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ORIGINAL ARTICLE

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Variability of cyclophosphamide uptake into human bronchial carcinoma: consequences for local bioactivation

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Abstract *Purpose*: The alkylating cytostatic prodrug cyclophosphamide is bioactivated by the human cytochrome P450 enzyme system. Since these enzymes are not only expressed in human liver, but also in extrahepatic tissue, local bioactivation of this drug may play an important role in its antineoplastic effects, e.g., chemotherapy of lung tumors. This would require uptake of significant amounts of cyclophosphamide into tumor tissue, which has not yet been demonstrated. *Methods*: We used a recently developed, ex vivo isolated, ventilated and perfused human lung model to study cyclophosphamide uptake into bronchial carcinoma and healthy lung tissue. Following a standard lobectomy, lung samples containing the tumor were perfused with buffer containing 2 m*M* cyclophosphamide for 2 h. Cyclophosphamide concentrations in perfusate and healthy peripheral tissue were measured during the perfusion and in tumors at the end of perfusion. *Results*: In all tissue samples, cyclophosphamide uptake was relatively poor, indicated by a tissue to perfusate ratio of 0.021. Moreover, in tumor samples, cyclophosphamide concentrations were significantly lower ($P < 0.05$) than in healthy lung tissue and showed pronounced interindividual variability. Median concentrations were 36.8 μg/g (26.9–44.2 μg/g) in healthy tissue and 5.1 μg/g (0.0–26.8 μg/g) in tumor samples. Tumor cyclophosphamide concentrations varied between 0 and 75% of those reached in healthy tissue. *Conclusions*: Our results indicate that CP tumor concentrations are modulated by factors different from dose and that expression of bioactivating enzymes in human lung or transfection of genes encoding these enzymes into tumor cells does not necessarily lead to local bioactivation of cyclophosphamide.

Key words Cyclophosphamide · Human lung tissue · Pharmacokinetics

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Introduction

The lack of tumor selectivity is one of the major limitations of cancer chemotherapy. To improve tumor response, doses of cytostatics have been escalated in the last years, resulting in multiple dose-limiting adverse effects [26]. Among various strategies to improve drug targeting, the use of non-cytotoxic prodrugs, which are mainly activated at the tumor site, appears to be promising.

One of the most common prodrugs is the oxazaphosphorine cytostatic cyclophosphamide, which is widely used in chemotherapy of various malignancies. It is well established that the parent compound is bioactivated, and also deactivated, by the cytochrome P450 (CYP) enzyme system in human liver. The first step, a 4-hydroxylation (ring oxidation), which is catalyzed by CYP 2B6, 2C, and to a lesser extent by CYP 3A [6], leads to the formation of 4-hydroxycyclophosphamide. This compound is in equilibrium with its ring-opened tautomer aldophosphamide, which spontaneously decomposes to yield phosphoramide mustard, the ultimate alkylating agent, and the urotoxic compound acrolein. Besides bioactivation, there are two other pathways that lead via oxidation of 4-hydroxycyclophosphamide or aldophosphamide to the inactive metabolites 4-ketocy-

clophosphamide and carboxyphosphamide, respectively [8, 10, 12]. Cyclophosphamide inactivation is also achieved by CYP 3A-mediated side-chain oxidation [2], yielding dechloroethylcyclophosphamide and the neurotoxic metabolite chloroacetaldehyde.

Cyclophosphamide is used in the chemotherapy of non-small cell lung cancer [11]. It is reported that the therapeutic effect of cyclophosphamide in extrahepatic tissue is achieved by the transportation of 4-hydroxycyclophosphamide/aldophosphamide to the target organ following cyclophosphamide ring oxidation in the human liver [9]. Because of the short plasma half-life of 15–20 min [22] for the free circulating metabolite and its stabilization by strong protein binding [23], it is questionable whether 4-hydroxycyclophosphamide/aldophosphamide could pass a number of biological membranes to eventually reach a tumor in a target organ far from liver.

However, it is well established that some of the enzymes involved in cyclophosphamide metabolism (CYP 2C, CYP 3A5, and to a lesser extent CYP 3A4) are expressed in human lung and bronchial carcinoma [1, 13, 20]. Therefore, local bioactivation of parent compound rather than transport of the active metabolite may contribute to the therapeutic effects of cyclophosphamide in lung tumors. New strategies for combined chemotherapy/gene therapy include the transfection of genes encoding CYPs involved in cyclophosphamide metabolism (e.g., CYP 2B6 gene) into tumor cells [7]. In both instances (bioactivation by enzymes expressed in situ or gene transfer), an uptake of significant amounts of cyclophosphamide into tumor tissue is a pivotal question.

To investigate cyclophosphamide uptake into healthy tissue and tumor, we therefore used a recently developed, ex vivo isolated, ventilated and perfused human lung model [14]. The applicability of this model has been shown for measurement of doxorubicin uptake from a glucuronide prodrug [19]. Whereas doxorubicin uptake has also been studied in various ex vivo or in vivo animal models (e.g., [3, 17]), there are no data available concerning the concentration of cyclophosphamide in solid tumors.

Materials and methods

Chemicals

All solvents used were of high-performance liquid chromatography (HPLC) quality; chemicals were of analytical grade. Cyclophosphamide monohydrate was obtained from ICN (Meckenheim, Germany). Sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2 * 2H_2O$), magnesium chloride ($MgCl_2 * 6H_2O$), monopotassium phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), glucose, and albumin were from Sigma (Deisenhofen, Germany).

Patients and preparation of lung samples

Six patients with bronchial carcinoma underwent a standard thoracotomy. Before the operation, each patient signed a written informed consent. The use of the resected lung samples for perfusion was approved by a local ethics committee. Details of patients and tumor characteristics are listed in Table 1. The patients' average age was 55.2 ± 6.0 years.

Perfusion experiments

The lung preparations were perfused as described previously [15, 19]. The experimental setup is depicted in Fig. 1. In summary, immediately after the operation, arteries were cannulated and the bronchus was connected to a bronchial tubing. The lung was placed in a heated waterbath (37 °C), ventilated with air and CO_2 using an Engström Erica 2 respirator (Engström Elektromedizin GmbH, Munich, Germany), and rinsed with 1.5 l perfusion buffer pH 7.4 consisting of 85 m*M* NaCl, 4.0 m*M* KCl, 1.0 m*M* $MgCl_2$, 2.5 m*M* $CaCl_2$, 2.5 m*M* KH_2PO_4, 25 m*M* $NaHCO_3$, 5.5 m*M* glucose, and 5% albumin. Cyclophosphamide was added to the buffer to reach a concentration of 2 m*M* (522 µg/ml). Out of a reservoir that was kept at 37 °C, the buffer was pumped through a heat exchanger, a blood filter, a bubble trap, and a valve into one to three segmental arteries. The perfusate leaving the open veins was collected and flowed back into the reservoir. The buffer flow rate was 300 ml/min.

During the perfusion, pH, pO_2, pCO_2, K^+ and Na^+ were continuously measured as parameters for the system's stability using an Eschweiler system 2000-D03 (L. Eschweiler & Co., Kiel, Germany).

Edema formation during the perfusion was controlled by weighing the lung preparations directly before and after perfusion.

Sample preparation

Samples from perfusate (2 ml) and peripheral tissue (1 g) from the surface of the specimen were taken directly before the perfusion was started ($t = 0$) and during the perfusion every 10 min for 2 h. They were immediately frozen in liquid nitrogen. Following the perfusion, lung tumors were investigated by a pathologist, and samples were cut from tumor, surrounding tissue, healthy tissue and lymph nodes ($n = 3$) and frozen in liquid nitrogen. All samples were stored at −80 °C until they were analyzed.

Determination of cyclophosphamide concentrations in tissue and perfusate using HPLC

Frozen tissue samples were homogenized using a Mikrodismembrator S from Braun Biotech International (Melsungen,

Table 1 Characteristics of patients, tumors and lung preparations

No.	Sex	Age (years)	Weight gain during perfusion	Tumor type	Tumor vascularization[a]
1	Female	50	16.2%	Squamous cell carcinoma	+ + +
2	Male	57	0.0%	Adenosquamous carcinoma	+ + +
3	Female	48	33.3%	Adenocarcinoma	+ +
4	Male	57	74.9%	Squamous cell carcinoma	+ +
5	Male	65	26.6%	Squamous cell carcinoma	+
6	Male	54	16.8%	Adenocarcinoma	+

[a] Poor (+), moderate (+ +) or high (+ + +) vascularization

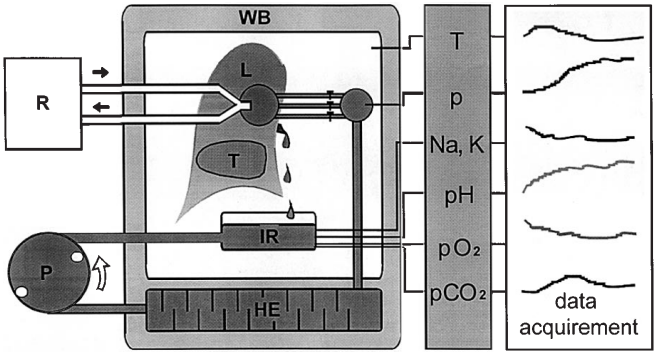

Fig. 1 Perfusion set-up and tubing system (*L* lung preparation, *WB* water bath, *P* perfusion pump, *HE* heat exchanger, *IR* intermediate reservoir, *R* respirator, *T* tumor). Modified from [15]

Germany) and thereafter suspended in albumin-free perfusion buffer. The samples were stored at −80 °C until analysis. Immediately before the analyses were started, samples were carefully thawed on ice. To an aliqout of 25 µl and 100 µl of perfusate and tissue homogenate, respectively, the internal standard ifosfamide 125 µg in 25 µl was added, and the mixture was extracted with 5 ml acetic acid ethylester by shaking overhead for 10 min. After centrifugation for 10 min at 4000 rpm, the organic phase was aspirated off, transferred to a conical tube, evaporated to dryness in a stream of nitrogen, and the residue dissolved in 100 µl of water. Fifty microliters of the extract was then analyzed by HPLC. The HPLC system consisted of a 110B pump from Beckman (Munich, Germany), a 785A variable wavelength UV detector from Abimed (Düren, Germany), and an SIL 9A auto injector from Shimadzu (Duisburg, Germany). HPLC was performed on a 125 × 4-mm Nucleosil-100 C18 (5 µm) column (Macherey & Nagel, Düren, Germany) with a 20 × 4-mm precolumn filled with the same packing material. The mobile phase consisted of acetonitril-water (12:88 v/v), the flow rate was 1.0 ml/min. The detector was set at 195 nm. Under these conditions, the retention times for ifosfamide and cyclophosphamide were 32.8 and 36.4 min, respectively. Calibration curves were prepared in the same manner as the samples by spiking the perfusion buffer with cyclophosphamide. Curves were linear over the entire concentration range investigated (0.5–50 µg/ml). Intra-day and inter-day variation were controlled during each sample series by analyzing quality controls. The coefficients of variation were less than 12.5% for 0.5 µg/ml (limit of quantification) and less than 6% for 10 and 25 µg/ml, respectively. CP concentrations in tissue are expressed as micrograms per gram of tissue.

Immunohistochemical staining for factor VIII

The immunohistochemical determination of factor VIII was used to measure the tumor vascularization. Immunostaining was performed by the avidin-peroxidase technique [13]. Factor VIII antibody (polyclonal rabbit antihuman von Willebrand factor from Dako Diagnostika GmbH, Hamburg, Germany) was diluted 1:400. Paraffin sections were pretreated with 0.1% pronase, and peroxidase was developed with diaminobenzidine/H_2O_2. The samples were then visually classified into poorly, moderately and highly vascularized tumors by a pathologist.

Statistical analysis

All statistical calculations were performed with GraphPad Prism (GraphPad Software, Inc., San Diego, Calif., USA). Concentrations in tumor vs healthy tissue were compared with paired *t*-test using an a priori significance level of 0.05.

Results

Quality of lung perfusions

For estimation of the stability of the lung preparations and the quality of the perfusion experiments, several physiological parameters described in the Materials and Methods section (pH, pO_2, pCO_2, K^+ and Na^+) were continuously monitored during the perfusion. All six experiments were included in the study, since physiological and biochemical conditions were similar to those in vivo. The system was stable over the whole period of time investigated. The mean net weight gain during the perfusion was 28.0 ± 25.6% for the six lung preparations investigated.

Cyclophosphamide concentration in the perfusate

The decline of cyclophosphamide in the perfusate during the perfusion is depicted in Fig. 2A. Cyclophosphamide concentrations decreased rapidly during the first minutes of the perfusion and reached a plateau level after 30–40 min.

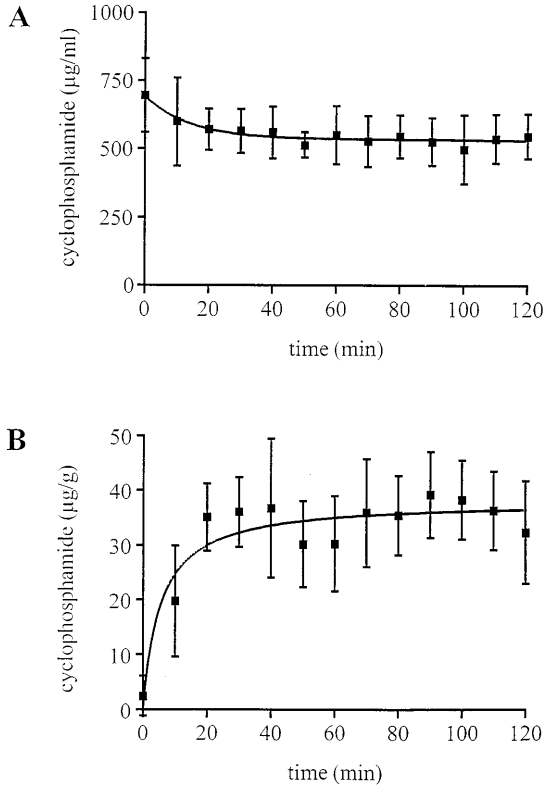

Fig. 2 **A** Cyclophosphamide concentrations in perfusate during perfusion of six lungs with buffer containing 2 m*M* (522 µg/ml) cyclophosphamide (mean ± SD). **B** Cyclophosphamide uptake into healthy peripheral tissue during perfusion of six lungs with buffer containing 2 m*M* (522 µg/ml) cyclophosphamide (mean ± SD)

Cyclophosphamide uptake into healthy tissue during perfusion

In the same manner as the cyclophosphamide concentration in the perfusate decreased, the amount of the drug in the peripheral tissue increased during the perfusion, as shown in Fig. 2B. Following a rapid increase in the first 20 min, a maximum is reached after 30–40 min. The final concentrations of cyclophosphamide averaged 32.5 ± 9.4 μg/g.

Cyclophosphamide uptake into different kinds of tissue

Since it was not possible to obtain tumor tissue during the perfusion, these samples were collected after the end of the perfusion. At the same time, samples of tumor-surrounding tissue ($n = 6$), healthy peripheral lung tissue ($n = 6$), and of lymph nodes ($n = 3$) were taken. Cyclophosphamide concentrations (median) were determined as 36.8 μg/g (26.9–44.2 μg/g) in healthy tissue, 23.4 μg/g (6.6–43.3 μg/g) in tumor-surrounding tissue, 5.1 μg/g (0.0–26.8 μg/g) in tumor tissue, and 31.3 μg/g (21.0–44.3 μg/g) in lymph nodes. The individual cyclophosphamide concentrations in tissue samples are depicted in Fig. 3.

Cyclophosphamide concentrations in tumor samples were significantly lower than those in healthy tissue ($P < 0.05$) and showed pronounced variability. The percentage ranged from 0.0 to 75.2% of the concentrations reached in healthy tissue. The area under the cyclophosphamide concentration-time curve (AUC) of the peripheral tissue samples taken during the perfusion did not correlate with the cyclophosphamide concentrations reached in the corresponding tumor samples ($r^2 = 0.263$, $P > 0.2$). The ratio of concentrations of cyclophosphamide in healthy lung tissue vs perfusion buffer averaged 0.021 ± 0.010 (0.013–0.040).

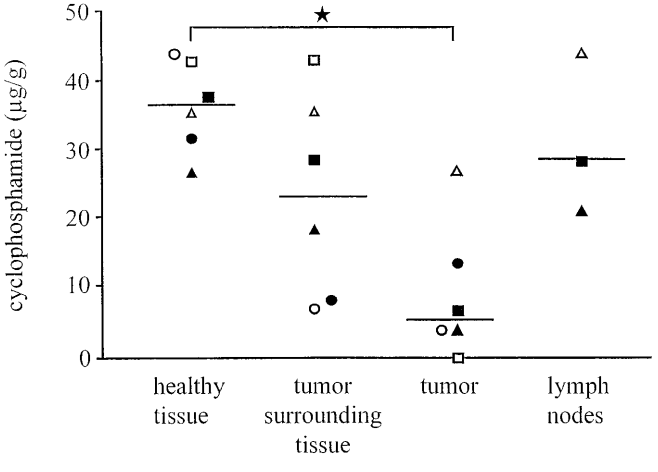

Fig. 3 Cyclophosphamide concentrations of individual tissue samples and median after lung perfusion with buffer containing 2 m*M* (522 μg/ml) cyclophosphamide. Each symbol represents one lung preparation (○, no. 1; □, no. 2; ●, no. 3; □, no. 4; ■, no. 5; △, no. 6); *$P < 0.05$

Using immunohistochemical staining for factor VIII, tumor samples were classified into poorly, moderately, and highly vascularized tumors (see Table 1). However, there was no detectable relation between vascularization and cyclophosphamide tumor concentrations.

Cyclophosphamide concentrations in lymph nodes were similar to those of peripheral tissue, whereas tumor-surrounding tissue showed extremely variable cyclophosphamide uptake (see Fig. 3).

Discussion

It is generally assumed that plasma drug concentrations are in equilibrium with the corresponding tissue concentrations. Although the ratio blood/tissue may differ depending on the particular drug, the tissue concentration achieved should be related to blood concentrations and should vary only within a rather narrow range. Many solid tumors that are sensitive to a specific drug in vitro show no or only minor response to the same drug in vivo. One reason for the poor response in vivo could be a restricted drug uptake into tumor tissue.

In this article, we present data on cyclophosphamide uptake into human bronchial carcinoma and healthy lung tissue following perfusion with 2 m*M* of the drug, which is in the range of clinically achieved plasma concentrations in high-dose anticancer chemotherapy [5]. A minor fraction of cyclophosphamide was taken up rapidly into healthy tissue during perfusion. Moreover, drug uptake into lung tumors was significantly lower, resulting in a median of less than 15% of that reached in peripheral tissue. As a consequence, tumor cyclophosphamide concentrations were below 1% of those in perfusate in five of six lung samples. In one case, no cyclophosphamide could be detected in the tumor. Our data further indicate that cyclophosphamide uptake into tumor tissue was highly variable, resulting in concentrations of between 0 and 75% of those reached in healthy tissue.

Moreover, tumor cyclophosphamide concentrations could not be predicted by physiological (e.g., vascularization) or pharmacokinetic parameters. There was no correlation of the AUC of peripheral tissue with tumor concentrations of cyclophosphamide observed. Furthermore, we determined tumor vascularization with immunohistochemical staining for factor VIII, since drug uptake may depend to a great extent on tumor vascularization and blood flow, which are known to be highly variable [24, 25]. However, there was no obvious relation between tumor cyclophosphamide concentrations and vascularization of the tumor, indicating that there are additional factors that modulate cyclophosphamide concentrations in tumor tissue.

The tumor-surrounding tissue showed the highest variability in cyclophosphamide concentrations, ranging between 15 and 100% of the concentrations reached in

peripheral tissue. In contrast, cyclophosphamide concentrations in lymph nodes were similar to those of peripheral tissue, indicating that the distribution of cyclophosphamide in the lymphatic system is the same as in healthy lung tissue.

In general, chemotherapy in patients with bronchial carcinoma has a poor outcome. One reason for this may be limited drug exposure of the tumor owing to insufficient drug targeting. In fact, a recent clinical trial investigating the intratumoral pharmacokinetics of 5-fluorouracil showed that the response of cancer chemotherapy of solid tumors is dependent on the drug concentration in the tumor [20]. Comparable investigations are rare because of lack of a suitable model. The isolated perfused human lung model recently developed and validated in our laboratories allows the investigation of drug uptake into both healthy lung tissue and lung tumors ex vivo [15, 19]. With this system, it is possible to perfuse a human lung preparation immediately after lobectomy for up to 3 h under physiological and biochemical conditions that approximate those in vivo.

In a recent study, we have shown that bioactivation of cyclophosphamide in man is highly variable [5], indicating that therapeutic drug monitoring would be a useful tool for the optimization of individual therapy. Similar to a previous investigation by Boyd et al. [4], Busse and coworkers used nuclear magnetic resonance (NMR) technology to monitor cyclophosphamide and metabolites. The data from the present study indicate that even under conditions of constant peripheral cyclophosphamide concentrations, tumor uptake of the drug is extremely variable. As a consequence, it is obvious that the determination of plasma levels of cyclophosphamide for therapeutic drug monitoring is not very useful for estimation of the efficacy of cyclophosphamide chemotherapy.

Similar conclusions were obtained from Müller et al. [18] who studied the clinical response as a function of 5-fluorouracil pharmacokinetics in the interstitial tumor space in breast cancer patients. The authors observed no association of subcutaneous or plasma AUC with tumor response, while an increased interstitial tumor AUC lead to a higher response of 5-fluorouracil chemotherapy. Thus plasma concentrations of cytostatics do not directly translate into drug action, since the transport from plasma into the tumor is an important yet unpredictable factor for the response of chemotherapy.

Cyclophosphamide bioactivation is mediated by the human cytochrome P450 enzyme system in liver. The major enzyme is CYP 2B6, whereas CYP 2C and 3A are involved to a lesser extent. We and others have shown that some of these enzymes (CYP 2C and CYP 3A) are also expressed in human lung and in bronchial carcinoma (CYP 3A5) [1, 14, 21]. Therefore, local bioactivation may play an important role in chemotherapy of these malignancies. However, in view of the rather limited concentration of the parent compound at the tumor site, no major contribution of local bioactivation can be expected.

Recently, new strategies have been developed that combine gene therapy and conventional chemotherapy [7, 16]. These strategies include the transfection of genes encoding the cyclophosphamide bioactivating enzymes, usually CYP 2B1, into tumor cells. These cells, which normally do not activate cyclophosphamide or its isomer ifosfamide, are sensitized to oxazaphosphorine therapy following gene transfection. Such therapy would only be successful if significant amounts of the prodrug are taken up into the tumor tissue, which, based on our data, appears to be questionable. As a consequence, only a limited number of patients with non-small cell bronchial carcinoma would benefit from a combined gene-/chemotherapy.

In summary, our results show that tumor concentrations of cyclophosphamide following perfusion of lobectomy preparations with buffer containing 2 m*M* of the drug are modulated by factors different from the dose administered. Our results further indicate that poor uptake of cyclophosphamide into tumors could be one explanation for the insufficient response of some classes of tumors to cyclophosphamide chemotherapy. Similarly, the interindividual variation in the chemotherapy response of similar tumors may result from variable uptake of the drug into tumor tissue.

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References

1. Anttila S, Hukkanen J, Hakkola J, Stjernvall T, Beaune P, Edwards RJ, Boobis AR, Pelkonen O, Raunio H (1997) Expression and localization of CYP3A4 and CYP3A5 in human lung. Am J Respir Cell Mol Biol 16: 242
2. Bohnenstengel F, Hofmann U, Eichelbaum M, Kroemer HK (1996) Characterization of the cytochrome P450 involved in side-chain oxidation of cyclophosphamide in humans. Eur J Clin Pharmacol 51: 297
3. Bongard RD, Roerig DL, Johnston MR, Linehan JH, Dawson CA (1993) Influence of temperature and plasma protein on doxorubicin uptake by isolated lungs. Drug Metab Dispos 21: 428
4. Boyd VL, Robbins JD, Egan W, Ludeman SM (1986) 31P nuclear magnetic resonance spectrometric observation of the intracellular transformations of oncostatic cyclophosphamide metabolites. J Med Chem 29: 1206
5. Busse D, Busch FW, Bohnenstengel F, Eichelbaum M, Fischer P, Opalinska J, Schumacher K, Schweizer E, Kroemer HK (1997) Dose escalation of cyclophosphamide in patients with breast cancer: consequences for pharmacokinetics and metabolism. J Clin Oncol 15: 1885
6. Chang TK, Weber GF, Crespi CL, Waxman DJ (1993) Differential activation of cyclophosphamide and ifosphamide by cytochromes P-450 2B and 3 A in human liver microsomes. Cancer Res 53: 5629
7. Chen L, Waxman DJ (1995) Intratumoral activation and enhanced chemotherapeutic effect of oxazaphosphorines following cytochrome P-450 gene transfer: development of a combined chemotherapy/cancer gene therapy strategy. Cancer Res 55: 581
8. Connors TA, Cox PJ, Farmer PB, Foster AB, Jarman M (1974) Some studies of the active intermediates formed in the micro-

somal metabolism of cyclophosphamide and isophosphamide. Biochem Pharmacol 23: 115
9. Domeyer BE, Sladek NE (1980) Kinetics of cyclophosphamide biotransformation in vivo. Cancer Res 40: 174
10. Domeyer BE, Sladek NE (1980) Metabolism of 4-hydroxycyclophosphamide/ aldophosphamide in vitro. Biochem Pharmacol 29: 2903
11. Eagan RT (1994) Management of regionally advanced (stage III) non-small cell lung cancer. LCSG 831. Chest 106: 340S
12. Fenselau C, Kan MNN, Rao SS, Myles A, Friedman OM, Colvin M (1977) Identification of aldophosphamide as a metabolite of cyclophosphamide in vitro and in vivo in humans. Cancer Res 37: 2538
13. Hsu SM, Raine L, Fanger H (1981) The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. Am J Clin Pathol 72: 816
14. Kivistö KT, Griese EU, Fritz P, Linder A, Hakkola J, Raunio H, Beaune P, Kroemer HK (1996) Expression of cytochrome P450 3A enzymes in human lung: a combined RT-PCR and immunohisto-chemical analysis of normal tissue and lung tumours. Naunyn-Schmiedebergs Arch Pharmacol 353: 207
15. Linder A, Friedel G, Kivistö KT, McClellan M, Toomes H (1996) The ex-vivo isolated, perfused human lung model: description and potential applications. Thorac Cardiovasc Surg 44: 140
16. Manome Y, Wen PY, Chen L, Tanaka T, Dong Y, Yamazoe M, Hirshowitz A, Kufe DW, Fine HA (1996) Gene therapy for malignant gliomas using replication incompetent retroviral and adenoviral vectors encoding the cytochrome P450 2B1 gene together with cyclophosphamide. Gene Ther 3: 513
17. Minchin RF, Johnston MR, Aiken MA, Boyd MR (1984) Pharmacokinetics of doxorubicin in isolated lung of dogs and humans perfused in vivo. J Pharmacol Exp Ther 229: 193
18. Müller M, Mader RM, Steiner B, Steger GG, Jansen B, Gnant M, Helbich T, Jakesz R, Eichler HG, Blocht-Daum B (1997) 5-Fluorouracil kinetics in the interstitial tumor space: clinical response in breast cancer patients. Cancer Res 57: 2598
19. Mürdter TE, Sperker B, Kivistö KT, McClellan M, Fritz P, Friedel G, Linder A, Bosslet K, Toomes H, Dierkesmann R, Kroemer HK (1997) Enhanced uptake of doxorubicin into bronchial carcinoma: β-glucuronidase mediates release of doxorubicin from a glucuronide prodrug (HMR 1826) at the tumor site. Cancer Res 57: 2440
20. Presant CA, Wolf W, Waluch V, Wiseman C, Kennedy P, Blayney D, Brechner RR (1994) Association of intratumoral pharmacokinetics of fluorouracil with clinical response. Lancet 343: 1184
21. Shimada T, Yamazaki HMM, Wakamiya N, Ueng YF, Guengerich FP, Inui Y (1996) Characterization of microsomal cytochrome P450 enzymes involved in the oxdiation of xenobiotic chemicals in human fetal livers and adult lungs. Drug Metab Dispos 24: 515
22. Voelcker G, Haeglsperger R (1982) Die Pharmakokinetik von Cyclophosphamid und Cyclophosphamid-Metaboliten bei der Maus und ihr Einfluß auf die therapeutische Wirkung von "aktiviertem" Cyclophosphamid (4-Hydroxycyclophosphamid). Arzneim ittelforschung 32: 639
23. Voelcker G, Giera HP, Jäger L, Hohorst HJ (1978) Zur Bindung von Cyclophosphamid und Cyclophosphamid-Metaboliten an Serum-Albumin. Z Krebsforsch Klin Onkol 91: 127
24. Warren BA (1979a) The vascular morphology of tumors. In: Peterson HI (ed) Tumor blood circulation. CRC Press, Boca Raton, p 1
25. Warren BA (1979b) Tumor angiogenesis. In: Peterson HI (ed) Tumor blood circulation. CRC Press, Boca Raton, p 49
26. Zorsky PE, Perkins JB (1993) Optimizing high-dose therapy using pharmacokinetic principles. Semin Oncol 20: 2