Pharmacokinetics of Gemcitabine when Delivered by Selective Pulmonary Artery Perfusion for the Treatment of Lung Cancer

Bart P. van Putte, Marco Grootenboers, Wim-Jan van Boven, Jeroen M. H. Hendriks, Paul E. Y. van Schil, Gunther Guetens, Gert De Boeck, Gerard Pasterkamp, Franz Schramel, and Gert Folkerts

Departments of Cardiothoracic Surgery (B.P.v.P., W.-J.v.B.) and Pulmonology (M.G., F.S.), Sint Antonius Hospital, Nieuwegein, The Netherlands; Department of Pharmacology, University of Utrecht, Utrecht, The Netherlands (G.F.); Department of Thoracic and Vascular Surgery, University Hospital, Antwerp, Belgium (J.M.H.H., P.E.Y.v.S.); Laboratory of Experimental Oncology, Catholic University Leuven, Leuven, Belgium (G.G., G.D.B.); and Department of Cardiology, Laboratory of Experimental Cardiology, University Medical Center, Utrecht, The Netherlands (G.P.)

Received January 2, 2007; accepted January 7, 2008

ABSTRACT:
Lung cancer represents a major health problem. Cytostatic and radiotherapeutic treatment is limited because of dose-limiting systemic toxicity and surgery as a result of its invasive nature. Therefore, we developed a catheterization model of selective pulmonary artery perfusion (SPAP) combining the properties of isolated lung perfusion and i.v. treatment to achieve higher local drug levels and equivalent systemic exposure. Sixteen pigs underwent SPAP using a clinically applied dose of gemcitabine (1 g/m²). They furthermore underwent thoracotomy for tissue sampling. Three groups were treated with SPAP for 2 min with normal pulmonary blood flow, 50 and 90% flow reduction. Another group had SPAP for 10 min with normal blood flow. All the SPAP groups underwent catheterization of the left pulmonary artery. An additional group (n = 4) was infused i.v. for 30 min using the same dose. Concentrations were analyzed with analysis of variance. Pulmonary peak concentrations (p = 0.01) and areas under the curve (AUC) (p = 0.001) of SPAP for 2 and 10 min were significantly higher compared with i.v., whereas SPAP for 10 min resulted in the highest AUC (p = 0.045) compared with SPAP for 2 min. Flow reduction during SPAP resulted in inhomogeneous distribution. Liver levels, AUC (serum), and wet-to-dry ratios of all the SPAP groups were not significantly different compared with i.v. SPAP resulted in higher lung concentrations, whereas systemic exposure was comparable with i.v. Therefore, we advocate SPAP as a new method to be tested clinically to achieve down-staging of the tumor and lymph node status in lung cancer.

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 (Jemal et al., 2006). 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 the cancer types, lung cancer has the highest death rate (Jemal et al., 2006).

Non–small cell lung cancer (NSCLC) is usually treated by surgical resection, radiotherapy, and/or cytostatic drug administration, depending on the disease stage. Stage I (a and b) and 2 (a and b) NSCLCs are currently treated by surgical resection, whereas (adjuvant) cytostatic therapy is applied to stage 1b, 2, and 3 disease, resulting in a 5-year survival of 75, 60, 40, 20, and 15%, respectively (Spiro and Silvestri, 2005).

An i.v. infusion is the desired route of cytostatic drug administration to achieve exposure of the primary lung tumor and distant disease, as well as resulting in a 5-year survival benefit of 4 to 14% compared with surgery alone (Betticher, 2005). 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, isolated lung perfusion 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, isolated lung perfusion results in significantly higher drug concentrations in both lung and tumor tissue compared with i.v. administration as shown by many experimental and human data (Pass et al., 1996; Ratto et al., 1996; Hendriks et al., 1998, 2004; Burt et al., 2000; van Putte et al., 2002). High-dose drug administration during isolated lung perfusion using drugs like gemcitabine and cisplatin is well tolerated by healthy lung tissue (Ratto et al., 1996; van Putte et al., 2003a, 2005). Furthermore, a recent phase I trial evaluating toxicity of isolated lung perfusion with melphalan showed a rapid pulmonary lymph drainage resulting in equivalent concentrations in either the lymph nodes and lung tissue, although this approach is applied only once or twice per patient because of its invasive nature (unpublished data). However, isolated lung perfusion is a local regional treatment modality developed for the treatment of lung metastases but not for the treatment of NSCLC.

ABBREVIATIONS: NSCLC, non-small cell lung cancer; SPAP, selective pulmonary artery perfusion; AUC, area under the curve.
Twenty-six to 32% of all the recurrences are local in patients with stage 1 and 2 NSCLC treated by lobectomy (Rivera et al., 2001). 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 selective pulmonary artery perfusion (SPAP) combining the properties of isolated lung perfusion and i.v. treatment to improve outcome of NSCLC with minimal side effects and to achieve down-staging of stage 3a and 3b NSCLC toward a surgical stage.

First, higher drug exposure of the diseased lobe is necessary to achieve tumor (T status) size reduction. Second, the residual lung lobes probably contain micrometastases that have to be treated more aggressively to prevent pulmonary recurrences. Third, lymph node status (N status) reduction is essential to achieve down-staging of stage 3a and 3b NSCLC. These lymph nodes are the first station for metastases after the lung lobes themselves and will be cotreated using high drug levels. Finally, SPAP aims to attack distant disease outside the lungs (M status) in an equivalent way as the currently applied i.v. administration does. More specifically, in this study, infusion variables of SPAP are optimized and compared with the standard i.v. route of drug administration.

Materials 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 (National Institutes of Health 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) i.m. Each pig received 4 mg/kg thiopental sodium through an i.v. line. After intubation, the animals were connected to a volume-controlled ventilator (8 ml/kg, 12 breaths/min guided by capnography) maintaining positive end-expiratory pressure of 5 cm of H2O and an inspiratory oxygen fraction of 0.5. Anesthesia was maintained by continuous infusion of midazolam (0.7 mg/kg/h). Analgesia was obtained with continuous infusion of sulfentanil citrate (10 μg/kg·h) and muscle relaxation with pancuronium (0.1 mg/kg·h). Furthermore, a continuous infusion of saline (300 ml/h) was administered during the operation. After finishing the experiment, animals were sacrificed with pentobarbital sodium (200 mg/kg) i.v. A central venous line was inserted for serum sampling during finishing 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, Reading, PA) 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 5th 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 (Fig. 1B).

A 12-mm flow probe was placed around the left pulmonary artery for blood flow measurements just distal of the tip of the balloon catheter and just proximal of the first side branches. A 12-mm flow probe was placed around the left pulmonary artery for blood flow measurements just distal of the tip of the balloon catheter and just proximal of the first side branches. After stabilization of the blood flow, gemcitabine was infused through the lumen of the balloon catheter (SPAP) into the left pulmonary artery or through the central venous line (i.v. administration) using an infusion pump. Tissue samples of the lung were obtained from the left lower lobe and stored in liquid nitrogen. At the end of the experiments, liver samples were obtained through an opening in the right hemidiaphragm.

Histology. After fixation in chloroform calcium at 90 min at room temperature, lung tissue was stored in a buffer (10 ml of distilled H2O, 1 g of CaCl2, 0.121 M cacodylate) at 4°C until further processing. Tissue samples for light microscopic investigations were dehydrated with isopropanol, cleared with toluol, and embedded in paraffin wax. Four-micrometer sections were stained with H&E for later assessment.

Gemcitabine. Gemcitabine (difluorodeoxycytidine) (Ely Lilly, Indianapolis, IN) solutions were prepared by reconstituting nonlyophilized powder in saline solution. All the animals were treated with gemcitabine in a dose and volume (1000 mg/m² body surface area, solved in 50 ml of 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 (difluorodeoxycytidine) in plasma, lung, and liver tissue. Standard samples of blank plasma were spiked with gemcitabine (100–100,000 ng) and extracted in the same way as the other samples and used for a calibration curve (De Boeck et al., 1997). Within-run and between-run precisions were less than 10%, and average accuracies were between 90 and 110%. Before analysis, the frozen tissue and serum samples are mixed with tetrahydrouridine to prevent metabolization by cytidine deaminase.

Gemcitabine Assay by High-Performance Liquid Chromatography/UV. Separation was achieved on a Chrompack Spherisorb ODS-2 reversed-phase column (25 × 4.6 mm, 5 μm). The mobile phase used was Pic B7 reagent (Waters, Milford, MA) 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 the concentrations and wet-to-dry ratios shown in this article are depicted as median ± S.E. Lung and serum concentrations are determined...
in function of time and calculated as areas under the curve (AUCs). The AUC values and the median concentrations at each single time point were compared between the different groups using analysis of variance 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) (Fig. 1A). All the groups received gemcitabine in a dose of 1 g/m², solved in a volume of 50 ml of saline.

To determine the optimal infusion time for SPAP, two groups underwent SPAP with a normal pulmonary artery blood flow for 10 min and for 2 min, resulting in 6 and 30 times higher drug concentrations delivered at the tip of the catheter, respectively, compared with i.v. infusion. A control group was treated i.v. according to a clinically applied regimen for the treatment of NSCLC, and gemcitabine was infused over a period of 30 min.

Two more groups underwent SPAP for 2 min with 50 and 90% flow reduction within the pulmonary artery as checked with the flow probe around the pulmonary artery, resulting in 60 and 300 times higher drug levels delivered at the tip of the catheter, respectively, compared with i.v. infusion. After SPAP for 2 min 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.

Liver samples and lung tissue were taken at 45 min after start of infusion. Liver samples and lung tissue were taken at 45 min for concentration analysis, wet-to-dry ratio measurements, and histology, respectively.

**Results**

In lung tissue, SPAP for 2 and 10 min resulted in significantly higher AUC when compared with i.v. infusion ($p = 0.001$), and SPAP for 10 min resulted in the highest lung AUC compared with SPAP for 2 min ($p = 0.045$) (Fig. 2B). In addition, the peak concentration of gemcitabine within the lung tissue was significantly higher after SPAP for 2 min when compared with i.v. administration ($p = 0.01$) (Fig. 2A).

Within the serum, the AUC was not significantly different between SPAP for 2 and 10 min and i.v. therapy (Fig. 3B). The peak concentration of gemcitabine within the serum was significantly higher after SPAP for 2 min compared with i.v. infusion ($p = 0.004$) (Fig. 3A).

Flow reduction during SPAP for 50 and 90% did not result in a significant different lung (Fig. 4, A and B) and serum (Fig. 5, A and B) AUC compared with SPAP without flow reduction. However, the S.D. 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) (Fig. 4, A and B). Liver concentrations of gemcitabine ($11.4 \pm 1.4 \mu g/g$) and wet-to-dry ratios ($8.3 \pm 0.5$) did not significantly differ between the five groups when determined at 45 min after the start of infusion.

Histologic examination of lung tissue after SPAP with gemcitabine suggests evidence of slight alveolar hyperplasia, which was more pronounced in the flow reduction groups with evident moderate congestion. No alveolar hyperplasia was present in the i.v. group (Fig. 6). No abnormalities were observed in the slight sections of the pulmonary artery in either the SPAP or the i.v. group. Physiologic functions like heart rate and systemic and pulmonary blood pressure did not change significantly during and after SPAP.

**Discussion**

To the best of our knowledge, this is the first report of a model of SPAP without complete blood flow occlusion using an endovascular catheterization technique that combined the properties of isolated lung perfusion and i.v. administration to improve the current treatment of NSCLC with minimal side effects and to achieve down-staging of stage 3a and 3b NSCLC. This approach resulted in significantly higher lung levels and equivalent serum and liver levels compared with the generally accepted i.v. route of cytostatic drug administration, whereas physiological functions did not change.

This study evaluated infusion time of SPAP and pulmonary artery flow reduction during SPAP and compared the results with i.v. injection of gemcitabine. Clinically, gemcitabine is administered in a dose of 1 g/m² solved in 50 ml of isotonic solution through an extremity infusion system during 30 min for the treatment of NSCLC. The infusion rate clinically applied is limited because of the occurrence of local toxicity at higher rates. In this study, gemcitabine dose and solvent volume were equal in all the groups.

All the SPAP groups showed significantly higher lung AUC and peak levels compared with i.v. infusion. First, this observation is partially explained by a dilutional effect. In contrast to i.v. infusion, SPAP is characterized by infusion of the left or right pulmonary artery, resulting in a 2 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 6 (SPAP, 10 min) and 30 times (SPAP, 2 min) higher local blood concentrations compared with i.v. infusion. Therefore, SPAP resulted in significantly higher peak pulmonary concentrations and AUCs. 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 plasma AUC levels that are in the same range compared with i.v. infusion.

However, pulmonary peak concentrations after SPAP for 2 min without blood flow occlusion were only 5 times higher compared with
i.v. infusion, whereas local blood concentrations delivered at the tip of the catheter during SPAP were 30 times higher. The most reasonable explanations are saturation of the first-pass capacity of the lung at 2-min infusion time or a too-short uptake interval. Interestingly, SPAP for 10 min resulted in either 6 times higher lung and 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 with SPAP for 2 min. In our opinion, SPAP for 10 min seems to be the most efficient strategy in saturating the lung because the time interval of the peak concentration after SPAP for 2 min is too short to achieve intracellular saturation.

Important first-pass capacity of the lung was shown in a former rat study in our laboratory (van Putte et al., 2003b). Significantly higher gemcitabine lung levels were achieved after pulmonary artery perfusion without control of the pulmonary veins, whereas significantly less systemic toxicity was observed compared with i.v. injection of even a higher dose during the same infusion interval (van Putte et al., 2003b).

Reduction of blood flow during SPAP up to 50 and 90% was applied and compared with normal blood flow 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 with normal blood flow, whereas 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 S.D. (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 to maintain pulmonary artery pressure, finally resulting in inhomogeneous distribution of the drug infused.

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 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 furthermore treating the systemic disease in an equivalent manner as i.v. therapy does. Furthermore, these studies are of limited significance probably because of inhomogeneous drug distribution.

First, Karakousis et al. (1981) performed Schwann-Ganz catheterization of lobe branches separately under fluoroscopic control in seven patients with recurrent pulmonary metastases who had already received the maximum dose of adriamycin. They received a dose of 10 to 20 mg diluted in 50 ml infused over a 1- to 2-min period followed by 5 min of blood flow occlusion. Fifty-six injections were given in lobar arteries in the seven patients treated. After metastasectomy, three patients were disease-free after 4 months, whereas 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 standardized between the patients, resulting in high variability of the intravascular drug concentration delivered.
Furthermore, they applied blood flow occlusion 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 (Karakousis et al., 1981).

Second, Furrer et al. (1998) compared endovascular pulmonary artery perfusion during blood flow occlusion with isolated lung perfusion and i.v. infusion of doxorubicin. Both selective techniques resulted in significantly higher lung concentrations and lower serum levels compared with i.v. administration. However, pulmonary artery perfusion was performed during blood flow occlusion that should have resulted in inhomogeneous distribution. Furthermore, they achieved lower serum levels compared with i.v. injection, whereas we believe that maximal systemic exposure during SPAP is essential to treat (micro)metastatic disease outside the lungs in lung cancer (Karakousis et al., 1981).

Third, Brown et al. (2006) recently published their results on SPAP during blood flow occlusion compared with i.v. infusion of cisplatin using a swine model. They concluded that no relation was observed between the infusion time and inflow concentration compared with the final lung tissue concentrations. They showed a very wide range (6.63–76.78 fmol/µg) of final lung concentrations, probably as a result of inhomogeneous drug distribution as shown in our study in a pulmonary blood flow reduction experiment (Fig. 3A) (Brown et al., 2006).

However, based on this article we modified the hypothesis from treating lung metastases using this catheter technique toward the hypothesis to achieve down-staging of the T and N status of primary lung cancer. In our study, we showed high peak concentrations in lung tissue after SPAP that were significantly higher compared with the i.v.-treated animals, suggesting (not tested in this study) that more efficacy could be achieved.

Furthermore, our study shows a rapid washout phenomenon after peak pulmonary concentrations were achieved at the end of the SPAP procedure (Figs. 2A and 4A). In a very recent rat study, we reported that a major part of the drug taken up during isolated lung perfusion is quickly exchanged into the circulation during the washout interval and during reperfusion (van Putte et al., 2006). 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 because of a too-short uptake interval. In the same article, we achieved stabilization of high lung peak levels after isolated lung perfusion by delayed restoration of normal blood circulation up to 30 min (van Putte et al., 2006). Further studies are necessary to confirm these findings in this endovascular SPAP model.

Patients suffering from NSCLC die as a result of local pulmonary or distant recurrences in 25 and 75%, respectively (Rivera et al., 2001). 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 to improve the current treatment of NSCLC with minimal side effects and to achieve down-staging of stage 3a and 3b NSCLC. This technique is characterized by the superior pharmacokinetic properties of isolated lung perfusion resulting in high local lung and lymph node drug levels and by the properties of i.v. infusion to achieve total body exposition. As shown in Figs. 2 and 3, significantly higher lung levels were achieved after SPAP, whereas plasma and liver levels did not significantly differ compared with i.v. infusion.

Interestingly, a significant portion of both lymph node and pulmonary tumor vasculature is fed by the pulmonary arterial circulation (Miller and Rosenbaum, 1967; Mochizuki et al., 2000). Therefore, patients suffering from stage 1, 2, and 3 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) to achieve down-staging of stage 3 NSCLC toward a surgical stage. Third, systemic serum and liver levels equivalent to i.v. therapy will treat systemic disease in the same way as achieved in currently applied i.v. schedules for NSCLC.

The currently applied i.v. schedule for the treatment of NSCLC consists of a combination of gemcitabine and cisplatin, resulting in synergistic activity (Bergman et al., 1996; Peters et al., 2006). Future studies have to find out whether coadministration of platin-based drugs to this SPAP gemcitabine model will be feasible. Subsequently, these results have to be validated in a phase I study because of the lack of large animal tumor models.

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

Acknowledgments. We thank Ellen Tromp for reviewing statistics. We also thank Cees Verlaan, Maringa Emoms, and Merel Schurink for excellent biotechnical support.

References

Address correspondence to: B. P. van Putte, Department of Cardiothoracic Surgery, Sint Antonius Hospital, Koekoekslaan 1, Nieuwegein, The Netherlands.
E-mail: bvanputte@yahoo.com