Cytostatic lung perfusion results in heterogeneous spatial regional blood flow and drug distribution: Evaluation of different cytostatic lung perfusion techniques in a porcine model

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Objectives: Comparison of doxorubicin uptake, leakage and spatial regional blood flow, and drug distribution was made for antegrade, retrograde, combined antegrade and retrograde isolated lung perfusion, and pulmonary artery infusion by endovascular inflow occlusion (blood flow occlusion), as opposed to intravenous administration in a porcine model.

Methods: White pigs underwent single-pass lung perfusion with doxorubicin (320 $\mu\text{g/mL}$), labeled $^{99\text{m}}\text{Tc}$ -microspheres, and Indian ink. Visual assessment of the ink distribution and perfusion scintigraphy of the perfused lung was performed. $^{99\text{m}}\text{Tc}$ activity and doxorubicin levels were measured by gamma counting and high-performance liquid chromatography on 15 tissue samples from each perfused lung at predetermined localizations.

Results: Overall doxorubicin uptake in the perfused lung was significantly higher ($P = .001$) and the plasma concentration was significantly lower ($P < .0001$) after all isolated lung perfusion techniques, compared with intravenous administration, without differences between them. Pulmonary artery infusion (blood flow occlusion) showed an equally high doxorubicin uptake in the perfused lung but a higher systemic leakage than surgical isolated lung perfusion ($P < .0001$). The geometric coefficients of variation of the doxorubicin lung tissue levels were 175%, 279%, 226%, and 151% for antegrade, retrograde, combined antegrade and retrograde isolated lung perfusion, and pulmonary artery infusion by endovascular inflow occlusion (blood flow occlusion), respectively, compared with 51% for intravenous administration ($P = .09$). $^{99\text{m}}\text{Tc}$ activity measurements of the samples paralleled the doxorubicin level measurements, indicating a trend to a more heterogeneous spatial regional blood flow and drug distribution after isolated lung perfusion and blood flow occlusion compared with intravenous administration.

Conclusions: Cytostatic lung perfusion results in a high overall doxorubicin uptake, which is, however, heterogeneously distributed within the perfused lung.

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Isolated lung perfusion (ILP) with cytostatic drugs is an attractive treatment concept for malignancies affecting the lung because it allows for a higher loco-regional cytostatic drug delivery with lower drug concentration in the systemic circulation compared with intravenous (IV) administration. It has been evaluated in clinical¹⁻⁸ and experimental⁸⁻¹² settings in this respect. Cytostatic ILP has been shown to result in significantly higher drug concentration in lung tissue and significantly lower concentration in plasma compared with systemic drug administration.⁸ However, the results emerging from clinical trials have not shown a

Abbreviations and Acronyms

a-ILP	= antegrade isolated lung perfusion
BFO	= blood flow occlusion
c-ILP	= combined antegrade and retrograde isolated lung perfusion
IV	= intravenous
ln	= natural logarithm
r-ILP	= retrograde isolated lung perfusion

survival advantage in patients with pulmonary malignancies, irrespective of the cytostatic agent administered. One explanation for this may be a heterogeneous spatial regional blood flow and distribution of the cytostatic agent during lung perfusion.

The current study was designed to compare different cytostatic ILP techniques with respect to the spatial distribution of ^{99m}Tc -labeled microspheres and doxorubicin levels within the perfused lung in a porcine model. Antegrade ILP (a-ILP) (perfusion through the pulmonary artery), retrograde ILP (r-ILP) (perfusion through the pulmonary veins), combined antegrade and retrograde ILP (c-ILP), and cytostatic pulmonary artery infusion with inflow occlusion of the pulmonary artery by a percutaneously placed balloon catheter were compared with IV administration in this respect.

Methods

Study Design

Tumor-free white pigs underwent a-ILP, r-ILP, or c-ILP isolated perfusion of the left lung. Each group consisted of 3 animals. Three animals had cytostatic pulmonary artery infusion with inflow occlusion of the pulmonary artery by a percutaneously placed balloon catheter (blood flow occlusion [BFO]), and 3 animals had IV administration of the perfusate. The perfusate consisted of 500 mL of 6% buffered hetastarch (HAES 6%, Fresenius, Stans, Switzerland) with 160 mg doxorubicin (320 $\mu\text{g}/\text{mL}$), 111 MBq ^{99m}Tc -labeled microspheres, and 1% ink of India.

Animals and Housing

White pigs with a body weight of 25 kg were used. All animals received humane care and were treated in accordance with the Animal Welfare Act, the National Institute of Health "Guidelines for the Care and Use of Laboratory Animals," and the guidelines of the Local Ethical Committee of the University of Lausanne.

Isolated Lung Perfusion

Induction of anesthesia was performed by intramuscular administration of ketamine (10 mg/kg body weight) followed by IV pentothal (10 mg/kg). After tracheal intubation, anesthesia was maintained with 0.50% $\pm$ 0.05% halothane and 70% nitrous oxide during ventilation. Continuous IV infusion of fentanyl 5 $\mu\text{g} \cdot \text{kg} \cdot \text{h}$ and pancuronium 0.5 mg $\cdot \text{kg} \cdot \text{h}$ was performed. The lungs were ventilated with a volume-controlled ventilator with positive end-

expiratory pressure of 3 to 4 cm H_2O throughout and therefore during lung perfusion. The tidal volume was 10 mL/kg, and ventilation frequency was adjusted to maintain PACO_2 between 4.5 and 5.5 kPa. All animals were placed on a heating pad while body temperature was maintained at 38°C. The ventilated gas and IV fluids were at room temperature.

A standard median sternothoracotomy extending to the right neck and into the left chest was performed. The right carotid artery and jugular vein were dissected out, and an arterial and central venous 6F catheter were inserted for arterial blood pressure monitoring, fluid perfusion, drug administration, and collection of blood samples. The hilar structures of the left lung were dissected, and the left pulmonary artery and superior and inferior pulmonary veins were individually dissected, encircled, and proximally clamped after IV administration of heparin (2 mg/kg). A custom-made silicon cannula was introduced into the pulmonary artery after arteriotomy. The inferior and superior pulmonary veins were separately cannulated with 2 identical cannulae that were joined with a Y-connector during infusion.¹¹

Single-pass, gravity-driven perfusion was performed. The bag containing the perfusion solution was positioned 50 cm above the level of the animal's heart, and the bag and the inflow cannula were connected using a light-shielded standard perfusion tube. Both lungs were ventilated during perfusion. a-ILP consisted of administration of the perfusate (500 mL) through the pulmonary artery over 20 minutes and collection of the effluent through the 2 venous cannulae. r-ILP consisted of the administration of the perfusate through the pulmonary veins (250 mL each) over 20 minutes and collection of the effluent by the arterial cannula. c-ILP consisted of the administration of 250 mL of the perfusate through the pulmonary artery over 10 minutes and 250 mL through the pulmonary veins over 10 minutes. The animals were sacrificed at the end of the perfusion, and the perfused and nonperfused lungs were harvested for visual inspection and assessment of ^{99m}Tc activity and doxorubicin tissue concentration measurements, respectively.

Cytostatic Lung Perfusion by Endovascular Blood Flow Occlusion Technique

Anesthesia, ventilation, dissection, and catheterization of the carotid artery and jugular vein (without sternotomy) were performed as previously described. A right-sided groin dissection was performed, and a Swan-Ganz catheter was introduced into the femoral vein. The tip of the catheter was placed at the level of the proximal left pulmonary artery under fluoroscopic control. The balloon of the Swan-Ganz catheter was inflated, which led to occlusion of the proximal left pulmonary artery, and on-table pulmonary angiography was performed to confirm the correct position of the catheter. Five-hundred milliliters of the perfusate was administered as a single-pass gravity-driven perfusion over 20 minutes¹² through the catheter distal to the inflated balloon. After completion of the perfusion, on-table pulmonary angiography was repeated to confirm the correct position of the catheter. The catheter was removed, and the femoral vein was ligated. The animals were sacrificed, and the lungs were harvested for visual inspection, assessment of ^{99m}Tc activity, and doxorubicin tissue concentration measurements.

Figure 1. Mapping for lung tissue harvesting. Each perfused lung was cut in half through the hilum and displayed as an “open book.” Fifteen samples were harvested according to the map for ^{99m}Tc activity and doxorubicin concentration measurements.

Intravenous Administration of the Perfusate (Controls)

Induction of anesthesia, dissection, and cannulation of the carotid artery and jugular vein was performed as previously described. Oxygen (2-4 L/min) was supplied by a use of a mask during spontaneous respiration. Five-hundred milliliters of the perfusate were administered through the catheter placed in the jugular vein by gravity. The animals were sacrificed, and the perfused and contralateral lungs were harvested.

Assessment of the Spatial Perfusion Pattern by Ink Staining and Perfusion Scintigraphy

After sacrifice of the animal, the whole perfused and contralateral nonperfused lungs were harvested. The pattern of ink distribution of the entire perfused lung was assessed by visual inspection and photo documentation of the visceral, mediastinal, and diaphragmatic pleura of the perfused lung. The perfused lung was cut in half through the hilum and displayed as an “open book.” Perfusion scintigraphy of both lungs was then performed with a γ -camera. Fifteen lung tissue samples were harvested in each perfused lung at predetermined locations according to a mapping as shown in Figure 1. ^{99m}Tc activity was assessed in a quantitative manner in each sample by gamma-counting followed by storing them at -80°C for doxorubicin concentration measurements.

Assessment of Doxorubicin Concentration in Lung Tissues and the Systemic Circulation

Systemic blood samples were collected in 5.5-mL EDTA S-Monovettes (Sarstedt, Nümbrecht, Germany) at 0, 5, 15, and 20 minutes for a-ILP, r-ILP, and c-ILP. The duration of the BFO and IV administrations was longer, and the blood samples were taken with an adapted schedule at 0, 5, 10, 15, and 30 minutes. The samples were stored on ice before centrifugation within 2 hours at 1500g for 10 minutes at $+4^\circ\text{C}$. The plasma was snap-frozen at -80°C . The harvested lung tissue samples were frozen after ^{99m}Tc activity measurements and stored at -80°C . Doxorubicin concentration measurements were performed by high-performance liquid chromatography as previously described.¹³

Evaluation of Doxorubicin Distribution in the Lung Tissue: Data Presentation and Statistics

Because the tissue concentrations of doxorubicin are distributed according to a log-normal distribution, the calculations and statistics have been done after transformation of the data in their natural logarithm (ln). The doxorubicin mean tissue concentration in the perfused lung was calculated for each pig separately and for each treatment group and expressed as geometric means:

$$\text{Geometric mean } (C_{\text{lung}} \text{ or } C_{\text{treat}}) = e^{\text{mean}(\ln \text{ data})}$$

Where $\text{mean}(\ln \text{ data})$ corresponds to the mean of the ln-transformed data. C_{lung} is the mean doxorubicin concentration in the lung tissue for a pig, and C_{treat} is the mean doxorubicin concentration in the lung tissue for a treatment group.

The homogeneity of doxorubicin distribution was evaluated by calculating the variance of the doxorubicin tissue levels in each perfused lung separately (s^2_{lung}), and by calculating the intra-lung variance for each treatment group ($s^2_{\text{intra-lung}}$). $s^2_{\text{intra-lung}}$ represents the mean variance of the doxorubicin levels of each lung separately (s^2_{lung}) for a treatment group. The overall variance (s^2_{tot}) of a treatment group can be described as follows:

$$S^2_{\text{tot}} = S^2_{\text{intra-lung}} + S^2_{\text{intra-lung}}$$

where $s^2_{\text{intra-lung}}$ corresponds to the variance between the lungs of a treatment group, that is, the inter-lung variance. Because $s^2_{\text{intra-lung}}$ should not be the result of the treatment effect, only the $s^2_{\text{intra-lung}}$ was calculated to describe adequately the variability of the doxorubicin distribution for a treatment group.

Then, as for the mean concentrations, the variances were obtained from the ln-transformed data and back-calculated, applying the exponential function, to get the geometric coefficients of variation (CV, %). CV_{lung} (%) is the geometric coefficient of variation for each lung separately, and $CV_{\text{intra-lung}}$ (%) is the intra-lung geometric coefficient of variation for a treatment group:

$$CV(\%) = \left[\left(e^{\sqrt{s^2_{\text{ln data}}}} \right) - 1 \right] \bullet 100$$

where: $s^2_{\text{ln data}}$ corresponds to the variance of the ln-transformed data

$\sqrt{s^2_{\text{ln data}}} = \text{sd}(\ln \text{ data})$, these corresponding standard deviation.

$e^{\text{sd}(\ln \text{ data})} = \text{pd}$, the corresponding percent deviation

$CV\% = (\text{pd} - 1) \bullet 100$

For statistical analysis, a 1-way completely randomized design analysis of variance was performed with the software Statistix version 8.0 for Windows (Analytical Software, Tallahassee, Fla). Statistical analysis was run with the ln-transformed data of (1) C_{lung} and (2) s^2_{lung} to determine the treatment effect on the (1) doxorubicin lung levels (C_{treat}) and (2) homogeneity of doxorubicin distribution ($CV_{\text{intra-lung}}$). If the criteria for a statistical difference were met, the Tukey's all-pairwise comparison test was performed to find out which groups differ from the others.

Results

Assessment of the Perfusion Pattern by Ink Staining and Perfusion Scintigraphy

Visualization of the ink distribution within the perfused lung revealed a heterogeneous pattern of ink distribution in

Figure 2. Visualization of ink staining after c-ILP demonstrating heterogeneous perfusion pattern not related to anatomy (lobar or segmental) or hydrostatic gradient. This heterogeneity was similarly observed after each of the perfusion techniques.

all animals for all modes of perfusion assessed. The most homogeneous perfusion was found after IV administration. The spatial distribution of ink was neither anatomic (lobar or segmental) nor explained by a hydrostatic gradient (Figure 2). Perfusion scintigraphy of the lung also revealed a heterogeneous pattern of ^{99m}Tc activity within the lung parenchyma without correlation with anatomic (lobar or segmental) structures. ^{99m}Tc activity measurements of each harvested sample revealed a marked heterogeneity for all modes of perfusion assessed (Figure 3) without correlation to anatomic structures or a hydrostatic gradient.

Doxorubicin Concentration Time Profile in Plasma

The concentration time profile of doxorubicin in plasma is shown in Figure 4. The maximum concentration of doxorubicin in plasma was significantly lower (~ 100 times) after ILP than after IV and BFO administration ($P < .0001$) without differences between the ILP techniques applied. There was no significant difference in plasma doxorubicin concentrations between IV administration and BFO perfusion.

Doxorubicin Distribution in the Perfused Lung

Table 1 shows the mean lung tissue levels and their geometric coefficients of variation with respect to the mode of lung perfusion depicted from the analyses of the 15 samples harvested from each perfused lung. With all treatment modes, there was a large variability in the doxorubicin lung tissue levels indicating a heterogeneous distribution of doxorubicin throughout the lung. There was no statistical significant difference between the different modes of perfusion (a-ILP, r-ILP, c-ILP, and BFO) with respect to doxorubicin lung tissue levels and the variability of doxorubicin tissue distribution. In contrast, a trend to a more homogeneous doxorubicin distribution within lung tissue was seen after IV administration ($P = .09$). However, IV doxorubicin administration resulted in significantly lower drug tissue levels compared with ILP ($P = .001$).

Correlation Between ^{99m}Tc Activity and Doxorubicin Tissue Levels

There was a significant correlation between ^{99m}Tc activity and doxorubicin level measurements in the different tissue samples of the perfused lung, for each mode of perfusion assessed ($P < .01$). The correlations (r) were 0.55, 0.73, 0.76, 0.61, and 0.77 for a-ILP, r-ILP, c-ILP, BFO, and IV administration, respectively (Figure 5).

Comment

Several publications have demonstrated the feasibility of cytostatic ILP in clinical trials.¹⁻⁸ Various cytostatic regimens were tested including doxorubicin,^{1,3,7} melphalan,⁵ platinum,^{2,4} and tumor necrosis factor- α combined with interferon- γ .⁶ The major indication for ILP was pulmonary metastases, and several reports considered complete metastasectomy before⁴ or after ILP.^{2,5} All trials demonstrated excellent separation between systemic and pulmonary circulations with minimal or undetectable systemic levels of cytostatic agents. ILP resulted in acceptable toxicity in patients and effectively delivered high doses of the cytostatic agent to the perfused lung. However, the clinical response was modest in those trials in which no metastasectomy was performed, which is in contrast with the results obtained after cytostatic limb perfusion¹⁴ or cytostatic ILP in a rodent model with sarcoma lung metastases.^{8,9} Effective shielding of the tumor tissue from the delivered drug or uneven drug distribution within the perfused lung may be responsible for these disappointing results after cytostatic ILP in patients.

Our experimental study was designed to study the spatial pattern of pulmonary blood flow and drug distribution according to the mode of perfusion technique applied in comparison with IV drug administration. The IV administration was performed during spontaneous breathing because it should reflect as closely as possible the clinical context of IV application of a cytostatic agent.

Most of the experimental work on ILP has been performed on a tumor-bearing rodent model allowing the assessment of a diversity of antineoplastic agents.⁸ However, the size of the lung in this model does not easily allow the assessment of the spatial pattern of blood flow and drug distribution during ILP, which requires the harvesting of various tissue samples at different localizations of the perfused lung. A tumor-free porcine model was therefore used in this respect, which has been used for ILP.^{8,11,12} A similar technique as described by Glenny and colleagues^{15,16} has been applied for the assessment of regional blood flow and doxorubicin distribution for different techniques of cytostatic lung perfusion.

Retrograde ILP has the theoretic potential to perfuse the parts of the lung supplied by both the pulmonary and bronchial artery. This might result in a more homogeneous

Figure 3. ^{99m}Tc activity measurements for each tissue sample harvested according to the mapping of Figure 1. A, a-ILP. B, r-ILP. C, c-ILP. D, BFO. E, i.v. administration. The left and right hemi-circle represent the lowest and highest ^{99m}Tc activity measured in each treatment group, respectively. The results demonstrate a marked heterogeneity of ^{99m}Tc activity for all modes of perfusion assessed.

Figure 4. Plasma doxorubicin concentration time profile after a-ILP: ▲, r-ILP: ●, c-ILP: ■, BFO: ○, and IV administration: ○.

drug delivery to all structures of the perfused lung. r-ILP has been assessed for paclitaxel-based ILP in sheep combined with hyperthermia and has been demonstrated to be feasible

TABLE 1. Mean doxorubicin lung tissue levels and their geometric coefficient of variation for each animal (C_{lung} and CV_{lung}) and each treatment group (C_{treat} and $CV_{intra-lung}$), respectively, for antegrade, retrograde, combined antegrade and retrograde isolated lung perfusion, pulmonary artery infusion with endovascular inflow occlusion, and intravenous administration

	C_{lung} ($\mu\text{g/g}$)	CV_{lung} (%)	C_{treat} ($\mu\text{g/g}$)	$CV_{intra-lung}$ (%)
a-ILP	158	244%	289	175%
	405	195%		
	377	86%		
r-ILP	589	84%	260	279%
	199	416%		
	150	349%		
c-ILP	417	236%	272	226%
	260	234%		
	186	209%		
BFO	577	129%	445	151%
	279	129%		
	547	195%		
IV	56	46%	42*	51%
	31	49%		
	44	58%		

*IV group was found to be statistically different from the other groups by Tukey's all-pairwise comparison test ($P = .001$). a-ILP, Antegrade isolated lung perfusion; r-ILP, retrograde isolated lung perfusion; c-ILP, combined antegrade and retrograde isolated lung perfusion; BFO, blood flow occlusion; IV, intravenous.

and associated with a substantial pharmacokinetic advantage compared with IV administration.¹⁰ In addition, results emerging from retrograde flushing of the donor lungs with the preservation solution during the procurement of the graft for lung transplantation suggest that there is improved flow to the airways compared with antegrade flushing.¹⁷ Combined ILP allows the delivery of the perfusate to the pulmonary arteries and veins and has been used during lung transplantation.¹⁸

Cytostatic lung perfusion with pulmonary artery infusion and inflow occlusion by a percutaneously placed balloon catheter (BFO)^{8,12,19,20} has 2 theoretic advantages. First it could alleviate any changes in perfusion pattern caused by the surgery itself. Second it allows repeated regional chemotherapy delivery without operation. Cisplatin-based BFO has been compared with ILP in a porcine model with lower lung and higher systemic drug levels and a more heterogeneous drug distribution compared with ILP.¹⁹ In a previous experimental study, we compared doxorubicin-based ILP with BFO in a porcine model.¹² Overall drug uptake was similar after both techniques, but BFO resulted in higher doxorubicin plasma concentrations than ILP. However, disappointing results were obtained in clinical trials using the BFO technique.²⁰

This experimental study revealed that all methods of ILP showed an excellent separation of the systemic and pulmonary circulations, and increased drug uptake of the perfused lungs compared with IV administration. Although r-ILP or c-ILP may theoretically result in an increased leakage because of retrograde flushing of the bronchial arteries, the plasma doxorubicin concentrations after r-ILP and c-ILP were not different from a-ILP in this model. r-ILP or c-ILP did not show an increased overall drug uptake compared

Figure 5. Relationship between doxorubicin concentration and ^{99m}Tc activity in the samples collected in the perfused lungs after ILP, BFO, and IV administration: $\blacktriangle$ a-ILP, $r = 0.55$; $\bullet$ r-ILP, $r = 0.73$; $\blacksquare$ c-ILP, $r = 0.76$; $\circ$ BFO, $r = 0.6$; and $\circ$ IV, $r = 0.77$.

with a-ILP, but the bronchial circulation represents only 5% of the pulmonary circulation. BFO resulted in a similar overall doxorubicin uptake in the perfused lung but revealed a significantly higher systemic leakage as observed after ILP. However, the small number of animals per group and the wide variation of mean drug lung tissue levels for each animal (CV_{lung}) and each treatment group ($CV_{\text{intra-lung}}$) render identification of any significant difference between methods of cytostatic perfusion difficult.

We found a significant correlation between ^{99m}Tc activity and doxorubicin level for each lung specimen in all treatment groups, indicating that doxorubicin distribution correlates with regional blood flow during ILP. However, all methods of cytostatic perfusions (including BFO) resulted in a marked spatial heterogeneity of regional ^{99m}Tc activity and doxorubicin levels in the perfused lung tissues, which was neither anatomic (lobar or segmental) nor explained by a hydrostatic gradient. These differences were apparent both macroscopically and microscopically. In contrast, IV administration resulted in a less heterogeneous (but not homogeneous) ^{99m}Tc activity and doxorubicin distribution within lung tissue but also in significantly lower drug tissue levels compared with ILP and BFO.

Spatial heterogeneity of regional blood flow during ILP may be related to anesthetic and ventilatory management, the presence of ventilation-perfusion mismatch and hypo-

thermia caused by thoracotomy and manipulation of the lung, and acute changes of oxygen tension.^{15,21,22} Despite the avoidance of sternotomy and manipulation of the lung during BFO, the spatial regional blood flow and drug distribution in the perfused lung was as heterogeneous as after ILP. We speculate that the heterogeneous spatial blood flow and doxorubicin distribution in the perfused lung after BFO may be related to (1) a hypoxia-induced regional redistribution of blood flow (caused by a temporary occlusion of the pulmonary artery), (2) an acute vascular reactivity of the pulmonary blood vessels exposed to the concentrated perfusion solution leading to localized vasoconstriction and to a consecutive redistribution of pulmonary blood flow, and (3) a difference in anesthetic and ventilatory management compared with IV administration (absence of myorelaxant, endotracheal intubation, and controlled ventilation in the IV group).

A wide inter-animal variation was not only observed for the overall doxorubicin uptake in the perfused lungs but also with respect to the regional ^{99m}Tc activity and doxorubicin levels in the perfused lung tissues, for all experimental groups assessed. IV administration also resulted in a marked inter-animal variability of regional blood flow and drug distribution with a geometric coefficient of variation for drug tissue levels of 51% for that treatment group. Previous experiments have studied the pattern of regional pulmonary blood flow distribution on ventilated dogs and demonstrated

a marked spatial heterogeneity of regional perfusion over time that varied between animals.¹⁵ However, a heterogeneous spatial pattern of pulmonary blood flow distribution was also found under physiologic conditions in standing awake dogs, which was stable over days suggesting inhomogeneous pulmonary blood flow distribution not only in anesthetized animals but also under physiologic conditions in awake standing animals at rest.¹⁶

Conclusion

a-ILP, r-ILP, c-ILP, and BFO resulted in a significantly higher doxorubicin uptake in the perfused lung than IV administration. The inter-animal variation and spatial heterogeneity of regional blood flow and drug uptake were more pronounced after “invasive” perfusion techniques than observed after IV drug administration in spontaneously breathing animals. Future investigations to try to alleviate this unequal perfusion pattern would include the stimulation of beta-adrenergic receptors on pulmonary vascular smooth muscles, pretreatment of the pulmonary circulation by vasodilators (prostacyclin, nitride oxide), avoidance of hypoxic vasoconstriction by hyperoxygenation before and during ILP, and recruitment of hypoperfused areas by increasing the cardiac output with catecholamines before ILP.

References

1. Johnston MR, Minchen RF, Dawson CA. Lung perfusion with chemotherapy in patients with unresectable metastatic sarcoma to the lung or diffuse bronchioalveolar carcinoma. *J Thorac Cardiovasc Surg.* 1995;110:368-73.
2. Ratto GB, Toma S, Civalleri D, Passerone GC, Esposito M, Zaccheo D, et al. Isolated lung perfusion with platinum in the treatment of pulmonary metastases from soft tissue sarcomas. *J Thorac Cardiovasc Surg.* 1996;112:614-22.
3. Burt ME, Liu D, Abolhoda A, Ross HM, Kaneda Y, Jara E, et al. Isolated lung perfusion for patients with unresectable metastases from sarcoma: a phase I trial. *Ann Thorac Surg.* 2000;69:1542-9.
4. Schröder C, Fisher S, Pieck AC, Müller A, Jaehde U, Kirchner H, et al. Technique and results of hyperthermic (41°C) isolated lung perfusion with high-doses of cisplatin for the treatment of surgically relapsing or unresectable lung sarcoma metastases. *Eur J Cardiothorac Surg.* 2002;22:41-6.
5. Hendriks JMH, Grootenboers MJH, Schramel FMNH, Van Boven WJ, Stockman B, Ter Beek TM, et al. Isolated lung perfusion with melphalan for resectable lung metastases: a phase I clinical trial. *Ann Thorac Surg.* 2004;78:1919-27.
6. Pass HI, Mew DJK, Kranda KC, Temeck BK, Donington JS, Rosenberg SA. Isolated lung perfusion with tumor necrosis factor for pulmonary metastases. *Ann Thorac Surg.* 1996;61:1609-17.
7. Putnam JB. New and evolving treatment methods for pulmonary metastases. *Semin Thorac Cardiovasc Surg.* 2002;14:49-56.
8. Weksler B, Burt ME. Isolated lung perfusion with antineoplastic agents for pulmonary metastases. *Chest Surg Clin N Am.* 1998;8:157-82.
9. Weksler B, Lenert J, Ng B, Burt B. Isolated lung perfusion with doxorubicin is effective in eradicating soft tissue sarcoma lung metastases in a rat model. *J Thorac Cardiovasc Surg.* 1994;107:50-4.
10. Schrupp DS, Zhai S, Nguyen DM, Weiser TS, Fisher BA, Terrill RE, et al. Pharmacokinetics of paclitaxel administered by hyperthermic retrograde isolated lung perfusion techniques. *J Thorac Cardiovasc Surg.* 2002;123:686-94.
11. Furrer M, Lardinois D, Thormann W, Altermatt HJ, Betticher D, Cerny T, et al. Isolated lung perfusion: single-pass system versus recirculating blood flow perfusion in pigs. *Ann Thorac Surg.* 1998;65:1420-5.
12. Furrer M, Lardinois D, Thormann W, Altermatt HJ, Betticher D, Triller J, et al. Cytostatic lung perfusion by use of an endovascular blood flow occlusion technique. *Ann Thorac Surg.* 1998;65:1523-8.
13. Kümmerle A, Krueger T, Dusmet M, Vallet C, Pan Y, Ris HB, et al. A validated assay for measuring doxorubicin in biological fluids and tissues in an isolated lung perfusion model: matrix effect and heparin interference strongly influence doxorubicin measurements. *J Pharm Biomed Anal.* 2003;33:475-94.
14. Lienard D, Ewalenko P, Delmotte JJ, Renard N, Lejeune FJ. High-dose recombinant tumor necrosis factor alpha in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. *J Clin Oncol.* 1992;10:52-60.
15. Glenny RW, Polissar NL, McKinney S, Robertson HT. Temporal heterogeneity of regional pulmonary perfusion is spatially clustered. *J Appl Physiol.* 1995;79:986-1001.
16. Glenny RW, McKinney S, Robertson HT. Spatial pattern of pulmonary blood flow distribution is stable over days. *J Appl Physiol.* 1997;82:902-7.
17. Baretti R, Bitu-Moreno J, Beyersdorf F, Matheis G, Francischetti I, Kreitmayer B. Distribution of lung preservation solutions in parenchyma and airways: influence of atelectasis and route of delivery. *J Heart Lung Transplant.* 1995;14:80-91.
18. Varela A, Cordoba M, Serrano-Fiz S, Burgos R, Montero G, Tellez G, et al. Early lung allograft function after retrograde and antegrade perfusion. *J Thorac Cardiovasc Surg.* 1997;114:1119-20.
19. Ratto GB, Esposito M, Leprini A, Civalleri D, De Cian F, Vannozzi MO, et al. In situ lung perfusion with cisplatin. An experimental study. *Cancer.* 1993;71:2962-70.
20. Karakousis CP, Park HC, Sharma SD, Kanter P. Regional chemotherapy via the pulmonary artery for pulmonary metastases. *J Surg Oncol.* 1981;18:249-55.
21. Walther SM, Domino KB, Glenny RW, Hlastala MP. Pulmonary blood flow distribution in sheep: effects of anesthesia, mechanical ventilation, and change in posture. *Anesthesiology.* 1997;87:335-42.
22. Melsom MN, Flatebo T, Nicolaysen G. Hypoxia and hyperoxia both transiently affect distribution of pulmonary perfusion but not ventilation in awake sheep. *Acta Physiol Scand.* 1999;166:151-8.