Evaluation of isolated lung perfusion as neoadjuvant therapy of lung metastases using a novel in vivo pig model: I. Influence of perfusion pressure and hyperthermia on functional and morphological lung integrity

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Abstract

Objective: Despite favorable experimental results and an encouraging early experience in humans, isolated lung perfusion (ILP) for treatment of metastases is still not established clinically. The complexity of the procedure as well as poor knowledge regarding the technical necessities of lung perfusion represents major limitations.

Methods: In this novel in vivo pig model, ILP of the left lung was performed for 40 min followed by the exclusion of the right lung. Survival and all monitored parameters of hemodynamics, ventilation and gas exchange were exclusively dependent on the previously perfused left lung for the 6-h reperfusion period. Furthermore, histological examination was assessed. In the first protocol influence of different perfusion pressures (PP) on the native lung tissue was investigated (LPG, n = 6; PP, ≤ 25 mmHg; HPG, n = 8; PP > 25 mmHg). In the second protocol the influence of normothermic (T-38; n = 5; t = 38 °C), mild (T-40; n = 5; t = 40 °C) and moderate hyperthermic (T-41.5; n = 5; t = 41.5 °C) perfusion temperature was evaluated. Results were compared to those of a sham-operated control group (SG, n = 5).

Results: ILP led to a slight deterioration of all functional as well as histological parameters in all groups. HPG resulted in impairment regarding all monitored parameters compared to LPG and SG. Significant differences between HPG and SG were found for cardiac index (P = 0.026) and pulmonary vascular resistance index (PVRI, P = 0.048). Histological scoring revealed significantly higher grade of lung injury for HPG animals (P = 0.001). Functional parameters did not differ between normothermic and hyperthermic perfusion groups. However, animals of the T-38 group demonstrated significantly increased PVRI (P = 0.004). Histological examination revealed significantly higher scores of acute lung injury for all perfusion groups compared to the Sham group (P < 0.001).

Conclusions: The results of this novel large animal model represent the first available demonstration that increased PP in a setting of ILP will result in deleterious effects on lung function and morphology. However, mild to moderate hyperthermia is well tolerated by the native lung tissue.

Keywords: Isolated lung perfusion; Lung metastases; Perfusion pressure; Hyperthermia; Lung function; Histology

1. Introduction

Surgical metastasectomy is therapy of choice for a wide spectrum of tumor entities, because of the low morbidity and mortality of this procedure. However, patients with multiple, simultaneous or recurrent metastases have a very poor prognosis [1]. Isolated lung perfusion (ILP) might be a successful alternative therapeutic strategy in these patients, especially in the light of failure of chemotherapy. Potential advantages of ILP compromise the opportunity to apply very high drug doses to the diseased lung with avoidance of systemic drug toxicity. Furthermore this concept allows the variation of concomitant conditions so that drug activity might be enhanced and/or drug toxicity limited [2].
For the therapeutic concept of ILP the application of hyperthermia seems very attractive. The anti-proliferative effect of hyperthermia on tumor tissue is well known for a long time. Hyperthermia itself induces a significant cytotoxic effect on the native tumor tissue [3,4]. Additionally, numerous experimental as well as preclinical studies demonstrated the synergistic effect of hyperthermia to cytotoxic agents [5,6]. In isolated hyperthermia, temperatures might be increased to levels, which would otherwise not have been tolerated by numerous organ systems. Due to the demand for an extracorporeal circuit in order to apply ILP, temperature changes of the perfusion fluid can be achieved very easily via the heat exchanger system.

Despite favorable results in experimental rodent tumor models [7–9] and an encouraging early experience in humans [10–15], ILP for the treatment of pulmonary metastases is still not established clinically. The complexity of the procedure as well as the poor knowledge regarding various technical details of optimal lung perfusion represents major limitations. Systematic studies regarding the optimal perfusion parameters in large animals do not exist in the literature so far.

In preparation of this study we established a novel in vivo pig model, which allows evaluation and quantification of the impact of different perfusion parameters on the functional and morphological integrity of the lung. In the first part, the influence of perfusion pressure (PP) and hyperthermia on the integrity of native lung tissue was assessed.

2. Materials and methods

2.1. Surgical procedure

The experimental model was adapted from an established lung transplantation model [16,17]. Domestic pigs (German Landrace) of 23–39 kg were anesthetized with propofol 2% (2–4 mg/kg), followed by a combined fentanyl (0.2–0.4 μg/kg per min), midazolam (16–20 μg/kg per min) and pancuronium bromide (6–10 μg/kg per min) infusion. After intubation animals were ventilated in a pressure-controlled mode (peak inspiratory pressure <20 mmHg, inspiratory/expiratory ratio 1:1, positive endexpiratory pressure 5 mmHg, FiO2 0.5). For monitoring the systemic arterial pressure and the arterial blood gas analysis a catheter was placed into the right carotid artery. A Swan-Ganz catheter was used for online monitoring of pulmonary hemodynamics and pulmonary blood gas analysis.

Left thoracotomy was performed in the fourth intercostal space. The left lung was mobilized and the structures of the left hilum were separated. In addition the right pulmonary arteries and the right main bronchus were dissected. A pressure catheter was introduced into the left atrium. Purse-string sutures were placed at the orifice of the pulmonary veins and in the pulmonary artery. After clamping of the pulmonary artery and the left atrium the two venous cannulae (18 Ch, Jostra, Germany) and the arterial cannula (16 Ch, Jostra, Germany) were placed. An additional pressure line (Cava fix, B. Braun, Germany) was inserted into the arterial cannula, which allowed for PP measurements in the pulmonary artery distally to the cannula tip. Isolated perfusion of the left lung was performed by recirculation of the perfusate in a closed system using a heart-lung machine (Stöckert, Germany). A combined oxygenator/heat exchanger (Sarns Turbo membrane oxygenator modul, Sarns, Germany) for temperature regulation was integrated into the extracorporeal circulation system.

After ILP and decannulation the purse-string sutures were tightened and both the atrial and the arterial clamps were removed. After a short period of reperfusion the contralateral right main bronchus and the right pulmonary arteries were clamped. If necessary, inotropic substances were used for hemodynamic improvement during this procedure. In this model survival of pigs and all parameters of lung function were exclusively dependent on the function of the previously perfused left lung. All experiments were terminated after 6 h of reperfusion with intracardial injection of magnesium sulfate.

2.2. Perfusion parameters

ILP was maintained for 40 min followed by a 5 min wash-out period. Perfusate consisted of buffered hetastarch (HAES 6%, Fresenius, Germany; pH 7.2–7.5), 5000 units heparin (Liquemin N 25000, Roche, Germany) and the variable amount of residual blood from the excluded left lung. Perfusion rate was gradually increased up to 800–1000 ml/min. In case of elevated PP > 30 mmHg in the pulmonary artery the perfusion rate was lowered to a minimum of 700 ml/min. The perfused lung was ventilated with an FiO2 of 0.5 to avoid the Euler-Liljestraat effect.

2.3. Experimental groups

All study groups were compared to a sham-operated group. Sham-operated animals (Sham group, n = 5) received an identical operation with exception of cannulation and perfusion of the left lung.

2.3.1. Perfusion pressure groups

After 30 min of ILP animals were differentiated by the pulmonary perfusion pressure (PPP). Animals with a PPP lower than 25 mmHg (LP group, n = 6) were compared with those with a PPP of 25 mmHg or higher (HP group, n = 8). Groups did not differ preoperatively regarding pulmonary hemodynamics and gas exchange parameters. However, sham-operated animals were significantly smaller then animals of the perfusion groups (body surface area, Sham 0.89 ± 0.13 m2; LP 1.04 ± 0.09 m2; HP 0.94 ± 0.06 m2) and had a lower dynamic lung compliance (Sham 17.4 ± 4.0, 28.7 ± 7.0, and 23.4 ± 4.0 ml/mmHg).
2.3.2. Hyperthermia groups

In animals of the normothermic group (T-38 group, \( n = 5 \)) perfusion of the left lung was performed at a perfusate temperature of \( 38.1 \pm 0.1 ^\circ C \). Perfusate temperatures of the mild (T-40 group, \( n = 5 \)) and moderate (T-41.5 group, \( n = 5 \)) hyperthermia groups were \( 40.2 \pm 0.2 \) and \( 41.6 \pm 0.1 ^\circ C \), respectively. Groups did not differ preoperatively regarding weight (\( 30.0 \pm 8.8 \text{ kg} \)) and body surface area (\( 1.0 \pm 0.1 \text{ m}^2 \)), pulmonary hemodynamics, ventilatory as well as gas exchange parameters.

2.4. Measurements of lung function

All atrial, systemic arterial as well as pulmonary arterial pressures were monitored continuously during the reperfusion period. Pulmonary vascular resistance was calculated after measurement of the cardiac output by means of a continuous thermodilution cardiac output computer (Vigilance, Edwards Lifescience, Germany). Effective ventricular temperature was measured and data taken from the ventilator (Evita 2, Dräger, Germany). Both, mixed venous and arterial blood gases were analyzed simultaneously using the automated blood gas machine (ABL 725, Radiometer, Denmark). The systemic proinflammatory response of the animal to the procedure was measured using porcine specific enzyme immunoassays for tumor necrosis factor-\( \alpha \) (TNF\( \alpha \), Quantikine P Porcine TNF\( \alpha \)/TNFSF2 Immunoassay, R&D Systems, USA), interleukin 1\( \beta \) (IL-1\( \beta \), Quantikine P Porcine IL-1\( \beta \) Immunoassay, R&D Systems, USA) and interleukin 6 (IL-6, Quantikine P Porcine IL-6 Immunoassay, R&D Systems, USA).

2.5. Measurement of temperature

Continuous measurement and monitoring of temperatures was performed using four temperature probes (Myocardial temperature probe 90038, Malinckrodt medical, Germany). Two probes were placed into the dorsal part of the upper and lower lobes of the left lung. Two additional probes were inserted into the afferent and efferent tubes of the perfusion circuit. The systemic temperature of the animal was measured by the Swan-Ganz catheter.

2.6. Wet–dry ratio

Prior to termination of the observation period lung tissue specimens from defined areas of the upper and lower lobes of the left lung were taken. To evaluate the wet-to-dry weight (W/D) ratio a native tissue specimen was weighted. After a period of 48 h at \( 80 ^\circ C \), samples were weighted again and W/D ratio was calculated.

2.7. Histological scoring

Lung tissue specimens from defined localizations of each lobe were dissected and immediately fixed in (4%) buffered formalin. After embedding and cutting, all sections were stained with haematoxylin/eosin. Additionally, Elastica-van Gieson staining as well as immunohistochemical staining of factor VIII, CD 68 and CD 15 were used in certain cases. Histological evaluation was performed in a blinded manner by a single pathologist (M.L.) without any information regarding grouping or treatment of the accompanying animals. Histological findings were compared using the established scoring system by Chiang et al. [18], which was initially developed for evaluation of the severity of acute lung injury due to ischemia/reperfusion injury of transplanted lungs. Each of the following pathological parameters led to a specific score, which correlates to the histopathological severity: perivascular edema 1; peribronchial edema 2, interstitial edema 2, perivascular cell infiltration 2, alveolar edema 3, interstitial cell infiltration 3, and alveolar cell infiltration 4. A total of 20 scope views were examined for each lung tissue specimen. The sum of all the pathological scores was the score for each scope, and then the mean score of 20 scopes was calculated as the injury score for this lung tissue. The mean of the injury scores of both lobes was considered as the final score for each lung.

2.8. Animal care

All animals received human care in compliance with the European Convention on Animal Care, and with the ‘Principles of Laboratory Animal Care’ formulated by the National Society for Medical Research, and the ‘Guide for the Care and Use of Laboratory Animals’, published by the National Institute of Health (NIH publication No. 86-23, revised 1985).

2.9. Statistical analysis

Values are expressed as mean ± standard deviation. Comparisons between all groups for a single parameter were carried out using one-way ANOVA (preoperative characteristics, wet–dry ratio, histological score) or the ANOVA with repeated measures (hemodynamic, ventilatory and gas exchange parameters). For post hoc testing the Dunnett method was applied. For description of correlation the Pearson’s coefficient (PC) was calculated. A \( P \)-value of \( < 0.05 \) was considered to represent a statistically significant difference. All data were analyzed using SPSS for MS Windows version 10.0 (SPSS Inc., Chicago, IL, USA).
3. Results

There was no animal death due to technical errors or the ILP procedure. Two experiments were excluded, due to severe pneumonia in the first and a persisting foramen ovale with consecutive severe right-to-left shunt in the second experiment. Perfusion rate did not differ between the groups (865 ± 173 ml/min, 28 ± 8 ml/kg per min, \( P = 0.621 \)). Mean loss of perfusion fluid was 241 ± 199 ml without any difference between the groups (\( P = 0.380 \)).

3.1. Perfusion pressure study

The high pressure group (HP) showed impaired values of all monitored parameters compared to the low pressure group (LP) and the Sham group (SG). Animals of the HP group developed an increasing PP over time (Fig. 1) due to an elevated pulmonary vascular resistance. This resulted in a significantly and continuously higher pulmonary vascular resistance index (PVRI; \( P = 0.048 \), Fig. 2a). Consecutively, HP group animals were found to have a significantly lower cardiac index (\( P = 0.026 \)). The correlation between PP and pulmonary vascular resistance was significant for the total reperfusion period. The Pearson’s coefficient ranged between 0.540 and 0.679, representing a \( P \)-value between 0.046 and 0.010. Both effective as well as dynamic compliance were insignificantly lower in the HP group compared to LP as well as Sham group (\( C_{\text{eff}}, P = 0.103; C_{\text{dyn}}, P = 0.331 \); Fig. 2b). A significant inverse correlation between PP and compliance could be demonstrated. The Pearson’s coefficient for both the effective as well as the dynamic lung compliance, ranged between −0.668 and −0.873 at the different times of measurement within the reperfusion period (\( P \)-values between <0.001 and 0.009). Consecutively, the resulting peak inspiratory pressure and plateau pressure were higher in the HP group. The pO2/FiO2 ratio was compromised in both perfusion groups with a trend to more deteriorated levels for HP group animals (post hoc test Sham vs. HP, \( P = 0.057 \)). Gas exchange parameter AADO2 confirmed the trend toward more deteriorated values for the HP group without statistical significance (\( P = 0.166 \)) (Fig. 3).

ILP led to mild histological signs of acute lung injury in all animals. Predominantly perivascular and interstitial edema could be observed. Rare perivascular cell infiltration was detectable. More severe signs of acute lung injury in terms of alveolar edema as well as interstitial cell infiltration parameter AADO2 confirmed the trend toward more deteriorated values for the HP group without statistical significance (\( P = 0.166 \)) (Fig. 3).

Fig. 1. Perfusion pressure in the left pulmonary artery during ILP. PP represents the time after placement of all monitoring catheters before the left pulmonary artery was clamped. \( P \)-value was calculated by ANOVA with repeated measures.

Fig. 2. Pulmonary vascular resistance index (PVRI) and effective lung compliance during the reperfusion period. Time ‘0’ represents the time point immediately after complete clamping of the right pulmonary arteries. (a) PVRI = PVR/body surface area (dyne s cm⁻² m⁻⁵). Post hoc testing (Dunnett T3) of PVRI revealed the following \( P \)-values: Sham–LP \( P = 0.828 \), Sham–HP \( P = 0.032 \), LP–HP \( P = 0.593 \). (b) Post hoc testing of effective lung compliance revealed no significances.

Fig. 3. Histological score of acute lung injury applying the method of Chiang [18]. Results of post hoc testing (Dunnett T3): Sham–LP \( P = 0.018 \), Sham–HP \( P = 0.002 \), LP–HP \( P = 0.436 \).
were found in only few scopes of all specimens. Histological scoring revealed significant differences between the groups ($P = 0.001$; Fig. 4).

### 3.2. Hyperthermia study

Within the first minutes of ILP the lung tissue temperature reached the temperature level of the corresponding perfusate (Fig. 4) showing a highly significant correlation between perfusion temperature and lung tissue temperature (Pearson’s coefficient 0.965, $P < 0.001$).

After clamping of the right pulmonary arteries the PVRI increased in all animals due to the reduction of perfused pulmonary vascular diameter. This increase of PVRI was higher in all perfusion groups compared to the Sham group (Fig. 5a). During the monitoring period the normothermic T-38 group demonstrated the highest PVRI compared to all other groups ($P = 0.004$). There were no significant differences between the groups regarding neither ventilatory nor gas exchange parameters. Animals of the Sham group represented with a slightly superior ($P = 0.170$) as well as dynamic lung compliance ($P = 0.148$) than the animals of all perfusion groups. Generally, animals of the moderate hyperthermic T-41.5 group were found to have the highest effective lung compliance of all perfusion groups, however this difference did not reach statistical significance. Evaluation of gas exchange parameters revealed a slow deterioration of pO$_2$/FiO$_2$-ratio as well as AADO$_2$ for all groups during the monitoring period. Although there was a better pO$_2$/FiO$_2$-ratio for Sham-operated animals this difference was not significant ($P = 0.216$, Fig. 5b).

Mild to moderate histological signs of acute lung injury were observed in all ILP treated animals. Observed morphological correlates of acute lung injury were perivascular and peribronchial edema as well as perivascular or interstitial infiltration with mononuclear cells. Signs of severe lung injury as alveolar edema or severe interstitial cell infiltration were not detected. The histological scores demonstrated significantly lower tissue damage in Sham-lungs as compared to lungs from each ILP group (Fig. 6). The animals of the T-40 group were characterized by a higher incidence of preoperative bronchitis and focal pneumonic infiltrates. Wet–dry ratio did not demonstrate any differences between the study groups.

### 4. Discussion

Despite the fact that ILP was performed in about 40 reported patients worldwide, the clinical use of this alternative strategy is discussed controversially. The operative and technical demands of ILP are challenging. Furthermore a potentially increased morbidity and the absence of proven efficacy of this procedure have to be taken into account. All human studies of ILP were characterized by complete heterogeneity regarding all perfusion parameters. The cause for this heterogeneity is the lack of systematic experimental large animal studies, which would allow the transformation of results into clinical practice. In the few available published animal studies in pigs or dogs either the pharmacokinetic of anti-neoplastic drugs [19,20] or the mid term survival with or without contralateral pneumonectomy were described [2,21,22]. However, with exception of the publications of Rickaby et al. [20] and Cowen et al. [22], who investigated the influence of temperature on the native lung tissue, no systematic studies regarding the perfusion parameters are available. Because of the lack of a suitable animal model, which would allow evaluating the extent of an acute lung injury, we established such an ILP model by modification of a pig lung transplantation model [16,17].
Due to the anatomic and genetic similarity, the transformation of results from this pig lung model to the clinical reality in humans is widely accepted.

So far, studies with focus on the influence of PP on the integrity of the lung are absent in world literature. Maron et al. [23] were able to demonstrate that in dogs a peak pulmonary arterial pressure of 80 mmHg for a period of 4 min led to a significant increase of the capillary filtration coefficient. Investigators of the ILP studies used a ‘physiological perfusion pressure’. However, the pressure in these publications ranged from 5 to 35 mmHg [2,21,22]. The pulmonary pressure is proportional to both the vascular resistance and the flow rate. It remains unclear, however, which flow rate is optimal for ILP. Under physiological conditions, about half of the cardiac output is passing the lung. It can be assumed that the flow rate influences the overall homogeneity of perfusion and consecutively the potential distribution of the chemotherapeutical drug. In their rodent model, Weksler et al. [7] were not able to demonstrate an influence of perfusion rate on the Doxorubicin uptake. However, the described perfusion conditions were completely different from the clinical modalities of human use. So far it is not well explained why the pulmonary vascular resistance was elevated in the one group of animals. Probably the application of vasodilators could solve this specific problem.

The profound anti-neoplastic effect of hyperthermia is well known in the literature [3,4]. Experimentally, hyperthermia leads to disturbances of the microvascular blood supply of tumors due to heat-dependent lesions of
the vascular endothelium with consecutive activation of adhesion molecules [24]. Subsequently, the damage of nutritive processes as well as the activation of the cytokine–macrophages system results in tumor destruction [25]. Besides this direct cytotoxic effect on the tumor tissue hyperthermia could increase the uptake of cytotoxic agents when used in ILP [21]. However, temperatures higher than 41.8°C might cause complete occlusion of nutritive tumor vessels with the consequence of non-uptake of the cytotoxic agents by the neoplastic tissue [26]. As a consequence the theoretical temperature optimum is thought to be in the range of 40–41°C.

There are only two experimental studies available evaluating the influence of hyperthermia on the lung function with use of an ILP model in dogs. Cowen et al. [22] perfused both lungs simultaneously for 1 h. The perfusion was performed without ventilation or oxygenation of the perfusate. Temperature of the study groups was set to 43–45°C or higher than 45°C. In the group with temperatures of more than 45°C all animals died due to fulminating pulmonary edema. Animals of the lower temperature group showed severe functional disturbances. Experimental results of Rickaby et al. demonstrated no detectable influence of temperature on the variable lung weight, extravascular water, vascular volume, serotonin uptake, urea permeability surface area product PP, and lung compliance when the temperature was less than 44°C. However, these experiments were influenced by several temperature-independent factors as type of perfusion fluid and a rather long perfusion period of 2 h with a high potential of ischemic lung injury [20].

The analyzed parameters of our study did not reveal a uniform influence of hyperthermia on the functional and histological characteristics of the normal lung tissue. Compared to sham-operated animals, ILP per se led to slight deterioration of all parameters. However, gas exchange as well as ventilatory parameters were not influenced significantly. Hyperthermia led to a significant reduction of pulmonary vascular resistance which might be caused by its relaxing effect on the vasculature. The significantly higher score of acute lung injury for the mild hyperthermia group might be due to the described higher preoperative incidence of infectious histological changes resulting in a higher vulnerability of the lung tissue.

In this study we were able to show that PP of more than 25 mmHg were negative on both the functional as well as the morphological integrity of the isolated perfused lung. Furthermore, moderate hyperthermia of 41.5°C, which is applied by ILP, does not result in negative effects on the native lung tissue. Due to the remarkable reduction of pulmonary vascular resistance, moderate hyperthermia might ever be beneficial with respect to outcome, especially when it is combined with cytotoxic agents. The rather limited number of animals in combination with high standard deviations for several parameters let to cautious interpretation of our data. The significant reduction of native pulmonary tissue damage is the essential prerequisite prior to introduction of ILP into clinical practice.

References

