A Randomized Phase II Study of Drug-Eluting Beads versus Transarterial Chemoembolization for Unresectable Hepatocellular Carcinoma

Hannah van Malenstein^{a*} Geert Maleux^{b*} Vincent Vandecaveye^b Sam Heye^b
Wim Laleman^a Jos van Pelt^a Johan Vaninbrouck^b Frederik Nevens^a Chris Verslype^a

^aDepartment of Hepatology,

^bDepartment of Radiology, University Hospitals Leuven, Belgium

Keywords

Hepatocellular carcinoma · Chemoembolization · Drug-eluting beads · Doxorubicin · Pharmacokinetics

Summary

Background: Transcatheter arterial chemoembolization (TACE) is the standard treatment in selected patients with unresectable hepatocellular carcinoma (HCC). Drug-eluting particles are developed to reduce side effects and improve efficacy. We present safety data of a prospective randomized phase II study with doxorubicin-eluting superabsorbent polymer (SAP) microspheres. **Material and Methods:** We prospectively included 30 HCC patients with different Barcelona Clinic Liver Cancer (BCLC) stages (A = 3, B = 19, C = 8) and randomly assigned them to receive conventional TACE (n = 14) (control group) or doxorubicin-eluting SAP microspheres (n = 16). The doxorubicin plasma level was assessed at different time points, biochemical analysis was performed, and side effects were reported following the Common Toxicity Criteria. Tumor response was assessed at 6 weeks according to the modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria. **Results:** There was a significantly lower plasma peak concentration ($C_{\max}$) of doxorubicin and smaller area under the curve (AUC) with SAP microspheres (mean $C_{\max}$ 495 ± 293.9 ng/ml, mean AUC 69.7 ± 26.9 ng/ml min) compared to controls (mean $C_{\max}$ $1,928 \pm 560.8$ ng/ml, mean AUC 165 ± 32.3 ng/ml/min; both $p < 0.001$). Furthermore, there were less grade 3 and no grade 4 adverse events in the SAP microsphere group. Tumor response was comparable between the groups. **Conclusions:** TACE with SAP microspheres leads to low plasma levels of the cytotoxic drug and therefore minimizes toxicity compared to conventional TACE.

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Schlüsselwörter

Hepatozelluläres Karzinom · Chemoembolisation · Drug-Eluting Beads · Doxorubicin · Pharmakokinetik

Zusammenfassung

Hintergrund: Die transarterielle Chemoembolisation (TACE) ist die Standardbehandlung für selektierte Patienten mit nichtresektierbarem hepatozelluläres Karzinom (HCC). Mittels sogenannter Drug-Eluting Beads können Nebenwirkungen reduziert und die Effektivität verbessert werden. Wir stellen die Sicherheitsdaten einer prospektiven, randomisierten Phase-II-Studie mit Doxorubicin eluierenden Superabsorber(superabsorbent polymer; SAP)-Mikrosphären vor. **Material und Methoden:** Insgesamt wurden 30 HCC-Patienten mit verschiedenen Barcelona-Clinic-Liver-Cancer(BCLC)-Stadien (A = 3, B = 19, C = 8) prospektiv untersucht und randomisiert einer Behandlung mit klassischer TACE (n = 14) (Kontrollgruppe) oder Doxorubicin eluierenden SAP-Mikrosphären (n = 16) zugeteilt. Der Doxorubicin-Plasmaspiegel wurde zu verschiedenen Zeitpunkten bestimmt. Außerdem wurden biochemische Untersuchungen durchgeführt und Nebenwirkungen entsprechend der Common Toxicity Criteria registriert. Das Tumoransprechen wurde nach 6 Wochen entsprechend der modifizierten «Response Evaluation Criteria in Solid Tumors»(RECIST)-Kriterien evaluiert. **Ergebnisse:** Mit SAP-Mikrosphären war die maximale Plasmakonzentration ($C_{\max}$) von Doxorubicin signifikant niedriger und die AUC (area under the curve) kleiner (mittlere $C_{\max}$ $495 \pm 293,9$ ng/ml, mittlere AUC $69,7 \pm 26,9$ ng/ml min), verglichen mit der Kontrollgruppe (mittlere $C_{\max}$ $1928 \pm 560,8$ ng/ml, mittlere AUC $165 \pm 32,3$ ng/ml/min; beide $p < 0,001$). Des Weiteren traten in der SAP-Mikrosphären-Gruppe weniger Nebenwirkungen 3. und 4. Grades auf. Das Tumoransprechen war in beiden Gruppen vergleichbar. **Schlussfolgerungen:** TACE mit SAP-Mikrosphären führt zu einem niedrigen Plasmaspiegel des zytotoxischen Medikaments, wodurch, verglichen mit klassischer TACE, die Toxizität herabgesetzt wird.

Introduction

Hepatocellular carcinoma (HCC) is the 6th most common malignancy worldwide and has a high mortality rate. The majority of patients with HCC are diagnosed at an advanced stage and are therefore no candidates for curative therapy. Patients with well-preserved liver function and multinodular HCC without clear extrahepatic spread are eligible for transcatheter arterial chemoembolization (TACE). TACE is widely used as primary therapy for unresectable intermediate HCC defined by the Barcelona Clinic Liver Cancer (BCLC) staging system [1]. Additionally, TACE can be used in some patients with early HCC, who have contraindications for radical therapies and in selected patients on the waiting list for a transplantation [2]. TACE yields response rates of 35–42% and prolongs life in comparison with best supportive care in selected patients with intermediate advanced HCC [3, 4].

The conventional interventional radiological technique is based on transcatheter infusion of a mixture of ethiodized oil and an antimitotic drug(s) into the hepatic artery, and can be followed by selective particulate embolization of the feeding artery if a residual contrast blush persists on completion angiography after the infusion [5]. Despite this interventional liver-directed therapy, a residual high concentration of cytotoxic agent is found in the systemic circulation, which leads to important side effects [6]. The side effects of TACE procedures include those of the chemotherapeutic agent [7, 8] and the complications of arterial embolization [9].

Recently, drug-eluting beads were developed [10, 11] which provide a potentially higher local effect of the anti-mitotic drug and less systemic side effects. To date, there are 2 different types of drug-eluting beads: polyvinyl alcohol-based (PVA) microspheres (DC Bead®, BioCompatibles Ltd., Farnham, UK) and superabsorbent polymer (SAP) microspheres (HepaSphere® (Europe) / QuadraSphere® (USA), Biosphere Medical, Rockland, MA, USA)). The first cohort studies [12, 13] have demonstrated a safe use and encouraging

clinical short-term results. A large study with DC beads on safety demonstrated a significantly reduced systemic exposure to doxorubicin [14]. The first randomized trial with DC beads showed a better tolerance compared to conventional TACE and a higher response rate in patients with Child B cirrhosis [15]. The safety and efficacy of drug-eluting SAP microspheres still remains to be determined. SAP microspheres have a number of different properties compared to DC beads in terms of loading of the beads and adaptation to the vessel wall [16].

In this study, we prospectively analyzed doxorubicin pharmacokinetics, post-procedural side effects, and short-term magnetic resonance imaging (MRI) follow-up assessment for the first time in a randomized manner.

Patients and Methods

Patients

We included patients with a confirmed diagnosis of HCC based on the European Association for the Study of the Liver (EASL) criteria [17]. Other inclusion criteria were age > 18 years, no candidate for curative treatments, Child A or B cirrhosis, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or ECOG < 3 if the restriction in status was not due to the HCC. Therefore, the patients with ECOG > 0 were not automatically considered as BCLC C. Exclusion criteria were Child C cirrhosis, extrahepatic spread, or extensive portal vein thrombosis. However, patients with an isolated segmental portal branch tumoral thrombosis but with a patent portal main branch were included [18–20].

Study Design and Objectives

This was a single-center, single-blind, randomized phase II study in which we prospectively included 30 patients with unresectable HCC (fig. 1). Patients were recruited from September 2006 to February 2009. All patients gave written informed consent, and the study design was approved by the Medical Ethics Committee of the Catholic University of Leuven, Belgium. The hypothesis of the study was that SAP microsphere TACE is superior to conventional TACE for safety. Therefore, the study objective was to demonstrate better doxorubicin pharmacokinetics (lower systemic exposure) and a decrease in adverse events. Patients were randomly assigned to receive conventional TACE with an emulsion of lipiodol, doxorubicin, and particle embolization versus doxorubicin-eluting SAP microsphere TACE. Patients received a single treatment, and we analyzed doxorubicin pharmacokinetics and side effects. For preliminary efficacy assessment, we performed a baseline MRI before treatment and a follow-up MRI after 6 weeks.

Doxorubicin Preparation and TACE Procedure

The dose of doxorubicin was determined for body surface area and bilirubin level. Patients with a bilirubin level of < 1.5 mg/dl received 75 mg/m² doxorubicin, patients with a bilirubin level of 1.5–3 mg/dl received 50 mg/m², and patients with bilirubin levels of 3–5 mg/dl received 25 mg/m². In the case of leukopenia, the dose was further adjusted.

In the case of conventional TACE, 2 syringes, each of them consisting of 5 ml NaCl 0.9%, 2.5 ml lipiodol (Laboratoire Guerbet, Aulnay-sous-Bois, France), and 1.25 mg doxorubicin (Pfizer UK, Sandwich, Kent, UK), and 1 infusion unit containing the remaining amount of doxorubicin dissolved in NaCl 0.9%, were prepared. In the case of drug-eluting SAP microspheres, 1 vial of 25 mg dry microspheres with a nominal diameter of 50–100 µm was mixed with doxorubicin (Pharmachemie, Haarlem, The Netherlands). The mix was then dissolved in 10 ml NaCl 0.9% and 10 ml of contrast medium (Visipaque®, GE Healthcare, Oslo, Norway).

Fig. 1. Study flowchart: 30 patients were included and all patients had magnetic resonance imaging before the transcatheter arterial chemoembolization (TACE) procedure; treatment was followed by biochemical analysis and toxicity assessment in all patients.

The interventional procedure was performed in a dedicated interventional angiography suite and follows the classical steps of hepatic artery catheterization and embolization [21]. After administration of local anesthesia in the right groin, a 4-French sheath was introduced in the right common femoral artery. Selective catheterization of the celiac trunk and superior mesenteric artery was followed by superselective catheterization of the right and the left hepatic artery, respectively. Depending on the randomization, an emulsion of lipiodol and doxorubicin followed by microparticle embolization or doxorubicin-eluting beads were administered by transcatheter technique under fluoroscopic guidance. After administration, a second hepatic angiography was performed in order to look for a residual vascular tumor blush. If present, additional embolization with microparticles (EmboSphere® 300–500 µm and 500–700 µm, Biosphere Medical) of this hypervascular nodule was performed. No prophylactic antibiotics were used.

Doxorubicin Pharmacokinetics

In all patients, we evaluated the pharmacokinetics of doxorubicin and determined the maximum peak ($C_{\max}$) of the doxorubicin plasma level and the area under the curve (AUC) to calculate the cumulative dose. Peripheral blood samples were taken at the end of the lipiodol chemoembolization or SAP microspheres injection and at 10, 20, 30 min, 1, 2 and 3 h after lipiodol chemoembolization or SAP microspheres injection. Plasma was separated immediately and doxorubicin levels were measured with high performance liquid chromatography and fluorescence detection (HPLC-FLUO). The method is based on the validated assay described by Kummerle et al. [22]. Briefly, following a single-step protein precipitation, the obtained supernatant was evaporated to dryness using a vacuum centrifuge. The solid residue was reconstituted in 0.5 ml water/acetonitrile (80/20). The sample was subjected to HPLC analysis onto a Waters Atlantis C18 column (5 µm, 30 × 150 mm; Waters Ireland, Dublin, Ireland) using a gradient elution acetonitrile and acetic acid (0.1%, pH 3). The analytes were detected by fluorescence detection at 480 and 560 nm.

Post Procedure Parameters and Toxicity Assessment

Patients were hospitalized for at least 1 night. Depending on the clinical status, antiemetic and/or analgesic therapy was given. Furthermore, we performed a laboratory analysis to check hematologic parameters and liver tests for all patients on the first day after the procedure and after 1 month. If patients were hospitalized longer, we continued the parameter assessment up to 3 days after their TACE.

Toxicity was assessed during the first days of follow-up and 1 month later. Adverse events were classified following the Common Toxicity Criteria (US National Cancer Institute, NCI-CTC version 2.0) [23]. Grade 3 and grade 4 adverse events were considered serious adverse events. Typical symptoms during the first days after the procedure, such as pain, mild fever, and nausea, were classified as post-embolization syndrome. If these post-embolization symptoms persisted for more than 5 days or if they evolved to grade 3 or 4 adverse events, they were reported as single entities. For biochemical adverse events, the baseline values were taken into account.

Tumor Response Assessment

Tumor response assessment was done with MRI at 6 weeks after the TACE procedure in correlation to the pretreatment MRI. The MRI was performed at 1.5 Tesla (SonataVision, Siemens, Erlangen, Germany). The imaging sequences consisted of 2 consecutive T2-weighted (T2w), half-Fourier acquisition single-shot turbo spin-echo (HASTE) sequences in the transverse plane (TR/TE = ∞/63 ms and TR/TE = ∞/360 ms, respectively) and a transverse T1-weighted (T1w) turbo-flash sequence with identical parameters of 30 slices, 6 mm slice thickness, 1.2 mm intersection gap, FoV of 31 × 38 cm², and a matrix of 220 × 256. A T1w in- and opposed-phase gradient echo (GRE) sequence was performed with 20 slices and otherwise identical geometry. Dynamic imaging was performed in all patients (eGFR > 60 ml/min/1.73m²) before and after the adminis-

tration of 0.05 mmol/kg gadolinium BOPTA (Multihance®, Bracco, Milan, Italy) in the arterial, portal venous, and venous phase, with a T1w 3D fat-suppressed GRE sequence (volumetric interpolated breath-hold examination, VIBE) with 60 contiguous slices of 4 mm, FoV 30 × 40 cm², matrix of 166 × 256, and TR/TE = 4.2/2.1 ms.

Tumor response assessment was done according to the modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The change in viable perfused tumor volume of the target lesions – as measured on the arterial phase images – before and after the TACE procedure was determined [17, 24, 25]. For further tumor response assessment, we determined the alpha-fetoprotein (AFP) levels before and after TACE.

Statistical Analysis

Statistical analysis between groups was performed with a t-test or Mann-Whitney U test when appropriate. Statistical analysis of biochemical parameters over time was performed with a paired t-test. Adverse events per group were tested with the Fisher's exact test. AUC was calculated in SlideWrite Plus, version 6.10 (Statcon, Witzenhausen, Germany). A p value < 0.05 was considered statistically significant.

Results

Patients

We included 30 patients with unresectable HCC. Of the 30 patients, 16 patients received SAP microspheres and 14 patients received conventional TACE. Baseline characteristics are presented in table 1. The only difference between the 2 groups was that patients in the SAP microsphere group were older. Most patients were male (n = 25) and the mean age was 62 years. The majority suffered from cirrhosis (73%), mainly due to viral hepatitis, alcohol abuse, or non-alcoholic fatty liver disease. Most patients had well-preserved liver function (93.3% Child-Pugh A, 6.7% Child-Pugh B) and a good performance status. At baseline, 63.3% of patients presented with multifocal disease and 26.7% showed vascular invasion of an intrahepatic portal vein branch (n = 5 in the SAP microsphere arm and n = 3 in the control arm). 13 patients (43.3%) had received previous treatment, of which 9 patients (30%) had surgical treatment, including resection, radiofrequency ablation, or transplantation.

Although the majority of patients had intermediate BCLC stage (63.3% stage B), we also included 3 patients (10%) with BCLC stage A and 8 patients (26.7%) with BCLC stage C. In the 3 patients with early stage disease, radiofrequency ablation was technically not possible. All patients with advanced disease showed vascular invasion of intrahepatic side branches of the portal vein.

Safety of the Procedure

Since the majority of patients suffered from multifocal disease, most patients underwent bilobar embolization. In the case of bilobar involvement, both lobes were injected in the same session. All patients received their full dose of doxorubicin. Because of residual tumor blush after the procedure, 8 patients in the control group needed additional bland embolization compared to only 1 patient in the SAP microsphere

Table 1. Baseline characteristics

	Total	Controls	SAP microsphere	p
Patients, n	30	14	16	
Sex (male/female), n	25/5	11/3	14/2	0.64
Age (mean ± SD), years	62.3 ± 12.6	56.6 ± 13.4	67.3 ± 9.8	0.02
Etiology (HBV/HCV/(N)ASH/others), n	8/4/14/4	4/0/8/2	4/4/6/2	0.24
Cirrhosis (yes/no/septal fibrosis), n	22/6/2	11/3/0	11/3/2	0.39
Number of nodules (1/2/1 + satellite/multifocal), n	5/1/5/19	1/1/4/8	4/0/1/11	0.18
Vascular invasion (yes/no/suspected), n	8/17/5	3/8/3	5/9/2	0.73
Portal vein invasion (yes/no/suspected/NA), n	6/18/5/1	3/8/3/0	3/10/2/1	0.73
BCLC (A/B/C), n	3/19/8	1/10/3	2/9/5	0.69
Child-Pugh (A/B), n	28/2	14/0	14/2	0.49
ECOG performance status (0/1/2), n	19/8/2	10/2/2	9/7/0	0.10
AFP (≤ 13.6/13.7–300/> 300), n	12/6/12	7/3/4	5/3/8	0.46
Albumin (mean ± SD), g/dl	39.5 ± 5.5	40.4 ± 5.1	38.7 ± 5.9	0.49
Bilirubin (mean ± SD), mg/dl	1.15 ± 0.57	1.13 ± 0.58	1.17 ± 0.58	0.83
Prothrombin time (mean ± SD), s	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	0.48
Creatinin (mean ± SD), mg/dl	0.82 ± 0.21	0.78 ± 0.19	0.86 ± 0.23	0.37
Previous treatment (surgery/systemic therapy), n	9/4	3/1	6/3	0.30

SAP = Superabsorbent polymer; SD = standard deviation; HBV = hepatitis B virus; HCV = hepatitis C virus; (N)ASH = (non-)alcoholic steatohepatitis; NA = not available; BCLC = Barcelona Clinic Liver Cancer; AFP = alpha-fetoprotein.

Fig. 2. Pharmacokinetics: **A** mean peak concentrations (C_{max} , ng/ml) in controls and superabsorbent polymer (SAP) microsphere patients; **B** mean area under the curve (AUC, ng/ml min) in controls and SAP microsphere patients; doxorubicin plasma levels were determined at different time points up to 3 h after injection.

Table 2. Doxorubicin pharmacokinetics per dose

	Mean C_{max} ± SD	Mean AUC ± SD
<i>Controls</i>		
Doxorubicin dose, mg/m ²		
Total (n = 13)	1,928.1 ± 560.8	165.0 ± 32.3
35 (n = 1)	1,482.7 ± –	105.3 ± –
50 (n = 2)	2,074.3 ± 91.4	172.8 ± 16.1
75 (n = 10)	1,943.4 ± 625.5	169.4 ± 30.5
<i>SAP microspheres</i>		
Doxorubicin dose, mg/m ²		
Total (n = 16)	495.5 ± 293.9	69.7 ± 26.9
50 (n = 6)	371.6 ± 168.9	60.0 ± 24.5
60 (n = 1)	1,082.5 ± –	98.7 ± –
75 (n = 9) ^a	513.0 ± 298.8	72.9 ± 28.2

^aData of 1 patient who received 75 mg/m² doxorubicin was missing.

C_{max} = Peak concentration; SD = standard deviation; AUC = area under the curve.

Fig. 3. Liver function: alanine transaminase (ALT) levels during follow-up; ALT was measured the first 3 days after TACE and 1 month later (solid line = mean ALT level in the control group; dashed line = mean ALT level in the SAP microsphere group; *p = 0.003, **p = 0.01, ***p = 0.04).

group, meaning that all patients in both groups had the same angiographic occlusive (= ischemic) end result: pruned tree image at the very end of the TACE procedure. In 1 patient in the control group, an intratumoral arteriportal shunt was closed during the procedure before the chemoembolization with the use of a mixture of lipiodol and n-butyl-2 cyanoacrylate (Histoacryl®, B. Braun, Melsungen, Germany). Almost all patients (n = 14, conventional TACE; n = 12, SAP microspheres) suffered from post-embolization syndrome which was treated with analgesia. One patient in the SAP microsphere group developed a cholecystitis, probably ischemically induced. Ultrasound showed thickening of the gallbladder wall and the patient recovered quickly after antibiotic treatment.

Doxorubicin Dose

The mean doxorubicin dose in the control group (68 ± 13.6 mg/m²) was similar to the SAP microsphere group

(65 ± 12.9 mg/m², $p = 0.56$). The mean total dose of doxorubicin given was 120 mg (range 74–150 mg). In the control group, the majority of patients ($n = 11$, 78.6%) received a doxorubicin dose of 75 mg/m², 2 patients received 50 mg/m², and 1 patient received unintentionally 35 mg/m² where it should have been 75 mg/m². In the SAP microsphere group, 9 patients (56.3%) received a doxorubicin dose of 75 mg/m², 1 patient (6.3%) received 60 mg/m², and 6 patients (37.5%) received 50 mg/m².

Doxorubicin Pharmacokinetics and Toxicity

In 29 patients, the pharmacokinetics of doxorubicin were analyzed. Mean $C_{\max}$ in control patients was $1,928.1 \pm 560.8$ ng/ml compared to 495.5 ± 293.9 ng/ml in SAP microsphere patients ($p < 0.001$) (fig. 2A). Also, the cumulative dose represented

by the AUC was significantly lower in the SAP microsphere patients (mean $AUC_{\text{control}} 165 \pm 32.3$ ng/ml min vs. mean $AUC_{\text{SAP}} 69.7 \pm 26.9$ ng/ml/min; $p < 0.001$) (fig. 2 B, table 2). Subanalysis with only the patients that received 75 mg/m² doxorubicin showed similar significant results.

Patients in the control group showed more pronounced liver enzyme elevations during the first days, but all patients showed recovery of their transaminases after 1 month (fig. 3). In both groups, there was some elevation of cholestatic parameters (table 3). For both the control group and the microsphere group, around 50% of the patients ($n = 7$, conventional TACE; $n = 7$, SAP microspheres) were admitted to hospital for more than 1 day. In these patients, liver enzymes on the 2nd and 3rd day after TACE were significantly more elevated in the control group.

Table 3. Biochemical analysis of all patients during the first 3 days after transcatheter arterial chemoembolization (TACE) and 1 month later

	Controls					SAP microsphere				
	Baseline	d1	d2	d3	m1	Baseline	d1	d2	d3	m1
AST, IU/l										
n	14	13	5	8	14	16	14	7	7	16
Mean	66.5	225.5	600.2	366.9	53.1	72.6	120.5	151.7	151	52.3
Median	62.5	192	466	206.5	57.5	51	88.5	134	97	43.5
SD	29.1	130.6	505.1	474.5	20.9	61	119.1	33.4	156.3	23.4
Range	24–140	56–515	203–1,464	75–1,509	9–85	27–253	45–506	118–189	68–502	32–115
p value							0.007 ^a			
ALT, IU/l										
n	14	13	5	8	14	16	14	7	7	16
Mean	52.7	128.2	553.4	355.6	39.1	44.1	59.9	126.3	107.1	38.5
Median	46.5	119	340	267	42	38	57	120	118	39.5
SD	29.2	64.2	437.9	261.3	19.7	26	29.6	55.2	41.6	16.7
Range	17–114	26–232	156–1,185	58–750	7–80	20–123	26–135	60–213	39–160	13–78
p value							0.003 ^a			
GGT, IU/l										
n	14	13	5	8	14	16	14	7	7	16
Mean	241.7	234.8	317	242.3	231.1	302.4	318.1	168.4	407.1	220.4
Median	250.5	221	248	201.5	212.5	104.5	152.5	88	286	154
SD	173.3	176.4	235.2	181.7	140.6	511.9	498.2	160.3	532.9	230.6
Range	34–750	45–713	70–316	61–613	58–537	39–2,129	46–1,966	39–470	37–1,580	39–960
p value							ns ^a			
ALKP, IU/l										
n	14	13	5	8	14	16	14	7	7	16
Mean	489.6	451.2	886.8	744.8	509.1	421.3	418.9	243.9	589.7	366.5
Median	333	283	587	447.5	385	236	231.5	216	361	301.5
SD	472.9	470	854.1	716.2	435	475.9	461.3	97	516.8	224.3
Range	159–2,002	179–1,901	230–2,318	194–2,180	203–1,871	136–1,850	142–1,672	149–421	189–1,407	200–1,124
p value							ns ^a			
Bilirubin, mg/dl										
n	14	13	5	7	13	16	15	7	7	15
Mean	1.13	1.72	2.06	1.98	1.04	1.17	1.8	2.08	3.18	1
Median	1.13	1.49	2.05	2.04	0.91	1.14	1.7	1.66	2.81	1.11
SD	0.6	0.84	0.79	1.05	0.59	0.58	0.95	1.25	1.82	0.51
Range	0.47–2.27	0.59–3.21	1.11–2.85	0.89–3.5	0.28–2.22	0.25–1.93	0.74–3.71	1.13–4.61	1.34–6.87	0.34–2.01
p value							ns ^a			
AFP, ng/ml										
n	14				12	16				14
Mean	8,069.2				1,380.1	1,803.4				193.1
Median	26.1				13.4	272.2				62.5
SD	23,342.3				3,009.9	5,409.2				239.5
Range	2.7–81,732				1.7–7,888	1.5–21,930				1.4–564.8
p value					ns ^b					0.021 ^b

^ap values for controls vs. SAP microspheres at day 1.

^bp value for AFP levels compared to baseline in patients with an elevated AFP.

SAP = Superabsorbent polymer; SD = standard deviation; d1–3 = days 1–3; m1 = 1 month; AST = aspartate transaminase; ALT = alanine transaminase; GGT = gamma glutamyl transpeptidase; ALKP = alkaline phosphatase; AFP = alpha-fetoprotein; ns = not significant.

In table 4, all adverse events are listed. There was no treatment-related mortality. Eight patients in the control group (57%) developed grade 3 or 4 adverse events, compared to 4 patients in the SAP microsphere group (25%). Significantly more patients in the control group developed grade 4 adverse events (controls $n = 5$ vs. SAP microspheres group $n = 0$; $p = 0.005$). Almost all patients ($n = 14$, conventional TACE; $n = 12$, SAP microspheres) suffered from post-embolization syndrome, as mentioned before. In the conventional TACE group, most of the patients ($n = 10$) had alopecia and more patients ($n = 14$) suffered from constitutional symptoms such as fatigue. The most remarkable difference between the 2 groups was the development of myelosuppression. In the control group, 3 patients showed grade 4 leukopenia and all of them developed neutropenic fever, whereas none of the SAP microsphere patients showed bone marrow toxicity. Furthermore, 1 patient in the control group developed decompensated liver disease with encephalopathy. Two of the 3 patients with leukopenia and the patient with decompensated liver disease had segmental portal branch involvement. However, the 5 patients in the SAP microsphere group with segmental portal branch involvement did not experience more side effects.

As described earlier, 1 patient in the SAP microsphere group developed cholecystitis. Another SAP microsphere patient had a proven streptococcus bacteriemia which resolved after intravenous antibiotics.

Efficacy Assessment

25 patients had a complete radiological assessment (12 control patients and 13 SAP microsphere patients). In 3 patients, response could not be assessed as a result of end-stage liver failure: 1 patient received a liver transplantation before the second radiological assessment and 2 patients died early after the chemoembolization. All 3 patients were initially treated with conventional TACE. The 2 patients that died had already advanced disease and were in poor general condition. However, death was not related to TACE toxicity. In another 2 patients, it was impossible to measure tumor volume because of extensive multinodularity extending into both liver lobes.

Tumor response (fig. 4) based on MRI revealed stable disease in 11 of 12 patients (92%) of the control group, and 1 patient had progressive disease (8%). In the SAP microsphere group, 10 of 13 patients had stable disease (77%) and

Table 4. Adverse events

	Conventional TACE			SAP microspheres		
	total	grade 3	grade 4	total	grade 3	grade 4
Post-embolization syndrome	14	–	–	12	–	–
Alopecia	10	–	–	–	–	–
Constitutional symptoms						
Fatigue	5	1	–	3	–	–
Anorexia	2	–	1	–	–	–
Weight loss	2	–	–	1	–	–
Fever	5	1	–	1	–	–
Myelosuppression						
Anemia	2	–	–	–	–	–
Thrombocytopenia	3	2	–	–	–	–
Leukopenia	3	–	3 ^a	–	–	–
Febrile neutropenia	3	3 ^a	–	–	–	–
Infection	–	–	–	1	1	–
Liver dysfunction						
Liver failure	1	–	1	–	–	–
AST	12	2	2	3	–	–
ALT	11	2	1	5	1	–
ALKP	3	1	–	–	–	–
GGT	2	1	–	1	–	–
Bilirubin	8	2	–	4	2	–
Gastrointestinal						
Nausea	2	–	–	1	–	–
Vomiting	–	–	–	1	–	–
Stomatitis	1	–	–	–	–	–
Cholecystitis	–	–	–	1	1	–
Dyspnea	–	–	–	1	–	–
Allergic reaction to contrast	–	–	–	1	–	–
Total	15	8		5	0	

^aThe patients with grade 4 leukopenia all developed grade 3 febrile neutropenia.

TACE = Transcatheter arterial chemoembolization; SAP = superabsorbent polymer;

AST = aspartate transaminase; ALT = alanine transaminase; ALKP = alkaline phosphatase;

GGT = gamma glutamyl transpeptidase.

Fig. 4. Tumor response: viable tumor volumes were measured at baseline and 6 weeks after TACE to determine changes, tumor response was comparable in both groups; **A** boxplots on changes in viable tumor volume; **B** percentages of patients in different response subgroups.

3 patients showed progression (23%). However, the difference was not statistically significant ($p = 0.54$).

The response rate was also reflected in the AFP measurements. Seven control patients had an elevated AFP, of which 6 patients had a complete follow-up. In the SAP microsphere group, 11 patients had an elevated AFP level, of which 9 patients had a complete follow-up. Eight out of 9 SAP microsphere patients (89%) showed a decrease in their serum levels. The decrease in AFP in these patients was significant ($p = 0.021$) (table 3). In the control group, 4 out of 6 patients (67%) showed a reduction, however, this was not significant. Two patients in the control group and 1 patient in the SAP microsphere group showed a clear increase in AFP serum levels.

Discussion

TACE is the best treatment option for selected patients with unresectable HCC [26]. The technique is based on the predominantly hepatic artery blood supply of HCC; when selective intra-arterial injection of a cytotoxic agent is performed, high concentrations can be obtained within the tumor [27]. In the conventional procedure, lipiodol is added to improve uptake of the chemotherapeutic agent within the tumor and to reduce systemic side effects [28]. The subsequent embolization using microparticles is not only effective through induction of tumor necrosis but also through preventing early washout of the chemotherapeutic drug. Nevertheless, there are still significant side effects associated with conventional TACE, which makes it unsuitable for certain cirrhotic patients with reduced liver function, since it can cause significant TACE-related morbidity or even mortality.

The presented data show that chemoembolization with use of drug-eluting SAP microspheres can reduce toxicity. Superabsorbent polymer microspheres are biocompatible, hydrophilic, acrylic copolymer microspheres which are non-resorbable and have the ability to absorb fluids up to 64 times their dry state volume [29]. SAP microspheres can be loaded with doxorubicin and oxaliplatin [12, 30]. Once injected in the tumoral feeding arteries, the working mechanism is most probably based on the slow release of doxorubicin from the beads within the liver and HCC tissue, resulting in a relatively high tumor concentration and a significantly lower systemic concentration compared to conventional TACE. The low peak concentration and the low cumulative dose lead to reduced side effects. In these series, none of the SAP microsphere patients showed grade 3 or 4 bone marrow toxicity and liver enzyme elevations were less pronounced. This reduced toxicity potentially enables consecutive treatments with TACE, even in patients with cirrhosis and reduced liver function, and therefore might lead to an overall better tumor control and potentially can be a safe treatment option for cirrhotic patients with reduced liver function.

We also assessed the short-term efficacy of TACE to control tumor progression after 6 weeks. Both techniques seem to have comparable tumor response at this time point. Clinical and radiological long-term outcome were beyond the scope of this study, but we are planning a long-term follow-up study. Moreover, a multicenter randomized controlled trial analyzing the long-term effect of SAP microspheres on tumor control and overall survival is strictly needed. Additionally, radiological response should be evaluated using the modified RECIST criteria, since these criteria take the amount of necrosis into account [17, 24]. Potentially, there might be a place for diffusion-weighted MRI in evaluating TACE efficacy, since diffusion MRI gives insight into the microstructure of the tumor and appreciates necrosis better [31].

SAP microspheres can induce necrosis since these conformable microspheres occlude very efficiently. In our study, only 1 patient in the SAP microspheres group needed additional embolization because of residual tumor blush, compared to 8 patients in the control group. However, the embolization of the feeding arteries can lead to complications. With both the conventional and the microsphere technique, almost all patients suffered from post-embolization syndrome with transient liver dysfunction, pain, and fever [32–34], and 1 patient in the SAP microsphere group additionally developed an ischemically induced cholecystitis [35]. In other studies, the development of hepatic abscesses or other ischemic injury as a consequence of embolization was described [14, 36–38]. These complications have been reported with the use of conventional TACE and other embolizing materials as well. There is no clear evidence for the routine use of prophylactic antibiotics in every patient undergoing TACE with both the conventional techniques and with drug-eluting bead technology to avoid serious complications such as liver abscesses [39]. However, some patients with hepaticojejunostomy, biliary sphincterotomy, or biliary stents have a higher risk of bacterial colonization of the biliary tree and should be prescribed prophylactic broad-spectrum antibiotics [40, 41].

Our data are in agreement with the results of a non-randomized prospective study using another type of drug-eluting beads: the DC beads. As with DC beads, the pharmacokinetic profile is superior to conventional TACE and the antitumoral effect at 6 weeks of follow-up does not seem to be inferior to conventional TACE [14]. The mid-term efficacy of the DC beads is already determined in a randomized matter and seems especially beneficial due to the possibility of repeated treatments with optimal doxorubicin doses [15]. The efficacy of SAP microspheres still has to be determined. Furthermore, a head-to-head comparison of data between SAP microspheres and DC beads would be interesting, since they have some different properties. First of all, the beads are loaded differently. With the DC bead, mainly the surface is loaded with doxorubicin, whereas SAP microsphere beads are completely loaded with doxorubicin. Theoretically, this should lead to an extended release of doxorubicin. Moreover, the

SAP microsphere adapts its shape to the vessel lumen, leading to an optimal contact between endothelium and microsphere surface. This enables the diffusion of more cytotoxic agent into the tumor and optimal embolization.

In conclusion, we demonstrated for the first time in a randomized manner that drug-eluting SAP microspheres have a superior toxicity profile compared to conventional TACE. Their ability to slowly release the cytotoxic drug into the tumor in combination with direct embolization leads to low systemic concentrations of the chemotherapeutic agent, thus

reducing adverse events. Future studies are warranted to establish its potential superiority in tumor control and overall survival compared to conventional TACE in patients with unresectable HCC.

Disclosure Statement

The authors declare that they have no conflict of interest.

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