

# Chemoembolization of the Lung Improves Tumor Control in a Rat Model

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## ABSTRACT

**Purpose:** The novel method of organ-specific drug application we present here is unilateral chemoembolization of the lung by injecting the pulmonary artery with degradable starch microspheres and cytotoxic drugs to improve tumor control in lung metastases.

**Experimental Design:** In a solitary metastasis rat model (CC531 adenocarcinoma), we studied the clinical and histological tumor response as well as subacute toxicity of the lung. Fourteen days after tumor induction, animals were randomized into five groups. Groups I and II served as controls. Group III received carboplatin i.v. (45 mg/kg). Isolated lung perfusion with buffered starch solution and carboplatin (15 mg/kg) was installed in group IV. Chemoembolization with carboplatin (15 mg/kg) was performed in group V.

**Results:** Seven days later, the difference in the tumor volume before and after treatment was +422 mm<sup>3</sup> (±226) in group I, +697 mm<sup>3</sup> (±423) in group II, +70 mm<sup>3</sup> (±31) in group III, -8 mm<sup>3</sup> (±17) in group IV, and -17 mm<sup>3</sup> (±16) in group V (*P* < 0.05 groups IV and V versus groups I, II, and III). No pleural spread was observed in groups IV and V. Histologically, the area of tumor necrosis was largest in group IV. Mild alveolar cell hyperplasia, pulmonary edema, and hemorrhage without subacute fibrotic changes were noted in all groups.

**Conclusion:** This is the first study to perform chemoembolization of the lung. Compared with i.v. therapy, chemoembolization was more effective without serious toxicity. Its efficacy was comparable with that of isolated lung perfusion but less stressful for a possible clinical application.

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## INTRODUCTION

Treating lung metastases of solid tumors is problematic, because only ~30% of the patients are eligible for resection. A 5-year survival rate can be expected in only 25–50%, 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). i.v. chemotherapy does not achieve a cure in these cases and is primarily limited by systemic toxicity. Surgical therapy should thus be supplemented by 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, ILP<sup>2</sup> 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 i.v. therapy (9). Experimental results and first clinical applications demonstrated ILP as a promising therapeutic approach, although an optimal treatment regimen has not yet been clinically established (10). On the other hand, ILP has the disadvantage that it cannot be repeated indefinitely, because it requires a thoracotomy and installation of an extracorporeal cardiovascular system (10–13).

Therefore, we are introducing unilateral chemoembolization of the lung with DSMs as a novel method for delivering regional chemotherapy. Unilateral chemoembolization requires an injection of DSMs combined with cytostatic drug into the pulmonary artery and can thus be achieved by interventional procedure. Recently, we performed chemoembolization of the lung with DSMs 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.<sup>3</sup> The aim of this study was to demonstrate the effect and subacute toxicity of unilateral chemoembolization of the lung in a solitary-metastasis rat model.

## MATERIALS AND METHODS

Experiments were carried out on 32 inbred male WAG/Rij rats weighing 200–280 g. The animals were housed individually in rooms maintained at 21° ± 1°C with a 12-h 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.

<sup>2</sup> The abbreviations used are: ILP, isolated lung perfusion; DSM, degradable starch microsphere.

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### Tumor Implantation

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 (Deutsches Krebsforschungszentrum, Heidelberg, Germany). Subpleural tumor inoculation was performed with a tumor suspension produced *in vitro*. The tumor cell line was cultivated at 37°C and 5% CO<sub>2</sub> in an incubator in 20 ml of complete medium RPMI 1640 (Life Technologies, Inc., Eggenstein, Germany), 10% FCS (Seromed, Biochrom, Berlin, Germany), and 1% Pen/Strep (Seromed). After 3 days, cells washed twice with PBS (2) were detached with 1 ml of trypsin. The trypsin was deactivated, adding 5 ml of complete medium. After centrifugation and 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  $2 \times 10^6$  vital cells/100  $\mu$ l suspension by recentrifugation and resuspension.

For tumor implantation, surgical anesthesia was induced with vaporized ether, followed by i.m. pentobarbital 20 mg/kg (Nembutal; Pharmazeutische Handelsgesellschaft, Garbsen, Germany) and i.m. ketamine 40 mg/kg (Ketanest; Parke Davis and Company, Berlin, Germany). The animals were then endotracheally intubated under visual control according to the technique of Weksler *et al.* (14). Mechanical ventilation with 35% O<sub>2</sub>/65% N<sub>2</sub> 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<sub>2</sub>O). The left chest was shaved, prepared with povidone iodine solution, and entered through the sixth intercostal space. The intercostal space was opened 5–7 mm with a miniretractor (Aesculap, Tuttingen, Germany) to access the lung surface. Using a 26-gauge injection needle on a 1-ml insulin syringe, tumor cell suspension was subpleurally injected *in situ* at a 15–20° angle to the lung surface. The subpleural lung parenchyma was injected with  $1.5 \times 10^6$  tumor cells in 0.075 ml of tumor cell suspension. The puncture site was slightly compressed for 2 min 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 ~30 min.

### Experimental Design

Fourteen days after tumor inoculation, the animals were randomized into five groups of five animals each. Group I had ILP with 6% buffered starch solution without carboplatin and served as controls. Group II had embolization of the left lung with DSM (Spherex; Pharmacia, Stockholm, Sweden) and served as second control group. Group III received carboplatin (Carboplat; Bristol, Munich, Germany) i.v. via a vena cava catheter. Group IV was submitted to ILP with carboplatin in 6% buffered starch solution. Group V underwent chemoembolization of the left lung with carboplatin and DSM. All animals were thoracotomized before treatment to evaluate the induced tumor. The tumor was macroscopically measured with a micrometer, and volume was calculated as described below. We excluded all

animals with macroscopic chest wall or pleural invasion and pericardial tumor spread.

**ILP-Control Group (Group I).** Animals in this group were anesthetized as described above, and ILP was performed as in group IV with 6% buffered starch solution without carboplatin.

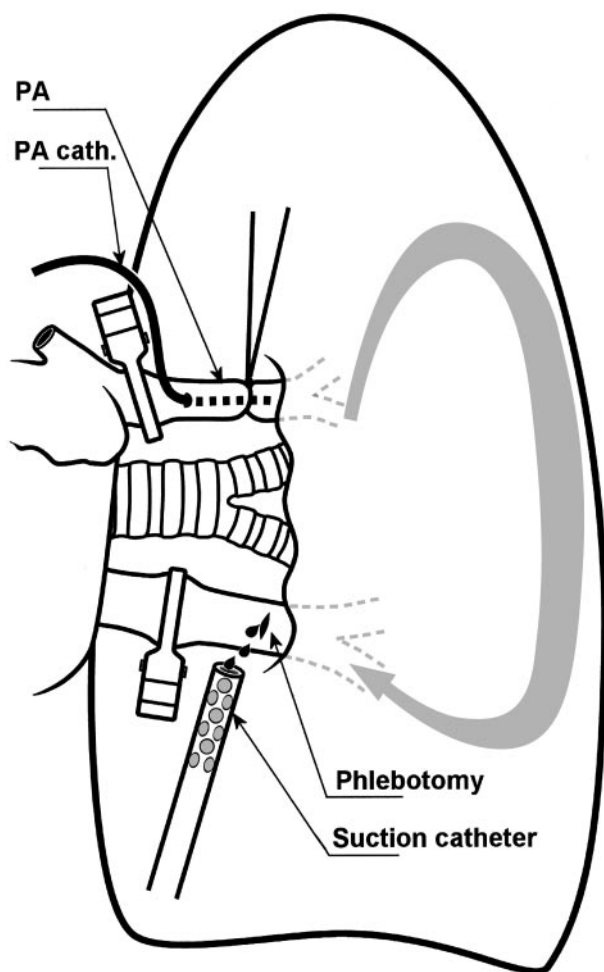
**DSM-Control Group (Group II).** Animals in this group were anesthetized as described above, and embolization was performed as in group V with 0.5 ml/kg of Spherex at a flow rate of 0.5 ml/min without carboplatin.

**i.v. Therapy (Group III).** Animals in this group were anesthetized as described above and thoracotomized for tumor measurement. After closure of the thorax, the right internal jugular vein was cannulated for a 15-min drug infusion of 45 mg/kg of carboplatin in 2 ml of saline solution. After the infusion period, the venal catheter was removed, and the vein was ligated.

**Isolated Lung Perfusion (Group IV).** Anesthesia was induced as described above. After endotracheal intubation, left rethoracotomy was done 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 placed at the base of the pulmonary artery, and 6-0 polypropylene 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 cannulated with a silicone catheter (0.3-mm inside diameter; 0.6-mm outside diameter; 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 was placed at the base. A short phlebotomy performed under the operation microscope was followed by a 15-min 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) at a flow rate of 0.5 ml/min. The perfusate escaped from the phlebotomy and was aspirated from the operating area by a suction catheter (Fig. 1). The catheter was removed from the artery after infusion, and the arteriotomy was 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 s after repositioning the lungs in their anatomical position (15). The thorax was closed as described above, and the animal was extubated after recovery of spontaneous respiration.

We used 6% buffered starch solution for the perfusate as recommended by Weksler *et al.* (16) for isolated lung perfusion. Perfusate was prepared sterilely in our laboratory and composed of 216 mM sodium, 4 mM potassium, 158 mM chloride, 2 mM magnesium, 37 mM PO<sub>4</sub>, 100 mg/dl glucose, and 60 g/l pentastarch (HAES-steril 6%; Fresenius Kabi, Bad Homburg, Germany) with a pH of 7.4 (16).

**Chemoembolization (Group V).** Anesthesia was induced as described above. The procedure for chemoembolization was initially the same as for ILP but without phlebotomy of the pulmonary vein (Fig. 1). Only the left pulmonary artery was

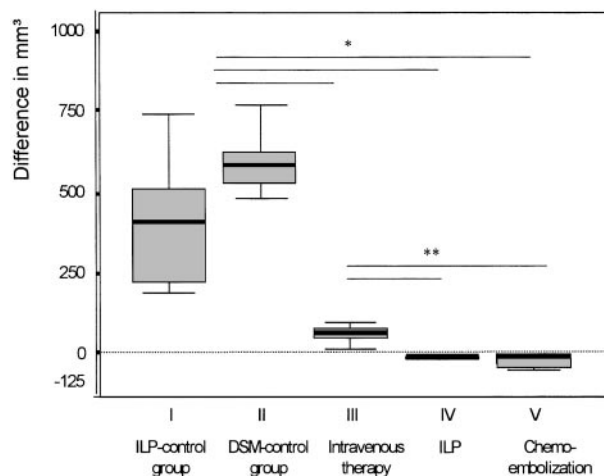


**Fig. 1** Isolated lung perfusion on the left side: positioning of a microvascular clamp at the base of the pulmonary artery (PA) and vein; cannulation of the pulmonary artery by silicone catheter (PA-cath.). The perfusate escaped by phlebotomy of the pulmonary vein and was aspirated by a suction catheter.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

cannulated as described before. Subsequent manual infusion of 15 mg/kg of carboplatin together with 0.5 ml/kg of Spherex at a flow rate of 0.5 ml/min was performed. The arteriotomy was closed after injection as described above. DSMs (amilomer 25/45) are prepared from partially hydrolyzed starch and have a mean diameter of  $45 \pm 7 \mu\text{m}$ , in which 95% of the microspheres are 20–70  $\mu\text{m}$ . Under physiological conditions, the microspheres have a half-life 20–30 min at 37°C and a pH of 7. DSM are nontoxic and provide temporary vascular occlusion (17).

#### Treatment Efficacy and Toxicity

All animals were sacrificed by an overdose of anesthetic 7 days after treatment and tumor size determined. The width of the greatest lesion and the maximum diameter perpendicular to the width were measured with a micrometer. Lesion volume ( $v$ ) was calculated using the formula  $v = 4 \times \pi \times ab^2/3$ , in which  $a$  and  $b$  are the radii of the measured axes. The difference of the tumor



**Fig. 2** Difference of tumor volumes in five therapeutic groups. \*,  $P < 0.05$  groups I and II versus groups III, IV, and V; \*\*,  $P < 0.05$  group III versus groups IV and V; (group I versus II and group IV versus V;  $P > 0.05$ ).

volumes before and after treatment was calculated and compared between the therapeutic groups. In addition, we documented pleural and pericardial tumor spread as well as direct tumor infiltration of the chest wall.

Subsequently, the treated lung was fixed in buffered 5% formalin for 2 days, embedded in paraffin, and stained with H&E and van Gieson. Histological examination to detect lung tissue injury as well as histological changes in the tumor tissue was performed in a blinded way by the same pathologist (C. L.).

#### Statistical Analysis

The mean tumor volumes  $\pm$  SD were calculated for each group. The differences between the tumor volumes in the groups were determined using the global Kruskal-Wallis test.  $P$ s were adjusted for multiple comparison according to Bonferroni. A probability value of  $<0.05$  was considered significant.

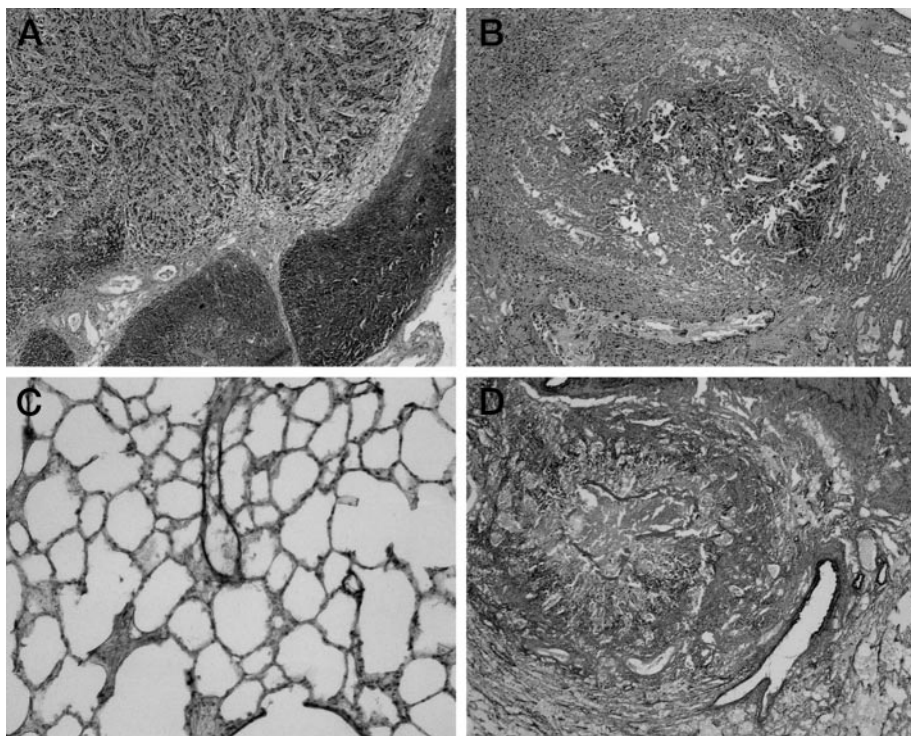
## RESULTS

#### General Observations

Surgical mortality was none in groups I and III, 1 of 6 in group II (fatal hemorrhage), 2 of 7 in group IV (fatal hemorrhage), and 1 of 6 animals in group V (contralateral pneumothorax). Five animals could be assessed in each group. Fourteen days after tumor implantation, all animals had a solitary  $19.4 \pm 19.1 \text{ mm}^3$  intrapulmonary tumor nodule at the injection site before therapy. Chest wall infiltration or pleural spread was also macroscopically detected in 3 of the 32 animals, which were excluded.

#### Tumor Volumes

The difference between the tumor volumes in the five groups 7 days after therapy are shown in Fig. 2. The greatest difference in tumor volumes was found in group I ( $+422 \pm 226 \text{ mm}^3$ ) and group II ( $+697 \pm 423 \text{ mm}^3$ ). This significantly differed from group III ( $+70 \pm 31 \text{ mm}^3$ ) and the regionally



**Fig. 3** Histological findings. **A**, H&E,  $\times 100$ . Hilar lymph node metastasis of the adenocarcinoma without tumor necrosis in the ILP-control group I. **B**, H&E,  $\times 100$ . Massive necrosis in a lung tumor treated with chemoembolization (group V) showing large confluent areas composed of necrotic debris, pyknotic and karyorrhectic cells, and eosinophilic ghosts of tumor cells. **C**, van Gieson,  $\times 200$ . A chemoembolized lung specimen of group V with mild alveolar cell hyperplasia disclosing no fibrosis of the alveolar septae is shown. **D**, H&E,  $\times 200$ . Acute vasculitis with fibrinoid necrosis, infiltrating inflammatory cells in the endothelium, and damage of myocytes in the media (group IV) are shown.

treated groups ( $-8 \text{ mm}^3 \pm 17$  in group IV and  $-17 \pm 16 \text{ mm}^3$  in group V;  $P < 0.05$ ). The regionally treated animals differed significantly from those treated i.v., but the difference between groups IV and V was not significant. Tumor size in regionally treated animals was significantly smaller after therapy than before. The tumor was macroscopically visible in all animals of group IV, but one animal in group V no longer had a macroscopically recognizable tumor.

#### Pleural and Local Tumor Infiltration

All animals in the control groups I and II showed pleural metastasis and chest wall infiltration. In the i.v.-treated group, pleural metastasis was found in two of five animals and chest wall infiltration in none. After regional chemotherapy (groups IV and V), neither pleural spread nor chest wall infiltration was found at sacrifice.

#### Histological Findings

**Efficacy Study.** In groups I and II, histology disclosed an intraparenchymal, unilocular, moderately differentiated adenocarcinoma with predominantly glandular growth pattern and an average spontaneous central necrosis of  $<20\%$  of the total tumor area. In two of five cases in group I and three of five cases in group II, lymph node metastasis was present (Fig. 3A). In groups III, IV, and V, the average area of tumor necrosis was 50, 35, and 65%, respectively. Two of five tumors in group V revealed only few residual viable tumor cells (Fig. 3B).

**Toxicity Study.** In the van Gieson stains, there were no fibrotic changes detected in all groups (Fig. 3C). Groups III–V showed evidence of alveolar cell hyperplasia, mild pulmonary edema, and hemorrhage adjacent to tumor necrosis. In group III

with i.v. therapy, there were perivascular accumulations of lymphocytes. In group IV, fibrinoid necrosis of a medium size pulmonary vessel occurred in one case (Fig. 3D). Various degrees of pleuritis and pleural fibrosis were found in all groups as postoperative sequelae.

#### DISCUSSION

In this model, the effect of chemoembolization 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 led to macroscopically recognizable tumor growth in all animals. This method has proven to be an effective tumor model and is even superior to percutaneous tumor cell injection in that tumor cells do not disseminate in the pleural cavity (18). In our tumor model, carboplatin was chosen as the antineoplastic agent because of its well-known activity against a variety of solid tumors. Treatment with carboplatin significantly inhibited tumor growth in all groups compared with controls. These findings confirm the sensitivity of the cell line CC531 to carboplatin reported by Los *et al.* (19). But there are only a few reports in the literature on doses used in i.v. or regional application. In their survival experiment, Ellis *et al.* (20) showed that 75% of the animals survived the contralateral pneumonectomy 21 days after ILP with 15 mg/kg carboplatin, but all animals died with 30 mg/kg carboplatin after contralateral pneumonectomy. The authors evaluated the high mortality as a result of irreversible lung damage caused by carboplatin. All animals survived i.v. therapy with 45 mg/kg carboplatin, but there was 50% mortality with 55 mg/kg carboplatin (20). Chemoembolization uses only one-third of the i.v. dose, which minimizes the risk of systemic

toxicity. Histotoxicity observed in our experiments was comparable in all therapeutic groups with mild alveolar cell hyperplasia, pulmonary edema, and hemorrhage. No subacute fibrotic changes were noted.

In a solitary metastasis model, we showed a tumor response comparable with ILP. Regional treatment procedures were clearly superior to systemic therapy. Not only were tumor volumes significantly smaller after ILP or chemoembolization, but pleural tumor dissemination and local chest wall infiltration were also less frequently observed than in the i.v. therapy. None of the ILP or chemoembolization cases showed tumor growth extending beyond the organ. On histological examination, the area of tumor necrosis was largest in the chemoembolization group with extensive pyknotic and karyorrhectic cells.

These results confirm reports in the literature insofar as tumor control in regional therapy is superior to systemic therapy. Lung metastases are supplied primarily from pulmonary circulation (21). Therefore cytostatic drugs delivered via the pulmonary artery could be extracted by the metastases. Weksler *et al.* (8) in 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, they were able to develop a model enabling the isolated perfusion of one lung without passage into systemic circulation and without irreversible lung damage. The concentration of doxorubicin was significantly higher in ILP than in i.v. therapy with a lower serum and cardiac concentration (9). The authors also showed its effectiveness by complete eradication of experimental sarcoma and colon metastases (22, 23). Other substances such as tumor necrosis factor- $\alpha$ , melphalan, and carboplatin have also been applied successfully (20, 24, 25). In a pig model, Ratto *et al.* (26) likewise confirmed the superiority of ILP with cisplatin. Compared with 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.

In contrast to chemoembolization as applied here, ILP requires cannulation of the pulmonary artery and vein, an intervention only feasible with a thoracotomy. That 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 an endovascular catheter, *i.e.*, without thoracotomy. The oncological results are comparable with ILP. As Furrer *et al.* (27) showed in a pig model, injecting only the cytostatic agent into the pulmonary artery increased its concentration in the lung, but Ratto *et al.* (26) did not obtain the tissue concentrations of ILP in a comparable study. For this reason, we recommend temporary embolization and BF retardation by coinjection of an embolic agent, which have been successfully used in the treatment of primary and secondary liver tumors. In a rat model, Johansson *et al.* (28) demonstrated the positive effect of temporarily reducing hepatic perfusion with DSMs. The injection of doxorubicin with DSMs into the hepatic artery increases cytostatic retention in hepatic microcirculation and concomitantly reduces the systemic load by 30%. Pauser *et al.* (29) found chemoembolization of VX2 liver tumor to be more effi-

cient than regional application without embolizates. Phase II and III clinical trials have demonstrated the efficacy of DSMs when coadministered with chemotherapeutic drugs; compared with drug therapy alone, a significantly greater tumor response is associated with chemoembolization for patients with either hepatocellular carcinoma or metastatic liver cancer (30–32). 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 BF retardation in a rat model.<sup>3</sup> Injecting DSMs into the pulmonary artery led to a 7.1-min interruption of perfusion with 14.3 min of BF retardation on capillary level. Early pulmonary toxicity did not occur.

In this study, chemoembolization of the lung was performed in a solitary-metastasis rat model, revealing that chemoembolization is more effective than systemic therapy and comparable with isolated lung perfusion. The carboplatin dose applied in the regional procedure is clearly less than that of the common i.v. dose, which minimizes the potential risk of systemic toxicity. Mild late sequelae were detected in the treated lung. Because this method can be performed percutaneously and repeated as needed, we believe that it is suitable for clinical application in the treatment of unresectable pulmonary metastases.

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