INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Efficacy of Contrast-Enhanced Ultrasonography in Radiofrequency Ablation for Hepatocellular Carcinoma

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Abstract

Objective Local recurrence after radiofrequency ablation (RFA) is a major problem that needs to resolved to increase the survival rate of hepatocellular carcinoma (HCC). CE-US with Sonazoid®, the second-generation contrast media, can detect smaller HCC lesions and the detection rate of ultrasonically unrecognized hypervascular HCC was improved by CE-US. The aim of the present study was to evaluate the role of CE-US with Sonazoid® in improving radicality and reducing local recurrence after RFA for HCC.

Patients and Methods A total of 102 nodules treated by RFA at our hospital from January 2006 to October 2009 were enrolled: 31 nodules were treated without CE-US, since CE-US was not yet available (Group A), and 71 nodules were treated with a combination of RFA and CE-US with Sonazoid® (Group B).

Results The clinical characteristics (sex, virus marker, Child-Pugh grade, with or without transcatheter arterial infusion chemotherapy with lipiodol, and T factor) did not differ significantly between group A and group B. Mean age was significantly older and tumor size was significantly larger in group B. Group B had significantly better radicality compared with group A. The non-local recurrence rate was significantly higher in group B as compared with group A.

Conclusion CE-US with Sonazoid® greatly helps to improve RFA efficacy in HCC treatment. We suggest that the ability of CE-US with Sonazoid® to detect an accurate area of HCC before RFA and to immediately detect a residual tumor during RFA might contribute to an increase of the radicality and reduction of local recurrence after RFA.

Key words: contrast-enhanced ultrasonography, radiofrequency ablation, hepatocellular carcinoma, radicality, local recurrence

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common and principal cancers worldwide. Surgical resection plays a pivotal role in the treatment of HCC, but underlying severe cirrhosis or tumor multicentricity often contraindicates surgery (1, 2). Percutaneous ethanol injection, microwave coagulation therapy, and radiofrequency ablation (RFA) are widely used in the treatment of HCC (3, 4). The 5-year survival rate following RFA was as high as 57% in patients registered in the Liver Cancer Study Group of Japan, and 83.8% (single 2 cm or smaller tumor) and 76.3% (2-5 cm liver tumor) in liver damage A cases, showing outcomes equivalent to those of resection (5). Although RFA is a powerful procedure for the treatment of HCC, local recur-

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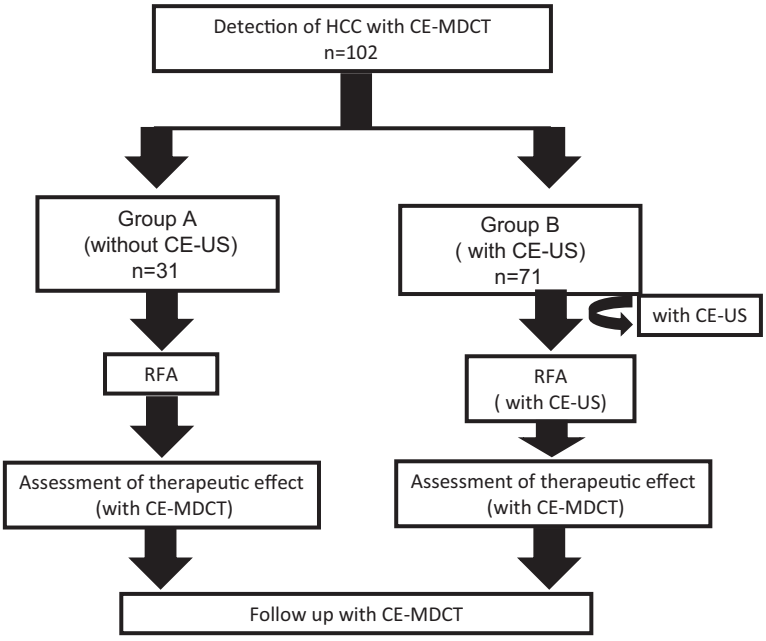


Figure 1. **Assessment of efficacy of RFA for HCC in our study.**

rence after RFA treatment is a major problem. The rate of local recurrence can range from as low as 2% to as high as 53% after RFA of HCC (6-11). Local recurrence should be resolved to increase the survival rate of HCC patients.

Contrast-enhanced multidetector-row computed tomography (CE-MDCT) is the most commonly used modality for assessing the efficacy of RFA, as the ablated margin is determined by CE-MDCT before and after RFA (12). Levovist®, a first-generation US contrast agent, improved the localization of sonographically unrecognized hypervascular lesions of the liver. However, because of the fragility of the Levovist® microbubbles, images must be obtained intermittently while the post-vascular phase must be obtained by a single sweep scan of the liver. Sonazoid® (GE Healthcare, Oslo, Norway), the second-generation contrast media which is composed of a hard shell containing bubbles, produces stable non-linear oscillations in the low-power acoustic field and supplies great details of the second harmonic signals in real time. This contrast agent provides detailed perfusion features of the microvascular bed of the liver parenchyma and tumor during the vascular phase. Moreover, the post-vascular phase, which is stable for at least up to 3 h after injection and tolerant for multiple scanning, can be obtained in the low-power acoustic field (13). CE-US with Sonazoid® can detect smaller HCC lesions more clearly than conventional US (14). The detection rate of ultrasonically unrecognized hypervascular HCC was improved by CE-US with Sonazoid® (15). CE-US with Sonazoid® will play a pivotal role in RFA treatment for HCC before and during RFA treatment using real-time vascular imaging and the post-vascular imaging, and it will facilitate improvement of the radicality and reduction of the local recurrence after RFA treatment.

The aim of the present study was to evaluate the role of CE-US with Sonazoid® in improving the radicality and decreasing the local recurrence after RFA for HCC.

Materials and Methods

Subjects

One hundred two nodules of 87 patients treated by RFA for HCC at Akita University Hospital from January 2006 to October 2009 were enrolled in this study. Diagnosis of HCC was confirmed by typical imaging findings of CE-MDCT. There were three limitations in this study: 1) sizes of nodules <35 mm, 2) numbers of nodules were less than five and 3) no invasion of portal vein, hepatic vein or bile duct. The patients were classified into two groups. The first 31 nodules of 27 patients were treated by RFA without CE-US since CE-US was not yet available for use (group A). The latter 71 nodules of 60 patients were treated by RFA under CE-US with Sonazoid® (group B). Transcatheter arterial infusion chemotherapy with lipiodol (Lip-TAI) was performed before RFA when HCC was located near surface of the liver or adjacent to the vessel, where it was difficult for us to treat with RFA. In those cases, we treated by epirubicin-lipiodol emulsion in Lip-TAI before RFA. Lip-TAI had direct anti-tumor effect and accumulated lipiodol in HCC. 20 nodules were treated with both Lip-TAI and RFA and 11 nodules were treated with only RFA in group A. 48 nodules were treated with Lip-TAI and RFA and 23 nodules were treated with only RFA in group B.

In group A and group B, the complete ablation of HCC nodules were evaluated with CE-MDCT using hepatic arterial phase in 3-5 days after last RFA session (Fig. 1). We classified the radicality of the procedure according to the extent of the ablated area around the nodule by Nishijima's

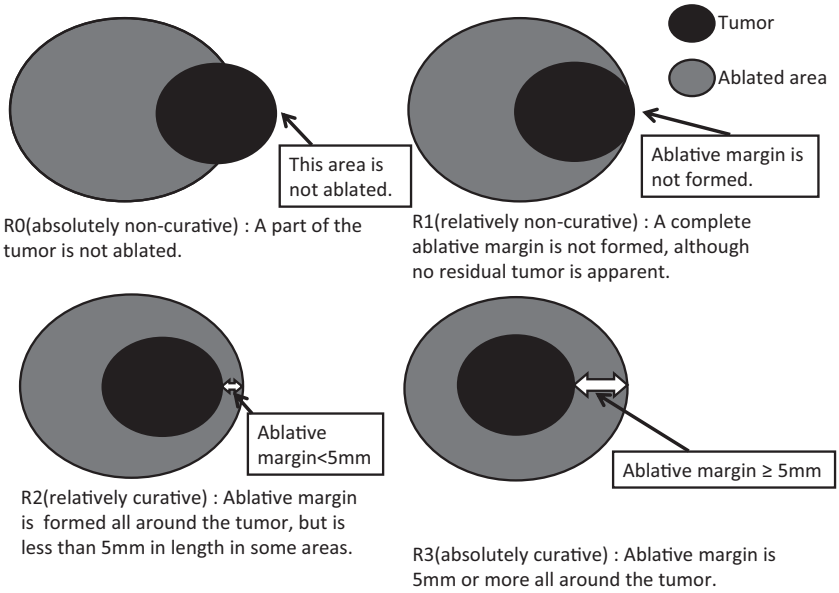


Figure 2. Radicality grade definition. R0 (absolutely non-curative); a part of the tumor is not ablated. R1 (relatively non-curative); a complete ablative margin is not formed, although no residual tumor is apparent. R2 (relatively curative); an ablative margin is formed all around the tumor, but it is less than 5 mm wide in some areas. R3 (absolutely curative); the ablative margin is 5 mm wide or more all around the tumor.

method (16) : R0 (absolutely non-curative); a part of the tumor is not ablated. R1 (relatively non-curative); a complete ablative margin is not formed, although no residual tumor is apparent. R2 (relatively curative); an ablative margin is formed all around the tumor, but it is less than 5 mm wide in some areas. R3 (absolutely curative); the ablative margin is 5 mm wide or more all around the tumor (Fig. 2). Mean radicality grade score was calculated using the formula: mean radicality grade score=0× number of R0 cases+1× number of R1 cases +2× number of R2 cases +3× number of R3 cases/total number of cases.

After treatment with RFA, CE-MDCT was performed every 4 months. Local recurrence was defined as the appearance of enhancement in the hepatic arterial phase adjacent to the original lesion.

Equipment and image analysis

US was carried out using ProSound α 10 (Aloka, Tokyo, Japan) and a micro-convex probe (UST9133, 3.5 MHz). We first assessed hepatic lesions using conventional US. CE-US was carried out in extended pure harmonic detection mode with a mechanical index (MI) level of 0.25-0.3. The liver was scanned at 15 frames per second. The focus point was just under the deep margin of the lesion. The contrast agent Sonazoid® was used at a dose of 0.5 mL by a manual bolus injection followed by a flush with 10 mL of normal saline via a peripheral venous line. We observed the post-vascular phase (approximately 10 minutes after the bolus intravenous injection of Sonazoid®) and searched the lesion depicted as a perfusion defect. Re-injection of Sonazoid® at a dose of 0.5 mL was then performed to confirm the presence of hypervascular enhancement within the perfusion defect. Enhancement within the perfusion defect from 30 to 120 seconds after the bolus re-injection of Sonazoid® was considered as evidence of the residual viable lesions. The site of the recurrent tumor was classified as extrazonal or intrazonal relative to the zone of the perfusion defect seen on CE-MDCT. CE-MDCT was performed using an Aquillion 64 scanner (GE Healthcare Japan, Tokyo, Japan). Triple-phase CE-MDCT scans were obtained after injection of 100 mL of Iopamiron 370 (Bayer, Tokyo, Japan) via an antecubital vein at a rate of 3 mL/s with a 5.0 mm slice thickness to obtain hepatic arterial, portal venous, and equilibrium phase images.

RFA technique

Local anesthesia was achieved by injecting 5 mL of 1% Procaine through the skin into the peritoneum along a predetermined puncture line before ablation. We inserted a 20-cm long, 17-gauge radiofrequency electrode equipped with a 2.0- or 3.0-cm long exposed metallic tip into the tumor guided by US. Cool-tip devices, including cool-tip electrodes and cool-tip system are provided by Valleylab (Boulder, CO, USA). In group A, 6 nodules were ablated with 2.0-cm long exposed metallic tip and the mean ablation time was 9.24 minutes, 25 nodules were ablated with 3.0-cm long exposed metallic tip and the mean ablation time was 16.73 minutes. In group B, 30 nodules were ablated with 2.0-cm long exposed metallic tip and the mean ablation time was 10.49 minutes, 41 nodules were ablated with 3.0-cm long exposed metallic tip and the mean ablation time was 17.95 minutes.

When the ablation of HCC nodule was evaluated as R0 or R1, we treated HCC with RFA again. The average number

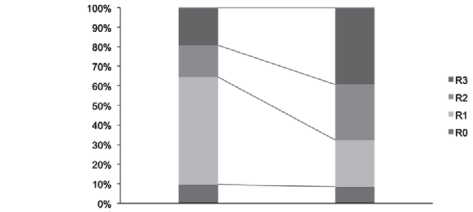
Table 1. Clinical Features

Variable		Group A	Group B
Sex	male/female	23/4	42/18
Age	years(mean ± SD)	68.4±6.8*	71.8±7.3*
Virus marker	B/C/NBNC[1]	2/19/6	2/40/18
Child-Pugh grade	A/B/C	22/5/0	38/21/1
Lip-TAI	+/-	20/11	48/23
T factor[2]	1/2/3	17/12/2	8/25/38
Tumor size	mm (mean ± SD)	16.8±5.4*	21.2±7.5**

Group A: 31 nodules of 27 patients
Group B: 71 nodules of 60 patients

*Age: p=0.012
**Tumor size: p=0.0011

[1]B, hepatitis B surface antigen (HBs-Ag) positive; C, anti-hepatitis C virus antibody (HCV-Ab) positive; NBNC, HBs-Ag negative and HCV-Ab negative. [2]T factor: (1. Solitary, 2. ≤ 20 mm, 3. No vascular or bile duct invasion) T1, fulfilling 3 factors; T2, fulfilling 2 factors; T3, fulfilling 1 factor; T4, fulfilling 0 factor. These are derived from the general rule of the liver cancer study group of Japan[17].



	Group A	Group B	
R3	6 (19.3%)	28 (39.4%)	
R2	5 (16.1%)	20 (28.2%)	
R1	17 (54.8%)	17 (23.9%)	
R0	3 (9.7%)	6 (8.5%)	
Radicality score mean ± SD	1.43 ± 0.91*	1.99 ± 0.99*	*p=0.014

Figure 3. Radicality in group A and group B.

of RFA sessions was 1.33 in group A, and 1.19 in group B. There were no significant differences in the number of RFA sessions between the 2 groups, but there was a tendency for a reduced number of sessions in group B. The procedures were performed by the same experienced physicians in both group A and group B.

Statistical analysis

Results are presented as the mean ± SD. Statistical comparisons between groups were made using student's t-test. A p value <0.05 was considered to indicate significance. Cumulative non-local recurrence rate curves were drawn using the Kaplan-Meier method and analyzed by the log-rank test. Univariable analysis for factors contributing to local recurrence was performed using the Kaplan-Meier method and the log-rank test.

Results

Clinical features of patients

The clinical characteristics are shown in Table 1: sex, virus marker, Child-Pugh grade, with or without Lip-TAI, and T factor (17) did not differ significantly between group A and group B. Mean age was 68.4 years old in group A and 71.8 years old in group B. Patients in group B were significantly older than patients in group A (p=0.012). Tumor size was 16.8 mm in group A and 21.2 mm in group B. Tumor size in group B was significantly larger than that in group A (p=0.0011).

Radicality in group A and group B

In group A, 9.7% of tumors were R0, 54.8% were R1, 16.1% were R2, and 19.3% were R3. In group B, 8.5% of tumors were R0, 23.9% were R1, 28.2% were R2, and 39.4% were R3. Mean radicality grade score was 1.43 in group A and 1.99 in group B. Group B had significantly improved radicality compared with Group A (p=0.014) (Fig. 3).

Cumulative non-local recurrence rate after RFA

In order to evaluate the role of CE-US with Sonazoid® in the treatment of HCC with RFA, we compared the non-local recurrence rate between group A and group B. In group A, the cumulative non-local recurrence rate in 1 year was 76.3%, and in 2 years 66.4%, whereas in group B, in 1 year it was 92.1%, and in 2 years 85.3%. The cumulative non-local recurrence rate was significantly higher in group B as compared with group A during the 24-months follow-up period (Fig. 4).

Factors contributing to local recurrence after RFA

We analyzed eight factors contributing to local recurrence after RFA (Table 2): sex, age (more than 65 years vs. under 65), virus marker (HCV vs. others), Child-Pugh grade (A vs. B or C), with or without Lip-TAI, T factor (T1-T2 vs. T3) and tumor size (more than 20 mm vs. under 20 mm) did not differ significantly in local recurrence. R2 or R3 radicality significantly reduced the local recurrence com-

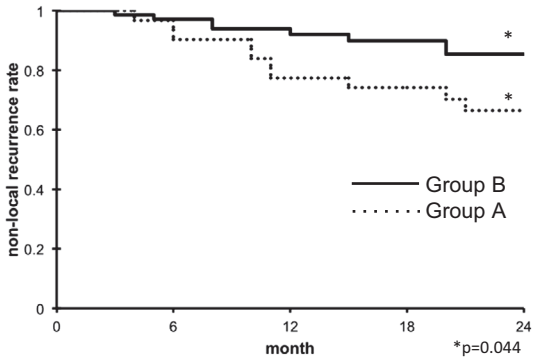


Figure 4. **Non-local recurrence rate after RFA in group A and group B.**

pared with R0 or R1 radicality (p=0.0429).

Table 2. **Factors Contributing to Local Recurrence after RFA**

Variable	p value
①Sex male (79) vs. female (23)	0.2168
②Age≥65y Yes (83) vs. No (19)	0.5208
③Virus marker HCV (74) vs. others (28)	0.1713
④Child-Pugh grade A (65) vs. B+C (37)	0.6836
⑤Lip-TAI with (68) vs. without (34)	0.1408
⑥T factor 1+2 (62) vs. 3(40)	0.8871
⑦Tumor size ≥ 20mm Yes (50) vs. No (52)	0.5091
⑧Radicality R0+R1 (43) vs. R2+R3 (59)	0.0429

Discussion

CE-US with Sonazoid® was useful in detecting small lesions and feeding vessels, and in displaying the relationship between the tumor and the major organs (18). CE-US with Sonazoid® could provide valuable clinical information for planning RFA treatment protocols and was reported to be a useful procedure for assessing the efficacy of RFA of HCC (19). Evaluation of the local recurrence with CE-US is comparable to CE-MDCT which is one of the commonly used modalities for assessing the safety margin after treatment of HCC with RFA (20). Treating the local recurrence is not easy because it is frequently difficult to differentiate between viable tumor and necrotic tissue on conventional US (21). Solbiati et al reported their experience in using CE-US with SonoVue® (sulphur hexaflouride microbubbles), before, during, and after RFA treatment, and their results indicated that CE-US represented a significant improvement in the detection of lesions, selection of patients for treatment, and all steps of tumor ablative treatment (22). But SonoVue® does not have a post-vascular phase, because SonoVue® is a blood pool agent. Sonazoid® enables real-time vascular imaging and the post-vascular imaging thanks to its stability under low acoustic pressure (23). Malignant hepatic tumors contain few or no reticuloendothelial cells and appear as perfusion defects on post-vascular phase (24, 25). The post-vascular phase lasts approximately 10-120 minutes using Sonazoid®, which is sufficient for performing RFA. Kudo et al have proposed defect reperfusion imaging to distinguish between necrotic areas and vascular tumor tissues (26). Re-injection of Sonazoid® was then performed to confirm the presence of hypervascular enhancement within the perfusion defect. Enhancement within the perfusion defect from 30 to 120 seconds after the bolus re-injection of Sonazoid® was considered evidence of local recurrence (25, 26). In RFA treatment, CE-US with Sonazoid® is very useful to ablate HCC with a fully ablative margin because it gives us sufficient perfusion defect time to detect HCC and we can repeat to confirm local recurrence of HCC using re-injection of Sonazoid®. A wider application of RFA guided by CE-US with Sonazoid® will help to confirm that there is a sufficient ablation margin which is critical for the prevention of local recurrence (27). Miyamoto et al studied 16 cirrhotic patients with 17 cases of hypervascular locally recurrent HCC and concluded that the CE-US with Sonazoid® appearance of local recurrences correlated well with those on MDCT and a wider use of CE-US to guide repeat of percutaneous RFA may be possible with Sonazoid® (28). In the present study, we evaluated numerous nodules and had a long observation period. Actually, we executed RFA for HCC under CE-US with Sonazoid® in 71 nodules and observed for 24-months.

In principle, we aimed that the goal of RFA arrived at more than R2 radicality. Because R2 or R3 radicality significantly reduced the local recurrence compared with R0 or R1 radicality in our study (Table 2), our goal of RFA is reasonable. In spite of repetition of RFA, some cases ended in R1 or R0 radicality. Before using CE-US, it was difficult to grasp insufficient ablated area by using only conventional US. After using CE-US we could repeat to confirm local residual of HCC using re-injection of Sonazoid®. Therefore, the rates of R2 and R3 radicality increased. The rates of R1 (relatively non-curative) were significantly low in group B, but the rates of R0 (absolutely non-curative) of the two groups seem to be equal. In R0 cases, HCC nodules were located near surface of liver or adjacent to the vessel. Because the rates of difficult cases in RFA were no significant differences in 2 groups, the rates of R0 seem to be equal. Some cases were still R1 or R0 radicality in group B. These nodules were fundamentally treated with Lip-TAI before RFA, and were carefully observed with CE-MDCT.

The rate of local recurrence can range from low to high after RFA of HCC from some studies (6-11). In the present study, it was difficult to grasp insufficient ablated area by using only conventional US, the rates of R0 and R1 were

high and non-local recurrence rate at 24 months was low in group A. But by using CE-US, the radicality score became significantly high confirming the presence of hypervascular enhancement of HCC before and during RFA and non-local recurrence rate increased obviously. Although the tumor size of HCC in the cases treated by RFA with CE-US was significantly larger than that in the cases treated by RFA without CE-US, the radicality score and the cumulative non-local recurrence rates were significantly higher in the cases treated by RFA with CE-US, suggesting that CE-US with Sonazoid® could detect HCC lesions clearly, confirm the presence of hypervascular enhancement of HCC before and during RFA treatment and take sufficient ablative margin in RFA.

Accordingly, the present results imply that RFA for HCC under CE-US with Sonazoid® contributed the higher non-local recurrence rates leading to the improvement of the prognosis of HCC patients. CE-US with Sonazoid® greatly helps improve RFA efficacy in HCC treatment. We suggest that the ability of CE-US with Sonazoid® to detect an accurate area of HCC before RFA and to immediately detect a residual tumor during RFA might contribute to the reduction of HCC local recurrence following RFA.

The authors state that they have no Conflict of Interest (COI).

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