Jpn J Radiol (2012) 30:62–68
DOI 10.1007/s11604-011-0010-2

ORIGINAL ARTICLE

Clinical trial of cisplatin-conjugated gelatin microspheres for patients with hepatocellular carcinoma

Tetsuya Toyama · Norihisa Nitta · Shinichi Ohta · Toyohiko Tanaka · Yukihiro Nagatani · Masashi Takahashi · Kiyoshi Murata · Hisanori Shiomi · Shigeyuki Naka · Yoshimasa Kurumi · Tohru Tani · Yasuhiko Tabata

Received: 14 November 2010 / Accepted: 11 August 2011 / Published online: 17 December 2011

Abstract
Purpose We report our initial experience with the transarterial chemoembolization (TACE) of unresectable hepatocellular carcinoma (HCC) using cisplatin-conjugated gelatin microspheres (Cis-GMS).
Methods and material Nineteen patients with 25 HCC nodules (mean diameter 23.0 mm) were treated by selective TACE using 50- to 100-μm Cis-GMS. Tumor necrosis and postembolization syndrome were assessed during the follow-up. The tumor response was evaluated on contrast-enhanced computed tomography images at 1 and 3 months after TACE using Cis-GMS.
Results All procedures were technically successful in all patients; following the TACE using Cis-GMS, there were no major complications, and postembolization syndrome was minimal. At the 1-month follow-up, the response rate was 12 of the 25 (48%) and 21 of the 25 (84%) HCC nodules based on RECIST 1.1 and EASL criteria, respectively; at the 3-month follow-up, it was 10 of the 25 (40%) and 14 of the 25 (56%) HCC nodules, respectively.
Conclusion Our initial experience with using Cis-GMS for TACE suggests that these drugs may represent an optimal treatment option for the treatment of advanced HCC and that the use of gelatin microspheres loaded with chemotherapeutic agents warrants further study.

Keywords Hepatocellular carcinoma · Cisplatin-conjugated gelatin microspheres · Transarterial embolization chemoembolization · Drug delivery system · Liver

T. Toyama (✉) · N. Nitta · S. Ohta · T. Tanaka · Y. Nagatani · M. Takahashi · K. Murata
Department of Radiology, Shiga University of Medical Science, Seta, Tsukinowa-cho, Otsu, Shiga, Japan
e-mail: toyama@belle.shiga-med.ac.jp

H. Shiomi · S. Naka · Y. Kurumi · T. Tani
Department of Surgery, Shiga University of Medical Science, Seta, Tsukinowa-cho, Otsu, Shiga, Japan

Y. Tabata
Institute of Frontier Medical Science, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, Japan

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and a major public healthcare problem worldwide; its incidence is also increasing [1]. Curative treatments, including surgical resection, liver transplantation, and ablation therapy, are well established, and patients with advanced or recurrent HCC are treated by surgery, percutaneous ablation, transarterial embolization (TAE)/chemoembolization (TACE), hepatic arterial infusion (HAI), and systemic chemotherapy with or without molecular targeting using agents such as the multikinase inhibitor sorafenib [2–6]. TAE/TACE is considered to be an effective approach because HCC is primarily nourished arterially [6, 7].

Various combinations of chemotherapeutic and embolic agents have been used in attempts to decrease the tumor burden [8, 9]. Novel particles, such as DC-beads (Biocompatible UK Ltd, Surrey, UK) and Hepaspheres (Biosphere Medical, Roissy, France), which enable local drug delivery have been introduced [9, 10]. These are nonabsorbable microspheres loaded with an anticancer drug. Konishi et al. [11, 12] developed gelatin microspheres impregnated with cisplatin (Cis-GMS). The dynamics of these slow-releasing microspheres in vivo have been examined, and accumulated clinical experience in the use

of gelatin microspheres as an embolization material had demonstrated that Cis-GMS may represent a novel embolization combination that facilitates slow drug release [13–15]. The drugs used in TACE performed with Cis-GMS consist of electrophysiologically bound cisplatin and gelatin molecules. This combination allows for the slow release of cisplatin due to enzymatic degradation of the gelatin [11, 12]. As such, Cis-GMS represents a new embolization material for slow drug release. Here we report our initial 1- and 3-month follow-up results on the use of Cis-GMS for TACE in patients with unresectable HCC.

Subjects and methods

Patients

This study was approved by the ethics committee of our institution. Prior informed consent was obtained from all patients. Beginning in 2006, data on each HCC patient treated with drugs for TACE were recorded prospectively. Basically, patients eligible for the TACE/Cis-GMS combination had a histological diagnosis of HCC untreatable by surgical resection, liver transplantation, or percutaneous therapy (radiofrequency or alcohol injection) for precariously located HCC nodules or organ dysfunction, including hepatic dysfunction and/or they agreed to the TACE/drug combination and refused surgical and ablation therapies. In addition, they were (1) able to provide prior written informed consent, (2) in good performance status [0–2 on the Eastern Cooperative Oncology Group (ECOG) scale], and (3) free of main portal vein thrombosis or invasion. Patients with abdominal lymph node and distant metastases were not excluded**.** Contraindications to entry included serum creatinine $\geq$2 mg/dl, serum total bilirubin $\geq$3.0 mg/dl, severe hypersplenism (based on an absolute neutrophil count of $\leq$1,500/μl or a platelet count of $\leq$25,000/μl), and any contraindication for angiography. The overall follow-up period was 12.5 months (range 6–18 months).

Materials

Gelatin microspheres were prepared according to the modified method of Tabata et al. [16–18] by glutaraldehyde crosslinking of an aqueous gelatin solution dispersed in an oil phase in the absence of a surfactant. Cisplatin was acquired from Nippon Kayaku Co. Ltd. (Tokyo, Japan). All other chemicals were of the highest commercially available purity. The gelatin microspheres, with a diameter of between 50 and 100 μm, were designed to degrade completely in extravascular tissue within 14 days. The conjugation of gelatin microspheres to cisplatin was as reported elsewhere [13]. Prior to use, the Cis-GMS were washed in doubly distilled water; they contained 18 mg platinum/1 g gelatin.

TACE procedure

The TACE/Cis-GMS procedure was performed in 19 patients (16 men and 3 women; age range 51–90 years, average age 67.1 years). Table 1 presents the patient characteristics. The procedure was carried out in one segment in four patients, in two segments in 12 patients, and in three segments in three patients. Hepatic angiography was performed by the femoral approach using a 5-Fr catheter and a 2.4-Fr microcatheter. After confirmation of the identify of the tumor-feeding arteries, the microcatheter was advanced as close to each tumor as possible. Prior to delivery, Cis-GMS (40 mg; equivalent to 0.72 mg cisplatin) in a single vial were resuspended in 5 ml of Iopamiron 370 and 5 ml of physiological saline. We confirmed that the solution containing the contrast medium persisted under fluoroscopy and that embolization was complete. Immediately after the embolization procedure, we acquired non-enhanced computed tomography (CT) images and evaluated the accumulation of the gelatin microspheres in the HCC nodules.

Evaluation of treatment efficacy

Treatment responses were assessed on CT images acquired 1 and 3 months post-treatment. The two-dimensional (2D) size of the treated HCC nodules on the first and subsequent CT images was recorded, and the response was evaluated using the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) [19, 20] and criteria promulgated by the European Association for the Study of the Liver (EASL). Both of these scales take into account the area of tumor necrosis [21].

Evaluation of adverse effects

Toxicity was evaluated 7 and 30 days after the TACE/Cis-GMS combination procedure according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) version 3.0 [22]. All other severe complications, such as liver infarction, biliary damage, liver abscess, and renal and pulmonary damage, were also recorded.

Results

Treatment efficacy

All 19 patients were effectively treated with the TACE/Cis-GMS procedure, and all were alive at the last follow-up.

Table 1 Patients enrolled in the study and hepatocellular carcinoma lesions

Patient no.	Age (years)	Sex	Etiology	Child–Pugh class	Stage	Number of target nodules	Tumor size (mm)
1	78	M	HCV	A	3	1	26
2	70	M	HCV	A	3	1	18
3	71	M	ALC	A	3	2	41, 23
4	68	M	HCV	B	3	1	28
5	75	M	HBV	A	2	1	22
6	67	M	HCV	A	3	1	50
7	72	M	HCV	A	2	1	20
8	72	F	HBV	A	1	1	10
9	60	M	HBV	B	4	1	20
10	61	F	PBC	A	1	1	17
11	58	M	HCV	A	3	4	10, 10, 10, 10
12	51	M	HCV	A	1	1	10
13	55	M	HBV	A	1	1	11
14	57	M	HCV	A	2	2	32, 23
15	72	M	N	A	2	2	20, 11
16	61	F	HBV + HCV	A	2	1	20
17	66	M	HCV	A	2	1	14
18	69	M	HCV	A	3	1	55
19	90	M	HCV	A	2	1	65

M Male, *F* female, *ALC* alcohol-induced, *PBC* primary biliary cirrhosis, *N* cryptogenic, *HCV* hepatitis C virus, *HBV* hepatitis B virus

We encountered no instances of microcatheter occlusion or disruption due to impaction with the gelatin microspheres. The mean diameter of the targeted HCC nodules was 23.0 (range 10–65) mm. The mean Cis-GMS dose was 48.0 mg per patient (range 10–165 mg; equivalent to 0.18–2.97 mg cisplatin). Based on the RECIST 1.1 criteria, at 1 month post-treatment five of the 25 nodules showed a complete response (CR), seven showed a partial response (PR), 12 showed stable disease (SD), and one was recorded as progressive disease (PD); based on EASL criteria, the outcomes were 14 CR, seven PR, three SD, and one PD (Figs. 1, 2). The treatment responses after 3 months according to RECIST 1.1 criteria were six CR, four PR, 14 SD, and one PD; according to EASL criteria, the outcomes were 13 CR, three PR, eight SD, and one PD. At 1 and 3 months, the response rate was 48% according to RECIST and 84% by EASL, and 40% by RECIST 1.1 and 64% by EASL, respectively (Table 2).

Adverse effects

Of the 19 patients receiving TACE/Cis-GMS therapy, 17 (89.5%) did not experience pain during the procedure. No patient manifested severe post-treatment side-effects. Adverse events occurred in 11 patients (57.9%), with six patients (31.6%) showing fever and ten patients (52.6%) complaining of abdominal pain (Table 3). All symptoms resolved within a few days of the procedure with or without medication. No patient suffered bone marrow toxicity. Although alanine aminotransferase (ALT) was higher 3–7 days post-TACE (mean 92.8 U/l, range 21–226 U/l) than at baseline (mean 64.2 U/L, range 21–161 U/l), no patient experienced relevant deterioration (Table 4).

Discussion

Our results demonstrate the feasibility and safety of Cis-GMS for TACE. Our technical success rate was 100%, and we encountered no major complications. At the 1-month follow-up, the objective response rate was 48 and 84% according to RECIST 1.1 and EASL criteria, respectively, which is higher than that for conventional TACE [23]. At the 3-month follow-up, it had remained high, at 40% (RECIST 1.1) and 64% (EASL) (Table 2). We calculated the gelatin index to predict treatment responses based on the results on the unenhanced CT scans performed immediately post-embolization. Although patients with CR, PR, and SD were included as grade 5, higher grades tended to indicate a better treatment response.

TACE in the setting of no vascular invasion or extrahepatic tumor spread can increase the survival interval by 1–2 years, which is a notable survival benefit to HCC patients [6, 23, 24]. However, there is currently no standard method to address HCC by TACE; embolization materials and chemotherapeutic agents vary from center to center.

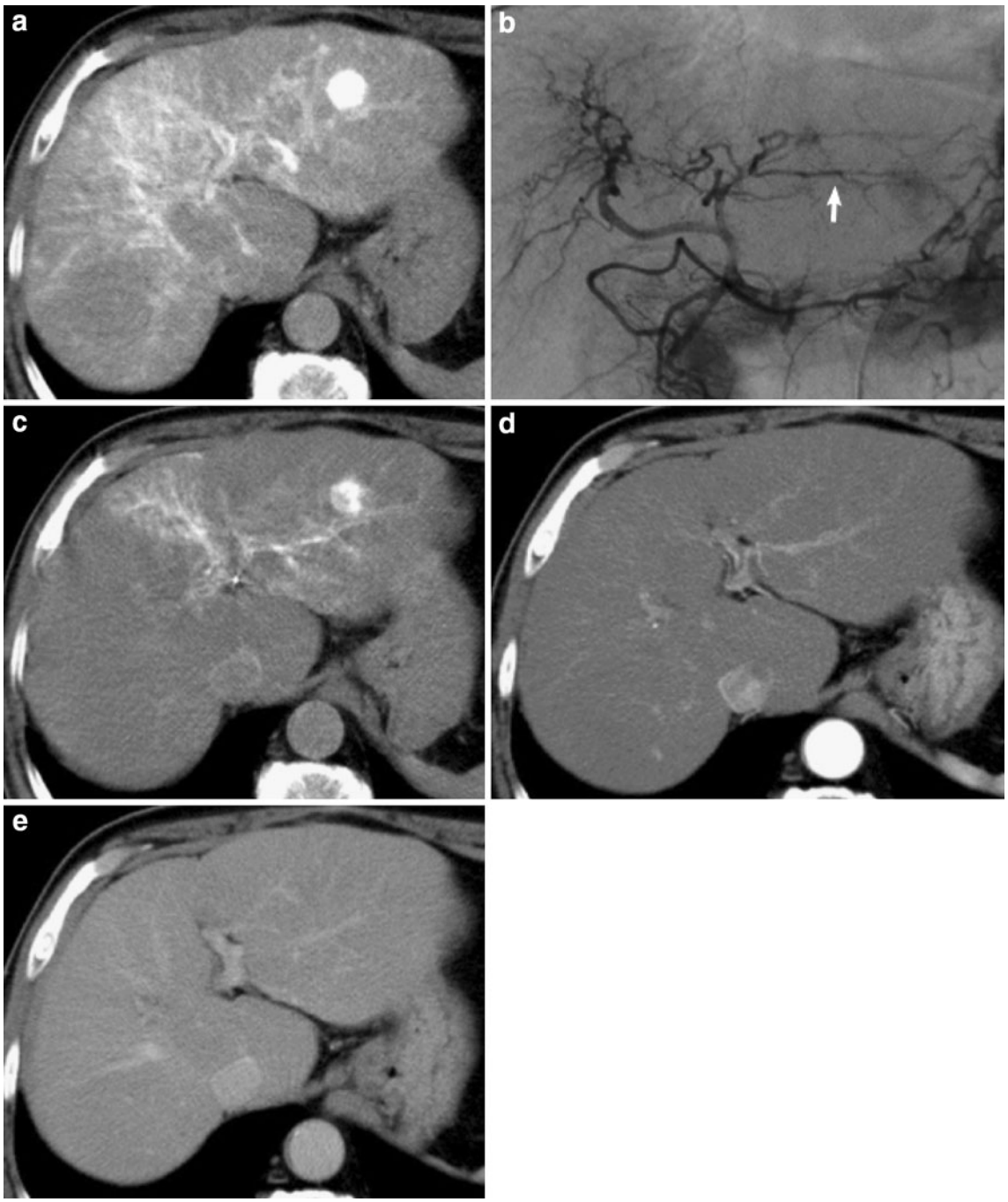

Fig. 1 A 72-year-old man with two hepatocellular carcinoma (HCC) nodules in the lateral segment. **a** Computed hepatic arteriography (CTA) showing a targeted HCC nodule of the drugs used in transarterial embolization chemoembolization (TACE). **b** Diagnostic angiograph depicting the HCC nodule. The TACE/Cis-GMS (gelatin microspheres impregnated with cisplatin) was selectively performed with the aid of a microcatheter (*arrow*). **c** Plain CT performed immediately after the TACE/Cis-GMS procedure showing contrast medium accumulation in the treated nodule. **d** Contrast enhanced CT (CECT) (arterial phase) performed at the 1-month follow-up shows a complete tumor response with disappearance of the treated tumor. **e** CECT (venous phase) at the 1-month follow-up

Of the many embolitic materials used clinically, such as gelatin sponges, lipiodol, and polyvinyl alcohol, we suggest that gelatin microspheres are optimal embolization materials. First, they are completely spherical, solid, and more dense than conventional gelatin sponges. Second, their degradation can be regulated by adjusting the level of cross-linkage, to about 3 days post-treatment to almost undegradable [25]. Third, particle size can be specifically determined to any size between 10 and 1,000 μm. Moreover, gelatin microspheres can be impregnated with

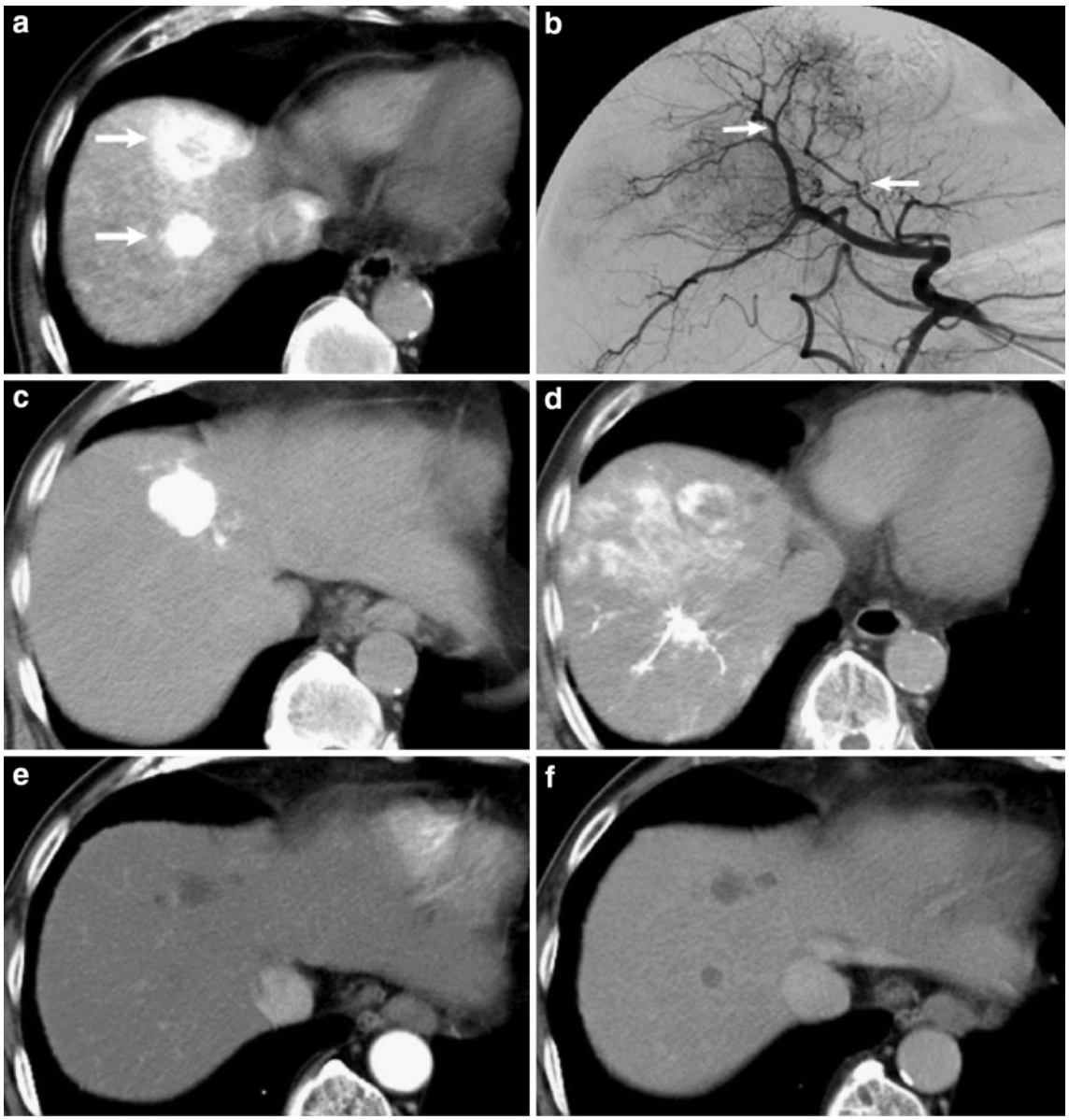

Fig. 2 A 71-year-old man with multiple HCC nodules. **a** Hepatic CTA showing target nodules (*arrows*) for the TACE/Cis-GMS in the anterior and medial segments. **b** The TACE/Cis-GMS procedure was successful at sectors A4 and A8 (*arrows*). **c**, **d** Plain CT performed immediately after the TACE/Cis-GMS procedure showing sufficient contrast accumulation in the treated nodule in the medial segment and insufficient accumulation in the anterior segment. **e**, **f** Contrast enhanced CT (CECT) (arterial and venous phase) performed at the 1-month follow-up showing nonenhanced tumors of reduced size, indicating a partial tumor response

cisplatin, allowing the slow release of this drug not only through the enzymatic degradation of gelatin but through the electrophysiological bond between cisplatin and gelatin [9, 14]. We used cisplatin for TACE because it is widely employed throughout the world to treat various types of cancer; in addition, it is the only anticancer drug that has been found to be better than doxorubicin in a nonrandomized trial [26]. In addition, using an in vivo model, Ohta et al. reported that Cis-GMS offers the advantage of increased anti-cancer effects with reduced toxicity compared to cisplatin alone [15].

The low rate of treatment-related complications and the absence of procedure-related deaths demonstrate the safety of our treatment. During the first month post-treatment, there were no severe side effects due to the Cis-GMS/TACE procedure, such as liver failure, liver abscess, biliary damage, gastrointestinal hemorrhage, and renal failure. Only 26–52% of patients undergoing the procedure manifested post-chemoembolization syndrome consisting of transient abdominal pain, fever, nausea, and vomiting (Table 3). Fever (temperature >38°C) was recorded in 31.5% of our patients during the first week after the

Table 2 Tumor response assessed by computed tomography imaging at the 1- and 3-month follow-up according to the RECIST and EASL criteria

Results	Post-treatment 1 month		Post-treatment 3 months	
	RECIST	EASL	RECIST	EASL
CR	5 (20%)	14 (56%)	6 (24%)	13 (52%)
PR	7 (28%)	7 (28%)	4 (16%)	3 (12%)
SD	12 (48%)	3 (12%)	14 (56%)	8 (32%)
PD	1 (4%)	1 (4%)	1 (4%)	1 (4%)
Total	25	25	25	25
OR	12 (48%)	21 (84%)	10 (40%)	16 (64%)

RECIST Response Evaluation Criteria in Solid Tumors criteria, *EASL* European Association for the Study of the Liver criteria, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *OR* objective response

Table 3 Adverse events after the Cis-GMS/TACE procedure according to CTCAE v3.0

Symptom	Grade[a] 0/1/2/3/4/5
Abdominal pain	9/8/2/0/0/0
Fever	13/5/1/0/0/0
Nausea/vomit	17/2/0/0/0/0

Cis-GMS/TACE Gelatin microspheres impregnated with cisplatin/transarterial embolization chemoembolization, *CTCAE v.3.0* Common Terminology Criteria for Adverse Events v3.0 (available at: http://ctep.cancer.gov)

[a] Data are given as the number of patients with the specific grade of symptom

Table 4 Changes in serum liver function parameters after the Cis-GMS/TACE procedure

Item	Baseline	Post-treatment 1–7 days	Post-treatment 1 month
ALT (U/l)	64.2 (21–161)	92.8 (21–226)	53.2 (17–139)
Bilirubin (mg/dl)	1.1 (0.5–2.6)	1.7 (0.6–3.3)	0.9 (0.3–2.0)
Albumin (g/dl)	3.7 (2.2–4.5)	3.3 (2.1–4.0)	3.7 (2.7–4.9)

Data are presented as the median level, with the range given in parenthesis

ALT Alanine aminotransferase

procedure, which is a much lower rate than that associated with conventional TACE. With respect to liver enzyme elevation, one patient manifested a transient increase during the first week and a return to pre-embolization levels thereafter (Table 4). We suggest that a further advantage of our Cis-GMS/TACE combination over conventional TACE is that fewer patients complained of abdominal pain.

The amount of cisplatin impregnated in Cis-GMS was as low as 18 mg per 1 g gelatin. The mean drug dose used in our study was very low, approximately 0.9 mg cisplatin. We have not yet been able to determine to what degree cisplatin augmented the effect of embolization. In previous basic experiments, tumor growth suppression was better with Cis-GMS/TACE than with GMS alone (84.5 vs. 241.4%) [15], suggesting that minute amounts of cisplatin augmented the antitumor effect of embolization. This antitumor effect is insufficient, and efforts are underway in our laboratory to determine the intratumoral concentration of cisplatin over time. Theoretically, as much as 45 mg of cisplatin can be impregnated in 1 g of gelatin, and it is possible to instill cisplatin close to the theoretically calculated highest concentration by impregnating gelatin microspheres in a cisplatin solution at as high a concentration as possible and at a high temperature. We confirmed that it is possible to impregnate 30 mg of cisplatin in 1 g of gelatin, but higher concentrations will require further trials and other devices. Nonetheless, our results suggest that the Cis-GMS/TACE is a safe, well-tolerated, and useful treatment modality for unresectable HCC. Further studies are needed to verify not only the efficacy of our Cis-GMS/TACE procedure, but also its role in maintaining the patients' quality of life.

In conclusion, despite the limited number of patients in our study, our initial experience suggests that the the Cis-GMS/TACE procedure described here may represent an optimal treatment option in patients with advanced HCC. A more extensive evaluation of the use of gelatin microspheres loaded with different chemotherapeutic agents is underway.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer. 2001;94:153–6.
2. El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology. 2008;134:1752–63.
3. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208–36.
4. Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis. 2005;25:181–200.
5. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359:378–90.
6. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. Hepatology. 2003;37:429–42.
7. Gunven P. Liver embolizations in oncology: a review. Part I. Arterial (chemo)embolizations. Med Oncol. 2008;25:1–11.
8. Brown DB, Pilgram TK, Darcy MD, Fundakowski CE, Lisker-Melman M, Chapman WC, et al. Hepatic arterial chemoembolization for hepatocellular carcinoma: comparison of survival rates with different embolic agents. J Vasc Interv Radiol. 2005;16:1661–6.

9. Grosso M, Vignali C, Quaretti P, Nicolini A, Melchiorre F, Gallarato G, et al. Transarterial chemoembolization for hepatocellular carcinoma with drug-eluting microspheres: preliminary results from an Italian multicentre study. Cardiovasc Intervent Radiol. 2008;31:1141–9.
10. Malagari K, Alexopoulou E, Chatzimichail K, Hall B, Koskinas J, Ryan S, et al. Transcatheter chemoembolization in the treatment of HCC in patients not eligible for curative treatments: midterm results of doxorubicin-loaded DC bead. Abdom Imaging. 2008;33:512–9.
11. Konishi M, Tabata Y, Kariya M, Hosseinkhani H, Suzuki A, Fukuhara K, et al. In vivo anti-tumor effect of dual release of cisplatin and adriamycin from biodegradable gelatin hydrogel. J Control Release. 2005;103:7–19.
12. Konishi M, Tabata Y, Kariya M, Suzuki A, Mandai M, Nanbu K, et al. In vivo anti-tumor effect through the controlled release of cisplatin from biodegradable gelatin hydrogel. J Control Release. 2003;92:301–13.
13. Ohta S, Nitta N, Sonoda A, Seko A, Tanaka T, Takahashi M, et al. Cisplatin-conjugated degradable gelatin microspheres: fundamental study in vitro. Br J Radiol. 2009;82:380–5.
14. Nitta N, Ohta S, Tanaka T, Takazakura R, Toyama T, Sonoda A, et al. An initial clinical study on the efficacy of cisplatin-releasing gelatin microspheres for metastatic liver tumors. Eur J Radiol. 2009;71:519–26.
15. Ohta S, Nitta N, Sonoda A, Seko A, Tanaka T, Takahashi M, et al. Prolonged local persistence of cisplatin-loaded gelatin microspheres and their chemoembolic anti-cancer effect in rabbits. Eur J Radiol. 2009;72:534–40.
16. Tabata Y, Hijikata S, Muniruzzaman M, Ikada Y. Neovascularization effect of biodegradable gelatin microspheres incorporating basic fibroblast growth factor. J Biomater Sci Polym Ed. 1999;10:79–94.
17. Tabata Y, Uno K, Ikada Y, Muramatsu S. Potentiation of antitumor activity of macrophages by recombinant interferon alpha A/D contained in gelatin microspheres. Jpn J Cancer Res. 1988;79:636–46.
18. Hong L, Miyamoto S, Hashimoto N, Tabata Y. Synergistic effect of gelatin microspheres incorporating TGF-beta1 and a physical barrier for fibrous tissue infiltration on skull bone formation. J Biomater Sci Polym Ed. 2000;11:1357–69.
19. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000;92:205–16.
20. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228–47.
21. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the study of the liver. J Hepatol. 2001;35:421–30.
22. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol. 2003;13:176–81.
23. Llovet JM, Real MI, Montana X, Planas R, Coll S, Aponte J, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet. 2002;359:1734–9.
24. Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology. 2002;35:1164–71.
25. Ozeki M, Tabata Y. In vivo degradability of hydrogels prepared from different gelatins by various cross-linking methods. J Biomater Sci Polym Ed. 2005;16:549–56.
26. Ono Y, Yoshimasu T, Ashikaga R, Inoue M, Shindou H, Fuji K, et al. Long-term results of lipiodol-transcatheter arterial embolization with cisplatin or doxorubicin for unresectable hepatocellular carcinoma. Am J Clin Oncol. 2000;23:564–8.