J Med Ultrasonics (2012) 39:79–86
DOI 10.1007/s10396-011-0342-7

ORIGINAL ARTICLE

Phase II clinical study of DD-723 (perflubutane): dose–response study in patients with breast tumors

Yukio Miyamoto · Toshikazu Ito · Etsuo Takada · Kiyoka Omoto · Toshiko Hirai · Ryuzo Sekiguchi · Toshitaka Okuno · Shinsaku Kanazawa · Norio Nakata · Takao Igarashi

Received: 30 August 2011 / Accepted: 15 November 2011 / Published online: 3 February 2012

Abstract
Purpose We compared the contrast effect of three doses of DD-723 in subjects with breast tumors to determine the recommended dose. We then evaluated differential diagnosis results using plain ultrasonography, contrast-enhanced ultrasonography (plain + enhanced), and contrast-enhanced magnetic resonance imaging (MRI) compared to the pathological diagnosis.

Methods To evaluate the contrast effect, contrast-enhanced ultrasonic images were independently evaluated in a randomized sequence by three blinded reviewers trained in the evaluation method beforehand. Multiple evaluation results from the three reviewers were used to assess the overall contrast effect. The differential diagnosis was evaluated independently by three blinded reviewers using contrast-enhanced ultrasonic images and contrast-enhanced magnetic resonance images in a randomized sequence; reviewers were also blinded to subject characteristics. Multiple evaluation results from the three reviewers were used to assess the overall differential diagnosis.
Results The recommended dose of DD-723 is an intermediate dose of 0.12 μL MB/kg. Accuracy, sensitivity, and specificity were improved more in the differential diagnosis by contrast-enhanced ultrasonography than in plain ultrasonography. Accuracy and specificity were better and sensitivity similar compared to contrast-enhanced MRI.
Conclusions An intermediate dose showed the highest efficacy in terms of overall contrast effect. Contrast-enhanced ultrasonography is safe and useful when used in differential diagnosis.

Keywords Ultrasound contrast medium · Breast tumors · Phase II clinical study · Sonazoid

Y. Miyamoto (✉) · N. Nakata · T. Igarashi
Department of Diagnostic Imaging, Jikei University Hospital, 3-19-18 Nishishinbashi, Minato, Tokyo 105-8471, Japan
e-mail: miyamoto@jikei.ac.jp

T. Ito
Department of Surgery, Rinku General Medical Center, Osaka, Japan

E. Takada
Center of Medical Ultrasonics, Dokkyo Medical University, Tochigi, Japan

K. Omoto
Department of Laboratory Medicine, Saitama Medical Center, Jichi Medical University, Saitama, Japan

T. Hirai
Department of Endoscopy and Ultrasound, Nara Medical University Hospital, Nara, Japan

R. Sekiguchi
Department of Diagnostic Imaging, Tochigi Cancer Center, Tochigi, Japan

T. Okuno
Kobe Urban Breast Clinic, Hyogo, Japan

S. Kanazawa
Department of Radiology, Toho University Omori Hospital, Tokyo, Japan

Introduction

DD-723 is a contrast medium for ultrasonic diagnosis produced by Nycomed from Norway (currently GE Healthcare AS). It is a freeze-dried product for injection that contains perflubutane, a chemically stable gas, with egg yolk phosphatidylserine and hydrogen in an internal capsule. By adding water for injection to this product

before administration, a suspension of microbubbles with a mean diameter of 2–3 μm is formed, and a contrast effect is obtained when ultrasonic waves undergo efficient reflection and dispersion from intravascular microbubbles after this agent is administered intravenously.

Since this product circulates systemically via the capillaries, it can enhance the blood vessels of various organs. Therefore, it is expected to be useful and has been applied clinically for the detection of abnormal venous structures and mass lesions in the contrast phase.

Since primary liver cancer secondary to viral hepatitis is common in Japan and the liver is also frequently a site of metastasis, development was carried out in Japan to evaluate the diagnostic capacity of this product for hepatic mass lesions and its detection of hepatic mass lesions based on characteristic incorporation by Kupffer cells of the liver [1]. As a result, this product was approved in October 2006 with "contrast enhancement of hepatic mass lesions during ultrasonography" as the indication. It has been marketed since January 2007 under the brand name of Sonazoid® for Injection.

Since this product provides contrast enhancement of blood vessels in various other organs as well as the liver, as described above, the possibility of additional indications was studied.

In Japan, breast cancer is the most common cancer among women and about 40,000 new cases occur every year [2]. This number has been increasing every year and it is expected to exceed 50,000 women annually by 2020 [3]. The mortality rate is also increasing [4]. In the United States and Europe, the number of cases is higher than in Japan, but the mortality rate has tended to decrease in recent years [5]. This is considered to be due to early detection and treatment because of a high breast cancer screening rate of 60–80% [6]. However, the screening rate in Japan is currently about 10%, and this low rate presents a problem [6].

In the diagnosis of breast cancer, inspection and palpation are performed initially, with mammography and ultrasonography used for imaging diagnosis. For differential diagnosis of benign and malignant lesions and for assessment of the extent of lesions, contrast-enhanced magnetic resonance imaging (MRI) and contrast-enhanced computed tomography (CT) are used, while the definitive diagnosis is done by pathological examination (cytodiagnosis and histodiagnosis).

Contrast-enhanced MRI and contrast-enhanced CT have problems related to lack of convenience and the need for caution in patients with reduced renal function. Contrast-enhanced CT is also associated with the problems of radiation exposure [7] and iodine allergy or shock.

Contrast-enhanced ultrasonography with this product will be useful for the differential diagnosis of benign and malignant breast lesions, assessing the extent of infiltration of lesions, and assessing the response of breast cancer to treatment. In comparison with contrast-enhanced MRI and contrast-enhanced CT, contrast-enhanced ultrasonography has the advantages of excellent spatial, temporal, and contrast resolution, as well as the ability to observe continuous real-time images during wash-in and wash-out of the contrast medium through the tumor vasculature. Since contrast-enhanced ultrasonography using this product is simple to perform, it is expected to become a new modality for the detailed examination of breast cancer.

Therefore, a dose–response study in patients with breast tumors was planned as a phase II clinical study to confirm the efficacy of this product for breast tumors and to investigate the optimum dose.

Subjects and methods

Subjects

The subjects were 86 patients who met all of the inclusion criteria, did not violate any of the exclusion criteria, and gave written informed consent of their own free will from among patients with breast tumors at five hospitals in Japan between April and December 2009. The study was approved by the institutional review boards of the hospitals and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

The inclusion and exclusion criteria were as follows. As described in the "inclusion criteria" section, subjects confirmed to have untreated masses (lesion of interest) and expected to undergo pathological examination were enrolled in the study. The benign/malignant nature of the lesion of interest was identified after performing the pathological examination. Therefore, no bias exists in subject enrollment. Subjects were registered and randomized by the central registration method.

Inclusion criteria:

1. Patients with untreated masses (lesions of interest) detected by plain ultrasonography.
2. Patients expected to undergo pathological examination (cytodiagnosis or histodiagnosis) of the lesion of interest.
3. Patients aged from 20 to 80 years when giving consent.

Exclusion criteria:

1. Patients with a history of allergy to eggs or egg products.
2. Patients with an arteriovenous shunt (right-left) in the heart or lungs.

3. Patients with serious heart disease.
4. Patients with serious lung disease.
5. Patients who were scheduled to undergo gastrointestinal investigations such as barium meal using a foaming agent or peritoneoscopic examination on the day of study drug administration.
6. Patients who are currently participating in another clinical study or who have done so within the past 180 days.
7. Patients who are pregnant, possibly pregnant, or breast-feeding.
8. Patients who are expected to undergo surgery between the time consent is obtained and the pathological examination is completed.
9. Patients who cannot undergo contrast-enhanced MRI (patients with pacemakers, etc.).
10. Patients who are receiving or are expected to receive treatments such as chemotherapy or radiation therapy between the time consent is obtained and the pathological examination is completed.
11. Patients with local recurrence of the lesion of interest.
12. Patients who are receiving or are expected to undergo examination using a contrast medium (iodinated contrast medium, MRI contrast medium, other ultrasonic contrast medium, etc.) from 2 days before until 2 days after administration of the study drug.
13. Patients who are expected to undergo pathological examination up to 2 days after administration of the study drug.
14. Patients who have previously undergone administration of DD-723 (Sonazoid® for Injection).
15. Any other patients who are considered to be unsuitable to participate in this clinical study by the investigator.

Methods

Ultrasonic imaging

Plain and contrast-enhanced ultrasonic imaging were performed using ultrasonography equipment with a built-in harmonic B mode and the images were recorded. Table 1 shows the recommended settings for ultrasonography.

The manufacturer, type of equipment, probe, and settings (mechanical index: MI, frame rate) for ultrasonography were recorded. After starting the examination, the imaging conditions (MI value, frame rate, focus, etc.) were not changed, as a rule.

For plain ultrasonography, one mass was selected as the lesion of interest, the probe was set over the center of this lesion, and images were obtained every 15 s and recorded. Next, the probe was placed in approximately the same position as that for plain imaging, and imaging was conducted in the harmonic B mode from 15 s before administration of the study drug. Imaging was continued for 1 min after study drug administration and contrast-enhanced images were recorded.

Table 1 Recommended settings for ultrasonography equipment

	Plain ultrasonography	Contrast-enhanced ultrasonography	
		Before study drug administration	After study drug administration
Ultrasonography equipment (manufacturer)	Aplio (Toshiba)/Logiq 7 (GE)/Logiq E9 (GE)/ ProSound α10 (Aloka)		
Imaging mode	Fundamental B mode Harmonic B mode	Harmonic B mode	
Mechanical index	Maximum acoustic pressure	0.1–0.4	
Focus site	Just below lesion		
Frame rate	–	–	5–21 fps

Dosage and evaluation method

In subjects with breast tumors, a single dose of 0.024, 0.12, or 0.36 μL MB/kg of DD-723 was administered into the antebrachial vein. For efficacy evaluation, the primary endpoint was the efficacy rate of the contrast effect obtained with each dose, and the recommended dose was investigated from the dose–response relation. Secondary endpoints included the evaluation of differential diagnostic capacity. Safety was also evaluated.

The efficacy evaluation was performed by six blinded reviewers (three each for randomized ultrasonic images and contrast MRI images).

Before evaluation was performed, a training session was held and the evaluation committee (using 15 subjects for training) met to confirm the reliability of the blinded reviewers in order to standardize evaluation among them and to ensure the reliability of the results. The images used for training were excluded from the efficacy analysis.

All patient characteristic information was blinded to the image reviewers, including age, familial history, findings by questioning, findings on inspection and palpation, findings on imaging, and results of pathological examination. The blinded reviewers separately evaluated the contrast effect on the images for each subject in randomized sequence.

Each of three blinded US reviewers separately reviewed the ultrasonography images for all the subjects except for

the training, and also each of three blinded MRI reviewers separately reviewed the MRI images for all the subjects except for the training. When there was a difference in the evaluation results among the three reviewers, the dominant result was used.

Contrast-enhanced MRI

Contrast-enhanced ultrasonography and contrast-enhanced MRI were performed within 30 days, with at least a 2-day interval, after the study drug was administered regardless of sequence.

The imaging condition was 1.5 T or more and the Gd product was used as the contrast medium. T1-weighted images, T2-weighted images, diffusion-weighted images, and dynamic MRI were done.

Pathological diagnosis

After study drug administration, contrast-enhanced ultrasonography, and contrast-enhanced MRI had been completed, cytodiagnosis or histological diagnosis was done for pathological testing.

Primary endpoint

Contrast efficacy rate evaluated by blinded reviewers:

Results of multiple evaluations by three blinded reviewers were used to assess the overall contrast effect as the primary endpoint. The contrast efficacy rate was calculated from the following expression: a/(a + b + c + d).

Evaluation criteria of contrast effect:

a: There were no artifacts that hindered diagnosis, and sufficient contrast enhancement of the lesion of interest and the surrounding blood vessels was obtained.
b: There were no artifacts that hindered diagnosis, but contrast enhancement of the lesion of interest and the surrounding blood vessels was insufficient.
c: Artifacts occurred or tissue enhancement by the contrast medium was too strong and it was difficult to assess the lesion of interest or the surrounding blood vessels.
d: A contrast effect was not obtained (incorrect imaging conditions, failure of the ultrasonography equipment, etc.).

Secondary endpoints

Results of multiple evaluations by three blinded reviewers were used for the overall differential diagnosis.

1. Taking the pathological diagnosis as the gold standard, the differential diagnostic capacity (benign vs. malignant) of plain ultrasonography, contrast-enhanced ultrasonography (plain + enhanced), and contrast-enhanced MRI was evaluated in comparison with the pathological diagnosis.
2. The differential diagnosis (benign vs. malignant) made by plain ultrasonography, contrast-enhanced ultrasonography (plain + enhanced), and contrast-enhanced MRI was evaluated.

Plain US images were exclusively stored on one DVD, and both plain and contrast-enhanced US images were stored on another DVD. The three blinded US reviewers individually reviewed the two DVDs separately to determine whether the lesion was benign or malignant. The three blinded MRI reviewers individually reviewed the contrast-enhanced MRI images to determine the malignant versus benign nature of the lesion. The sequence of the images was randomized for review by the three reviewers. The three reviewers reviewed the US/MRI images in different sequences.

Main evaluation criteria for differential diagnosis by contrast-enhanced ultrasonography [8]:

1. Benign
    a. No enhancement
    b. Homogeneous enhancement
    c. Clear vasculature or "tree-like branching" in the lesion
    d. Ring-shaped enhancement of peripheral blood vessels in the lesion
2. Malignant
    a. Heterogeneous enhancement with defect
    b. Heterogeneous enhancement without clear defect
    c. Linear, curled, meandering, or irregular vasculature in the lesion
    d. "Crab-claw"-like enhancement of peripheral blood vessels in the lesion
    e. Multiple vascular enhancements entering linearly toward the lesion from many directions
    f. Pulsation in the lesion

Evaluation criteria for differential diagnosis by contrast-enhanced MRI:

Assessment of differential diagnosis by the same methods as in routine diagnosis.

Safety endpoints

The safety endpoints were adverse events, laboratory tests, and vital signs within 2 days after administration of the study drug.

Results

Efficacy

As shown in Fig. 1, 86 subjects were randomized in this study, 83 of whom received the study drug (28 in the low dose group, 28 in the intermediate dose group, and 27 in the high group). The primary endpoint was analyzed in 67 patients (23 in the low dose group, 23 in the intermediate dose group, and 21 in the high dose group from the efficacy analysis set). The secondary endpoints (differential diagnosis) were analyzed in 66 subjects (22 in the low dose group, 23 in the intermediate dose group, and 21 in the high dose group), after excluding 15 subjects who were used for the training session and the evaluation committee meeting (five from each group). Subject demographics are shown in Table 2.

Primary endpoint

In the efficacy analysis set for analysis of the primary endpoint, the efficacy rate for the overall contrast effect was 26.1% [6/23, 95% confidence interval (CI) 8.1–44.0] in the low dose group, 95.7% (22/23, 95% CI 87.3–100.0) in the intermediate dose group, and 81.0% (17/21, 95% CI 64.2–97.7) in the high dose group (Table 3). The highest efficacy rate for overall contrast effect was found in the intermediate dose group, and the maximum response was seen at the intermediate dose [Cochrane–Armitage test using contrast coefficients (−2, 1, 1): $P < 0.001$].

Failure of visualization of not only the lesion of interest but also surrounding tissues has been taken into account for the calculation of efficacy rate.

From the above findings, the highest efficacy rate for overall contrast effect was achieved in the intermediate dose group, and the dose–response profile showed a maximal response at the intermediate dose.

Secondary endpoints

Tables 4 and 5 show the accuracy, sensitivity, and specificity of the differential diagnosis in the efficacy analysis set. Pathological examination of 66 subjects in the efficacy analysis set revealed a malignant tumor in 26 cases and a benign tumor in 40 cases.

The accuracy of the differential diagnosis in the efficacy analysis set was 90.9% (60/66, 95% CI 84.0–97.8) for contrast-enhanced ultrasonography (plain + enhanced), 78.8% (52/66, 95% CI 68.9–88.7) for plain ultrasonography, and 75.8% (50/66, 95% CI 65.4–86.1) for contrast-enhanced MRI. The difference in accuracy between contrast-enhanced ultrasonography (plain + enhanced) and plain ultrasonography was 12.1% (95% CI 2.3–22.0), while the difference from contrast-enhanced MRI was 15.2% (95% CI 3.8–26.5). Significant differences were found among the groups (McNemar's test: $P = 0.021$, 0.012), and the accuracy was improved by contrast ultrasonography.

The sensitivity of the differential diagnosis was 96.2% (25/26, 95% CI 88.8–100.0) for contrast-enhanced ultrasonography (plain + enhanced), 84.6% (22/26, 95% CI 70.7–98.5) for plain ultrasonography, and 96.2% (25/26, 95% CI 88.8–100.0) for contrast-enhanced MRI. The difference in sensitivity between contrast-enhanced ultrasonography (plain + enhanced) and plain ultrasonography was 11.5% (95% CI −0.7 to 23.8). No significant difference was found between the two groups (McNemar's test: $P = 0.083$). The difference in sensitivity between contrast-enhanced ultrasonography (plain + enhanced) and contrast-enhanced MRI was 0.0% (95% CI −10.7 to 10.7), so the sensitivity was the same.

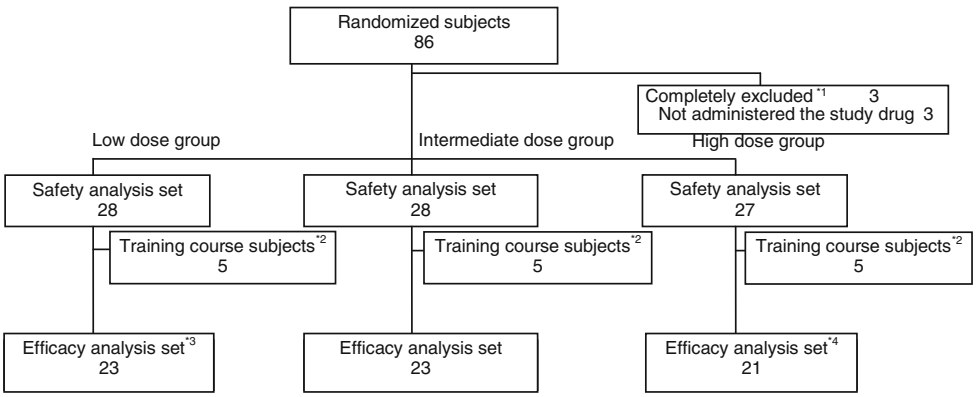

Fig. 1 Disposition of the subjects. *1The three patients were withdrawn from the study before study drug administration because they asked to leave the study for their own reasons. *2Subjects used for the training session and the evaluation committee meeting to confirm the reliability of the blinded reviewers. *3One subject was excluded from analysis of the secondary endpoints of differential diagnosis because the pathological diagnosis was unclear. *4One subject was excluded from the efficacy analysis because the subject had no recorded image

Table 2 Demographic and other baseline characteristics (efficacy analysis set)

Item	No. of subjects evaluated	Low dose group 23	Intermediate dose group 23	High dose group 21	All subjects 67
Age	<65	18 (78.3)	21 (91.3)	15 (71.4)	54 (80.6)
	≥65	5 (21.7)	2 (8.7)	6 (28.6)	13 (19.4)
	Mean ± SD	48.3 ± 16.0	51.2 ± 8.7	54.0 ± 13.2	51.1 ± 13.0
	Median	45.0	50.0	54.0	49.0
	Min, max	25, 74	34, 69	29, 74	25, 74
Body weight	<50 kg	9 (39.1)	6 (26.1)	7 (33.3)	22 (32.8)
	≥50 kg	14 (60.9)	17 (73.9)	14 (66.7)	45 (67.2)
	Mean ± SD	54.27 ± 12.60	55.13 ± 7.30	52.94 ± 7.37	54.15 ± 9.38
	Median	52.00	54.40	51.20	53.00
	Min, max	38.0, 104.0	40.6, 73.5	38.4, 70.0	38.0, 104.0
Treatment status	Inpatient	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Outpatient	23 (100.0)	23 (100.0)	21 (100.0)	67 (100.0)
Size of the lesion of interest (long diameter)	<1 cm	9 (39.1)	4 (17.4)	6 (28.6)	19 (28.4)
	≥1 cm	14 (60.9)	19 (82.6)	15 (71.4)	48 (71.6)
	Mean ± SD	1.27 ± 0.73	1.52 ± 0.88	1.36 ± 0.57	1.38 ± 0.74
	Median	1.10	1.30	1.30	1.20
	Min, max	0.3, 3.4	0.4, 4.2	0.6, 2.6	0.3, 4.2
Pathological examination	Cytodiagnosis	2 (8.7)	4 (17.4)	4 (19.0)	10 (14.9)
	Histodiagnosis	21 (91.3)	19 (82.6)	17 (81.0)	57 (85.1)
	Both cytodiagnosis and histodiagnosis	3 (13.0)	1 (4.3)	1 (4.8)	5 (7.5)
Pathological diagnosis[a]	Malignant	10 (45.5)	7 (30.4)	9 (42.9)	26 (39.4)
	Benign	12 (54.5)	16 (69.6)	12 (57.1)	40 (60.6)

[a] For this subject (low dose group), the pathological specimen was not assessable. This subject was excluded from the differential diagnosis population for the secondary endpoint

Table 3 Overall contrast effect (efficacy analysis set)

Treatment group	a	b	c	d	Total	Efficacy rate[a] [95% CI]	Test[b]
Low dose	6 (26.1)	17 (73.9)	0 (0.0)	0 (0.0)	23	26.1 [8.1, 44.0]	$P < 0.001$
Intermediate dose	22 (95.7)	1 (4.3)	0 (0.0)	0 (0.0)	23	95.7 [87.3, 100.0]	
High dose	17 (81.0)	1 (4.8)	3 (14.3)	0 (0.0)	21	81.0 [64.2, 97.7]	

No. of subjects (%)

[a] Efficacy rate of contrast effect = a/(a + b + c + d)

[b] Cochrane–Armitage test using contrast coefficients (−2, 1, 1)

The specificity of the differential diagnosis was 87.5% (35/40, 95% CI 77.3–97.7) for contrast-enhanced ultrasonography (plain + enhanced), 75.0% (30/40, 95% CI 61.6–88.4) for plain ultrasonography, and 62.5% (25/40, 95% CI 47.5–77.5) for contrast-enhanced MRI. The difference in specificity between contrast-enhanced ultrasonography (plain + enhanced) and plain ultrasonography was 12.5% (95% CI −1.7 to 26.7), which was not significant (McNemar's test: $P = 0.096$). The difference in specificity between contrast-enhanced ultrasonography (plain + enhanced) and contrast-enhanced MRI was 25.0% (95% CI 8.4–41.6). There was a significant difference between the two groups (McNemar's test: $P = 0.008$), and specificity was greatly improved by contrast ultrasonography.

Although contrast-enhanced ultrasonography (plain + enhanced) showed no significant differences from plain ultrasonography, the overall differential diagnostic capacity was improved in all dose groups (100% sensitivity in the intermediate dose group for both examinations). In comparison with contrast-enhanced MRI, there were no significant differences from the low and intermediate dose groups, but the high dose group showed a significant difference. In all contrast-enhanced ultrasonography groups, the accuracy and specificity were improved while the sensitivity remained the same.

The above results indicate that the differential diagnostic capacity of contrast-enhanced ultrasonography (plain + enhanced) is good. In comparison with plain ultrasonography, the accuracy, sensitivity, and specificity are all improved, while the accuracy and specificity are improved and the sensitivity is the same when compared with contrast-enhanced MRI.

Safety

All 83 subjects who received the study drug (28 in the low dose group, 28 in the intermediate dose group, and 27 in the high dose group) were included in the safety analysis set.

Table 4 Overall differential diagnosis: number of subjects with benign and malignant lesions (efficacy analysis set)

Treatment group Pathological examination	All subjects		
	Malignant	Benign	Total
Contrast-enhanced ultrasonography			
Malignant	25	5	30
Benign	1	35	36
Total	26	40	66
Plain ultrasonography			
Malignant	22	10	32
Benign	4	30	34
Total	26	40	66
Contrast-enhanced MRI			
Malignant	25	15	40
Benign	1	25	26
Total	26	40	66

The overall incidence of adverse events was 6.0% (5/83). The incidence of adverse events was 7.1% (2/28) in the low dose group, 3.6% (1/28) in the intermediate dose group, and 7.4% (2/27) in the high dose group. Adverse events included headache, diarrhea, and rash in two subjects from the low dose group (headache and rash in the same subject), injection site pain and malaise in one subject from the intermediate dose group, and upper abdominal pain and injection site pain in one subject each from the high dose group.

The overall incidence of adverse drug reactions was 4.8% (4/83). The incidence was 7.1% (2/28) in the low dose group, 3.6% (1/28) in the intermediate dose group, and 3.7% (1/27) in the high dose group. The adverse drug reactions were diarrhea and rash in one subject each from the low dose group, and injection site pain in one subject each from the intermediate and the high dose groups.

The severity of adverse events was mild in all cases. Upper abdominal pain was treated, but the other events recovered without treatment. No serious adverse events were observed.

Discussion

For evaluation of contrast effect in this study, contrast-enhanced ultrasonic images were independently evaluated in a randomized sequence by three blinded reviewers who received training in the evaluation method beforehand, and the results of multiple evaluations by the three reviewers were used to assess the overall contrast effect. The overall contrast effect in the efficacy analysis set (the primary endpoint) was 26.1% (6/23) in the low dose group, 95.7% (22/23) in the intermediate dose group, and 81.0% (17/21) in the high dose group. The highest efficacy rate was found in the intermediate dose group, and maximum efficacy at an intermediate dose of 0.12 μL MB/kg was confirmed [Cochrane–Armitage test using contrast coefficients (−2, 1, 1): $P < 0.001$].

For assessment of the differential diagnostic capacity, contrast-enhanced ultrasonic images and contrast-enhanced MRI images were independently evaluated in a randomized sequence by three blinded reviewers. The results of multiple evaluations by the three reviewers were used for overall evaluation (three blinded reviewers each for the ultrasonography and MRI evaluations).

Table 5 Overall differential diagnostic capacity (efficacy analysis set)

Treatment group	Overall differential diagnostic capacity	Contrast-enhanced ultrasonography	Plain ultrasonography	Contrast-enhanced MRI
All subjects	Accuracy	90.9 (84.0, 97.8)	78.8 (68.9, 88.7)	75.8 (65.4, 86.1)
	Sensitivity	96.2 (88.8, 100.0)	84.6 (70.7, 98.5)	96.2 (88.8, 100.0)
	Specificity	87.5 (77.3, 97.7)	75.0 (61.6, 88.4)	62.5 (47.5, 77.5)

Statistic (%) (95% CI)

Assessment of secondary endpoints showed that the accuracy was 90.9% (60/66), the sensitivity was 96.2% (25/26), and the specificity was 87.5% (35/40) for the overall differential diagnostic capacity of contrast-enhanced ultrasonography in the efficacy analysis set. The difference in accuracy was 12.1% (95% CI 2.3–22.0), the difference in sensitivity was 11.5% (95% CI −0.7 to 23.8), and the difference in specificity was 12.5% (95% CI −1.7 to 26.7) for the overall differential diagnostic capacity between contrast-enhanced ultrasonography and plain ultrasonography. This suggested that the differential diagnosis was improved by using the contrast medium.

In Japan, plain ultrasonography is considered to be useful for differentiation between malignant and benign mass lesions of the breast, and the accuracy is reported to be 75.6–88.4% [9]. In this study, blinded reviewers performed differential diagnosis using only the images without being given any medical information about the subjects. The accuracy of the overall differential diagnosis by plain ultrasound was 78.8% (52/66), which is similar to that reported before. These are reproducible results in terms of data obtained in routine clinical practice.

Analysis of safety showed that the adverse events detected in this study were similar to the adverse drug reactions already reported for this product [10]. No dose-dependency was found in terms of the incidence of adverse events, and no clinical problems related to safety occurred at the high dose.

Conclusions

Based on the results of comparison of the contrast effect of this product at three doses, the recommended dose for contrast-enhanced ultrasonography (plain + enhanced) in patients with breast tumors is the intermediate dose of 0.12 μL MB/kg. It was also suggested that the differential diagnostic capacity of contrast-enhanced ultrasonography (plain + enhanced) is good. In comparison with plain ultrasonography, the accuracy, sensitivity, and specificity were improved by contrast ultrasonography. In addition, the accuracy and specificity were better and sensitivity was similar compared with contrast MRI. Analysis of safety revealed no dose-dependence in terms of the incidence of adverse events, and all of the adverse events that occurred were mild.

Acknowledgments We thank Professor Fuminori Moriyasu (Tokyo Medical University) for useful suggestions and Satoshi Tatsuno (Tokyo Dental College Ichikawa General Hospital), Masanori Nagase (Tokyo Rosai Hospital), and Takako Shirakawa (JR Tokyo General Hospital) for MRI evaluation.

References

1. Watanabe R, Matsumura M, Munemasa T, et al. Mechanism of hepatic parenchyma-specific contrast of microbubble-based contrast agent for ultrasonography. Invest Radiol. 2007;42:643–51.
2. Matsuda T, Marugame T, Kamo K, The Japan Cancer Surveillance Research Group, et al. Cancer incidence and incidence rates in Japan in 2002: based on data from 11 population-based cancer registries. Jpn J Clin Oncol. 2008;38(9):641–8.
3. Oono Y, Nakamura T, Murata K, et al. Future estimations of cancer prevalence in Japan. White paper on cancer statistics. Tokyo: Shinohara Press; 2004. p. 201–17.
4. Changes in the number of deaths caused by major malignant neoplasms (database on the Internet) 2007 Demographics (data from Ministry of Health, Labour and Welfare). http://www.mmjp.or.jp/kawakami-clinic/data/h19suii.htm. Updated 19 Sept 2008; cited 11 July 2011.
5. National Cancer Center (homepage on the Internet). Tokyo: Center for Cancer Control and Information Services (Cancer Information Service) Breast cancer. http://ganjoho.ncc.go.jp/professional/statistics/digest/digest12.html. Updated 1 Oct 2006; cited 11 July 2011.
6. National Cancer Center (homepage on the Internet). Tokyo: Center for Cancer Control and Information Services (Cancer Information Service) Cancer screening. http://ganjoho.jp/professional/pre_scr/screening/screening.html#03. Updated 5 Apr 2010; cited 11 July 2011.
7. de Gonzalez AB, Darby S. Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries. Lancet. 2004;363:345–51.
8. Du J, Li FH, Fang H, et al. Microvascular architecture of breast lesions: evaluation with contrast-enhanced ultrasonographic micro flow imaging. J Ultrasound Med. 2008;27:833–42.
9. Japanese Breast Cancer Society, editor. Breast cancer management guidelines. vol. 4. Screening and diagnosis. Kanehara Shuppan; 2008:18–19.
10. Sonazoid® for Injection (package insert). Daiichi Sankyo Co., Ltd. June 2011.