Evaluation of isolated lung perfusion as neoadjuvant therapy of lung metastases using a novel in vivo pig model: II. High-dose cisplatin is well tolerated by the native lung tissue

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Abstract

Objective: Efficacy of in vivo isolated lung perfusion (ILP) with cisplatin could be shown in different rodent tumor models. Despite the use of this alternative therapeutical strategy in very few patients with lung metastases, there are no systematic studies regarding the tolerance of the native lung tissue in large animal models or humans. Methods: In a novel ILP pig model, groups with two different concentrations of cisplatin (group CP150: 150 mg/m² cisplatin, n = 5; group CP300: 300 mg/m² cisplatin, n = 5) were compared with a control group (n = 5) and a Sham group (n = 5) concerning the influence on hemodynamic, ventilatory and gas exchange parameters as well as on structural integrity of the lung. In the additional CP300-HT group the potentially cumulative effect of hyperthermia and high-dose cisplatin perfusion was evaluated (300 mg/m² cisplatin, 41.5 °C, n = 5). Following the ILP of the left lung for 40 min, right main bronchus and right pulmonary arteries were clamped and survival as well as lung function parameters were dependent on the previously perfused lung for the 6-h-reperfusion period. Quantification of histological acute lung injury was performed using the score of Chiang. ANOVA, ANOVA with repeated measures and Pearson’s correlation estimation were applied for statistical evaluation. Results: All animals survived ILP and the entire reperfusion period. Platinum levels of the perfusate and lung tissue showed a significant correlation with the dose given (P < 0.001) but no correlation with the very low plasma levels in all groups (P = 0.825). ILP resulted in a slight deterioration of most functional parameters compared to the Sham group. Although there were no differences between the perfusion groups regarding hemodynamic and ventilatory parameters, gas exchange parameters (pO2/FiO2-index, pCO2, AADO2) demonstrated a trend toward dose-related functional impairment. Histological evaluation confirmed a dose-depended damage of lung tissue (P < 0.001, correlation coefficient 0.670). The hyperthermic ILP with high-dose cisplatin led to improved gas exchange parameters and a reduction of morphological lung damage. Conclusions: In vivo ILP with high-dose cisplatin represents a safe procedure in this pig model. Hyperthermic perfusion up to 41.5 °C was beneficial to reduce the acute lung injury. The promising results of this study might be used for initiation of clinical trials as an alternative treatment in patients with a very poor prognosis.

Keywords: Isolated lung perfusion; Lung metastases; Cisplatin; Lung function; Acute lung injury; Hyperthermia

1. Introduction

High-dose application of chemotherapeutics using isolated organ perfusion has been established in soft tissue sarcoma and in-transit melanoma of the limb as therapy of choice [1–5]. Superior results with remission rates up to 100% were obtained using melphalan alone or in combination with tumor necrosis factor-α [6,7]. Further chemotherapeutic agents used in this therapeutical concept were doxorubicin and carboplatin [8–10].

In the clinical setting, isolated lung perfusion (ILP) for the therapy of lung metastases was performed in only few patients so far. In these patients, doxorubicin as well as
cisplatin was applied \[11–14\]. In rodent tumor models these chemotherapeutic agents as well as melphalan, 5-FU and gemcitabine were proved to be effective in the therapy of lung metastases \[15–22\]. Particularly cisplatin seems to be attractive for use in ILP because of its high cytotoxic potency for the most tumors metastasizing to the lung as well as its nephrotoxic dosage limit in systemic application. Although cisplatin was applied in the ILP of 12 patients, studies evaluating the tolerance of the native lung tissue against high-dose cisplatin concentrations are not yet published.

In preparation of clinical trials, our study evaluates the influence of different cisplatin concentrations on the functional and histological integrity of the native lung tissue using a novel large animal model of ILP.

**2. Materials and methods**

**2.1. Experimental model**

The experimental model was previously described in detail \[23\]. In domestic pigs of 23–40 kg the vessels of the left lung were isolated. ILP was performed using an arterial cannula, which was inserted into the pulmonary artery, and two venous drainage catheters, which were placed via the left atrium into left lung veins. After cannulation and declamping ILP of the left lung was followed by a short period of reperfusion. Consecutively, the contralateral right main bronchus and the right pulmonary arteries were clamped. Animal survival and functional parameters were therefore entirely dependent on the previously perfused left lung. Monitoring was stopped after 6 h of reperfusion.

**2.2. Perfusion parameters**

ILP was maintained for 40 min. at normothermia (38.0 °C) followed by a 5 min wash-out period. Perfusate consisted of buffered hetastarch (HAES 6%, Fresenius, Germany; pH 7.2–7.5) with 5000 units heparin (Liquemin N 25000, Roche, Germany) and the residual amount of blood from the excluded left lung. Perfusion rate was gradually increased up to 800–1000 ml/min. The perfused lung was ventilated with an oxygen fraction of 0.5 to avoid the Euler-Liljestraat-effect. At stable perfusion conditions the defined cisplatin dose was added to the recirculating perfusion fluid.

**2.3. Experimental groups**

Three different study groups were compared to a control group as well as to a Sham-operated group. Whereas animals of the low-dose group received 150 mg cisplatin into the perfusion fluid (CP150 group, \( n = 5 \)), corresponding to \( 147 \pm 19 \) mg/m\(^2\) body surface area or \( 4.6 \pm 0.9 \) mg/kg body weight. Animals of the high-dose cisplatin group received 300 mg cisplatin (CP300 group, \( n = 5 \)), corresponding to \( 288 \pm 10 \) mg/m\(^2\) or \( 8.9 \pm 0.5 \) mg/kg. Additionally a further high-dose group received 300 mg cisplatin (CP300-HT group, \( n = 5 \)) at a perfusion temperature of 41.5 °C for evaluation of the combination effects of both, high-dose cisplatin and hyperthermia. Animals of the control group (control group, \( n = 5 \)) were perfused without any additional drug. Sham operated animals (Sham group, \( n = 5 \)) received an identical operation with exception of cannulation and perfusion of the left lung. Groups did not differ preoperatively regarding weight (31.3 ± 4.5 kg) and body surface area (1.0 ± 0.1 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 lung compliance was calculated by the formula \( C_{eff} = \frac{TV}{PP - PEEP} \) (TV, tidal volume; PP, ventilatory plateau pressure; PEEP, positive endexpiratory pressure). Dynamic lung compliance was measured and data taken from the ventilator (Evita 2, Dräger, Germany). Both mixed venous as well as arterial blood gas analysis was performed using an automated blood gas machine (ABL 725, Radiometer, Denmark).

**2.5. Gas exchange parameters**

Oxygen partial pressure of arterial (paO\(_2\)) and venous blood (pvO\(_2\)) were measured simultaneously. Alveolo-arterial oxygen partial pressure difference (AADO\(_2\)) represents the necessary oxygen pressure of alveolar air for oxygenation of the arterial blood. A low value of AADO\(_2\) characterizes a favorable gas exchange.

**2.6. Platinum measurement**

Dried lung tissue was weighted and subsequently digested using an acid mixture of nitric and perchloric acid. After mineralization at 260 °C the residual substance was dissolved in ammonium nitrate. Concentration of platinum was measured using the atom absorption spectrometry. The detection limit and precision of this method were 0.02 μmol/l and 1.5%, respectively.

**2.7. Wet–dry ratio**

Just before termination of the observation period lung tissue specimens from defined positions of the upper and lower
lobes of the left lung were taken. To evaluate the wet-to-dry weight (W/D) ratio a tissue specimen was weighted. After drying to a constant weight for 48 h at 80 °C, samples were weighted again and W/D ratio was calculated.

2.8. Histological scoring

One lung tissue specimen from defined positions of each lobe was dissected and immediately fixed in (4%) buffered formalin. After embedding and cutting all sections were stained with haematoxylin/eosin. Additionally Elastica–vanGieson 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 one pathologist (M.L.) without any information regarding grouping or treatment of the accompanying animals. Histological findings were evaluated using the scoring system developed by Chiang et al. [23,24].

2.9. 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).

2.10. Statistical analysis

Values are expressed as mean ± standard deviation. Comparisons between groups for preoperative characteristics, wet–dry ratio and the histological score were performed using one-way ANOVA. Hemodynamic, ventilatory and gas exchange parameters were compared using ANOVA with repeated measures. For post hoc testing the Dunnett method was applied. For description of correlation the Pearson 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

All animals survived the operative procedure and the subsequent monitoring period.

3.1. Platinum concentration

At the end of perfusion there was a highly significant correlation of the applied cisplatin doses with the platinum concentration of the perfusate (PC 0.933; P < 0.001) and the lung tissue (PC 0.863; P < 0.001, Fig. 1). However, platinum serum measurements were very low and independent of the administered cisplatin dose (PC 0.062; P = 0.825, Fig. 2).

3.2. Hemodynamic and ventilatory parameters

There were no significant differences between the groups regarding hemodynamic as well as ventilatory parameters. Pulmonary vascular resistance showed a slight deterioration for all perfusion groups compared to the Sham group (P = 0.359). However, the highest value of PVRI was found for the animals of the control group, which were perfused without cisplatin (Fig. 3a). Animals of the Sham group had a slightly better effective (P = 0.668) as well as dynamic lung compliance (P = 0.236) than the animals of all perfusion groups. Control group animals showed the lowest lung compliance (Fig. 3b).
3.3. Gas exchange parameters

Evaluation of gas exchange revealed the worst parameters in animals of the high-dose cisplatin group at normothermia (CP300 group) and the best values for the Sham group. The pO2/FiO2-ratio showed a slow deterioration during the monitoring period for all groups. However, the decrease was more pronounced for animals of the cisplatin groups. The pO2/FiO2-ratio of animals of the hyperthermic high-dose cisplatin group (CP300-HT) was higher than that of animals with the same cisplatin dose at normothermia and comparable to those of the lower dose cisplatin group (CP150; Fig. 3c). These differences were not statistically significant. Similar results were obtained for AADO2 (Fig. 3d).

ILP led to mild histological signs of acute lung injury in all animals. Correlates of a severe acute lung injury like alveolar edema as well as interstitial cell infiltration were
found in all specimens of the CP300 and occasionally in specimens of the CP300-HT (Fig. 6). Animals of the CP150 group demonstrated only signs of mild acute lung injury comparable to those of the control group. Subsequently, histological scoring revealed highly significant differences between the groups ($P < 0.001$; Fig. 7). In post hoc analysis the score of the Sham group was significantly lower as compared to all perfusion groups. Both high-dose cisplatin groups (CP300, CP300-HT) had a significantly higher lung injury score than the control group. However, the Chiang-score of the CP300-HT was comparable to that of the CP150-group. There was a highly significant correlation of the cisplatin dosage with the histological score of acute lung injury ($PC = 0.864; P < 0.001$; Fig. 8). In contrast, wet–dry ratio did not demonstrate any differences between the study groups.

![Fig. 5. Correlation of applied cisplatin dosage and measured arterial pCO$_2$ after 300 min of reperfusion. Correlation was statistically highly significant for the entire reperfusion period beginning 60 min after reperfusion (PC, Pearson’s coefficient).](image)

![Fig. 6. Histological signs of moderate to severe acute lung injury. (a) Moderate acute lung injury: periartrial edema with concomitant slight bleeding. Moderate interstitial infiltration. Specimen from CP300-group with a concomitant Chiang-score of 6.4 (HE staining, 500×). (b) Severe acute lung injury: interstitial infiltration of neutrophiles and few eosinophiles as well as macrophages. Moderate interstitial and severe alveolar edema and beginning of alveolar infiltration. Specimen of the animal with the strongest acute lung injury (CP300-group, Chiang-score 10.05; HE staining, 312.5×).](image)

![Fig. 7. Comparison of the histological score of acute lung injury [24]. Post hoc analysis revealed the following $P$-values: Sham–control, $P = 0.026$; Sham–CP150, $P = 0.029$; Sham–CP300, $P < 0.001$; Sham–CP300-HT, $P < 0.001$; control–CP300, $P = 0.001$; control–CP300-HT, $P = 0.003$; CP150–CP300, $P = 0.003$; CP150–CP300-HT, $P = 0.115$; CP300–CP300-HT, $P = 0.071$.](image)

![Fig. 8. Correlation of applied cisplatin dosage and the score of histological acute lung injury (PC, Pearson’s coefficient).](image)
4. Discussion

With respect to the favorable experiences of isolated limb perfusion the alternative therapeutic concept of ILP might cause similar superior results for patients with advanced or recurrent lung metastases. Although there is an extensive knowledge regarding efficacy and pharmacokinetics of several chemotherapeutical drugs in different rodent tumor models, transformation of ILP into the clinical practice is difficult. Whereas the technique of isolated antegrade perfusion of a single lung is well described and easy to perform, the most limiting factor for implementation of ILP is the poor knowledge regarding the optimal perfusion conditions and the dosage limits of the drugs. Therefore, systematic studies in large animal ILP models are necessary to investigate these indispensable basics.

Cisplatin was used in clinical ILP application in three different studies [11,12,14]. The dosage of this drug ranged from 70 to 200 mg/m², resulting in a platinum concentration of the perfusate in between 20 and 200 µg/ml. Therefore, the results of these clinical trials are not comparable.

The only large animal study using cisplatin compared ILP with two different pulmonary infusion techniques [12]. The authors were able to demonstrate high platinum concentrations of the perfusate and, simultaneously, very low systemic platinum concentrations. This important result of low systemic platinum concentrations and subsequently poor systemic toxicity was confirmed by our investigations. Additionally, we could observe a strong correlation between the applied cisplatin dosage and the resulting platinum concentration in the native lung tissue. These findings are somewhat different compared to the rodent models, where a dosage–tissue level relationship could not be described [18,25]. In humans, Ratto et al. were able to demonstrate an additional correlation of platinum levels of lung tissue with the perfusion time. They reported a continuous increase of platinum tissue levels during the perfusion period up to their perfusion stop after 60 min.

All authors of the clinical trials described an impairment of ventilatory parameters and lung function. An interstitial and alveolar edema appeared in the subsequent days following ILP, which dissolved within the next few weeks [12,14]. However, this functional deterioration is the result of several contributing factors of ILP. However, for systematic evaluation of the optimal perfusion conditions and the standardized definition of dosage limits for the various cytostatic drugs a systematic large animal study is mandatory prior to clinical use. Our novel large animal model allows for reliable quantification of deteriorated lung function as well as evaluation of changes in lung morphology by extensive monitoring of various parameters during the reperfusion period.

Cisplatin-treated animals had similar pulmonary hemodynamic and ventilatory parameters as compared to non-treated ILP animals. However, the gas exchange was compromised by the cisplatin application. The resulting values of AADO₂ as well as pCO₂ suggest an increased respiratory demand.

We were able to demonstrate a dosage-dependent effect of cisplatin on the integrity of the lung structure. The significantly higher histological acute lung injury score indicates that a cisplatin perfusate concentration of about 80 µg/ml, corresponding to a dosage of about 300 mg/m², probably represents the dose limit of normothermic ILP. Hyperthermia at 41.5 °C seems to improve the results of cisplatin ILP, reflected by a normalization of all parameters to the values of lower dose cisplatin ILP. However, this difference did not reach statistical significance due to the limited number of animals observed. Why the hyperthermia did not cause deleterious effects on the native lung tissue is not explained. There are few animal studies which consider the finding that perfusion temperatures up to 43 °C were well tolerated by the lung tissue [26]. The clinical use of hyperthermic perfusion confirmed this observation [14].

In conclusion the results of this study suggest, that in vivo ILP with high-dose cisplatin is a safe procedure. Hyperthermic perfusion up to 41.5 °C led to a reduction of the acute lung injury observed, which was caused by the cisplatin treatment. The promising results might allow for the clinical application in selected patients with a poor prognosis. Further experimental studies should focus on survival studies to evaluate the assessing long-term function of the perfused lungs.

References

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Appendix A. Conference discussion

Dr T. Krueger (Lausanne, Switzerland): The pulmonary circulation is known to be a very complex system. And in a physiological situation, you have a regional blood flow which is dependent on a multitude of factors. Do you think for your perfusate you have a regional perfusion or regional flow which is similar in all parts of the lung? And don’t you think this would be a condition to offer this treatment to patients?

Dr Franke: We discussed this topic already this morning. We observed the distribution of the perfusion simultaneously by thermal probes placed in the upper and in the lower lobe. We saw an earlier increase of temperature of the lower lobe in the first 2 to 5 min. However, there was a very similar temperature distribution for the remaining 40 min of perfusion. We have not performed examinations regarding the fine evaluation of this distribution into the lung tissue. Maybe the retrograde perfusion, as suggested by a lot of groups, would improve the quality of perfusion.

Dr A. Wechsler (Philadelphia, PA, USA): Are you planning to do some studies that perhaps survive the animals over a few weeks? I mean, this certainly proves your point about acute injury, but I’d be very concerned about what might happen a few days later.

Dr Franke: We plan to perform survival experiments. We agree, that the 6 h monitoring period is very short to evaluate the influence of toxic agents on the lung tissue. From our experiments regarding the ischemia/reperfusion injuries in lung transplantation we know, that there is no additional effect later. However, the application of cisplatin might induce damages remarkable after this time.