tumor effect of ILuP with melphalan as a single therapy or in combination with TNF-α, in comparison to IV-treatment.

Nawata et al. reported a significant reduction on the number of lung lesions after treatment with melphalan through ILuP, when compared to melphalan IV or buffered hetastarch ILuP: 7 versus 60 versus 84 [4]. Left lung melphalan ILuP resulted in again significant reduction of tumor nodules, left versus right: 7 versus 185.

Hendriks et al. tested survival after ILuP with melphalan in a rat model of unilateral metastatic pulmonary adenocarcinoma [18]. They found median survival of ILuP-treated animals to be significantly longer compared to the groups treated with melphalan IV and no treatment: 81 versus 37 versus 28 days, respectively.

Based on these promising results of ILuP with melphalan in rodent models, a dose-finding phase I trial in humans with melphalan with normothermia and hyperthermia was performed by Hendriks et al. in the University Hospital of Antwerp, Belgium and St. Antonius Hospital, Nieuwegein, The Netherlands [13]. Before describing the perfusion procedure itself, we would now like to report on some studies concerning ILuP perfusion and hyperthermia, as this concept is implemented in our protocol.

Isolated lung perfusion and hyperthermia

Hyperthermia has been used in the treatment of a variety of malignant tumors and its usage dates back as far as Hippocrates (400 BC) and Galen (200 AD). Hyperthermia in combination with anticancer drugs achieves significant pharmacokinetical advantage by enhancing cytotoxicity [22] or antineoplastic drug uptake [23], resulting in tumor growth delay. A number of anticancer drugs, among which melphalan and platinum compounds, show enhanced cytotoxicity with hyperthermia. Tumoricidal activity appears to increase at higher temperatures, and hyperthermia seems to obtain its optimal effect at the highest tolerable dose [24]. Organ tolerance to hyperthermia seems to be variable with some evidence showing that brain, liver, skeletal muscle and peripheral nerves may be particularly heat sensitive. The normal lung appears to be tolerant to fairly severe hyperthermia, though.

Rickaby et al. performed a study in dogs to determine the short-term tolerance of the lung to hyperthermia on isolated lung lobes perfused with autologous blood or an artificial salt solution during a 2-h period [25]. Hyperthermia had no detectable influence on variables, such as lung weight, extravascular water, perfusion pressure, and lung compliance, when the temperature was kept below 44.4°C. A significant increase in perfusion pressure was noted at the end of perfusion above 44.4°C. Several changes independent of temperature occurred in

the lobes during perfusion, such as an increase in wet weight, extravascular volume and wet-to-dry ratio, and a decrease in lung compliance. The authors concluded that temperatures as high as 44.4°C for approximately 2 h produced little short-term damage in isolated perfused dog lung.

Cowen et al. performed a study to investigate the effects of hyperthermia on lung function in an intact animal model in which both acute and subacute toxicity were monitored. Their findings showed tolerance in perfused dog lungs up to about 44°C for 1 h [24]. No evidence of thermal injury was reported at 2 weeks. Fulminating pulmonary oedema was described at temperatures over 45°C.

Notably, a mere four studies exist of human ILuP with hyperthermia, namely, the previously mentioned studies from Johnston [7], Pass [8], Schröder [12], and Hendriks et al. [19] (Table 2).

Year	Author	Drug	Perfusion temperature (°C)	Perfusion time (min)	Operable
1958	Creech *et al.*	Mitomycin C + nitrogen mustard + 5FU	NA	30	No
1984	Minchin *et al.*	Doxorubicin	37	50	No
1995	Johnston *et al.*	Doxorubicin/ Cisplatin	25- 40	45 – 60	No
1996	Pass *et al.*	TNF-α	38 - 39.5	90	No
1996	Ratto *et al.*	Cisplatin	37	60	Yes
2000	Burt *et al.*	Doxorubicin	37	20	No
2000	Putnam	Doxorubicin	37	NA	No
2002	Schröder *et al.*	Cisplatin	41	21 – 40	Both
2004	Hendriks *et al.*	Melphalan	37 or 42	30	Yes
2007	Grootenboers *et al.* (to be published)	Melphalan	42	30	Yes

NA : not available

Table 2. Perfusion conditions in human ILuP-trials

No conclusions can be drawn on efficacy of hyperthermia, as all these studies are pilot studies or phase I trials. Interesting is the fact, that in the study by Johnston et al. [7], only one patient was perfused under moderate hyperthermic conditions with cisplatin, and neither of the two reported major complications occured in this patient. Furthermore, the only other patient treated with cisplatin for a shorter period of time at 25°C had similar lung levels, but higher

tumor levels. We must add, though, that these two patients had different malignancies: bronchioloalveolar carcinoma and chondrosarcoma of the lung.

Pass et al. published their results of a phase I trial with ILuP with TNF-α, interferon-γ and moderate hyperthermia (38-39.5°C) [8]. Sixteen perfusions in 15 patients with unresectable metastatic disease were performed during 90 minutes. Direct toxicities reported were hepatitis A and pancreatitis in one patient, and TNF toxicity with hypotension, pulmonary insufficiency with bilateral infiltrates in another patient. Furthermore, one late complication of a longabscess was documented. No deaths were reported. No remarks on hyperthermia-induced toxicity can be made, as it was not reported which patients were actually treated with (moderate) hyperthermia.

As described earlier, Schröder et al. performed a feasibility study in four patients with pulmonary sarcoma metastases [12]. Metastasectomy was followed by ILuP of cisplatin (70 mg/m^2) at 41°C, with an average perfusion time of 32 minutes. All patients developed transient pulmonary toxicity, occurring as non-cardiogenic oedema and ischemic bronchial mucosal changes, but these complications improved in the next 12 weeks. Three patients were alive and disease-free after a median follow-up of 12 months, one patient died from cerebral metastases without autopsy evidence of local recurrence. Pulmonary toxicity reported in this study seems to warrant further investigations, though.

Hendriks et al. performed, as will be described more extensively later in this paper, a phase I study in patients with resectable pulmonary metastases [13]. Patients were treated with ILuP with melphalan according to a dose-escalation schedule followed by metastasectomy. Patients were perfused under normothermic (37°C) and hyperthermic (42°C) conditions.

Despite the hypothesis that hyperthermic conditions could enhance cytotoxic effects of chemotherapeutics, and in vivo hyperthermic ILuP procedures in dogs had no obvious deleterious influence, there is no evidence of superiority of hyperthermic ILuP in human trials.

Development of ILuP procedure

History

The first request for ILuP in our hospital was made in 1997. A physician diagnosed with rectal cancer metastasized to both lungs and liver, made a request for possible treatment. Experience in our group was primarily in isolated limb perfusion, and the decision was made to adopt such a system for ILuP to treat pulmonary metastases. The system was composed of a pump (roller), heat exchanger, oxygenator, reservoir, tubing and cannulae. The oxygenator used was a Cobe VPCML (Cobe Inc., Arvada, USA). Due to the small volumes, the smaller compartment of the oxygenator was used with a membrane surface of 0.4 m^2 and total priming of 270 mL. A conservative strategy was chosen because of lack of clinical information on the concentration of cytostatic agent. Temperature was set at 42°C, and the patient was treated with a bilateral

pulmonary metastasectomy followed by a unilateral ILuP with 4 mg of melphalan. Patient recovery was uneventful and discharge followed 9 days after admittance to the hospital. Ten months later, recurrence of bilateral pulmonary metastases was established, and the patient was treated with a bilateral metastasectomy and bilateral ILuP with 5 mg of melphalan without complications. Lungfunction assessments two years after the first ILuP procedure were unchanged. Unfortunately, one year later, a further recurrence of pulmonary metastases occurred and a re-metastasectomy was performed with follow-up in another medical centre. Over a period of 20 months, 5 ILuP procedures were performed in combination with metastasectomies in two patients. The decision was made to perform a phase I dose-finding study based on these promising results, in order to establish the toxic dose of melphalan in ILuP. In the process of formulating the study criteria, a number of questions arose on the optimisation of the technique as a whole, such as the need for an oxygenator, treatment with heparin, safety features and blood loss/volume management.

Oxygenator and current system

After the first ILuP procedures, the necessity of using the artificial lung brought on a discussion, because of the relatively small contribution to oxygenation by the extracorporal oxygenator. While some degree of pulmonary oxygenation may be possible through the bronchial circulation, a small debt in oxygen supply might even be considered beneficial as hypoxia increases vulnerability to cytostatic drugs. Potential benefit of discontinuation of the oxygenator is the loss of the binding effect of cytostatic drugs to the membrane, making pharmacologic analysis easier. Furthermore, the exclusion of the oxygenator reduces priming volume. Monitoring small changes in circulating volume in an "open venous reservoir" is difficult with a single lung containing only approximately 100 mL of circulating blood volume. Terminating the use of the VPCML also resulted in the elimination of the "open" cardiotomy reservoir. A self-constructed airseparator (Figure 1), which could expel air in case of calamities during the isolated lung procedure, was implemented into the system.

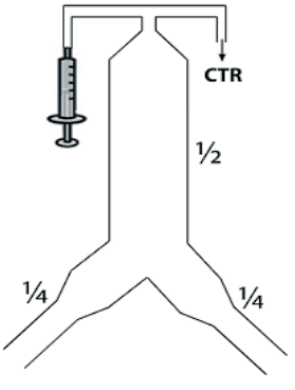

Figure 1. Airseparator

This separator device has an afferent and efferent leg to enable flow to and from the device, respectively. The tubing diameter of the afferent leg increases from 1/4" to 1/2" and the diameter decreases from 1/2" to 1/4" in the efferent leg. The rising column is 1/2" in diameter. Due to the use of toxic agents, it is essential to maintain a closed circulation. Therefore, air is manually evacuated by means of a syringe through a line into the cardiotomy reservoir (CTR), which holds the washing volume.

The commercially available system used for the ILuP procedure is the Temet system (First Circle Medical Inc., Minneapolis, USA). This system has a CE marking for hyperthermic techniques and basically uses a centrifugal blood pump (comparable to a pediatric Biomedicus unit) with incorporation of a pediatric Pall arterial line filter to prevent air embolic events. A Medtronic Cardiotherm heat exchanger (Medtronic Inc., Minneapolis, USA) and PVC tubing 1/4" with 3/32 wall thickness were used. This commercially available system was completed by incorporating a waste line, a washing line, and a recirculation line.

Unfortunately, the production of these systems was discontinued during the course of the phase I dose-finding study. Therefore, the centrifugal pump was replaced by the Jostra Rotaflow (Jostra, Hirrlingen, Germany). The arterial filter was replaced by the airseparator previously mentioned, and the Jostra heat exchanger HEC 44 was incorporated (Figure 2).

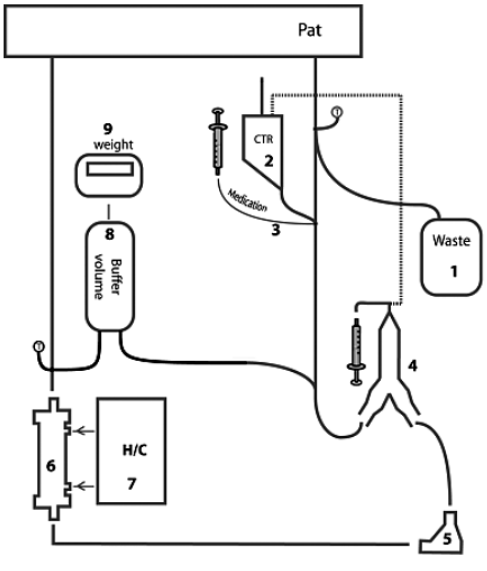

Figure 2. Isolated lung perfusion system

Perfusion pressure, flow and temperature

The accepted maximum pulmonary artery pressure during ILuP is less or equal to the systolic pulmonary pressure before isolation. This parameter is easy to measure by placing a monitor line just prior to cannulation of the pulmonary artery (PA). Pressures are also monitored at the exit of the heat exchanger. In general, the PA pressure during operation is approximately 25 mmHg. During the procedure, it is essential to monitor fluctuations in PA pressure. The circulating blood volume will be reduced because of volume loss due to third spacing/pulmonary edema and leakage from cannula sites, and the intravascular volume should be restored (by the recirculation system) in order to maintain optimal flows.

Concerning perfusion pressure, our train of thought was the following. As normal cardiac output of about 4000 to 5000 mL/min is transferred to both lungs, normal physiological flow should be about 2000 mL/min. In general, surgeons would like to use the smallest cannula possible, because cannulation of both pulmonary veins just prior to the left atrium is a rather difficult task. Normally, we would use a 14 or 16 F Medtronic single stage venous cannula for cannulating the PA. For cannulating the pulmonary vein, however, a 12 or 14 F Medtronic cannula is used. Because of the limited size of cannulae, it is hard to obtain a flow of 2000 mL/min. From a practical point of view, our goal is to maintain at least half of the normal physiological blood flow, resulting in the pre-trial procedures in blood flows of approximately 600 to 1800 mL/min. These values are now accepted as minimal flows, and, usually, perfusion procedures result in blood flows between 1200 and 1500 mL/min. It is also important to take the amount of circulating volume in consideration, as the actual intravascular blood volume of a single lung is small and estimated to be about 75-100 mL. The priming volume of the extracorporal system is 300 mL.

In the dose-finding study, perfusion procedures were performed under normothermic (37°C) and hyperthermic (42°C) conditions. Isolated lung perfusion was performed with melphalan at a dose determined by an escalation schedule, and for each dose-level three patients underwent perfusion at 37°C and 42°C, respectively. Perfused melphalan concentrations were increased until dose-limiting toxicity was encountered and procedures were performed without a specific heating protocol. Temperature differences were kept to <7 °C. Because the lung is a low-volume and relatively high-bloodflow organ, the warming procedure is predictable, fast, and easily controllable. The heat-exchanger of our choice is the Jostra HEC 44 (Jostra) because of bloodflow rates running up to 1.5 L/min, polyethylene surface area of 0.4 m^2, and small priming volumes of 40 mL. The heat-exchanger used in our system is the Temet system 1000. As the lung remains inflated and ventilated during the procedure, it is important to heat ventilation gases during the hyperthermic procedures by means of a nebulizor, in our case the Fisher & Paykel MR-730 (Fisher & Paykel, Panmure, New Zealand) set to 42°C.

Perfusate and rinsing volume

Priming volume consists of 1000 mL Voluven (6% hydroxyethyl starch 130/0.4) and 500 mL Ringer's Lactate solution. Because a mere 300 mL of priming volume is needed instantly, the remainder is kept as buffer volume in the recirculation bag for replacement of lost intravascular volume during the isolated perfusion period. Melphalan is washed out of the lung with 3 L of the priming solution after 30 minutes of perfusion. Loss of volume during the isolated period can theoretically be explained by vasodilatation, to some extend, during hyperthermic therapy, increased volume shift to the interstitium and alveolar space because of an increase in intravascular volume due to low haematocrit, and, finally, leakage at the cannula sites, especially the sites of the pulmonary veins. Loss of volume should be compensated by the infusion of volume to ensure optimal hemodynamic conditions throughout the entire lung. As melphalan has a short initial half-life time, there is no need to recirculate some of the flow via the recirculation bag. Preferably, we try to expand intravascular volume to its maximum point just prior to the injection of melphalan. Usually, we can maintain circulation for 10 min before the need for additional volume. Loss of volume is measured by pressure changes in the PA. The circulation between CTR and waste bag is interrupted during rinsing of the lung, and volume will be taken from the CT reservoir. Volume coming from the patient will be drained into a waste bag. In our protocol, there is no specified isolated-to-systemic circulation leakage testing. Because we use vascular clamps to interrupt circulation, chances of leakage between circulations are minimal. Initially, a ligature around the trachea was used to discontinue flow to the lung through the bronchial artery, but this maneuver was abandoned in the last few cases. Currently, this procedure is used to supply the lung with some oxygenated blood.

Autotransfusion therapy and airseparation

Because blood loss during cannulation can be severe, autotransfusion therapy is implemented. Volume from the surgical field will not be reinfused to the patient after the introduction of melphalan. Usage of autotransfusion equipment during oncology procedures is not absolutely contraindicated. From the available literature, it is evident that the majority of malignant cells will be washed away. Some cells, however, do remain in the circulation, but, apparently, the malignant potency of these cells is very low. Leucocyte-depletion filtration is used on the volumes of erythrocyte concentrate after being processed. One of the major problems of extracorporeal circulation is the introduction of air through venous lines, and the prevention of infusing air back into the patient through the arterial circulation. Air handling with the current system is, basically, very limited because of the absence of a venous reservoir, cardiotomy reservoir, oxygenator and arterial line filtration. In order to expel air from the system, a vertical leg with a large diameter was incorporated into the system. Initially, we tested this procedure with two legs in a row. By introducing air with blood flows of 1500 mL/min, we noticed that all air was evacuated by the first leg itself.

Isolated lung perfusion procedure

After arrival in the operation room, the patient is connected to the electrocardiograph and an arterial line is introduced for pressure monitoring. Temperature probes and a urine catheter are placed. All patients were intubated with a double-lumen endotracheal tube and turned to the lateral position. An anterolateral or posterolateral thoracotomy was performed in a standard fashion. After isolation of the main PA and both pulmonary veins, the pericardium is opened and previously mentioned vessels clamped centrally. The patient is anticoagulated with heparin up to an activated clotting time up of >200 sec. The PA is cannulated with a 14-16 F Medtronic DLP cannula and both pulmonary veins are cannulated with 12–14 F Medtronic DLP cannulae by standard techniques. The perfusion circuit is primed with a mixture of voluven and heparin. Target blood flows in the isolated lung are 750 mL/m^2. Maximum pulmonary perfusion pressure is equal to or less than systolic PA pressure before isolation. Temperature is regulated according to study protocol to 37°C or 42°C. After an initial period of stabilising perfusion pressure and temperature (if applicable), melphalan is injected into the perfusion circuit. Subsequently, a perfusion period of 30 minutes is started, followed by washing of the lung with 3 L of priming solution. At the end of the wash-out period, air is removed from the pulmonary vasculature by sequentially removing the PA cannula, repairing the arteriotomy, removing the pulmonary vein cannulae, and removing the PA clamp until bleeding from the pulmonary veins has vented all air. Blood flow to the lung is restored after repairing the venotomies and removal of the clamps. Complete metastasectomy is performed after correction of the activated clotting time with protamine. After the ILuP procedure and metastasectomy, the patient is transferred to the intensive care unit.

Comment

The scope of this article is to give a mini-review of trials of ILuP, and, more specifically, to describe the perfusion procedure and its development in our institute. Nevertheless, we would like to present some results concerning our work to date with ILuP and melphalan. Five patients were treated outside of protocol from 1997 to 1998. From May 2000 to December 2004, a total of 29 procedures of ILuP with melphalan followed by metastasectomy were performed in a phase I dose-finding study and extension trial in both Antwerp, Belgium and Nieuwegein, the Netherlands. The results of the dose-finding phase I trial were published by Hendriks et al., reporting a total of 21 procedures of ILuP with melphalan followed by surgical resection of all metastatic disease [13]. Sixteen patients with primary tumors, such as colorectal, renal cell, salivary gland carcinoma and sarcoma were treated, of which five patients had bilateral disease who underwent staged thoracotomies. Dose-limiting toxicity (defined as any common toxicity criteria grade 3 hematologic or non-hematologic toxicity at any time except for pulmonary toxicity, any radiographic change of the perfused lung resembling a

chemical pneumonitis involving the whole lung, or grade 3 or more pulmonary toxicity on day 28) was set at 60 mg melphalan at 37°C. Systemic melphalan concentrations after ILuP in this study were still much lower than previously reported levels after IV treatment of 10-20 mg/m^2 melphalan [14], or reported levels for high dose (180 mg/m^2) treatment with melphalan combined with bone marrow substitution [12]. There was no perioperative mortality. Re-thoracotomy was required in one patient because of postoperative bleeding. Two patients experienced lung edema and radiographic changes suggestive of a chemical pneumonitis of the entire perfused lung, and recovered slowly. These patients could leave the hospital at the 16th and 21st day postoperatively. On average, patients returned to the ward after 2 days and were discharged from hospital after 15 days. The study describes a short clinical follow-up in which all patients were still alive after a mean follow-up period of 14 months. Seven out of 16 patients developed recurrent metastatic disease. Extended follow-up of these patients is still under investigation, and pharmacokinetic analyses of this study will be published shortly. Recently, a protocol for a phase II study of ILuP with melphalan and metastasectomy has been submitted.

In this comment, we would like to address some minor problems we encountered during the perfusion procedures. Of all patients treated so far, we encountered perfusion problems only once in a patient treated in 1998. These problems originated from cannulation difficulties combined with blood leakage from venous cannulation sites. Overall blood loss in this patient was 4200 mL, with 2200 mL of erythrocyte concentrate. In this specific case, maximal blood flow of 500 mL/min was obtained, and the decision was made to continue ILuP despite the troublesome procedure. Patient recovery and follow-up were uneventful. Another point of interest is the slight differences in outcome of perfusion procedures between the two hospitals. Any form of leakage during ILuP is not tolerated in the other hospital, and, therefore, the stabilising period before cytostatic agent injecton is longer compared to ours. However, the direct effect of loss of perfusate on toxicity is still under investigation. We feel it is important, however, to formulate to what extend blood loss is acceptable and to document the exact amount of lost volume, making further analyses on acute and long-term toxicity possible.

In summary, ILuP is an exciting experimental technique providing an opportunity to dose intensify local chemotherapy while minimizing systemic toxicity. Previous trials in humans have shown this method to be safe, feasible and reproducible. Further perfusion studies are needed, however, to document the optimal environment for cytostatic agents with regard to pH strategy, temperature and priming volumes. Until now, few human trials evaluating this procedure have been published, and most studies have been performed on patients with inoperable primary or metastatic pulmonary disease of different primary tumors treated with a variety of perfusate drugs. We feel confident, though, to implement this technique as a treatment module for a selection of patients with lung metastases, together with surgical intervention. The first results of our phase I study appear to be promising, but, obviously, more research is needed to improve techniques and reveal efficacy.

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Chapter 3

Isolated lung perfusion technique

Grootenboers MJ, ter Beek HT, van Boven WJ, Heeren JJ, Knibbe CA, Seldenrijk CA, Slee PH, Schramel FM. A phase I clinical trial of isolated lung perfusion. In: Van Schil P, editor. Lung metastases and isolated lung perfusion. New York: Nova Science, 2007; pp 219-27.

Introduction

This chapter describes the protocol of a dose-finding phase I trial in patients with pulmonary metastases treated with melphalan via isolated lung perfusion (ILuP) followed by pulmonary metastasectomy. This study is performed in conjunction with the University Hospital of Antwerp, Belgium. The used perfusion circuit has previously been documented in a number of animal experiments with ILuP [1,2]. Few adjustments have been made to this circuit, the most important one being the absence of an oxygenator. The study's objectives are primarily to determine the maximum tolerated dose (MTD) and the dose limiting toxicity (DLT) of melphalan in patients treated with ILuP and pulmonary metastasectomy and to determine melphalan concentrations in lung tissue, tumor tissue, blood and perfusate samples. Secondary objectives are to determine pulmonary toxicity by pulmonary function testing, to define the safety profile of melphalan using ILuP with pulmonary metastasectomy for further phase II studies and to perform pharmacokinetic studies.

Preoperative evaluation

Preoperative investigations include medical history, clinical examination, blood hematology and chemistry (with tumor markers, if indicated), electrocardiography, chest roentgenogram, thoracic, abdominal and cerebral computed tomography, bone scintigraphy and PET scan (if available). Pulmonary function testing (spirometry and carbon monoxide diffusion capacity), arterial bloodgas analysis, exercise testing (VO2), bronchoscopy and lung ventilation/perfusion scintigraphy are also included.

Anesthetic management

After local routine for thoracotomy, anesthesia is induced through a peripheral intravenous line. Antibiotic prophylaxis is administered, and maintenance of anesthesia is assured with propofol IV or anesthetic gas, ventilation with FiO2 30%. After positioning a single lumen nasogastric tube, the patient is intubated with a left sided double-lumen endobronchial tube (female 37, male 39). A double-lumen intravenous catheter is placed into the right internal jugular vein and used for pressure monitoring. The arterial pressure is monitored in the contralateral radial artery. Once the chest wall is opened, ventilation on that side is discontinued to facilitate cannulation. After ensuring pleural and intrapulmonary hemostasis, systemic anticoagulant is given with heparin IV in a dosage to reach an activated clotting time of > 200 seconds. Arterial and venous cannulation is instituted and perfusion is started. Ventilation is restarted on both lungs with warmed (38°C) room air, 30% O^2. The perfusion pressure in the distal pulmonary artery is monitored through a needle introduced by the surgeon. After starting the perfusion procedure, a fiberoptic bronchoscopy is performed to establish the endobronchial situation and, thereby, to exclude edema or bleeding. Both lungs

are continuously ventilated with normal room air 30% O^2 after the lung perfusion. During the pulmonary metastasectomy, ventilation is discontinuated if required. At the end of procedure, when standard criteria are fulfilled, the patient is extubated either in the operating room or in the ICU. Blood gases have to be acceptable with 20-30% O^2 and spontaneous breathing must occur.

Surgical procedure

After positioning the patient into the lateral decubitus position, the pleural space is opened by means of a muscle-sparing lateral thoracotomy. The main bronchus is isolated. Subsequently, lobar and hilar pulmonary vessels and bronchial arteries are identified and carefully dissected to prevent systemic leakage. The pericardium is opened to isolate the pulmonary artery (PA). Next, the venous return is isolated (on the right side Waterstons groove: Satinsky clamping, on the left side pulmonary vein occlusion: either clamping or snaring). Before cannulation purse-string stitches are placed with prolene 4x0 or 5x0 on the PA and pulmonary veins. Cannulae are positioned into the isolated pulmonary artery (arterial inflow cannula: Bardic 16-22 F) and beyond the pulmonary venous take off to drain venous return (venous cannula: 16-24 F, Y-connector for venous return). Occlusion of the main PA by atraumatic clamping is followed by pressure monitoring and occlusion of the venous return, respectively. The perfusion procedure is started. Under PEEP and CO^2 insufflation, if desired, the PA and then the pulmonary veins are decannulated in order to avoid air emboli. Complete pulmonary metastasectomy is performed, intended with a margin of 5 mm normal lung tissue. After irrigation of the thoracic cavity and hemostasis, the chest is closed after insertion of two chest tubes. Staged thoracotomies are performed, except in case of unilateral metastases. During the first operation, an one-sided (left or right) thoracotomy is performed followed by a contralateral thoracotomy after 4-8 weeks. This interval between thoracotomies allows adequate observation of (sub)acute toxicity and leaves time for patient recovery. Isolated lung perfusion is followed by radical metastasectomy.

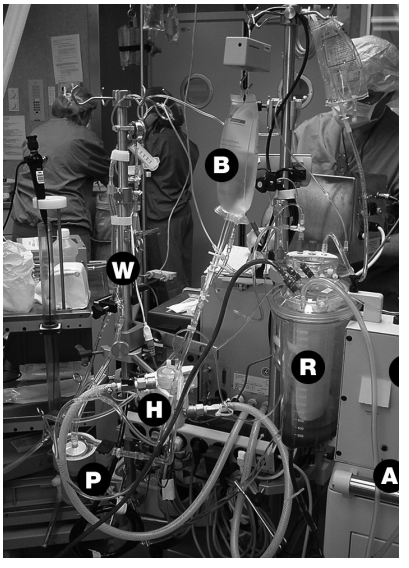
Lung perfusion management

During the procedure, the systemic circulation is isolated from the pulmonary circulation and, as mentioned before, low dose heparin is administered into the patient's systemic circulation. After cannulation of the PA (Bardic 22 F (Lifestream, 007328)) and the pulmonary vein (DLP 16 F (Medtronic, 69316) or comparable cannula), both cannulae are connected to the extracorporal circuit (ECC). The PA pressure is measured before cross-clamping the PA. The systolic pulmonary pressure will be the maximum perfusion pressure during the isolated perfusion period, in order to avoid pulmonary hypertension. Before starting ILuP, the perfusion circuit is primed with a volume of approximately 1000 mL (500 mL Voluven 6% (Fresenius) +

500 mL Ringer's solution + 2 mL heparin (5000 IU/mL)). Perfusion flow target will be 0.7 l/m^2 of body surface area at the start of the extracorporal circulation. Total ILuP time with melphalan is 30 minutes. The cytostaticum is introduced into the circuit as a bolus injection. Perfusion temperature is chosen according to the dose regimen (table 1). The active prime volume of the system is approximately 275 mL (excl. buffervolume). At the end of ILuP, the circuit was re-primed with wash-volume in approximately 5 minutes. The wash volume is approximately 3000 mL (500 mL Voluven 6% (Fresenius) + 500 mL Ringer's solution + 2 mL heparin (5000 IU/mL). After perfusion with melphalan, the treated lung is flushed with a balanced fluid (Ringer's solution), and the flushed volume is collected in a waste bag.

System for isolated lung perfusion

The extracorporal circuit is a CE approved system designed for hyperthermic therapy consisting of a centrifugal pump, a heat exchanger, a filter and special tubing (figure 1). Notably, the oxygenator is not incorporated into the system, thereby avoiding contact between blood and the foreign body with a relatively large surface area. Slight hypoxia is anticipated and some lowering of the pH is accepted. By leaving out the oxygenator and choosing a pump technique that enables a primarily volume dependent circulation, some other adjustments in the circuit were made. The venous reservoir was no longer needed and the venous return was directly routed towards the pump unit, pumping the blood through the heat-exchanger directly to the patient. Bleeding and leakage from the cannulation sites were managed by using an autotransfusion device.



P = Pump unit
W = Weight system
H = Heat-exchanger
B = Buffer reservoir
A = Autotransfusion device
R = Reservoir

Figure 1. Perfusion circuit

Main circuit components are:

- pump unit (Biomedicus, First Circle Medical, Temet system)
- heat-exchanger (Avecor, First Circle Medical, Temet system)
- filter (Avecor, First Circle Medical, Temet system)
- tubing system (Medtronic, First Circle Medical, Temet system)
- waste bag (Sorin, 007-002-001, Arvada, C.O.)
- weight system (electronic unster)
- autotransfusion device (Sorin BRAT-2, Arvada, C.O.)

ILuP pulmonary toxicity

Dose limiting toxicity (DLT) is defined as:

- any common toxicity criteria (CTC) grade 3 or more on day 28 pulmonary toxicity
- any CTC grade 3 or more hematologic or non-hematologic toxicity
- an absolute neutrophil count less than $1x10^9$/L
- a platelet count less than $50x10^9$/L

Three procedures are entered at each dose level. Dose and temperature escalation according to table 1 is performed if none of the first three procedures exhibits DLT. If DLT occurs in one of the first three procedures, at least three more procedures are enrolled. Dose escalation is performed if no further DLT is encountered in the expanded cohort. If at least 2 out of 3, or at least 3 out of 6 patients developed DLT, no more patients would be treated at that dose- and temperature level, thereby terminating the trial. MTD was defined as the dose level just below the one at which DLT was encountered.

Pharmacokinetic Evaluation

Blood, perfusate, tumor and lung tissue are sampled during ILuP. Before the start of ILuP and at 5, 15, and at 30 minutes off bypass, systemic arterial blood and perfusate samples are collected. These samples are acquired in serum tubes and directly stored at 0°C.

Level 1: Unilateral perfusion of the lung with 15 mg at 37°C
Level 2: Unilateral perfusion of the lung with 15 mg at 42°C
Level 3: Unilateral perfusion of the lung with 30 mg at 37°C
Level 4: Unilateral perfusion of the lung with 30 mg at 42°C
Level 5: Unilateral perfusion of the lung with 45 mg at 37°C
Level 6: Unilateral perfusion of the lung with 45 mg at 42°C
Level 7: Unilateral perfusion of the lung with 60 mg at 37°C
Level 8: Unilateral perfusion of the lung with 60 mg at 42°C

Table 1. Dose regimen and sequencing of melphalan and perfusion temperature

Samples are spinned at 4000 rpm/min for 5 minutes in a cooled centrifugator and stored at minus -70°C until analysis is performed. Analyses of concentrations of melphalan are performed in perfusate, systemic arterial blood, as well as in lung tissue and tumor tissue. These tumor tissue samples and lung tissue samples are obtained during metastasectomy. Half of the samples is directly frozen into liquid nitrogen and stored at -70 °C in the pathology laboratory. Melphalan levels are measured in both normal lung and tumor tissue. The other half of the tumor tissue sample is fixed in buffered formaldehyde and used for light microscopy investigations to confirm the classification of malignancy and assess the level of toxicity by histological changes.

Safety evaluation

Clinical examination and recording of toxicities (CTC according to the National Cancer Institute (NCI)) [3] are performed on days 1 to 7, 9, 14, 21, 28 and 180 by means of a chest roentgenogram, arterial blood gas analysis, blood chemistry and hematology, electrocardiography and a transthoracic cardiac ultrasound on days 0 and 2.

Follow-Up

Besides standard blood hematology and chemistry and chest X-ray, the following investigations are made during follow-up:

- Pulmonary function testing (spirometry, diffusion capacity) on day 28
- Exercise testing (VO2) on day 28, if indicated
- Ventilation/perfusion scintigraphy on day 28, if indicated
- Transthoracic cardiac ultrasound on day 0 and day 2
- High resolution thoracic CT
- Bronchoscopy, if indicated.

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Chapter 4

Isolated lung perfusion with melphalan for resectable lung metastases: a phase I clinical trail

Hendriks JM, Grootenboers MJ, Schramel FM, van Boven WJ, Stockman B, Seldenrijk CA, ten Broecke P, Knibbe CA, Slee P, De Bruijn E, Vlaeminck R, Heeren J, Vermorken JB, van Putte B, Romijn S, Van Marck E, Van Schil PE. Isolated lung perfusion with melphalan for resectable lung metastases: a phase I clinical trial. Annals of Thoracic Surgery 2004;78(6): 1919-27.

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Abstract

Background. Current 5-year survival after complete resection of pulmonary metastases is 20-40%, and many patients develop intrathoracic recurrences. Isolated lung perfusion (ILuP) is an experimental technique to deliver high-dose chemotherapy to the lung without systemic exposure. A phase I trial of ILuP with melphalan combined with pulmonary metastasectomy for resectable lung metastases was conducted in order to define the dose limiting toxicity (DLT) and maximum tolerated dose (MTD).

Methods. From May 2001 to August 2003, 16 patients underwent ILuP with melphalan, followed by surgical resection of lung metastases. Patients were treated with increasing melphalan doses (levels 15, 30, 45, 60 mg). For each dose level, normothermia (37°C) and hyperthermia (42°C) were evaluated (n=3 per level). Serum samples were obtained during the procedure. Pulmonary, hematologic and non-hematologic toxicities were recorded. The primary tumor was colorectal in 7 patients, renal in 5, sarcoma in 3, and salivary gland in 1. ILuP was performed unilaterally in 11 patients, and staged bilaterally in 5.

Results. In total, 21 procedures of ILuP with complete metastasectomy were performed without technical difficulties. Operative mortality was 0%, and no systemic toxicity was encountered. Grade 3 pulmonary toxicity developed at a dose of 60 mg of melphalan at 37°C in 2 out of 3 patients in this level, terminating the trial.

Conclusions. ILuP with melphalan combined with pulmonary metastasectomy is feasible. DLT occurred at a dose of 60 mg melphalan at 37°C and MTD was set at 45 mg melphalan at 42°C.

Introduction

The current 5-year survival after complete surgical resection of pulmonary metastases remains low at 20-40% [1]. Although preoperative selection of the surgical candidate was able to improve this survival rate, the resistance to current chemotherapy and the inability to deliver intravenous (IV) chemotherapy in an appropriate dose without systemic toxicity could not increase this survival rate over the last two decades [2]. In addition, many patients develop intrathoracic recurrences soon after pulmonary resection, probably due to micrometastatic disease present at the time of initial operation [1]. Many new therapies focusing on better local drug delivery are studied, of which isolated lung perfusion (ILuP) is a surgical one. ILuP is able to deliver an agent in a high dose to the lung while minimizing systemic toxicity and avoiding drug metabolisation through the liver or kidneys. Based on promising results of ILuP with melphalan (Alkeran®, Glaxo Smith Kline) in rodent models [3,4], we started a dose-escalating phase I trial evaluating pulmonary, hematologic and non-hematologic toxicity of ILuP with melphalan combined with complete resection of all visible and palpable pulmonary metastatic disease. The purpose of the present study was to define the dose limiting toxicity (DLT) and maximum tolerated dose (MTD) of melphalan during ILuP. This study ran simultaneously in the St. Antonius Hospital, Nieuwegein, the Netherlands and the University Hospital Antwerp, Edegem, Belgium.

Patients and Methods

Endpoints of the study

Primary objectives were to determine the DLT and MTD of melphalan in patients treated with ILuP and pulmonary metastasectomy (see dose escalation scheme).
The study was approved by the ethical committee of Antonius Hospital in March 2001 and of the University Hospital Antwerp in September 2001, and written informed consent was obtained from each patient.

Inclusion Criteria

Patients with pulmonary metastases from melphalan-sensitive tumors were included if general and specific criteria were met. General criteria to perform a procedure were fourfold: all metastatic disease assessed by radiologic examination was resectable, metastatic disease was confined to the lungs, patients had adequate pulmonary and cardiac reserve, and no comorbid conditions that preclude an operation were present. All inclusion criteria are listed in table 1.

1. Cytological/histological diagnosis of pulmonary metastases
2. No other systemic metastases present than pulmonary metastases
3. Primary site has been radically treated without signs of recurrence
4. Patient is a candidate for pulmonary metastasectomy
5. No standard treatment options available, except pulmonary metastasectomy
6. Normal pulmonary function and normal diffusion capacity
7. Sufficient cardiopulmonary reserve to undergo pulmonary metastasectomy
8. No clinical or radiographic signs of interstitial lung disease
9. Performance status ECOG 0-1
10. Normal kidney and liver function
Urine creatinine clearance higher than 60 mL/min
Serum creatinine less than 130 μmol/L
ALAT and ASAT less than 3 times normal
11. Adequate bone marrow reserve
Absolute neutrophil count more than 2x10E9/L
Platelet count more than 150x10E9/L
12. Written informed consent

Table 1: Inclusion Criteria.

Exclusion criteria were pregnancy or lactation, uncontrollable infectious disease, liver or kidney insufficiency, severe comorbidity, and previous thoracotomy or pleuropulmonary disease resulting in obliteration of the pleural space.

Dose escalation scheme

ILuP with melphalan was performed at a dose determined by an escalation schedule. For each dose level, three patients were perfused at 37°C and three patients at 42°C. The different levels are depicted in table 2.

Level	Dose of melphalan (mg)	Temperature (°C)
1	15	37
2	15	42
3	30	37
4	30	42
5	45	37
6	45	42
7	60	37
8	60	42

mg: milligram; °C: degrees Celsius

Table 2: Dose Regimen.

DLT was defined as any CTC (common toxicity criteria according to National Cancer Institute) grade 3 hematologic or non-hematologic toxicity at any time point except for pulmonary toxicity, any radiographic change of the perfused lung resembling a chemical pneumonitis involving the whole lung, or any CTC grade 3 or more pulmonary toxicity on day 28. Only if

no patient in a level experienced DLT, 3 patients were subsequently treated in the next level. If one patient in a group experienced DLT, three more patients were treated within the same dose- and temperature-level. If at least 2 out of 3, or at least 3 out of 6 patients developed DLT, no more patients would be treated at that dose- and temperature level, thereby terminating the trial. MTD was defined as the dose level just below the one at which DLT was encountered.

Preoperative examinations

All patients underwent a work-up consisting of electrolyte studies, complete blood count, tumor markers if indicated, and liver and renal function tests; chest roentgenogram; computed tomographic (CT) scan of the brain, chest, and abdomen; bone scan; and positron emission tomography (PET) scan if available. Pulmonary function was analysed by means of quantitative lung perfusion scintigraphy and pulmonary function tests including spirometry and diffusion capacity, while cardiac function was tested by electrocardiography and transthoracic cardiac ultrasound. If indicated, a colonoscopy was also performed in case of colorectal metastases. Pathologic diagnosis of suspected lung metastases was obtained preoperatively or during the operation by frozen section analysis.

Surgical procedure

Complete metastasectomy was performed after ILuP. In case of bilateral disease, staged thoracotomies were planned with an interval of 4-8 weeks. This interval allowed adequate observation of (sub)acute toxicity, leaving time for the patient to recover. All patients were intubated with a double-lumen endotracheal tube and turned to a lateral position. An antero- or posterolateral thoracotomy was performed in a standard fashion. After inspecting the thoracic cavity, contraindications for a complete metastasectomy were excluded. All nodules were palpated before perfusion and their anatomic localisation was documented before perfusion. ILuP can induce pulmonary edema making accurate identification after perfusion more difficult. However, we choose to perform ILuP before surgical resection since perfusion is more homogeneous throughout the lung tissue, and since no bleeding will occur at the sites of resection because heparin is corrected with protamin. In case no preoperative histologic diagnosis was present, frozen section of one of the tumor nodules was performed to obtain pathological confirmation of metastatic disease.

Next, the main pulmonary artery and both pulmonary veins were isolated. The pericardium was opened to clamp the pulmonary artery and veins centrally. The patient was systemically anticoagulated with IV administration of heparin sodium up to an activated clotting time of above 200 seconds. The pulmonary artery and veins were clamped proximally and cannulated by standard techniques; the main bronchus was snared in order to occlude bronchial arterial blood flow. A perfusion circuit consisting of a centrifugal pump, a heat exchanger, and special

extracorporeal circuit tubings was primed with a mixture of 6% Voluven (6% hydroxyethyl starch 130/0.4) and 2% heparin. The total volume of this circuit was less than 300 mL. ILuP was carried out for a period of 30 minutes, during which the lung was reventilated with warmed (38°C) room air. The flow rate was calculated preoperatively (0.7 l/m^2), but adjusted to a mean pulmonary artery pressure below 30 mmHg. After stabilisation of temperature, flow and no signs of leakage (loss of priming volume out of the circuit), melphalan was injected into the perfusion circuit through the pulmonary artery line. After 30 minutes of perfusion, melphalan was washed out of the lung with a balanced fluid consisting of 3 liters of the priming solution. The flushed volume was collected in a waste bag. At the end of the washing period, the lung and pulmonary veins were de-aired by sequentially removing the pulmonary artery cannula, repairing the arteriotomy, removing the cannulae from the pulmonary veins, and removing the pulmonary artery clamp until bleeding from the pulmonary veins had vented all air. The venotomies were repaired and the clamps removed, restoring blood flow to the lung.

After correcting the activated clotting time with protamin, a complete metastasectomy was performed. Metastases were resected with a margin of 5 mm of normal lung tissue. Subsequently, a hilar and mediastinal nodal sampling were performed. Before the start of ILuP, at 5, 15, 30 minutes during ILuP, and at 30 minutes off bypass, systemic arterial blood samples were collected in serum tubes and directly stored at 0°C. Samples were spinned at 4000 rpm for 5 minutes in a cooled (4°C) centrifugator and sera were stored at -70°C until analysis was performed.

Postoperative evaluation

Clinical examination and recording of CTC were performed on days 1 to 7, 14, 28 and 180 by means of electrolyte studies, complete blood count, chest roentgenogram, arterial blood gases, and electrocardiography. A transthoracic cardiac ultrasound was performed on day 2, while pulmonary function testing (FEV1, DLCO) and a high resolution CT scan of the thorax were performed on day 28. Further follow-up was performed according to the specific scheme of the primary tumor.

Measurement of melphalan

High performance liquid chromatographic assay with fluorescence detection based on the assay of Wu et al (5), was used for the quantization of melphalan. Serum samples were stored at -70°C until required for analysis. Samples were clarified by centrifugation before the supernatant was brought on the column. After the samples were completely absorbed by the column, the columns were washed and dried. Finally, the compounds of interest were eluted and the extract was evaporated to a volume of approximately 200 µL. Finally, the extract was filtered and ready for injection. A calibration curve was prepared using blank serum. Dilutions

of 100 µg/mL, 10 µg/mL and 1 µg/mL for melphalan were prepared. Calibration standards of 50, 100, 300, 500, 1000, 3000, 5000, 10000 and 30000 ng/mL were prepared by mixing an appropriate volume of the melphalan dilutions to 50-500 µL blank serum and 100 µL of the internal standard solution (10 µg/mL).

Results

Patient characteristics (table 3)

Between May 2001 and August 2003, 16 patients with resectable pulmonary metastases fulfilled the entry criteria of this phase I trial. Five patients had bilateral disease and underwent staged procedures. Of these, 3 had bilateral disease at the time of their first procedure, while the second procedure was performed at an interval of 5 to 8 weeks.

	Number
Number of patients	16
Gender: Male	10
Female	6
Age	54 years (range, 34-72)
Primary tumor	
Colorectal carcinoma	7
Renal cell carcinoma	5
Sarcoma	3
Salivary gland carcinoma	1
Disease-free interval	48 months (range, 0 – 228)
Number of lung metastases	2 (range, 1 – 6)
Diameter	24 mm (range, 7 – 150)
Access	
Anterolateral thoracotomy	10 procedures
Posterolateral thoracotomy	11 procedures
Resection: Wedge	20
Lobectomy	1
Lung perfusion	
Unilateral	11
Staged bilateral	5
Left side	8
Right side	13

Table 3: Patient characteristics.

Two patients had unilateral disease initially, but developed metastatic disease in the other lung during follow-up. They underwent their second ILuP 4 and 11 months later. So, in total, 21 combined surgical procedures of ILuP followed by complete surgical resection of all metastatic disease were performed.

Ten patients were male and 6 patients female. Mean age was 54 years, mean length 174 cm (range, 154 to 199), and mean weight 79 kg (range, 60 to 108).

All metastatic nodules were resected by a wedge resection in all patients, but one. This patient, with complete replacement of the right lower lobe by a large metastasis of a colorectal tumor, had a lobectomy followed by perfusion of the right upper and middle lobe. Tumor nodules were located in the right upper lobe in 5 patients, the right middle lobe in 5, the right lower lobe in 7, the left upper lobe in 6, and the left lower lobe in 6.

Toxicity (table 4 and 5)

In total, seven levels of ILuP were finalised. There was no operative or postoperative mortality. In one patient, postoperative bleeding required re-intervention (level 6). Two patients in level 7 (60 mg at 37°C) developed lung edema (grade 3 CTC) and radiographic changes resembling a chemical pneumonitis of the whole perfused lung (Figure 1).

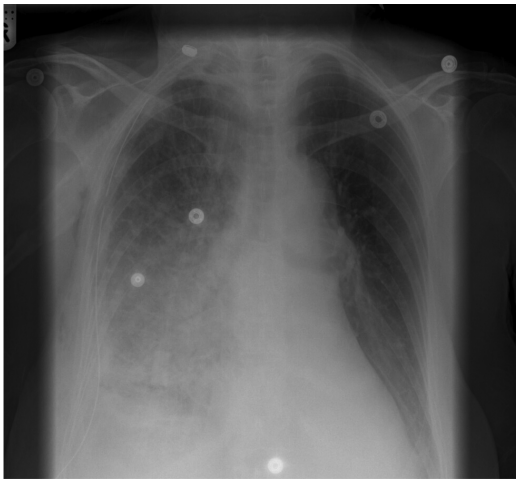

Figure 1. Chemical pneumonitis at day 2 post-isolated lung perfusion at the right side in patient no 21.

Both patients recovered slowly, but could leave the hospital at the 16th and 21st day postoperatively. Therefore, the DLT was set at 60 mg melphalan at 37°C and the MTD at level 6, meaning 45 mg of melphalan at a temperature of 42°C.

The highest cardiac toxicity recorded was a CTC grade 2 in level 6. Postoperative cardiac decompensation resulted in ankle edema, which necessitated therapy with diuretics for one week. Different degrees of radiographic pulmonary changes were seen. In three patients minimal diffuse edema was present during the first week around the site of resection of the lung metastases (procedures nos. 6, 12 and 14). One patient was treated for a lobar pneumonia (procedure no. 9) with antibiotics soon after ILuP. In two patients a small apical pneumothorax developed after removal of the chest tubes. In one of these 2 patients a new chest tube was inserted with good results. The mean duration on intensive care was 2 days (range, 2 to 6) and the mean hospitalisation was 14 days (range, 9 to 23).

Level	ILuP	FEV1 (% N)	FEV1 d28 (% N)	DLCO (% N)	DLCO d28 (% N)
		Pre ILuP	Post ILuP Day 28	Pre ILuP	Post ILuP Day 28
Level 1	**1**	98	70	103	88
	2	90	71	100	70
	3	122	89	90	96
Level 2	**4**	118	83	92	75
	5	83	83	75	71
	6	138	103	100	89
Level 3	**7**	103	89	89	102
	8	109	83	119	96
	9	102	92	85	78
Level 4	**10**	100	84	86	60
	11	101	82	95	78
	12	77	69	73	50
Level 5	**13**	121	100	99	72
	14	106	84	94	86
	15	101	67	96	72
Level 6	**16**	100	69	72	62
	17	79	61	102	77
	18	115	79	84	68
Level 7	**19**	150	69	68	72
	20	74	65	108	96
	21	91	61	70	58

FEV1: forced expiratory volume in one second in % of normal
DLCO: diffusing capacity not corrected for alveolar volume in % of normal

Table 4: Lungfunction assessments.

ARDS	Absent				Present
Apnea	None			Present	Intubation
Grade					
Toxicity	**0**	**1**	**2**	**3**	**4**
DLCO + FEV1	>90%N	>75%- <90%N	>50%- <75%N	>25%- <50%N	<25%N
Pleural effusion	None	Asymptomatic	Need for diuretics	Need for oxygen and chest tube	Life-threatening
Pneumothorax	None	No intervention	Chest tube required	Surgery or slerosis required	Life-threatening

Table 5: Pulmonary Common Toxicity Criteria (CTC) according to the WHO.

Melphalan levels (table 6)

In the first four levels, all patients but one had undetectable systemic levels of melphalan at 30 min after perfusion. At the final three levels, all patients had systemic leakage, but far below the levels known from IV therapy [6]. These systemic levels were not different between the groups with a range from 0.16 to 0.57 µg/mL. Although limited systemic exposure was present, no systemic toxicity was seen up to the dose of 60 mg. (0-0.39)

Level	Systemic Level (µg/mL)			
	5 min	15 min	30 min	30 min post-ILuP
1	0	0	0	0
2	0.4 (0-1.2)	0.4 (0-1.2)	0.37 (0-1.1)	0
3	0.39 (0–1.18)	0.23 (0–0.7)	0.43 (0–1.3)	0
4	0.33 (0-1)	0.38 (0-1.14)	0.37 (0-1.1)	0.13 (0-0.39)
5	0.26 (0-0.79)	0.36 (0.1-0.76)	0.3 (0.13-0.54)	0.35 (0.3-0.44)
6	0.45 (0-0.9)	0.5 (0-1.19)	0.22 (0.12-0.32)	0.27 (0.25-0.28)
7	0.03 (0-0.08)	0.02 (0-0.06)	0.08 (0-0.25)	0.31 (0.16-0.57)

µg: microgram; mg: milligram; mL: milliliter; ILuP: isolated lung perfusion

Table 6: Mean systemic levels (and range) of melphalan during ILuP (at 5, 15 and 30 min), and at 30 min post – ILuP

Clinical follow-up

All patients are alive after a mean follow-up of 14 months (range: 8 to 33 months). Of the 16 patients, recurrent metastatic disease developed in seven. Three patients had pulmonary metastases after a mean disease-free interval of 9 months (range: 7 to 11 months). Of these three patients, only one presented with pulmonary metastases in the lung that was previously perfused (procedure no. 12), while in two metastatic disease was in the non-perfused lung. One patient underwent a pulmonary metastasectomy with ILuP of the untreated lung (procedure 2, level 1). One patient underwent a completion pneumonectomy 9 months after right lower lobectomy and ILuP (procedure no. 12, level 4). He is still disease-free 6 months later. The third patient is treated with systemic chemotherapy. Of the seven patients with recurrent metastatic disease, four had disease outside the lung. Two patients treated for a renal cell carcinoma developed bone metastases after a disease-free interval of 5 and 11 months, respectively (procedures nos. 15 and 21). The two other patients developed metastatic disease in the mediastinum, 7 months after ILuP for which palliative chemotherapy is given (procedures nos. 13 and 16).

Comment

Isolated lung perfusion is a surgical technique developed in response to the low 5-year survival rate of 20 to 40 % after complete surgical resection of all metastatic disease of solid tumors [1]. ILuP has the advantage to deliver a high dose of chemotherapy to the lung at a controlled

rate while minimizing systemic exposure (2). The technique was already used with success in large animals and humans since 1983 by Johnston, both for single and double lung perfusion [7,8]. ILuP can be combined with resection in a single stage without increasing morbidity or mortality [9]. Tumor eradicating studies were started since 1994 with the development of rodent models of ILuP [10, 11]. Several agents were tested with success, and these studies showed that ILuP is superior to IV infusion [3,4,12-15]. Some of these agents, such as doxorubicin, tumor necrosis factor alpha (TNF-α) and cisplatin, were subsequently tested in human phase I trials, and in some studies the MTD levels were defined (Table 7).

Year	Author	Ref.	Drug	Lung temp (°C)	No. of pts	Perfusion time (min)	Operable	MTD
1958	Creech	16	Mitomycin C + nitrogen mustard + 5FU	NA	24 isolated organ procedures, with 1 ILuP	NA	Yes	NA
1984	Minchin	7	Doxorubicin	37	3	50	No	NA
1995	Johnston	8	Doxorubicin/ Cisplatin	NA	8	45 - 60	No	NA
1996	Pass	19	TNF-α	38 - 39.5	19	90	No	6 mg
1996	Ratto	9	Cisplatin	37	6	60	Yes	200 mg/m^2 (fixed)
2000	Burt	17	Doxorubicin	37	8	20	No	40 mg/m^2
2000	Putnam	18	Doxorubicin	37	16	NA	No	60 mg/m^2
2002	Schröder	20	Cisplatin	41	4	21 - 40	Both	70 mg/m^2 (fixed)
2004	Present study		Melphalan	37 - 42	16 (21 procedures)	30	Yes	45 mg – 42°C

NA : not available

Table 7: Human ILuP studies.

One of the first reports of clinical ILuP was published in 1958 by Creech [16]. Next, Minchin and coworkers reported on ILuP with doxorubicin in tree patients [7]. No systemic leakage was present while increasing lung levels of doxorubicin with time of perfusion were seen. Johnston et al. continued experimental work with doxorubicin and performed both single and total lung perfusion in patients with inoperable pulmonary metastases and primary lung cancer [7,8]. Drug concentrations in normal lung and tumor increased with higher doses, although lung levels were higher than tumor levels. Two major complications occurred in 8 patients: one

patient developed a pneumonia with subsequent a sternal dehiscence, and one patient developed respiratory failure several days after lung perfusion and died at postoperative day 81. Burt et al. described their results of ILuP with doxorubicin after extensive laboratory research [17]. Eight patients with inoperable lung metastases underwent single-lung perfusion in a phase I protocol. Again, intrapulmonary concentrations of doxorubicin correlated with the dose given while systemic levels were minimal or undetectable, but tumor levels were lower compared to lung levels. The MTD dose for this study was defined at 40 mg/m^2 of doxorubicin since an important chemical pneumonitis developed in one patient at a dose of 80 mg/m^2.
More recently, Putnam and coworkers presented their phase I study of isolated single-lung perfusion of doxorubicin in 16 patients with unresectable pulmonary metastatic disease [18]. Systemic levels were minimal or undetectable, while two patients developed a grade 4 pulmonary toxicity at a dose of 75 mg/m^2, therefore defining the MTD at 60 mg/m^2 of doxorubicin.
Results of ILuP with tumor necrosis factor-alpha (TNF-α) were published by Pass et al. in 1996 [19]. Nineteen patients underwent a 90 minute perfusion with TNF-α combined with γ-interferon and moderate hyperthermia. No deaths occurred and no significant systemic changes in systemic blood pressure or cardiac output were seen. Isolation of the lung was complete in 10 patients with 0% leak. For a dose up to 6 mg of TNF-α, maximal systemic TNF-α level was 3 ng/mL, which is far below the systemic MTD of 150 µg/m^2 in humans.
ILuP with cisplatin was tested by Ratto et al. in a series of 6 patients [9], and by Schröder et al. in a series of 4 patients [20]. Ratto et al. showed that ILuP combined with surgical excision of sarcoma lung metastases was technically feasible. The dose was fixed at 200 mg/m^2 of cisplatin while ILuP was performed for 60 min at normothermia. No deaths were seen while in two patients diffuse lung edema developed 48 hours after treatment. Schröder et al. tested the combination of hyperthermic ILuP with cisplatin at a fixed dose of 70 mg/m^2 for both resectable and unresectable disease [20]. Perfusion was variable between 21 and 40 min and surgical resection of lung metastases was done after ILuP. No deaths were seen, although all patients developed non-cardiogenic edema and ischemic changes in the treated lung.

Melphalan, an alkylating agent used for the treatment of many tumors like ovarian cancer, rhabdomyosarcoma, pancreatic carcinoma, osteogenic sarcoma, and multiple myeloma has rendered its attractivity for local organ perfusion after being investigated with success in isolated limb perfusion for melanoma when combined with TNF-α [21,22]. Experimental work of ILuP in rodent models was able to achieve lung levels of melphalan that were several times higher, while systemic concentrations were significantly lower with ILuP compared to IV injection [3,4]. In addition, ILuP with 2 mg/kg was able to eradicate carcinoma and sarcoma metastases in the lung which also resulted in a prolonged survival compared to control animals [23,24].

No other human or large animal trials of ILuP with melphalan were published so far, and therefore the initial dose to start with was unknown. The starting dose of 15 mg of melphalan was calculated from rat studies and estimated to result in one-third of the effective melphalan concentration of the rat studies in the human perfusion circuit [4]. As it was of utmost importance to avoid leakage into the systemic circulation, which could result in gastrointestinal and bone marrow toxicity, any level increase was only performed after the results of systemic melphalan levels were analysed.

If systemic melphalan levels were detected after ILuP, these were much lower compared to the levels obtained after IV infusion or after high-dose IV melphalan combined with bone marrow substitution [6,25]. For a dose of 10-20 mg/m^2 of melphalan given IV, reported systemic levels are 0.5 to 7.2 µg/mL. For high-dose melphalan (180 mg/m^2) with bone marrow eradication, systemic levels are 4.8 to 11.5 µg/mL.

No systemic, hematologic or non-hematologic DLT was seen in this study up to a dose of 60 mg at 37°C. All patients were scored a cardiac CTC grade 1 because the pericardium was opened for central clamping. This resulted in an asymptomatic pericardial effusion.

Only for a dose of 60 mg at 37°C severe pulmonary changes were seen in two out of three patients. The radiographic picture resembled a vascular leakage syndrome or chemical pneumonitis and was controlled with oxygen and diuretics. This picture was similar to the one seen in the study by Ratto and Burt [9, 17], although permanent extensive collapse and consolidation, as seen by Burt, was not present in our two patients who fully recovered.

Schröder et al. recommended to perform surgery before ILuP based on their experience with 4 patients. They had difficulty to identify metastatic nodules due to the edematous lung tissue after ILuP [20]. In our experience, we chose to identify and record all metastatic disease before cannulating the pulmonary artery and veins. Next, ILuP was performed in order to have a homogenous perfusion throughout the lung. Pharmacokinetic analysis of experimental work of ILuP in rats [4], of isolated liver perfusion [26], and of isolated limb perfusion [27], showed that melphalan is only slowly removed from the perfused tissue after an initial high uptake phase that extends up to 30-40 min.

Clinical follow-up results presented in this paper have to be interpreted with caution. In this phase I trial, survival is not a secondary end point, while responses in the perfused lung cannot be assessed since macroscopic disease was completely resected at the time of perfusion. In addition, in each dose level there were only three patients with different primary tumors lacking uniformity. Since ILuP is a local therapy, recurrent disease outside the lung or disease in the non-treated lung cannot be prevented. From this point of view, only one of the 16 patients developed recurrent disease within the perfused lung, 9 months after perfusion.

In conclusion, hyperthermic ILuP with melphalan followed by surgical resection of pulmonary metastatic disease is feasible. A dose of melphalan of 45 mg given at a temperature of 42°C

was defined as the MTD. A higher dose of 60 mg of melphalan at 37°C resulted in substantial, but reversible, injury to the lung. Further clinical studies of ILuP with melphalan in patients with sensitive lung metastases are justified to determine its long-term efficacy and possible survival benefit.

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Chapter 5

Pharmacokinetics of isolated lung perfusion with melphalan for resectable pulmonary metastases, a phase I and extension trial

Grootenboers MJ, Hendriks JM, Van Boven WJ, Knibbe CA, Van Putte B, Stockman B, Vlaeminck R, Heeren J, De Bruijn E, Vermorken JB, Van Schil PEY, Schramel FM. Pharmacokinetics of isolated lung perfusion with melphalan for resectable pulmonary metastases, a phase I and extension trial. Journal of Surgical Oncology 2007;96(7):583-9.

Abstract

Background: Isolated lung perfusion (ILuP) with melphalan was performed under normo- and hyperthermic conditions combined with pulmonary metastasectomy for patients with resectable lung metastases. We present the results of a phase I and extension trial developed to perform pharmacokinetic analysis.

Methods: Twenty-one procedures of ILuP with melphalan were performed in the phase I trial according to a dose-escalation schedule under normothermic and hyperthermic conditions followed by surgical resection of pulmonary metastases. In an extension trial, 8 additional procedures of ILuP with 15 and 45 mg melphalan were performed under hyperthermic conditions followed by pulmonary metastasectomy. Samples of serum, perfusate, lung and tumor tissue were obtained during and after the, in total, 29 procedures.

Results: High perfusate concentrations of melphalan were recorded in contrast to low systemic concentrations, while marked interindividual variability was observed in melphalan concentrations in perfusate, tumor and lung tissue. Statistically significant correlations between melphalan dose and perfusate area under the concentration-time curve (R^2=0.578, p=0.001) and lung tissue concentrations (R^2=0.459, p=0.028) were observed. No significant correlation between melphalan dose and tumor tissue concentrations could be established.

Conclusion: Isolated lung perfusion effectively delivers high doses of melphalan to the lung and tumor tissues with minimal systemic levels. Significant correlation between perfused melphalan dose, perfusate area under the concentration-time curve and lung tissue melphalan concentrations were observed.

Introduction

Pulmonary metastases are a common manifestation of metastatic disease, and even after complete surgical resection prognosis is poor with a 5-year survival between 16% and 42% [1]. Many of these patients develop intrathoracic recurrences, possibly because of micrometastatic disease at the time of initial procedure [2]. Systemic chemotherapy has proven to be little beneficiary, probably because of genetic drug resistance or the incapacity of delivering an effective dosage of local chemotherapy. Regional chemotherapy in combination with surgical resection could be a treatment modality in resectable pulmonary metastases, based on the concept of a distant spread phase in which metastases are confined to the lungs only, as e.g. in soft tissue sarcoma [3]. These findings would support the need for a multimodality approach to the treatment of pulmonary metastases, consisting of surgical resection of the macroscopic burden of tumor and chemotherapy to eradicate residual clinical undetectable micrometastases. As in isolated limb and liver perfusion, isolated lung perfusion (ILuP) is a promising surgical technique and an example of a locoregional procedure to deliver high-dose chemotherapy with minimal systemic toxicity [4]. To date, a relatively small number of human trials with ILuP have been performed in patients with both operable and inoperable metastatic disease. Exact pharmacokinetics in ILuP remain unclear because of the limited number of studies, small and heterogeneous groups of patients with operable and inoperable malignancies of different histological types, and the usage of different drugs under various perfusion conditions [3,5-11]. Previously, we have reported a phase I trial of ILuP with melphalan (Alkeran, Glaxo Smith Kline, Zeist, The Netherlands) in humans with a maximum tolerated dose (MTD) set at 45 mg melphalan at 42°C [12]. Subsequently, an extension trial was performed because of large interindividual variability in melphalan concentration levels in order to obtain insight in the exact pharmacokinetics of melphalan during ILuP. The purpose of this study is to describe the pharmacokinetics of melphalan in serum, perfusate, lung and tumor tissue at different doses and different perfusion temperatures during and after ILuP. Both phase I and extension trials ran simultaneously in the University Hospital Antwerp, Edegem, Belgium and the St. Antonius Hospital, Nieuwegein, the Netherlands and were approved by the Ethics Committee of both hospitals.

Patients and methods

Patient eligibility

Patients with pulmonary metastases from melphalan-sensitive tumors were included if general and specific criteria were met [12]. General criteria to perform the procedure were: all metastatic disease assessed by radiologic examination was resectable and confined to the lungs, patients had adequate pulmonary and cardiac reserve, and no comorbid conditions precluding an operation were present. The specific inclusion criteria are listed in Table 1. Investigations were performed after approval by a local Human Investigations Committee and in accord with an assurance filed with and approved by the Department of Health and Human Services. Informed consent was obtained from each participant.

Cytological or histological diagnosis of pulmonary metastases
No systemic metastases present other than pulmonary metastases
Radically treated primary site without signs of recurrence
Patient is a candidate for pulmonary metastasectomy
No standard treatment options available, except pulmonary metastasectomy
Normal pulmonary function and normal diffusion capacity
Sufficient cardiopulmonary reserve to undergo pulmonary metastasectomy
No clinical or radiographic signs of interstitial lung disease
Performance status ECOG 0-1
Normal kidney, liver function and adequate bone marrow reserve

Table 1. Inclusion Criteria.

Dose regimen

During the initial phase I trial, 16 patients underwent 21 procedures of ILuP according to a dose escalation schedule from 15 to 60 mg of melphalan with normothermia or hyperthermia tested for each dose-level. Because of considerable variations in melphalan concentration levels in perfusate, lung and tumor tissue in the results of the initial phase I trial, we chose to perform 8 more procedures (in 7 patients) in order to expand data for pharmacokinetic analysis (Table 2). Preliminary analysis of individual serum and tissue levels from the 15 and 45 mg melphalan groups did not reveal a trend towards higher melphalan concentrations in tumor tissue at higher melphalan doses. Thus, additional patients in the extension trial were treated with either 15 mg or 45 mg of melphalan, the latter being the previously reported MTD. Furthermore, as hyperthermia is one of the treatment modalities of the ILuP procedure, these additional patients were all (unintentionally with the exception of one) perfused under hyperthermic conditions.

Level	Dose of melphalan (mg)	Temperature (°C)	No of procedures phase I trial	No procedures extension trial
1	15	37	3	1
2	15	42	3	5
3	30	37	3	0
4	30	42	3	0
5	45	37	3	0
6	45	42	3	2
7	60	37	3	0

mg: milligram; °C: degrees Celsius

Table 2. Dose regimen and number of procedures.

ILuP technique and surgical procedure

In summary, an antero- or posterolateral thoracotomy is followed by inspection of the thoracic cavity with all nodules palpated and documented before ILuP. Next, the main pulmonary artery (PA) and both pulmonary veins are isolated and the pericardium is opened to clamp the PA and veins. Cannulae are positioned into the isolated PA and both pulmonary veins distal to their entry in the left atrium to drain venous return. After ensuring hemostasis, adequate systemic anticoagulant therapy is given with heparin. Occlusion of the main PA by atraumatic clamping is performed under pressure monitoring followed by occlusion of the venous return, respectively. The perfusion circuit consists of a centrifugal pump, heat exchanger and extracorporeal tubings. The cytostatic drug is introduced into the circuit as a bolus and perfusion temperature is chosen according to the dose regimen. Total ILuP time with melphalan is 30 minutes followed by a wash-out of the lung. The blood flow is restored after removing the cannulae, repairing the arteriotomy and venotomies, and removing the clamps. A complete pulmonary metastasectomy was performed after the ILuP procedure with staged thoracotomies planned with an interval of 4-8 weeks in case of bilateral disease.

Pharmacokinetics and measurement of melphalan

Before the start of ILuP, and at 5, 15, 30 and at 30 minutes off bypass, systemic arterial blood and perfusate samples are collected. These samples are acquired in serum tubes and directly stored at 0°C. Tumor and lung tissue samples are obtained during metastasectomy. Half of the samples is directly frozen into liquid nitrogen and stored at –70 °C in the pathology laboratory. The other half of the tumor tissue samples is fixed in buffered formaldehyde and used for light microscopical investigations. Samples are spinned at 4000 rpm/min for 5 minutes in a cooled centrifugator and stored at –70°C until analysis is performed. Based on the assay of Wu and colleagues high-performance liquid chromatography assay with fluorescence detection is utilized to quantify melphalan concentrations [13]. Samples were clarified by centrifugation

before the supernatant was brought on the column. After the samples were completely absorbed by the column, the columns were washed and dried. The compounds of interest were eluted and the extract was evaporated to a volume of approximately 200 µL. Finally, the extract was filtered and ready for injection. A calibration curve was prepared using blank serum. Dilutions of 100 µg/mL, 10 µg/mL and 1 µg/mL for melphalan were prepared. Calibration standards of 50, 100, 300, 500, 1000, 3000, 5000, 10000 and 30000 ng/mL were prepared by mixing an appropriate volume of the melphalan dilutions to 50-500 µL blank serum and 100 µL of the internal standard solution (10 µg/mL).

Statistical analysis of results

Pharmacokinetic data were analysed with univariate analysis of variance and presented as mean ± standard deviation or the median followed by the range. The areas under the concentration-time curves (AUC) were calculated with the trapezoïdal rule. Two-sided p-value ≤ 0.05 was considered statistically significant.

Results

Patient and ILuP procedure characteristics

Between May 2001 and December 2004, 29 procedures of ILuP were performed in 23 patients who fulfilled the criteria of this phase I and the extension trial (Table 3). Six out of these 23 patients had bilateral pulmonary disease and underwent staged thoracotomies. Four out of 6 patients presented with bilateral disease at the time of the first procedure and underwent their second, contralateral perfusion and thoracotomy at an interval of 5 to 12 weeks. Initial unilateral disease was present in 2 out of these 6 patients, who developed contralateral metastatic pulmonary disease during the follow-up period and underwent their second procedure 4 and 11 months later. Wedge resections were performed in all but one patient, who had a large metastasis with complete replacement of the right lower lobe, and who was treated with a lobectomy followed by perfusion of the upper and middle lobes. Ten patients had tumor nodules located in the right upper lobe, 6 in the middle lobe, 8 in the right lower lobe, 8 in the left upper lobe and 8 in the left lower lobe. Mean duration of ICU ward admittance was 2 days (range 1-9 days). Mean hospital stay was 16 days (range 8-46 days).

Melphalan pharmacokinetics

Table 4 lists the data of mean systemic melphalan concentrations (and range) at different time points during and after the ILuP procedure. No systemic concentrations of melphalan could be recorded in the first three levels. Although some systemic leakage occurred in the next four levels, serum concentrations remained well below levels known from intravenous therapy [14]. Data on individual melphalan pharmacokinetics are recorded in table 5, on melphalan

	Number
Number of patients	23
Number of procedures	29
Gender: Male	14
Female	9
Age (y)	56 (range, 34-72)
Primary tumor	
Colorectal carcinoma	10
Renal cell carcinoma	8
Sarcoma	4
Salivary gland carcinoma	1
Disease-free interval (mo)	42 (range, 0 – 228)
Number of lung metastases	3 (range, 1 – 13)
Diameter (mm)	21 (range, 4 – 150)
Resection: Wedge	28
Lobectomy	1
Lung perfusion	
Unilateral	17
Staged bilateral	6
Left side	13
Right side	16

Numbers in means (and range)

Table 3. Patient and ILuP procedure characteristics

concentrations in the perfusate and in the systemic circulation at 45 mg melphalan (MTD) in figure 1, and on melphalan concentrations in lung and tumor tissue per dose level in figure 2. Our data show that pharmacokinetics of melphalan during ILuP are linear with dose, although melphalan concentrations in the perfusate, lung and tumor tissue were found to vary considerably between the patients. The AUC in the perfusate reflects melphalan exposure to the lung and pulmonary metastases. A statistically significant correlation was found between the melphalan dose and the mean AUC in perfusate (R^2=0.578, p=0.001).

Mean peak concentrations and AUC of melphalan in perfusate of patients treated with the maximum tolerated dose of 45 mg of melphalan at 42°C were 108.6 µg/mL and 53.4 µg/mL respectively, while systemic mean peak concentrations and systemic AUC were 0.44 µg/mL and 5.4 µg/mL, respectively. Melphalan concentrations in lung and tumor tissue were documented in 23 and 17 procedures respectively. Melphalan lung tissue concentrations ranged from 1.1 to 26.6 µg/g, and melphalan tumor tissue concentrations ranged from 0.78 to 11.5 µg/g in the different dose levels. A statistically significant correlation between the dose of melphalan and lung tissue concentrations of melphalan (R^2=0.459, p=0.028) was found. However, no significant correlation between melphalan dose and tumor tissue concentrations could be documented (R^2=0.255, p=0.600). No significant correlations could be established between tumor or lung tissue melphalan concentrations and variables, such as temperature, histology,

or diameter of metastases. Furthermore, additional statistical analyses revealed no correlation between drug concentrations in perfusate, lung, and tumor, and pulmonary or systemic toxicity.

Level	No of patients	Systemic Level (µg/mL)			
		5 min	15 min	30 min	30 min post-ILuP
1	4	0	0	0	0
2	8	0.15 (0-1.2)	0.15 (0-1.2)	0.14 (0-1.1)	0
3	3	0.39 (0–1.18)	0.23 (0–0.68)	0.43 (0–1.26)	0
4	3	0.33 (0-1)	0.38 (0-1.14)	0.38 (0-1.13)	0.13 (0-0.39)
5	3	0.26 (0-0.79)	0.36 (0.1-0.76)	0.3 (0.13-0.54)	0.35 (0.3-0.44)
6	5	0.27 (0-0.9)	0.36 (0-1.19)	0.13 (0-0.32)	0.16 (0-0.28)
7	3	0.03 (0-0.08)	0.02 (0-0.06)	0.08 (0-0.25)	0.31 (0.16-0.57)

µg: microgram; mL: milliliter; ILuP: isolated lung perfusion

Table 4. Mean systemic levels (and range) of melphalan during ILuP (at 5, 15 and 30 min), and at 30 min post – ILuP.

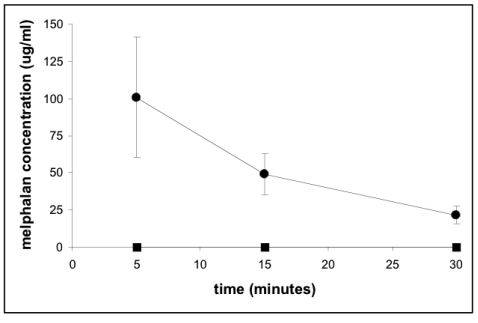

Figure 1. Melphalan concentrations (µg/mL) measured in the ILuP perfusate (circle) and in the systemic circulation (square) at the maximum tolerated dose of 45 mg melphalan (mean ± SD)

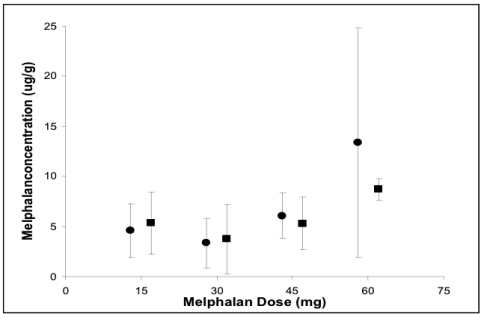

Figure 2. Melphalan concentrations (µg/g) in lung (circle) and tumor (square) tissue for the different melphalan doses (15, 30, 45 and 60 mg) during ILuP (mean ± SD)

Procedure number	Level	MN dose	Perfusate temperature in °C	Cmax systemic µg/mL	Cmax perfusate µg/mL	AUC systemic	AUC perfusate	Lung MN µg/g	Tumor MN µg/g	LN MN µg/g
1	1	15	37	0	4.3	0	1.9	5.28	1.53	-
2	1	15	37	0	2.9	0	1.6	9.03	3.84	-
3	1	15	37	0	7.9	0	4.3	-	-	-
4	1	15	37	0	62.9	0	38.1	2.02	6.24	-
5	2	15	42	1.21	11.1	15	4.9	-	-	-
6	2	15	42	0	6.3	0	6.8	3.34	4.14	-
7	2	15	42	0	6.5	0	Nd	-	-	-
8	2	15	42	0	30.0	0	14.7	6.37	-	-
9	2	15	42	0	24.1	0	10.2	3.61	4.60	-
10	2	15	42	0	39.2	0	18	7.63	11.5	-
11	2	15	42	0	58.7	0	32.9	2.59	-	1,85
12	2	15	42	0	26	0	23	1.31	5.5	2,55
13	3	30	37	0	6.2	0	4	4.66	1.71	2,59
14	3	30	37	1.26	161	10	38.7	1.41	3.8	-
15	3	30	37	0	120	0	26.7	1.1	0.78	-
16	4	30	42	1.14	75.1	21	31.4	6.18	8.18	-
17	4	30	42	0	22.3	0	20.1	-	-	-
18	4	30	42	0	114	0	46.2	-	-	2,35
19	5	45	37	0.79	64.6	12	33.7	8.53	4.44	-
20	5	45	37	0.44	57.5	7	44.6	5.12	6.76	-
21	5	45	37	0.30	140.7	6	78.7	5.99	6.57	-
22	6	45	42	0.44	113.1	7	63.3	6.63	9.07	-
23	6	45	42	1.19	107.9	14	60.8	-	-	-
24	6	45	42	0.28	72.2	4	41.2	7.44	2.4	-
25	6	45	42	0.27	173.3	2	61.9	7.19	-	-
26	6	45	42	0	76.3	0	40	1.58	2.6	-
27	7	60	37	0.20	64.2	1	45.2	7.46	-	11,1
28	7	60	37	0	54.7	0	46.1	6.09	7.98	6,71
29	7	60	37	0.57	249.0	6	169.3	26.6	9.5	-

MN: melphalan, Nd=below limit of detection, LN=lymph node

Table 5. Individual melphalan pharmacokinetics

Isolated, resectable pulmonary metastases represent an unique situation in tumor biology with evidence that these patients do not have the same poor prognosis as patients with metastases in multiple sites [15]. Combinations of resection of macrometastases with treatment of clinical undetectable micrometastases may be best in improving disease-free and overall survival [3]. Regional chemotherapy with ILuP can be seen as a one-course adjuvant chemotherapy, possibly enhancing therapy for patients with pulmonary metasases in combination with surgery. In our study we chose melphalan as antineoplastic drug, because (1) melphalan anti-tumor activity has been reported for a great variety of tumors and renders its attractiveness for local organ perfusion, such as isolated liver perfusion for metastases of colorectal origin [16-18] or isolated limb perfusion for melanoma [19,20], and (2) since 1995 ILuP experiments with melphalan in a rat model were extensively performed showing pharmacokinetic superiority, low operative mortality and neglible long-term lung injury, as well as significantly higher eradication of tumor nodules and longer survival time in comparison to intravenous treatment [21-23]. The rationale for hyperthermia is based on both preclinical and clinical studies showing enhanced chemotherapeutic drug uptake and increased tumor growth delay, as well as in vivo increased cytotoxicity in many anti-neoplastic drugs [24,25]. These findings

encouraged us to pursue a dose-escalating phase I trial of hyperthermic ILuP with melphalan combined with complete resection of all pulmonary metastatic disease to define dose limiting toxicity and MTD [12].

This pharmacokinetic study demonstrates that ILuP with melphalan effectively delivers high doses of melphalan to the lung and tumor tissues with minimal systemic levels. Systemic melphalan concentrations after ILuP were still much lower than previously reported levels of 0.5 to 7.2 µg/mL after intravenous treatment of 10 to 20 mg/m^2 melphalan [13], or reported levels of 4.8 to 11.5 µg/mL for high dose (180 mg/m^2) treatment with melphalan combined with bone marrow substitution [26]. In our opinion, these conclusions justify the concept of ILuP as a means of delivering and exposing the lung to high-dose local chemotherapy with minimal systemic levels. Reviewing the literature on human ILuP studies, a high degree of lung vascular isolation in human patients is obtained in numerous other reports of ILuP [6-11].

Our data show a statistically significant correlation between the melphalan dose and the AUC in perfusate, as well as lung tissue concentrations of melphalan. The fact that no significant correlation between melphalan dose and tumor tissue concentrations was noted is relevant, but the concept of ILuP as a (neo)adjuvant chemotherapeutic modality is to treat residual clinical undetectable micrometastases in the lung and not macroscopic pulmonary metastases. Establishing exposure (with the AUC) and uptake in the lung tissue is therefore of utmost importance. In our opinion, these conclusions justify the concept of ILuP followed or preceded by surgical resection as a treatment modality for both microscopic and macroscopic pulmonary localized malignancies.

Melphalan concentrations in lung and tumor tissue were documented and showed marked variability per individual. Possible causes for this phenomenon are leakage at the cannula sites during the procedure, heterogeneous perfusion, use of standard doses of melphalan regardless of patients' weight, length or pulmonary vascular volume, and differences in number, diameter, histology and localization of pulmonary metastases. The absence of significant correlation between perfusate and tumor tissue concentrations suggests the uptake of melphalan in tumor tissue to be independent of perfusate melphalan concentrations, possibly because of a maximum uptake or plateau of chemotherapeutic concentration in tumor tissue. Another possible explanation could be differences in vascularisation of the tumor, dependent on histological type and localization. Heterogeneous perfusion can not be the sole explanation for this phenomenon because of the previously reported statistical correlation between AUC and lung tissue concentrations of melphalan. In order to explain the absence of correlation between melphalan perfusate concentration and tumor levels, a number of subanalyses were performed. However, no significant correlations could be established between tumor or lung tissue melphalan concentrations and variables, such as temperature, body weight, number, histology, or diameter of pulmonary metastases. Considering the number of patients per subgroup, it is impossible to draw definite conclusions upon these topics. When reviewing

other ILuP studies on correlation between dose of chemotherapy and lung and tumor tissue levels, some studies report increased drug concentrations in lung tissue only [10], or both lung and tumor tissue [7]. In our study, as in Ratto and associates' study [9], we did not find significant differences in cytostatic levels between lung and tumor tissue. Other human ILuP studies found lower or a trend towards lower drug concentrations in tumor tissue [6,10].

In conclusion, this study represents to the best of our knowledge the first report on pharmacokinetics of patients treated with melphalan in ILuP to date. Previously, we have reported ILuP with melphalan followed by surgical resection of pulmonary metastatic disease to be a feasible procedure [12]. This study demonstrates the great pharmacokinetic advantage of ILuP with melphalan in achieving high-dose local chemotherapy with minimal systemic concentrations. Furthermore, we demonstrated a significant correlation between the melphalan dosage, AUC of perfusate, and lung tissue melphalan concentrations, justifying the concept of isolated lung perfusion as one-course adjuvant chemotherapeutic treatment modality for microscopic residual malignant disease. A multicentered, phase II trial has been submitted to the Ethical Commitees, hopefully providing us with additional information on pharmacokinetics and toxicity, but also long-term efficacy and possible survival benefit in carefully selected patients with resectable pulmonary metastases.

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Chapter 6

Re-evaluation of toxicity and long-term follow-up of isolated lung perfusion with melphalan in patients with resectable pulmonary metastases, a phase I and extension trial.

Grootenboers MJ, Schramel FM. Van Boven WJ, Van Putte B, Hendriks JM, Van Schil PE. Re-evaluation of toxicity and long-term follow-up of isolated lung perfusion with melphalan in patients with resectable pulmonary metastases, a phase I and extension trial. Annals of Thoracic Surgery 2007;83(3):1235-6.

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Abstract

Background: Isolated lung perfusion (ILuP) with melphalan combined with pulmonary metastasectomy for patients with resectable pulmonary metastases is a feasible procedure with a maximum toleterated dose (MTD) of 45 mg at 42°C. Toxicity and clinical follow-up of a previously described phase I and extension trial are the subjects of this study.

Methods: From May 2001 to December 2004, 16 patients were treated in a phase I trial with ILuP with escalating doses of melphalan under normo- and hyperthermic conditions followed by surgical resection of pulmonary metastases. In the extension trial, 7 additional patients were treated with ILuP with 15 or 45 mg of melphalan at 42°C followed by pulmonary metastasectomy. One patient was treated off-protocol with MTD of melphalan in ILuP under normo- and hyperthermic conditions. Patients were evaluated for pulmonary and systemic toxicities including pulmonary function assessments, as well as for clinical follow-up.

Results: Twenty-nine procedures in 23 patients included in the clinical trials were performed without technical difficulties. Three out of 8 procedures in the extension trial under hyperthermic conditions were complicated with empyema, rhabdomyolysis and postoperative bleeding, respectively. Pulmonary function assessments show a statistically significant decline with partial reversibility in all parameters. With a mean follow-up of 25 months, 8 out of 23 patients are alive and disease-free. Fourteen patients, of which 3 patients are deceased, developed recurrent disease. One patient died of non-malignancy related disease. Off protocol, one patient was treated with MTD of melphalan in ILuP under normo- and hyperthermic conditions. The procedure under hyperthermic conditions was complicated by chemical pneumonitis, massive pulmonary hemorrhage, and eventually death.

Conclusion: Under hyperthermic conditions, considerable pulmonary and systemic morbidity was encountered in the extension trial patients. Furthermore, a hyperthermic procedure in a patient treated off-protocol with MTD was complicated by diffuse alveolar damage and death. In contrast to our previous report, MTD of melphalan in ILuP should be 45 mg under normothermic conditions.

Introduction

Prognosis of patients with pulmonary metastases remains poor with a 5-year survival of approximately 20-40% after complete surigical resection [1]. Preoperative selection has improved the survival rate initially, but despite current chemotherapy there has been no increase in survival rate over the last 20 years [2]. Furthermore, many patients develop intrathoracic recurrences probably because of micrometastatic disease at the time of initial procedure [1]. ILuP is an experimental surgical technique enabling the delivery of high-dose local chemotherapy with minimal systemic toxicity and thereby avoiding drug metabolism through the kidneys and liver. A multimodality approach was developed, including ILuP with melphalan combined with pulmonary metastasectomy for patients with resectable lung metastases. Hendriks and colleagues reported clinical toxicity of a human phase I study of ILuP with melphalan followed by pulmonary metastasectomy and performed a total of 21 procedures without technical difficulties or operative mortality [3]. The authors concluded, based on the development of chemical pneumonitis in 2 patients treated with 60 mg melphalan at 37°C, the MTD of melphalan in ILuP to be 45 mg at 42°C. At the final pharmacokinetic analysis of this study, marked interindividual variability in drug concentrations was observed, encouraging us to pursue an extension trial of hyperthermic ILuP with melphalan combined with complete resection of pulmonary metastatic disease [4]. We now report on pulmonary and systemic toxicity, serial pulmonary function assessments and clinical follow-up after ILuP with melphalan followed by pulmonary metastasectomy in the series of Hendriks and co-workers [3] together with the additional procedures of the extension trial. These studies ran simultaneously in the St. Antonius Hospital, Nieuwegein, the Netherlands and the University Hospital Antwerp, Edegem, Belgium.

Patients and methods

The study was approved by the ethical committee of the University Hospital Antwerp, Edegem, Belgium and the St. Antonius Hospital, Nieuwegein, the Netherlands. Written informed consent was obtained from each patient.

Patient eligibility

Patients with pulmonary metastases from melphalan-sensitive tumors were included if general and specific criteria were met, as previously described by Hendriks and colleagues [3]. In summary, metastatic disease had to be resectable (as assessed by radiologic examination), and confined to the lungs. Furthermore, patients had to have adequate pulmonary and cardiac reserve, and no comorbid conditions precluding an operation. Preoperative examinations,

anesthetic management, perfusion and surgical procedure, and postoperative evaluation were also extensively documented in the paper by Hendriks and associates [3].

Dose regimen

Dose and temperature of the individual procedures are documented in table 1. In the phase I trial, patients were treated with ILuP with melphalan according to a dose escalation scheme under normo- (37°C) or hyperthermic conditions (42°C) [3]. The additional patients of the extension trial were all (with the unintentional exception of one case) perfused under hyperthermic conditions and treated with either 15 mg or 45 mg of melphalan via ILuP.

ILuP technique and surgical procedure

Our technique of ILuP has been described in detail previously [3]. In summary, a complete pulmonary metastasectomy is performed after the ILuP procedure. In case of bilateral disease, staged thoracotomies were planned with an interval of 4-8 weeks. The pleural space is opened by means of a muscle-sparing lateral thoracotomy. After inspection of the thoracic cavity, all nodules were palpated and documented before ILuP. Next lobar and hilar pulmonary vessels and bronchial artery/arteries are identified and carefully dissected to prevent systemic leakage. The pericardium was opened to clamp the pulmonary artery and veins centrally. Cannulae were positioned into the isolated pulmonary artery and beyond the pulmonary venous take off to drain venous return. Melphalan was introduced into the circuit as a bolus and washed out after 30 minutes of perfusion.

ILuP toxicity and pulmonary function assessments

In retrospect, histologic samples of lung and tumor tissue obtained at the metastasectomy after 30 minutes of ILuP were examined from all procedures. Clinical examination and recording of toxicities on days 1 to 7, 9, 14, 21, 28 , 90, 180 and subsequently every 3 months by means of blood hematology and chemistry, arterial bloodgas analysis, electrocardiography and chest x-ray were obtained and analysed. Toxicity (pulmonary and non-pulmonary) was graded by using the CTC according to the National Cancer Institute (CTC Version 2.0). Pulmonary function assessments consisted of forced expiratory volume in 1 second (FEV1), inspiratory vital capacity (IVC), total lung capacity (TLC), diffusing capacity of the alveolocapillary membrane (DLCO), and the transfer factor of the lungs for carbon monoxide per unit alveolar volume (KCO). Assessments were measured on days 28, 90, 180, 360 and from there on every single year. Thoracic computed tomography was performed on days 90 and 180.

Statistical analysis of results

Two-sided p-value ≤ 0.05 was considered statistically significant. Statistical analysis to compare changes in lung function to pretreatment values was performed by analysis of variance in a repeated-measurement design.

Results

Patient and ILuP procedure characteristics

Twenty-nine procedures of ILuP were performed in 23 patients who fulfilled the criteria of this phase I and extension trial between May 2001 and December 2004. The primary tumor was colorectal in 10 patients, renal in 8, sarcoma in 4, and salivary gland in 1. Fourteen patients were male and 9 patient female with a mean age of 56 years (range, 34 to 72 yrs). Seventeen out of 23 patients patients were treated with unilateral thoracotomies. Six patients had bilateral pulmonary disease and underwent staged thoracotomies. Wedge resections were performed in all but one procedure. Because of a large metastasis completely replacing the right lower lobe, this patient was treated with a lobectomy followed by perfusion of the upper and middle lobes. The mean number of pulmonary metastases was 3 (range 1-13) with a mean diameter of 21 mm (range 4-15). Mean duration of ICU ward admittance was 2 days (range 1-9 days) and mean hospital stay was 16 days (range 8-46 days).

Toxicity

Histologic examinations of all procedures (phase I and extension trial) did not reveal any signs of pulmonary toxicity or tumor lysis. Table 1 describes the perfusion conditions, encountered pulmonary and non-pulmonary toxicity, and patients' status in all procedures included in the phase I and extension trial. In the phase I trial, we observed in the first 21 procedures: 2 patients with chemical pneumonitis (procedures nos. 27 and 29), 3 patients with minimal edema (procedures nos. 7, 18, and 20), 2 patients with pneumothorax necessitating insertion of a chest tube in one patient with good results (procedures nos. 22 and 24), 1 patient with postoperative bleeding requiring surgical intervention (procedure no. 23) and 1 patient with a lobar pneumonia treated with antibiotics (procedure no. 15). All patients recovered uneventfully, and no peri- or postoperative mortality was encountered. In the extension trial, 8 additional procedures were performed in 7 patients with again no operative or postoperative mortality but considerable pulmonary and systemic toxicity in 3 patients. We will now, briefly, discuss these three complicated procedures. The first patient underwent bilateral procedures with 15 mg and 45 mg of melphalan both under hyperthermic conditions (procedures nos. 9 and 26, respectively). The first procedure was complicated by reperfusion edema of the right lower lobe and subcutaneous emphysema, not requiring any intervention (Figures 1 and 2). Histologic examination of the tumor and lung tissue, obtained directly after 30 minutes after perfusion, revealed no signs of pulmonary toxicity (Figure 3).

Proc. No	Phase I (I) or extension (E)	MN dose	Perf temp	Pulmonary toxicity	CTC pulm	Other toxicity	CTC other	Status
1	I	15	37	-	-	-	-	Alive without disease
2	I	15	37	-	-	-	-	Alive without disease
3	I	15	37	-	-	-	-	Alive without disease
4	E	15	37	-	-	-	-	Alive with disease
5	I	15	42	-	-	-	-	Alive without disease
6	I	15	42	-	-	-	-	Alive without disease
7	I	15	42	Minimal edema	1	-	-	Alive without disease
8	E	15	42	-	-	-	-	Deceased
9	E	15	42	Pneumothorax, empyema	3	-	-	Alive with disease
10	E	15	42	-	-	-	-	Alive without disease
11	E	15	42	Pneumonia	3	Rhabdomyolysis	4	Alive with disease
12	E	15	42	Bleeding chest tube and pulmonary consolidations	3	Hypotension requiring inotropics	4	Deceased
13	I	30	37	-	-	-	-	Alive without disease
14	I	30	37	-	-	-	-	Alive with disease
15	I	30	37	Pneumonia	2	-		Alive with disease
16	I	30	42	-	-	-	-	Alive without disease
17	I	30	42	-	-	-	-	Alive without disease
18	I	30	42	Minimal edema	1	-	-	Alive with disease
19	I	45	37	-	-	-	-	Deceased
20	I	45	37	Minimal edema	1	-	-	Alive without disease
21	I	45	37	-	-	-	-	Alive with disease
22	I	45	42	Pneumothorax	2	-	-	Deceased
23	I	45	42	Bleeding chest tube	2	-	-	Alive with disease
24	I	45	42	Pneumothorax pneumonia	2	-	-	Alive with disease
25	E	45	42	-	-	-	-	Deceased
26	E	45	42	Chemical pneumonitis, Subcutaneous emphysema	2	-	-	Alive with disease
27	I	60	37	Chemical pneumonitis	3	-	-	Alive with disease
28	I	60	37	-	-	-	-	Alive without disease
29	I	60	37	Chemical pneumonitis	3	-	-	Alive with disease

Table 1. Details of individual perfusion conditions, toxicity and patients status

After the second, contralateral procedure a small pneumothorax was initially accepted, but did require reinserting of a chest tube a few days later. Unfortunately, an empyema developed, treated with intravenous antibiotics and drainage of the infected pleural effusion. Patients' recovery was uneventful, but hospitalisation period was prolonged to 46 days.

The second patient (procedure no. 11) was treated with 15 mg of melphalan at 42°C and developed a lobar pneumonia and respiratory insufficiency requiring antibiotics and prolonged mechanical ventilatory support. The 9-days ICU admittance was complicated by rhabdomyolysis without evident cause with uneventful recovery. Patient's discharge could be documented after 20 days.

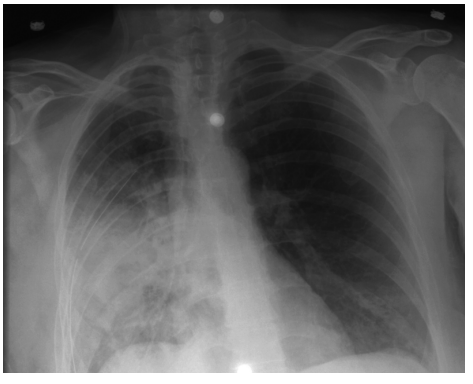

Figure 1. Procedure no. 26 with 45 mg melphalan at 42°C: chemical pneumonitis right lower lobe.

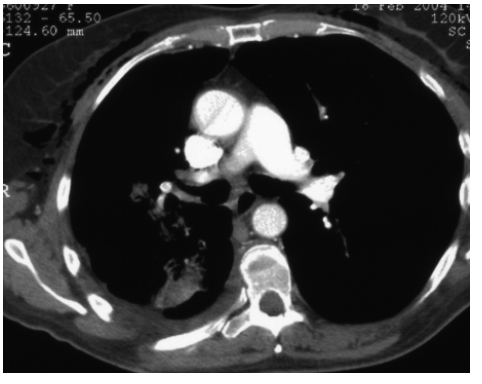

Figure 2. Procedure no. 26. CT thorax one month later: minor consolidation right lower lobe

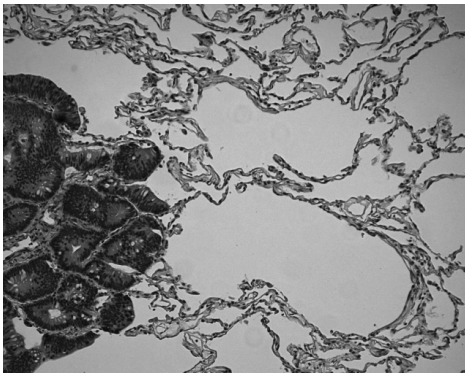

Figure 3. Procedure no. 26: Adenocarcinoma and normal lung tissue after ILuP

The last patient with significant toxicity (procedure no. 12) was treated with 15 mg of melphalan at 42°C and suffered from hemodynamic instability during operation with excessive blood loss through the chest tubes, requiring multiple blood transfusions. Intravenous antibiotic therapy was initiated because of development of pulmonary consolidations. Admittance on intensive care lasted 3 days, and duration of hospitalisation was 18 days. Unfortunately, this patient deceased 4 months later after a contralateral pulmonary metastasectomy without ILuP due to cardiac complications.

Pulmonary function assessments

Assessments of lung function in time are described in table 2. Because of progressive lack of follow-up, one has to be careful in reviewing the data. Despite the relative small

Lung function	Baseline	Months after procedure	Change from baseline	% Change from baseline	Number of patients	p-value
DLCO	84.4	1	-23.2	-27	23	0.00
	-	3	-20.3	-24	12	0.00
	-	6	-18.1	-21	11	0.00
	-	12	-10.3	-12	9	0.02
	-	24	-7.4	-9	3	0.29
KCO	93.2	1	-7.9	-8	23	0.00
	-	3	-9.8	-12	12	0.00
	-	6	-11.1	-13	11	0.00
	-	12	-2.0	-2	9	0.59
	-	24	-2.8	-3	3	0.65
FEV 1	3202	1	-721	-23	24	0.00
	-	3	-402	-13	12	0.00
	-	6	-292	-9	13	0.01
	-	12	-253	-8	8	0.08
	-	24	-362	-11	4	0.11
VC	4151	1	-971	-23	24	0.00
	-	3	-674	-16	12	0.00
	-	6	-453	-11	13	0.00
	-	12	-277	-7	8	0.13
	-	24	-335	-8	4	0.23
TLC	6294	1	-1339	-21	19	0.00
	-	3	-981	-16	9	0.00
	-	6	-428	-7	8	0.04
	-	12	-454	-7	7	0.07

Table 2. Summary of changes of lungfunction assessments in time

number of pulmonary function assessments, it is evident that after the procedure (both perfusion and metastasectomy) a significant decline in DLCO (first 12 months), KCO , FEV1 , VC and TLC (all in the first half year) was documented. This decline in lung function seems to be partially reversible as seen from the changes from baseline, although one has to be careful for selection bias. Subanalysis of the group of patients with serious toxicity (procedure nos. 9, 11, 12, 27 and 29) could not be investigated thoroughly, because of loss of follow-up (procedure no. 29) and occurence of death (procedure no. 12).

Radiographic follow-up

Unfortunately, the number of patients with evident pulmonary toxicity with long-term radiographic follow-up is limited. The reason for this phenomenon is the fact that a significant number of patients were originally sent by another medical centre and returned there after the procedures. In practice, one has to say, that these patients were more or less lost for follow-up, making it hard to draw definite conclusions. Taking into account the limited number of patients available for radiologic folllow-up, there were no assessments of interstitial lung disease, even after four years. We would like to illustrate this with the CT scan of a patient treated with 45 mg of melphalan under normothermic conditions (Figure 4), the perfusion conditions of the phase II investigation now running.

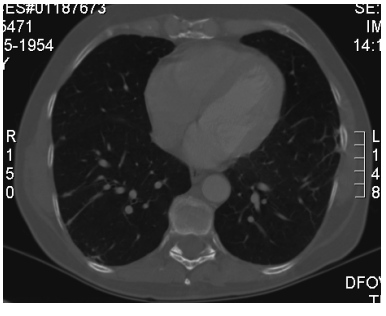

Figure 4. Procedure no 20. CT chest four years after the procedure without signs of recurrence or interstitial lung disease.

Clinical follow-up

Nineteen of the 23 patients are still alive after a mean follow-up of 25 months (range, 3-52 months). Mean disease-free interval for the total study group of 23 patients was 18 months (range, 0-43 months). Two patients were lost to further follow-up, because of referral to their own specialist. One patient did not wish to continue trial investigations after 6 months of follow-up. One patient died after a thoracotomy (without ILuP) because of cardiac

complications. Fourteen patients developed recurrent metastatic disease of which 3 patients deceased due to their illness. Mean disease-free interval for the patients with recurrent disease was 10 months (range, 0-26 months). Eight out of 14 patients developed pulmonary metastases after a mean disease-free interval of 13 months (range, 0-26 months). Four out of 8 patients with pulmonary metastases presented with metastases in the non-perfused lung, 2 patients in the perfused lung and 2 patients bilaterally. The 6 patients with non-pulmonary recurrences presented with bone metastases, brain metastases, and metastases in the kidney and liver. Palliative chemotherapy was given to 7 out of the 11 patients with recurrent disease, the other patients have a wait-and-see policy. Until March 2006, 8 patients are still disease-free with a mean disease-free interval and follow-up period of 34 months (range, 20-43 months).

Serious adverse event outside of trial

A 74-year-old man with bilateral pulmonary metastases of colorectal origin was treated off protocol with ILuP with 45 mg of melphalan, followed by resection of pulmonary metastases with staged, bilateral thoracotomies. In anticipation of the definitive evaluation of toxicity of previously ILuP-treated patients by a multidisciplinary case discussion, the decision was made to perform the first procedure with 45 mg melphalan under normothermic conditions (37°C). The left-sided lLuP with wedge resection of one metastasis of the left upper lobe was complicated only by a mild postoperative skin infection treated with antibiotics. After multicentered interim-analysis of toxicity, the decision was made to continue with the previously reported MTD [3], and 2 months later a right-sided thoracotomy under hyperthermic conditions (42°C) was performed. Due to an unstable perfusion circuit before the actual chemotherapeutic perfusion, the hyperthermic perfusion time was prolonged from 30 to 50 minutes. Melphalan perfusion was performed for 30 minutes, similar to previously performed procedures. No further perioperative complications were recorded, loss of perfusion circuit volume was a mere 650 mL. A chemical pneumonitis involving the entire right lung developed one day after the procedure. Production of pleural fluid through the thoracostomy was 2.5 L in the first 24 h and diminished afterwards. Bronchoscopy revealed edematous and easily hemorrhaging mucosa on the right side. Computed tomography of the thorax demonstrated subtotal consolidation of the right lung with a small pneumothorax, and infiltration of the basal parts of the left lung (Figure 5). Despite treatment with antibiotics, steroids, inotropics, and mechanical ventilation, the patient's condition deteriorated, culminating in a pneumonia with massive pulmonary hemorrhage 10 days after the procedure. The patient deceased despite the efforts of cardiopulmonary resuscitation. Post-mortem examination was performed and concluded both lungs to show signs of diffuse alveolair damage with extensive infiltration of granulocytes and hemorrhage on the right side (Figures 6 and 7).

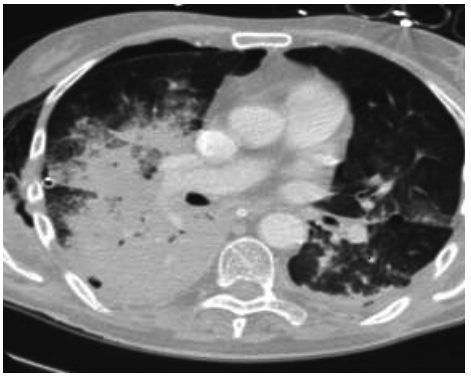

Figure 5. Consolidation and partial pneumothorax of the right lung

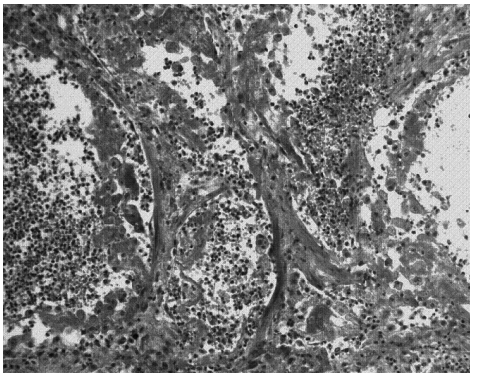

Figure 6. Right lung with extensive inflammatory infiltrate of granulocytes with focal hyaline membranes

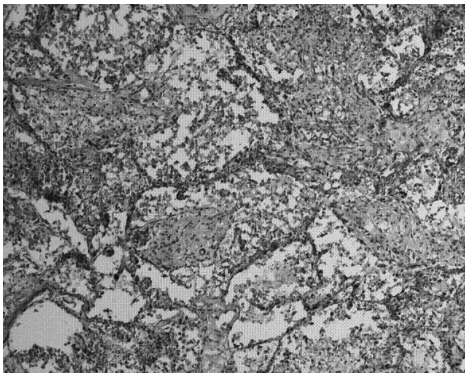

Figure 7. Left lung with organising pneumonia with fibroblasts proliferation in the alveoli with inflammation

Discussion

Pulmonary toxicity remains an important issue with ILuP in contrast to perioperative or systemic complications. Johnston and associates perfused 8 patients with either metastastic sarcoma to the lung or diffuse bronchoalveolair carcinoma with cisplatin or doxorubicin and reported one patient with pneumonia and another patient with respiratory insufficiency, who deceased [5]. Pass and colleagues performed ILuP with TNF-α, interferon-γ, and moderate hyperthermia for patients with unresectable pulmonary metastases of different histological types and documented one patient with TNF-α toxicity presenting with hypotension and pulmonary insufficiency with bilateral infiltrates [6]. Ratto and co-workers reported two postoperative complications with radiologic signs of interstitial en alveolar edema after ILuP with platinum in

6 patients with metastatic soft tissue sarcoma [7]. Burt and colleagues treated 8 patients with metastatic sarcoma with ILuP with doxorubicin and reported a chemical pneumonitis in one patient with complete destruction of the perfused lung at follow-up [8]. Putnam and co-workers reported marked toxicity in 2 out of 16 patients developing pulmonary toxicity grade 4 and reported an overall operative mortality of 3 out of 16 [9]. Schröder and colleagues treated 4 patients with lung sarcoma metastases with hyperthermic ILuP with cisplatin. All patients experienced transient pulmonary edema [10]. The data from these reports suggest, that lung intolerance and pulmonary toxicity seems to be the limiting factor in human ILuP studies in contrast to systemic complications.

Hendriks and colleagues reported only minor clinical toxicity besides the two patients with chemical pneumonitis in 21 procedures [3]. In contrast, in the extension trial, 3 of the 8 additional procedures in the group of patients treated with 15 mg of melphalan at 42°C were complicated by serious toxicity, namely pulmonary CTC grade 3 after 28 days (empyema in procedure no. 9), and 2 times non-pulmonary CTC grade 4 (rhabdomyolysis in procedure no. 11 and shock requiring inotropics in procedure no.12). One might argue that most complications could not directly be associated with local toxic effects from perfusion with melphalan. For instance, the procedure complicated by persistent pneumothorax and empyema was after resection of 5 metastatic lesions. The procedure complicated by hemodynamic instabillity and chest tube bleeding had an extensive cardial medical history with multiple infarctions and a PTCA procedure. (The cardiologist evaluated the patient before the operation.) The deceased patient treated outside of protocol was the first fatal incident in over 30 ILuP procedures performed in our institutions. Synergy between melphalan and hyperthermia in combination with a relative hypoxic perfusate might be the cause of serious pulmonary toxicity in this patient. The patient's prolonged perfusion time performed under hyperthermic condition possibly enhanced the pulmonary toxicity. We would like to add that in our institutions other procedures of ILuP with lower dosages of melphalan under hyperthermic conditions have been performed with similar perfusion periods without significant differences in toxicity. In terms of reproducibility, an initially unstable perfusion circuit prolonging the perfusion time (before the actual cytostatic infusion) is not an uncommon finding. Because of the spontaneous degration of melphalan, melphalan is ordered at the pharmacy just right after stabilisation of the perfusion circuits. The fact remains that ILuP with melphalan in the same patient was performed initially on the left side under normothermic conditions uneventfully, and later contralaterally under hyperthermic conditions with serious pulmonary complications and eventually death, suggesting a significant role of (prolonged) hyperthermia in pulmonary toxicity in ILuP.
Previously, we have reported hyperthermic ILuP with melphalan followed by surgical resection of pulmonary metastatic disease to be a feasible procedure at a MTD of melphalan of 45 mg

at 42°C without significant toxicity or (post)operative mortality. In contrast, this report clearly demonstrates considerable toxicity in the hyperthermic group of the extension trial and the first fatal toxicity in the patient treated off protocol with hyperthermia with a previous uneventful procedure under normothermic conditions. We therefore conclude that continuation with a phase II trial requires adjustment of the MTD of melphalan in ILuP from 45 mg at 42°C to 45 mg at 37°C.

This study furthermore describes a significant decline in assessment of lung function in time such as FEV1, VC, TLC, KCO and DLCO, as described in table 2. No statistical differences were observed in decline of lung function between the groups with or without serious toxicity, although there was serious lack of follow-up as time progressed. Data on pulmonary function assessments after ILuP with melphalan has never been published before. The influence of mechanical ventilation on decline of pulmonary funcion assessments in this study is unknown, but a decline in parameters has been described previously in non-ILuP studies [11]. Part of the changes in pulmonary function assessments could be the result of metastasectomy with manipulation of the lung. When reviewing previous publications on ILuP, few reports comment on pulmonary function assessments. Pass and colleagues [6] and Ratto and co-workers [7] assessed pulmonary function tests before and after the procedure and reported significant differences with a return to near-normal, findings similar to are own results. Burt and colleagues also evaluated pulmonary function tests and reported a statistically significant difference between the preoperative and 2 months' postoperative values for FEV1, and a trend towards significance in the same period for DLCO [8]. Schröder and colleagues did perform these tests and used them for a toxicity grading scale, but details on when these tests were performed and the exact results were not published [10].

As survival is not a secondary endpoint and responses in the lung cannot be assessed because of complete metastasectomy, results from this phase I and extension trial must be interpreted with caution, especially in small study groups. We found 19 of the 23 patients were still alive after a mean follow-up of 25 months, and mean disease-free interval for the total study group of 23 patients was 18 months. The survival after complete metastasectomy was 82% after 1 year (n=21), 78% after 2 years (n=18), and 71% after 3 years (n=14). In comparison, Pastorino and associates investigated survival in 4572 patients with complete pulmonary metastasectomy in a comparable group and found survival rates of approximately 60%, 40% and 25% after 1, 2 and 3 years, respectively [1].

In conclusion, we report unforeseen and considerable toxicity in the extension trial and the first fatal toxicity in a patient treated off protocol, procedures all performed under hyperthermic conditions. In our opinion, adjustment of the maximum tolerated dose of melphalan in ILuP from 45 mg at 42°C to 45 mg at 37°C seems to be justified. A multicentered, phase II trial under normothermic conditions will hopefully provide us with additional information on toxicity, but also long-term efficacy and possible survival benefit in carefully selected patients with resectable pulmonary metastases.

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PART 2
SELECTIVE PULMONARY ARTERY PERFUSION

Chapter 7

Selective pulmonary artery perfusion: a novel method for the treatment of pulmonary malignancies

Grootenboers MJ, Schramel FM, Hendriks JM, Van Boven WJ, Van Schil PE, Van Putte BP. Selective pulmonary artery perfusion: a novel method for the treatment of pulmonary malignancies. Acta Chirurgica Belgica 2007;107(4):361-7.

Abstract

Selective pulmonary artery perfusion (SPAP) is a modality of regional chemotherapy first investigated in the 1950's. A number of studies in animal models documented pharmacokinetic superiority with high-dose local cytostatic drug concentrations when compared to intravenous (IV) administration. Blood flow occlusion of the pulmonary artery before or after drug injection results in further increase in local drug concentrations. Animal tumor models with sarcoma and coloncarcinoma confirm anti-tumor efficacy in cytostatic SPAP. In human investigations, feasibility and safety of chemotherapeutic SPAP has been documented. Recent encouraging investigations of SPAP with gemcitabine and blood flow occlusion in a porcine model emphasize the need for further investigations in humans with pulmonary malignancies for safety and efficacy assessments.

Introduction

The treatment of pulmonary malignancies, such as non-small cell lung cancer (NSCLC) and pulmonary metastases from solid tumors, still remains a major clinical problem. Management of pulmonary metastases from solid tumors, even after surgical intervention with curative intent, results in 5-year survival rates between 25-50% [1]. For NSCLC, surgery provides the only chance for cure, but, unfortunately, only a minority of about 30% of these patients is eligible for resection at presentation. Chemo(radio)therapy of unresectable NSCLC is often dose-limited and provides modest benefit in terms of improvement in survival with a poor median survival of 9 to 11 months in advanced NSCLC (stage IIIB en IV) with good performance score [2]. The minimal impact of systemic chemotherapy on survival in patients with unresectable NSCLC or pulmonary metastases of non-primary lung cancer, resulted in the development of alternative chemotherapeutic modalities such as regional chemotherapy. Regional cytostatic lung perfusion is a treatment concept enabling high-dose local chemotherapy for the primary tumor mass and eradication of additional, local microscopic foci. Isolated lung perfusion (ILuP) and selective pulmonary artery perfusion (SPAP) are examples of regional chemotherapy to the lung in order to achieve more efficient drug delivery via the pulmonary artery, potentially overcoming tumor cell resistance by exposing the lung to high-dose local chemotherapy while minimizing systemic exposure and toxicity. First described by Creech and colleagues in the 1950's, ILuP is a surgical technique requiring thoracotomy for cannulation of both pulmonary artery and veins, enabling arterial perfusion of a chemotherapeutic agent with collection of the venous effluent [3]. The therapeutic concept of ILuP was developed by Weksler and colleagues [4] and proved to be pharmacokinetically superior to intravenous administration resulting in higher local-regional drug delivery and lower systemic drug concentration in various clinical studies [5-11]. Isolated lung perfusion is indicated primarily for patients with pulmonary metastases of non-primary lung cancer and has currently not shown any survival benefit in human clinical trials. Furthermore, in contrast to SPAP, ILuP is an invasive procedure which necessitates a thoracotomy limiting repetitive application.

The first studies of SPAP without venous control were described in the 1950's by Blades and Hall [12]. To emphasize the potential of SPAP as an effective palliative treatment, Morris and Goldberg reported a study of SPAP with nitrogen mustard during blood flow occlusion (BFO) in patients with unresectable bronchogenic carcinoma [13]. Subjective benefits were noted in 10 of 18 patients with rapid relief of chest pain or definite improvement observed in chest roentgenograms. In the last two decades, an increased interest in research of SPAP resulted in a growing number of publications in animal models with and without pulmonary tumors. Pharmacokinetic superiority of SPAP when compared with IV treatment has been confirmed in

several animal studies, and anti-tumor effects have been shown in a sarcoma tumor model [14, 15]. Only one human SPAP study has been published in the last three decades, demonstrating feasibility of BFO of a lobar pulmonary artery in humans without serious toxicity [16]. The results of these animal and human studies, in our opinion, justifies further exploration of SPAP as a promising technique of regional chemotherapy resulting in equivalent systemic cytostatic drug exposure comparable with the currently used IV administration, and high pulmonary concentrations comparable to the invasive, once-only performed ILuP procedure. Besides usage in a palliative setting, cytostatic SPAP could be an encouraging method potentially downstaging and improving prognosis in patients with stage 3A or 3B NSCLC.

Selective pulmonary artery perfusion.

SPAP is a mode of regional chemotherapy, allowing delivery of the biological agent through a catheter into the pulmonary artery with or without BFO (via an inflated balloon), without diverting or collecting the venous pulmonary effluent (Figure 1). This technique has been investigated in numerous ways, such as (sub)segmental or lobar perfusion, with or without thoracotomy or application of BFO during the perfusion. The published SPAP studies are limited in number and heterogeneous in method (Table 1). In this review, SPAP will be defined as both perfusion of the proximal pulmonary artery (and thus the entire lung), as well as segmental or lobar artery perfusion. The application of SPAP with or without thoracotomy will be reported as surgical SPAP or endovascular SPAP, respectively. Furthermore, a significant number of SPAP studies have been performed during BFO. Our group is the first to report on SPAP followed by BFO, as will be described later in this paper.

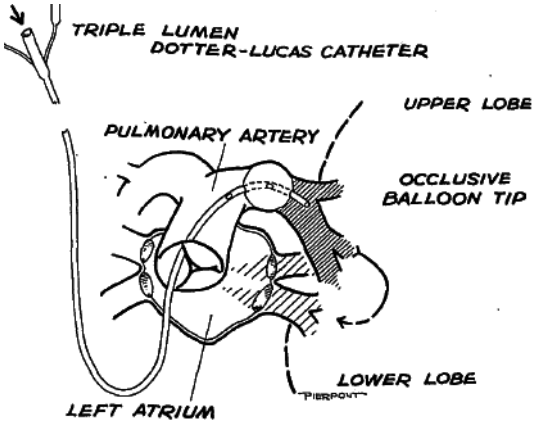

Figure 1. Schematic drawing of the balloon catheter infusion technique. The biological agent is injected through the distal lumen of the catheter. Notice the leak, via the left atrium, into the systemic circulation as there is no outflow occlusion. (From Smyth NPD, Pierpont HC, and Blades BB. Selective chemotherapy to the lung. Bull Soc Int Chir 1961;20:576-83; with permission)

Doxorubicin (Adriamycin)

Initially discovered 30 years ago, doxorubicin is an anthracycline antibiotic with activity against a number of malignancies, such as carcinomas of the breast, lung, and sarcomas of bone and soft tissue [26]. The combination of excellent clinical activity against (soft tissue) sarcoma's and cardiac toxicity limiting extensive systemic treatment justifies doxorubicin as an attractive drug to explore for locoregional application.

Year	Author	Ref	Drug	Thoracotomy	BFO	Subject and No (incl IV/ILuP)	Tumor Model
1958	Blades et al.	12	Nitrogen mustard	Yes	Yes	24 dogs	No
1960	Smyth et al.	17	Nitrogen mustard	Dogs: Yes Humans:No	Yes	14 dogs and 3 humans	Tumor-free dogs, humans with pulmonary metastases from sarcoma and primary lung cancer
1961	Morris	13	Nitrogen mustard	No	Yes	18 humans	Bronchogenic carcinoma
1981	Karakousis et al	16	Adriamycin	Dogs: Yes Humans: No	Yes	12 dogs and 7 humans	Tumor-free dogs, humans with pulmonary metastases from sarcoma
1993	Ratto et al.	19	Cisplatin	Yes	Yes	16 pigs	No
1995	Wang et al.	14	Doxorubicin	Yes	Yes	76 rats	Sarcoma
1996	Wang et al.	15	Doxorubicin	Yes	Yes	96 rats	Sarcoma
1998	Furrer et al.	20	Doxorubicin	Both	Yes	12 pigs	No
2003	Van Putte et al.	18	Gemcitabine	Yes	Yes	58 rats	No, additional study of a colorectal carcinoma cell line treated in vitro
2004	Brown et al.	21	Cisplatin	No	Yes	12 pigs	No
2006	Brown et al.	22	Cisplatin	No	Yes	13 pigs	No
2006	Krueger et al.	23	Doxorubicin	No	Yes	15 pigs	No
2007	Van Putte et al.	24	Gemcitabine	Yes	No	20 pigs	No
2007	Grootenboers et al. (submitted)	25	Gemcitabine	Yes	Yes	18 pigs	No

Table 1 human and animal SPAP studies

Karakousis and colleagues documented pharmacokinetic superiority of surgical SPAP during BFO with adriamycin in a canine model [16]. Three groups of dogs underwent a left-sided thoracotomy and dissection of the left lower lobe artery. The first group of dogs was treated intravenously, the second group received adriamycin into the lobar artery, and the third group was treated with adriamycin into the lobar artery during BFO by using a vascular clamp. The average level of adriamycin in the left lower lobe was significantly higher in the SPAP with BFO

group, when compared to the other groups. More importantly, feasibility and relative safety of endovascular SPAP with BFO in humans was documented in the same paper. The authors investigated seven patients with pulmonary metastases of soft tissue sarcoma presenting with pulmonary recurrences. All patients had been pretreated with systemic dose of adriamycin, methotrexate or cisplatin. In this human study of SPAP, the only one published in the last three decades, a total of 56 injections of adriamycin in small doses of 10 to 20 mg were administered into the individual lobar arteries after occlusion with an inflated balloon. Three small, spontaneously resolving infiltrates and one pneumothorax was documented, only one partial objective regression was noted. This paper is very interesting, as it indicates SPAP during BFO in humans to be feasible without serious side-effects. Nevertheless, as the authors themselves mentioned, no judgment can be made upon efficacy. The reproducibility of treating these patient with varying dosis of chemotherapy with different number of treatment sessions in various lobar arteries is questionable.

Wang and co-workers evaluated surgical SPAP during BFO with doxorubicin in a metastatic sarcoma model in rats and demonstrated pharmacokinetic and therapeutic superiority with minimal local and systemic toxicity, when compared to IV administration [14]. With regards to toxicity, animals in the IV group failed to gain weight after the procedure with one animal dying in contrast to SPAP-animals with normal growth patterns, all surviving the procedure. Histologic analysis of lung samples in the SPAP group and a control group (treated with saline through the pulmonary artery and IV) showed no significant differences with mild to moderate interstitial thickening and (sub)pleural inflammation. Regarding efficacy, a significant reduction or even no tumors were observed in the lungs treated in the SPAP-group, when compared to either the untreated right lung or the IV and control groups. In another publication by the same authors, surgical SPAP with doxorubicin during BFO was evaluated in a sarcoma model in the rat and proved, again, to be pharmacokinetically and therapeutically superior to IV treatment [15]. Significantly higher drug levels in tumor nodules and lung tissue were achieved in the SPAP group, with serum drug levels comparable to IV administration. All animals survived the initial procedure and resumed normal growth without differences in daily weights. Nevertheless, mortality rate after right pneumonectomy suggests that 0.5 mg/kg of doxorubicin via SPAP may be the maximum tolerated dose in the rat. Tumor weight was significantly decreased after SPAP with doxorubicin in contrast to the saline control and IV-groups, in which no effect on tumor growth could be observed.

Furrer and associates evaluated ILuP, surgical SPAP during BFO, and IV treatment with doxorubicin in a porcine model and demonstrated insignificantly higher lung tissue levels of doxorubicin in the surgical SPAP with BFO group compared to ILuP, with a six- to nine-fold higher level compared to IV [20]. ILuP serum concentrations were not detectable in contrast to the IV and the surgical SPAP groups. An additional endovascular BFO group of three pigs was included to assess feasibility and pulmonary toxicity after 1 month, comparing the perfused

with the non-perfused lung. The procedure of endovascular BFO perfusion was technically feasible with successful performance in all animals. The authors state that no signs of cytostatic-induced histologic changes were observed after one month. Unfortunately, one animal died because of catheter-induced thrombophlebitis with an occluded vena cava and bilateral lung abcesses. Furthermore, in the two remaining animals bilateral hemorrhagic edema and focal atelectasis was noted, raising questions upon the consequences of subacute toxicity of the endovascular SPAP procedure during BFO whether or not are directly attributable to the cytostatic agent. In another study, Krueger et al. compared ILuP, endovascular SPAP during BFO, and IV treatment in a porcine model [23]. These authors demonstrated a similar, but significantly higher, overall doxorubicin uptake in the perfused lung in ILuP and SPAP compared to IV. No significant difference in serum doxorubicin levels could be documented between SPAP and IV administration. Furthermore, a comparison of drug distribution was made by performing a visual assessment of ink distribution and perfusion scintigraphy. Variations of drug levels in cytostatic lung perfusion procedures indicated a trend to more heterogeneous regional blood flow and drug distribution in both ILuP and SPAP when compared to IV. Within the perfusion groups (including ILuP), there was a trend of more homogeneous flow and distribution in the group treated with SPAP with endovascular occlusion.

In conclusion, SPAP with BFO with doxorubicine is a feasible procedure in humans with minor toxicity, proven to be pharmacokinetic superior compared to SPAP alone and IV in an animal model [16]. When compared to ILuP, similar pharmokinetic properties of the different SPAP techniques have been reported in tumor-free porcine models [20,23]. Furthermore, anti-tumor efficacy has been documented in a rat sarcoma model [14,15].

Cisplatin/carboplatin

Cisplatin was introduced into clinical practice in the early 1970s and has a well-known activity against a number of solid tumors [27]. Carboplatin, an analog of cisplatin, has substituted cisplatin in certain chemotherapeutic regimens, such as suboptimally debulked ovarian cancer and is characterized by a better toxicity profile. The most troublesome common side-effects of cisplatin-based chemotherapy are renal impairment, neurotoxicity and ototoxicity [28].

Ratto and colleagues investigated the feasibility of local regional lung perfusion with cisplatin in a tumor-free porcine model by using different surgical techniques: stop-flow, stop-flow and out-flow occlusion, and ILuP [19]. Stop-flow occlusion equals the previously defined BFO and was achieved by placing a tourniquet proximal to the site of catheter insertion in the pulmonary artery. Out-flow occlusion was attained by application of two pulmonary vein tourniquets immediately after stop-flow and before infusion of cisplatin. Stop-flow occlusion resulted in higher systemic and lower pulmonary effluent and tissue levels when compared to the other treatment modalities. Stop-flow and out-flow occlusion, as well as ILuP attained

comparable regional and systemic platinum exposure. Platinum values were significantly lower in bone marrow and significantly higher in mediastinal nodes in the ILuP-groups. Platinum lung tissue exposure was dependent on the concentration of infused cisplatinum without significantly increasing systemic toxicity. According to the authors, significantly more homogeneous distribution of cisplatin was achieved in the ILuP groups compared to the SPAP groups, although these data were not reported in the actual article. Four hours after cisplatin infusion, histological changes of lung parenchyma were similar in all groups.

Brown and colleagues reported on endovascular SPAP during BFO with high-dose cisplatin in swine compared to IV cisplatinum treatment with sham pulmonary artery perfusion [21]. Immediately after completion of SPAP, pulmonary platinum adducts were more than 17 times elevated, while the renal cisplatin adducts actually were lower when compared to IV. All pulmonary platinum levels were at least six times higher in the SPAP group. The area under the adduct-time curve, as a measure of systemic exposure, was seven times greater when compared to IV. Serum cisplatin levels were significantly higher in the SPAP group, with an area under the curve of free serum platinum more than four times elevated compared to the IV group. Interestingly, one swine from each group experienced shortness of breath. At necropsy, one animal from the study group revealed a saddle embolus in the main pulmonary artery, while the other animal from the control group had an aspiration pneumonia. At pathologic examination of the lungs in the other swine, there were no signs of SPAP-related toxic injury.

In another report, Brown and co-workers evaluated variables that affect adduct formation in swine undergoing endovascular SPAP with high-dose cisplatin [22]. One group was treated with cisplatin during BFO and compared to IV administration, the other group was sequentially perfused in both lungs via SPAP during BFO with different concentrations of perfusate. In the first group, mean cisplatin concentrations were more than ten times higher after SPAP compared to IV. In the second group, no significant correlation between inflow concentrations and pulmonary adduct levels was observed, possibly due to the large range of measured pulmonary adduct levels. Pulmonary uptake in all 20 procedures was significantly correlated with an increased time of infusion. The authors therefore concluded that, in contrast to inflow concentrations, longer cisplatin infusion times are correlated with higher pulmonary adduct formation. The lack of correlation with inflow concentration potentially has consequences for future investigations, as it might enable reduction of perfusate volumes and thus limit fluid overload. These investigations in SPAP with cisplatin again confirm pharmacokinetic superiority in SPAP and ILuP, when compared to IV treatment in tumor-free porcine models.

Gemcitabine

Gemcitabine is a fluorine-substituted cytarabine analogue with significant clinical activity against a variety of malignancies, such as lung and breast cancer. Concentration- and time-dependency of tumor cell kill is documented by Van Putte and co-workers, rendering its

potential as locoregional chemotherapeutic agent [29]. Dose-limiting toxicity in systemic therapy is myelosuppression, although gemcitabine is well tolerated overall.

The first investigations of surgical SPAP with gemcitabine were reported by Van Putte and colleagues [18]. These authors studied survival of adenocarcinoma cells in vitro and surgical SPAP with BFO in a tumor-free rat model. In vivo, rats underwent SPAP with BFO with gemcitabine with different flow rates and exposure times, and results were compared to IV and ILuP. No significant differences in gemcitabine lung levels were observed at any exposure time point between the different flow levels. In SPAP with BFO, lung levels were comparable to the ILuP-group, while serum levels were lower than IV. Perfusion with gemcitabine demonstrated a significant first-pass effect of the lung, one of the main arguments for choosing this agent in SPAP. Van Putte and co-workers further explored gemcitabine pharmacokinetics in the setting of surgical SPAP with evaluation of infusion flow and time in a pig model [24]. Animals were treated with SPAP with normal pulmonary blood flow without BFO with gemcitabine (in a clinically applied dose of 1g/m^2 as used in therapy for NSCLC), with varying infusion time, while serum and lung concentrations were compared with IV treated pigs receiving the same dose and volume. In lung tissue, SPAP resulted in significantly higher AUC and peak gemcitabine concentrations compared to IV administration. Systemic exposure was comparable in all groups. Two more groups were treated with SPAP while pulmonary blood flow was reduced by insufflating the balloon catheter, resulting in inhomogeneous distribution with increased standard deviations of lung concentrations together with increased reduction of flow rates. Furthermore, flow reduction did not result in significantly different lung and serum AUC compared to SPAP without flow reduction.

Grootenboers and associates evaluated SPAP of gemcitabine with BFO and dose-escalation in a tumor-free porcine model [25]. To the best of our knowledge, these are the first investigations reported on SPAP followed by BFO, and not during BFO, to delay arterial wash-out. SPAP with 1 g/m^2 of gemcitabine was performed during 2 minutes followed by different times of BFO. These results were compared with an IV group receiving the same dose and volume. All SPAP-groups had significantly higher pulmonary AUC compared to IV administration. Extension of BFO times up to 30 minutes resulted in significantly higher pulmonary AUC with comparable serum AUC values. Dose escalation of gemcitabine in two additional groups resulted in a further significantly increased lung and serum AUC. At histologic examination, no SPAP related toxicity could be observed after 1 hour sacrifice. Equivalent serum AUC and 10-times higher lung tissue AUC values were achieved after SPAP of 1.25 g/m^2 of gemcitabine followed by 30 minutes of BFO compared to IV. In our opinion, this non-invasive technique might be a potentially promising, treatment modality for patients suffering from either pulmonary metastases or NSCLC to achieve more effective local tumor cell kill while maintaining systemic equivalent efficacy and safety when compared to currently used gemcitabine IV treatment.

Discussion

This review focuses on pulmonary intra-arterial administration of cytostatics in subjects suffering from pulmonary malignancies. Pulmonary metastases of solid tumors are primarily supplied by either the pulmonary arterial system [30], or both the pulmonary and bronchial circulations [31]. SPAP may serve as a means to reduce the tumor itself, treat pulmonary (micro)metastatic disease, or possibly reduce the extent of a subsequent parenchymal resection. In case of primary pulmonary malignancies, selective arterial perfusion has been studied by perfusion of the bronchial and not the pulmonary circulation [32, 33]. The rationale for pulmonary artery perfusion in NSCLC is supported by: (1) documented physiological anastomoses and shunts between the bronchial and pulmonary circulations [34] enlarging in case of pathologic conditions [35], (2) the results from the SPAP study by Morris [2], (3) and increased drug concentrations in mediastinal lymph nodes during ILuP using the pulmonary circulation [19]. We hypothesize that SPAP for the treatment of NSCLC could potentially convert unresectable disease to resectable disease by downstaging from stage 3 to 2.

Endovascular SPAP has a number of theoretical advantages to ILuP. First, this non-invasive, relatively simple procedure might be applied for several times without the use of a thoracotomy. Second, perfusion patterns are not disturbed by the operation itself and by BFO during SPAP, and third, high-dose local chemotherapy is combined with equivalent systemic drug exposure as in systemic administration. Besides the theoretical advantages, a number of unwanted events may occur in high-flow intra-arterial perfusion, such as dilution of the concentration of the anticancer agent and a too short time interval of passage through the capillary with inadequate diffusion. BFO performed during the actual selective perfusion ('inflow-occlusion') has the potential advantages of delaying the systemic washout and prohibiting the dilutional effect. Furthermore, there are indications that BFO may actually inhibit the growth of pulmonary metastases in a animal model [36]. Unfortunately, regional blood flow and drug distribution in SPAP during BFO seem to be as heterogeneous as in ILuP, despite the absence of thoracotomy and manipulation of the lung. Possible explanations for this phenomenon are redistribution caused by hypoxia after the occlusion site and vascular response to either the chemotherapeutic agent in the perfusate and/or the subsequent flow reduction after BFO, resulting in localised vasoconstriction.

In our currently described experimental studies of SPAP with gemcitabine, we are the first authors to report BFO after, and not during, SPAP to retard arterial washout and maintain pulmonary drug distribution as homogeneous as possible. Obviously, the exact timing of the BFO after SPAP by vascular clamping or insufflating of the balloon for maximum exposure, minimal dilution and adequate diffusion remains a major challenge. The number of publications on SPAP (without chemoembolization) is modest with only one recent article on

human investigations reported in PubMed with disappointing results. Main limitations of SPAP studies in general, and more specific in this article by Karakousis, are the limited number of subjects with non-standardized methods of (lobar) perfusion and the use of drug injection after BFO promoting heterogeneous distribution of the cytostatic agent. One should bear in mind though, that the aim of this study was not to demonstrate efficacy, but feasibility and toxicity. Furthermore, these patients had been extensively pretreated with systemic chemotherapy and had failed to these drugs. One may also argue about the different number of procedures per patient and the heterogeneity of type and extent of soft tissue sarcoma metastases with different chemosensitivity, making comparison and evaluation of efficacy a hazardous task. Nevertheless, 56 injections of a chemotherapeutic agent were injected through a pulmonary lobe artery during 5 minutes of BFO without serious toxicity, warranting further (phase I) investigations of cytostatic SPAP in humans.

In summary, endovascular SPAP with BFO is a simple, feasible procedure in humans [16] and animals without serious toxicity, proven pharmacokinetic superiority when compared to IV, and similar pharmacokinetic advantages when compared to ILuP [20] without the use of thoracotomy. Furthermore, anti-tumor effects are documented in surgical SPAP in metastatic sarcoma in animal models [14, 15]. In our opinion, these promising results warrant further investigations in SPAP focusing on toxicity and efficacy, preferably in humans.

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Chapter 8

Selective pulmonary artery perfusion with gemcitabine and flow reduction

Van Putte BP, Grootenboers MJ, Van Boven WJ, Schramel FM, Guetens G, de Boeck G, de Bruijn EA, Van Schil PE, Hendriks JM, Pasterkamp G, Folkert G. New method for delivering cytostatic drugs to the lung: selective pulmonary artery perfusion for the treatment of non-small cell lung cancer. Accepted for publication in for Drugs, Metabolism and Disposition.

Abstract

Introduction: Lung cancer represents a major health problem. Cytostatic and radiotherapeutic treatment are both limited due to dose-limiting systemic toxicity, and surgery due to its invasive nature. Therefore, we developed a catheterisation model of selective pulmonary artery perfusion (SPAP) combining the properties of isolated lung perfusion (ILuP) and intravenous (IV) treatment to achieve higher local drug levels and equivalent systemic exposure.

Material and Methods: Sixteen pigs underwent SPAP using a clinically applied dose of gemcitabine (1g/m^2). They furthermore underwent thoracotomy for tissue sampling. Three groups (n=4, each) were treated with SPAP for two minutes with normal pulmonary blood flow, 50% and 90% flow reduction. Another group (n=4) had SPAP for ten minutes with normal blood flow. All SPAP groups underwent catheterisation of the left pulmonary artery. An additional group (n=4) received IV treatment for thirty minutes using the same dose. Concentrations were analysed with ANOVA.

Results: Pulmonary peak concentrations (p=0.01) and areas under the curve (AUC) (p=0.001) of SPAP for two and ten minutes were significantly higher compared to IV, while SPAP for ten minutes resulted in the highest AUC (p=0.045) compared to SPAP for two minutes. Flow reduction during SPAP resulted in inhomogeneous distribution. Liver levels, AUC (serum), and wet-to-dry ratios of all SPAP groups were not significantly different compared to IV.

Conclusion: SPAP resulted in higher lung concentrations, while systemic exposure was comparable with IV. Therefore, we advocate SPAP as a new method to be tested clinically to try to achieve down-staging of the tumor and lymph node status in lung cancer.

Introduction

Cancer is the leading cause of death before the age of 85 years, resulting in more than half a million deaths per year in the United States [1]. In 2005, primary lung cancer was the second leading cancer type in the United States with approximately 190.000 new cases to be estimated for 2006. Among all cancer types, lung cancer has the highest death rate [1].

Non-small cell lung (NSCLC) cancer is usually treated by surgical resection, radiotherapy and/or cytostatic drug administration depending on the disease stage. Stage I (A and B) and II (A and B) NSCLC are currently treated by surgical resection, while (adjuvant) cytostatic therapy is applied to stage IB, II and III disease resulting in a 5-year survival of 75, 60, 40, 20 and 15%, respectively [2].

IV treatment is the desired route of cytostatic drug administration in order to achieve exposure of the primary lung tumor and distant disease as well, resulting in a 5-year survival benefit of 4-14% compared to surgery alone [3]. However, this method is dose-limited by the occurrence of systemic toxicity, like bone-marrow suppression, that limits exposure of the primary tumor and pulmonary (lymph node) metastases.

In contrast, ILuP with cytostatic drugs is an experimental surgical technique for the treatment of lung metastases, that aims to destroy pulmonary (lymph node) micrometastatic disease probably present at the moment of surgery. This technique is characterized by some properties that could improve current treatment of NSCLC. First, ILuP results in significantly higher drug concentrations in both lung and tumor tissue compared to IV administration as shown by many experimental and human data [4-8]. High-dose drug administration during ILuP using drugs like gemcitabine and cisplatin is well tolerated by healthy lung tissue [9-11]. Furthermore, a recent phase I trial evaluating toxicity of ILuP with melphalan showed a rapid pulmonary lymph drainage resulting in equivalent concentrations in either the lymph nodes and lung tissue, while this approach is applied only once or twice per patient due to its invasive nature [unpublished data]. However, ILuP is a local regional treatment modality developed for the treatment of lung metastases, but not for the treatment of NSCLC.

Twenty-six to thirty-two percent of all recurrences are local in patients with stage I and II NSCLC treated by lobectomy [12]. Recurrent disease probably originates from micrometastases present at the moment of surgical resection. In fact, local control is not achieved in these patients after initial surgical and cytostatic treatment. Therefore, this study aimed to develop a hybrid model of SPAP combining the properties of ILuP and IV treatment in order to improve outcome of NSCLC with minimal side-effects and to achieve down-staging of stage III A and B NSCLC towards a surgical stage.

First, higher drug exposure of the diseased lobe is necessary in order to achieve tumor (T status) size reduction. Second, the residual lung lobes probably contain micrometastases, that have to

be treated more aggressively in order to prevent pulmonary recurrences. Third, lymph node status (N status) reduction is essential to achieve down-staging of stage III a and b NSCLC. These lymph nodes are the first station for metastases after the lung lobes themselves and will be co-treated using high drug levels. Finally, SPAP aims to attack distant disease outside the lungs (M status) in an equivalent way as the currently applied IV administration does. More specifically, in this study, infusion variables of SPAP are optimalized and compared to the standard IV route of drug administration.

Material and Methods

Animals

Twenty female Dutch Landrace pigs (mean weight: 60 ± 3.7 kg) were used. Animals were fed with a normal diet and were treated in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" (NIH Publication 86-23, revised 1985). The experimental protocol was approved by the animal experimentation committee of the Utrecht University (04/220).

Anesthesia and Euthanasia

Anesthesia was induced with ketamine (10 mg/kg), midazolam (0.5 mg/kg) and atropine (0.04 mg/kg) intramuscularly. Each pig received thiopental natrium 4 mg/kg through an intravenous line. After intubation, the animals were connected to a volume-controlled ventilator (8 mL/kg, 12 breaths/minute guided by capnography) maintaining positive end-expiratory pressure of 5 cm of H20 and an inspiratory oxygen fraction of 0.5. Anesthesia was maintained by continuous infusion of midazolam (0.7 mg/kg x h). Analgesia was obtained with continuous infusion of sufentanil citrate (10 mg/kg x h) and muscle relaxation with pancuronium (0.1 mg/kg x h). Furthermore, a continuous infusion of saline (300 mL/h) was administered during the operation. After finishing the experiment, animals were sacrificed with pentobarbital natrium (200 mg/kg) intravenously. A central venous line was inserted for serum sampling during the experiment, and a catheter was introduced into the right femoral artery for arterial blood pressure monitoring.

Surgery

Initially, a balloon catheter (Balloon Wedge Pressure Catheter, 7 French, 110 cm, Arrow International, USA) was introduced through the left internal jugular vein. The catheter was positioned into the left pulmonary artery under blood pressure guidance. Subsequently, a left sided anterolateral thoracotomy was performed through the fifth intercostal space. The left pulmonary artery was dissected free and the position of the balloon catheter was checked manually. The tip of the catheter was positioned in the left main stem pulmonary artery just

proximal of the first side-branches. A 12 mm flow probe was placed around the left pulmonary artery for blood flow measurements. In addition, these measurements were necessary to check reduction of the pulmonary artery flow (0%, 50% and 90% blood flow reduction). This flow reduction was realized by insufflating the balloon of the balloon catheter.

After stabilisation of the blood flow, gemcitabine was infused through the lumen of the balloon catheter into the left pulmonary artery (SPAP), or through the central venous line (IV administration) using an infusion pump. Tissue samples of the lung were obtained from the left lower lobe and stored in liquid N_2 for later analysis. Furthermore, serum samples were collected from the central venous line and stored in tubes filled with 500 µl K2-EDTA to prevent clotting. At the end of the experiments, liver samples were obtained through an opening in the right hemidiaphragm.

Gemcitabine

Gemcitabine (difluorodeoxycytidine, Ely Lilly, Indianapolis, USA) solutions were prepared by reconstituting non-lyophilized powder in saline solution. All animals were treated with gemcitabine in a dose and volume (1000 mg/m^2 body surface area, solved in 50 mL saline) as clinically applied for the treatment of NSCLC.

Gemcitabine processing and measurement

A high-performance liquid chromatographic method has been used and validated for the determination of gemcitabine in serum, lung and liver tissue. Standard samples of blanc serum were spiked with gemcitabine (100 ng-100.000 ng) and extracted in the same way as the other samples and used for a calibration curve [13]. Within-run and between-run precisions were less than 10% and average accuracies were between 90 and 110%.

Gemcitabine assay by HPLC-UV

Separation was achieved on a Chrompack Spherisorb ODS-2 reversed phase column (25 xm x 4.6 mm, 5 µm). The mobile phase used was Pic B7 reagent (Waters Corporation) in 15% methanol (pH = 3.1) with a flow rate of 1.0 mL/min. Gemcitabine is detected by UV detection at 270 nm.

Statistics

All concentrations and wet-to-dry ratios shown is this paper are depicted as median ± standard error. Lung and serum concentrations are determined in function of time and calculated as areas under the curve (AUC). The AUC values and the median concentrations at each single time point were compared between the different groups using ANOVA analysis, followed by comparison between two individual groups using Student's t-test. Statistical significance was accepted at $p < 0.05$.

Experiment

Twenty pigs were randomized into five groups (n=4, each) (figure 1). All groups received gemcitabine in a dose of 1 g/m^2 solved in a volume of 50 mL saline. In order to determine the optimal infusion time for SPAP, two groups underwent SPAP with a normal pulmonary artery blood flow for ten minutes and for two minutes. A control group was treated intravenously according to a clinically applied regime for the treatment of NSCLC, and gemcitabine was infused over a period of 30 minutes. Two more groups underwent SPAP for two minutes with 50% and 90% flow reduction within the pulmonary artery, as checked with the flow probe around the pulmonary artery.

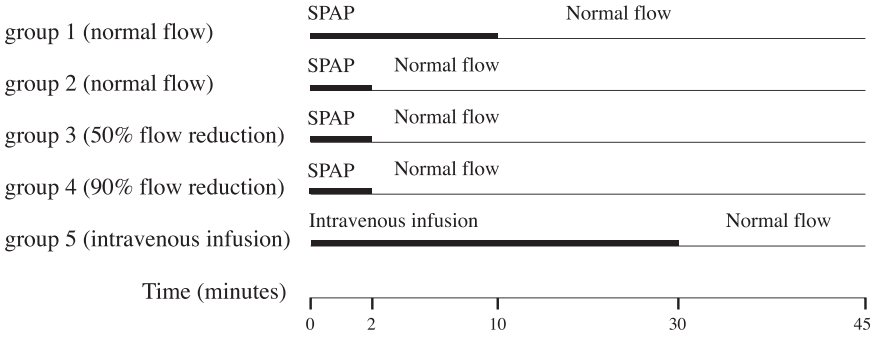

Figure 1. Experimental setting.

After SPAP for 2 minutes at normal or reduced flow rate, the balloon was desufflated, and a normal blood flow within the pulmonary artery was maintained throughout the further duration of the experiment. Lung and serum samples were obtained at 2, 10, 30, 45 minutes after start of infusion. Liver samples and lung tissue for concentration and wet-to-dry ratio measurements, respectively, were taken at 45 minutes.

Results

In lung tissue, SPAP for two and ten minutes resulted in significantly higher AUC when compared to IV treatment ($p = 0.001$), and SPAP for ten minutes resulted in the highest lung AUC compared to SPAP for two minutes ($p = 0.045$) (Figure 2a). In addition, the peak concentration of gemcitabine within the lung tissue was significantly higher after SPAP for two minutes when compared to intravenous administration ($p= 0.01$) (Figure 2b).

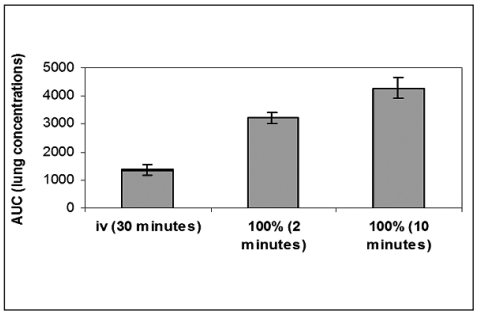

Figure 2a. Median AUC levels of gemcitabine lung concentrations (± standard error) of standard IV treatment during thirty minutes, SPAP two and SPAP ten minutes.

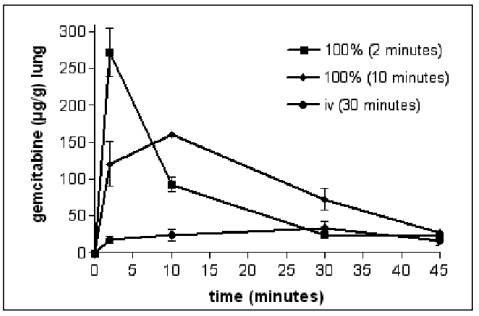

Figure 2b. Median gemcitabine lung concentrations (± standard error) of standard IV treatment during thirty minutes, SPAP two and SPAP ten minutes in function of time.

Within the serum, the AUC was not significantly different between SPAP for two and ten minutes and IV therapy (Figure 3a). The peak concentration of gemcitabine within the serum was significantly higher after SPAP for two minutes compared to IV treatment ($p = 0.004$) (Figure 3b).

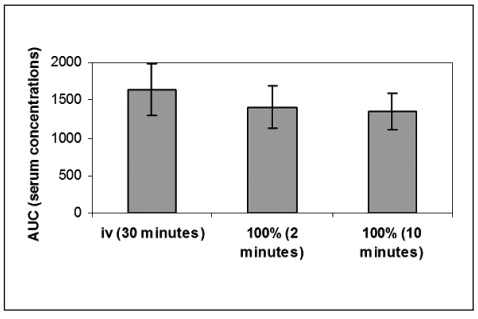

Figure 3a. Median AUC levels of gemcitabine serum concentrations (± standard error) of standard IV treatment during thirty minutes, SPAP two and SPAP ten minutes.

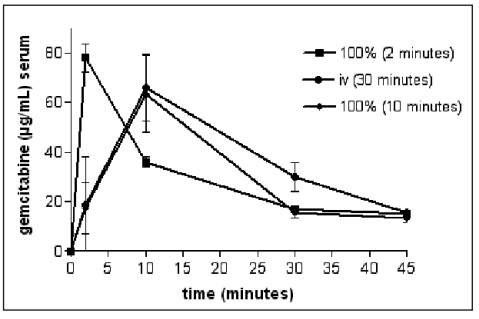

Figure 3b. Median gemcitabine serum concentrations (± standard error) of standard IV treatment during thirty minutes, SPAP two and SPAP ten minutes in function of time.

Fifty and ninety percent of flow reduction did not result in a significant different lung (Figures 4a-b) and serum (Figures 5a-b) AUC compared to SPAP without flow reduction. However, the standard deviation of lung concentrations increased significantly as a higher flow reduction was installed (47%, 62% and 79% for normal flow, 50 and 90% flow reduction, respectively) (Figures 4a-b).

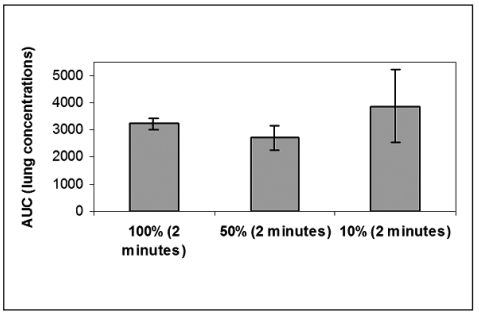

Figure 4a. Median AUC levels of gemcitabine lung concentrations (± standard error) of SPAP during two minutes without, with 50% and 90% pulmonary blood flow reduction.

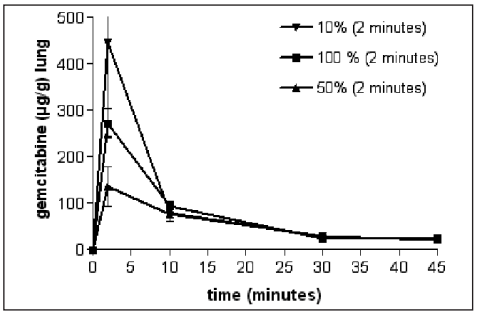

Figure 4b. Median gemcitabine lung concentrations (± standard error) of SPAP during two minutes without, with 50% and 90% pulmonary blood flow reduction.

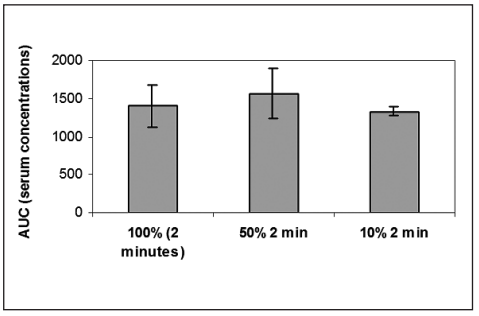

Figure 5a. Median AUC levels of gemcitabine serum concentrations (± standard error) of SPAP during two minutes without, with 50% and 90% pulmonary blood flow reduction.

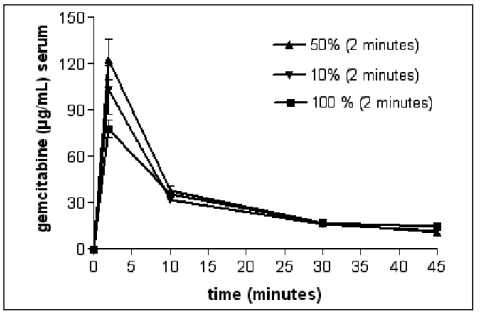

Figure 5b. Median gemcitabine serum concentrations (± standard error) of SPAP during two minutes without, with 50% and 90% pulmonary blood flow reduction.

Liver concentrations of gemcitabine (11.4 ± 1.4 µg/g) and wet-to-dry ratios (8.3 ± 0.5) did not significantly differ between the five groups when determined at 45 minutes after the start of infusion. Histologic examination of lung tissue after pulmonary artery perfusion with gemcitabine suggest evidence of alveolar hyperplasia, which was more pronounced in the flow reduction group with evident moderate congestion (Figure 6).

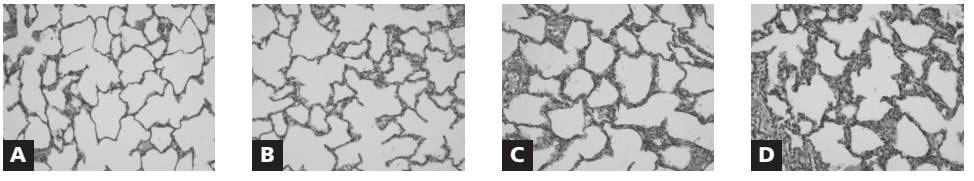

Figure 6. Histologic findings of the perfused groups: SPAP IV (A), SPAP 10 min (B), and SPAP 2 min (C), and SPAP 50% flow reduction (D).

Discussion

To the best of our knowledge, this is the first report of a model of SPAP without complete BFO using an endovascular catheterisation technique, that combined the properties of ILuP and IV administration in order to improve, theoretically, the current treatment of NSCLC with minimal side-effects and to achieve down-staging of stage III A and A NSCLC. This approach resulted in significantly higher lung levels and equivalent serum and liver levels compared to the generally accepted IV route of cytostatic drug administration.

This study evaluated infusion time of SPAP and pulmonary artery flow reduction during SPAP and compared the results to IV injection of gemcitabine. Clinically, gemcitabine is administered in a dose of 1 g/m^2 solved in 50 mL isotonic solution through an extremity infusion system during thirty minutes for the treatment of NSCLC. The clinically applied infusion rate is limited due to the occurrence of local toxicity at higher rates. In this study, gemcitabine dose and solvent volume were equal in all groups.

All SPAP groups showed significantly higher lung AUC and peak levels compared to IV treatment. First, this observation is partially explained by a dilutional effect. In contrast to IV infusion, SPAP is characterized by infusion of the left or right pulmonary artery resulting in a two times higher blood concentration entering the treated lung. Furthermore, the blood concentration delivered at the tip of the SPAP catheter was increased by augmentation of the infusion rates resulting in six (SPAP, ten minutes) and thirty times (SPAP, two minutes) higher local blood concentrations compared to IV treatment. Therefore, SPAP resulted in significantly higher peak pulmonary concentrations and areas under the AUC's. Second, the high pulmonary peak and AUC levels during and after SPAP are explained by the important first-pass capacity of the lung, resulting in systemic serum AUC levels that are in the same range compared to IV treatment.

However, pulmonary peak concentrations after SPAP for two minutes without blood flow occlusion were only five times higher compared to IV treatment, while local blood concentrations delivered at the tip of the catheter during SPAP were thirty times higher. Saturation of the first-pass capacity of the lung at two minutes infusion time or a too short uptake interval are the most reasonable explanations.

Interestingly, SPAP for ten minutes resulted in either six times higher lung ánd local blood levels delivered at the catheter tip, suggesting that lack of first-pass saturation is present. Furthermore, pulmonary AUC levels were significantly higher compared to SPAP for two minutes. In our opinion, SPAP for ten minutes seems to be the most efficient strategy in saturating the lung, because the time interval of the peak concentration after SPAP for two minutes is too short to achieve intracellular saturation.

Important first-pass capacity of the lung was shown in a former rat study in our laboratory [14].

Significantly higher gemcitabine lung levels were achieved after pulmonary artery perfusion without control of the pulmonary veins, while significantly less systemic toxicity was observed compared to IV injection of even a higher dose during the same infusion interval [14].
Reduction of blood flow during SPAP up to 50 and 90% was applied and compared to normal blood flow in order to investigate the relation between blood flow and cytostatic drug uptake into the lung. In contrast to what we expected, SPAP with 50% flow reduction resulted in even lower pulmonary drug levels compared to normal blood flow, while the blood concentration delivered at the catheter tip was even twice as high. Furthermore, 90% flow reduction resulted in higher levels compared with normal blood flow. Obviously, flow reduction during SPAP resulted in increasing standard deviations (47%, 62% and 79% for normal flow, 50 and 90% flow reduction, respectively). We hypothesize, that this phenomenon may be explained by compensatory pulmonary vasoconstriction during flow reduction in order to maintain pulmonary artery pressure resulting in inhomogeneous distribution of the infused drug.

Three former studies evaluated feasibility of endovascular pulmonary artery perfusion for the treatment of pulmonary metastases using adriamycin and cisplatin. These studies aimed to achieve higher local pulmonary drug levels and less systemic toxicity in order to treat pulmonary metastatic disease more aggressively. In contrast, we believe that this endovascular method is an ideal strategy to treat local NSCLC and lymph node metastases more intensively, while treating the systemic disease in an equivalent manner as IV therapy does. In addition, these studies are of limited significance probably due to inhomogeneous drug distribution.
First, Karakousis et al. performed Schwann-Ganz catheterisation of lobe branches separately under fluoroscopic control in seven patients with recurrent pulmonary metastases who had already received the maximum dose of adriamycin [15]. They received a dose of 10-20 mg diluted in 50 mL infused over a 1- to 2-minute period during five minutes of BFO. A total of 56 injections were given via lobar arteries in the seven patients treated. After metastasectomy, three patients were disease-free after four months, while the other patients had tumor progression. The disappointing results in this study can be explained by some major limitations. First, the dose and infusion rate are not standardised between the patients resulting in high variability of the intravascular drug concentration delivered. Furthermore, they applied BFO during infusion that should have resulted in inhomogeneous distribution as shown in our study. During each injection, only one of different lobar branches was selected resulting in varying pulmonary distribution volumes and therefore varying local tissue levels.
Second, Furrer et al. compared endovascular pulmonary artery perfusion during BFO with ILuP and IV infusion of doxorubicin [16]. Both selective techniques resulted in significantly higher lung concentrations and lower serum levels compared to IV administration. However, pulmonary artery perfusion was performed during BFO that should have resulted in

inhomogeneous distribution. Furthermore, they achieved lower serum levels compared to IV injection, while we believe that maximal systemic exposure during SPAP is essential to treat (micro)metastatic disease outside the lungs in lung cancer.
Third, Brown et al. recently published their results on SPAP during BFO compared to IV infusion of cisplatin using a swine model [17]. They concluded, that no relation was observed between the infusion time and inflow concentration compared to the final lung tissue concentrations. They reported a very wide range (6.63-76.78 fmol/µg) of final lung concentrations probably due to inhomogeneous drug distribution, as shown in our study in a pulmonary blood flow reduction experiment (figure 3a) [17].
Our study furthermore shows a rapid washout phenomenon after peak pulmonary concentrations were achieved at the end of the SPAP procedure (figure 2a and 4a). In a very recent rat study, we reported that a major part of the drug taken up during ILuP is quickly exchanged into the circulation during the washout interval and during reperfusion [18]. Main part of the drug is returned from the interstitium into the vascular compartment based on simple diffusion, suggesting that part of the drug did not enter the cells due to a too short uptake interval. In the same article, we achieved stabilization of high lung peak levels after ILuP by delayed restoration of normal blood circulation up to thirty minutes [18]. Further studies are necessary to confirm these findings in this endovascular SPAP model.

Patients suffering from NSCLC die due to local pulmonary or distant recurrences in 25 % and 75%, respectively [12]. In fact, in 25% of these patients local control is not achieved after initial surgical and cytostatic treatment. In this study, we created a model of endovascular SPAP as a hybrid modality for the treatment of patients suffering from NSCLC to treat the primary tumor (T status) and pulmonary lymph nodes (N status) more aggressively, in order to improve the current treatment of NSCLC with minimal side-effects and to achieve down-staging of stage III A and B NSCLC. This technique is characterized by the superior pharmacokinetic properties of ILuP resulting in high local lung and lymph node drug levels, and by the properties of IV infusion in order to achieve total body exposition. As shown in figures 2 and 3, significantly higher lung levels were achieved after SPAP, while serum and liver levels did not significantly differ compared to IV treatment.
Interestingly, a significant portion of both lymph node and pulmonary tumor vasculature is fed by the pulmonary arterial circulation [19,20]. Therefore, patients suffering from stage I, II and III NSCLC will be the main target population for treatment with endovascular SPAP. First, SPAP aims to result in primary tumor reduction (T status) before surgical treatment. Second, SPAP results in higher drug exposure of the residual lobes and of local lymphogenic metastatic disease (N status) in order to achieve down-staging of stage III NSCLC towards a surgical stage.

Third, systemic serum and liver levels equivalent to IV therapy will treat systemic disease in the same way as achieved in currently applied IV schedules for NSCLC.

In conclusion, we created an endovascular SPAP model that results in higher local pulmonary drug levels with equivalent serum and liver concentrations compared to IV treatment. This new approach could potentially improve prognosis of NSCLC by reducing the primary tumor size and by down-staging of the lymph node status.

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Chapter 9

Selective pulmonary artery perfusion with gemcitabine and blood flow occlusion and dose escalation

Grootenboers MJ, Schramel FM, Van Boven WJ, Pasterkamp G, Folkerts G, Hendriks JM, Van Schil PE, Van Putte BP. Selective pulmonary artery perfusion with gemcitabine and blood flow occlusion and dose escalation. Submitted.

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Abstract

Introduction: Selective pulmonary artery perfusion (SPAP) is an experimental endovascular technique for the treatment of pulmonary malignancies. This study evaluated blood flow occlusion (BFO) after SPAP and dose-escalation in order to delay washout of gemcitabine from the lung tissue, to augment pulmonary drug exposure and to maintain serum concentrations equivalent to intravenous (IV) administration.

Material and Methods: Twenty-two pigs underwent left-sided SPAP using gemcitabine in a clinically applied dose of 1-1.5 g/m^2 after balloon catheterisation.

BFO experiment: Four groups (n=4, each) were treated with SPAP with 1 g/m^2 of gemcitabine during two minutes followed by BFO for 0, 10, 20 and 30 minutes, respectively.

Dose-escalation experiment: Two more groups (n=3, each) received SPAP with 1.25 g/m^2 and 1.5 g/m^2 of gemcitabine during two minutes followed by 30 minutes BFO.

All pigs underwent left thoracotomy with sampling of lung, liver and blood. The animals were sacrificed after one hour. The lung and serum areas under the curve (AUC) were calculated for each group and ANOVA and, t-test was used for comparison.

Results: Thirty minutes BFO resulted in the highest lung AUC compared to 0, 10 and 20 minutes BFO ($p<0.001$), while no significant differences in serum AUC and liver levels were observed. Gemcitabine dose-escalation up to 1.25 g/m^2 resulted in significantly higher lung AUC ($p=0.02$) compared to 1 g/m^2, while serum AUC was equivalent with IV treatment. Further dose-escalation to 1.5 g/m^2 did not result in significantly higher lung levels compared to 1.25 g/m^2.

Conclusion: BFO after SPAP delays the washout of gemcitabine from lung tissue. Dose-escalation resulted in higher lung concentrations, while serum levels were equivalent with IV administration. We advocate two minutes of SPAP with 1.25 g/m^2 of gemcitabine followed by 30 minutes of BFO to be investigated as a new treatment modality for pulmonary malignancies.

Introduction

Lung cancer remains the leading cause of cancer deaths in the United States [1]. Incidence and mortality rates of lung cancer increased markedly through most of the last century with an estimated number of 174.470 new cases in 2007 and 162.460 deaths in 2006. Non-small cell lung cancer (NSCLC) accounts for almost 80% of all deaths from lung carcinoma. Surgical resection remains the cornerstone of treatment for early-stage NSCLC, but, unfortunately, only 30% of patients with NSCLC presents with resectable disease. Chemotherapy plays a prominent role in the treatment of advanced or metastastic NSCLC, but provides modest improvement in survival. Systemic chemotherapy in dosages high enough to improve prognosis is often limited by host toxicity. Regional chemotherapy with high local regional cytostatic drug concentrations has, when used in a neoadjuvant setting, the theoretical advantage of an increase in resectability of malignant laesions technically unresectable at the time of diagnosis, with mediastinal downstaging, and/or reduction of tumor mass. As a locoregional treatment, however, it is unsuitable for metastasized malignant disease.

Isolated lung perfusion (ILuP) with cannulation of the pulmonary artery and veins is a feasible surgical technique for the treatment of pulmonary metastases, achieving high-dose local chemotherapy to the lung, while minimizing systemic exposure [2]. Most recently, we demonstrated pharmacokinetic advantage of ILuP with melphalan with a significant correlation between cytostatic dosage, area under the curve (AUC) of perfusate, and lung tissue concentrations [3]. Furthermore, cytostatic concentrations were considerably elevated in resected pulmonary metastases but also in mediastinal lymph node tissues [unpublished data]. The invasive character of ILuP, however, limits repetitive chemotherapeutic intervention. Less invasive procedures, such as pulmonary artery perfusion through a catheter without control of the venous circulation, are more suitable for repeated cytostatic lung perfusion.

Selective pulmonary artery perfusion (SPAP) is an attractive non-surgical modality of regional chemotherapy with the advantages of delivering high-dose chemotherapy to the lung, while maintaining adequate systemic exposure of the cytostatic agent for extrapulmonary (micrometastatic) disease. Most recently, we developed an endovascular catheterisation technique of SPAP with a clinically applied dose of gemcitabine using a porcine model [4]. Pulmonary peak concentrations and AUC of gemcitabine in lung tissue were significantly higher in SPAP compared to intravenous (IV) infusion. However, a rapid washout phenomenon after peak pulmonary concentrations was observed at the end of the SPAP procedure. Pulmonary artery blood flow occlusion (BFO) may overcome this limitation by preventing excessive dilution and extending diffusion time. Thus, this experiment of SPAP was designed to evaluate pharmacokinetics of BFO and dose escalation with gemcitabine in order to delay washout of gemcitabine from the lung tissue after SPAP, to augment pulmonary drug exposure, but still maintain serum concentrations equivalent to IV administration.

Material and Methods

Study Design

Twenty-two pigs were randomized into six groups and treated with SPAP of the left lung (Figure 1). All animals received gemcitabine solved in a volume of 50 mL saline. After SPAP for two minutes with fysiologic pulmonary artery flow without occlusion, the balloon was insufflated and desufflated at time points according to the study design. Normal blood flow within the pulmonary artery was maintained until sacrifice. Lung and serum samples were obtained at 2, 10, 30 and 45 minutes after start of infusion. Additional lung samples were taken at 60 minutes. Wet-to-dry ratios were obtained at 45 minutes.

Experiment 1: Blood flow occlusion

Four groups (n=4, each) were treated with SPAP with gemcitabine in a dose of 1 g/m^2 for two minutes with BFO for 0 (group 1), 10 (group 2), 20 (group 3) and 30 minutes (group 4), to determine the optimal BFO time after SPAP.

Experiment 2: Gemcitabine dose-escalation

Two groups (n=3, each) were treated with SPAP for two minutes with 1.25 g/m^2 (group 5) and 1.5 g/m^2 (group 6) of gemcitabine followed by 30 minutes of BFO, to determine the optimal gemcitabine dosage with 30 minutes of BFO.

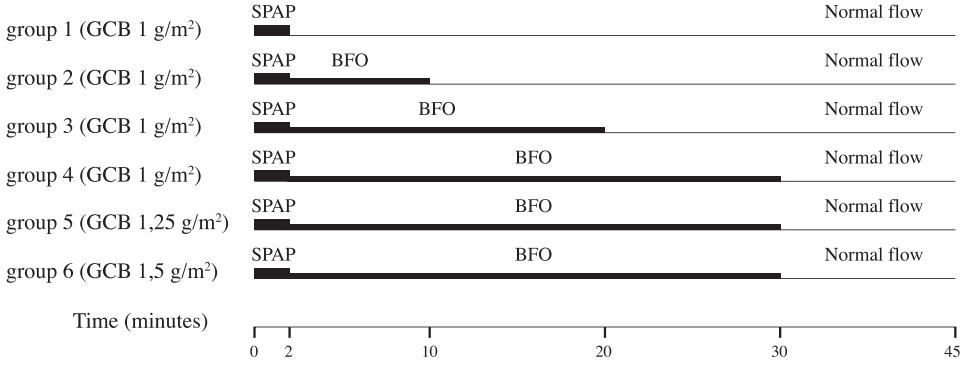

Figure 1. Study design

Animals and Housing

Twenty-two female Dutch Landrace pigs with a mean weight of 60 kg were used. Animals were treated in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" (NIH Publication 86-23, revised 1985) and fed with a normal diet. The animal experimentation committee of the University of Utrecht (04/220) approved the experimental protocol.

Anesthesia and euthanasia

Animals were anesthesized with intramuscular treatment of ketamine (10 mg/kg), midazolam (0.5 mg/kg) and atropine (0.04 mg/kg) followed by thiopentalnatrium 4 mg/kg intravenously. After intubation, the animals were ventilated with a volume-controlled ventilator (8 mL/kg, 12 breaths/minute guided by capnography) maintaining positive end-expiratory pressure of 5 cm of H_20 and an inspiratory oxygen fraction of 0.5. Anesthesia was maintained by continuous infusion of midazolam (0.7 mg/kg x h). Analgesia was obtained with continuous infusion of sufentanil citrate (10 mg/kg x h) and muscle relaxation with pancuronium (0.1 mg/kg x h). During the operation, a continuous infusion of saline (300 mL/h) was administered. Serum samples were obtained through a central venous line inserted during the experiment, and a catheter was introduced into the right femoral artery for arterial blood pressure monitoring. The animals were sacrificed with pentobarbitalnatrium (200 mg/kg) intravenously after finishing the experiment at 60 minutes.

Pulmonary artery perfusion procedure

A balloon catheter (Balloon Wedge Pressure Catheter, 7 French, 110 cm, Arrow International, USA) was introduced through the left internal jugular vein and positioned into the left pulmonary artery under blood pressure guidance. A left-sided anterolateral thoracotomy was performed through the fifth intercostal space. The left pulmonary artery was dissected free, enabling a manual check of the position of the balloon catheter. The tip of the catheter was placed at the level of the proximal left main stem pulmonary artery. BFO was obtained by insufflating the balloon of the catheter and confirmed by a 12 mm flow probe placed around the left pulmonary artery. Gemcitabine was infused through the lumen of the balloon catheter into the left pulmonary artery. Tissue samples of the lung were obtained from the left lower lobe and stored in liquid N_2 for later analysis. Histological samples were stored in buffered formaldehyde for further staining. Serum samples were collected from the central venous line and stored in tubes filled with 500 µl K2-EDTA to prevent clotting. At the end of the experiments just before sacrifice, liver samples were obtained through an incision of the right hemidiaphragm after entering the right pleural space.

Gemcitabine processing and measurement

Gemcitabine (difluorodeoxycytidine, Ely Lilly, Indianapolis, USA) perfusate solutions were prepared by reconstituting non-lyophilized powder in saline solution. All animals were treated with gemcitabine in a dose and volume (1 g/m^2 body surface area solved in 50 mL saline) as clinically applied for the treatment of NSCLC in humans. A high-performance liquid chromatographic method measured gemcitabine in serum, lung and liver tissue. Standard samples of blanc serum were spiked with gemcitabine (100 ng-100.000 ng), extracted in the

same way as the other samples and used for a calibration curve [11]. Separation was achieved on a Chrompack Spherisorb ODS-2 reversed phase column (25 xm x 4.6 mm, 5 µm). The mobile phase used was Pic B7 reagent (Waters Corporation) in 15% methanol (pH = 3.1) with a flow rate of 1.0 mL/min. Gemcitabine is detected by UV detection at 270 nm.

Statistics

All data are presented as median ± standard error, unless stated otherwise. Lung and serum concentrations are determined in function of time and calculated as AUC. The AUC values and the median concentrations at each single time point were compared between the different groups using ANOVA analysis and Student's t-test. Significance was defined as a p-value < 0.05.

Results

Table 1 shows the mean AUC and standard error in lung tissue and serum for all SPAP- groups.

Group	Number	AUC lung tissue	AUC plasma
BFO 0	4	3218 ± 196	1588 ± 278
BFO 10	4	4074 ± 158 *	1118 ± 58
BFO 20	4	5207 ± 95 *	1032 ± 19
BFO 30	4	5782 ± 254 *	1005 ± 16
125% GCB	3	18893 ± 4613 **	1842 ± 211 **
150% GCB	3	22627 ± 7690 **	2150 ± 431 **

All values in mean ± standard error. *P-value < 0.05 compared to BFO 0.

** P-value < 0.05 compared to BFO 30.

Table 1: Area under the curve in lung tissue and serum

Experiment 1: Blood flow occlusion

Significantly higher AUC of lung tissue could be documented by extending BFO time, when comparing 0 minutes with 10, 20, and 30 minutes BFO (p=0.015, p=0.001 and p=0.001, respectively) (Table 1 and Figure 2). In serum, all BFO groups with gemcitabine in a dose of 1 g/m^2 had equivalent low AUC values (Table 1 and Figure 3).

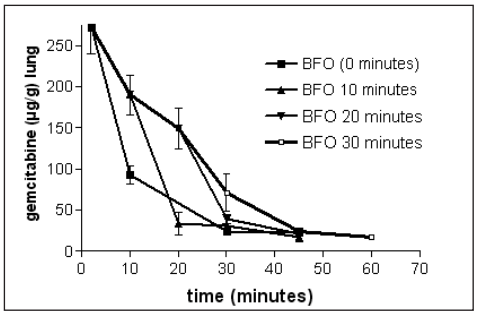

Figure 2. Median gemcitabine lung concentrations (± standard error) of SPAP for two minutes followed by BFO for 0, 10, 20 and 30 minutes.

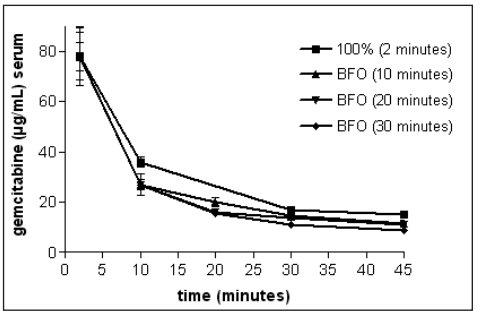

Figure 3. Median gemcitabine serum concentrations (± standard error) of SPAP for two minutes followed by BFO for 0, 10, 20 and 30 minutes.

Experiment 2: Gemcitabine dose-escalation

When the dosage of gemcitabine was increased from 1 g/m^2 to 1.25 g/m^2 and 1.5 g/m^2 of gemcitabine, a significantly higher AUC of the lung could be documented (p=0.020 and p=0.047, respectively) (Table 1 and Figure 4). In serum, all BFO groups with escalating dosages had equivalent AUC values. When compared to 1 g/m^2, increased dosages of 1.25 g/m^2 and 1.5 g/m^2 of gemcitabine with 30 minutes BFO resulted in significantly higher AUC of serum gemcitabine (p=0.005 and p=0.025 respectively) (Table 1 and Figure 5).

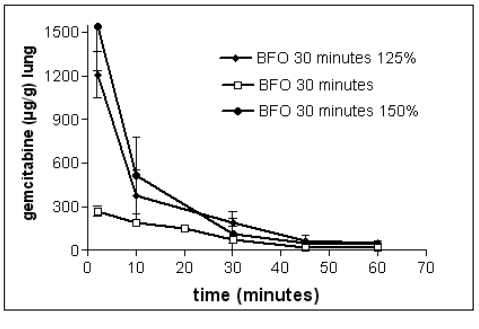

Figure 4. Median gemcitabine lung concentrations (± standard error) of SPAP for two minutes with 1 g/m^2 (100%), 1.25 g/m^2 (125%) and 1.5 g/m^2 (150%) of gemcitabine followed by 30 minutes BFO.

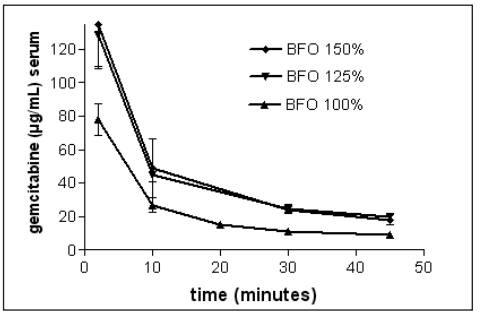

Figure 5. Median gemcitabine serum concentrations (± standard error) of SPAP for two minutes with 1 g/m^2 (100%), 1.25 g/m^2 (125%) and 1.5 g/m^2 (150%) of gemcitabine followed by BFO for 30 minutes.

No significant differences in wet-to-dry ratio were observed. Liver concentrations of gemcitabine and wet-to-dry ratios did not significantly differ between the six groups, when determined at 60 minutes after the start of infusion.

Discussion

We report an unique model of SPAP with high-dose local, pulmonary drug concentrations by use of BFO after arterial perfusion, but also adequate systemic concentrations by dose-escalation of the perfused cytostatic agent. In this study, BFO after (and not during) SPAP alllows for more homogeneous distribution of the perfused drug [4], but also increased pulmonary uptake by delaying pulmonary washout and extending diffusion time. Dose escalation of the perfused drug results in systemic circulation drug concentrations comparable to 'common' IV treatment, as it compensates for first pass effect causing increased pulmonary drug extraction and, consequently, reduced serum drug concentrations.

SPAP is an experimental treatment modality of regional chemotherapy delivering higher locoregional cytostatic drug concentrations with minimal systemic drug concentrations and thus minimal side-effects [5-8]. As a relatively safe and simple non-surgical procedure, SPAP has the advantage over ILuP of allowing repeated regional chemotherapy with minimal volume administration. Van Putte et al. studied pharmacokinetics of SPAP during (and not before) BFO with gemcitabine with different exposure times and flow rates in rats and compared these data with ILuP and IV administration [5]. Lung levels after SPAP during BFO were comparable to ILuP and higher than the IV group. Also, systemic exposure after IV injection was higher compared to BFO and ILuP. These results show an important first-pass capacity of the lung. In a more recent study, we report on pharmacokinetics of infusion flow and time in SPAP with gemcitabine and compared the results to IV injection, using a clinically applied dose of gemcitabine in the same pig model as used in this experiment [4]. SPAP resulted in significantly higher peak pulmonary concentrations and AUC's. Systemic serum AUC levels were in the same range when compared to IV infusion. Finally, flow reduction during SPAP resulted in inhomogeneous pulmonary drug distribution. In order to prevent inhomogeneous distribution, we perfused the lung with intact pulmonary circulation followed by BFO, and not during BFO as in all published SPAP studies in the last 25 years [6-11].

Our current experimental study was designed to study pharmacokinetics of SPAP with gemcitabine with variable BFO time and dose escalation. These results were compared with the data of previous investigations in a porcine model under exact same conditions, including a group with 30 minutes IV treatment of 1 g/m^2 gemcitabine [4]. In our BFO experiment (experiment 1), we report higher local regional cytostatic drug delivery by extending blood flow occlusion from 0 to 30 minutes with a 75% increase of the mean AUC values. Furthermore, we observed that all SPAP groups treated with 1 g/m^2 with or without BFO had significantly

higher AUC of lung tissue, when compared to IV infusion (p=0.000-0.042). Basically, we conclude that lung tissue AUC in IV treatment is doubled by SPAP alone and quadroubled by SPAP followed by 30 minutes of BFO. Serum AUC were not significantly different in all BFO groups with gemcitabine in a dose of 1 g/m^2, when compared to the IV group. In order to find the optimal SPAP circumstances resulting in higher local drug exposition but also equivalent systemic drug exposure comparable to IV treatment, gemcitabine dose escalation with BFO was performed in our 2nd experiment.

From our dose-escalation experiment (experiment 2), we observed that dose-escalation with 30 minutes BFO results in significantly higher AUC values in lung tissue with values up to 4 times higher when comparing 1.5 g/m^2 to 1.0 g/m^2 of administered gemcitabine. Furthermore, all SPAP groups with escalating dosages of gemcitabine and 30 minutes BFO had significantly higher AUC in lung tissue in comparison to IV infusion (p=0.000-0.021) with values up to 15 times higher. With regards to serum AUC, dose-escalation of gemcitabine via SPAP followed by 30 minutes BFO results in significantly higher values when compared to 1 g/m^2 gemcitabine. Most importantly, dose escalation of gemcitabine results in equivalent AUC serum values, when compared to the IV group. Based on the mean values of AUC as documented in table 1 and the data from a previously investigated IV group with AUC values of 1780 (± 343) in serum and 1373 (± 195) lung tissue, we conclude SPAP with 1.25 g/m^2 gemcitabine followed by 30 minutes BFO to be pharmacokinetically superior to IV treatment with equivalent systemic and statistically signicant higher local drug exposure.

Several studies documented the feasibility and relative safety of cytostatic SPAP during BFO. First, Karakousis et al. investigated pulmonary artery perfusion with adriamycin in different individual lobar arteries in patients with recurrent pulmonary metastases from soft tissue sarcomas [6]. Results were disappointing, though, with only one partial objective regression noted. Possible explanations for these results are the non-standardized treatment regimen (variable dose, infusion rate and localisation) resulting in variable distribution volumes and tissue levels. The use of BFO during infusion might have resulted in inhomogeneous distribution, as we previously documented [4]. Furthermore, the patients selected were relatively chemotherapy resistent as they had been pretreated with several chemotherapy protocols, including adriamycine. Wang et al. evaluated pulmonary artery perfusion during BFO with different concentrations of doxorubicin in a metastatic sarcoma model in rat [7]. The authors reported pharmacokinetic superiority with higher drug levels in tumor and perfused lung and lower drug levels in non-perfused lung and serum. No tumors or a significant reduction in nodules were noted in the lungs treated with perfusion, when compared with the untreated lung and IV groups. Furrer et al. compared endovascular pulmonary artery perfusion during BFO to ILuP and IV infusion of doxorubicin in a porcine model [8]. Both selective techniques resulted in significantly higher lung concentrations and lower serum levels, when

compared to IV administration. Brown and co-workers developed a swine model with endovascular lung perfusion using high-dose cisplatin and reported significant higher cisplatin exposure to lung and serum without evidence of direct pulmonary toxicity [10]. Furthermore, they evaluated variables affecting adduct formation and concluded longer infusions lead to greater adduct formation in pulmonary tissues. Changes in infusion concentrations or hemodynamic parameters, however, did not affect uptake of cisplatin.

Gemcitabine was selected as the agent of choice in SPAP because of its high first-pass pulmonary extraction rate and proven tolerance in ILuP in previous investigations [5, 11]. Another argument for selecting gemcitabine in this experiment is its widely acceptance as an agent in combination chemotherapy for advanced NSCLC, and therefore a potential candidate in a combined-modality treatment strategy with surgery in patients with advanced NSCLC. In fact, regional chemotherapy can now be a potentially effective tool for induction therapy when tumors are apparently inoperable, as it can lead to sufficient shrinkage to make such tumors resectable. Extrapulmonary malignant disease would no longer be undertreated, as this approach results in comparable systemic drug exposure as IV administration. Several publications have demonstrated the safety and feasibility of thoracic regional chemotherapy techniques combined with systemic chemotherapy in advanced NSCLC [12,13]. To date, pharmacokinetics of combination chemotherapy with platinum and gemcitabine in pulmonary artery perfusion experiments remain undefined. As this a frequently used regimen for NSCLC, future SPAP investigations with platinum and gemcitabine are warranted, hopefully identifying a definitive combination of agents for use in humans.

In summary, BFO after SPAP and dose-escalation result in significantly higher loco-regional cytostatic drug delivery with equivalent systemic exposure as in 'standard' IV chemotherapy. We advocate SPAP with 1.25 g/m^2 of gemcitabine followed by 30 minutes of BFO to be investigated as a potential new treatment modality for advanced NSCLC.

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Chapter 10

Discussion and future directions

General Discussion

The aim of the work described in this thesis is to explore the potential of ILuP with (normothermic) melphalan and to provide the pharmokinetic rationale of SPAP with gemcitabine in conjunction with BFO. Patients with either (non)resectable pulmonary metastatic disease or, possibly, primary lungcarcinoma might benefit from one of these two techniques. The techniques we have applied are both to be discussed as phase I studies and both leave many questions unanswered. In this general discussion relevant points are addressed on aspects of both ILuP and SPAP, in the form of question and answer.

Part I Isolated lung perfusion with melphalan

Which patients will benefit from ILuP?

The criteria that will identify patients benefiting from ILuP remain still rather poorly delineated. First of all, pulmonary artery perfusion has been primarily indicated (and investigated) in the treatment of pulmonary metastases of non-primary lung cancer, albeit without proven efficacy. As mentioned before, pulmonary metastases receive most of their blood exclusively from the pulmonary circulation [1], in contrast to bronchogenic carcinoma supplied by a dual circulation including both pulmonary and bronchial circulation [2]. The clinical finding of our phase I study is the demonstrated feasibility of the procedure and the acceptable toxicity, when using the adjusted maximum tolerated dose of 45 mg under normothermic conditions. No subjective and/or hematologic, systemic side effects were found. Thus, there seems to be a clear physiologic and pharmacokinetic rationale for further investigations with ILuP using melphalan in a more homogeneous study group of patients with pulmonary metastases to assess toxicity and efficacy.

What causes variation in ILuP pharmacokinetics?

The marked variability in drug concentrations is evident, despite the documented statistically significant correlations between melphalan dose and perfusate AUC curve, and lung tissue concentrations. Furthermore, no significant correlation between melphalan dose and tumor tissue concentrations could be established. Questions concerning these findings remain to be answered. Perfusion related variables can be leakage at the cannula sites during the procedure, possible heterogeneous perfusion, and the use of a standard dose of melphalan regardless of patients' weight. Other factors involved include: localization and volume of the tumor, as well as the biological, growth characteristics of the malignant process apart from its histology. Also, differences in vascularisation may be of importance, as reported, for instance, in renal cell carcinoma, which are highly vascularized tumors. Regarding tumor localizations, the standard

technique of antegrade ILuP resulted in lower melphalan levels in the hilum, higher levels in apex and base, and no significantly different concentrations in the periphery of the lung when compared to retrograde ILuP (including the bronchial system) [3]. These results suggest that tumor localization in the lung is yet another variable determining drug concentrations with possible less uptake in central lesions. Tumor volume might be another variable in explaining the variations in concentration: in an animal model of ILuP with cisplatin, smaller tumor volumes corresponded with higher tumor concentrations [4]. The absence of a significant correlation between perfusate and tumor tissue concentrations suggests that the tumor-uptake of melphalan is independent of the perfusate concentrations, possibly because of a maximum uptake by the tumor or a plateau of chemotherapeutic concentration in the tumor tissue.

Is toxicity in ILuP acceptable?

Histologic findings of tumor as well as of normal lung tissue after ILuP with melphalan were unremarkable. Thus, toxicity could not be documented in morphologic studies. Furthermore, no histologic differences were noticed when comparing normothermic to hyperthermic treatment modalities, or, when comparing patients with and without serious clinical complications. It should be stressed that tissue was removed by pulmonary metastasectomy immediately after the 30 minutes ILuP procedure. Radiographic findings did not reveal persistent pleural effusions, infiltrates or interstitial disease after ILuP procedures, irrespective of normothermic or hyperthermic perfusion conditions. We must add, though, that, unfortunately, a number of patients was lost to follow-up, not only because of complications, but also because of further treatment in the referring institutes. As do the follow-up radiographic findings, the pulmonary function assessments also suffer from progressive lack of follow-up with only small numbers having one or two years follow-up. Follow-up bias in the pulmonary function assessments may skew the results reported in normalization of pulmonary function over time. We think it is absolutely necessary to compare similar postoperative lung function tests for patients undergoing wedge resections to further support our claim of slow improvements in, i.e. recovery of lung function over time.

Unfortunately, we had to document one fatal pulmonary toxicity in a patient treated with hyperthermic ILuP with 45 mg of melphalan, a single death in over 30 procedures. This event had serious consequences, resulting in the amendment of the previously reported maximum tolerated dose of melphalan in ILuP. The exact cause of death in this case of serious pulmonary toxicity remains unclear. One might argue, that the duration of the perfusion period with relative hypoxia amplified the toxic response. However, previous procedures with similar perfusion periods were completed without complications. During the course of the phase I trial, we abandoned snaring the bronchial artery. Thus, the potential detrimental effect –

related to tissue hypoxia - of this manoeuver cannot be a factor in this particular case. Furthermore, no hypoxic complications have been reported as long as the lungs are ventilated during the procedure. Fatal toxicity, as caused by the procedure itself, may have been related to a combination of hyperthermia, relative hypoxia and drug induced toxicity, further complicated by respiratory infection, ultimately culminating into a diffuse lung hemorrhage with diffuse alveolar damage. Clearly, the disproportionate morbidity encountered in the extension trial and off protocol patients justifies retracting the originally documented MTD under hyperthermic conditions.

Is there improved survival in ILuP?

Results of clinical follow-up have to be interpreted with caution in a phase I trial. Survival was not a secondary endpoint, while responses in the perfused lung cannot be adequately assessed after resection of all macroscopic disease at the time of perfusion. Since survival is probably related to the number of metastases and histology as well, it is difficult to draw inferences from our data and compare them to historical controls, especially in this small series. In each dose level, there were only three patients with different primary tumors, lacking uniformity. Since ILuP is a regional therapy, recurrent disease outside the lung or disease in the non-treated lung cannot be prevented. As a single-course treatment, the effectiveness of ILuP with melphalan thus remains unknown.

Part II Selective pulmonary artery perfusion with gemcitabine

Which patients will benefit from SPAP?

An often-cited criticism of pulmonary artery perfusion of primary lung malignancies relates to tumor blood supply. The rationale for pulmonary artery perfusion in NSCLC is supported by documented physiological anastomoses and shunts between the bronchial and pulmonary circulations [5], their number being increased in case of pathologic conditions [6]. The results of the SPAP study by Morris with observed radiographic response in bronchogenic carcinoma [7], and the significant levels of the infused drug in the tumor in cases of primary lung carcinoma after ILuP in the study by Johnston [8], prove that both primary and metastatic cancers in the lung will, at least partially, be perfused through the pulmonary circulation. We hypothesize, that SPAP in the treatment of NSCLC could potentially convert unresectable to resectable disease by downstaging patients from stage III to stage II.

First, SPAP aims at primary tumor reduction (T status), before surgical treatment. Second, SPAP will result also in drug exposure in the other, ipsilateral lobes and of the involved lymphatic system of the affected lobe. Thereby, local lymphogenic metastatic disease (N status) could be

down-staged towards surgical cure. Finally, systemic serum and hepatic levels equivalent to IV therapy, will attack potential systemic disease in the same way as achieved in currently applied IV schedules for NSCLC.

There are some points of criticism, though. In advanced NSCLC – where the N status will dictate prognosis - the issue is to reach adequate control of lymphatic disease at the N2 level and, possibly, N3. Moreover, multinodal involvement at N2 has been shown to be an unfavourable factor. To date, it has not been proven that neoadjuvant chemotherapy will convert an unresectable T compartment to resectable tumor. In addition, since N2 disease - and even more so N3 - is considered to be a systemic manifestation of the malignant process, the question remains if SPAP will have additional value treating possible distant sites as compared to IV infusion. The efficacy in downstaging the nodal status is therefore crucial, and further examinations should focus on mediastinal lymph node sampling and drug levels analysis. In this SPAP model, we propose to "improve the current treatment of NSCLC with minimal side effects". However, with comparable drug concentrations through SPAP and IV infusions in the serum, one would expect exactly the same incidence of untoward events. With systemic serum and hepatic levels equivalent to IV therapy, SPAP will treat systemic disease in the same way as achieved in currently applied IV schedules. If, and when SPAP is to be considered as a treatment modality in a human model for NSCLC, the pre-eminent investigations are to be in patients with stage IV NSCLC, aiming at better local control (with higher local drug levels) and comparable systemic treatment for mediastinal and systemic disease. This would not only be appropriate for a phase I study, but as a secondary endpoint the radiographic response should be taken into account as well.

The question arises, whether SPAP could potentially be a treatment modality for resectable pulmonary disease of non-primary lung carcinoma. In patients with resectable pulmonary metastases without extrapulmonary disease, the rationale for ILuP selective pulmonary perfusion was high dose local chemotherapy without, or with minimal systemic drug levels. Indeed, in our phase I study, we reported the use of ILuP as an adjuvant, single course treatment in this exact study population. We feel SPAP needs to be investigated, as it has the clear advantage over ILuP of treating undetectable contralateral pulmonary and extrapulmonary micrometastases with, to IV administration comparable, adequate systemic concentrations of chemotherapy, besides high-dose local chemotherapy as a (neo)adjuvant therapy.

In patients presenting with irresectable and extrapulmonary disease, SPAP may also have potential as a treatment modality. Larger study populations could be studied for the anti-neoplastic efficacy of this procedure, particularly in non-academic hospitals, as the procedure is not as highly specialized as ILuP, and there is the possibility of repeating the procedure. Also, the procedure could easily be performed (sequentially) bilaterally. The pharmacokinetic properties of SPAP would further justify research in these patients, as we already have

established the maximum tolerated dose for pulmonary artery perfusion with melphalan, limiting significant pulmonary toxicity in future human trials. The total concentration represented by the AUC in lung tissue, though, needs to be translated into a single-pass system of SPAP. The additional systemic effects could, potentially, have a anti-tumor effect upon extrapulmonary disease, but will limit the dosage of the cytostatic agent in the perfusate as significant leakage will occur with potential systemic toxicity.

Can we grasp SPAP pharmacokinetics?

The drug

Gemitabine is a nucleoside analogue of deoxycytidine, in which two fluorine atoms are incorporated in the deoxyribofuranosyl ring. All investigations to date, in both ILuP and SPAP, measured, analyzed, and reported pharmacokinetics of gemcitabine levels. However, after entering the cell, gemcitabine is phosphorylated to gemcitabine di- and triphosphate. Studies using radioactive gemcitabine showed gemcitabine triphosphate to be its major active metabolite [9]. Furthermore, gemcitabine elimination is dependent on the cellular concentrations of this triphosphate [10]. These studies suggest that in order to investigate pharmacokinetics of gemcitabine, measurement of the most active metabolite is of the greatest importance. When the tissues are saturated with gemcitabine, further accumulation of gemcitabine is induced due to higher triphosphate levels with a prolonged terminal elimination phase. In other words, from an oncologic point of view, ''the higher (concentration of gemcitabine) is not the better", as it does not reflect increased clinical activity, but merely less activity of the elimination route. Currently, measurements of tri-phosphate from our reported experiments are being analyzed, which is a highly specialized procedure only available in a limited number of centers.

SPAP has pharmacokinetic properties superior to IV administration, with all SPAP groups showing significantly higher lung AUC and peak levels compared to IV infusion. First, this observation is partially explained by a dilutional effect. In contrast to IV treatment, SPAP is characterized by infusion into the left or right pulmonary artery resulting in a two times higher blood concentration entering the treated lung. Furthermore, the blood concentration delivered at the tip of the SPAP catheter was increased by augmentation of the infusion rates resulting in six (with a ten minutes infusion time) and thirty times (two minutes infusion time) higher local blood concentrations compared to IV infusion. As in ILuP, heterogeneous distribution remains an important issue, as we documented this phenomenon in flow reduction in our experiments. In contrast to what we expected, SPAP with 50% flow reduction resulted in even lower pulmonary drug levels compared to normal blood flow, while the blood concentration delivered at the catheter tip was twice as high. Furthermore, 90% flow reduction resulted in higher levels as compared to normal blood flow. Obviously, flow reduction during SPAP

resulted in increasing standard deviations. We hypothesize that this phenomenon may be due to compensatory pulmonary vasoconstriction during flow reduction in order to maintain pulmonary artery pressure resulting in inhomogeneous distribution of the drug infused. These data suggest that blood flow reduction as a means of improving first-pass uptake, should be abandoned, in contrast to BFO.

The use of BFO

Besides the apparent advantages of endovascular SPAP, namely non-invasiveness, the possibility of repeated procedures, and pharmacokinetic superiority, there are a number of unwanted events. In high-flow intra-arterial perfusion, clinical application is hampered by dilution of the concentration of the anticancer agent and a too short time interval of passage through the capillary with inadequate diffusion. BFO performed during the actual selective artery perfusion, that is injection of the chemotherapeutic agent distal to its occlusion by a balloon-tipped catheter, has the potential advantages of delaying the systemic washout and prohibiting the dilutional effect. Pharmacokinetic properties of SPAP during BFO are superior to SPAP without BFO. For instance, Karakousis et al. investigated SPAP with adriamycin in dogs and documented significantly higher pulmonary drug levels in the group treated with SPAP during BFO when compared to SPAP without occlusion. Results of his human trial were disappointing, though, with only one partial objective regression noted. Possible explanation for these results could be inhomogeneous distribution, which is an issue in both ILuP and SPAP with BFO. In our model, we chose to perfuse not during, but after SPAP, which is in contrast to all PubMed cited articles on SPAP with BFO in the last 25 years. The rationale for this different approach is the hypothesis, that physiologic pulmonary flow during the selective perfusion yields more homogeneous distribution of the cytostatic agent. To the best of our knowledge, there are no publications of data comparing drug distribution in SPAP during BFO, with SPAP followed by BFO. There are some studies, though, suggesting the importance of pulmonary flow in maintaining a more homogeneous distribution of the perfused cytostatic agent inside the lung. Indeed, a study by Smyth and Blades investigating the diffusion of methylene blue in the visceral pleura of all lobes in dogs after injection with and without occlusion of the pulmonary artery, clearly showed un uneven distribution of the indicator in the group with occlusion [13]. Ratto and co-workers documented a more heterogeneous drug distribution in cisplatin-based BFO compared with ILuP in a porcine model [14]. In their excellent evaluation of different cytostatic lung perfusion techniques in a porcine model, Krueger and associates addressed heterogeneous spatial regional blood flow and drug distribution in both ILuP, and SPAP during BFO, when compared with IV administration [15]. We recently reported of a catheterisation model of SPAP without BFO, in which flow reduction during SPAP was investigated [16]. Flow reduction during SPAP resulted in increasing standard deviations. We hypothesize that this phenomenon may be explained by compensatory

pulmonary vasoconstriction during flow reduction in order to maintain pulmonary artery pressure finally resulting in inhomogeneous distribution of the drug infused. In our opinion, by using BFO after instead of during SPAP, the distribution of the drug itself is more homogeneous by using, in that timepoint, the physiologic flow of the pulmonary artery. Furthermore, we speculate on less exposure of highly concentrated perfusate, and therefore less local vascular reactivity and consequently heterogeneous distribution.

Is SPAP toxicity acceptable?

Histologic examination of lung tissue after SPAP with gemcitabine suggests evidence of alveolar hyperplasia, which was more pronounced in the flow reduction group. The latter could be explained by higher pulmonary peak concentrations and AUC value. As this mild to moderate congestion occurred just after 45 minutes after the perfusion, further examinations in SPAP with gemcitabine without flow reduction in a porcine model should be performed to investigate long-term toxicity, before application in a human setting.

Future directions in pulmonary artery perfusion

Selective pulmonary artery perfusion is a pharmacokinetically interesting and sound concept of minimally-invasive, regional chemotherapy. Besides the limited toxicity in a small number of human studies, one must be attentive to locoregional toxicity. Further research in a porcine model is warranted with emphasis on chronic toxicity with, for instance, sacrifice after 6 weeks followed by extensive pathological examination.

Future research in patients with ILuP as a pulmonary adjuvant chemotherapeutic modality should focus on a homogeneous group of patients with resectable, metastatic disease from tumors susceptible to chemotherapy with, as indicated by reported studies, improved prognosis after pulmonary metastasectomy, such as sarcomas and colorectal carcinoma. Since November 2006, this study population is being examined in a phase II trial of ILuP with 45 mg of melphalan under normothermic conditions followed by pulmonary metastasectomy. This trial is conducted by the University of Antwerp, Leidsch University Medical Center and the St. Antonius Hospital Nieuwegein.

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Chapter 11

Summary/Samenvatting

Dankwoord

List of Publications

Curriculum Vitae

Summary

The purpose of this thesis was to study two modes of regional chemotherapy of the lung: Isolated Lung Perfusion (ILuP) and Selective Pulmonary Artery Perfusion (SPAP).
In our ILuP studies in humans with resectable pulmonary metastatic disease, we evaluated feasibility, maximum tolerated dose (MTD), pharmacokinetics and toxicity of melphalan under normo- and hyperthermic conditions, followed by pulmonary metastasectomy.
Furthermore, we investigated SPAP by using a non-tumor porcine model. The primary objective was to evaluate pharmacokinetics while varying blood flow, perfusion time and blood flow occlusion (BFO) interval. Moreover, we performed a dose escalation study administering gemcitabine.

Chapter 1
This general introduction briefly reviews the two techniques of pulmonary artery perfusion (ILuP and SPAP) and identifies (future) study populations. Furthermore, we offer a description and motivation of the chosen perfusion temperature and of the used biological agent.

ILuP is presented in chapters 2 - 6 (Part I), while SPAP is discussed in chapters 7 - 9 (Part II)

Part I: Isolated lung perfusion

Chapter 2
The available information regarding human and animal studies in ILuP with melphalan is reviewed. Furthermore, we discuss the development of the isolated perfusion technique in our institute, reporting the first patient perfused with such a circuit. Adjustments of the perfusion system, such as discontinuation of the use of an oxygenator, application of a self-constructed air-separator, as well as the influences of perfusion pressure, flow and temperature are discussed.

Chapter 3
This chapter describes the protocol of a dose-finding phase I trial in patients with resectable pulmonary metastases treated with melphalan through ILuP, followed by pulmonary metastasectomy. This study is performed in conjunction with the University Hospital of Antwerp, Belgium.

Chapter 4

This chapter reports the results of a phase I trial of ILuP, in which patients underwent lung perfusion with melphalan combined with pulmonary metastasectomy for resectable lung metastases. The study was conducted in order to define the dose limiting toxicity and MTD. Patients were treated with ILuP with increasing melphalan doses under normothermic and hyperthermic conditions. In total, 21 procedures of ILuP with complete metastasectomy were performed without technical difficulties. Operative mortality was 0%, and no systemic toxicity was encountered. Grade 3 pulmonary toxicity developed at a dose of 60 mg of melphalan at 37°C in 2 out of 3 patients in this level, terminating the trial. We concluded that ILuP with melphalan, combined with pulmonary metastasectomy, was a feasible procedure. Fourty-five mg of melphalan perfused at a temperature of 42°C was defined as the MTD.

Chapter 5

A pharmacokinetic analysis of 29 procedures of ILuP followed by pulmonary metastasectomy is discussed. The patients treated by ILuP with melphalan in the phase I trial, and eight additional procedures in the extension trial of ILuP treated with 15 and 45 mg melphalan under hyperthermic conditions, are included.

This study demonstrated the great pharmacokinetic advantage of ILuP with melphalan in achieving high-dose local chemotherapy with minimal systemic concentrations. Furthermore, we demonstrated a significant correlation between the melphalan dosage, AUC of perfusate, and lung tissue melphalan concentrations, justifying the concept of isolated lung perfusion as a one-course adjuvant chemotherapeutic treatment modality for microscopic residual malignant disease.

Chapter 6

This chapter further clarifies safety issues of ILuP using melphalan under hyperthermic conditions. The evaluation of pulmonary and systemic toxicities as well as the clinical follow-up of 29 procedures in 23 patients are reported. One patient was treated off-protocol with 45 mg of melphalan under normo- and hyperthermic conditions. Remarkably, in contrast to the results of the initial phase I trial, three out of eight procedures in the extension trial under hyperthermic conditions were complicated by empyema, rhabdomyolysis and postoperative bleeding, respectively. Pulmonary function assessments with progressive lack of follow-up showed a statistically significant decline with partial reversibility of all parameters. With a mean follow-up of 25 months, 8 out of 23 patients are alive and disease-free, 14 patients developed recurrent disease, of which 3 patients deceased. Of these 23 patients, one patient died of non-malignancy related disease during contralateral thoracotomy several months after the ILuP procedure. Off protocol, one patient was treated with MTD of melphalan in ILuP under normo- and hyperthermic conditions. The procedure under hyperthermic conditions was complicated by chemical pneumonitis, massive pulmonary hemorrhage, and eventually death.

Because of the considerable pulmonary and systemic morbidity encountered in the extension trial and the fatal toxicity off protocol, we have to conclude that the previously reported MTD of melphalan in ILuP is 45 mg under normothermic, and not under hyperthermic conditions.

Chapter 7

This chapter reviews the published investigations of SPAP. The pharmacokinetic superiority resulting in high-dose local cytostatic drug concentrations, as compared to IV administration, was shown in a number of animal models. BFO of the pulmonary artery before or after drug injection results in a further increase in the local drug concentrations. Anti-tumor efficacy in animal tumor models with sarcoma and colon carcinoma were also confirmed. More importantly, feasibility and safety of chemotherapeutic SPAP was documented in humans.

Chapter 8

In this chapter, we developed a catheterisation model of SPAP. By selective perfusion of one lung, using a pulmonary artery catheter, substantially high local drug levels were obtained. Moreover, simultaneously, systemic concentrations were reached which were similar to those obtained by standard IV (i.e. systemic) administration.

Sixteen pigs underwent SPAP with gemcitabine (1g/m^2) followed by thoracotomy. Three groups received an infusion for two minutes: in one group with normal pulmonary blood flow, in the two others applying a 50%, and a 90% flow reduction. This reduction was obtained by inflating the balloon of the balloon catheter. Another group received SPAP with an infusion time of ten minutes with normal blood flow. A non-SPAP group received gemcitabine intravenously (peripheral infusion) using a thirty minutes infusion time. Pulmonary peak concentrations and areas under the concentration-curve (AUC) of SPAP were significantly higher using the 2- and 10 minutes infusion time as compared to IV administration, while SPAP for ten minutes resulted in the highest AUC. Flow reduction during SPAP led to inhomogeneous distribution of the cytotoxic agent. In this animal model, SPAP resulted in higher lung concentrations, while systemic exposure was comparable with IV administration.

Chapter 9

The study described in this chapter was designed to evaluate SPAP with BFO and dose escalation of gemcitabine in a porcine model. Four groups of animals underwent SPAP with 1g/m^2 gemcitabine using an infusion time of two minutes, followed by BFO for 0, 10, 20 and 30 minutes. Two further groups received SPAP using an infusion time of 2 minutes followed by 30 minutes of BFO: one group receiving 1.25 g/m^2 of gemcitabine and the other 1.5 g/m^2. BFO resulted in significantly higher lung AUC. When comparing 10, 20 and 30 minutes, the longest duration of flow occlusion gave the highest local concentrations of the cytotoxic agent. Selective pulmonary artery perfusion with dose escalation –from 1 to 1.25 to 1.5 g/m^2

followed by 30 minutes BFO- resulted in higher lung and serum AUC, although the differences between 1.25 and 1.5 g/m^2 did not reach statistical significance. We concluded, that SPAP with escalating dosages of gemcitabine applying BFO resulted in significantly higher lung and equivalent serum AUC. We propose SPAP with 1.25 g/m^2 of gemcitabine followed by 30 minutes of BFO, as a potentially promising treatment modality for lung carcinoma.

Chapter 10

The general discussion focuses on the advantages and disadvantages of ILuP versus SPAP. ILuP is a rather invasive technique using extensive instrumentation. In most patients, it will be a single treatment modality. In contrast, SPAP, which we consider to be the natural "successor" to ILuP, can be repeated. Moreover, we have shown that it has the advantage of resulting – apart from high local concentrations - in adequate systemic drug levels that will contribute to the over-all efficacy of this perfusion method. The application of BFO further significantly enhanced the local effectiveness of this technique. Moreover, the various groups of patients who might benefit from these different techniques are discussed.

Samenvatting

Het doel van de onderzoeken beschreven in dit proefschrift is het bestuderen van twee vormen van lokale chemotherapie, dwz behandeling met cytostatica van alleen het orgaan waar zich de uitzaaiingen bevinden. De twee onderzochte methodes zijn geïsoleerde longperfusie (ILuP) en selektieve arteria pulmonalis perfusie (SPAP).
ILuP met melfalan werd verricht onder normotherme (37°C) en hypertherme (42°C) omstandigheden gevolgd door chirurgische verwijdering van de longuitzaaiingen, een zgn pulmonale metastasectomie. In de onderzoeken met ILuP evalueerden we uitvoerbaarheid, maximaal tolereerbare dosis (MTD), farmacokinetiek en toxiciteit bij patiënten met uitzaaiingen in de longen die in aanmerking voor chirurgische verwijdering.
SPAP met gemcitabine werd bestudeerd in een varkensmodel zonder tumor. Het hoofddoel van deze studie was het beschrijven van farmacokinetiek bij variabele bloeddoorstroming van de longslagader, perfusietijd en tijdsduur van bloedstroomonderbreking (BFO). Tevens onderzochten we de farmacokinetische effecten van SPAP met steeds hogere doseringen gemcitabine (ook wel dosis-escalatie genoemd).

De studies van ILuP worden beschreven in hoofdstukken 2 t/m 6.
De studies van SPAP zijn beschreven in hoofdstukken 7 t/m 9.

Hoofdstuk 1
De algemene introduktie geeft een korte beschrijving van de twee technieken van lokale chemotherapie, ILuP en SPAP, en identificeert (toekomstige) patiënten populaties die baat zouden kunnen hebben bij deze behandelingen. Verder beschrijven en motiveren we de gekozen perfusietemperatuur en de keuze van het chemotherapeuticum.

Hoofdstuk 2
Dit hoofdstuk geeft een samenvatting van alle humane en dierexperimentele studies in ILuP met melphalan. Daarnaast wordt de ontwikkeling van de ILuP procedure in ons instituut beschreven aan de hand van de eerste patiënt die deze behandeling kreeg. Veranderingen in het perfusiesysteem, zoals het weglaten van de oxygenator, de ontwikkeling van een systeem voor luchtscheiding, alswel de invloed van perfusiedruk, bloeddoorstroming en temperatuur staan ter discussie.

Hoofdstuk 3
Het protokol van de fase I ILuP studie met melfalan onder normotherme en hypertherme omstandigheden gevolgd door pulmonale metastasectomie wordt hierin beschreven. Deze studie werd verricht in samenwerking met de afdelingen Thorax en Vaatheelkunde, Longziekten en Medische Oncologie van de Universiteit van Antwerpen, Belgie.

Hoofdstuk 4

Dit hoofdstuk beschrijft de resultaten van het fase I onderzoek om de dosisbeperkende toxiciteit en MTD van melfalan in ILuP te definiëren. Patiënten werden behandeld met ILuP in steeds hogere doseringen melfalan onder normotherme en hypertherme omstandigheden. In total werden 21 ILuP procedures (gevolgd door metastasectomie) verricht zonder technische problemen. Het operatieve sterftecijfer was 0%, er werd geen systemische toxiciteit geobjectiveerd. Graad 3 pulmonale toxiciteit werd gedocumenteerd bij gebruik van een dosis van 60 mg melfalan onder normotherme omstandigheden in twee van de drie patiënten in dezelfde dosis level, waarna het onderzoek werd afgesloten conform protokol. ILuP met melfalan gevolgd door pulmonale metastasectomie is een uitvoerbare procedure. De MTD werd gedefinieerd als zijnde 45 mg melfalan onder hypertherme condities.

Hoofdstuk 5

Een farmacokinetische analyse van 29 ILuP procedures met melfalan gevolgd door metastasectomie wordt beschreven in dit hoofdstuk. De patiënten van het fase I onderzoek naast 8 additionele procedures van een extensie onderzoek werden geïncludeerd. Deze studie demonstreert het farmacokinetische voordeel van ILuP met melfalan resulterend in lokaal hoge doseringen en systemisch lage doseringen van het chemotherapeuticum. Daarnaast werd een statistisch significante correlatie beschreven tussen de melfalan dosering, area under the curve (AUC) van het perfusaat (als een maat van melfalan expositie), en longweefsel concentraties, hetgeen het concept van ILuP als een eenmalige adjuvante chemobehandeling voor microscopische residuale ziekte onderbouwt.

Hoofdstuk 6

Veiligheidsaspecten van ILuP met melfalan onder hypertherme omstandigheden worden behandeld. De evaluatie van pulmonale en systemische toxiciteit, naast de klinische follow-up van 29 procedures wordt beschreven. Een patiënt werd buiten protokol behandeld met 45 mg melfalan onder normotherme en hypertherme omstandigheden. In tegenstelling tot de resultaten van de fase I studie, werden drie van de acht procedures in de extensie studie gecompliceerd door respectievelijk empyeem, rabdomyolyse en postoperatieve bloeding. Longfunktiebepalingen met een beperkte follow-up lieten een statistisch significante daling met partiële reversibiliteit zien in alle parameters. Met een gemiddelde follow-up van 25 maanden waren 8 van de 23 patiënten in leven en ziektevrij; 14 patiënten ontwikkelden een recidief, waarvan 3 patiënten overleden. Van de in totaal 23 patiënten, overleed één patiënt vier maanden na de ILuP procedure door cardiale complicaties tijdens een thoracotomie (zonder ILuP). De hypertherme procedure van de patiënt behandeld buiten studieverband werd gecompliceerd door een chemische pneumonitis, longbloeding en uiteindelijk overlijden.

Gezien de toxiciteit in de hypertherme procedures in het extensie onderzoek en de patiënt buiten studieverband, concluderen we dat de MTD van melfalan in ILuP 45 mg is onder normotherme omstandigheden.

Hoofdstuk 7

Gepubliceerde onderzoeken naar SPAP worden besproken in dit hoofdstuk. De farmacokinetische superioriteit van SPAP, resulterend in hoge lokale en lage systemische concentraties is aangetoond in een aantal dierexperimentele studies. Tijdelijk onderbreken van de doorstroming van de pulmonale slagader, oftewel BFO, tijdens de injectie van de chemobehandeling resulteert in een verdere verhoging van de lokale concentraties van het gebruikte medicijn. Anti-tumor effecten in dierexperimenteel onderzoek met weke delen tumoren en darmkanker zijn aangetoond. Uitvoerbaarheid en veiligheid van SPAP met een chemotherapeuticum is beschreven in een humaan model.

Hoofdstuk 8

In dit hoofdstuk beschrijven we een catheterisatiemodel van SPAP. Zestien varkens werden behandeld met SPAP met gemcitabine in een dosis van 1 g/m^2 gevolgd door een thoracotomie. Drie SPAP groepen (n=4) werden gedurende twee minuten geperfundeerd: één groep met normale bloeddoorstroming van de longslagader, de andere twee groepen (n=4) met 50% en 90% verminderde doorstroming. Een andere SPAP groep (n=4) werd behandeld met normale bloeddoorstroming gedurende 10 minuten. Een andere groep werd niet met SPAP, maar intraveneus behandeld met gemcitabine gedurende 30 minuten. Piek concentraties van gemcitabine in het longweefsel en AUC van de SPAP groepen met 2 en 10 minuten durende perfusie waren signifikant verhoogd in vergelijking met intraveneuze behandeling. SPAP met gemcitabine gedurende 10 minuten resulteerde in de hoogste AUC waarde. Reduktie van de bloeddoorstroming leidde tot inhomogene verdeling van de gemcitabine in de long.
Door selektieve perfusie van de longslagader via een pulmonaliscatheter werd een substantieel hogere lokale dosering van gemcitabine bereikt. Daarnaast werden systemische concentraties gemeten die vergelijkbaar zijn met de 'standaard' intraveneuze behandeling.

Hoofdstuk 9

De studie beschreven in dit hoofdstuk was ontwikkeld om SPAP gevolgd door BFO, en SPAP met hogere dosering gemcitabine te onderzoeken in een varkensmodel. Vier groepen varkens (n=4, elk) werden behandeld met SPAP van 1 g/m^2 gemcitabine gedurende 2 minuten, gevolgd door 0, 10, 20 en 30 minuten BFO. Twee andere groepen (n=3, elk) werden behandeld met SPAP gedurende 2 minuten met respectievelijk 1.25 g/m^2 en 1.5 g/m^2 gemcitabine, gevolgd door 30 minuten BFO. BFO resulteerde in een signifikant hogere long AUC. Bij vergelijking van de 10, 20, en 30 minuten groepen, resulteerde de langste BFO tijd in de hoogst lokale gemcitabine concentraties.

SPAP met 1.25 g/m^2 en 1.5 g/m^2 gemcitabine gevolgd door 30 minuten BFO resulteerde in hogere AUC waarden in long en serum, alhoewel dit geen statistisch signifikante resultaten waren. We concluderen dat SPAP met hogere doseringen gemcitabine gevolgd door 30 minuten BFO leidt tot signifikant hogere long AUC en gelijkwaardige serum AUC waarden (in vergelijking met intraveneuze behandeling). Op basis van deze gegevens, stellen we voor SPAP met 1.25 g/m^2 gemcitabine gevolgd door 30 minuten BFO te onderzoeken als een interessante behandelingsmodaliteit voor long carcinoma.

Hoofdstuk 10

In dit hoofdstuk, de discussie, worden de voor- en nadelen van ILuP versus SPAP besproken. ILuP is een invasieve techniek met uitgebreide instrumentatie. Bij de meeste patiënten wordt deze procedure gebruikt als een eenmalige procedure. Dit in tegenstelling tot de SPAP procedure, die we beschouwen als een natuurlijke opvolger van ILuP en meerdere malen (non-invasief) gebruikt kan worden. Daarnaast hebben we aangetoond dat naast de hogere lokale geneesmiddelen concentratie, een adequate systemische concentratie kan worden bereikt, bijdragend aan de extrapulmonale effectiviteit van deze non-invasieve techniek. Het toepassen van BFO versterkt de lokale effectiviteit met nog hogere long concentraties van het chemotherapeuticum. De verschillende studiepopulaties die mogelijk gebaat zouden kunnen zijn bij behandeling met deze lokale technieken worden besproken.

Dankwoord

Hierbij wil ik iedereen die (in)direkt een bijdrage heeft geleverd aan het tot een succesvol einde brengen van het proefschrift hartelijk danken.

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List of Publications

Hendriks JM, **Grootenboers MJ**, Schramel FM, Van Boven WJ, Stockman B, Ter Beek HT, Seldenrijk CA, Ten Broecke P, Knibbe CA, Slee P, De Bruijn EA, Vlaeminck R, Heeren J, Vermorken JB, Van Putte BP, Romijn S, Van Marck E, Van Schil PE. Isolated lung perfusion with melphalan for resectable lung metastases: a phase I clinical trial. Ann Thorac Surg 2004:78(6):1919-27.

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Curriculum Vitae

De auteur van dit proefschrift werd geboren op 1 juli 1973 te Breda. Na het volgen van het VWO op de Rijksscholengemeenschap Graaf Engelbrecht te Breda, starte hij met de studie geneeskunde aan de Erasmus Universiteit Rotterdam (huidige Erasmus Medisch Centrum). Het artsexamen werd behaald in 1999. Na 1.5 jaar AGNIO-schap volgde in 2001 de stap naar de opleiding Interne Geneeskunde van het Baronie Ziekenhuis te Breda (huidige Amphia lokatie Langendijk) onder leiding van Dr. PJ Stijnen. In 2003 maakte hij de overstap naar de longziekten, waar de opleiding werd genoten van 2003 tot 2007 in het Sint Antonius Ziekenhuis te Nieuwegein onder leiding van Prof. dr. JMM van den Bosch. Het in dit proefschrift beschreven ILuP onderzoek werd reeds gestart in 1999 en is afgerond in 2005. De studies naar SPAP werden verricht in 2006 en 2007. De auteur is sinds februari 2007 werkzaam als longarts in het Amphia Ziekenhuis te Breda. Hij is getrouwd met Arianne Visser en samen hebben ze een zoon, Stijn.