P-Glycoprotein Modulation by Valspodar and Cyclosporin Does Not Increase Tumor Uptake of Doxorubicin Administered *via* Isolated Lung Perfusion to Rats Bearing Sarcoma Lung Metastases

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Abstract. *Background: Isolated lung perfusion (ILP) with doxorubicin allows a regional increase in drug exposure while sparing unaffected tissues, but clinical results have so far been disappointing, presumably in part because of the limited tumor penetration of doxorubicin. The aim of this study was to assess whether tumor uptake of doxorubicin, administered locoregionally by ILP, would be increased by the administration of P-glycoprotein (P-gp) modulators. Materials and Methods: Single-pass antegrade ILP (A-ILP) was performed with doxorubicin in rats bearing a pulmonary sarcoma nodule which were either untreated or received P-gp inhibitors cyclosporin, valspodar or the vehicle, Cremophor®, only. Doxorubicin concentrations in tumor, lung and effluent were measured by high performance liquid chromatography (HPLC) coupled to spectrofluorimetric detection and the expression of P-gp was examined by Western blot in tumors and lungs. Results: Doxorubicin concentrations in tumors were 5- to 10-fold lower than those measured in lungs tissues. Doxorubicin penetration in tumors, expressed as tumor retention ratios (TR60min), were not different between the groups. Western blot analysis did not show any evidence of baseline or doxorubicin-induced P-gp expression in the tumor model. Conclusion: P-gp modulation with cyclosporin or valspodar fails to increase the tumor uptake of doxorubin administered by A-ILP. Other reasons for low doxorubicin penetration in tumor, such as high interstitial fluid pressure or tumor vasculature barrier, or alternate cell membrane drug transporters, need to be examined for a better understanding of impaired doxorubicin delivery to tumor.*

Isolated lung perfusion (ILP) is an attractive treatment approach for locoregional diseases affecting the lung, by allowing the selective administration of a therapeutic agent to the target organ while sparing non-affected tissues. The principal indication for cytostatic ILP is the presence of pulmonary metastases. Pulmonary metastases occur in approximately 30% of patients dying of cancer and in one third, the lung is the only site of metastasis (1, 2). Despite encouraging results in preclinical studies in two rodent models of pulmonary soft tissue sarcoma (3, 4), there was only a low tumor response to ILP with doxorubicin in phase I trials (5, 6). The reasons for the poor responses observed in these clinical settings still remain unclear. Heterogeneous drug disposition within the perfused lung and a lower doxorubicin intake in tumors than in healthy lung tissue have been observed (7, 8).

In the ILP rodent model developed at the Department of Thoracic Surgery at CHUV (7, 8), the tumor and lung tissue sections obtained after ILP examined by fluorescence microscopy showed that doxorubicin was virtually absent from tumor cells, whereas doxorubicin fluorescence was observable in the surrounding healthy lung tissue and also in

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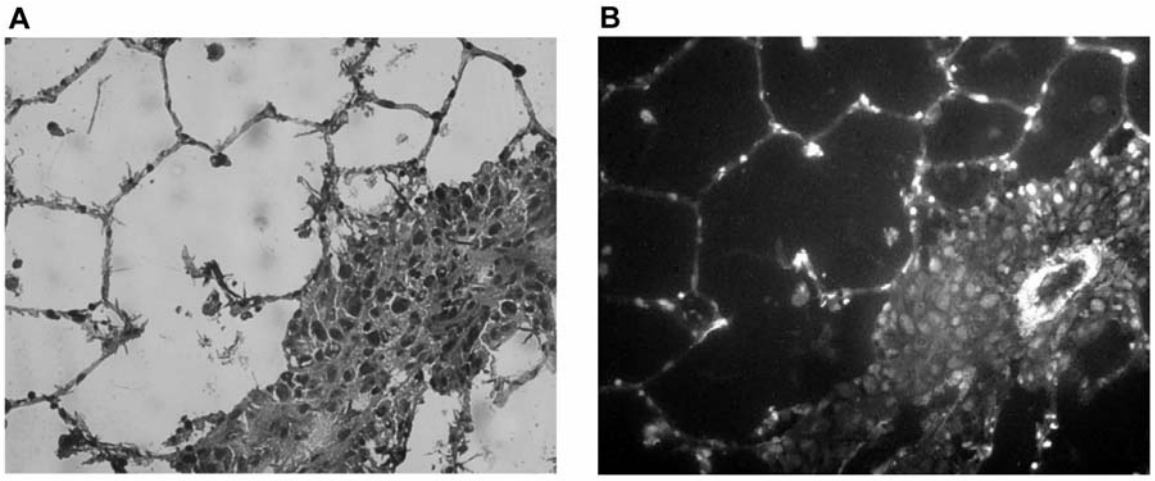

Figure 1. *Pulmonary sarcoma tumors surrounded by normal lung tissue after A-ILP with doxorubicin as assessed by haematoxylin-eosin staining (a) and fluorescence microscopy (b), demonstrating that doxorubicin is localised in normal tissue, including the wall of the tumor-feeding vessel, but not in the tumor itself. Original magnification, ×400. From Krueger et al. 2006 (7) with permission.*

the tumor-feeding vessels (Figure 1) (7). A possible reason for the limited drug penetration in tumor cells is the occurrence of tumors with a multidrug resistance (MDR) phenotype, notably due to the overexpression of transmembrane drug transporter proteins, such as P-glycoprotein (P-gp), the product of the *MDR1* gene that expels the cytotoxic drug out of the cell. The induction of P-gp expression has been notably described to occur very rapidly in patients undergoing ILP with doxorubicin for soft tissue sarcoma lung metastases (9). Its overexpression confers resistance to many cytotoxic anticancer drugs, including anthracyclines (10), and many attempts have been made to revert this mechanism pharmacologically. In clinical trials however, regimens combining cytotoxic anticancer agents such as doxorubicin with P-gp inhibitors, aiming at increasing drug levels in tumors, were generally accompanied by an overall increase in systemic toxicity, as the cytotoxic drug likely penetrates several other deep compartments (*i.e.* mostly central nervous system and bone marrow) normally protected by the physiologic role of P-gp (11, 12).

By contrast, with a locoregional approach such as ILP, doxorubicin is distributed for the most part into the target tissue (*i.e.* the lung) only, and its combination with a P-gp inhibitor should therefore have minimal influence on systemic doxorubicin toxicity especially cardiac toxicity. To the best of our knowledge, only one group has evaluated the potential advantage of this locoregional approach in ILP using cyclosporin, a known P-gp inhibitor, administered to animals in association with doxorubicin (13).

The aim of our investigations was to assess whether the tumor uptake of doxorubicin, administered locoregionally by ILP, would be increased by the addition of the P-gp modulators cyclosporin and its nonsuppressive analog valspodar (SDZ PSC-833). The impact of the ILP procedure and doxorubicin administration on P-gp expression in tumor and lung tissue was also examined.

Materials and Methods

Animals and housing. Male Fischer 344 rats, weighting 150-300 g (Charles River, France) were used. They had free access to standard laboratory rat food and water and were housed with a 12:12-h light-dark cycle under controlled temperature. This animal experimentation was approved on 6/6/2004 by the Competent Veterinary Authority. The animals were treated in accordance with Swiss legislation (Loi fédérale sur la protection des animaux du 9 mars 1978 (LPA), section 6 et ordonnance sur la protection des animaux du 27 mai 1981 (OPAn), art. 58 à 64b).

ILP procedure and generation of a pulmonary sarcoma tumor nodule. The surgical procedure for single-pass antegrade ILP (A-ILP) and the generation of a solitary pulmonary nodule of a methylcholanthrene (MCA)-induced sarcoma cell line, syngeneic to our rats are described in detail elsewhere (7). Briefly, once rats were bearing an adequate pulmonary nodule and after completion of the surgical procedure, A-ILP was performed during 20 minutes using a peristaltic pump (MBV AG; Staefa, Switzerland) set at a flow-rate of 0.25 ml/min. Doxorubicin inflow in the pulmonary artery and collection of the effluent from the pulmonary vein lasted 20 min (see Figure 2). Cytostatic perfusion was followed by a 20 min wash-out phase of the isolated lung with 5 ml of hetastarch without doxorubicin at the same perfusion conditions. Throughout the perfusion, the lung was ventilated with a positive end expiratory pressure of 2-3 cm H_2O.

Modulation by the P-gp inhibitors cyclosporin and valspodar. The influence of two known P-gp inhibitors on doxorubicin tissular and tumor distribution was assessed *in vivo* after systemic administration of the P-gp inhibitors by *i.v.* injection in the jugular vein over 20

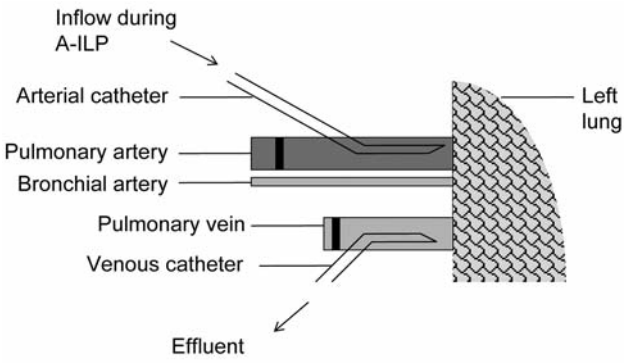

Figure 2. *Schematic illustration of the antegrade isolated lung perfusion (A-ILP) procedure. Two hours after the injection of the P-gp modulation, A-ILP was performed during 20 min with doxorubicin inflow in the pulmonary artery followed by a 20 min wash-out phase.*

minutes, two hours before the A-ILP procedure. The P-gp inhibitors used in this study were cyclosporin (Sandimmun®; Novartis Pharma AG, Basel, Switzerland) at a dose of 30 mg/kg and valspodar (kindly provided by Novartis Pharma AG) at a dose of 12.5 mg/kg. Sandimmun® was diluted with NaCl 0.9% (1:1 v/v) aqueous solution before use. Valspodar was dissolved in a vehicle analogous to that used in Sandimmun® pharmaceutical preparation, containing 6.5 g Cremophor® ELP (BASF, Ludwigshafen, Germany), 2.61 g of ethanol and diluted to 10 ml with NaCl 0.9% (w/v) aqueous solution. As for Sandimmun®, this preparation was further diluted with NaCl 0.9% aqueous solution (1:1 v/v) before use. The P-gp inhibitors were injected in a total volume of 120 µl/100 g of rat weight. The same vehicle without drugs was used in the control group.

Experimental design and sample collection schedule. Ten days after tumor implantation, 19 rats underwent A-ILP with doxorubicin at a level of 20 µg/ml, corresponding to a total doxorubicin dose of 100 µg. Doxorubicin HCl (Adriblastin®; Pharmacia & Upjohn, Dübendorf, Switzerland) was supplied as solution ready for use (2,000 µg/ml) and was diluted to 5 ml with hetastarch (HAES 6%® (hydroxyethyl amidon solution, hydroxyethyl starch; Fresenius, Switzerland).

Six animals underwent A-ILP without any P-gp modulation pre-treatment (blank group). 13 animals underwent A-ILP with pre-treatment: 5 with the vehicle solvent only (Cremophor® group); 4 with 30 mg/kg of cyclosporin (cyclosporin group); and 4 with 12.5 mg/kg of valspodar (valspodar group). The doxorubicin levels in infusion, effluent, lung tissues and tumor nodule were measured using a validated analytical method by high performance liquid chromatography (HPLC) coupled to spectrofluorimetric detection (as described in details elsewhere (14)). The doxorubicin concentrations were determined in perfusion solutions, in effluent samples collected continuously during the entire cytostatic perfusion (*i.e.* periods: 0-2 min, 2-4 min, 4-10 min, 10-18 min and 18-20 min) and during the wash-out of the isolated lung with hetastarch (*i.e.* periods 20-22 min, 22-24 min, 24-30 min, 30-38 min and 38-40 min). The animals were sacrificed one hour after blood recirculation. Healthy perfused lung tissue and the tumor nodule were collected, together with the contra-lateral non-perfused lung, the heart, the chest wall, the mediastinum and the liver for doxorubicin assay in tissue.

Pharmacokinetic analysis. a) Doxorubicin retention in the lung during A-ILP. The amount of doxorubicin (*A*, µg) calculated from the doxorubicin concentration (µg/ml) and the measured volume of sample (ml) in the infusion solution and in each effluent sample was determined in order to assess the doxorubicin retention during A-ILP and the spillage during wash-out. The amount of doxorubicin retained by the lung tissue during A-ILP can be determined by substracting the amount of drug recovered in the effluent during the infusion and subsequent wash-out procedure from the total amount of doxorubicin infused into the lung. This allows an extrapolation of the drug retention in the perfused lung during A-ILP without the need for lung biopsy sampling. The initial fraction of drug retained by the perfused lung tissue, $f_{initial;0\text{-}20}$, is calculated as follows:

$$f_{initial;0\text{-}20} = 1 - \left(\frac{A_{t0\text{-}20}}{D} \right)$$

where D corresponds to the applied drug dose (µg) accurately determined in the infusion solution, and $A_{t0\text{-}20}$ is the amount of doxorubicin collected in the effluent during the 20 min of the cytostatic perfusion. The fraction of doxorubicin, previously retained during the initial drug retention, which was removed during the 20 min of wash-out with hetastarch, *i.e.* $f_{spillage}$, was evaluated as follows:

$$f_{spillage} = \frac{A_{t20\text{-}\infty}}{\left(D - A_{t0\text{-}20} \right)}$$

where $D - A_{t0\text{-}20}$ corresponds to the amount of drug retained in the lung before the wash-out was initiated and $A_{t20\text{-}\infty}$ is the amount of drug collected in the effluent during wash-out with hetastarch, extrapolated to infinity, based on the terminal half-life of the doxorubicin flow decrease. Finally, the final fraction of drug retained by the lung after wash-out, $f_{final;\,0\text{-}\infty}$, can thus be calculated as follows:

$$f_{final;0\text{-}\infty} = 1 - \left(\frac{\left(A_{t0\text{-}20} + A_{t20\text{-}\infty} \right)}{D} \right)$$

b) Lung retention ratio one hour after blood flow restoration: The doxorubicin retention ratio (RR_{60min}) was calculated using the total dose of doxorubicin (D, µg) administered by A-ILP, the mean final doxorubicin concentration in the perfused lung tissue (C_{lung}, µg/g) one hour after blood circulation restoration to the lung and the weight of the perfused lung (W, g):

$$RR_{60min} = \frac{C_{lung} \times W}{D}$$

c) Tumor to lung ratio one hour after blood flow restoration: The doxorubicin tumor to lung ratio (TR_{60min}) was calculated by dividing the tumor concentration (C_{tum}, µg/g) by the mean final

doxorubicin concentration in the perfused lung tissue (C_{lung}, μg/g) one hour after blood flow restoration:

$$TR_{60min} = \frac{C_{tum}}{C_{lung}}$$

d) Leakage to systemic compartments: Leakage of doxorubicin to systemic compartments from the ILP circuit was determined one hour after blood flow restoration in the tissues collected just after sacrifice (contra-lateral non-perfused lung, heart, chest wall, mediastinum and liver).

Data analysis. If not specified, data were expressed as geometrical means and geometrical coefficients of variation (CV, %). When the doxorubicin levels were lower than the lower limit of detection (LLOD) of the analytical method, the LLOD/2 was used for the calculations.

After transformation of the data into its natural logarithm, a two-way factorial analysis of variance (ANOVA) was carried out with the software Statistix version 8.0 for Windows (Analytical Software, Thallahassee, FL, USA) to determine the differences among the groups. Statistical significance was accepted at a *p*-value smaller than 0.05. Doxorubicin levels from A-ILP into systemic tissues were compared using Tukey's all pairwise test. Statistical difference was accepted at a *p*-value <0.05.

Determination of cyclosporin blood levels. In the 4 animals from the cyclosporin group, cyclosporin concentrations in the total blood were measured in samples collected 60 min after blood recirculation by use of an Enzyme Multiplied Immunoassay Technique (EMIT) run on an Integra 400 Plus instrument (Roche Diagnostics, Basel, Switzerland). Due to very high cyclosporine levels, the rat blood samples were diluted 1/100 with human whole blood.

P-gp expression study. The expression of P-gp was evaluated by Western blot in the tumors and the lung tissues. The mouse monoclonal anti-P-gp antibody (Mab) C219 (DakoCytomation, Carpinteria, USA) was used in these experiments.

A Western blot analysis was performed on the tumor cell culture, and on implanted tumor and lung samples of rats which underwent the three following treatments: no ILP, A-ILP without doxorubicin and A-ILP with doxorubicin. For the ILP treated groups, the samples were collected 60 minutes after the procedure. All the samples were rapidly frozen and kept at –80˚C until analysis.

Raw membrane fractions were prepared from the tumors, the lungs and the cell culture medium. The different tissues obtained were crushed and homogenised in homogenization buffer containing 250 mM sucrose, 5 mM EDTA, 25 mM Tris HCl (pH 8) and the protease inhibitor Complete without EDTA (Roche, Mannheim, Germany), while the tumor cell culture was suspended in this buffer. The homogenates were centrifuged through QIAshredder tubes (Quiagen, Hilden, Germany) at 20,000× g for 20 min at 4˚C. The pellets containing the crude membrane fraction were washed twice in 1.5 M NaCl in the homogenization buffer and were centrifuged at 20,000 g for 20 min at 4˚C. The washed pellets were finally suspended in 8 M urea, 1% (w/v) sodium dodecyl sulphate (SDS), 10 mM Tris/HCl (pH 7.5) and Complete without EDTA. To serve as positive control, crude membranes of rat intestine were prepared in an analogous manner. Protein concentrations of the samples were measured using the bicinchoninic acid protein assay (BCA; Pierce, Perbio, Rockford, IL, USA).

The samples were diluted with NuPAGE® LDS Sample buffer (Invitrogen, Carlsbad, CA, USA) to a final concentration of 0.5 μg/μl. The proteins were denaturated at 70˚C for 10 min. Aliquots of 10 μg proteins by lane were loaded and separated by a NuPAGE® 4-12% Bis-tris gel (Invitrogen) electrophoresis with NuPAGE® MOPS SDS running buffer (Invitrogen). After gel resolution, proteins were transferred electophoretically on a 0.45 μm pore size polyvinyldene difluoride (PVDF) Immobilon-P transfer membrane (Millipore, Bedford, MA, USA).

The membrane was then incubated overnight at room temperature with Mab C219 at a dilution of 1/500 in 3% dry milk (w/v) in TBS-T. The washed membrane was further incubated for 2 hours at room temperature with a goat anti-mouse IgG-(HRP)-conjugated antibody (Bio-Rad, Hercules, CA, USA) at a dilution of 1:2500. The chemiluminescence signal was generated using the ECL Western Blotting Detection Kit (Amersham, Little Chalfont, UK) and the blots were exposed onto Hyperfilm^{IM} ECL (Amersham) for 4 min.

Results

Animal experiments. A total of 19 rats underwent A-ILP with doxorubicin, 6 without any pre-treatment (blank group). Pg-p modulation was studied in 13 rats after administration of one of the three following pre-treatments: Cremophor® (n=5), cyclosporin (n=4) and valspodar (n=4). All perfusions were performed successfully. However, one rat of the blank group was excluded because of insufficient effluent recovery.

Pharmacokinetics. a) Doxorubicin retention in the lung during A-ILP: The profiles of doxorubicin flow *versus* time in the lung tissue effluents during the A-ILP for the different treatment groups are shown in Figure 3. Doxorubicin retention amounts and rates in the lung are shown in Table I. For $A_{t0\text{-}20}$ and $A_{t20\text{-}\infty}$, no difference was observed among the different treatment groups. Thus, the fraction of initial retention ($f_{initial;0\text{-}20}$) and the fraction of final retention ($f_{final;0\text{-}\infty}$) did not show any statistically significant difference nor did the fraction spilled out ($f_{spillage}$).

b) Lung levels (C_{lung}), lung retention ratio (RR_{60min}) and tumor to lung ratio (TR_{60min}) one hour after blood flow restoration of doxorubicin: The C_{lung}, the RR_{60min} and the TR_{60min} are reported in Table II. Neither the C_{lung}, TR_{60min} nor the RR_{60min} were significantly different amongst treatment groups. TR_{60min} ranged from 0.094 to 0.19, indicating that the concentrations measured simultaneously in the tumors were 5- to 10-fold lower than those measured simultaneously in the perfused lung tissue.

Cyclosporin blood levels. Mean cyclosporin blood levels measured in rats at the end of the experiment (*i.e.* 60 minutes after the end of A-ILP) were 17.1 μg/ml, a fairly high value, with low inter-animal variability (CV 14.7%, n=4).

Table I. *Doxorubicin retention in the lung during antegrade isolated lung perfusion. The results are expressed as geometrical means (CV, %).*

Group	$A_{t0\text{-}20}$ (μg)	$A_{t20\text{-}\infty}$ (μg)	$A_{t0\text{-}\infty}$ (μg)	$f_{initial;\ 0\text{-}20}$	$f_{final;\ 0\text{-}\infty}$	$f_{spillage}$
Blank	36 (87%)	10.8 (18%)	48 (60%)	0.54 (36%)	0.41 (52%)	0.21 (54%)
Cremophor®	36 (33%)	7.9 (43%)	44 (26%)	0.60 (19%)	0.51 (22%)	0.14 (45%)
Cyclosporin	37 (32%)	7.1 (13%)	44 (29%)	0.59 (21%)	0.51 (27%)	0.13 (35%)
Valspodar	46 (21%)	8.9 (34%)	54 (22%)	0.49 (24%)	0.38 (40%)	0.20 (67%)
Statistical analysis						
p-value	0.794	0.131	0.753	0.559	0.427	0.219

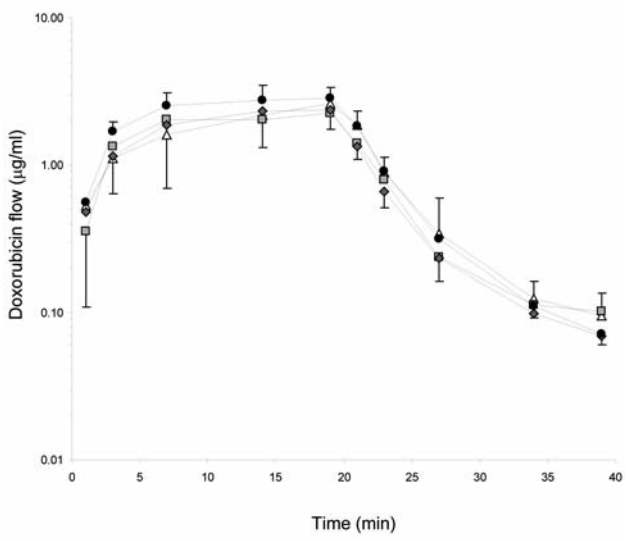

Figure 3. *Doxorubicin time-flow profile in the lung tissue effluents in a tumor bearing-rat model. Doxorubicin (100 μg) was administered the first 20 minutes without pre-treatment in the blank (▲) or with pre-treatment of 20 min with Cremophor® (■), cyclosporine (◆) or valspodar (●). A washing solution was perfused from 20 to 40 minutes in all groups. Error bar: ± CV%.*

Table II. *Perfused lung concentration (C_{lung}) (μg/g), lung retention ratio (RR_{60min}) and tumor to lung ratio (TR_{60min}) one hour after blood flow restoration. The results are expressed as geometrical means (CV, %).*

Group	C_{lung} (μg/g)	RR_{60min}	TR_{60min}
Blank	13.0 (39%)	0.14 (63%)	0.19 (60%)
Cremophor®	14.6 (41%)	0.12 (39%)	0.14 (82%)
Cyclosporin	13.9 (37%)	0.11 (15%)	0.094 (166%)
Valspodar	10.8 (32%)	0.086 (35%)	0.15 (27%)
Statistical analysis			
p-value	0.563	0.242	0.399

Impact of Pg-P modulation on systemic doxorubicin tissular distribution. The doxorubicin spillage from A-ILP into systemic tissues was measured in various tissues from animals sacrificed 60 min after circulation restoration. (Figure 4). Statistically significant differences in doxorubicin levels were found in the cardiac tissue (valspodar *vs*. blank control group) and in the chest wall (cyclosporin *vs*. blank control group) ($p<0.05$).

P-gp expression study. P-gp expression in the tumors and the surrounding lung tissues was evaluated qualitatively by Western blot. In crude membranes of intestine used as a positive control, Mab C219 specifically recognised a band around 160 kDa (Figure 5). This is in accordance with the molecular mass of P-gp, which may vary between 130 and 180 kDa, reflecting differences in glycosylation (15, 16). The P-gp signal found in the lung extract not subjected to ILP was as intense, or only slightly weaker, than that in the intestine extract. By contrast, there was only a very low expression of P-gp, if any, in the tumor extracts. Similarly, P-gp was not detected in the tumor cell culture extracts.

Discussion

Fluorescence microscopic examination of tumor and lung tissue sections obtained after ILP reveals a very low doxorubicin uptake in tumor cells, despite doxorubicin fluorescence being ascertained both in the surrounding healthy lung tissue and in the tumor feeding vessels (Figure 1) (7). Among the numerous factors that are capable of altering disposition of anticancer agents in tumors, resulting in a resistant phenotype, the (over)expression of P-gp has been frequently evoked and has been the focus of considerable interest in *in vitro* experiments and in the clinical setting. The induction of P-gp expression was reported to occur very rapidly in patients undergoing ILP with doxorubicin for soft tissue sarcoma lung metastases [9]. Thus, the potential for inducing expression of P-gp by doxorubicin treatment, and its possible impact on tumor uptake, has been examined in this experimental tumor model. Secondly, the present 'proof of concept' pre-clinical experiment was initiated to study whether pharmacological modulation of P-gp is able to enhance doxorubicin levels in

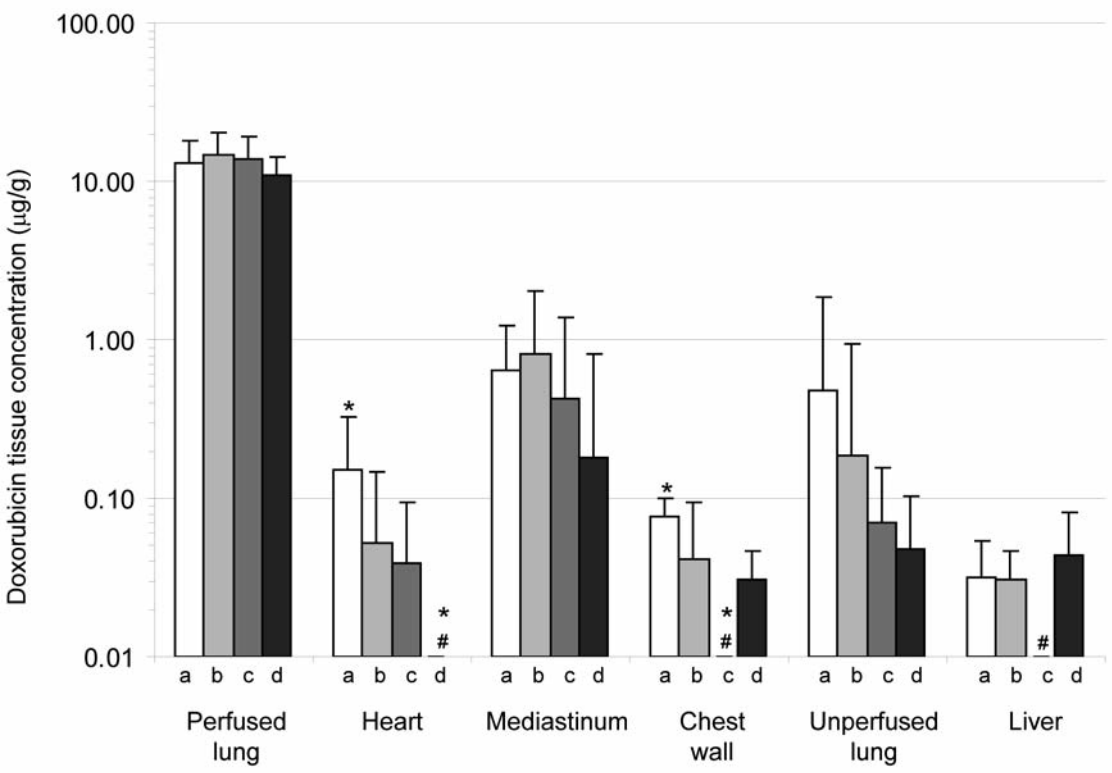

Figure 4. *Tissue concentrations of doxorubicin measured 60 minutes after circulation restoration. Doxorubicin (100 µg) was administered the first 20 minutes without pre-treatment in the blank group (a) or with pre-treatment in the Cremophor® group (b), the cyclosporine group (c) and the valspodar group (d). Error bar: ± CV%. #Doxorubicin level was below LLOD in all samples; *statistically significantly different (p<0.05) according to the Tukey's all pairwise test (heart levels: valspodar group vs. blank group; chest wall levels: cremophor group vs. blank group).*

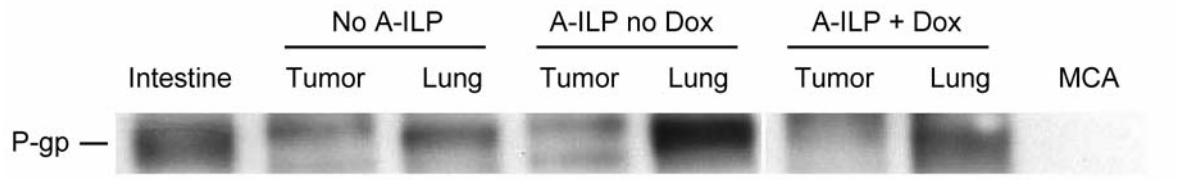

Figure 5. *Immunodetection of P-gp from crude membrane extracts of intestine, tumor and lung tissues control (no A-ILP), after A-ILP without doxorubicin (Dox), after A-ILP with doxorubicin and, extreme right, of MCA-induced sarcoma cell line (MCA).*

tumors and in lung tissue, in the perspective of the clinical use of P-gp inhibitors for locoregional chemotherapy.

In these experiments, we failed to find any differences in the tumors uptake of doxorubicin expressed as the TR_{60min}, between the animal groups pre-treated with cyclosporin or valspodar, and the control group. This cannot be ascribed to insufficient circulating concentrations of P-gp inhibitors as the mean blood level of cyclosporin measured at the end of the experiment (*i.e.* 60 min after A-ILP after a cyclosporine dose of 30 mg/kg) was 17.1 µg/ml, about 10-fold higher than the IC50 of 1.6 µg/ml of cyclosporin reported for P-gp inhibition (17). Valspodar was administered at a dose of 12.5 mg/kg, and being 10-fold more potent than cyclosporin, the level of valspodar attained in blood should also have been sufficient.

There are some uncertainties as to the level of expression of P-gp in the tumor model. P-gp expression was examined by both Western blot analysis and immunohistochemical staining (data not shown) with the Mab C219 antibody. Pg-P expression in the normal lung tissue was as intense, or slightly lower than that in the intestine used as positive control (Figure 5). However, inhibition of P-gp by cyclosporin or valspodar did not translate into an increase in doxorubicin retention in these healthy lung tissues (Table I).

Accordingly, there was no striking difference in the flow-time profile of doxorubicin in the lung tissue effluents (Figure 3) between animals receiving or not the P-gp-modulating treatment. The distribution of the small doxorubicin spillage from ILP into the systemic compartment was examined: valspodar and cyclosporin were found to significantly reduce the doxorubicin levels in cardiac and chest wall tissues (Figure 4), but the significance of such an observation remains to be comfirmed.

Unlike previous clinical reports (9), we did not observe any rapid induction of P-gp expression in this sarcoma tumor model following doxorubicin administration. In our experiments, the inhibition of P-gp did not have any impact on doxorubicin retention, neither in the tumor nor in the healthy lung tissue. In fact, the implanted tumors may express one or several drug efflux proteins, other than P-gp, such as the multidrug resistance-associated protein (MRP1), breast cancer resistance protein (BCRP) or lung resistance protein (LRP), also implicated in the MDR phenotype (18). In such a case, no major effect on doxorubicin tissue distribution should be expected using cyclosporin and valspodar, since these two modulating drugs, although known not to be specific inhibitors for a single drug transporter, are only weak inhibitors of drug-efflux pumps other than P-gp (19). Finally, it is also possible that the low doxorubicin concentrations observed in tumor may be due to barriers of the tumor vasculature or because of an increased oncotic pressure that would reduce drug access to the solid tumor compartment, therefore rendering cancer cells less vulnerable to cytotoxic drugs (20, 21).

In conclusion, the uptake of doxorubicin by the tumor was not found to be affected by P-gp modulation. The doxorubicin levels inside the tumors remained constantly 5 to 10-fold lower than in the surrounding healthy lung tissue. P-gp modulation with cyclosporin and valspodar neither increased the doxorubicin tumor retention ratio (TR_{60min}) nor affected overall lung retention of doxorubicin. These results are supported by the Western blot analysis, which did not show any evidence of baseline or treatment-induced P-gp expression in the tumor model. Nevertheless, the expression of another MDR drug efflux protein and/or a high tumor interstitial pressure cannot be excluded and may explain these low tumor retention ratios. Such factors need to be examined for a better understanding of the mechanism of impaired doxorubicin tumor delivery.

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