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Experimental Study of the Stability of Sonazoid™

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ABSTRACT

Purpose : The purpose of this study was to evaluate the physical characteristics of Sonazoid™ and its stability in comparison with Levovist™ and to clarify its usefulness for producing enhancement effects in superficial areas.

Materials and Methods : We evaluated changes in particle-size distribution over time. We also examined the stability of Sonazoid™ during ultrasound examination using the B mode and the harmonic imaging method. In addition, we observed changes in each contrast agent flowing through the lumen of a tissue-equivalent phantom.

Results : Differential interference optical microscopy revealed microbubbles that were uniform in size (1-2 μm) and dispersal pattern. No changes in the particle size distribution were observed 150 minutes after preparation. The density of microbubbles tended to decrease over time. Microbubbles of Sonazoid™ were more readily destroyed than were those of Levovist™, both when ultrasound beams with a low mechanical index value were directly applied to the contrast agent solution and when they were applied to the phantom containing contrast medium.

Conclusion : The results of our experiments suggest the usefulness of the enhancement effects of Sonazoid™ on superficial organs. On the other hand, Levovist™, a first-generation contrast agent, has no enhancement effects on these organs. (Jikeikai Med J 2010 ; 57 : 55-60)

Key words : ultrasound, perfluorobutane, galactose/palmitic acid, physicochemical properties, acoustic properties, contrast efficacy

INTRODUCTION

Sonazoid™ (GE Healthcare AG, Oslo, Norway), a second-generation ultrasound contrast material consisting of perflubutan microbubbles, was approved by the Japanese Ministry of Health, Labour and Welfare in January 2007. Since then, Sonazoid™-enhanced ultrasonography of the liver has been performed in many institutions. Sonazoid™ and Levovist™ (Bayer Schering AG, Berlin, Germany) are composed of microbubbles. Levovist™ is a first-generation ultrasound contrast material consisting of a galactose/palmitic acid microparticle mixture. The microbubbles of Levovist™ are destroyed by ultrasound, and favorable contrast effects are obtained by imaging harmonic components occurring at the time

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of their destruction[1]. In contrast, ultrasound causes Sonazoid™ microbubbles to resonate without being destroyed, providing adequate harmonic components necessary for imaging. Therefore, Sonazoid™ does not require ultrasound application with the high mechanical index (MI) necessary for bubble destruction and can adequately enhance contrast with ultrasound having a low MI. Sonazoid™ with these properties might be used to enhance the contrast of superficial organs whose contrast cannot be enhanced with Levovist™. Therefore, many clinicians have requested that health insurance coverage be expanded to include Sonazoid™. With a high-frequency transducer used for the visualization of superficial organs, ultrasound application with a high MI required for adequate microbubble destruction is impossible, and, therefore, adequate harmonic components necessary for enhancement effects cannot be obtained with Levovist™, a first-generation contrast agent requiring a high MI for microbubble destruction.

Several experimental studies have examined the stability of Sonazoid™ microbubble distribution and the physicochemical characteristics of Sonazoid™ [2,3]. Zhang evaluated perfusion defects of superficial lesions on high-frequency contrast-enhanced ultrasound by comparing them with the results of low-frequency contrast-enhanced ultrasound using SonoVue™ (Bracco AG, Milano, Italy), a second-generation ultrasound contrast material[4]. However, to our knowledge, no studies have examined the use of high-frequency transducers with Sonazoid™ and Levovist™.

We performed a basic *in vitro* study to evaluate the physical characteristics of Sonazoid™, a second-generation ultrasound contrast agent, and its stability in comparison with Levovist™, a first-generation contrast agent, and examined its clinical usefulness for producing enhancement effects in superficial areas.

MATERIALS AND METHODS

1. Evaluation of changes in particle size distribution over time

Sonazoid™ was diluted 1 : 100, and the obtained dilution was observed with the following 3 methods : visual observation, observation with a differential interference optical microscope, and measurement of the particle-size distribution with the dynamic scattering method. Data sampling was performed while each method was applied at 15-minute intervals for 150 minutes.

2. Test of the stability of Sonazoid™ under ultrasound exposure

We prepared 0.009375 mL of undiluted Sonazoid™ solution/2.5 L deaerated water and 0.125 mL of undiluted Levovist™ solution/2.5 L deaerated water, applied ultrasound to each contrast agent solution, and observed the state of microbubble destruction using the B mode and the harmonic imaging method. In addition, a tissue-equivalent phantom (ATS Laboratories, Inc., Bridgeport, USA) (Fig. 1) was produced with a consideration of ultrasound attenuation, and changes in each contrast agent flowing through the lumen of the phantom were observed. The employed system was a prototype (Aloka Co., Ltd., Tokyo, Japan) with a convex transducer (frequency, 2 MHz) and an electronic linear transducer (5.4 Hz ; harmonic components, 13 MHz).

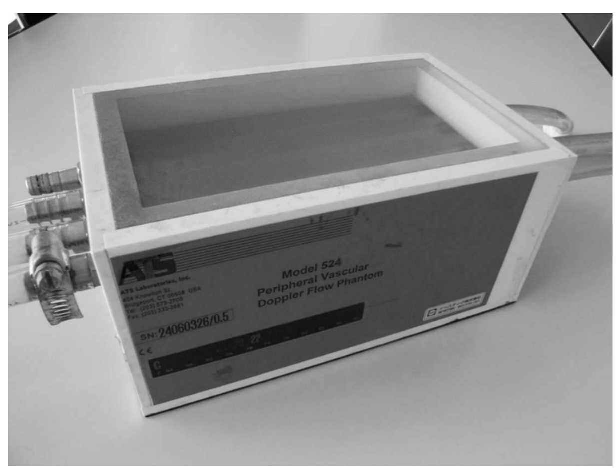

Fig. 1. The tissue-equivalent phantom used in these experiments.
This phantom contains 4 flow channels simulating superficial vasculature. The simulated vessels are located 15.0 mm below the scan surface.

Results

1. Evaluation of changes in particle-size distribution over time

Sonazoid™ solution separated into 2 layers 15 minutes after preparation, and bubbles disappeared over time (Fig. 2).

Differential interference optical microscopy revealed microbubbles that were uniform in size (1–2 μm) and dispersal pattern (Fig. 3). No changes in the particle-size distribution were observed 150 minutes after preparation, suggesting that Sonazoid™ microbubbles have high dispersion stability. The density of microbubbles tended to decrease over time (Fig. 3).

The particle-size distribution determined with the dynamic light-scattering method was consistent with the particle size of bubbles (1–2 μm) observed with optical microscopy. No changes in the particle-size distribution were observed 150 minutes after preparation, suggesting that Sonazoid™ microbubbles have high dispersion stability (Fig. 4).

2. Test of the stability of Sonazoid™ under ultrasound exposure

When ultrasound beams with a low MI were directly applied to the contrast agent solution, microbubbles of Sonazoid™ were more readily destroyed than were those of Levovist™ regardless of the frequency of the transducer (Table 1).

When ultrasound beams with a low MI were applied to the phantom containing contrast medium, microbubbles of Sonazoid™ were also more readily destroyed than were those of Levovist™ regardless of the frequency of the transducer (Table 2).

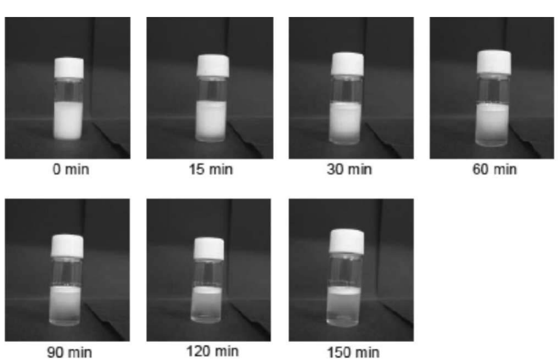

Fig. 2. Visual observation
The Sonazoid™ solution separated into 2 layers 15 minutes after preparation. The microbubbles disappeared over time.

Discussion

We performed an experimental study on the stability of Sonazoid™ microbubbles. We found that over time the microbubble diameter was stable and that the number of microbubbles decreased. However, in vivo, because the contrast medium is constantly stirred by such mechanisms as heart beats, Sonazoid™ microbubbles themselves are constantly replaced by new ones in the blood vessels. Therefore, the number of microbubbles in blood vessels may be stable.

We found that during ultrasound examination Sonazoid™ microbubbles were destroyed even at an MI of 0.2 and were destroyed more easily than we had expected. Thus, Sonazoid™ microbubbles readily resonate and are destroyed by ultrasound even with a low MI. Levovist™ microbubbles are harder to resonate than are Sonazoid™ microbubbles and are more difficult to destroy. Such phenomena were confirmed to occur with both a low-frequency transducer employed in routine abdominal ultrasonography and a high-frequency transducer used for superficial organs. Therefore, in Levovist™-enhanced ultrasonography with a low MI and a high-frequency transducer for superficial organs, microbubbles are difficult to destroy, as expected, and because harmonic components are difficult to generate, adequate enhancement effects are difficult to obtain even with the harmonic imaging method.

In Sonazoid™-enhanced ultrasonography with a low MI value and a high-frequency transducer, Sonazoid™ microbubbles were destroyed, contrary to our expectations. However, Sonazoid™ microbubbles were more difficult to destroy by means of ultrasound with a low MI than with a high MI.

Compared with Levovist™ microbubbles, Sonazoid™ microbubbles were more readily destroyed by ultrasound with a low MI. Thus, when ultrasound with a low MI is applied, echoes due to the destruction

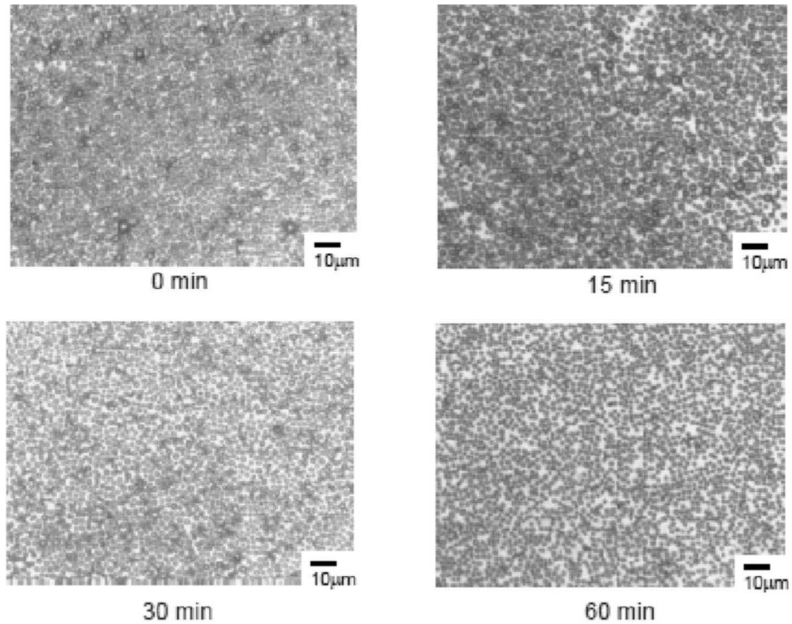

Fig. 3A.

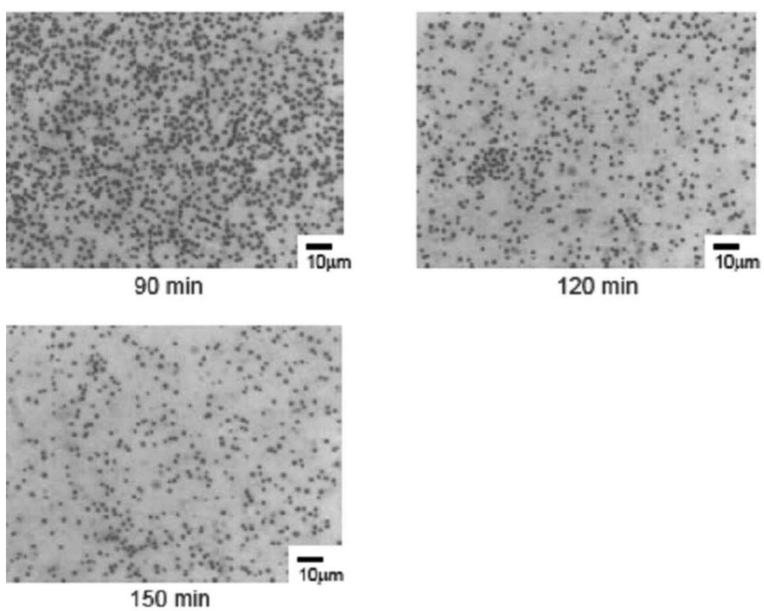

Fig. 3B.

Fig. 3A, B. Observation using a differential interference optical microscope
Observation was performed at 15-minute intervals for 150 minutes after preparation. The serial images revealed microbubbles that were uniform in size (1–2 μm) and dispersal pattern. No changes in the particle-size distribution were observed 150 minutes after preparation. The density of microbubbles tended to decrease over time.

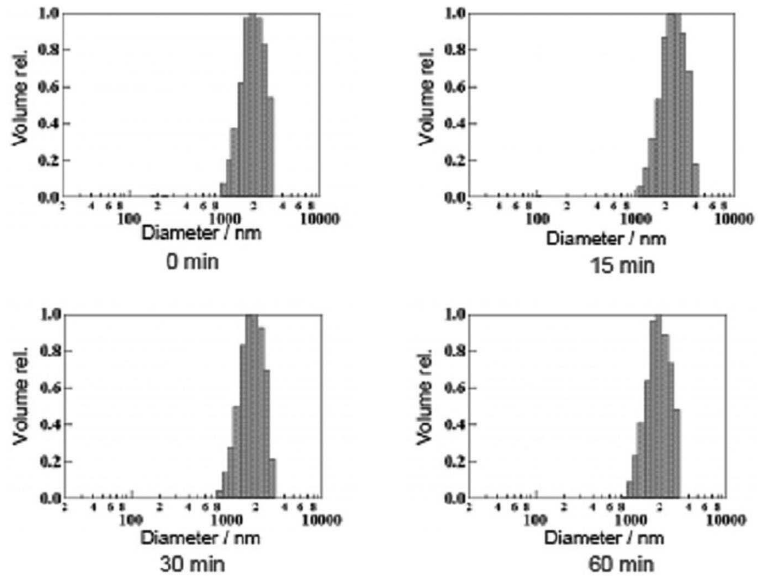

Fig. 4A.

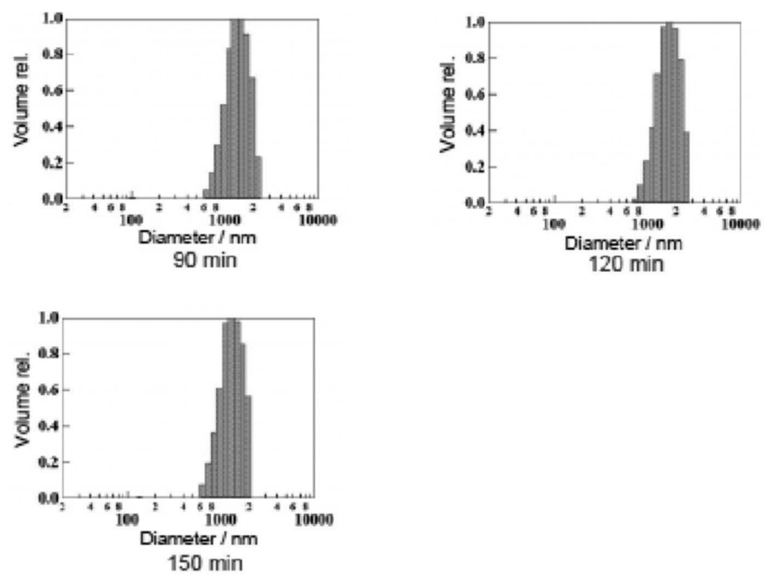

Fig. 4B.

Fig. 4A, B. Measurement of particle-size distribution with the dynamic scattering method
The particle-size distribution determined with the dynamic light-scattering method was consistent with the particle size of bubbles (1-2 μm) observed on optical microscopy. Observation was performed at 15-minute intervals for 150 minutes after preparation. No changes in the particle-size distribution were observed 150 minutes after preparation.

Table 1. Test of contrast agents stability without stir (Ultrasound beams applied directly to the contrast agent)

Method	MI value	Sonazoid™	Levovist™
B mode	0.2	gradually decreasing	no change
	0.5	decreasing rapidly	gradually decreasing
	1.0	decreasing rapidly	gradually decreasing
Harmonic imaging method	0.2	gradually decreasing	no change-gradually decreasing
	0.5	decreasing rapidly	gradually decreasing-decreasing rapidly
	1.0	decreasing rapidly	decreasing rapidly

Table 2. Test of contrast agents stability with pump off (Ultrasound beams applied to the phantom containing contrast agent)

Method	MI value	Sonazoid™	Levovist™
B mode	0.2	no change	no change
	0.5	decreasing rapidly	no change
	1.0	decreasing rapidly	decreasing rapidly
Harmonic imaging method	0.2	gradually decreasing	immesuarable
	0.5	decreasing rapidly	decreasing rapidly
	1.0	decreasing rapidly	decreasing rapidly

of Sonazoid™ microbubbles contain richer harmonic components than do echoes due to the destruction of Levovist™ microbubbles. In Sonazoid™-enhanced ultrasonography with a low MI, favorable enhancement effects of superficial organs can be obtained due to the synergistic effects of harmonic components associated with microbubble resonance and those associated with microbubble destruction.

At present, the use of Sonazoid™ for superficial organs is not covered by the Japanese health insurance system ; only its use for hepatic tumors is covered. The results of the present experiments suggest that Sonazoid™ has enhancement effects on superficial organs. In contrast, Levovist™, a first-generation contrast agent, has no enhancement effects on these organs. Therefore, insurance coverage should be expanded to include Sonazoid™ as early as possible.

Conflict of Interest Statement

Authors have no conflict of interest.

References

1. Yagisawa K, Moriyasu F, Miyahara T, Miyata Y, Iijima H. Phagocytosis of ultrasound contrast agent microbubbles by Kupffer cells. Ultrasound Med Biol 2007 ; 33 : 318-25.
2. Sontum PC. Physicochemical characteristics of Sonazoid™, a new contrast agent for ultrasound imaging. Ultrasound Med Biol 2008 ; 34 : 824-33.
3. Sontum PC, Ostensen J, Dyrstad K, Hoff L. Acoustic properties of NC100100 and their relation with the microbubble size distribution. Invest Radiol 1999 ; 34 : 268-75.
4. Zhang JQ. High-frequency contrast-enhanced ultrasound in evaluating perfusion of superficial lesions : limitations and countermeasures. Acad J Sec Mil Med Univ 2007 ; 28 : 1193-6.