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ORIGINAL ARTICLE

Low mechanical index contrast mode versus high mechanical index contrast mode: which is a more sensitive method for detecting Sonazoid microbubbles in the liver of normal subjects?

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Abstract
Purpose Using the low mechanical index (MI) contrast mode and the high MI contrast mode of contrast-enhanced ultrasonography, we evaluated which method is more sensitive for detecting Sonazoid microbubbles in the liver of normal subjects.
Methods Thirteen normal subjects received an intravenous bolus injection of 0.2 mL of Sonazoid. We defined the intensity difference as the intensity post-injection minus the intensity pre-injection. We evaluated the intensity difference at the portal vein using both the low MI (0.21–0.23) and the high MI (0.7–1.2) at 1 min, at every 10 min between 10 to 60 min, and at every 30 min between 60 to 300 min post-injection. The intensity difference at the liver parenchyma was also evaluated at eight points (1, 10, 30, 60, 120, 180, 240, and 300 min) using the low MI and at three points (1, 10, and 300 min) using the high MI.
Results The intensity differences at the portal vein measured using high MI were significantly higher than those measured using the low MI at each point between 1 and 240 min ($P < 0.01$) and at 270 min post-injection ($P < 0.05$). The intensity differences at the liver parenchyma measured using the high MI were also significantly higher than those measured using the low MI at each time point ($P < 0.01$).
Conclusion Compared with the low MI, the high MI is more sensitive for detecting Sonazoid microbubbles in the liver of normal subjects.

Keywords Contrast-enhanced ultrasonography · Low mechanical index contrast mode · High mechanical index contrast mode · Intensity difference · Sonazoid microbubbles

Introduction

Sonazoid (Daiichi Sankyo, Tokyo, Japan), a second-generation formulation of a lipid-stabilized suspension of a perfluorobutane gas microbubble contrast agent, has been used clinically in Japan for patients with liver tumors and for harmonic gray-scale ultrasonography (US) since January 2007 [1–6]. Sonazoid microbubbles have a higher stability [6, 7] than other second-generation ultrasound contrast agents, such as SonoVue (Bracco, Milan, Italy) [8]. The two contrast agents mentioned above can be used to evaluate vascular phase images. In addition to the vascular phase, Sonazoid can be used to scan the entire liver repeatedly using a low mechanical index (MI) contrast mode, providing detailed post-vascular phase images [9]. In contrast, SonoVue, which requires a low MI contrast mode, has a delayed phase (120 s post-injection), but it does not have a post-vascular phase [8]. This difference can likely be explained by the fact that only 7.3 % of injected SonoVue was phagocytosed by reticuloendothelial (Kupffer) cells in rats, whereas 99 % of Sonazoid were phagocytosed [10].

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Table 1 Characteristics of normal subjects

Characteristics	
No. of subjects	13
Age (mean ± SD, range; years)	42.8 ± 9.7, 25–62
Sex male/female	11/2
Body weight (mean ± SD, range; kg)	65.1 ± 7.8, 51–83
Body height (mean ± SD, range; m)	1.69 ± 0.05, 1.61–1.77
Body mass index (mean ± SD, range; kg/m^2)	22.5 ± 2.0, 19.7–26.5

There are two ways to demonstrate a contrast effect on contrast-enhanced (CE) US with Sonazoid: a low MI contrast mode at a low MI, and a high MI contrast mode at a high MI [6, 11–16]. The former method enables repeated observations of the liver in a real-time manner because the microbubbles undergo less breakdown. However, the evaluation of contrast findings for deeply located hepatic lesions might not be feasible because of the attenuation of the US beam. Another potential limitation of this mode is the difficulty in assessing perfusion defects in hyperechoic nodules since the pre-enhancement echogenicity may affect the post-enhancement appearance. On the other hand, the latter makes microbubble breakdown easier and could eliminate the B-mode signal from background tissue. A previous study showed the usefulness of a high MI technique for deeply located hepatic lesions and hyperechoic nodules [6, 12–16]. Although there might be a difference in the sensitivity for the detection of Sonazoid microbubbles between the low MI and the high MI contrast modes, little is known about the detectability under different contrast modes at different MI settings.

Against this background, we performed CEUS with Sonazoid using both the low MI and the high MI contrast modes in the liver of normal subjects and compared the intensity difference before and after the Sonazoid injection. The aim was to investigate MI-related differences (low MI contrast mode at a low MI versus high MI contrast mode at a high MI) in microbubble detectability when performing CEUS with Sonazoid.

Materials and methods

Subjects

This prospective study was approved by our institutional review board, and written informed consent was obtained from each subject. This study was conducted between September 2012 and May 2014. A total of 13 normal volunteers (11 males and 2 females) who were at least 20 years old were enrolled in this study. None of the subjects had cardiovascular-respiratory diseases or liver diseases (including fatty liver). All of the subjects' asparate aminotransferase, alanine aminotransferase, and γ-glutamyl transpeptidase values were within the normal ranges. Apparently obese subjects were not included in this study. Subject characteristics are summarized in Table 1.

Methods

CEUS procedure

To avoid the influence of increases in portal venous flow caused by diet [17], all the examinations were performed after the subjects had fasted. No foods or liquids with calories were ingested until the end of the examination. During the study, all the subjects remained lying down quietly.

CEUS was performed using the LOGIQ7 ultrasound system (GE Healthcare, Milwaukee, WI) with a 3.5-MHz convex probe. The focus position was set at under the right branch of the portal vein (Fig. 1c). We used the low MI contrast mode (coded phase inversion mode) at a low MI (transmission power, 20–35 %; MI, 0.21–0.23) and the high MI contrast mode (coded harmonic angio mode) at a high MI (transmission power, 100 %; MI, 0.7–1.2) at 2 frames per second: we called this method "high MI intermittent imaging" [6, 12–16]. Sonazoid was used as the contrast agent. All 13 subjects

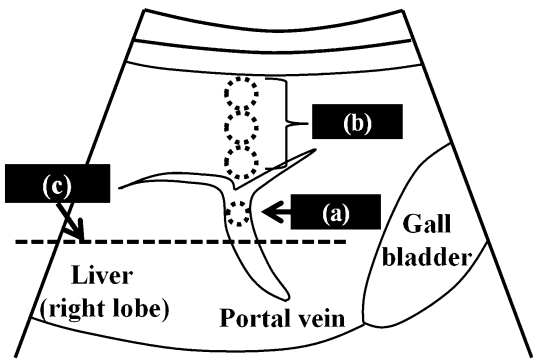

Fig. 1 Measurement of the intensity at the portal vein and in the liver parenchyma. **a** For CEUS images obtained using the low MI and the high MI contrast modes, the operator manually set a region of interest (ROI) with a diameter of 0.59 cm within the portal vein. **b** Three ROIs, each with a diameter of 1.2 cm, were set vertically, and the intensity of the liver parenchyma was measured; the ROIs were placed so as to avoid relatively large vascular structures, such as the portal vein or the hepatic vein. **c** We set the focus position at a depth that enabled a ROI to be placed on the right branch of the portal vein

received an intravenous bolus injection of 0.2 mL of Sonazoid, followed by 2 mL of 5 % glucose solution [6, 12–16].

Quantitative analysis of US images

Recording of US images

Using conventional US, we confirmed that none of the subjects had a fatty liver. Using CEUS with the low MI and the high MI contrast modes, US images (including those for the portal vein and the liver parenchyma) were recorded on the hard disk of a LOGIQ 7 ultrasound system by one operator (H.N.) before and after Sonazoid injection. To minimize the destruction of the Sonazoid microbubbles due to repeated scanning, we recorded images of the portal vein using cine clips of 2–3 s. Still images were captured from the cine clips, and the following evaluation was performed using the still images.

Intensity difference

Using each of the CEUS images obtained using the low MI and the high MI contrast modes, the operator manually set each of the ROIs within the right branch of the portal vein (Fig. 1a) or within the liver parenchyma (Fig. 1b). Using the intensity obtained from each ROI, we defined the "intensity difference" as the post-injection intensity minus the pre-injection intensity. Because of slight variations in the ROI intensity, we performed all measurements three times and calculated the average intensity, which was used as the final value [18].

Measurement of the intensity difference of the portal vein

Before and after Sonazoid injection, we set an ROI (with a diameter of 0.59 cm) at the right branch of the portal vein and measured the intensity within this ROI at 15 time points: before Sonazoid injection, 1 min post-injection, every 10 min up to 60 min (10, 20, 30, 40, 50, and 60 min post-injection), and then every 30 min up to 300 min (60, 90, 120, 150, 180, 210, 240, 270, and 300 min post-injection). For both the low MI and the high MI contrast modes, we compared the peak value of the intensity difference and the intensity difference obtained at other measurement times. We then compared the intensity differences at the portal vein as measured using the low MI contrast mode with that measured using the high MI contrast mode at the 15 different time points described above.

Measurement of the intensity difference in the liver parenchyma

It was difficult to avoid relatively large portal or hepatic veins if the ROIs were placed laterally at a depth of 2–4 cm from the skin surface. To solve this problem, we measured the intensity of the liver parenchyma using three ROIs, each with a diameter of 1.2 cm, placed vertically from the liver surface using both the low MI and the high MI contrast modes (Figs. 1b, 2a, b, d, e). The average value of these three ROIs was then defined as the intensity of the liver parenchyma. Using each of the CEUS images obtained using the low MI and the high MI contrast modes, the intensity difference before and after Sonazoid injection was then calculated.

Comparison of time course of intensity differences in the liver parenchyma as measured using the low MI contrast mode at a low MI

Using the low MI contrast mode, we scanned the liver parenchyma in Segment 3 at seven time points between 10 min and 300 min post-injection (10, 30, 60, 120, 180, 240, and 300 min). We then compared the peak value of the intensity difference and those differences obtained at the other measurement times.

Comparison of the time course of intensity differences in the liver parenchyma as measured using the high MI contrast mode at a high MI

Using the high MI contrast mode, we scanned the liver parenchyma in Segment 8 twice (1 and 10 min post-injection) and in Segment 3 once (300 min post-injection). We then compared the intensity difference of the liver parenchyma at each measurement time (1 versus 10, 1 versus 300, and 10 versus 300 min post-injection).

Comparison of the intensity difference in the liver parenchyma as measured using the low MI contrast mode at a low MI and using the high MI contrast mode at a high MI

For the CEUS images obtained using the low MI and the high MI contrast modes, we scanned the liver parenchyma at 1 min and at 10 min post-injection in Segment 8 and at 300 min post-injection in Segment 3. We compared the intensity difference of the liver parenchyma as measured using the low MI contrast mode with that measured using the high MI contrast mode at the three measurement times.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). The intensity differences between groups (low MI contrast mode vs. high MI contrast mode) were analyzed using the independent-samples t test. Individual data obtained using the low MI and the high MI contrast modes

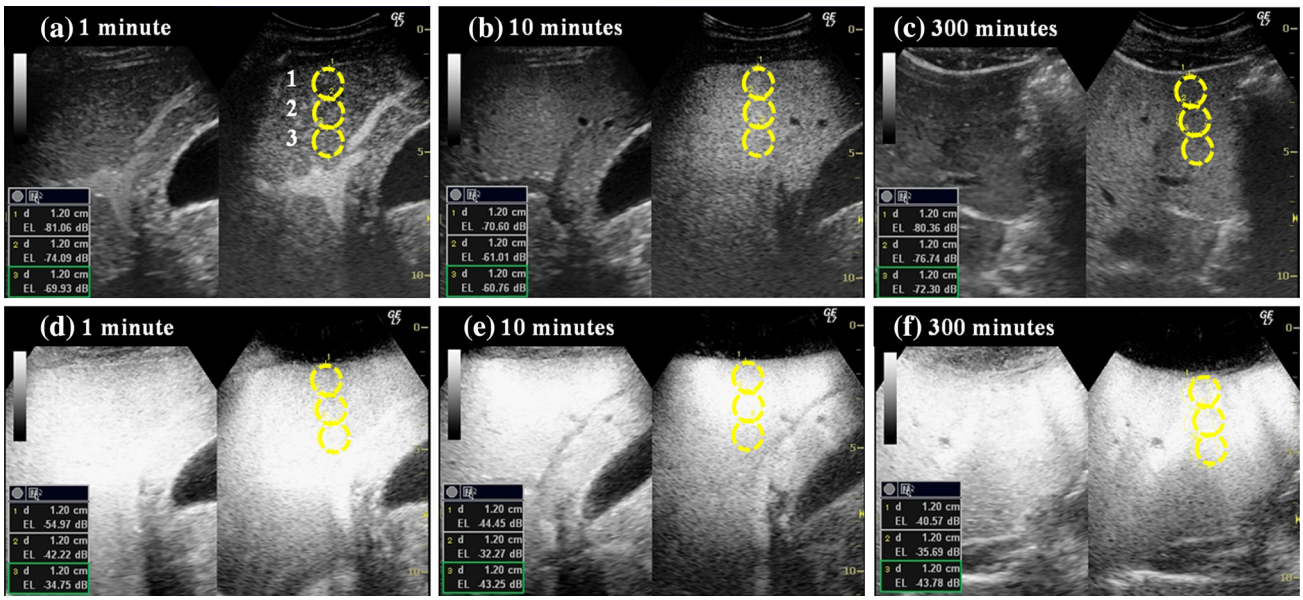

Fig. 2 Measurement of the intensity difference in the liver parenchyma. The *upper section* shows an image of the liver parenchyma obtained using the low MI contrast mode at a low MI. The *lower section* shows an image of the liver parenchyma obtained using the high MI contrast mode at a high MI. The images in **a**, **d** show Segment 8 at 1 min post-injection. The images in **b**, **e** show Segment 8 at 10 min post-injection. The images in **c**, **f** show Segment 3 at 300 min post-injection. Using the high MI contrast mode, the intensity differences in the liver parenchyma were similar at 1, 10, and 300 min post-injection. On the other hand, the intensity difference of the liver parenchyma at 10 min post-injection using the low MI contrast mode was higher than those obtained at 1 and 300 min post-injection. All the intensity differences in the liver parenchyma that were evaluated using the high MI contrast mode were higher than those evaluated using the low MI contrast mode at three measurement times (1, 10, and 300 min post-injection)

(intensity difference at the portal vein, intensity difference in the parenchyma) were analyzed using a paired *t* test. The level of statistical significance was set at $P < 0.05$. All the statistical analyses were performed using SPSS software version 21 (SPSS, Inc., Chicago, Ill., USA).

Results

Intra-observer variability

This study was evaluated by one examiner and one measurer. The intensities at the portal vein and the liver parenchyma were each measured three times. The range of the three intensity measurements was within 3 %.

Intensity difference at the portal vein

Low MI contrast mode at a low MI

The intensity differences at the portal vein as evaluated using the low MI contrast mode exhibited a peak value (mean ± SD: 21.1 ± 5.9 dB) at 1 min after the injection of Sonazoid. Significant differences were observed between the intensity difference obtained at 1 min post-injection and that obtained at every other measurement time from 10 to 300 min post-injection ($P < 0.01$, each) (Fig. 3).

High MI contrast mode at a high MI

The intensity differences at the portal vein as evaluated using the high MI contrast mode at a high MI exhibited a peak value (mean ± SD: 40.6 ± 4.7 dB) at 1 min post-injection. Significant differences were observed between the intensity difference obtained at 1 min post-injection and that obtained at every other measurement time from 10 to 300 min post-injection ($P < 0.01$, each) (Fig. 3).

Comparison of intensity difference at the portal vein as evaluated using the low MI contrast mode at a low MI and using the high MI contrast mode at a high MI

The intensity differences at the portal vein as evaluated using the high MI contrast mode were significantly larger than those evaluated using the low MI contrast mode at each time point from 1 to 240 min ($P < 0.01$) and at 270 min post-injection ($P < 0.05$). No significant difference was observed at 300 min post-injection (Fig. 3).

Low MI contrast mode at a low MI

The peak value of the intensity differences in the liver parenchyma in Segment 3 as evaluated using the low MI contrast mode occurred at 10 min post-injection. No

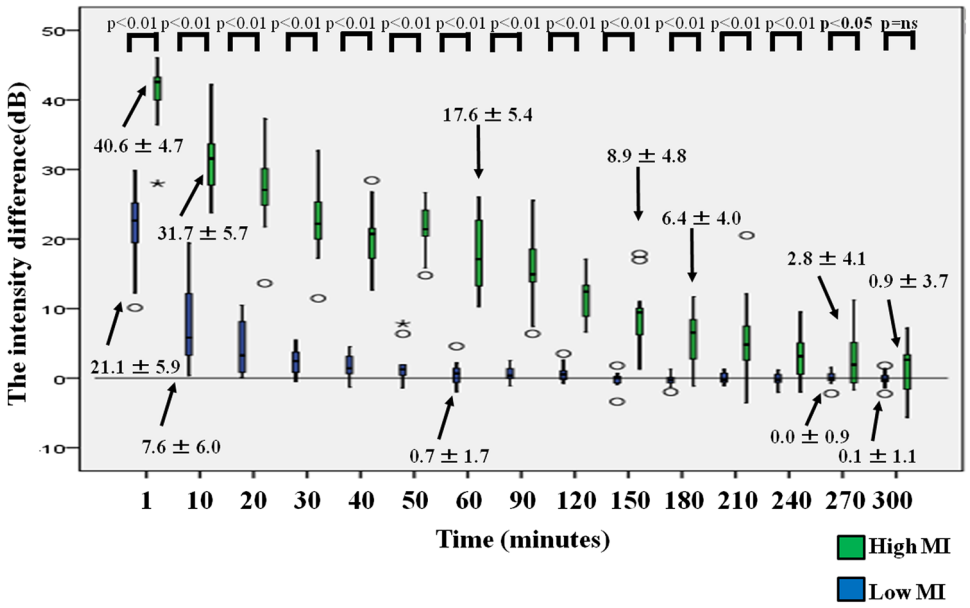

Fig. 3 Intensity differences at the portal vein as evaluated using the low MI and the high MI contrast modes ($n = 13$). Significant differences in the intensity differences at the portal vein were observed using the low MI contrast mode and the high MI contrast mode between 1 and 270 min post-injection ($P < 0.05$). The numbers in the graph show the mean ± SD. *ns* not significant, *low MI* low mechanical contrast mode, *high MI* high mechanical contrast mode

significant differences in the intensity difference of the liver parenchyma were observed from 30 to 120 min post-injection, compared with the value at 10 min post-injection. Significant differences in the intensity difference of the liver parenchyma were observed at 180 ($P < 0.05$), 240 ($P < 0.01$), and 300 min post-injection ($P < 0.01$), compared with that obtained at 10 min post-injection (Fig. 4).

High MI contrast mode at a high MI

Using the high MI contrast mode, no significant differences in the intensity difference of the liver parenchyma were observed at three measurement times (1 versus 10, 1 versus 300, and 10 versus 300 min post-injection) (Figs. 2, 5).

Comparison of intensity difference in the liver parenchyma as evaluated using the low MI contrast mode at a low MI and using the high MI contrast mode at a high MI

The intensity differences in the liver parenchyma as evaluated using the high MI contrast mode were significantly larger than those evaluated using the low MI contrast mode at three measurement times ($P < 0.01$) (Figs. 2, 5). The mean value of the intensity difference in the liver parenchyma at 10 min post-injection as evaluated using the low MI contrast mode was about 14 dB [Segment 3: 13.0 ± 2.7 dB, Segment 8: 15.7 ± 3.2 dB (mean ± SD)] (Figs. 4, 5). The mean value of the intensity difference in the liver parenchyma at 10 min post-injection as evaluated using the high MI contrast mode was about 40 dB [Segment 8: 39.3 ± 4.8 dB (mean ± SD)] (Fig. 5).

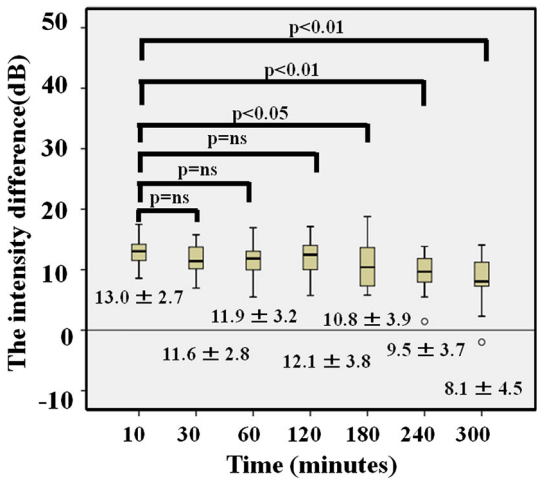

Fig. 4 Comparison of the intensity differences in the liver parenchyma as evaluated using the low MI contrast mode at each measurement time in Segment 3 ($n = 13$). Ten min versus each time: the intensity difference obtained at 10 min post-injection was compared with that obtained at each time point. The intensity differences in the liver parenchyma as evaluated using the low MI contrast mode showed the maintenance of enhancement from 10 min post-injection (mean ± SD: 13.0 ± 2.7 dB) to 120 min post-injection (mean ± SD: 12.1 ± 3.8 dB). The numbers in the graph show the mean ± SD. *ns* not significant

Discussion

In this study, the intensity differences at the portal vein and in the liver parenchyma were significantly higher when evaluated using the high MI contrast mode at a high MI, compared with the values evaluated using the low MI contrast mode at a low MI. Furthermore, the high MI

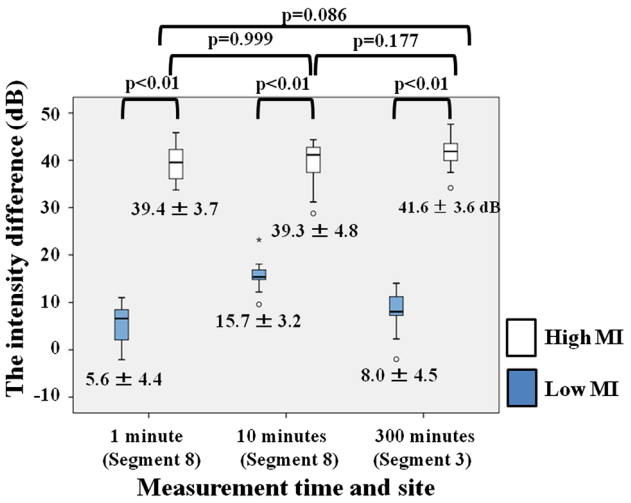

Fig. 5 Comparison of the intensity differences in the liver parenchyma in Segment 3 and Segment 8 as evaluated using the low MI and the high MI contrast modes at each measurement time ($n = 13$). Significant differences in the intensity differences were observed between the values obtained using the low MI and the high MI contrast modes ($P < 0.01$). The intensity differences in the liver parenchyma observed at three time points using the high MI contrast mode were not significantly different. The numbers in the graph show the mean ± SD. *low MI* low mechanical contrast mode, *high MI* high mechanical contrast mode

contrast mode at a high MI was more sensitive than the low MI contrast mode at a low MI for detecting Sonazoid microbubbles in the liver of normal subjects.

Sasaki et al. [19] reported that the portal vein was enhanced at between 15 s and 10 min using the low MI contrast mode after the injection of 0.0075 mL/kg of Sonazoid. In our study, all the subjects received an intravenous bolus injection of 0.2 mL/body of Sonazoid. The peak intensity difference at the portal vein was observed at 1 min post-injection using both the low MI and the high MI contrast modes. Thereafter, the intensity difference at the portal vein declined gradually. However, at each time point from 1 to 270 min post-injection, the intensity differences at the portal vein were significantly larger when evaluated using the high MI contrast mode at a high MI, compared with evaluations using the low MI contrast mode at a low MI.

Using the advanced dynamic flow mode (ADF; high MI contrast mode, MI of 1.6), Sasaki et al. scanned different planes at eight times (5, 10, 20, 40, 60, 120, 180, and 240 min post-injection) in normal subjects after the injection of a very small amount (0.000015 mL/kg) of Sonazoid and reported that the enhancement of the liver parenchyma lasted from 5 to 120 min [19]. According to their series of figures [19], the procedure for obtaining ADF-mode images might induce Sonazoid microbubble destruction in the liver parenchyma, even though they scanned different planes. In our study, however, no significant differences in the intensity difference of the liver parenchyma were observed at three measurement times (1, 10, and 300 min post-injection) when evaluated using the high MI contrast mode at a high MI since we did not scan the liver parenchyma between 10 and 300 min post-injection.

In the present study in subjects with normal livers, the intensity difference in the liver parenchyma obtained at 10 min post-injection (the same time as the post-vascular phase) using the high MI contrast mode at a high MI was about three times higher than that obtained using the low MI contrast mode at a low MI (40 dB versus 14 dB).

Skyba et al. [20] reported that 0.015 % of all the microvessels (≦7 μm in diameter, mostly capillaries) in rats were damaged after the destruction of microbubbles containing a mixture of perfluoropropane and air (Optison, GE Healthcare, Princeton, NJ) during examinations using the high MI contrast mode (MI 1.0). However, the actual MI value at the site of microvascular damage in the rats could not be specified, and interspecies differences in terms of tissue vulnerability remain unknown [20]. In the case of clinical examinations, there have not been any reports describing microvessel damage after examinations using the high MI contrast mode in human subjects. However, we cannot completely rule out the possibility of damage after examination using the high MI contrast mode. Thus, the low MI contrast mode is generally preferable for CEUS of the liver. In cases where diagnostic information can only be obtained using the high MI contrast mode, the benefits versus the possible damage should be weighed, and the most appropriate mode should be selected so as to benefit the subject [21].

After a bolus injection, Sonazoid is carried to the hepatic artery and the portal vein and flows into the sinusoid, where most of the Sonazoid microbubbles are phagocytosed by Kupffer cells [4]. Sonazoid microbubbles that are not phagocytosed by Kupffer cells are discharged into aspirated air. In rats, more than 50 % of the injected Sonazoid microbubbles are discharged into aspirated air within 20 min post-injection, and more than 96 % of the injected Sonazoid microbubbles are discharged into aspirated air within 24 h post-injection [3]. In humans, we know that Sonazoid microbubbles are recirculated to the hepatic artery and the portal vein [6]. In this study, the intensity differences in the liver parenchyma as evaluated using the low MI contrast mode did not decrease from 10 to 120 min post-injection, perhaps because the percentage of recirculating Sonazoid microbubbles was greater than the percentage that were lost as a result of the destruction caused by repeated scans, exhaled in air, and so on.

The recommended dose of Sonazoid for liver tumor enhancement is 0.015 mL/kg. This dose was determined based on clinical research conducted approximately 12 years ago [11]. The development of US equipment has enabled the image quality of CEUS with Sonazoid to be sufficiently good at doses lower than this recommended

dose [6]. Numata et al. reported that the use of Sonazoid at a dose of 0.2 mL/body was sufficient for the evaluation of liver tumors [6, 12–16]. Therefore, we used this dose in the present study. If the recommended dose of Sonazoid had been used in this study, the Sonazoid microbubbles at the portal vein and in the liver parenchyma might have been detectable for an even longer time.

Our study had some limitations. First, this study was conducted prospectively; however, the overall number of normal subjects included was relatively small. Second, patients with fatty liver and liver cirrhosis were not included. Future research examining a larger number of patients with chronic liver disease is needed to expand on our results. Third, we did not evaluate several segments of the right lobe to avoid the influence of microbubble destruction caused by the scanning of segment 8 using the high MI contrast mode at 10 min post-injection or later. Therefore, we only compared the intensity difference of the liver parenchyma between the low MI and the high MI contrast modes at three measurement points during this study.

Conclusion

Compared with the low MI contrast mode at a low MI, the high MI contrast mode at a high MI was more sensitive for detecting Sonazoid microbubbles in the livers of the normal subjects evaluated in this study.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Human rights statement and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent was obtained from all subjects for being included in the study.

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