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ORIGINAL ARTICLE

Modified transarterial chemoembolization with locoregional administration of sorafenib for treating hepatocellular carcinoma: feasibility, efficacy, and safety in the VX-2 rabbit liver tumor model

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PURPOSE
We aimed to assess the feasibility, efficacy and safety of a local application of sorafenib within a conventional transarterial chemoembolization in the VX-2 tumor-bearing rabbit model.

METHODS
VX-2 tumors were induced in the left liver lobe of 10 New Zealand White rabbits. After two weeks, growth was verified by contrast-enhanced computed tomography (CT). Five rabbits were treated by transarterial chemoembolization using an emulsion of sorafenib and ethiodized oil (referred to as SORATACE; n=5). Rabbits receiving oral sorafenib for two weeks (n=2) and untreated rabbits (n=3) served as controls. After two weeks, contrast-enhanced CT was performed, followed by animal necropsy.

RESULTS
The change in tumor diameter between baseline and follow-up was significantly different in the SORATACE group compared with the other groups; tumor shrinkage was observed in the SORATACE group only (P = 0.016). In both control groups, preserved hypervascularity was seen in the follow-up CT in all but one tumor. All tumors in the SORATACE group were devascularized in the follow-up CT. Importantly, substantial parenchymal damage in nontargeted areas of the tumor-bearing liver lobe was seen in rabbits treated with SORATACE.

CONCLUSION
SORATACE demonstrated high efficacy in the treatment of experimental VX-2 liver tumors but was also associated with substantial liver parenchymal toxicity.

Treating hepatocellular carcinoma (HCC) remains a challenge; its incidence is increasing worldwide, and the disease is frequently diagnosed in an advanced stage in which curative treatment cannot be provided. According to the Milan criteria and Barcelona Clinic Liver Cancer staging scheme, only a minority of patients are candidates for potentially curative liver transplantation or, in patients without evidence of severe liver cirrhosis, for resection or radiofrequency ablation (1–4). Transarterial chemoembolization (TACE) is accepted as the therapy of choice (1, 2, 5–7) in intermediate disease stages or as a bridging treatment for patients on the waiting list for transplantation. When TACE is contraindicated and in advanced disease, systemic therapy with sorafenib, a multikinase inhibitor, is the only available effective treatment for patients with preserved liver function (8). Depending on patient selection and the embolic agent used, the median survival after TACE ranges from 15.6 to 23.2 months, and the treatment has demonstrated a proven benefit in controlled studies (compared with best supportive care), especially in early intermediate stage patients (5–7, 9–12). In a phase III trial comparing sorafenib with a placebo in advanced-stage HCC in Child A patients, treatment with sorafenib yielded a median survival benefit of 10.7 vs. 7.9 months (8).

Through its embolic effect, TACE may induce the release of angiogenic factors, which in turn may promote the outgrowth of surviving tumor cells into tumor recurrence and metastatic spread. Therefore, combining TACE with systemic sorafenib treatment may be an attractive concept (13, 14). Clinical data still remain inconclusive (15–17). Although the toxicity of such a combined regimen (TACE plus sorafenib) does not seem to exceed the toxicity conferred by each of the two modalities alone, the overall toxicity of systemic sorafenib remains high. Common adverse events include diarrhea, hand-foot skin reaction,

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and fatigue, presenting at grade 3 intensity in approximately 25% of cases (8, 18). These observations have led to the idea of locally administering sorafenib in combination with ethiodized oil to increase the sorafenib concentration in the tumor while reducing the systemic concentration, and thus, toxicity. Recent pilot experiments by Gaba et al. (19) and Chatziioannou et al. (20) revealed transarterial sorafenib chemoembolization to be feasible in non-tumor-bearing rabbits, where favorable pharmacokinetics were observed. The sorafenib concentrations found in the treated lobe were significantly higher than the concentrations reported for systemic therapy, while no immediate histopathologic tissue toxicity was found in animal necropsy (19, 20).

In this study, we performed a modified TACE using an emulsion of sorafenib and ethiodized oil (referred to as SORATACE) for the treatment of experimental VX-2 liver tumors in rabbits. Efficacy and safety were measured using contrast-enhanced computed tomography (CT) and histopathologic examinations. Two groups of rabbits receiving either no treatment or oral sorafenib served as controls.

Methods

Ethical considerations and animals

This study was approved by the competent supervising authorities according to the State's animal protection act. All applicable institutional and national guidelines for the care and use of animals were followed.

Main points

- Transarterial chemoembolization (TACE) with varying agents/carriers is considered as the treatment of choice in selected patients with hepatocellular carcinoma in the intermediate stage (according to BCLC). However, TACE alone may not be sufficient for complete treatment.
- Additional systemic treatment with sorafenib is not able to increase the efficacy of TACE, as shown in various trials.
- The efficacy of locally applied sorafenib within a TACE procedure (SORATACE) is not known. Preclinical studies in non-tumor models indicate a high liver-to-serum ratio.
- The present study demonstrates the feasibility and high efficacy of SORATACE in a tumor bearing animal model (VX-2 tumor of the rabbit).
- However, associated local hepatotoxicity may limit the clinical use of the technique.

Female adult New Zealand White rabbits were used (Crl:KBL NZW, Charles River Laboratories International Inc.). After delivery, a minimum of two weeks of acclimatization was allowed, with free alimentation (as it was for the whole duration of the experiment). Ten rabbits were used for the experiments as described below. The weight at the start of the experiments ranged between 3.5 and 5.0 kg (mean, 4.3 kg).

Anesthesia and analgesia

For tumor implantation into the hind leg, tumor harvesting from the hind leg, tumor implantation into the liver, imaging, and TACE, the following anesthesia and analgesic protocol was followed: 0.5 mg atropine sulfate (Atropinsulfat 0.5 mg/mL, B. Braun Melsungen AG) was injected subcutaneously for premedication. A mixture of 50 mg/kg ketamine (Ketavet 100 mg/mL, Pfizer Deutschland GmbH) and 4 mg/kg xylazine (Rompun 20 mg/mL, Bayer AG) was injected intramuscularly. For maintaining anesthesia, ketamine was administered in repeated 20 mg injections through a 24-gauge line in the auricular vein. Immediately before surgical incision, intracutaneous infiltration with lidocaine (Xylocitin 1%, Mibe GmbH Arzneimittel) was performed. To reduce postsurgical pain, 4 mg/kg carprofen (Rimadyl, Pfizer Deutschland GmbH) was injected subcutaneously at the end of all surgical procedures.

Tumor induction and transfer to the liver

Propagation of the VX-2 tumor was performed according to descriptions in the literature (21). Deep-frozen solid tumor particles (courtesy of Bayer Pharma AG) were gently defrosted and washed in cold Roswell Park Memorial Institute medium (RPMI, Biochrome AG). A small incision was made and an 11-gauge coaxial needle (T-Lok bone marrow biopsy needle, Angiotech) was used to place two pieces (each of size approximately 2 mm^3) of the tumor tissue into both thigh muscles. After two weeks, tumors of approximately 1 to 2 cm in size were harvested (22). At the same time, an upper midline laparotomy of the recipient rabbits was performed, and the left liver lobe was exposed and gently brought up to the skin level. The VX-2 tumor from the donor rabbits was then stripped from the surrounding tissue and minced into small pieces of approximately 2 mm^3 under permanent cooling with ice-cold phosphate-buffered saline (PBS) solution. Two of these tumor pieces were then implanted intraparenchymally through an 11-gauge coaxial needle into the left liver lobe of the recipient rabbits. To avoid peritoneal leakage of tumor cells, the injecting channel was occluded with an absorbable gelatin sponge hemostat (Gelita-Spon Standard, Gelita Medical) while the needle was being retracted (23). Finally, the laparotomy was closed in two layers. Baseline CT imaging (details below) and further treatments were conducted after a mean (±standard deviation) of 14.8±2.9 days after tumor implantation.

Experimental groups

The rabbits were divided into three groups. Five rabbits received modified TACE (SORATACE) with sorafenib (sorafenib tosylate, by courtesy of Bayer Healthcare) and ethiodized oil (Lipiodol Ultra fluid, Guerbet), two rabbits received oral sorafenib (n=2), and three rabbits were left untreated (n=3).

SORATACE group: for rabbits treated by SORATACE, the procedure was conducted with an Artis Zeego angiography system (Siemens) immediately after baseline CT. The right femoral artery was exposed surgically and secured with two sutures. A T-shaped cut of the femoral artery was performed with microtomy scissors and a 22-gauge venous line was slowly inserted. Through a stiff 0.025-inch mini-guidewire, the venous line was exchanged to a 4F introducer sheath (Radiofocus Introducer II, Terumo Medical). A hydrophilic-coated 4F cobra-shaped catheter (Radiofocus Glidecath Cobra 2, Terumo Medical) was advanced over a hydrophilic-coated 0.035-inch guidewire (Radiofocus Guidewire M, Terumo Medical) to the celiac trunk. A celiac angiogram was performed with identification of the tumor-feeding artery (using an iodine contrast agent: Imeron 300, Bracco Imaging). In the coaxial technique, a hydrophilic-coated 3F microcatheter (Micro Ferret 18, Cook Medical) with a 0.014- or 0.016-inch guidewire (Cirrus 14 Microwire, Cook Medical or Radiofocus Guidewire GT, Terumo Medical) was introduced over the 4F catheter. The microcatheter was advanced in an attempt to perform the embolization as selectively as possible, at best exclusively in the tumor-feeding arteries; however, owing to the small size of these vessels, a lobar embolization had to be performed.

A well-mixed emulsion of ethiodized oil (0.5 mL), saline solution (0.5 mL), and sorafenib (25 mg) was established using the pumping method, with at least 20 pushes and pulls through a lipiodol-resistant stopcock between two disposable 1 mL syringes immediately before infusion. The intended sorafenib concentration was 5 mg/kg body weight to reach a local concentration of approximately 10 µg/mL, on the basis of dosing assumptions according to Gaba et al. (19). The solution was very slowly applied under continuous fluoroscopic guidance to maintain an antegrade flow for as long as possible. The predefined endpoint was a complete embolization of the tumor vessels. The mean applied sorafenib concentration was 6.26 mg/kg body weight (range, 2.04–10.2 mg/kg body weight). Finally, the catheters and the sheath were removed, the femoral artery was ligated, and the wound was closed in two layers. Immediately after the SORATACE treatment, an unenhanced CT scan was performed to verify the location of the injected ethiodized oil/sorafenib emulsion.

The ethiodized oil /sorafenib embedment in the tumor was considered to cover the complete tumor in three of the five rabbits and to be incomplete (defined as less than 100% but more than 50% coverage) in two of the five rabbits, as assessed by the post-treatment CT. CT also revealed minor and diffuse embedding of ethiodized oil/sorafenib in the liver parenchyma surrounding the tumor in all the rabbits treated.

Oral sorafenib group: in this group, sorafenib was administered orally at a dose of 5 mg/kg body weight twice a day, starting immediately after baseline CT imaging, until follow-up CT imaging.

Control group: control rabbits underwent only baseline and follow-up CT imaging without intraarterial or systemic drug application.

Computed tomography imaging

CT was performed at baseline and after an interval of 12.6±1.7 days after the initiation of tumor treatment/surveillance (Somatom AS+, Siemens). After an unenhanced scan, an arterial and a venous contrast-enhanced scan were started with a delay of 15 and 40 s, respectively, after starting the injection of 5 mL of contrast agent (Imeron 300, Bracco Imaging) through a 24-gauge venous line into the auricular vein at an injection rate of 1 mL/s, followed by injection of 5 mL saline solution at 1 mL/s.

The tumor diameter was measured as the maximum overall tumor diameter. Tumor volumes were obtained by segmentation using Osirix Imaging software.

Histopathology and TUNEL assay

After the final imaging procedure, euthanasia of the rabbits was performed by intensifying anesthesia by intravenous injection of 200 mg ketamine and 20 mg xylazine, followed by 10 mL of 7.45% potassium chloride solution (B. Braun Melsungen AG). The liver was removed. Specimens of the tumor were obtained and fixed in 4% formaldehyde solution. Hematoxylin-eosin staining was performed for characterization of the tissues (tumor and liver) of the representative rabbits.

Additionally, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was performed to detect apoptosis and cell death from formalin-fixed tissue using the In situ Cell Death Detection Kit Flurescein (Roche Diagnostics GmbH). Tissues were cut into slices of 5 µm, deparaffinized with xylol and permeabilized with Triton X-100 (Sigma Aldrich). After incubation for 60 min at 37°C in a humidified dark atmosphere, the slides were washed twice in PBS. Nuclear staining and mounting was done with Vectashield and 4′,6-diamidino-2-phenylindole mounting medium (Vector Laboratories).

Statistical analysis

Statistical analysis was performed using SPSS (SPSS 21, IBM Corp.). Descriptive analyses of the tumor characteristics and findings were performed by displaying continuous variables as means or medians with the standard deviation or range; frequency data were displayed as counts. Group comparisons regarding the induction period, follow-up period, tumor size, tumor volume, and tumor vascularity at baseline and under treatment were conducted using the Mann-Whitney U test and Fisher's exact test, as appropriate. A P value below 0.05 was considered statistically significant.

Results

The mean initial overall tumor size of the induced liver tumor was 16.0±6 mm (control group, 16.3±1.3 mm; oral sorafenib group, 10.5±5.5 mm; SORATACE group, 18±6.5 mm) with no significant difference between the groups. After treatment, the overall tumor size as measured by CT was 36.7±19.4 mm for the control group (+125.2% compared with baseline), 18.5±6.5 mm for the oral sorafenib group (+76.2%), and 15.5±8.8 mm for the SORATACE group (–13.9%); Fig. 1. The absolute diameter at follow-up did not differ significantly between the groups (SORATACE compared with the other groups), but the tumor growth between baseline and follow-up was significantly different between the groups, with a tumor shrinkage observed in the SORATACE group only ($P = 0.016$) (Table). Concordant results were found when the tumor volumes were compared between the groups (regarding baseline, follow-up, and the difference between baseline and follow-up) (Table).

At baseline CT scan, all tumors showed a hypervascular pattern of the tumor rim with central hypovascularity (Fig. 1a, 1f). At follow-up, all tumors in the SORATACE group were devascularized (Fig. 1e). The vascularity at follow-up was found to be significantly different when the SORATACE group was compared with the other groups ($P = 0.048$) (Table).

The liver parenchyma of the tumor-bearing left liver lobe showed regional hypovascularity in the follow-up CT, indicating liver parenchymal damage after SORATACE ($P = 0.008$) (Table). At liver procurement, all these liver lobes showed a focal yellowish color, i.e., necrosis. Histopathologic examination revealed areas of necrosis; no signs of foreign-body reaction were seen. Fig. 2 shows an example of the histopathologic appearance after SORATACE treatment. Obviously, no such changes were seen in the other groups.

Histopathology of selected subjects in the control group revealed viable tumor tissue at the periphery of the lesions (mainly arranged in a perivascular pattern), with major parts of the central tumor being inhomogeneous and necrotic (Fig. 3a–3c). Similar findings were seen in subjects in the oral sorafenib group. In the SORATACE group, all the tumor specimens analyzed were totally necrotic, with no perivascular viable tumor tissue left (Fig. 3d–3f).

Discussion

In this study, we report for the first time the feasibility of the locoregional application of sorafenib as an adjunct to conventional tumor embolization with ethiodized oil in a preclinical setting of VX-2 tumor-bearing rabbits.

The rationale for local (as opposed to systemic) application of an antiangiogenic chemotherapeutic agent is the increased tumoricidal effect due to higher drug con-

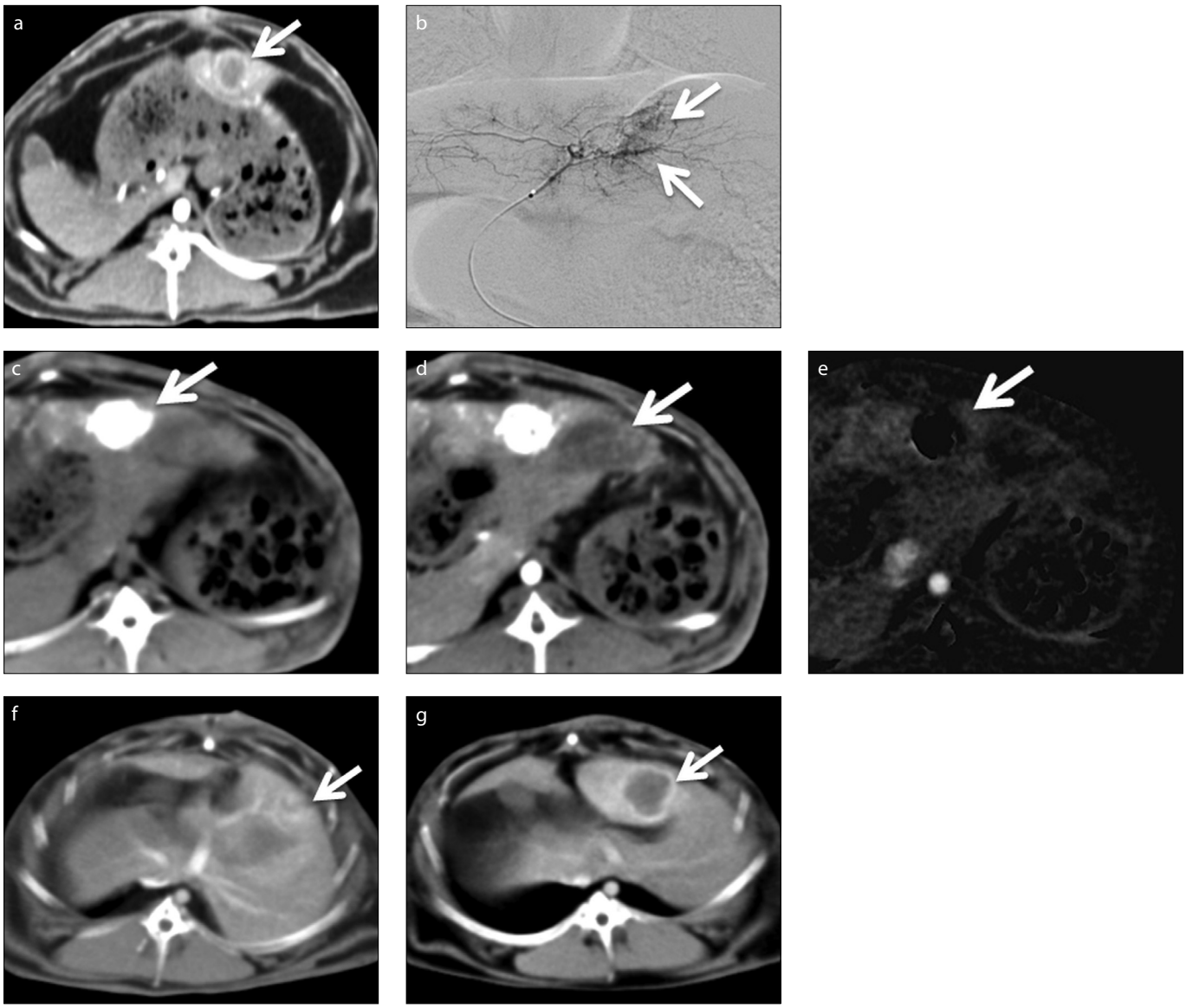

Figure 1. a–g. Exemplary images from the SORATACE group **(a–e)** and the control group **(f, g)**. Baseline arterial phase contrast-enhanced CT **(a)** shows a peripherally enhancing tumor in the left liver lobe *(arrow)*, diameter 12 mm. Angiogram of the left liver lobe **(b)** shows an arterial blush in the location of the tumor *(arrows)*. Unenhanced CT at two weeks after the SORATACE procedure **(c)** shows strong accumulation of ethiodized oil/sorafenib in the entire tumor *(arrow)*, diameter 12 mm. Arterial phase contrast-enhanced CT at two weeks after the SORATACE procedure **(d)** indicates no detectable new or remnant vascularized tumor. Hypovascularized liver parenchyma in the left liver lobe *(arrow)* indicates parenchymal damage. Subtraction of **(c)** from **(d)** shown in panel **(e)** verifies the absent hypervascularity of the embolized tumor *(arrow)*. Baseline arterial phase contrast-enhanced CT **(f)** shows a peripherally enhancing tumor in the left liver lobe *(arrow)*, diameter 10 mm. Arterial phase contrast-enhanced CT at two weeks after baseline **(g)** shows a persistent peripherally vascularized tumor *(arrow)*, which is increased in size compared with baseline (from 10 to 18 mm).

centrations at the tumor site. Furthermore, the concomitant application of an antiangiogenic drug may attenuate the effect of the release of angiogenic factors induced by conventional TACE. Induction of tumor hypoxia by embolization has also been shown to result in membrane instability, which may increase the uptake of concomitantly applied chemotherapeutic agents (24). Finally, the systemic toxicity of sorafenib may be reduced if the drug is applied locally (8, 13, 14, 18).

First attempts to increase the efficacy of chemoembolization by combination with an antiangiogenic drug were recently undertaken by Deng et al. (25) in VX-2 tumor-bearing rabbits with the use of endostatin. These investigators reported a significant reduction of the tumor diameter and growth rate compared with controls. However, changes in size were comparable to those in a group treated by conventional TACE, whereas the microvascular density and the expression of vascular endothelial growth factor were significantly reduced in the group that received concomitant endostatin (25).

In line with the results reported by Deng et al. (25), we were able to demonstrate an impressive tumor response after local application of a combined regimen of an embolic agent (ethiodized oil) and an antiangiogenic drug (sorafenib), which was significantly more pronounced than the response in a control group or in a group receiving oral sorafenib. However, the present study was designed as a feasibility and proof-of-concept study only and thus did not include a planned head-to-head comparison with conventional chemoembolization, e.g.,

Table. Tumor characteristics and imaging findings at baseline and follow-up

Variable	Mean±SD or frequency	*P**
Induction period (days)	14.8±2.9	0.151
Tumor size (and volume) at baseline, mm (cm^3)		
Overall (n=10)	16.0±6.0 (3±3.1)	0.841 (0.841)
Control (n=3)	16.3±1.3 (2.3±0.7)	
Sorafenib oral (n=2)	10.5±5.5 (1.1±1.5)	
SORATACE (n=5)	18.0±6.5 (4.3±4.6)	
Vascularity at baseline, overall (n=10) (hyper/hypo)	10/0	
Follow-up period (days)	12.6±1.7	0.556
Tumor size (and volume) at follow-up, mm (cm^3)		
Overall (n=10)	23.2±16.2 (16.2±38)	0.286 (0.151)
Control (n=3)	36.7±19.4 (50.1±75.5)	
Sorafenib oral (n=2)	18.5±6.5 (4.5±5.1)	
SORATACE (n=5)	15.5±8.8 (3.1±4.5)	
Tumor size (and volume) change under treatment, mm (cm^3)		
Overall (n=10)	6.6±14.9 (14.7±40)	0.016 (0.016)
Control (n=3)	20.3±22.4 (47.8±75)	
Sorafenib oral (n=2)	8.0±1.4 (3.4±3.7)	
SORATACE (n=5)	−3.3±3.2 (−1.1±0.6)	
Vascularity at follow-up, (hyper/hypo)		
Overall (n=10)	4/6	0.048
Control (n=3)	3/0	
Sorafenib oral (n=2)	1/1	
SORATACE (n=5)	0/5	
Tumor embolization after SORATACE (n=5)		
Complete coverage	3/5	
Incomplete coverage	2/5	
Liver parenchymal embedment of the embolisate after SORATACE (n=5), (yes/no)	5/0	
Liver parenchymal damage seen on follow-up CT, (yes/no)		
Overall (n=10)	5/5	0.008
Control (n=3)	0/3	
Sorafenib oral (n=2)	0/2	
SORATACE (n=5)	5/0	

SD, standard deviation; SORATACE, transarterial chemoembolization with lipiodol and sorafenib; hyper, hypervascularized; hypo, hypovascularized; CT, computed tomography.
*Comparison of SORATACE group with the other groups (control and sorafenib oral group combined). *P* values in parentheses indicate volume comparisons.

with ethiodized oil/doxorubicin. Nevertheless, the capacity of SORATACE to induce tumor necrosis was morphologically documented by tumor shrinkage and devascularization, and was also emphasized by the histopathologic analysis, which revealed total tumor necrosis in all specimens.

To date, no data on liver tumor treatment by transarterial sorafenib chemoembolization have been published. Gaba et al. (19) recently reported pharmacokinetics and early histopathologic findings after transarterial sorafenib chemoembolization of the left liver in non-tumor-bearing rabbits. A high local hepatic sorafenib concentration of up to 94.2±48.3 µg/mL was found (19). Systemic drug levels were not measured. Chatziioannou et al. (20) also measured local drug concentrations and pharmacokinetics after transarterial sorafenib chemoembolization in non-tumor-bearing rabbits and found therapeutic levels of local sorafenib concentration in the liver 24 h after administration (mean, 794±240 ng/mL). The measurement of systemic concentrations revealed a mean liver-to-serum ratio of 14±7 at that time (20). Compared with literature values, the sorafenib concentrations in the liver reported by these two authors are slightly lower (20) or higher (19) than the typical systemic therapeutic drug levels of 2–10 µg/mL achieved by oral sorafenib treatment (26, 27). The different tissue concentrations in the two studies (94.2 µg/mL vs. 794 ng/mL) most probably result from a difference in the doses applied; Gaba et al. (19) used an intended dose of 3 mg/kg body weight, while Chatziioannou et al. (20) used 0.1 mg/kg body weight.

Interestingly, according to Gaba et al. (19), these relatively high concentrations in the liver parenchyma were not accompanied by acute severe hepatocyte damage; this was confirmed in their work by histopathologic analysis of embolized liver specimens immediately after treatment. Only mild to moderate ballooning degeneration in zone 3 hepatocytes was seen in the treated liver lobe. Moreover, these changes were also seen in one animal that had been treated by embolization alone (without sorafenib). Blood parameters for liver damage were not evaluated in this study (19). Longer follow-up data (up to 72 h after treatment) are available from the study of Chatziioannou et al. (20). The authors reported mild to moderate histopathologic signs of liver damage. Moreover, measurement of blood parameters revealed an increase in aspartate and alanine transaminases (AST and ALT) up to one hundred times their baseline values, indicating substantial hepatocyte damage (20). It is surprising that the authors did not rate this finding as an indicator of liver parenchymal damage, especially in light of recently published data on the remarkable impact of hepatocyte damage (as measured by AST, as part of the ART score) on survival after TACE (28). Our observation of severe liver parenchymal damage after SORATACE suggests that the reported elevations of serum transaminase activities constitute a true indicator of liver damage, and that early histopathologic changes might lead to an underestimation of the accompanying toxic effects of SORATACE (although a different mixture of the oil/sorafenib emulsion has

to be regarded as an influencing factor). In contrast to the studies of Gaba et al. (19) and Chatziioannou et al. (20), we detected a considerable toxic effect on the tumor-bearing liver lobe after the combined application of an embolic agent and sorafenib. Taking into account the observed partial nontarget embedding of the embolisate in the liver parenchyma adjacent to the liver tumor, we conclude that this finding is associated with the SORATACE procedure. However, because we had no control group with bland ethiodized oil embolization only or with a local application of sorafenib only, we cannot state whether the parenchymal damage derives from the bland embolization effect or from a direct toxicity of sorafenib. It has to be kept in mind that the liver has a dual blood supply and that isolated embolization of the arterial vascular bed usually does not lead to parenchymal necrosis unless the embolisate does not pass through to the portal venous system, and we cannot definitely exclude the possibility that this may have occurred in our experiments. Interestingly, comparable toxicities after chemoembolization of VX-2 liver tumors in an experimental setting, as reported here, have not been found in other studies (using various embolic mixtures, whereby the carrier was always ethiodized oil) (21, 25, 29, 30). Thus, a direct toxic effect of locally applied, highly concentrated sorafenib cannot be excluded according to our observations. It is unlikely that this was merely caused by the relatively high local mean dose of 6.26 mg/kg body weight used in the present study, as substantial elevations of transaminases after SORATACE treatment using sorafenib at a dose of only approximately 0.1 mg/kg body weight have also been observed, as reported by Chatziioannou et al. (20).

A number of limitations need to be mentioned. First, no hepatoma model for rabbits (as for rats or mice) exists, so this preclinical study was performed in VX-2 tumor-bearing rabbits, which are a well described HCC tumor model (31, 32). VX-2 is a virus-induced

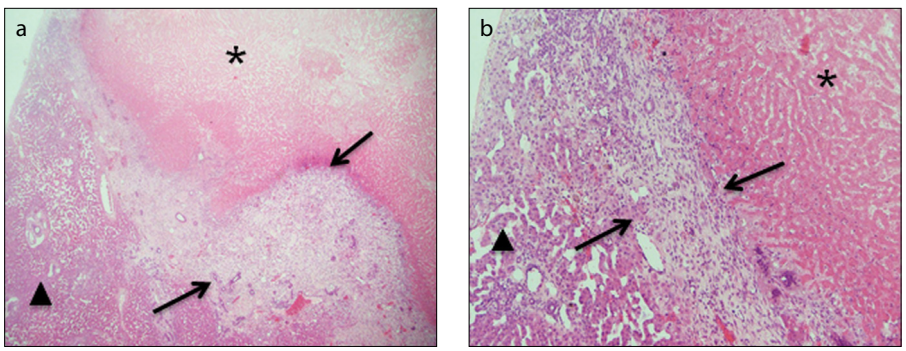

Figure 2. a, b. Histopathologic specimen, hematoxylin and eosin (HE) stain. Panel **(a)** shows overview of embolized liver tissue with necrosis *(asterisk)* and viable reaction at the margin *(right arrow)* next to fibrous tissue with proliferations of the bile ducts *(left arrow)* and normal liver tissue *(arrowhead)*. Original magnification: 25×. Panel **(b)** shows a higher magnification of **(a)** with necrosis *(asterisk)*, viable margin *(right arrow)*, fibrous areas *(left arrow)*, and normal liver tissue with sinusoidal ectasia *(arrowhead)*. Original magnification: 100×.

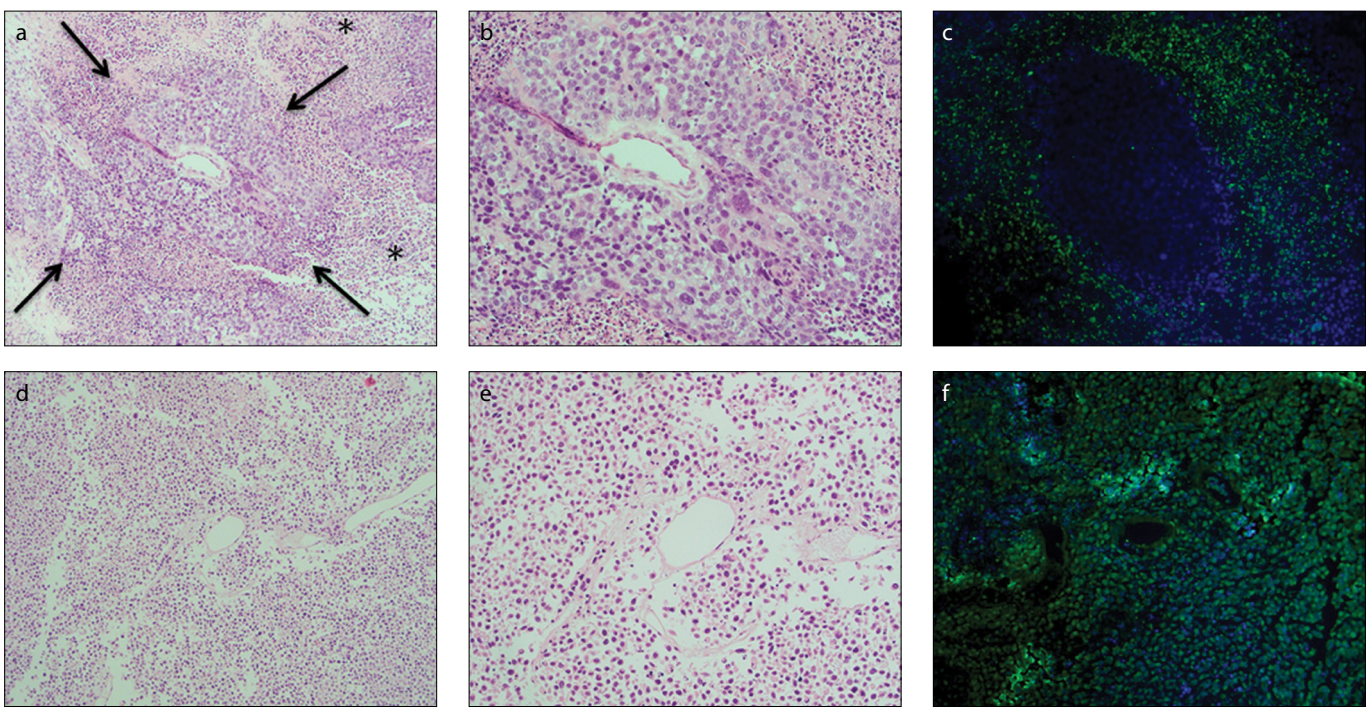

Figure 3. a–f. Histopathologic specimens. HE stain **(a, b, d, e)** and TUNEL stain **(c, f)** from the sorafenib oral group **(a–c)** and the SORATACE group **(d–f)**. Sorafenib oral group: HE staining (original magnification: 100×) **(a)** shows a viable tumor in the perivascular region (area marked by *arrows*). At a greater distance from the vessel, the tumor tissue shows necrotic areas *(asterisk)*. Panel **(b)** shows a higher magnification (200×) of the perivascular tumor tissue from **(a)**, displaying a capillary vessel in the center surrounded by viable tumor tissue with highly pleomorphic cells and multiple mitoses. Panel **(c)** shows TUNEL staining corresponding to **(a)**; in the perivascular region the cells are not apoptotic (stained blue). The cells at a greater distance from the blood vessel appear apoptotic (stained green). SORATACE group: HE staining (original magnification: 100×) **(d)** shows necrotic tumor tissue around a capillary vessel. Panel **(e)** shows a higher magnification (200×) of **(d)** displaying necrotic changes with pycnosis and karyorrhexis. Panel **(f)** shows TUNEL staining corresponding to **(d)**; large area of apoptotic cells (stained green) around the central blood vessel. Compared with **(c)**, no viable cells (stained blue) are visible around the central blood vessel.

papilloma transforming into an aggressive anaplastic carcinosarcoma of the rabbit. However, owing to percutaneous vascular accessibility in the rabbit and as the pattern of vascularization is similar to HCC, the VX-2 rabbit tumor is a widely accepted model for HCC (21, 33, 34). Although the VX-2 tumor model is commonly accepted and used as an HCC model, the transferability of the findings obtained with this model to HCC is not proven. Whether data drawn from the VX-2 model can be applied to HCC treatment remains unclear since reports on *in vitro* comparability between VX-2 and hepatoma cells are scarce, with only one study published in this field. In that study, the glucose metabolism of VX-2 cells was compared with that in hepatoma cells (AS-30D cell line), and a comparable pattern of glucose utilization and metabolization was shown (34). However, there is no other HCC TACE model available to date. Second, this was a preliminary feasibility study without control groups receiving conventional chemoembolization (e.g., doxorubicin and ethiodized oil), a bland embolization only (with ethiodized oil), or local application of sorafenib only. Third, the sample size was small, so that conclusions should be drawn cautiously. Fourth, blood measurements of angiogenic factors and liver function parameters were not performed.

In conclusion, this study confirms the technical feasibility and the efficacy of sorafenib chemoembolization in rabbits. However, the observed local hepatotoxicity may limit the clinical use of the technique.

Conflict of interest disclosure

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