



Evaluation of the therapeutic efficacy of high-intensity focused ultrasound ablation of hepatocellular carcinoma by three-dimensional sonography with a perflubutane-based contrast agent

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ABSTRACT

Objective: We performed contrast-enhanced three-dimensional sonography (CE 3D US) with a perflubutane-based contrast agent to immediately evaluate the completeness of ablation of small hepatocellular carcinoma (HCC) lesions by extracorporeal high-intensity focused ultrasound (HIFU).

Subjects and methods: Twenty-one HCC lesions were treated by a single ultrasound-guided HIFU ablation session, and CE 3D US was performed before, immediately after, and 1 week, and 1 month after HIFU, and contrast-enhanced CT (CE CT) or contrast-enhanced MRI (CE MRI) was performed before HIFU, 1 week and 1 month after HIFU, and during the follow-up period.

Results: Immediately and 1 month after HIFU, 17 lesions were evaluated as adequately ablated by CE 3D US, and the other 4 lesions as residual tumors. One month after HIFU, 18 were evaluated as adequately ablated by CE CT or CE MRI, and the other 3 as residual tumors. The evaluation by CE 3D US immediately after HIFU and by CE CT or CE MRI 1 month after HIFU was concordant with 20 lesions. The kappa value for agreement between the findings of CE 3D US and other modalities by two blinded observers was 0.83. When the 1-month CE CT or CE MRI findings were used as the reference standard, the sensitivity, specificity, and accuracy of CE 3D US immediately after HIFU for the diagnosis of the adequate ablation were 100%, 75%, and 95%, respectively.

Conclusion: CE 3D US appears to be a useful method for immediate evaluation of therapeutic efficacy of HIFU ablation of HCC lesions.

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1. Introduction

Extracorporeal high-intensity focused ultrasound (HIFU) ablation has been introduced clinically as a minimally invasive treatment for hepatocellular carcinoma (HCC) lesions that does not involve inserting needles into the lesions [1–10]. HIFU ablation provides a method of precise ablation of entire tumors of different sizes and shapes without damaging overlying and surrounding vital structures [1–10]. Moreover, HIFU can be used to safely ablate HCC lesions located adjacent to large vessels, such as the portal vein or hepatic veins [10], and it does not increase the risk of metastasis by HCC lesions [11,12].

Guidance and monitoring of HIFU ablation is based on magnetic resonance imaging (MRI) and US. In Japan, guidance of HIFU by US is

preferable to guidance by MRI because of its lower cost, compactness, and real-time nature. During the HIFU ablation procedure, hyperechoic changes on gray-scale US images obtained after each exposure are used to evaluate the extent of the area ablated on each slice [2–6]. However, since the appearance of hyperechoic changes do not always mean complete tumor necrosis [11–14], another monitoring method is needed to evaluate the adequacy of HCC lesion necrosis and to decide when to stop the HIFU session.

A newly developed perfluorobutane-based contrast agent, Sonazoid (Daiichi Sankyo, Tokyo, Japan) has recently enabled the performance of contrast-enhanced 3D sonography (CE 3D US) to evaluate the vascularity of HCC lesions and anatomical relationships between HCC lesions and relatively large adjacent vessels [15–17]. In this study, we performed CE 3D US by using the automatic scan function of a volume transducer to evaluate immediate therapeutic effectiveness of a single HIFU ablation session in patients with small HCC lesions.

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Table 1
Clinical characteristics of the cases with an HCC lesion treated by HIFU ablation retrospectively reviewed in this study.

Characteristics	
Number of patients	21
Sex (male/female)	12/9
Age (mean, range, years)	73.4, 65–80
Cause of the cirrhosis (alcohol, hepatitis B, hepatitis C)	2/0/19
Child-Pugh class (A/B/C)	18/3/0
Diameter of lesion (>2 cm, < or =2 cm)	2/19
Length of the lesion (mean, range, mm)	16.3, 10–26
Width of the lesion (mean, range, mm)	12.6, 8–20
Height of the lesion (mean, range, mm)	14.5, 10–24
Distance from skin surface (mean, range, mm)	52.8, 26–83
Location of the lesion in the right lobe/the left lobe	10/11
Diagnosis confirmed by biopsy/radiological imaging	2/19

2. Materials and methods

2.1. Subjects

Institutional review board approval and informed consent from all patients was obtained for this retrospective study. Between July 2007 and October 2008, 365 HCC patients were admitted to one of two institutions (Yokohama City University Medical Center and Naruto General Hospital). Forty of the 365 patients were screened for eligibility to enter to this study by CE CT or CE MRI, conventional US, and CE 3D US. The inclusion criteria of for this study were: (1) Child-Pugh grade A or B liver cirrhosis; (2) platelet count more than 50,000; (3) a solitary nodular-type HCC lesions without tumor thrombosis of the branches of the portal vein; (4) less than 3 cm in maximum diameter. The exclusion criteria of for this study were: (1) HCC lesion located 9 cm or more below the skin surface, because of poor visualization by monitoring US of HIFU equipment; (2) HCC lesion located adjacent to the bowel or immediately behind the scar from a previous operation or to avoid bowel damages or skin burns. In contrast to radiofrequency ablation (RFA), a lesion located behind the portal vein or bile duct was not a contraindication of HIFU ablation.

Seventeen patients did not fulfill the above criteria. The other 23 patients were enrolled in this study and underwent HIFU ablation under epidural anesthesia. Two patients were excluded, because epidural anesthesia was not effective in reducing the pain caused by HIFU ablation in 1 patient, and because the other patient's lesion was not targeted with HIFU since dysplastic nodule lesion that was almost the same size and shape lesion was present in the almost same area and was targeted by mistake. Therefore, ultimately, 21 HCC patients who had been treated by HIFU ablation were included in this study. They consisted of 12 men and 9 women, and their ages ranged from 65 to 80 years (mean, 73 years). HCC was diagnosed on the basis of the diagnostic imaging findings ($n = 19$) or pathological findings in biopsy specimens ($n = 2$). It was possible to establish the diagnosis of HCC without a biopsy when the lesions were 20 mm or less in size because both CE US and CE CT or CE MRI showed typical imaging findings ($n = 19$) [18,19]. Biopsy was performed with a 21-gauge fine-needle (Sonopsy; Hakko, Tokyo, Japan) under conventional US guidance. The size of the hepatic lesions was assessed by fundamental US. HCC dimensions ranged from 10 to 26 mm, and their mean maximum diameter \pm SD was 16.3 ± 3.6 mm. All patients had cirrhosis, and the diagnosis was made histologically and/or clinically. The etiology of the cirrhosis was alcohol in 2 cases and hepatitis C in 19 cases. According to child's classification, 18 patients had Class A cirrhosis and 3 had Class B. The characteristics of the subjects are shown in Table 1.

2.2. HIFU ablation procedure

The HIFU ablation procedure was guided by real-time US. A HIFU system (designed by Chongqing Hifu Co., Ltd., Chongqing, China) was used to perform the HIFU ablation. An HIFU6150S US imaging unit was installed on the HIFU system to obtain real-time US images during HIFU ablation. A 2–5-MHz imaging probe is located in the center of the HIFU transducer and it is mounted in a reservoir of degassed water [4,5]. Therapeutic US energy was produced by a HIFU transducer with 1.0 MHz operating frequency. Both imaging and therapeutic ultrasound beams are directed upward. The degassed water provides acoustic coupling between transducer and patient. Each patient in this study underwent a single HIFU ablation. When copious body hair was in the path of the HIFU beam, we removed the hair with depilatory cream instead of a razor to avoid making small cuts because of the a possibility that they would facilitate skin burns during exposure to HIFU. The patient's skin in the path of the HIFU beam was degassed before the ablation. After anesthesia was induced, the patient was carefully positioned in either the prone position ($n = 13$) or right lateral position ($n = 8$), so that the skin overlying the lesion to be treated would be easily put into contact with degassed water. The vertical ultrasound mode was chosen for the treatment. Before HIFU treatment, the dimensions of the region to be ablated were estimated and recorded in the cranial-caudal (length), transverse (width), and anteroposterior (height) directions to provide reference values to assess the CE 3D US and CE CT findings after the session. First, we made a treatment plan. For example, when the tumor was 20 mm long, we planed ablation in 5 planes 5 mm apart. We selected the plane which exhibited the largest portion of the lesion to ablate first. Ultrasound power was increased stepwise until a power of 300–450 W was reached. We performed HIFU ablation in degassed water at 16–21 °C and replaced it when the temperature of the water rose above 22 °C. Treatment consisted of a combination of single ultrasound pulses and multiple overlapping ultrasound pulses directed at the target tumor, but we mainly used single pulses. Gray-scale changes visualized by B-mode diagnostic US allowed the tissue response during treatment. Namely, after ablation, echogenicity of gray-scale increased. We then ablated the other planes. After we ablated the bottom area of all planes, we changed the targeted areas from bottom to the upper portion of the lesion and continued ablation [5]. We temporally stopped HIFU ablation when the increased echogenicity of gray-scale (transient hyperechoic zone) extended beyond the tumor margin. After the increased echogenicity of gray scale recovered to almost the same echogenicity as before treatment, CE 3D US was performed to evaluate the therapeutic effect and decide when to stop the HIFU ablation. When the tumor was diagnosed as residual tumor, we continued the HIFU ablation. When the tumor had been adequately ablated, we ended the HIFU ablation (Fig. 1). We measured "treatment time" as the interval between the start of treatment planning and the completion of the ablation session, and we measured "sonication time" as the total duration of exposure to HIFU. When the patient could not endure the treatment any more, we ended the ablation even when the therapeutic effectiveness was evaluated as residual tumor by CE 3D US.

2.3. CE 3D US imaging

CE 3D US was performed before HIFU ablation and immediately, 1 week, and 1 month after HIFU ablation. The 3D images were acquired with a LOGIQ 7 ultrasound machine (GE Healthcare, Milwaukee, WI) and a convex volume 4D3C-L probe with 2.0–5.5-MHz frequency. The probe automatically swept the region contained in a volume of interest (VOI), which had been determined prior to obtaining the volume according to the size and position of the

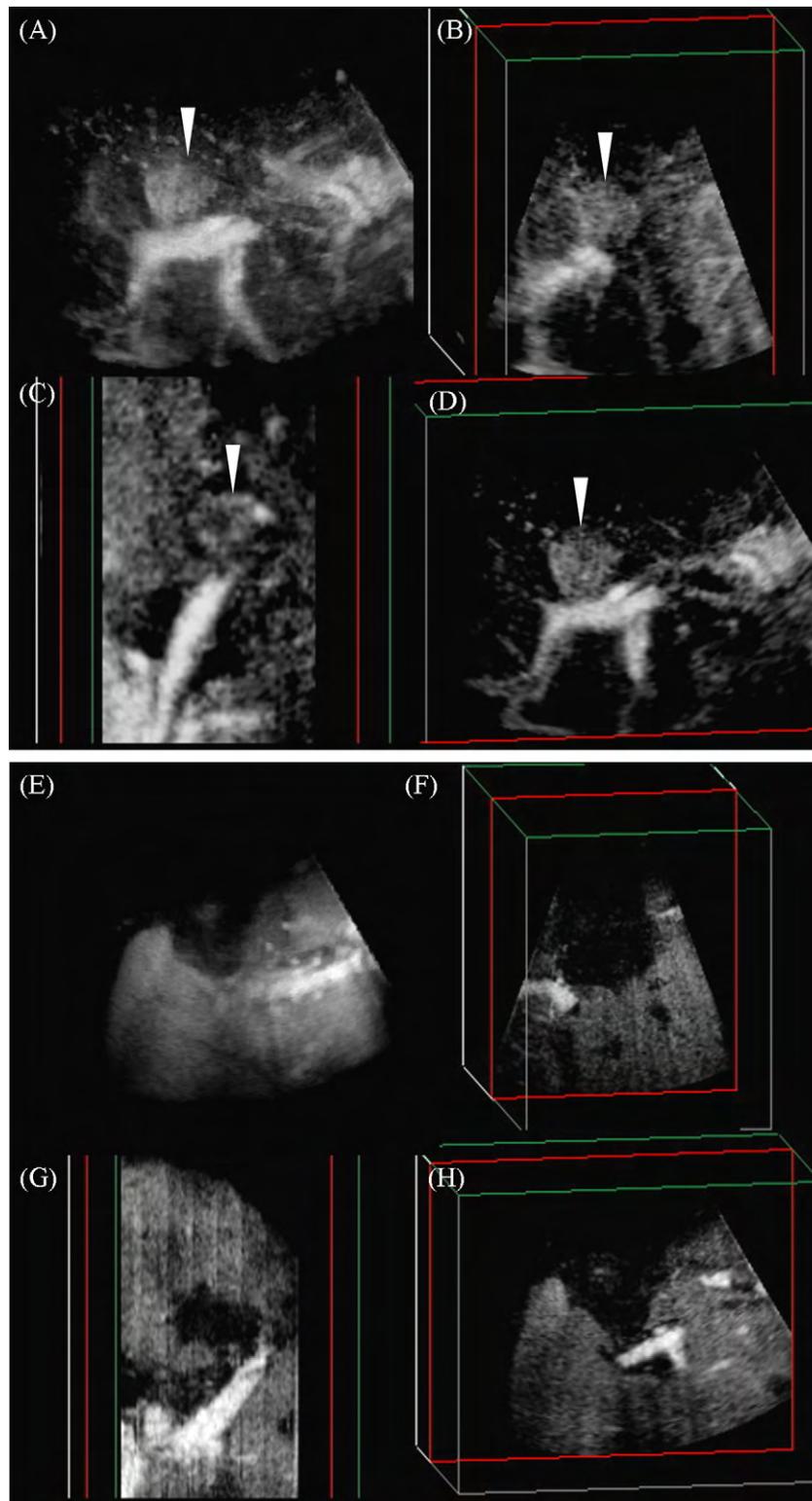


Fig. 1. A 69-year-old man with hepatocellular carcinoma (maximum diameter 18 mm) in segment V of liver. (A–D) Display of a sonographic angiogram rendered by using maximum intensity (A) and 3D plane B, which could be translated from right to left (B), plane C, which can be translated from down to up (C), plane A, which could be translated from front to back (D) on early phase contrast-enhanced three-dimensional ultrasonography images obtained before HIFU ablation shows homogeneous enhanced tumor adjacent to the portal vein. Arrowheads indicate the margin of tumor. (E–H) Display of sonographic angiogram rendered by using average intensity (E) and 3D plane B, which could be translated from right to left (F), plane C, which could be translated from down to up (G), plane A, which can be translated from front to back (H) on middle phase contrast-enhanced three-dimensional ultrasonography images obtained immediately after the HIFU ablation shows necrotic areas as perfusion defects adjacent to the portal vein. (I–J) Before the HIFU ablation, arterial phase contrast-enhanced CT image shows the HCC lesion as a high-attenuation area (arrowheads) (I). One month after HIFU ablation, adequate ablation was detected as an area of low attenuation in the arterial phase contrast-enhanced CT image (J). No local tumor progression was detected on contrast-enhanced CT images during the 18-month follow-up period.



Fig. 1. (Continued).

tumor. A scan angle of 45° was selected for use in this study because all lesions were small, and the mean scanning time was about 6 s.

Prior to obtaining the volume, all subjects received an intravenous bolus injection of 0.2 mL Sonazoid via a 24-gauge cannula into a forearm vein, followed by 2 mL of a 5% glucose solution and subsequent infusion of a 5% glucose solution at 10 mL/min. The CE 3D US procedure was divided into three phases: an early phase, 10–60 s after injection of the contrast agent; a middle phase, 80–120 s after injection of the contrast agent and a late phase, more than 5 min after injection of the contrast agent. The coded harmonic angio (CHA) mode with high-mechanical index (0.5–0.9) at 8–13 frames per second was used for CE 3D US in the early, middle, and late phase. The focus point was set beneath the tumor. The focus point was set at the bottom of the tumor. The data acquired were stored as cineloops in the hard disk of the ultrasound imaging system.

After CE 3D scanning, the 3D images were reconstructed using the functionalities of the ultrasound imaging system. In each contrast phase tomographic ultrasound images (TUI) in view of parallel slices were reconstructed in three orthogonal planes, i.e., plane A, which could be translated from front to back in the VOI, plane B, which could be translated from right to left, and plane C, which could be translated from up to down. The distance between two adjacent slices could be adjusted in order to show the desired regions. The mean times of these procedures were about 20 s. Sonographic angiograms were reconstructed in angio-like views during the early phase and middle phase by using various rendering modes. The maximum intensity mode for displaying the maximum intensity gray value of the VOI, mixed with the surface mode for displaying the gray value on the surface of the object, was used to visualize tumor vessels and early tumor enhancement before HIFU ablation and to detect residual viable portions of the HCC lesion after HIFU ablation, while the average-intensity mode for displaying the average-intensity gray value of the VOI, mixed with the surface mode, was used to describe the unenhanced areas, such as ablated areas with perfusion defects after treatment. The mean duration of this reconstruction procedure was about 45 s. TUI in three orthogonal planes and sonographic angiogram images together with raw volume data were stored on the hard disk of the ultrasound imaging system [15–17].

2.4. CE CT imaging

Before the HIFU ablation, 1 week and 1 month after the HIFU ablation, and during the follow-up period, CT scanning was performed with a commercially available CT scanner (8- or 16-channel multi-detector-row CT scanner; Aquilion TSX-101A or Aquilion 16, Toshiba Medical Systems Co., Ltd., Tokyo, Japan) with the following protocol: tube voltage 120 kV; tube current, auto mA exposure setting; reconstruction section and interval thickness, 5 mm; detec-

tor configuration, 16×1 mm; pitch, 15; and 0.5 s per rotation. The subjects were divided into a group weighing under 70 kg, who were injected with a 300-mgI/mL dose of the non-ionic contrast medium iopamidol (Iopamiron 300; Bayer Healthcare, Osaka, Japan) and a group weighing 70 kg or more, who were injected with a 370-mgI/mL dose of iopamidol. A catheter placed in the peripheral vein of the antecubital fossa was used to administer 100 mL of contrast medium at a rate of 3 mL/s with a power injector (Dual shot GX, Nemoto Kyorindo, Tokyo, Japan). The trigger point for starting the arterial phase scan was set at a 230-Hounsfield unit enhancement over the baseline attenuation of the abdominal aorta, and was confirmed with an automatic-bolus-tracking program (Real-Prep; Toshiba Medical Systems Co, Ltd., Tokyo, Japan). Scanning for the portal venous phase and the equilibrium phase was performed at 70 and 180 s, respectively, after the start of the contrast agent injection.

2.5. MRI

Before the HIFU ablation, 1 week and 1 month after the HIFU ablation, and during the follow-up period, MRI was performed at 3.0T with a superconductive Signa HDX 3.0T system (GE Healthcare, Milwaukee, WI). The MRI examination consisted of acquisition of precontrast and postcontrast images using high-resolution gradient-echo (GRE) sequences with T1 fat saturation images were obtained with 4.8/1.9 (repetition time (TR) ms/echo time (TE) ms), flip angle of 12° , matrix size of 320×192 , field of view of ≤ 100 mm, section thickness of 4 mm, intersection gap of 0–2 mm, and one breath-hold. The scan delay times were 22 s, 60 s, and 30 min after bolus injection of a 0.025 mmol dose of gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (d-EOB-DTPA) (Primovist; Bayer Schering Pharma)/kg body weight via an antecubital vein flushed with 20 mL of bolus sterile saline solution.

2.6. Image analysis

Three to seven days prior to the HIFU ablation, CE 3D US and CE CT ($n = 18$) or CE MRI ($n = 3$) were performed to determine tumor location and tumor enhancement. Immediately after the HIFU ablation, CE 3D US alone was performed to evaluate the therapeutic effects and when to end the HIFU ablation. CE 3D US and CE CT or CE MRI were performed about 1 week and 1 month after the HIFU ablation to evaluate the therapeutic effect. If no residual tumor was detected on the 1-month CE 3D US and CE CT or CE MRI images, follow-up CE CT or CE MRI was performed every 3 months thereafter. If residual tumor was detected, an RF ablation procedure was performed. The follow-up period of this study was until July 2009.

To evaluate the effect of treatment, the images acquired by the two modalities before and after HIFU ablation were reviewed by two experienced gastrointestinal radiologists, neither of whom was

involved in the HIFU procedure and both of whom were blinded to clinical information and other radiological findings. The images were reviewed independently. As they reviewed the CE 3D US images, the two radiologists had the option of interactively translating the multi-planar images and changing the angle of view of the angiograms, if necessary. Finally, the two radiologists conferred and arrived at a consensus.

When the lesion sites were evaluated on the CE 3D US images after the HIFU, the ablation was evaluated as adequate if after ablation a non-enhancing area seen in the early phase, middle phase, and late phase covered the hypervascular enhancement (typical HCC case) or hypovascular area (atypical HCC case) seen in the early phase before HIFU. Residual tumor on CE 3D US images was diagnosed when an area within the tumor was detected with hypervascular enhancement in the early phase and homogeneous enhancement in the middle phase, and was hypoechoic in the late phase of CE 3D US after ablation in typical HCC case. In atypical HCC case, residual tumor on CE 3D US images was diagnosed when an area within the tumor was detected with isoechoic in the middle phase, and was isoechoic in the late phase of CE 3D US after ablation. When the lesion sites were evaluated on CE CT or CE MRI after HIFU, the images were evaluated as showing adequate ablation if an ablated area covered the HCC lesion seen before HIFU. Residual tumor on CE CT or CE MRI images was diagnosed an ablated area did not cover the HCC lesion seen before HIFU.

The findings on the CE CT or CE MRI images acquired every 3 months as follow-up examinations were evaluated as indicating local tumor progression if hypervascular enhancement in the margin of the ablated areas in the arterial phase showed wash out in the equilibrium phase.

2.7. Statistical analysis

The Kappa test was calculated to evaluate agreement between the two modalities and inter-reader agreement and graded as poor (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), good (0.61–0.80), or excellent (0.81–1.00). Numerical data were expressed as means \pm SD. Group data were compared by one-way analysis of variance. Differences within the same group were evaluated by the paired *t*-test. Relationships between nominal variables and rates were analyzed by Fisher's exact test. A *p*-value less than 0.05 was considered statistically significant. Statistical analyses were performed using a computer software package (SPSS 11.0, Tokyo, Japan).

3. Results

3.1. Treatment by means of a single HIFU ablation

Table 2 shows the results of treatment by a single HIFU ablation. Mean treatment time was 203 ± 81.9 (44–407) min. Mean sonication time was 833 ± 365 (296–1691) s. Based on the 1-month CE CT

or CE MRI findings as the reference standard, adequate ablation was achieved in 18 (86%) of the 21 HCC lesions. The difference between the distance between the skin surface and the bottom of the lesion was significantly smaller in the adequate ablation group ($n = 18$) than in the residual tumor group ($n = 3$) ($p < 0.05$).

3.2. CE 3D US before HIFU ablation

In the CE 3D US images before HIFU ablation, 19 of the 21 HCCs appeared as hypervascular areas with homogeneous enhancement in the early phase, as homogeneous enhancement in the middle phase, and as hypoechoic areas in the late phase, and on the CE CT images ($n = 16$) or CE MRI images ($n = 3$) they appeared as hypervascular in the arterial phase and as washed out areas in the equilibrium phase. The remaining 2 HCCs appeared as hypovascular areas in the early and middle phase of CE 3D US images and as isoechoic areas in the late phase, and they appeared as hypo-attenuation areas in the arterial phase CE CT images and as low-attenuation areas both in the portal and equilibrium phases. Both lesions were diagnosed as well differentiated HCC pathologically.

3.3. CE 3D US during HIFU ablation

Four of the 21 HCC lesions were evaluated as having been adequately ablated by CE 3D US during the HIFU ablation. The other 17 HCC lesions were evaluated as residual tumors and the HIFU session was continued.

3.4. CE 3D US after HIFU ablation

No subjects were excluded from the analysis during the follow-up period (range 9–24 months; mean, 15.6 months).

As shown in Table 3, immediately after the single HIFU session, 17 lesions were evaluated as adequately ablated by CE 3D US images, and the other four lesions were evaluated as residual tumors.

One week after the HIFU ablation, both the CE 3D US images and the CE CT or CE MRI images showed adequate ablation of 18 of the 21 lesions and the remaining three lesions as residual tumors. One lesion evaluated as a residual tumor immediately after the HIFU ablation was re-evaluated as having been adequately ablated by CE 3D US images and no local tumor progression was observed during the follow-up period.

One month after the HIFU ablation, another lesion was evaluated as a residual tumor based on the CE 3D US images, but this tumor was not detected on the CE CT images. At 1 month, 4 residual tumors were observed on the CE 3D US image. One of the 4 lesions showed obvious residual tumor. The patient with this tumor could not tolerate HIFU ablation because of difficulty in immobilizing the patient's body, and the patient was treated by RFA within 3 months thereafter. Two of the 4 lesions were diagnosed as small residual

Table 2
HCC lesions adequate ablation and residual unablated tumor with high-intensity focused ultrasound.

Characteristics	Adequate ablation a ablation	Residual tumor	<i>p</i> -Value
Number of lesions	18	3	
Location in the right lobe/left lobe	7/11	3/0	0.090
Segment number 1/2/3/4/	0/1/8/2	0/0/0/0	
Segment number 5/6/7/8	4/1/1/0	0/1/1/1	
Tumor size (mean \pm SD)	16.2 \pm 3.9	16.3 \pm 1.5	0.962
Distance between skin surface and the deepest margin of the lesion (mean \pm SD, mm)	49.6 \pm 15.9	72.0 \pm 9.8	0.031
Sonication time (mean \pm SD, s)	813 \pm 388	953 \pm 173	0.553
Treatment time (mean \pm SD, min)	195 \pm 85	255 \pm 19.7	0.247

"Sonication time" means total time of exposure of HIFU ablation. "Treatment time" means the interval between the start of treatment planning and completion of the ablation session.

Table 3
Evaluation by contrast-enhanced three-dimensional US and contrast-enhanced CT after HIFU ablation to small HCC lesions.

Number of lesions	Immediate CE 3D US	One-week CE 3D US	One-week CE CT or CE MRI	One-month CE 3D US	One-month CE CT or CE MRI	Follow-up CE CT or CE MRI
<i>n</i> = 16	Adequate ablation	Adequate ablation	Adequate ablation	Adequate ablation	Adequate ablation	No local tumor progression during follow-up period (range 9–24 months)
<i>n</i> = 3 ^a	Residual tumor	Residual tumor	Residual tumor	Residual tumor	Residual tumor	Local tumor progression on 3-month CE CT ^a
<i>n</i> = 1 ^b	Adequate ablation	Adequate ablation	Adequate ablation	Residual tumor	Adequate ablation	Local tumor progression on 12-month CE CT ^b
<i>n</i> = 1	Residual tumor	Adequate ablation	Adequate ablation	Adequate ablation	Adequate ablation	No local tumor progression during follow-up period (11 months)

"Immediately CE 3D US", "One-week CE 3D US", and "One-month CE 3D US" mean contrast-enhanced three-dimensional ultrasonography performed immediately, 1 week, and 1 month after high-intensity focused ultrasound (HIFU) ablation. "One-week CE CT or CE MRI", "One-month CE CT or CE MRI" and "Follow-up CE CT or CE MRI" mean contrast-enhanced CT or contrast-enhanced MRI performed 1 week after HIFU ablation, 1 month after HIFU ablation and that performed every 3 months during the follow-up period, respectively.

^a One of the 3 lesions was obviously residual tumors and the patient was treated with radiofrequency ablation within 3 months after the HIFU ablation. Two of the 3 lesions were diagnosed as small residual tumors. They were treated by radiofrequency ablation between 3 and 6 months after HIFU ablation.

^b The lesion with local tumor progression on the follow-up CE CT was treated by radiofrequency ablation.

tumors on the CE CT images and showed local tumor progression 3 months after HIFU ablation (Fig. 2). Because these residual tumors were located behind the costal bone or near the right lung, both of which would attenuate the HIFU beams, they were treated by RFA between 3 and 6 months after the initial HIFU ablation. The remaining lesion, which was recognized as residual tumor 1 month after the HIFU ablation, exhibited local tumor progression 12 months after the HIFU ablation. None of the 17 lesions evaluated as adequately ablated including the lesions located behind the portal vein and bile duct showed local tumor progression during the follow-up period.

Inter-reader variation in the evaluations of the effects of HIFU ablation was minimal. The two readers' evaluations of 20 of the 21 lesions were the same, and they arrived at a consensus regarding evaluations of the other 1 lesion.

There was discordance between the evaluations of one of the 21 lesions on the CE 3D US images immediately after the HIFU ablation and the evaluations on the CE CT or CE MRI images obtained at 1 month after HIFU ablation. The evaluations of the other 20 lesions on the CE 3D US and CE CT or CE MRI images were concordant. The kappa value for agreement between the findings of two blinded observers based on the CE 3D US images and images obtained by the other modalities was 0.83.

When the CE CT or CE MRI findings 1 month after the HIFU ablation were used as the reference standard, the sensitivity, specificity, and accuracy of CE 3D US immediately after the HIFU ablation for the diagnosis of adequate ablation were 100%, 75%, and 95%, respectively.

4. Discussion

A novel second-generation ultrasound contrast agent, Sonazoid, which has been commercially available in Japan since January 2007, was used for CE 3D US imaging in our study. Sonazoid consists of microbubbles of perfluorobutane gas with phospholipid monolayer shells. The stable Sonazoid microbubbles have been reported to be phagocytosed by reticuloendothelial cells in the liver parenchyma 5 min after administration, and thus they support a protracted contrast imaging [20–25]. Occurrences of side effects of Sonazoid are low (albuminuria in 1.6%, diarrhea in 1.6% of 193 cases) [25]. We used the CHA mode and high-mechanical index contrast conditions,

which reduce the concentration of microbubbles in microvessels, but not in relatively large vessels, such as tumor vessels and portal veins and thereby allow clear visualization of tumor vessels and tumor enhancement in the early phase without interference from normal liver parenchyma enhancement [15–17,20]. It also eliminated the background B-mode and emphasized the microbubbles in the vessels, and we were able to differentiate the residual tumors from necrotic areas after ablation. Using this method, we recently demonstrated that CE 3D US with Sonazoid by demonstrating the ablated areas and residual tumor in three dimensions was shown to be useful for early evaluation of therapeutic efficacy of percutaneous RFA of HCC lesions [26]. In the present study, the increased echogenicity of HCC lesions caused by HIFU recovered to almost the same echogenicity as before treatment within a few minutes after the HIFU ablation, and we were able to perform CE 3D US to evaluate the therapeutic effect and immediately decide when to stop the HIFU ablation. We also used CE 3D US to evaluate treated areas and to detect untreated portions of the HCC, and we were then able to perform additional HIFU ablation of the untreated portions.

It took a longer HIFU treatment time than expected to adequately ablate even small HCC lesions. We decided not to treat HCC lesions located more than 9 cm below the skin surface by HIFU ablation in this study because these lesions were not visualized clearly by the monitoring US of HIFU equipment. However, it was possible to ablate the HCC lesions located behind the portal vein or a bile duct, for which RFA is contraindicated. CE 3D US under these contrast conditions enabled us to evaluate the correlation between the location of the HCC lesions and adjacent relatively large vessels.

The evaluations by CE 3D US immediately after the HIFU ablation and by CE CT or CE MRI 1 month after the HIFU ablation were concordant for 20 of the 21 lesions. The other lesion, which was evaluated as a residual tumor immediately after the HIFU ablation was re-evaluated as adequately ablated by CE 3D US, and no local tumor progression was observed during the follow-up period. We concluded that the margin of the lesion was influenced by reactive hyperemia caused by exposure to HIFU. Another lesion was evaluated as a residual tumor on CE 3D US images 1 month after the HIFU ablation, but was not detected on the CE CT images. In both cases the margins of the areas ablated by HIFU were almost the same as the margins of the targeted HCC lesions. To prevent misdiagnosis, such as false positive diagnoses and false negative diagnoses by CE 3D US, it will be necessary to ablate wider areas and sufficiently

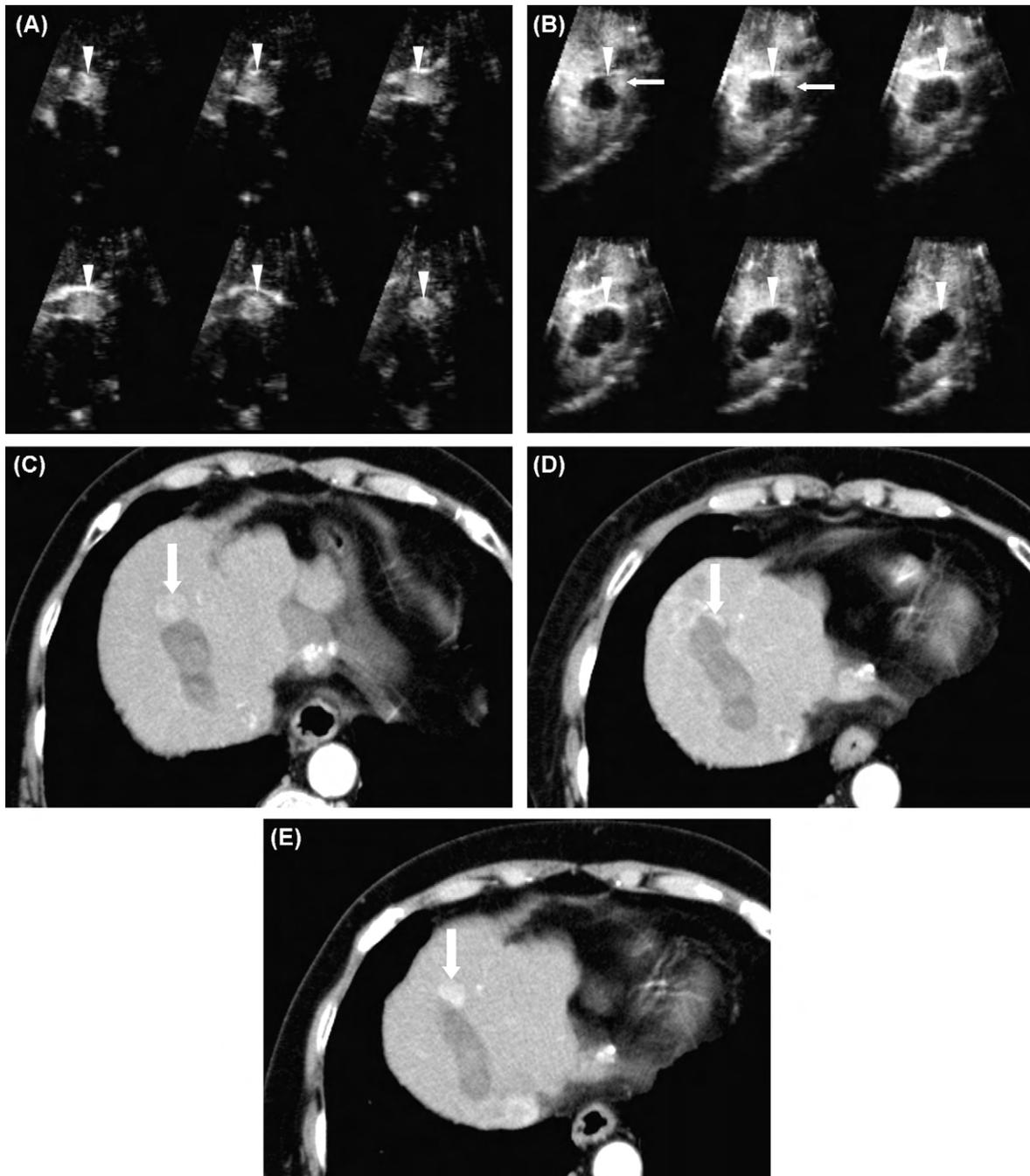


Fig. 2. A 70-year-old woman with hepatocellular carcinoma (maximum diameter 18 mm) in segment VIII of liver. (A) Tomographic ultrasound images in plane B, which could be translated from right to left, on early phase contrast-enhanced three-dimensional ultrasonography images obtained before the HIFU ablation show homogeneous enhanced tumor adjacent to the hepatic artery. Arrowheads indicate the margin of tumor. (B) Tomographic ultrasound images in plane B, which could be translated from right to left, in middle phase contrast-enhanced three-dimensional ultrasonography images obtained immediately after HIFU ablation show a small enhanced area adjacent to the hepatic artery (arrows). Arrowheads indicate the margin of tumor. (C–E) Before the HIFU ablation, arterial phase contrast-enhanced CT shows the HCC lesion as a high-attenuation area (arrowheads) (C). One month after HIFU ablation, arterial phase contrast-enhanced CT shows almost the entire area of the lesion as a hypo-attenuation area, but a small high-attenuation area is observed in the peripheral region of tumor (arrow) (D). This arterial phase contrast-enhanced CT image 3 months after the HIFU ablation shows an enlarged high-attenuation area (arrow) (E).

cover the HCC lesion seen on the images before the HIFU ablation [26].

The use of ultrasound contrast media in the experimental models made it possible to increase the volume ablated by HIFU [27–31]. In the present study, 17 of the 21 HCC lesions were evaluated as residual tumors by CE 3D US examination during the HIFU ablation, and we continued it. There were no side effects such as skin burns in these 17 patients after HIFU ablation. However, 4 of the 17 lesions were judged to have been incompletely ablated.

Based on to these results, we concluded that 0.2 mL per body of Sonazoid, which was small concentration of ultrasound contrast media compared with that of experimental models, did not emphasize the increase of ablated areas caused by HIFU. More clinical cases need to confirm whether a clinical usage of the ultrasound contrast media has an influence on the HIFU ablation or not.

After HIFU ablation of the bottom of the lesion, we changed the target point from the bottom to the upper portion of the

lesion 5 mm apart and shot the ultrasound beams toward the target. Precise measurements of tumor size are needed for adequate planning and adequate ablation by HIFU. 3D US provides real-time images and it is easy to measure tumor size in different directions by a single automatic sweep scan. In the future, if it becomes possible to expose all bottom areas of the lesion to ultrasound beams using by plane C, which can be translated from down to up, it would be easy to ablate the whole tumor. Decreasing the number of plane changes would shorten treatment time.

The results of this study showed significantly smaller distance between the skin surface and the bottom of the lesion in the adequate ablation group and than in the residual tumor group. Adequate ablation of all 13 HCC lesions treated with the patient in the prone position was achieved. However, adequate ablation of 3 of the 8 lesions treated with the patient in the right lateral position was not achieved. There are several reasons for the occurrence of residual HCC lesions by HIFU. First, lesions located deep in the liver, far from the skin surface, are more likely to be insufficiently ablated because of attenuation of ultrasound beams. Interposition of the intra- and extra-peritoneal abdominal fat itself also attenuates ultrasound beams. Second, the costal bones and right lung reduce penetration by the ultrasound beams, especially when the patient is in the right lateral position. Saline injection of the pleural cavity may be useful in producing an acoustic window that prevents reflection to the lungs [6]. Partial rib resection is another means of creating a better acoustic pathway for HIFU ablation [9]. Third, the right lateral position is an unstable position when the patient is awake because of the difficulty of immobilizing the patient's body. Patients tend to move because of the pain caused by exposure to the ultrasound beams. Slight changes in the patient's position result in a failure to strike the targeted HCC lesion accurately. Improvement of the immobilization apparatus and effective pain prevention is needed to solve this problem. Fourth, patients' respiratory movements also slightly alter the location of the HCC lesions, resulting in a failure to accurately strike the targeted areas of HCC lesions. To minimize this problem, patients must hold their breath for at least 10 s despite the pain caused by exposure to the ultrasound beams. We are looking forward to the development of software that automatically synchronizes firing of the HIFU beams with patients' respiratory movements. Fifth, the grade of vascularization of the HCC lesions themselves affects the results of HIFU ablation. It is easier to induce complete necrosis of hypovascular HCCs by HIFU ablation than hypervascular HCCs because of the differences in tumor blood flow between these two types of HCC.

Since almost all of the HCC lesions in this study were small, less than 2 cm in the maximum diameter, and were located in suitable positions in the liver for HIFU ablation, they were clearly not observed due to cardiac or respiratory motion, abdominal gas, or costal bone shadows, that were occasionally seen the CE 3D US images [17].

5. Conclusions

CE 3D US can be used to immediately evaluate the therapeutic effect of HIFU on small HCC lesions. This modality has the potential to enable us to detect residual portions of HCCs and perform additional HIFU ablation of the untreated portions.

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