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LABORATORY INVESTIGATION

Toward Transpulmonary Chemoembolization with Degradable Starch Microspheres: Systematic Analysis of Local and Systemic Effects in a Porcine Model

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Abstract

Purpose To investigate local and systemic effects of transpulmonary chemoembolization (TPCE) with degradable starch microspheres (DSM) and doxorubicin. The long-term goal is to establish DSM-TPCE as a treatment option for pulmonary malignancies.

Materials and Methods Nine pigs underwent TPCE of either the right or left lower lobe pulmonary artery (LLPA) and bland embolization (TPE) of the contralateral LLPA. Before the procedures, macroaggregated albumin (MAA) particles were injected into both LLPAs, to exclude systemic shunting. Pulmonary arterial pressure, heart rate and oxygenation were recorded immediately before and at 1, 3, 5 and 10 min after treatment.

To investigate possible nontarget embolization, animals underwent cerebral MRI (cMRI). We killed the animals after a contrast-enhanced chest computed tomography (CT) and performed a pathologic examination at 12 h (3), 24 h (3) and 72 h (3) after treatment.

Results All experiments were technically successful. Mean injected DSM dose until stasis was similar in TPCE and TPE (4.3 ± 1.4 vs. 4.0 ± 1.4 mL). Pulmonary arterial pressure increased significantly 3 min after treatment (TPE: 17 ± 5 vs. 27 ± 7 mmHg; TPCE: 22 ± 6 vs. 36 ± 8 mmHg). No significant changes in heart rate or peripheral oxygenation levels occurred. We observed no evidence of structural lung damage or permanent perfusion disruption on CT. MAA test injection and cMRI revealed no shunting or nontarget embolization. The pathologic assessment revealed nonspecific local inflammation of the lung parenchyma.

Conclusion In this large-animal model, TPCE and TPE appear feasible and safe. We observed a mild increase in pulmonary arterial pressure. Nontarget embolization did not occur. TPCE, as well as TPE, did not cause structural damage to the normal lung parenchyma.

Introduction

Lung cancer is the leading cause of cancer death both in men and in women worldwide [1]. Surgical resection is the treatment of choice for non-small-cell lung cancers (NSCLCs), while systemic chemotherapy is the primary treatment option for small-cell lung cancer (SCLC). Usually, this treatment is combined with radiochemotherapy or radiotherapy. Unfortunately, between 74 and 85% of patients diagnosed with NSCLC are not amenable to primary resection [2]. Pulmonary metastases from primaries other than lung cancer occur in up to 25% of cancer patients. In the majority of cases, these limit treatment options significantly, thus leading to an impaired survival [3, 4].

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In some patients, where surgical resection is not an option, local ablations, such as radiofrequency ablation (RFA), microwave ablation (MWA) and cryoablation (Cryo), that allow minimally invasive and repeatable treatment in selected patients, represent alternative treatment options [5]. Due to an impaired clinical outcome, lung tumors exceeding 3 cm are not good candidates for percutaneous tumor ablation [5].

However, the vast majority of patients with unresectable lung tumors are exceeding these limitations due to the advanced stage of their disease. For these patients, isolated lung perfusion (ILP) and transpulmonary chemoembolization (TPCE) are alternative local treatment options, both with the advantage of higher local chemotherapy concentrations [4–6]. However, none of these are established treatment methods yet.

ILP is an experimental technique to establish a closed circulation system using cannulation of the pulmonary artery and veins to achieve high concentrations of the chemotherapeutic agent in the lungs while minimizing systemic side effects [7]. ILP is an invasive and very complex procedure that requires thoracotomy and extracorporeal circulation. Hence, patients are usually only treated once. Additionally, this technique is limited to specialized cancer care centers which can perform invasive thoracic surgery with extracorporeal circulation [8, 9].

In TPCE, chemotherapeutic agents as well as embolic materials are administered into the tumor-feeding branch of the respective pulmonary artery via a catheter that is placed via the femoral vein. With this approach, chemotherapy concentrations as high as in ILP were reached in experimental studies and tumor response was superior to systemic chemotherapy [3]. However, besides experimental data, only a few clinical single-center studies on patients are published until now [4, 10–13]. In a recently published retrospective, a clinical study by Vogl et al. authors describes the treatment of 143 patients with unresectable pulmonary metastases refractory to systemic chemotherapy using TPCE. Response to treatment (i.e., partial response and stable disease) were observed in 77.3% of cases [9].

Embolotherapy is prolonging contact time between tumor and infused chemotherapy, and hence, higher concentrations of the chemotherapeutic agent can be achieved within the tumor. For lung tumor embolization, both in published experimental and in clinical studies starch microspheres (Spherex^{TM}, EmboCept^{TM}, Pharmacept GmbH Berlin, Germany) are the preferred embolic agents, since they only induce a transient embolic effect, however, still significantly prolonging contact time between tumor and infused chemotherapeutic agent [14]. This is in contrast to the role of hepatic TACE in HCC patients, where complete devascularization is the crucial aspect of therapy success [15]. Degradable starch microspheres (EmboCept^{TM} S, Pharmacept, Berlin, Germany) (DSM) are a temporary embolic agent with a half-life of 35 min and a mean size of 50 µm.

Our study aims to investigate safety as well as local pathologic and cardiovascular effects of TPCE. Therefore, we performed a large-animal study that intraindividually compares these aspects in TPCE using doxorubicin with bland lung embolization (i.e., transpulmonary embolization—TPE).

Material and Methods

Animal Preparation

In nine female domestic pigs with a mean weight of 60 kg, experiments were performed in accordance with and after allowance by the local regulatory board (Ministry of Environment, Nature and Consumer Protection). All interventions were done under general anesthesia after orotracheal intubation and mechanical ventilation. Both femoral veins were punctured using ultrasound guidance, and 6F sheaths (Terumo Radifocus Introducer 2, Terumo, Japan) were inserted. Pulmonary artery cannulation was performed using a 7F pulmonary artery Grollman catheter (Torcon NB Advantage Catheter, Cook, USA) with a 0.035″ guidewire (Radifocus Guide Wire M, Terumo, Japan). For embolization, a straight 4F catheter (Radifocus Glidecath, Straight, Terumo, Japan) was exchanged over a stiff guidewire in J-configuration (Amplatz Super Stiff, Boston Scientific, Costa Rica) and positioned in both proximal lower lobe pulmonary arteries (LLPAs).

MAA Test Injection

To evaluate a possible safety step, we injected a body weight-adjusted dose (mean 150 ± 46 Mbq) of [99mTc] MAA in each catheter, which was in embolization position. Subsequently, whole-body planar scans were acquired on a dual-head SPECT/CT camera (Symbia T16, Siemens Gammasonics Inc. Hoffman Estates, IL, USA). The images were visually inspected for abnormal tracer uptake outside the lungs by an experienced physician specialized in nuclear medicine.

Embolization Procedures

Immediately after the SPECT/CT, the animals were brought back to the angiographic suite to perform the embolization procedure: First, correct catheter positioning in both LLPAs was verified, and catheter repositioning was performed, if needed. TPE was performed at first in all

cases. For TPE, 7.5 ml EmboCept^{TM} S (450 mg; 50 µm) was mixed with 3.5 ml contrast agent (Ultravist 370, Bayer Healthcare Pharmaceuticals, Berlin, Germany) and 1.5 ml saline solution. For TPCE, 7.5 ml EmboCept^{TM} (450 mg; 50 µm) was mixed with 50-mg doxorubicin powder that was diluted in 3.5 ml iodine (Ultravist 370, Bayer Healthcare Pharmaceuticals, Berlin, Germany) and 1.5 ml saline. Both embolization procedures, i.e., TPE and TPCE, were performed under fluoroscopic guidance and intermittent DSA control to avoid backflow. When initial stasis was reached, embolization was continued after a five-minute interval. After having reached the second stasis in the treated vessel, the procedure was considered finished.

For vital parameter monitoring, we measured right ventricular systolic pressure (RVSP), heart rate and peripheral blood oxygenation levels using a monitoring system (Philips IntelliVue MP50, Philips, Boeblingen, Germany) immediately before the embolization procedures as well as one, three, five and ten minutes after each embolization procedure.

Cerebral MRI

All MRI procedures were performed on a 3 T system (Philips Achieva, Philips, Best, The Netherlands). Animals were placed in a supine position on the MRI table. The following sequences were acquired: T2w-FLAIR sequences in axial, coronal and sagittal orientation; DWI sequences (b0-b1000). Brain MRI was performed at least 2 h after embolization.

Chest CT

Animals were placed in a supine position on the CT table. A contrast-enhanced computed tomography (CT) in pulmonary arterial contrast phase (bolus tracking) of the lung was acquired in standard fashion.

After that, anesthesia was ceased, and animals were woken up.

Follow-Up

Depending on the length of follow-up, animals were divided into three follow-up groups consisting of three animals each. First three animals were killed after 12 h, next three animals after 24 h and finally three animals after 72 h, after the experiment. Before animals were killed, an additional chest CT was acquired with a protocol that is identical to the above-mentioned chest CT.

Pathological Evaluation

Lungs were explanted immediately after animals deceased. Lung explantation specimens after 12 h, 24 h and 72 h follow-up ($N = 3$ each) were fixed for a minimum of 4 weeks in 4% neutral buffered formalin. Samples from central and peripheral lung tissue were dehydrated, paraffinized, cut into 4-µm cuts and mounted on coated microscope slides (Dako, K8020). Periodic acid Schiff's staining (PAS) and Elastica van Gieson staining (EvG) were performed on an autostainer (Leica Autostainer XL). The staining with hematoxylin–eosin (H&E) and covering of the slides with coverslipping film were performed on a second autostainer (Sakura, 6132 Prisma®).

Slides were scanned in an automated slide scanner (Aperio VERSA, Leica). Whole slide images were generated. Mean alveolar area was quantified by creating a binary image from an 8-bit grayscale image followed by pixel analysis using Fiji software (NIH). The total lung tissue area in lung lobes after the intervention was compared with a control lobe of the respective lung. Morphological changes, such as alveolar thickening, atelectasis and granulocytic infiltration, were examined in TPE/ TPCE and control tissue in a semiquantitative fashion.

Statistical Evaluation

RVSP, heart rate and administered volume of the embolic agent and doxorubicin are expressed as mean values ± standard deviation (SD). RVSP, peripheral oxygenation levels and heart rate were compared for all time points (immediately before embolization, 1, 3, 5 and 10 min after embolization) using *t* test after performing Levene's test for equality of variances. Administered volumes of the embolic agent of TPCE and TPE experiments were compared in both groups using *t* test after completing Levene's test for equality of variances. Statistical evaluation was performed using SPSS 24 (IBM Deutschland GmbH, Ehningen, Germany).

Results

All experiments were technically successful, and no major complications occurred.

Pulmonary arterial MAA test injection revealed no systemic shunting of the tracer. Therefore, all experiments could be conducted as planned. For an example of MAA scintigraphy, please refer to Fig. 1.

After performing SPECT/CT and animals were brought back to the angiographic suite control, DSA revealed the exact placement of the catheters in each lower lobe

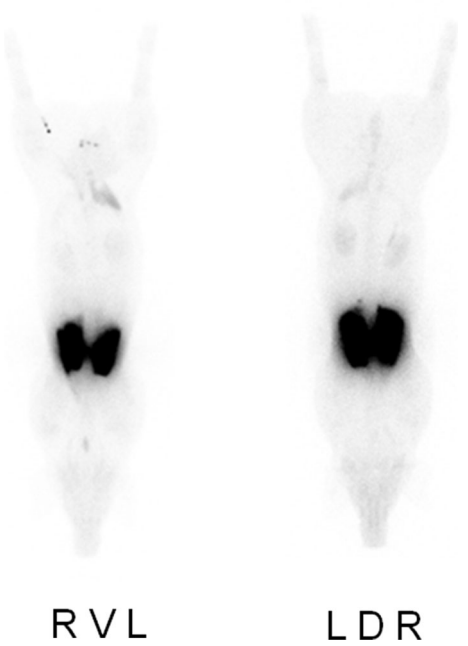

Fig. 1 MAA scintigraphy showing MAA accumulation in the lungs and no extrapulmonary tracer accumulation

pulmonary artery with no relevant difference to the DSA images acquired before SPECT/CT was performed.

For TPCE, a mean of 7.1 ± 2.3 ml of the doxorubicin–DSM mixture (i.e., 28.4 ± 9.3 mg doxorubicin and 4.3 ± 1.4 ml DSM) was injected into the respective LLPA until stasis was reached (Fig. 2). For TPE, a mean of 6.7 ± 2.4 ml DSM mixture (i.e., 4.0 ± 1.4 ml DSM) was injected into the contralateral LLPA. For both embolization volume and DSM volume, no significant difference was observed ($p > 0.05$).

Baseline pulmonary arterial pressure was significantly higher for all TPCE procedures compared to TPE procedures (20 ± 5 vs. 16 ± 5 mmHg). Mean maximum rise of RVSP was 15 ± 6 mmHg after TPCE and 12 ± 5 mmHg after TPE. Ten minutes after completion of embolization, mean pressure rise decreased to 12 mmHg after TPCE versus 9 mmHg after TPE. No significant differences in the increase in RVSP for TPCE and TPE could be observed at all time points ($p > 0.05$; see also Fig. 3).

The embolization procedures did not have any impact on heart rate (Fig. 4) and peripheral blood oxygenation levels, which stayed normal at all times.

The brain MRI performed after the experiments showed no signs of ischemia. CT of the chest immediately after the experiments demonstrated no atelectasis, congestion or signs of pulmonary infarction.

In the follow-up chest CT before killing the animals, no signs of dystelectasis, congestion, infiltrates or infarction were depicted. For CT imaging of the chest and MRI in the follow-up, please refer to Fig. 5.

In the histologic evaluation, one animal (24 h) had to be excluded from the analyses due to severe fixation artifacts. All other animals were included in the histologic and statistical analyses.

At histology, the lung tissue of the intervention lung lobes showed more prominent hemorrhage in comparison with the control lobes. Alveolar disruption or inflammation was not noticeable in any of the specimens examined at histologic evaluation. Intraalveolar edema was visible only very focally (less than 5% of the alveolar area). In three of

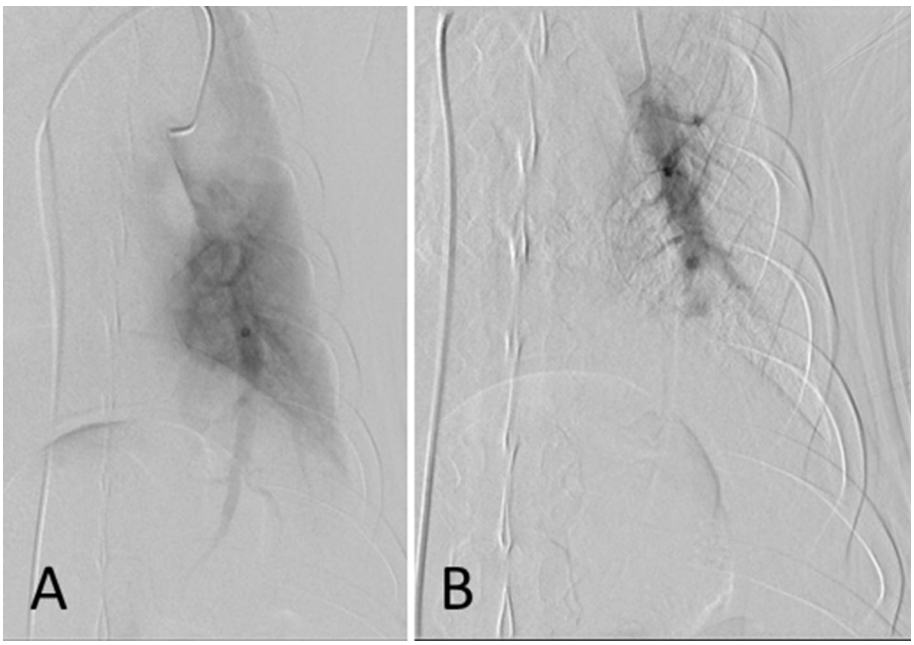

Fig. 2 Angiographic findings of TPCE procedure of the left lower lobe pulmonary artery (LLPA). A: Pre-embolization: DSA image demonstrates free blood flow to the left LLPA; B: post-embolization: DSA image shows stasis after TPCE was finished

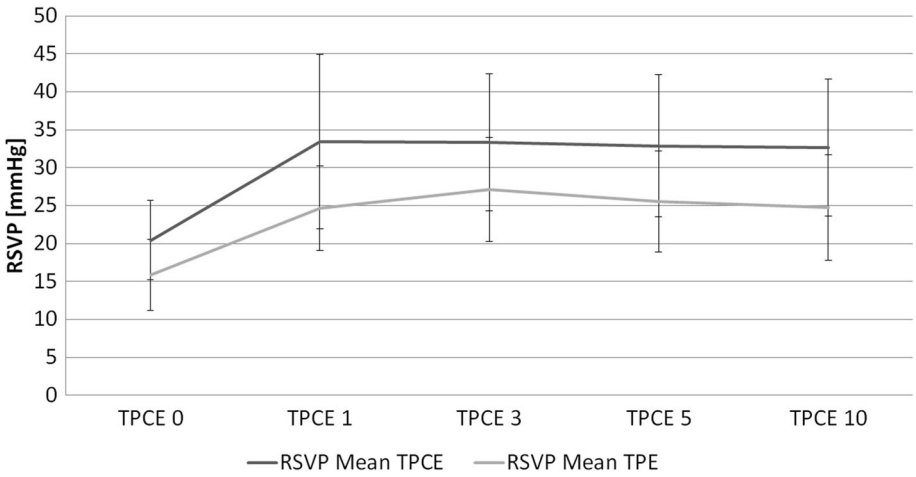

Fig. 3 Changes in mean right ventricular pressure (RVSP) immediately after the experiment (TPCE 0), as well as one (TPCE 1), three (TPCE 3), five (TPCE 5) and ten minutes (TPCE 10) after the embolization procedure in TPCE and TPE groups

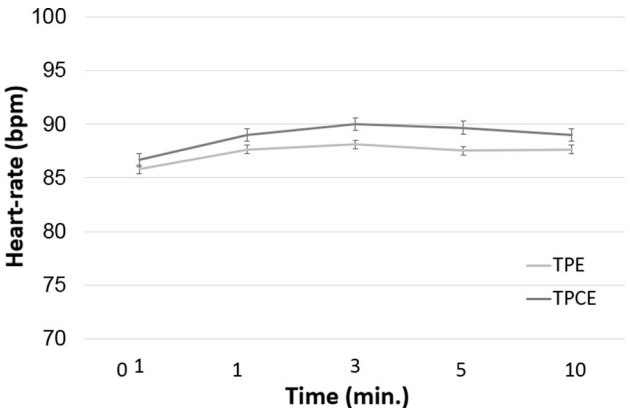

Fig. 4 Changes in mean heart rate in experiment

nine animals (one of the animals was killed after 12 h and two of the animals were killed after 72 h), foreign body granulomas within the arterial vessels were found. These granulomas were found in the TPE-treated lobes of all three animals (mean number of granulomas 3) and in two of the three animals in the TPCE-treated lobe (mean number of granulomas 9). For histologic results also refer to Fig. 6.

Discussion

This large-animal study shows that TPCE with degradable starch microspheres with optional delivery of doxorubicin is a feasible and safe technique.

Up to now, besides animal studies, only a few patient series are reported in the literature. In a rat model, pharmacokinetics and toxicity of DSM and carboplatin were studied, demonstrating that TPCE can deliver higher doses of carboplatin to the tumor tissue compared to ILP while sparing the surrounding lung parenchyma (7).

Systemic effects of TPCE with carboplatin and DSM were investigated by the same authors in a subsequent large-animal model [11]. Similar to our results, the authors observed a mean peak pressure increase of 12.5 mmHg 5 min after an embolization—we measured a peak pressure increase of 15 mmHg in the TPCE group and 9 mmHg in the TPE group. In contrast to Pohlen et al., we performed intraindividual comparisons with bland DSM embolization of the contralateral LLPA to evaluate the effect of doxorubicin. All RVSP measurements were higher in the TPCE group, but differences did not translate into significant results. Doxorubicin-induced vasospasms may play a role in this context [16].

Long-term histologic changes have been studied in another large-animal trial demonstrating normal lung parenchyma in all treated lobes 6 months after embolization. Imminent alterations of pulmonary microcirculation after transpulmonary application of DSM alone and combined with carboplatin (i.e., up to 30 min post-embolization) were studied by in vivo videomicroscopy in a rat model. In both groups, no signs of alveolar capillary membrane disorder, an early indicator for pulmonary toxicity, were observed [24]. To the best of our knowledge, subacute histologic alterations (i.e., between 12 and 72 h post-embolization) of the lungs by TPCE and TPE alone have not yet been studied in a large-animal model. Besides local lymphocyte infiltration and rare depiction of foreign body granulomas (in three of the nine animals), no relevant findings were observed. These foreign body granulomas were observed within the pulmonary arteries, most likely due to an immunologic reaction to the starch microspheres and not the doxorubicin as these were observed in both TPE- and TPCE-treated lobes. However, as long-term experiments reveal normal lung parenchyma, only these histologic changes are most likely transient reactions [4].

This study has proven the technical feasibility of MAA test injection and TPCE performed in one procedure. In our

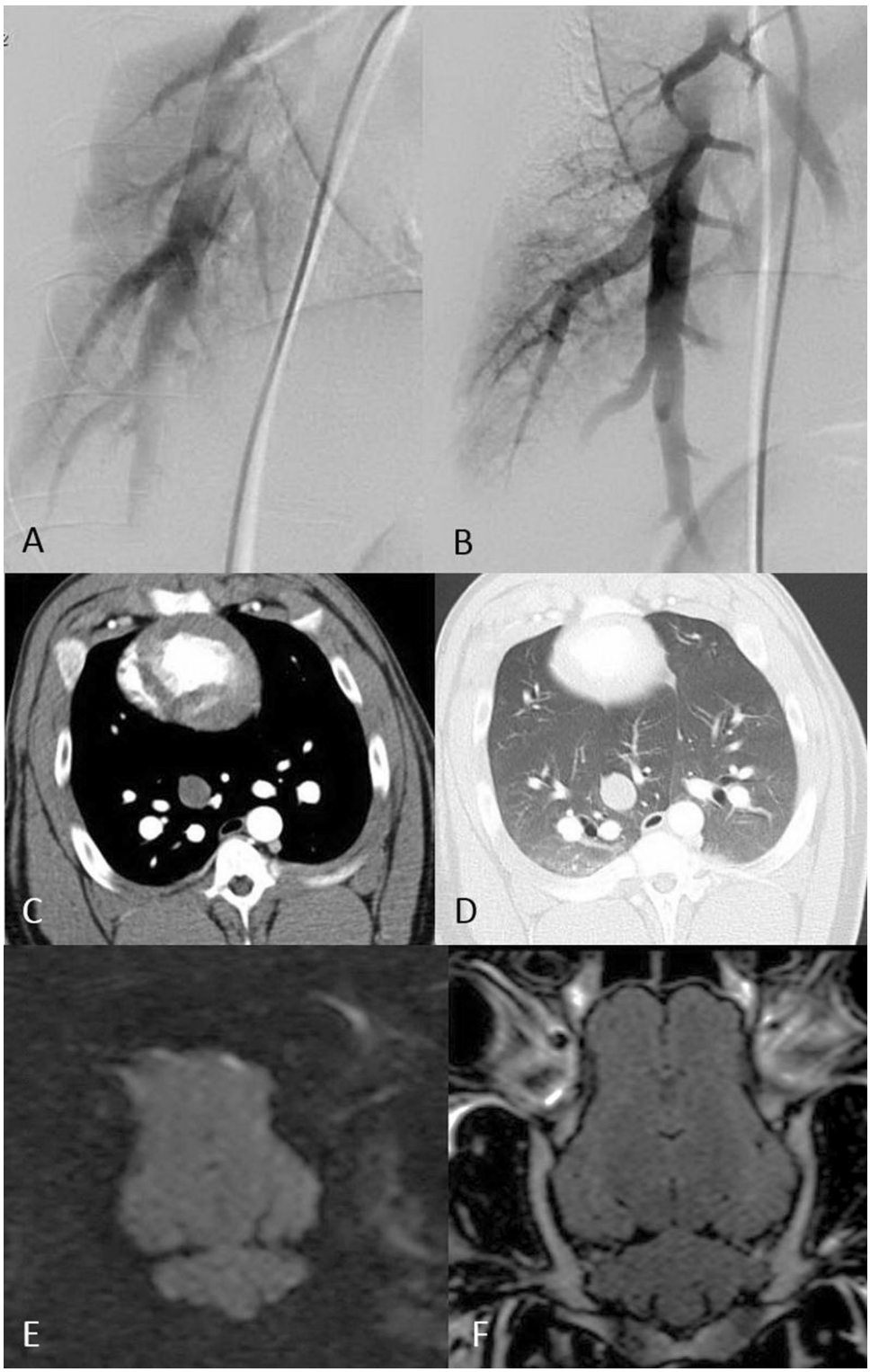

Fig. 5 Imaging during and after the experiments: **A**: Angiography of right lower lobe pulmonary artery before embolization; **B**: angiography of right lower lobe pulmonary artery after embolization (note more pronounced opacification of PA branches due to reduced flow rate; **C/D**: CT 12 h after embolization showing no signs of congestion, infiltration or infarction; **E/F**: MRI (E: diffusion-weighted MRI (*b* value: 1000) and F: FLAIR sequence) of animal's brain showing no evidence of nontarget embolization

experimental study, we did not expect any pulmonary arterial systemic shunting, as the pigs did not suffer from any lung tumor or vessel anomalies. However, if patients with lung tumors undergo TPCE in the future, although a rare situation, these tumors might have induced pulmonary arterial systemic shunting [14]. Hence, the goal here was to evaluate whether this "safety step," MAA test injection and SPECT/CT scan, can safely be performed in between angiographic-guided catheter placement in both lower lobe pulmonary arteries and the final embolization procedure, as

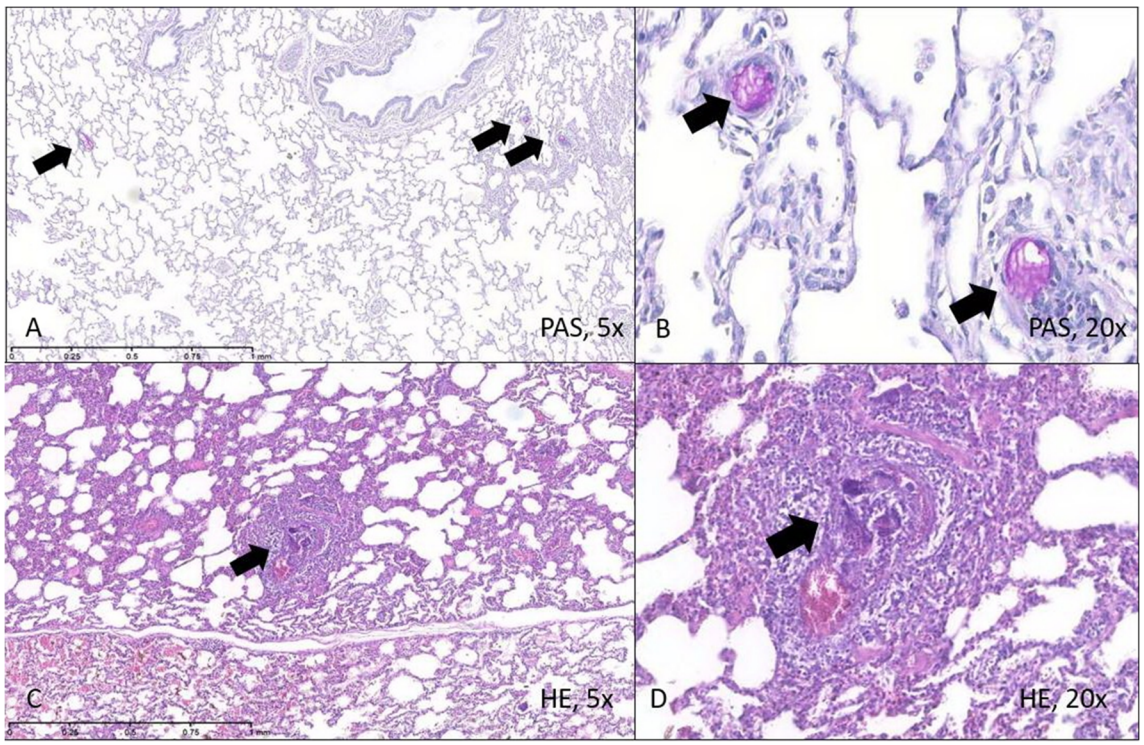

Fig. 6 Histologic evaluation: A/B: 12 h after embolization discrete signs of local inflammation are visible, as well as small arterioles filled with starch microspheres (black arrows); C/D: 72 h after embolization increasing but still mild signs of local inflammation are visible. In three animals, a low number of foreign body granulomas were found (black arrows)

this usually involves transfer of the patient to the nuclear medicine department and placement in the scanner, with the possible risk of catheter dislocation. Therefore, we suggest considering MAA test injection before performing TPCE in a clinical scenario.

There are several limiting factors in our study. First, we included nine animals only, and as we wanted to study subacute histologic alterations at different time points, we divided them into three groups consisting of three animals each, which again limits the results observed. Another limiting factor is that we used a large-animal model rather than a small-animal tumor model since our main interest was to investigate the local and cardiovascular effects of TPCE using DSM, rather than evaluating its efficacy on tumor growth.

Vital parameters were monitored for ten minutes after the embolization procedures according to the work of Pohlen et al. [11] We did not see the need to monitor vital parameters longer than 10 min, since RVSP will drop inevitably after this period of time and our measurements until ten minutes are concordant to the measurements of Pohlen et al. For the same reason, we have chosen a more short-term follow-up scheme for histologic evaluation—since these short-term data are still missing.

The goal of the present work was not to evaluate the MAA test injection as a possible safety algorithm for the exclusion of pulmonary–systemic shunting. In these cases, subjects with actual shunts have to be investigated, which was not part of this study. Additionally, as we did not monitor pulmonary arterial pressure, but only heart rate and blood oxygenation levels, before DSM was administered, we cannot rule out that there were synergistic effects of the MAA injection and embolization with DSM.

Another limiting factor is that we performed TPCE and TPE sequentially in the same individual so that the cardiovascular effects of the prior performed procedure will potentially have confounding effects on the cardiovascular effects of the following procedure. However, we acknowledged these by measuring "baseline parameters" before each lower lobe pulmonary artery embolization, so that intraindividual differences were calculated. Still, there will be effects of the prior performed procedure on the following procedure, which potentially distort our results.

In conclusion, we were able to demonstrate that TPCE and TPE can safely be performed without encountering relevant cardiovascular effects. We were furthermore able to demonstrate an algorithm that may be used to exclude pulmonary arterial shunting before performing pulmonary artery embolization procedures. Finally, histologic results confirmed that no irreversible pathologies, i.e., signs of ischemia, infarction or necrosis, occur after TPCE both in the embolized and in the non-embolized lung parenchyma.

Regarding a growing body of evidence concerning its oncological efficacy, we, therefore, promote performing TPCE in patients that are not amenable for surgery and have no appropriate systemic treatment option.

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Compliance with Ethical Standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed Consent For this type of study, informed consent is not required.

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