Chemoembolization of Lung Metastases – Pharmacokinetic Behaviour of Carboplatin in a Rat Model

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Abstract. *Aim: To improve tumor control in lung metastases using a novel method: unilateral chemoembolization of the lung by instillation of degradable starch microspheres (DSM) and cytotoxic agents via the pulmonal artery. Materials and Methods: A rodent model of solitary metastasis (CC531 adenocarcinoma) was studied. The animals were randomized into three groups: the control group receiving carboplatin (45 mg/kg) intravenously, an isolated lung perfusion (ILP) group recieving buffered starch solution and carboplatin (15 mg/kg) and a third group receiving chemoembolization with carboplatin (15 mg/kg) and DSM (2 ml/kg). The total platinum concentration in serum, lung and lung tumor at defined times (15, 30, 60, 120 min) was measured using an inductively coupled plasma mass spectrometer (ICP-MS). Results: The area under concentration (AUC) versus time curves showed a 7.9- to 42.6-fold higher concentration in the tumor tissue comparing the ILP and chemoembolization group to the control group ($p<0.01$). In the comparison of the AUCs of ILP versus chemoembolization, the tumor tissue of the lung showed a 5.4-fold higher concentration in the chemoembolization group ($p<0.01$). Conclusion: This is the first study to measure the concentration of carboplatin during chemoembolization of the lung. Compared to intravenous therapy, chemoembolization produced higher tumor tissue concentrations. Comparing chemoembolization to ILP, there was also an increase of carboplatin in the tumor tissue, without histological damage of the surrounding lung parenchyma.*

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The treatment of lung metastases of solid tumors is problematic, since only about 30% of the patients are eligible for resection. A 5-year survival rate can be expected in only 25-50% of patients, even after surgery with a curative aim (1-3). Many patients have extensive unresectable disease or pulmonary recurrence in the resected or the contralateral side after complete resection (4). Intravenous chemotherapy does not achieve a cure in these cases and is primarily limited by systemic toxicity. Surgical therapy should thus be supplemented by treatment with improved cytostatic agents and a more efficient drug delivery method. The regional application of cytostatic agents with exclusion of systemic circulation is thus an encouraging method for improving the therapy of unresectable lung metastases.

As a regional therapy, isolated lung perfusion (ILP) has a high level of cytostatic activity in the lung and tumor without manifest systemic toxicity (5-8). It is also more effective in the tumor model than intravenous therapy (9). Experimental results and first clinical applications demonstrated ILP as a promising therapeutic approach, though an optimal treatment regimen has not yet been clinically established (10-12). On the other hand, ILP has the disadvantage that it cannot be repeated indefinitely, since it requires a thoracotomy and installation of an extracorporeal cardiovascular system (12-15).

Therefore, we are introducing unilateral chemoembolization of the lung with degradable starch microspheres (DSM) as a novel method for delivering regional chemotherapy. Unilateral chemoembolization requires an injection of degradable DSM combined with cytostatic agents into the pulmonary artery and can thus be achieved by interventional procedure. Recently, we performed chemoembolization of the lung with DSM in a rat model and found that embolization occurred on the arteriolar and capillary level and was reversible. There was no interstitial edema indicative of early toxicity (16). The pharmacokinetics

of intra-arterial carboplatin with or without DSM are undefined and required further evaluation. The aim of this study was to measure the pharmacokinetics of carboplatin administrated *via* different applications (*i.v. vs.* ILP *vs.* chemoembolization) and demonstrate the acute toxicity of unilateral chemoembolization of the lung in a solitary-metastasis rat model.

Materials and Methods

Experiments were carried out on 60 inbred male WAG/Rij rats weighting 200-280 g. The animals were housed individually in rooms maintained at 21˚C±1˚C with a 12-hour dark cycle. They were fed a standard rat chow with free access to water. Care was provided in accordance with the national guidelines for the care and use of laboratory animals. The study was approved by the local ethics committee.

Tumor cell preparation. The tumor cell line (CC531) is a moderately differentiated adenocarcinoma originating from the colon of rats exposed to methylazoxymethanol. The cells were obtained from the German Cancer Research Center (DKFZ), Heidelberg, Germany. Subpleural tumor inoculation was performed by the injection of a tumor suspension produced *in vitro*. The tumor cell line was cultivated at 37˚C and 5% CO_2 in an incubator in 20 ml complete medium, RPMI-1640 (Gibco, Life Technologies, Eggenstein, Germany), 10% fetal calf serum (Seromed, Biochrom, Berlin, Germany), and 1% Pen/Strep (Seromed). After 3 days, cells were washed twice with PBS and were detached with 1 ml trypsin. The trypsin was deactivated by adding 5 ml complete medium. After centrifugation, washing and resuspension with PBS, vitality was evaluated in a Bürker hematocytometer after addition of trypan blue. After vital counting, the suspension was adjusted to 98% vitality with a density of $2x10^6$ vital cells/100 µl suspension by recentrifugation and resuspension.

Tumor implantation. Surgical anesthesia was induced with vaporized ether followed by intramuscular injection of pentobarbital 20 mg/kg (Nembutal®, Pharmazeutische Handelsgesellschaft, Garbsen, Germany) followed by intramuscular application of ketamine 40 mg/kg (Ketanest®, Parke Davis & Company, Berlin, Germany). The animals were then endotracheally intubated under visual control according to the technique of Weksler *et al.* (8). Mechanical ventilation with 35% O_2 / 65% N_2 was installed using a small-animal ventilator (KTR4, Hugo Sachs, March-Hugstetten, Germany). Oxygenation was achieved by pressure-limited ventilation (70 cycles/min, 12.5 cm H_2O). The left chest was shaved and prepared with polyvidone iodine solution. Thoracotomy was performed through the sixth intercostal space which was opened 5-7 mm with a miniature retractor (Aesculap, Tuttlingen, Germany) to access the lung surface.

Using a 26-gauge injection needle on a 1 ml insulin syringe, tumor cell suspension ($1.5x10^6$ tumor cells in 0.075 ml) was subpleurally injected *in situ* at a 15-20˚ angle to the lung surface. The puncture site was then slightly compressed for 2 minutes with a cotton-tip applicator to prevent tumor cell leakage. To facilitate lung expansion, a 16-gauge catheter was inserted into the thorax before closing the thoracotomy, led out of the wound and connected to a water seal. After recovery of spontaneous respiration, the animal was extubated and the pleural catheter removed. The procedure lasted about 30 minutes.

Experimental design. Fourteen days after tumor inoculation, the animals were randomized into 3 groups of 5 animals each and 4 times of measurement: 15, 30, 60 and 120 min.

(i) Intravenous group (n=20). This group received carboplatin (Carboplat®, Bristol, München, Germany) intravenously. The right internal jugular vein was cannulated for a drug infusion of 45 mg/kg of carboplatin in 2 ml saline solution. After the infusion period, the venous catheter was removed and the vein ligated. Following the *i.v.* therapy, 5 animals were sacrificed at 15, 30, 60 and 120 min and specimens of serum, lung tissue and lung tumor tissue were aservated for the measurement of the platinum concentration *via* flameless atomic absorption spectrophotometry.

(ii) lsolated Lung Perfusion (ILP) group (n=20). In this group, endotracheal intubation was followed by left rethoracotomy in the fifth intercostal space. A small thorax retractor was introduced into the chest wall and the left lung was retracted with cotton-tip applicators. The pulmonary artery was exposed by sharp and blunt dissection techniques according to the experiments of Weksler *et al.* (8). A microvascular clamp was then placed at the base of the pulmonary artery and 6-0 polypropylene suture was passed twice around the artery. Under an operative microscope (Zeiss OPMI 6-S, Aalen, Germany) an arteriotomy of the pulmonary artery was performed with microvascular scissors and was cannulated with a silicone catheter (0.3 mm ID, 0.6 mm OD) (Vygon, Aachen, Germany). Traction was applied to tighten the loop around the catheter. After arteriotomy and cannulation of the artery, the pulmonary vein was dissected and a microvascular clamp placed at the base.

A short phlebotomy performed under the operation microscope was followed by an application of 15 mg/kg of carboplatin mixed with 7.5 ml of 6% buffered starch with an infusion pump Perfusor® Secura FT (Braun Melsungen, Melsungen, Germany). The perfusate escaped from the phlebotomy and was aspirated from the operating area by a suction catheter (Figure 1). The catheter was removed from the artery after infusion and the arteriotomy transversally closed under the operative microscope with a microsurgical suture 9-0 (Ethicon, Norderstedt, Germany). The phlebotomy was not closed with sutures, but hemostasis was achieved by slight compression for 30 seconds after repositioning the lungs in their anatomical position (17). The thorax was closed as described above and the animal extubated after recovery of spontaneous respiration. A 6% buffered starch solution for the perfusate was used as recommended by Weksler *et al.* for isolated lung perfusion (18). The sterile perfusate was prepared in our laboratory and consisted of 216 mmol/l Na, 4 mmol/l K, 158 mmol/l Cl, 2 mmol/l Mg, 37 mmol/l PO_4, 100 mg/dl glucose and 60 g/l pentastarch (HAES-steril 6%®, Fresenius Kabi, Bad Homburg, Germany) with a pH of 7.4 (16). ls After ILP therapy the 5 animals were sacrificed at 15, 30, 60 and 120 min. Serum, lung tissue and lung tumor tissue were aservated for the determination of platinum concentration *via* inductively coupled plasma mass spectrometry (ICP-MS).

(iii) Chemoembolization group (n=20). Anesthesia was induced as described above. The operative procedure for chemoembolization was initially the same as for ILP, but without phlebotomy of the pulmonary vein (Figure 1). Only the left pulmonary artery was cannulated as described above. Subsequent manual infusion of 15

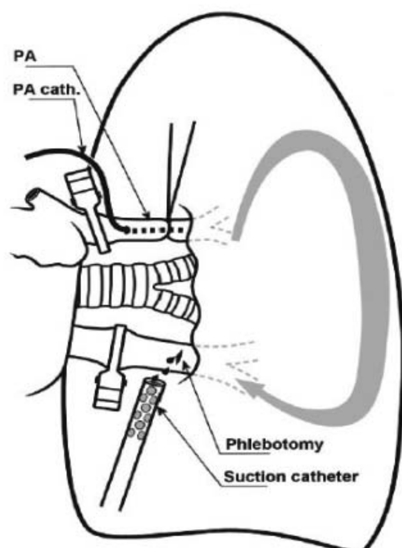

Figure 1. *Isolated lung perfusion on the left side: positioning of a microvascular clamp at the base of the pulmonary artery (PA) and vein; cannulation of the pulmonary artery by silicone catheter (PA-cath). The perfusate escapes by phlebotomy of the pulmonary vein and is aspirated by a suction catheter. During chemoembolization, no manipulation of the pulmonary vein is required.*

mg/kg of carboplatin in combination with 2 ml/kg of degradable starch microspheres (DSM amilomer 25/45) was performed. The arteriotomy was closed after injection as described above. DSM was prepared from partially hydrolyzed starch, with a mean diameter of 45±7 μm, in which 95% of the microspheres were 20-70 μm. Under physiological conditions, the microspheres show a half-life 20-30 minutes at 37˚C and a pH of 7. DSM are nontoxic and provide temporary vascular occlusion (19). The animals were sacrificed 5 at a time at 15, 30, 60 and 120 min and specimens of serum, lung tissue and lung tumor tissue were aservated for the determination of platinum concentration *via* inductively coupled plasma mass spectrometry (ICP-MS).

Platinum measurements. An Agilent 7500ce (Agilent Technologies, Waldbronn, Germany) ICP-MS was used for the analytical method development and platinum determination. The interface of the instrument contains a Ni sampler and skimmer cones with an orifice diameter of 1.0 and 0.7 mm, respectively. For sample introduction, a Babington nebulizer with a Peltier-cooled Scott spray chamber (5˚C) (Agilent Technologies) was used. The ICP mass spectrometer was equipped with a Cetac ASX-510 Autosampler (Cetac Technologies, Omaha, USA) for automation of the analyses. These were optimised to obtain the highest signal/background ratio for ^{195}Pt. The samples (80-350 mg) were dissolved with 0.5 ml 38% (V/V) HNO_3 and 1.5 ml 30% (V/V) HCl (ultrapure quality; Merck, Darmstadt, Germany) at 80˚C in 25 ml quartz glass flasks. After dissolution, the solutions were brought to room temperature, 1 ml of the internal standard solution with 10 μg/L ^{159}Tb (Merck, Darmstadt, Germany) as an internal standard was added and finally the solutions were made up to 25 ml with deionised water and mixed well. These solutions were directly introduced into the ICP-MS (20, 21).

Histological analysis. Treated lung tissue was fixed in buffered 5% formalin for 2 days, embedded in paraffin and stained with hematoxylin/eosin and van Gieson. Histological examination to detect lung tissue injury as well as histological changes in the tumor tissue was performed blind by the same pathologist (C.L.).

Statistical analysis. The areas under the concentration-time curves (AUC) were calculated over 15-120 min after administration of the carboplatin using the trapezoidal rule. The mean platinum concentration±SD in each organ and at the different time points were calculated for each group. The difference between the platinum concentrations in the groups were determined using the Mann-Witney *U*-test. Significance was defined as $p<0.05$.

Results

Pharmacokinetics. Figures 2A-C show the total platinum concentrations in the serum, lung and tumor tissue after intravenous therapy, isolated lung perfusion and chemoembolization at 15, 30, 60 and 120 min after application of carboplatin. The maximal carboplatin concentration (C_{max}) was observed after 15 min in serum, tumor tissue and lung tissue, only the chemoembolization group showed a C_{max} after 30 min in the lung tissue. In the serum the C_{max} of the intravenous group was 4766±266 μg/kg and so 1.8-fold higher than in the ILP-group with 2688±199 μg/kg ($p<0.01$) and 2.4-fold higher than in the chemoembolization group with 1999±μg/kg ($p<0.01$). In the lung tissue, the chemoembolization group reached C_{max} with 10512±8244 μg/kg after 30 min, the two other groups after 15 min. Comparing the C_{max} values in the lung tissue, the C_{max} of the chemoembolization group was 1.9-fold higher than in the intravenous group (5644±233 μg/kg) ($p<0.01$) and 2.6-fold higher than in the ILP-group (4048±212 μg/kg) ($p<0.01$). In the tumor tissue, the C_{max} in the chemoembolization group was 28594.4±8544 μg/kg, in the ILP group 9686±779 μg/kg and in the intravenous group 473.6±68.3 μg/kg. The C_{max} in the chemoembolization group was 60-fold higher than in the intravenous group and 3-fold higher than in the ILP-group. These differences were significant ($p<0.01$).

Figure 3 shows the area under the concentration-time curves (AUC) for the 3 groups. The lung tumor AUCs were 22536±1003.6, 176971±4684.7 and 960007±38446.3 μg/kg min^{-1} for the intravenous, ILP and chemoembolization groups respectively. For the lung tissue AUCs were 253433±11626.6, 148592±8838.6 and 455846±12382.1 μg/kg min^{-1} for the intravenous, ILP and chemoembolization groups.

For the serum, the following AUCs were measured 258622+33961.5, 99252+6120.6 and 83475+2456.4 μg/kg min^{-1} for intravenous, ILP and chemoembolization group.

The differences of the AUCs in lung tissue and tumor proved statistically significant ($p<0.01$). In the serum the difference between the intravenous and the ILP and also between the intravenous and the chemoembolization group

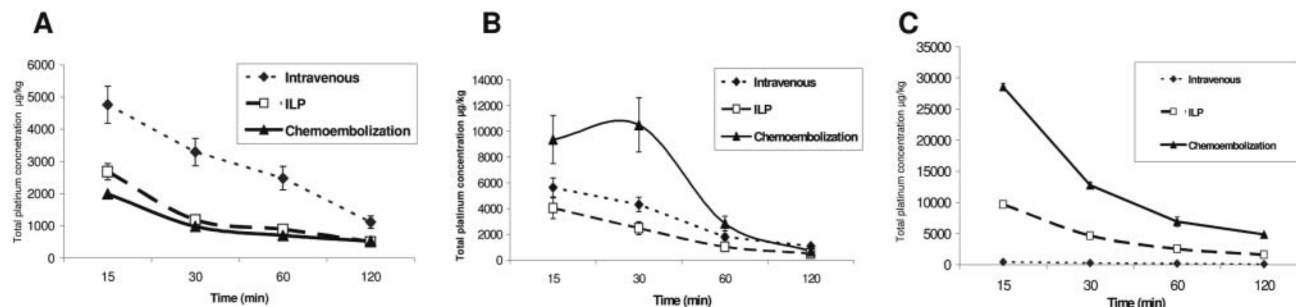

Figure 2. *Concentration-time profile of Platinum concentration in A: serum, B: lung tissue, C: lung tumor tissue in the 3 groups. The difference in platinum concentration in the 3 groups is significant for each organ p<0.01.*

were statistically significant ($p<0.01$), whilst that between the ILP and the chemoembolization group was not.

Histological analysis. On van Gieson staining there were no fibrotic changes detected in any group (Figure 4A, B). The ILP and chemoembolization group showed evidence of mild alveolar cell hyperplasia and pulmonary edema.

Discussion

In this model the pharmacokinetic effect of chemoembolization with carboplatin in the lung of tumor-bearing rats is demonstrated. The CC531 model was chosen because tumor inoculation by subpleural injection of tumor cells of the adenocarcinoma cell line CC531 leads to macroscopically recognizable tumor growth in all animals. In our tumor model, carboplatin as the chemotherapeutic agent was chosen as the CC531 the cell line is sensitive to carboplatin (22). In former experiments we used 45 mg/kg carboplatin for the intravenous therapy and 15 mg/kg carboplatin for ILP and chemoembolization. At these doses almost all animals survived therapy. This observation was also shared by other groups (23). Chemoembolization uses only 1/3 of the intravenous dose, which minimizes the risk of systemic toxicity. Burt and coworkers at the Memorial Sloan-Kettering Cancer Center systematically developed ILP as a therapeutic concept. By inserting a catheter into the pulmonary artery and by phlebotomizing the pulmonary vein for perfusate leakage, the authors were able to develop a model enabling the isolated perfusion of one lung without passage into systemic circulation and without irreversible lung damage (8). The concentration of doxorubicin was significantly higher in ILP than in intravenous therapy with a lower serum and cardiac concentration (9). The authors also showed its effectiveness by complete eradication of experimental sarcoma and colon metastases (24, 25). Other substances like TNF-α, melphalan and carboplatin have also been applied successfully (23, 26, 27). In a pig model,

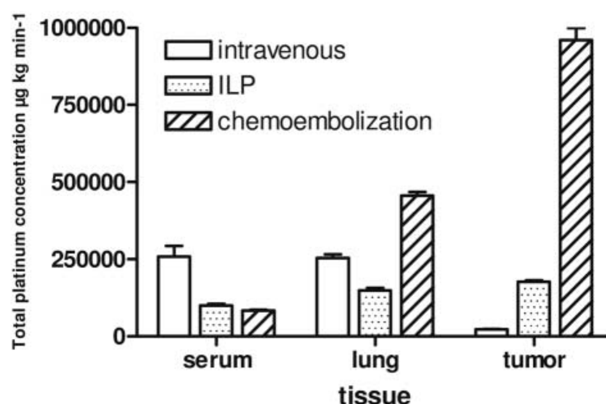

Figure 3. *AUC values of total platinum in serum, lung tissue and lung tumor tissue. All AUC values were determined from the concentration-time curves shown in Figure 2 15 min after drug administration up to the time of the last sampling (120 min). All values are signficantly different p<0.01. (**p<0.01 in comparison to i.v. administration, Mann Whitney U-test).*

Ratto *et al.* likewise confirmed the superiority of ILP with cisplatin. Compared to other regional application methods, *e.g.* the less invasive stop-flow technique and the stop-flow with outflow occlusion technique, ILP achieved the highest cytostatic concentration in the lung and in the mediastinal lymph nodes with the lowest systemic concentration (28). In contrast to chemoembolization as applied here, ILP requires cannulation of the pulmonary artery and vein, an intervention only feasible with a thoracotomy. This is why the procedure is stressful for patients and cannot be indefinitely repeated. In our opinion, the greatest advantage of our method is that in a possible clinical application it can be performed percutaneously with a endovascular catheter, *i.e.* without thoracotomy. As Furrer *et al.* (29) showed in a pig model, injecting only the cytostatic agent into the pulmonary artery increased its concentration in the lung, but Ratto *et al.* did not obtain the tissue concentrations of ILP in a comparable study (28).

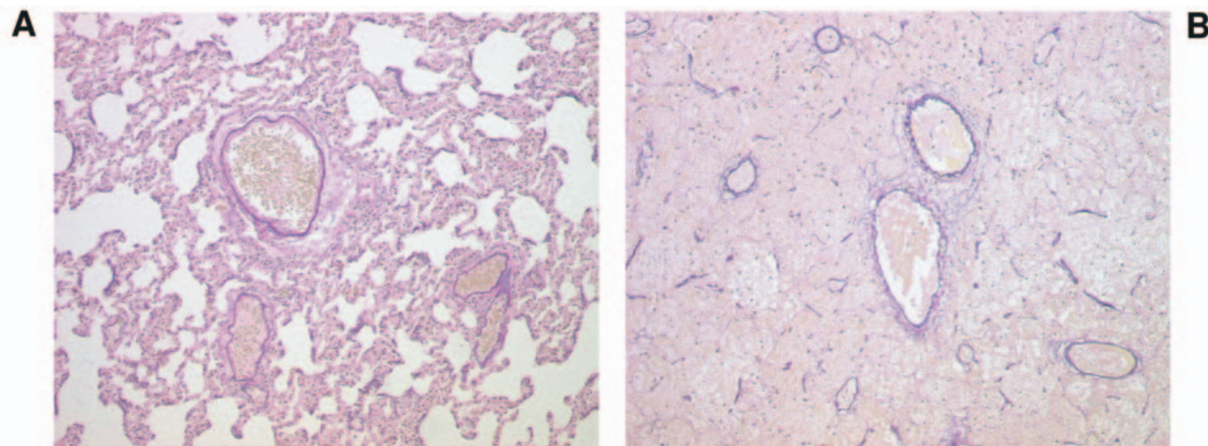

Figure 4. *Histological findings: Lung specimen of the ILP group (A) with mild alveolar cell hyperplasia and chemoembolization group (B) with alveolar edema (van Gieson, x100).*

For this reason, we recommend temporary embolization and blood flow retardation by coinjection of an embolic agent, which has been successfully used in the treatment of primary and secondary liver tumors. In a rat model, Johansson *et al.* demonstrated the positive effect of temporarily reducing hepatic perfusion with DSM (30). The injection of doxorubicin with DSM into the hepatic artery increased cytostatic retention in hepatic microcirculation and concomitantly reduced the systemic load by 30%. Pauser *et al.* found chemoembolization of VX2 liver tumor to be more efficient than regional application without embolizates (31). Phase II and III clinical trials have demonstrated the efficacy of DSM when coadministered with chemotherapeutic drugs: compared with drug therapy alone (32), a significantly greater tumor response is associated with chemoembolization for patients with either hepatocellular carcinoma or metastatic liver cancer (33-35).

Our aim was to apply the promising results of chemoembolization in the liver to the treatment of lung metastases. Recently, we performed temporary unilateral embolization of the lung and blood flow retardation in a rat model (16). Injecting DSM into the pulmonary artery led to a 7.1-minute interruption of perfusion with 14.3 minutes of blood flow retardation on capillary level. Early pulmonary toxicity did not occur. The first step was to perform an chemoembolization of the lung in a solitary-metastasis rat model and we measured the tumor volume and the delayed toxicity. We could show that chemoembolization was more effective than systemic therapy and comparable to isolated lung perfusion (36). The second step was a pharmacokinetic study to detect carboplatin in serum, lung tissue and lung tumor tissue leading to an explanation for the high rate of tumor regression after chemoembolization in our former experiments. The comparison of C_{max} shows an 60-fold higher concentration in the lung tumor after chemoembolization compared to the mere intravenous application. The advantage of a regional therapie is also obvious by looking at the serum concentrations. The C_{max} in the intravenous group was 1.8-fold higher as in the ILP-group and 2.4-fold higher as in the chemoembolization group ($p<0.01$). C_{max} in the lung tissue showed 2.6-fold higher values after chemoembolization and 1.9-fold higher values after ILP in comparison to the intravenous group ($p<0.01$). And we could prove histologically that the higher concentrations didn't damage the lung tissue.

The maximal carboplatin concentration (C_{max}) was observed after 15 min. in serum, tumor tissue and lung tissue, only the chemoembolization group showed a C_{max} after 30 min in the lung tissue. This effect could be explaned by the starch microspheres which embolize the lung tissue for 30 min. Similar effects were noticed during the chemoembolization of liver tumors (37).

The AUC of the lung tumor was 42.6-fold higher ($p<0.01$) after chemoembolization compared to intravenous therapy. Also the comparison of the AUC between ILP and the chemoembolization group was significant ($p<0.01$) and shows an 7.9-fold higher concentration of carboplatin in the lung tumor. This high concentration in the solitary lung tumor is a good explanation for the regression of tumor volume in our former experiment because most chemotherapeutic agents demonstrate step dose-responsive curves and therefore the exposure of the tumor to high drug concentrations is mandatory for eradication of both sensitive and drug resistant tumour cells (38, 39).

The results of our histological analysis with mild alveolar cell hyperplasia and pulmonary edema in the ILP and Chemoembolization groups were similar to the observations of other investigators (40).

Conclusion

The chemoembolization of lung tumors leads to a high concentration of carboplatin without significant damage of the surrounding normal lung tissue. The described method shows advantages in that it can be performed percutaneously and if necessary repeated without a thoracotomy. We therefore find this approach suitable for clinical application in the treatment of unresectable pulmonary metastases. Because of nonexisting clinical data, a prospective randomized studie should prove the advantage of chemoembolization of lung tumors.

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