We thank Dr. Lo for his comments on our paper "Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection" (1). We appreciate his concerns. In this study, we calculated the propensity score (PS) estimated for all patients treated with entecavir (ETV). Variables used in the model did not include alcohol consumption. We recalculated a PS including alcohol consumption and conducted PS matching using this revised PS. The revised PS matching process resulted in a matched sample size that consisted of 316 patients in each group. There was no difference in baseline characteristics between ETV and control groups, as was the case in our original cohort. We compared the incidence of hepatocellular carcinoma (HCC) with ETV vs. untreated control groups. The result was similar to our original report. Cumulative HCC incidence rates were significantly lower in ETV group than in the control group (Figure). Seven factors were associated with HCC development as determined by Cox proportional hazard regression at 5-year: age, alcohol consumption, cirrhosis, hepatitis B e antigen (HBeAg), platelet count, and ETV treatment. The multivariate adjusted hazard ratio of ETV treatment was 0.27 (95% confidence interval; 0.12-0.62). These results were re-analyzed using the update data until Jun. 2013. Median follow-up duration in the PS matched ETV group were extended to 4.5 years (original: 3.3 yrs).

Because prothrombin time was lacking in our pooled data, we could not present cirrhotic severity in this study. This study end point is HCC incidence over 1 year after the start of observation. Patients who developed HCC within 1 year were included in those with follow-up duration < 1 year. Therefore, patients with HCC or suspicion of HCC on enrollment were excluded from this study. It is impossible to avoid any bias because this study is a retrospective cohort study. We think that the method of this study is the next best method of ensuring that the experimental and control groups are similar in the absence of randomization as Prof. Sherman described (2). Our findings are consistent with those recently published from Hong Kong (3). A recent meta-analysis, that included our results, demonstrated a reduction in HCC incidence with oral antiviral agents (4). The risk reduction of HCC by nucleos(t)ide analogues needs to be confirmed in other long-term studies of ETV or tenofovir with high antiviral potency.

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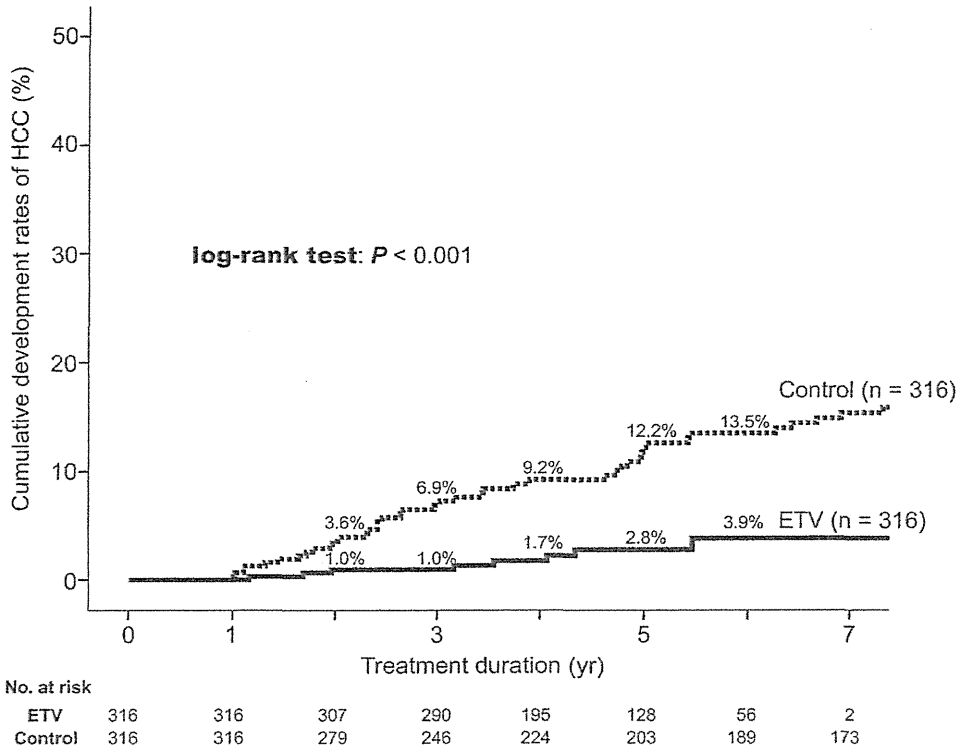
Figure legend

Figure. Comparison of HCC cumulative incidence rates between the entecavir-treated group and the non-treated control group after propensity score matching (alcohol consumption included) using update data until Jun. 2013. The long-rank test revealed a statistically significant difference between the ETV and the control group in the incidence of HCC at 5 years time.



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Hepatology Research

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Short Communication

Potential of a no-touch pincer ablation procedure for small hepatocellular carcinoma that uses a multipolar radiofrequency ablation system: An experimental animal study

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Aim: Treatment of hepatocellular carcinoma located on the liver surface is frequently difficult because direct puncture of the tumor must be avoided during needle insertion. The aim of this study was to investigate the utility of a no-touch pincer ablation procedure that uses a multipolar radiofrequency ablation (RFA) system for a tumor located on the liver surface.

Methods: The experimental animals were three pigs, and RFA was performed with two internally cooled bipolar electrodes. Three ablative procedures were compared: linear insertion at regular 13-mm intervals (pattern 1; virtual target tumor size, <10 mm); fan-shape insertion, maximum interval 20 mm (pattern 2; virtual target tumor size, <15 mm); and 25 mm (pattern 3; virtual target tumor size, <20 mm). All electrodes were inserted at a 30-mm depth. For patterns 1 and 2, ablation was performed on three other parts of the liver, and for pattern 3, ablation was performed on two other parts.

Results: For the median transverse and longitudinal diameter to the shaft, with the pattern 1 procedure, the ablative areas were 32 mm × 30 mm, and with the pattern 2 procedure, the ablative areas were 27 mm × 30 mm with carbonization of the liver surface. In contrast, with the pattern 3 procedure, the ablative areas were 45 mm × 26 mm; however, the ablative margin did not reach the surface, and carbonization was not apparent.

Conclusion: The no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small nodules that protrude from the liver surface.

Key words: bipolar, hepatocellular carcinoma, multipolar, no-touch ablation, radiofrequency ablation

INTRODUCTION

AMONG THE AVAILABLE treatment options for hepatocellular carcinoma (HCC), surgical resection is generally considered to be a local eradication method that can provide a satisfactory long-term outcome.[1–8] Recent advances in imaging procedures have led to increased detection of early-stage HCC and to improved survival due to the increased identification of patients in whom hepatic resection is possible.[9,10]

For patients who are not eligible for surgery for various reasons (e.g. lack of sufficient liver function for surgical resection), percutaneous local therapy is a viable therapeutic option. Several local ablation therapies are available, including percutaneous ethanol injection, percutaneous acetic acid injection, cryotherapy, percutaneous microwave coagulation therapy and radiofrequency ablation (RFA). In addition to surgical resection, local ablation therapies, particularly RFA, are considered to be local eradication methods for HCC that can provide good long-term outcomes.[11] Therefore, in recent years, RFA has become a widely used option for the primary treatment of small-size HCC. However, we often

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encounter cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver surface. In addition, a relationship between percutaneous local approaches to HCC (including tumor biopsy) and tumor seeding has been reported previously,[12,13] and with regard to the risk of treatment-related tumor seeding, the following risk factors have been reported: tumor size, tumor location (subcapsular portion), α-fetoprotein level, tumor stage and histopathological grade.[14,15] Therefore, a no-touch approach to local therapy may be considered an ideal treatment method for HCC.

Recently, a multipolar ablation system became available. Until now, in Japan, monopolar electrodes have typically been used, and the present cases are usually treated with some technical arrangement. For example, in the case of using a multi-tined expandable electrode, after obliquely inserting the electrode to avoid direct puncture of the target tumor, the multi needles are expanded toward the target tumor via non-tumor tissue, or in the case of using an internally cooled electrode, multiple insertions are made to avoid direct puncture of the target tumor, and RFA is performed after each insertion. However, these methods do not always provide enough of a treatment effect due to the influence of uncertain treatment procedures and natural, direct puncture to a tumor is indispensable. In contrast, a multipolar ablation system that uses an internally cooled bipolar electrode can combine the use of one to three electrodes at the same treatment session. When three electrodes are used, this system can treat large tumors; however, in the case of small tumors, it is not really necessary to use three electrodes to treat the target tumor. In addition, when we used this multipolar ablation system, usually electrodes were inserted into HCC, but in theory, this system can use no-touch ablation. However, to our knowledge, there are no technical reports that describe a non-direct punctual RFA method that uses a bipolar ablation system for HCC located on the liver surface. In this experimental animal study, we assumed that a small (<20 mm) HCC nodule protruded from the liver surface, and examined proper pincer ablation methods using two internally cooled bipolar electrodes.

METHODS

Summary of experimental procedures

WE USED A bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH [Telto, Germany]) and two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). RFA was applied in the livers of three normal female domestic pigs (each pig's weight was 60 kg) under general anesthesia maintained until killing. The abdomen was opened so that the needle could be inserted under an ultrasonography (US) guide directly into the upper region of the liver where the thickness was larger than 3.5 cm. As a pig liver consists of five thin lobes, RFA sessions were performed two to three times in each liver for evaluation of the "no-touch pincer ablation procedure". After the experiments were completed, the animal was killed, and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Toranomon Hospital.

Protocol of the no-touch pincer ablation procedure

We used a bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH), and all ablation procedures were performed with two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). Internal liquid circulation of the applicator enables the efficiency of coagulation to be increased. The delivery rate was set to 30 mL/min of saline solution at room temperature. The liquid flow was provided by a triple peristaltic pump, which is part of the system. The electrodes were operated by a power control unit working at 470 kHz and providing a maximum output power of 250 W (OLYMPUS Winter & Ibe GmbH). In this study, output power and total energy in each session were fixed at 60 W and 25 kJ, respectively, according to the dosimetry table for the bipolar RFA system (CelonPOWER System; OLYMPUS Winter & Ibe GmbH).

With regard to the ablation protocol, we performed the following three types of ablation procedure: linear insertion, at regular 13-mm intervals (pattern 1); fan-shape insertion, maximum interval of 20 mm (pattern 2); and 25 mm (pattern 3). All electrodes were inserted at a 30-mm depth from the liver surface under a US guide (Fig. 1). Each ablation procedure was performed for the following number of times: pattern 1, three sessions; pattern 2, three sessions; and pattern 3, two sessions. In this study, we assumed that the size of the virtual target tumor was less than 10 mm in pattern 1,

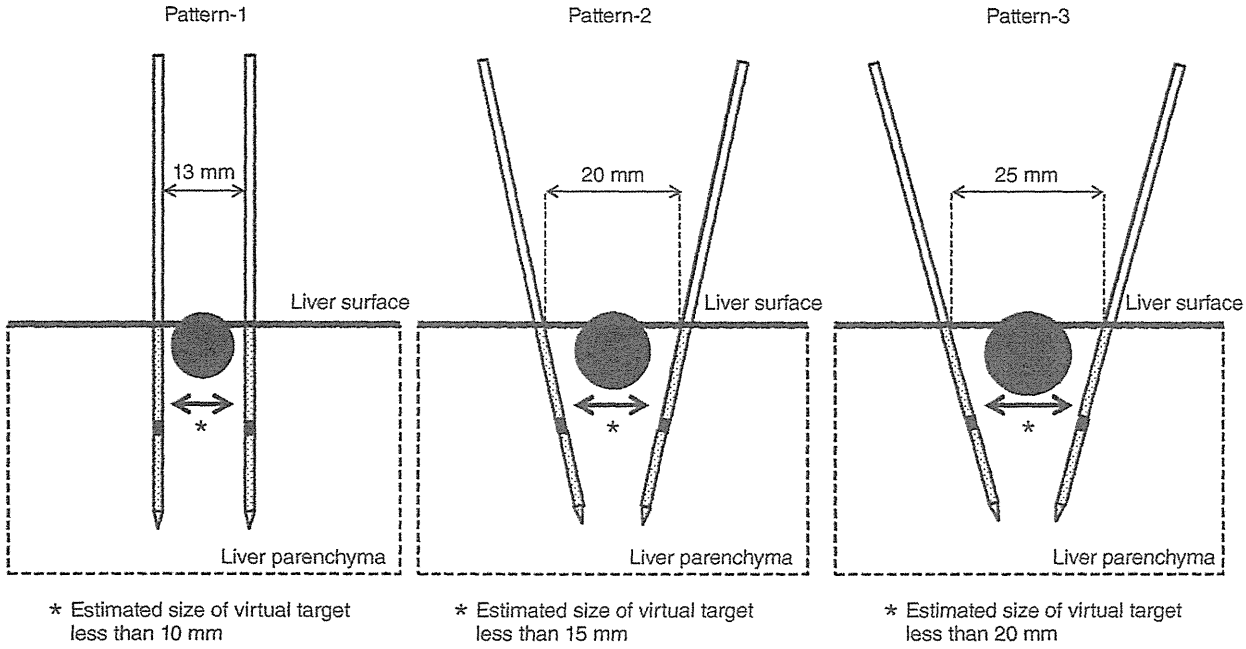


Figure 1 Protocol for a pincer ablation procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface.

less than 15 mm in pattern 2 and less than 20 mm in pattern 3.

Measurement procedure of the ablative margin

After completion of the experiments, the animal was killed and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone).

Statistical analysis

The size of the ablated zone and the duration of ablation were compared among the three groups with the Kruskal–Wallis test. All values are expressed as medians. A *P*-value of less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Features of the no-touch pincer ablation procedure

THE THREE TYPES of pincer ablation procedure applied to the pig liver were performed in the area shown in Figure 2(a).

Table 1 summarizes the features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface.

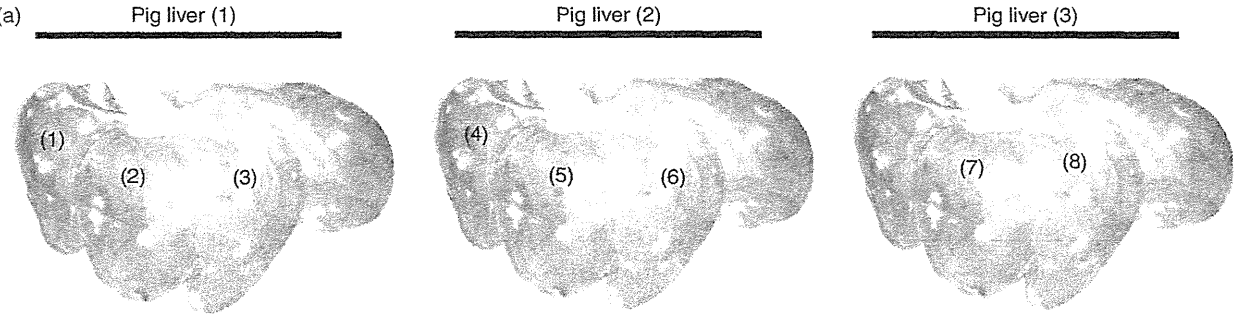
In the median (range) transverse and longitudinal diameter to the shaft, ablative areas were: pattern 1, 32 (27–35) mm × 30 (30–35) mm; pattern 2, 27 (25–35) mm × 30 (30–32) mm; and pattern 3, 45 (40–50) × 26 (25–27) mm. There were no significant differences in the size of each ablative area among the three ablation procedures. However, with the pattern 3 procedure, the transverse diameter to the shaft was larger than with the other procedures, and as a result, the ablative form was flatter. On the other hand, patterns 1 and 2 acquired sufficient ablative areas that covered the liver surface with carbonization of the surface; however, with pattern 3, the ablative areas did not reach the liver surface, and carbonization of the liver surface was not apparent (Fig. 2b–d).

In addition, there were no significant differences among ablation procedures in the duration of ablative time.

DISCUSSION

WE OFTEN ENCOUNTER cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver

(a)



Ablative pattern	*Location*
Pattern-1: linear insertion, at regular 13-mm intervals	(1)(2)(5)
Pattern-2: fan-shape insertion, maximum interval, 20 mm	(3)(4)(7)
Pattern-3: fan-shape insertion, maximum interval, 25 mm	(6)(8)

(b) Representative ablative images: pattern-1

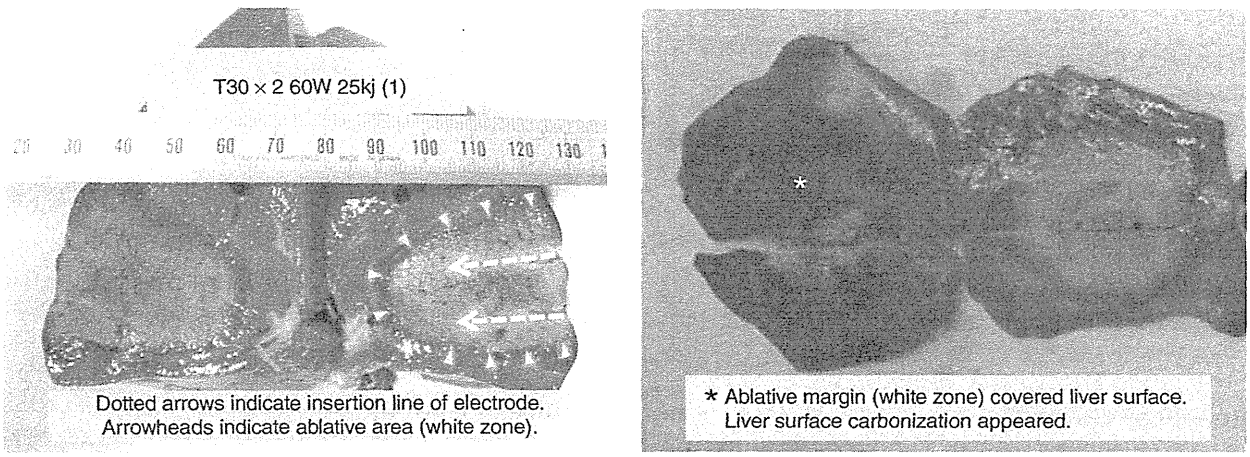
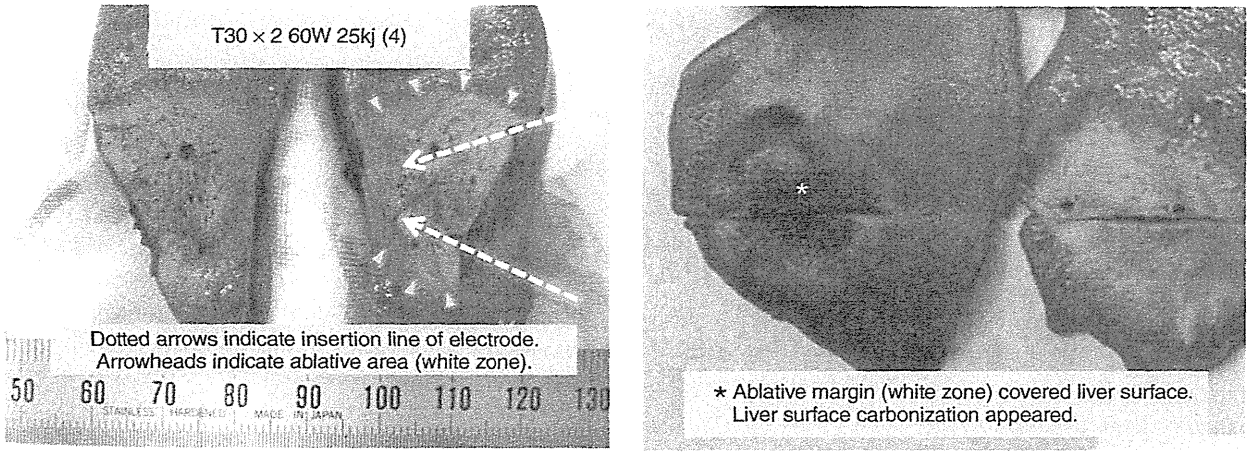


Figure 2 (a) Schema of the ablative areas of each pincer ablation procedure in the three pig livers. (b) One of the ablative shapes and the margin achieved with the pattern 1 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we inserted the electrodes linearly (maximum interval for each electrode was 13 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (c) One of the ablative shapes and the margin achieved with the pattern 2 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 20 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (d) Ablative shape and margin achieved with the Pattern 3 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 25 mm). The ablative area close to the liver surface was larger than with the other procedures. However, the ablative margin did not cover the liver surface, and carbonization of the liver surface was not apparent.

(c) Representative ablative images: pattern-2



(d) Representative ablative images: pattern-3

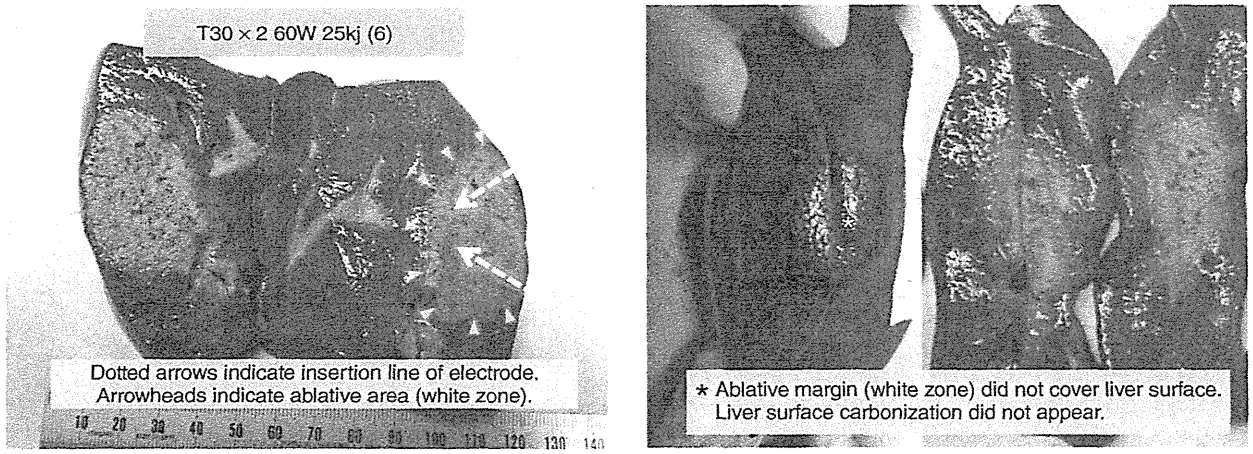


Figure 2 *Continued*

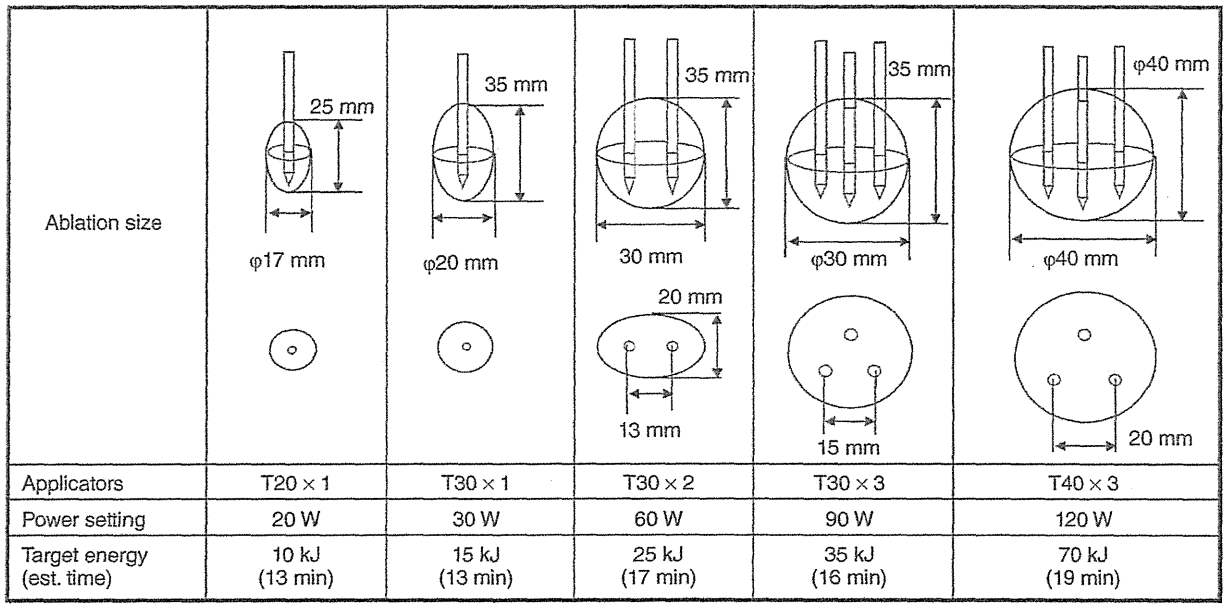
surface. In these situations, a multipolar ablation system that uses internally cooled bipolar electrodes may be suitable for treatment. With a multipolar ablation system, we can combine the use of one to three electrodes at the same treatment session, and when three electrodes are used, this system can treat a large tumor. However, in the case of small tumors (<20 mm), it is not really necessary to use three electrodes for treatment of the target tumor. However, in the dosimetry table of this bipolar system in Figure 3, which was made from previously reported early clinical data[16] and basic analysis, when two internally cooled bipolar electrodes are used (30 mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH), the recommended interval of each electrode in this system was 13 mm. With this regulation, we can treat only small tumors (<13 mm) when we perfume no-touch pincer ablation using two electrodes. Therefore, in this study we assumed a virtual target tumor with a tumor diameter less than 20 mm, and investigated the efficacy of a no-touch pincer ablation procedure and the maximum size of the tumor using two internally cooled bipolar electrodes for nodules that

Table 1 Features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface

	Pattern 1			Pattern 2			Pattern 3		*P*
	1	2	3	1	2	3	1	2	
Duration	13′46″	13′16″	12′58″	14′38″	13′50″	13′30″	13′05″	12′40″	$P = 0.151$
Ablated area									
Transverse diameter, mm	27	35	32	25	27	35	45	40	$P = 0.113$
Longitudinal length, mm	35	30	30	32	30	30	27	25	$P = 0.102$
Ablated area covered liver surface	Yes	Yes	Yes	Yes	Yes	Yes	No	No	
Liver surface carbonization appeared	Yes	Yes	Yes	Yes	Yes	Yes	No	No	

protrude from the liver surface. In addition, we investigated only the fan-shape insertion method at a maximum interval of 20–25 mm. The reason for this is that in an actual RFA procedure, it is occasionally difficult to insert two electrodes in the same intercostal space for slightly large nodules that protrude from the liver surface; therefore, in this study, we examined a fan-shape ablation method that assumed two different intercostal approaches. Our results showed that with the pattern 3 treatment procedure, we could not acquire a sufficient ablative margin to the side of the liver surface. From these results, tumors of 20 mm or more may not be suitable for a no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

In contrast, with the pattern 1 and 2 treatment procedures, we acquired a sufficient ablative margin to the side of the liver surface with carbonization of the liver



- The data are based on Frericks et al., Radiology (2005) 237: 1056–1062. The reported average efficacy was ~0.5 millitre ablation volume per kilojoule.
  From these data, the required energy for an ablation sphere or ellipsoid of given diameter was calculated.
- The application of blood flow interruption (e.g. Pringle's manoeuvre, embolization) allows for a significant reduction of the target energy.

Disclaimer: this dosimetry table does not replace the monitoring of actual ablation sizes. The ablation diameters are approximations based on statistical data; they are not guaranteed for individual clinical cases. Ablation size and shape as well as the procedure time may significantly vary due to tumor physiology and vascular structure. A deviation from the recommended applicator distances may also have an impact on the ablation dimensions.

Figure 3 Dosimetry table for the CelonPOWER system (in Japan).

surface. These results may indicate that tumors of less than 15 mm are candidates for the no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

Finally, this experimental animal study had some limitations. First, the number of animals was very small, and the target tumor was a virtual tumor. Second, an additional examination regarding a no-touch linear insertion procedure for maximum intervals of 20 mm and 25 mm for each electrode was not enforced. Third, we could not investigate the same fan-shape ablation procedure using monopolar RFA in this study, because we assumed it would be too difficult to carry out a two-step insertion method using a monopolar electrode under the influence of a first ablation for nodules that protrude from the liver surface. Fourth, we could not investigate the pathological changes in the ablative area in this study. Therefore, with only these study results, it may not be possible to draw conclusions regarding the utility of the fan-shape insertion method using a bipolar RFA device. To solve these problems, we must carry out an additional large-scale study that includes pathological examination in the near future.

Finally, to summarize the points to be noted at the time of performing the pincer ablation procedure, first, we should insert the needle carefully under US guidance, because in this procedure, measuring the distance of the needle tip from the liver surface and the two needle intervals on the liver surface correctly is the most important point.

Second, with this procedure, we should pay attention to the risk of thermal damage to the visceral peritoneum. Therefore, if possible, thermal protection using measures such as artificial ascites should be considered.

Third, in this study, we did not observe a portal or hepatic vein thrombus in the ablative area. However, this study was performed mainly in the vicinity of the liver surface, and usually this area does not include large vessels. Therefore, we need to use caution as with monopolar ablation when we ablate near large vessels.

In conclusion, the no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small HCC tumors that protrude from the liver surface.

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ORIGINAL ARTICLE

Gut and Liver, Vol. 7, No. 5, September 2013, pp. 576-584

What Is the Most Effective Drug Delivery System for Cisplatin during the Treatment of Hepatic Tumors with Single-Session Transcatheter Chemotherapy? A Pilot Study

Yusuke Kawamura, Kenji Ikeda, Taito Fukushima, Yuya Seko, Tasuku Hara, Hitomi Sezaki, Tetsuya Hosaka, Norio Akuta, Masahiro Kobayashi, Satoshi Saitoh, Fumitaka Suzuki, Yoshiyuki Suzuki, Yasuji Arase, and Hiromitsu Kumada

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Background/Aims: The aim of this study was to determine the pharmacodynamics of cisplatin following three different treatment procedures for intrahepatic arterial infusion therapy for hepatocellular carcinoma (HCC). **Methods:** We divided 13 HCC patients into the following three groups: group A, lone injection of cisplatin (n=3); group B, combined injection of cisplatin and lipiodol, with embolization using small gelatin cubes (GCs) (n=5); and group C, injection of suspended lipiodol with cisplatin powder, with embolization using small GCs (n=5). In each group, the free cisplatin concentration in the hepatic vein was measured at 0, 5, 10, and 30 minutes. **Results:** The mean free cisplatin concentrations were as follows. For group A, the mean was 48.58 μg/mL at 0 minute, 7.31 μg/mL at 5 minutes, 5.70 μg/mL at 10 minutes, and 7.15 μg/mL at 30 minutes. For the same time points, for group B, the concentrations were 8.66, 4.23, 3.22, and 1.65 μg/mL, respectively, and for group C, the concentrations were 4.81, 2.61, 2.52, and 1.75 μg/mL, respectively. The mean area under the curve $(AUC)_{0\text{-infinity}}$ for the free cisplatin concentration was 7.80 in group A, 2.48 in group B, and 2.27 in group C. The $AUC_{0\text{-infinity}}$ for the free cisplatin concentration gradually decreased, from group A to group C. **Conclusions:** These results indicate that the combination of lipiodol and small GCs may be useful for delaying cisplatin drainage from the liver. **(Gut Liver 2013;7:576-584)**

Key Words: Cisplatin; Attention; Carcinoma, hepatocellular; Drug delivery

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in Africa and in Asia, including Japan. It was established recently that more than 80% of cases with HCC have liver cirrhosis, and therefore a routine check-up for cirrhotic patients using ultrasound (US) usually detects small HCCs. However, due to the association between cirrhosis and tumor multiplicity, surgical resection is performed in only 20% of cases or less.[1,2] Transcatheter arterial chemoembolization (TACE) has been reported to be an effective palliative treatment for patients with unresectable HCC.[3-10] Although repeated TACE is one of the most potent therapies for unresectable HCC, resistance to this therapy often results after repeated therapy, with the long-term survival rates achieved after 3 years not being sufficiently high.

Platinum analogues are effective against many malignant tumors, and in recent years have been used in the treatment of HCC. For example, there are numerous reports that cisplatin is effective for advanced HCC and that combination therapy of cisplatin and lipiodol may be especially effective.[11-18]

Our group has reported previously that the rate of complete or partial response in cases of epirubicin TACE-resistant patients was significantly higher in patients treated with a platinum-analogue used TACE compared with a single hepatic arterial injection (HAI) without embolization.[19]

It is thought that the measurement of cisplatin concentration in samples collected from the hepatic veins after intrahepatic infusion is a useful method for determining differences in the curative effect of different treatment methods for cisplatin.

However, to our knowledge, there is no information on cisplatin concentration in the hepatic vein following different treatment methods. The aims of this study were therefore to measure total (protein-bound and unbound) and free (protein unbound)-cisplatin concentration in the hepatic vein and to carry out a pharmacokinetic analysis on the three kinds of drug delivery

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methods.

MATERIALS AND METHODS

1. Study population and ethical considerations

From 2007 to 2008, we carried out a prospective study on total and free cisplatin concentration in samples collected from the hepatic and peripheral veins during transcatheter arterial cisplatin chemotherapy in 13 patients with HCC. All the patients were considered to have an unresectable HCC at the time of diagnosis. Before treatment with the platinum analogue, all the patients underwent an evaluation consisting of a medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver imaging (computed tomography [CT], US, and digital subtraction angiography [DSA]), complete blood count, and blood chemistry. The diagnosis of HCC was established on the basis of the findings of the US, CT, and DSA.

A total of 13 patients were enrolled in the study using the following inclusion criteria: 1) typical hypervascular HCC observed in all imaging modalities; 2) Child-Pugh A or B classification; 3) performance status of 0 to 1; 4) adequate liver function with a bilirubin level ≤5 mg/dL; 5) sufficient hematopoietic function with a platelet count of >25,000 mm^3 and leukocyte count >2,000 mm^3; 6) an expected survival time of at least 3 months.

At first, if the patients had advanced portal vein invasion (tumor thrombus reaching the main trunks of the portal vein) or a severe arterioportal shunt, they were treated using only transcatheter arterial infusion of cisplatin (group A). The remaining patients were informed of the two other methods for administering cisplastin and the appropriate method was then chosen. One group received a combined injection of cisplatin and lipiodol, with embolization in small gelatin cubes (GCs) (group B), while the other group received an injection of suspended lipiodol with cisplatin powder, with embolization in small-GCs (group C). As a result, three patients were assigned to group A, five to group B, and five to group C (Fig. 1). The clinical background, laboratory data, and tumor characteristics of the patients are summarized in Tables 1 and 2.

The physicians in charge explained the purpose and method of this clinical trial to each patient, who provided their informed consent prior to participation.

The study was approved by Institutional Review Board of our hospital.

2. Details of treatment procedures

Hydration of the patients was performed through a peripheral line. The femoral artery was catheterized under local anesthesia, and a catheter then inserted superselectively into the hepatic artery that supplied the target tumor, followed by injection of cisplatin (IA-call; Nippon Kayaku, Tokyo, Japan) with or without lipiodol (Lipiodol Ultrafluide; Laboratoire Guerbet, Aulnay-sous-Bois, France) and 1-mm GCs (Gelpart; Nippon Kayaku). The dose of cisplatin was 100 mg/body administered over 20 minutes under careful fluoroscopic guidance.

In group A, only cisplatin was administered using transcatheter arterial infusion; in group B, cisplatin and lipiodol were first divided into six to eight parts and injected mutually, followed

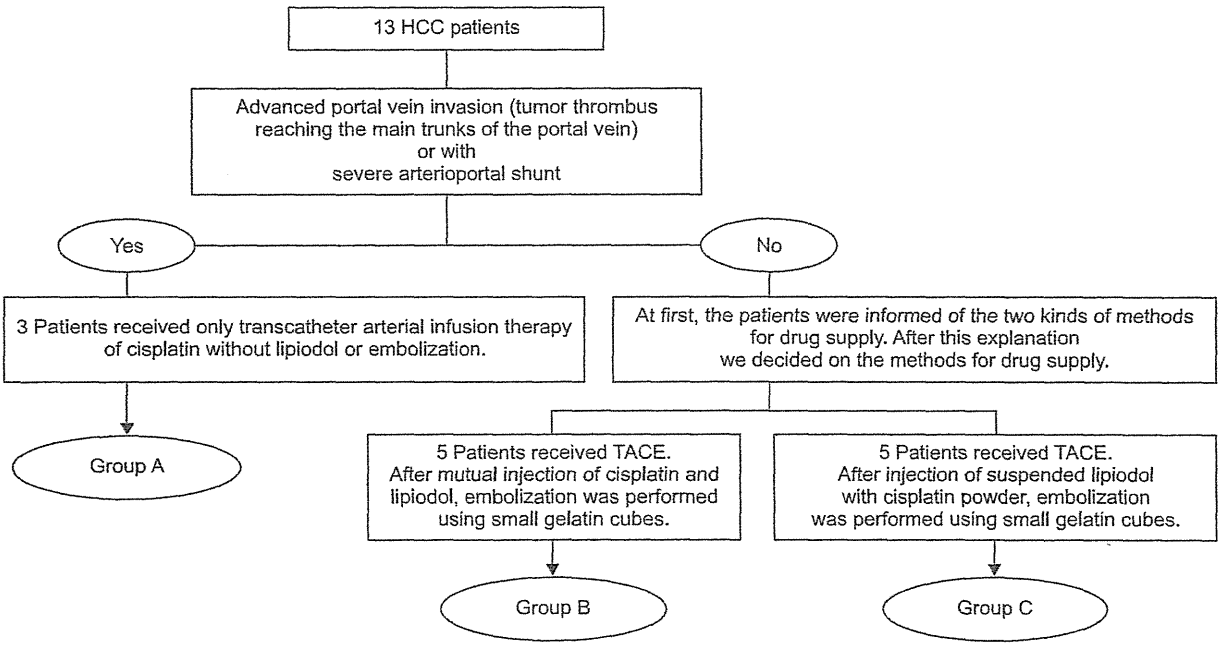

Fig. 1. Distribution of patients receiving cisplatin by three different administration procedures.
HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

Table 1. Demographic and Laboratory Data for 13 Patients with Unresectable Hepatocellular Carcinoma Who Underwent Blood Sampling from the Hepatic and Peripheral Veins for the Measurement of the Cisplatin Concentration after Transcatheter Arterial Chemotherapy Using Cisplatin

Parameter	Group A (n=3)	Group B (n=5)	Group C (n=5)
Patient characteristics			
Gender, male:female	2:1	4:1	5:0
Age, yr*	58 (46-73)	67 (57-87)	69 (63-77)
Backgrounds of liver disease			
Hepatitis B surface antigen positive	2	1	1
Anti-HCV antibody positive	1	4	4
Both negative	0	0	0
Liver function status			
Child-Pugh classification, A/B	2/1	5/0	5/0
Laboratory data			
Albumin, g/dL*	3.4 (2.8-3.7)	3.1 (2.9-4.0)	3.7 (3.2-3.9)
Bilirubin, mg/dL*	0.6 (0.4-1.8)	0.7 (0.5-1.4)	1.0 (0.5-1.1)
Prothrombin time, %*	94.7 (72.6-100.3)	86.8 (72.2-97.3)	82.1 (63.0-89.5)
AFP, μg/L*	55.9 (31.7-114,560.0)	1,664.0 (38.9-98,200.0)	116.0 (6.8-3,702.0)
DCP, AU/L*	3,065.0 (2,139.0-12,391.0)	141.5 (32.0-137,420.0)	98.5 (14.0-190.0)

*Data are presented as median (range).
HCV, hepatitis C virus; AFP, α-fetoprotein; DCP, des-γ carboxyprothrombin.

Table 2. Profiles of 13 Patients with Unresectable Hepatocellular Carcinoma Who Underwent Blood Sampling from the Hepatic and Peripheral Veins for the Measurement of the Cisplatin Concentration after Transcatheter Arterial Chemotherapy Using Cisplatin

Profiles of liver cancer	Group A (n=3)	Group B (n=5)	Group C (n=5)
Tumor size, median (range), mm	139 (79-187)	65 (16-140)	26 (5-76)
Intrahepatic multiplicity			
Solitary	0	0	1
Multiple, localized to one segment	0	0	0
Multiple, localized to one lobe	0	2	0
Multiple, extended to both lobes	3	3	4
Portal vein invasion, no/yes	1/2	3/2	4/1

by embolization using 1-mm GCs; and in group C embolization was performed using 1-mm GCs after injection of suspended lipiodol with cisplatin powder. In patients treated with lipiodol, its volume ranged from 2.0 to 5.0 mL, with the dose being determined according to tumor size and degree of liver dysfunction.

3. Method of drug and pharmacokinetic analyses

A pharmacokinetic study of cisplatin was performed after transcatheter arterial chemotherapy on day 1. After administration of cisplatin, blood samples were collected from the hepatic and peripheral veins. Total and free platinum concentration was measured in each sample, with the detailed pharmacokinetic study being performed only on the hepatic vein samples. The time the arterial infusion finished represented the observation starting point (0 minute), with blood samples collected at 5, 10, and 30 minutes. A sample was also collected from a peripheral vein 120 minutes after the completion of cisplatin infusion (Fig. 2). The blood samples were collected into heparinized syringes for measurement of plasma ultrafilterable platinum levels. Each sample was centrifuged at 3,000 rpm for 10 minutes and the plasma then placed in an ultrafiltration kit (Contrifree, MMPS-3; Amicon Inc., Tokyo, Japan), followed by centrifugation at 1,700×g for 20 minutes. This plasma ultrafiltrate was frozen immediately and stored at <-20°C. Platinum concentrations were analyzed using flameless atomic absorption spectrophotometry using a Hitachi polarized Zeeman atomic absorption spectrometer (Model Z-8000 with graphite furnace, temperature controller and autosampler; Hitachi Factor, Tokyo, Japan). The sample volumes were 10 μL. The oven was programmed using the following steps: 1) drying, 40 seconds at 80°C to 100°C; 2) drying, 50 seconds at 100°C to 130°C; 3) drying, 15 seconds at 130°C to 600°C; 4) charring, 15 seconds at 1,800°C; 5) atomization, 10 seconds at 3,000°C; 6) cleaning, 3 seconds at 3,000°C.

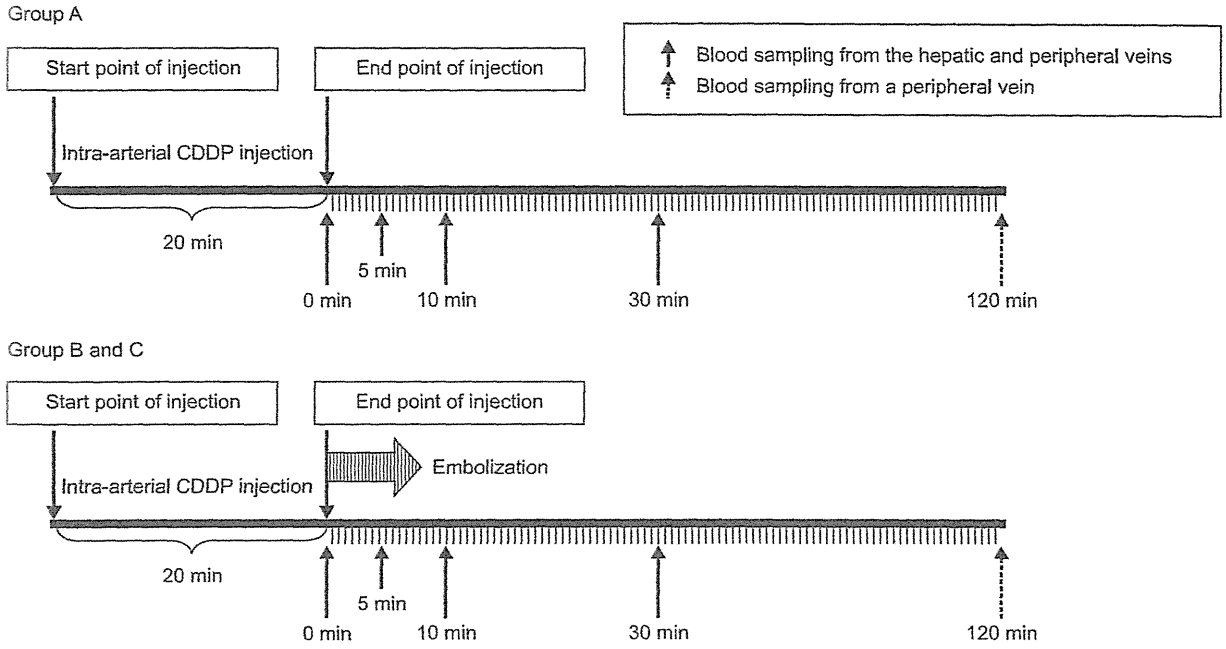

Fig. 2. Study protocol of cisplatin injection and blood sampling.
CDDP, cisplatin.

The absorbance of the samples was then measured at 265.9 nm. Standardization was performed using cisplatin saline solutions up to 1 μg/mL, with a detection limit of 10 ng/mL. Using this ultrafiltration kit almost all protein-bound cisplatin was eliminated and only free cisplatin (protein-unbound) could be measured. The measurement of cisplatin was carried out by NAC Co., Ltd., Tokyo, Japan. The AUC of total and free-cisplatin was calculated by the Automated Pharmacokinetic Analysis System computer program.[20]

4. Toxicity evaluation

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria version 4.0. The following toxicity evaluations were made within the 2 week period before treatment was started, and 3 to 7 days (three times during this period) and 2 weeks after treatment was started: hematological (leukocyte and thrombocyte counts) and clinical chemistry assessments (serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], total bilirubin, and serum creatine).

RESULTS

1. Total and free-cisplatin concentration in hepatic vein samples following each treatment procedure

The mean±SD total and free-cisplatin concentrations in hepatic vein samples were 68.08±30.30 and 48.58±41.56 μg/mL at 0 minute, 8.18±0.92 and 7.31±1.46 μg/mL at 5 minutes, 6.48±1.95 and 5.70±1.65 μg/mL at 10 minutes, and 9.46±8.59 and 7.15±7.12 μg/mL at 30 minutes in patients in group A; 10.35±4.89 and 8.66±5.36 μg/mL at 0 minute, 5.35±1.04 and 4.23±1.39 μg/mL at 5 minutes, 5.23±1.79 and 3.22±0.91 μg/mL at 10 minutes, and 3.36±0.67 and 1.65±0.33 μg/mL at 30 minutes in patients in group B; and 5.54±5.21 and 4.81±4.95 μg/mL at 0 minute, 3.30±1.28 and 2.61±1.19 μg/mL at 5 minutes, 3.75±1.97 and 2.52±1.13 μg/mL at 10 minutes, and 2.55±1.37 and 1.75±1.05 μg/mL at 30 minutes in patients in group C (Fig. 3).

With the exception of the 30 minutes time point, free-cisplatin concentration and the mean concentration of total and free-cisplatin were higher in the order of group A, B, and C at each measurement point.

2. Total and free-cisplatin concentration from a peripheral vein following each treatment procedure

Mean±SD total and free-cisplatin concentration of samples collected from a peripheral vein were 12.35±3.01 and 11.94±2.67 μg/mL at 0 minute, 6.75±1.00 and 5.87±0.35 μg/mL at 5 minutes, 5.54±1.01 and 4.92±0.61 μg/mL at 10 minutes, 3.91±1.40 and 2.69±0.68 μg/mL at 30 minutes, and 1.59±0.76 and 0.66±0.23 μg/mL at 120 minutes in patients in group A; 5.54±1.47 and 3.80±0.68 μg/mL at 0 minute, 4.31±0.55 and 3.04±0.51 μg/mL at 5 minutes, 4.33±1.08 and 2.65±0.45 μg/mL at 10 minutes, 3.34±0.76 and 1.66±0.17 μg/mL at 30 minutes, and 2.48±0.54 and 0.39±0.15 μg/mL at 120 minutes in patients in group B; and 2.30±0.88 and 1.70±0.95 μg/mL at 0 minutes, 2.49±0.68 and 1.93±0.58 μg/mL at 5 minutes, 2.21±0.93 and 1.58±0.51 μg/mL at 10 minutes, 1.85±0.77 and 1.07±0.40 μg/

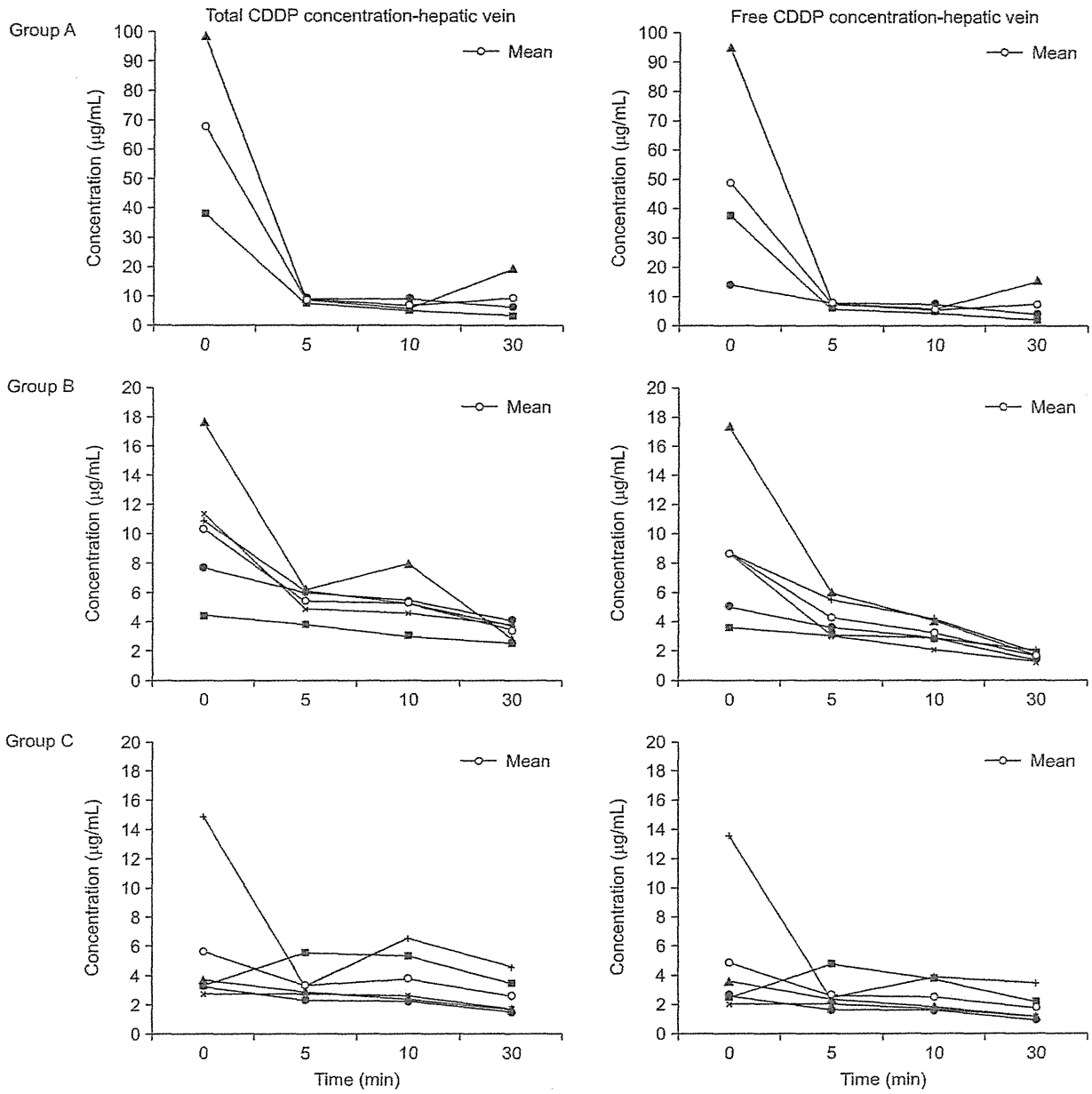

Fig. 3. Total and free cisplatin concentrations in samples collected from the hepatic vein after injection.
CDDP, cisplatin.

mL at 30 minutes, and 1.37±0.75 and 0.46±0.47 μg/mL at 120 minutes in patients in group C (Fig. 4).

The mean concentrations of total and free-CDDP were higher in the order of group A, B, and C at each measurement point, with the exception of the 120 time point.

3. Pharmacokinetic analysis of total cisplatin concentration in samples from the hepatic vein, following each treatment procedure

The pharmacokinetic analysis showed mean±SD maximum concentration (Cmax) of cisplatin in hepatic vein samples was 68.08±30.30 μg/mL in group A, 10.35±4.89 μg/mL in group B, and 5.99±5.06 μg/mL in group C.

Mean±SD $AUC_{0\text{-last}}$ was 6.43±2.70 μg/mL in group A, 2.52±0.65 μg/mL in group B, and 1.71±0.87 μg/mL in group C, while mean±SD $AUC_{0\text{-infinity}}$ was 11.84±6.16 μg/mL in group A, 5.93±2.00 μg/mL in group B, and 3.77±1.73 μg/mL in group C. The mean Cmax, $AUC_{0\text{-last}}$, and $AUC_{0\text{-infinity}}$ of total and free-cisplatin concentration were all higher in the order of group A, B, and C at each measurement point. The mean±SD of terminal half-life ($t_{1/2}Z$) was 0.53±0.17 hours in group A, 0.68±0.33 hours in group B, and 0.59±0.13 hours in group C (Table 3).

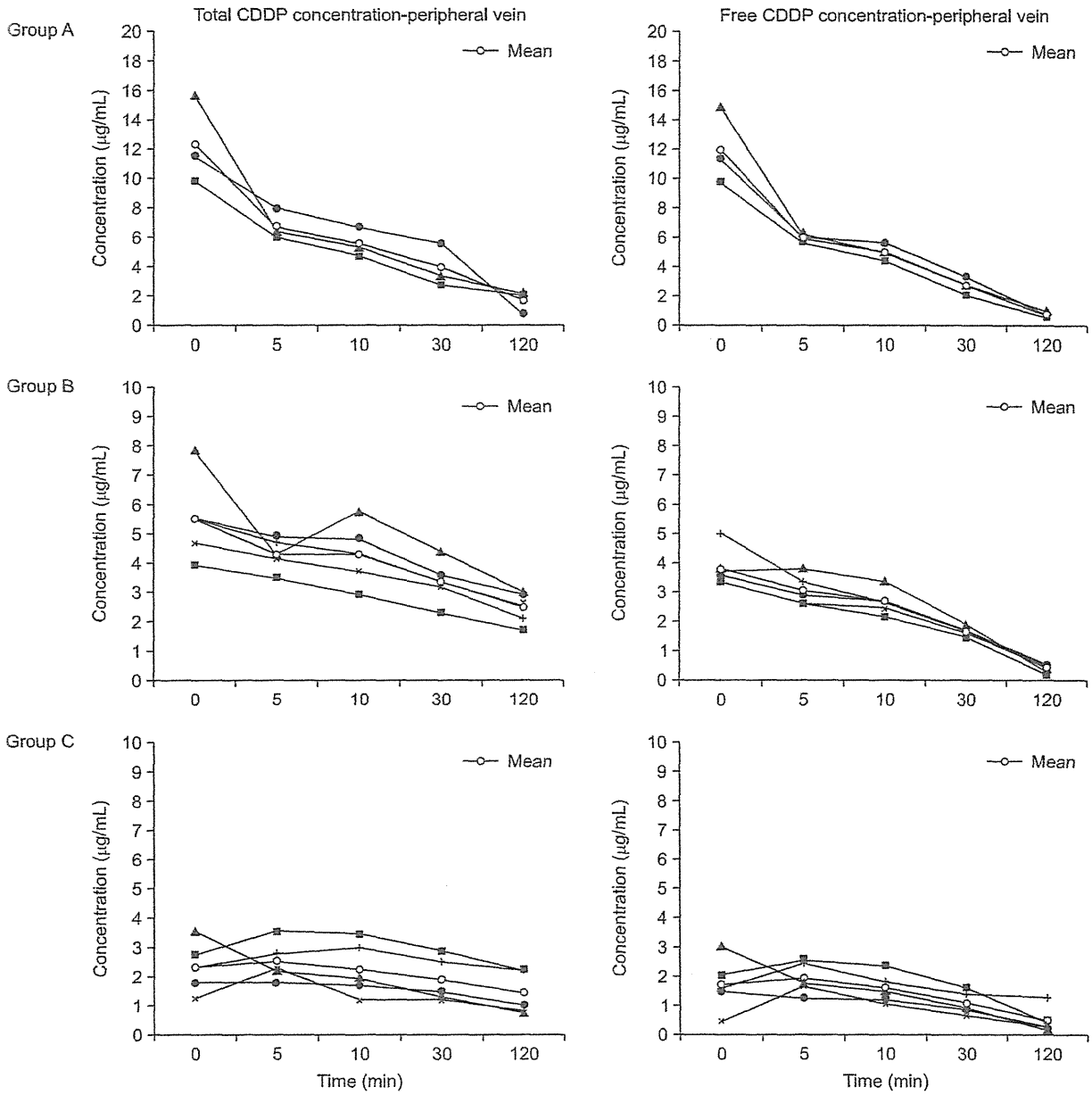

Fig. 4. Total and free cisplatin concentrations in samples collected from the peripheral vein after injection.
CDDP, cisplatin.

4. Pharmacokinetic analysis of free cisplatin concentration in samples from the hepatic vein following each treatment procedure

Pharmacokinetic analysis of free cisplatin in hepatic vein samples showed mean±SD Cmax was 48.58±41.56 μg/mL in group A, 8.66±5.36 μg/mL in group B, and 5.27±4.77 μg/mL in group C; $AUC_{0\text{-last}}$ was 5.01±2.81 μg/mL in group A, 1.66±0.51 μg/mL in group B, and 1.24±0.61 μg/mL in group C, and $AUC_{0\text{-infinity}}$ was 7.80±4.96 μg/mL in group A, 2.48±0.53 μg/mL in group B, and 2.27±1.10 μg/mL in group C. The means of Cmax, $AUC^{0\text{-last}}$, and $AUC_{0\text{-infinity}}$ for total and free cisplatin concentration was higher in the order of group A, B, and C at each measurement point. The mean±SD $t_{1/2}Z$ was 0.36±0.05 hours in group A, 0.35±0.10 hours in group B, and 0.45±0.06 hours in group C (Table 4).

5. Toxic effects

In this study, grade 4 side effects were not observed, although the following grade 3 events were observed: decreased hemo-

Table 3. Pharmacokinetic Parameters of Total Cisplatin

Parameter	Group A (n=3)	Group B (n=5)	Group C (n=5)
Hepatic vein			
Cmax, μg/mL*	68.08±30.30	10.35±4.89	5.99±5.06
$t_{1/2}Z$, hr†	0.53±0.17	0.68±0.33	0.59±0.13
$AUC_{0\text{-last}}$, μg/hr/mL‡	6.43±2.70	2.52±0.65	1.71±0.87
$AUC_{0\text{-infinity}}$, μg/hr/mL§	11.84±6.16	5.93±2.00	3.77±1.73

Data are presented as mean±SD.
*Cmax, maximum concentration (units, μg equation of cisplatin/mL); †$t_{1/2}Z$, terminal half-life (units, hour); ‡$AUC_{0\text{-last}}$, area under the curve from zero to the last measurable time point (units, μg equation of cisplatin/hr/mL); and §$AUC_{0\text{-infinity}}$, area under the curve from zero to infinity (units, μg equation of cisplatin/hr/mL).

Table 4. Pharmacokinetic Parameters of Free Cisplatin

	Group A (n=3)	Group B (n=5)	Group C (n=5)
Hepatic vein			
Cmax, μg/mL*	48.58±41.56	8.66±5.36	5.27±4.77
$t_{1/2}Z$, hr†	0.36±0.05	0.35±0.10	0.45±0.06
$AUC_{0\text{-last}}$, μg/hr/mL‡	5.01±2.81	1.66±0.51	1.24±0.61
$AUC_{0\text{-infinity}}$, μg/hr/mL§	7.80±4.96	2.48±0.53	2.27±1.10

Data are presented as mean±SD.
*Cmax, maximum concentration (units, μg equation of cisplatin/mL); †$t_{1/2}Z$, terminal half-life (units, hour); ‡$AUC_{0\text{-last}}$, area under the curve from zero to the last measurable time point (units, μg equation of cisplatin/hr/mL); and §$AUC_{0\text{-infinity}}$, area under the curve from zero to infinity (units, μg equation of cisplatin/hr/mL).

globin level in one patient (8%), decreased platelet counts in one patient (8%), increased AST in five patients (38%), increased ALT in two patients (15%), and increased bilirubin level in two patients (15%). All these abnormalities resolved within two weeks. In this study group, no other serious complications or treatment-related deaths were observed after administration of cisplatin.

DISCUSSION

Cisplatin is one of the effective carcinostatic agents for HCC. When HCC is treated using transcatheter chemotherapy we usually use a combination of lipiodol and carcinostatics. TACE is now established as a method for administering chemotherapy in cases of HCC. Lipiodol has the characteristic of accumulating in a tumor vessel of HCC, and therefore carcinostatics are usually used in combination with lipiodol when performing TACE. It has been reported that water in an oil type emulsion is useful for steady accumulation and sustained release of carcinostatics.[21,22] However, cisplatin was prepared conventionally for use in intravenous drips using dosage increases in small steps, making preparation of the suspended injection with lipiodol difficult.

Until recently, mutual injections of cisplatin and lipiodol were used as one of the methods for administrating cisplatin in HCC patients. This method was reported previously as "sandwich therapy."[13] Now, "IA-call" which is a preparation of fine cisplatin powder, has been developed for use as an intrahepatic artery injection, with the fine powder being added easily to lipiodol to make a suspension.

In the present study we measured the concentration of total and free-cisplatin in hepatic vein and peripheral vein samples, and determined whether the treatment procedure influenced delay of drug delivery. Our data showed both total and free-cisplatin concentration increased in the order of group A, B, and C. These results may indirectly indicate that cisplatin was slowly released from liver tissue and decreased in the order of group A, B, and C. Regarding these results, we interpreted that lipiodol mainly affected the slow elution of cisplatin, and GCs augmented drug retention in the liver and tumor tissues by a temporary shut off of arterial blood flow. However, in this study, we could not directly investigate cisplatin concentrations in liver and tumor tissues. Although, one recent animal experimental study reported that suspended lipiodol with cisplatin powder mostly retained the cisplatin concentration as compared to other treatment methods (HAI and combined use of GCs without lipiodol) in VX-2 tumor tissues of rabbits.[23] Thus, we will need additional studies on human liver and tumor tissues. At this time, in order to retain cisplatin in liver tissue for a long duration, it was useful that the methods for administering cisplatin, lipiodol, and embolization also affected cisplatin concentration in liver and tumor tissue, and in the case of HCC patients treated with cisplatin, the use of TACE using Lipiodol and small-GCs provided additional benefits based on this study and previously reported experimental study results.[23] In recent years, we have used third-generation platinum compounds that do not have cross-resistance to cisplatin. Repeated use of cisplatin often causes drug resistance and allergic reactions such as anaphylaxis. The risk of allergic reactions increases from the third session of TACE with cisplatin,[24] and therefore, miriplatin can be considered as a second-line chemoembolization agent in patients who exhibit hypersensitivity or resistance to cisplatin. On the other hand, the development of drug-eluting microspheres (DEMs) provides a new treatment method for drug delivery.

Preclinical and clinical studies on TACE using DEM have demonstrated greater and longer retention times of drug within tumors and a lower systemic concentration compared with conventional TACE using lipiodol.[25-27]

To date, two types of microspheres capable of being loaded with a drug are commercially available: superabsorbent polymer microspheres (HepaSphere; Merit Medical Systems, Salt Lake City, UT, USA) and polyvinyl alcohol-based microspheres (DC

Bead; Biocompatibles, Farnham, UK). HepaSphere has a reservoir effect after loading with some chemotherapeutic agents, with two *in vitro* studies confirming that it efficiently loads and elutes doxorubicin, irinotecan, and cisplatin.[28,29]

In accordance with our previous report,[19] Seki and Hori[30] reported it was useful to switch anticancer therapy from epirubicin to cisplatin for treatment of HCC that had become refractory to TACE using epirubicin-loaded microspheres.

We therefore consider that it is necessary for future studies to carry out additional investigations on DEM.

Finally, this study had several limitations. First, the study sample size was too small and we could not examine liver and tumor tissues. In addition, tumor characteristics were different for each treatment method. Therefore, tumor characteristics (i.e., portal vein invasion, severe arterioportal) may have greatly affected the cisplatin concentrations in the hepatic and peripheral veins in group A. Regarding this point, we intend to investigate the same study protocol for patients with similar tumor characteristics in the future. Second, we only investigated useful drug delivery methods under single session transcatheter therapy. Therefore, we did not investigate continuous hepatic arterial infusional chemotherapy (i.e., combined use of cisplatin and 5-fluorouracil [5-FU]).

Primarily in Asian countries, many patients in group A are selected for continuous hepatic arterial infusional chemotherapy if they have adequate liver function. Therefore, additional studies will be needed under continuous hepatic arterial infusional chemotherapy with or without lipiodol. Also, we usually use epirubicin for first line treatment of HCC by TACE in Japan. Therefore, it is difficult to compare the actual efficacy of each anticancer drug (i.e., mitomycin-C, 5-FU) at the same level. Thus, we will need a prospective study to investigate this.

In conclusion, combined use of lipiodol and small-GCs clearly reduced the $AUC_{0\text{-infinity}}$ of total and free cisplatin concentrations in samples collected from the hepatic vein. In other words, free cisplatin concentrations in the liver were retained to a greater extent in the patient group administered lipiodol and small-GCs together. We consider that these results strongly support the combined use of embolization for treatment of HCC without advanced portal vein invasion or with severe arterioportal shunt that uses cisplatin at the time of injection into the hepatic artery.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

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