Bolus versus Continuous Infusion of Microbubble Contrast Agent for Liver Ultrasound by Using an Automatic Power Injector in Humans: A Pilot Study

Emilio Quaia, MD, Antonio Giulio Gennari, MD, Roberta Angileri, MD, Maria Assunta Cova, MD

Department of Radiology, Cattinara Hospital, University of Trieste, Strada di Fiume 447, Trieste, Italy 34149

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ABSTRACT: Purpose. To evaluate the feasibility of using continuous infusion, in comparison with bolus injection, of a sulfur hexafluoride–microbubble contrast agent to prolong the duration of hepatic parenchymal enhancement in humans during sonographic examination.

Methods. This pilot study was approved by our institution’s ethics committee. Ten patients (5 men and 5 women; mean age ± SD, 65 ± 10 years) each received two injections: a bolus injection (2 ml/s) and then continuous infusion (0.5 ml/min) of the contrast agent by using an automatic injector. Acquired cine clips were transferred to a personal computer, and the video intensity was quantified by dedicated software.

Results. From the time of the first microbubble visualization in the scanning plane, maximal enhancement was reached in 6.3 ± 0.94 seconds after bolus injection and in 13.9 ± 1.44 seconds during continuous infusion (p = 0.002, Wilcoxon’s test for paired data). Compared with bolus injection, continuous infusion prolonged the duration of contrast enhancement (4.3 minutes ± 42 seconds versus 7.3 minutes ± 40 seconds; p = 0.002), although no statistically significant difference in maximal enhancement was observed (45 ± 18% for bolus injection and 39 ± 6% for continuous infusion; p = 0.62).

Conclusions. Continuous infusion of sulfur hexafluoride–filled microbubbles via an automatic power injector prolongs hepatic contrast enhancement without significantly modifying the maximal enhancement over that at baseline. These data, coming from a pilot study, can be used to design a larger study with adequate statistical power. © 2015 Wiley Periodicals, Inc. J Clin Ultrasound 44:136–142, 2016; Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jcu.22293

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INTRODUCTION

Contrast-enhanced ultrasound (CEUS) is now considered a reliable first-line imaging modality in clinical practice, particularly for assessing focal hepatic lesions.1 CEUS has several advantages over CT and MRI, including lower cost, portability, availability, lack of restrictions in performing serial examinations at short intervals, and absence of exposure to radiation or nuclear tracers.1

Perfusion of the liver parenchyma, corresponding to blood flow normalized for the volume or weight of the perfused tissue, influences the grade of hepatic contrast enhancement after injection of a contrast agent.2,3 The more prolonged the parenchymal contrast enhancement, the higher the chance for the operator to accurately explore all the liver regions, which is critical in the detection of hepatic metastases.1,4

The usual way to inject a microbubble contrast agent is by manual bolus injection; continuous infusion of the agent, especially if performed with an automatic injector, is used much less often. One previous study showed that infusion of sulfur hexafluoride–filled microbubble contrast agent allows the duration of the Doppler signal to be...
increased as long as desired and that peak enhancement increases with the infusion rate. A further study showed that continuous infusion of air-filled microbubbles with a shell of galactose-palmitic acid markedly prolongs but slightly diminishes hepatic enhancement. To our knowledge, no previous study has assessed whether continuous infusion of a sulfur hexafluoride–filled microbubble contrast agent by using an automatic injector may prolong the hepatic parenchymal enhancement if insonated in a contrast-specific mode.

Thus, the aim of this study was to evaluate the feasibility of using continuous infusion, in comparison with bolus injection, of a contrast agent containing sulfur hexafluoride–filled microbubbles to prolong the duration of hepatic parenchymal enhancement in humans during a CEUS examination.

PATIENTS AND METHODS

This pilot study conformed to the ethical guidelines of the Declaration of Helsinki, as reflected in prior approval by the ethics committee at our institution. Informed consent was obtained from all patients at the time of scanning after the nature of the research procedure was fully explained.

We included 10 consecutive noncirrhotic patients (five of each sex) who were undergoing routine liver imaging during a follow-up examination after surgery for a primary cancer. In each patient, who had been fasting for at least 6 hours, the right lobe of the liver was scanned along the longitudinal plane after intravenous injection of a contrast agent containing sulfur hexafluoride–filled microbubbles (SonoVue; Bracco, Milan, Italy). All sonographic (US) scanning of the liver was performed by one board-certified diagnostic radiologist with 10 years of experience in US of the abdomen; this investigator, who was blinded to the patients’ identification, was also the physician responsible for the study.

For the CEUS examination, we used an automatic power injector system (Echosid 2008; Sidam Medical Devices, Modena, Italy) dedicated to microbubble contrast–agent injection (Figure 1). This device consists of a double syringe pump capable of infusing the contrast agent and saline in sequence. The two 10-ml syringes, one containing the contrast agent and the other containing saline solution, are connected to a Y line, and the injection speed (0.1–3 ml/s) is selectable through manual or pedal control; the system allows multiple infusion speeds. The microbubble syringe is automatically shaken to avoid microbubble sedimentation until microbubble injection, when the shaking stops and effective pressure is applied to the syringe piston.

Each study patient received two injections of the microbubble contrast agent—one bolus injection of 2.4 ml administered at 2 ml/s, followed by one continuous infusion of 2.4 ml administered at 0.5 ml/min for a total infusion time of approximately 5 minutes—into the antecubital or a forearm vein through an 18-gauge intravenous catheter. The 18-gauge calipers of the catheter prevent destruction of the microbubbles. Liver insonation was continued for up to 10 minutes after the bolus injection, until the microbubbles had completely disappeared from the peripheral circulation. At that point, continuous infusion of the microbubble agent was started.

We used a Sequoia 512 US scanner (Siemens-Acuson, Mountain View, CA) with a convex-array probe (5C1; 2–5 MHz) equipped with frequency compounding. The US contrast-specific software corresponded to cadence contrast pulse sequencing (CPS) technology, which detects all nonlinear responses from the microbubble contrast agent, including nonlinear fundamental frequencies. The technical insonation parameters used were low acoustic power expressed by a mechanical index of
0.06–0.08; dynamic range, 65 dB; temporal resolution between frames, 75–100 milliseconds (ie, 10–13 frames per second); echo-signal gain, below noise visibility; signal persistence turned off; and two focuses placed at the level of the middle zone of the liver parenchyma between the skin and the diaphragm. For the entire duration of the CEUS examination, we used CPS contrast–specific imaging with effective suppression of tissue background.

The transducer was maintained in the same position for the entire duration of liver scanning. From 5 seconds after microbubble injection, the liver parenchyma was continuously insonated in real time with the patient breathing normally. The liver parenchyma was insonated on the longitudinal plane, which allows the lowest liver motion during breathing, by selecting an acoustic subcostal view including both the sixth and the seventh hepatic segments. For each type of contrast administration (bolus or continuous infusion), 20 consecutive uncompressed DICOM (digital imaging and communications in medicine) multiframe cine clips (15 frames per second; each clip of 30 seconds’ duration) were transferred to the picture archiving and communication system (PACS) of the radiology department.

**Quantitative Image Analysis**

Within 2 days after scanning was completed, the radiologist responsible for the study (blinded to all patient-identification information) performed a quantitative analysis of each sweep by using dedicated software (Q-ontrast, e-AMID [Advanced Medical Imaging Development] release 4.0; distributed by Bracco, Milan, Italy) to calculate the grade of contrast enhancement, expressed as the percentage increase in video intensity in grayscale level from that at baseline scanning. This software can quantify the increase in video intensity (ie, the contrast enhancement) after injection of the microbubble contrast agent from a sequence of perfusion frames and generate a chromatic parametric map, pixel by pixel, that allows immediate evaluation of the perfusion properties of regions of interest (ROIs) selected by the operator.

The digital cine clips registered after microbubble injection, after removal of all patient-identification data, were transferred to a personal computer (Intel Pentium 4, Santa Clara, CA) connected to the PACS and used for the quantitative analysis. The color map from the CPS technique was automatically converted to a gray-scale–level map. In the first cine clip of each patient, a manually defined polygonal ROI (4,100–40,110 pixels; mean, 21,430 pixels) was positioned on the right liver lobe, including the sixth and/or the seventh segment (Figure 2), and copied in the same position in the subsequent cine clips. Each ROI was drawn at approximately the same depth, avoiding blood vessels, artifacts, and the echogenic walls of the portal vessels. To eliminate the influence of slight movements of the transducer and the patient’s breathing, each frame was aligned with...
Continuous infusion (CI) for each parameter. For administration methods (bolus injection and evaluate the differences between the two signed-rank test for paired data was used to 2010.5.08 (Addinsoft, New York, NY). Wilcoxon’s analysis by using XLSTAT software, version A biostatistician participated in the statistical 139

Statistical Analysis

A biostatistician participated in the statistical analysis by using XLSTAT software, version 2010.5.08 (Addinsoft, New York, NY). Wilcoxon’s signed-rank test for paired data was used to evaluate the differences between the two administration methods (bolus injection and continuous infusion) for each parameter. For all tests, p < 0.05 was considered to indicate a statistically significant difference.

RESULTS

Our 10 patients had a mean (± SD) age of 65 ± 10 years, a mean body weight of 70 kg (range, 52–80 kg), and a mean height of 168.2 cm (range, 150–180 cm). They had undergone surgery for primary colon carcinoma (n = 6), breast carcinoma (n = 2), renal carcinoma (n = 1), and cutaneous melanoma (n = 1).

The continuous-infusion method of administration produced an increase in the video-intensity profile with a more gradual rise of the time-intensity than was seen with the bolus injection (Figure 3), followed by a plateau phase (defined as a horizontal curve over at least 4 minutes with <10% enhancement variation) that lasted until the end of the continuous infusion.

Table 1 presents the results of quantitative analysis of the digital cine clips acquired after administration of the contrast agent. No difference in the maximal enhancement of normal hepatic parenchyma was observed between the two administration methods, with a 45% increase over baseline for the bolus injection and a 39% increase for the continuous infusion (p = 0.62). The mean time to maximal enhancement from the time of microbubble visualization in the scanning plane was shorter after the bolus injection (6.3 ± 0.94 seconds [range, 5–8 seconds]) than it was after the start of the continuous infusion (13.9 ± 1.44 seconds [range, 10–15 seconds]; p = 0.002, Wilcoxon’s test for paired data) (Figure 4). Furthermore, the continuous infusion prolonged the mean duration of contrast enhancement (7.3 minutes ± 40 seconds), compared with the bolus injection (4.3 minutes ± 42 seconds; p = 0.002) (Figure 5).

DISCUSSION

In this study, we found that administration of a continuous infusion of sulfur hexafluoride–filled microbubble contrast agent (SonoVue) by means of an automatic injector, in comparison with its administration via bolus injection, prolonged the duration of contrast enhancement in the human liver parenchyma without significantly reducing the level of hepatic enhancement achieved. In contrast, one previous study showed that continuous infusion of an air-filled–microbubble contrast agent (Levovist) markedly prolongs but slightly reduces hepatic enhancement. Our results confirmed the usefulness of continuous infusion of contrast agent in this aspect and may enhance the utility of continuous infusion in several clinical applications.

the previous one by using a motion-compensation algorithm included in the software.

The US video intensity was measured in gray-scale levels from 0 (black pixels) to 255 (white pixels) through histogram analysis and expressed as the mean (± SD) video intensity of the pixels comprising each ROI, assuming a linear relationship at low contrast concentrations between the video intensity (log-compressed) domain and the microbubble concentration. The log-compressed video intensity can be calculated by applying the equation 10 * log10 (I/I_ref), where I is the acoustic intensity and I_ref is an intensity level determined through equipment gain. Time-intensity curves were fitted according to the following formula: SI(t) = A t e–αt + C, where t = time; SI(t) = signal video intensity versus time; A = amplitude of the curve above baseline; α = initial slope of the ascending track of the curve; and C = echo-signal intensity at baseline (ie, zero on the y-axis).

From these time-intensity curves, the semi-quantitative kinetics parameters—maximal enhancement, time to maximal enhancement, and duration of contrast enhancement—of the hepatic parenchyma were extracted and compared between the bolus injection and the continuous infusion. The maximal enhancement, corresponding to the percentage of the highest possible achievable value of video intensity in gray-scale levels, was calculated for each patient through analysis of the consecutive cine clips. The time to achieve maximal enhancement was calculated from the time of initial microbubble visualization in the scanning plane to the time of peak enhancement. The duration of contrast enhancement in the liver parenchyma was calculated from the time to achieve 10% of the maximal enhancement until the time when the enhancement was reduced to below the level of 10% of the maximal enhancement. According to our empiric evidence, this threshold of 10% of maximal enhancement corresponds to the lowest detectable increase in video intensity on visual analysis.

In this study, we found that administration of a continuous infusion of sulfur hexafluoride–filled microbubble contrast agent (SonoVue) by means of an automatic injector, in comparison with its administration via bolus injection, prolonged the duration of contrast enhancement in the human liver parenchyma without significantly reducing the level of hepatic enhancement achieved. In contrast, one previous study showed that continuous infusion of an air-filled–microbubble contrast agent (Levovist) markedly prolongs but slightly reduces hepatic enhancement. Our results confirmed the usefulness of continuous infusion of contrast agent in this aspect and may enhance the utility of continuous infusion in several clinical applications.
An automatic-injector device for continuous infusion of microbubbles was previously tested in rabbits in a study that produced results similar to ours by prolonging the US signal period in the heart. This is extremely important in the assessment of the liver during CEUS because the more prolonged the contrast enhancement in the liver parenchyma, the higher the chance for the sonographer to accurately explore all liver regions, especially with the aim of detecting hepatic metastases. The use of an automatic injector instead of a bolus injection of the contrast agent allows a constant injection speed of microbubbles, which is essential for prolonging the duration of the hepatic parenchymal contrast enhancement and maintaining that enhancement at approximately the same level during the entire scanning period. Moreover, an automatic power injector is especially well suited for use in hospitals that have a small staff because no additional person is needed in the US unit: the operator can activate infusion of the contrast agent by using the pedal control on the device.

In this study, we used only the slow infusion speed of 0.5 ml/min. We could have tested other speeds (eg, 0.4, 0.6, or 0.7 ml/min) but the use of one single infusion speed is acceptable for a pilot study. Also, we did not randomize the order in which the two administration methods were used, instead always giving 1 bolus injection (2.4 ml at 2 ml/s) followed by one continuous infusion (2.4 ml at 0.5 ml/min). Consequently, the existence of a possible effect of the bolus injection on the results of the continuous infusion cannot be denied, even though we stopped US scanning until the microbubbles from the bolus injection had completely disappeared from the peripheral circulation.
As expected, we found that the time to peak enhancement was shorter after the bolus injection than it was after the beginning of continuous infusion. In fact, our findings are similar to those from a previous study in which Doppler intensity was measured and the time-intensity curves exhibited a rapid first pass, followed by a slower washout, with higher peak enhancement after bolus injection. A difference in this pilot study is that we calculated the time to peak enhancement from the first visualization of microbubbles in the scanning plane instead of from the time of injection of the contrast agent. This method eliminates the influence of the microbubble arrival time, which may be quite different from patient to patient owing to individual differences in cardiac output and kinetics parameters. Moreover, even though the maximal enhancement we obtained after the bolus injection was higher than that after the continuous infusion, the difference was not statistically significant. This was the result of the progressive achievement of maximal video intensity on US screening to the point of video saturation by both administration methods, despite the fact that maximal enhancement was reached more quickly after the bolus injection. This finding implies that continuous infusion of the microbubble contrast agent would ensure a longer period of maximal enhancement as well as intense contrast enhancement, which are both essential for a complete assessment of the liver parenchyma.

The introduction of quantification software packages has enabled objective measurement of echo-signal intensity after injection of microbubble contrast agent. Such quantitative analysis allows a more precise assessment of parenchymal enhancement with less dependence on operator experience. US scanners apply nonlinear modifications to the US video data for visualization purposes, whereas the proprietary software we used in this study quantified the video intensity on the screen in gray-scale and did not perform anti-log conversion of the video log-compressed data. This is a limitation of our study, even though a linear relationship with video intensity can be assumed at low microbubble concentrations. The continuous infusion method of administration should be used for the detection of hypovascular metastases because the lengthier duration of contrast enhancement is essential for better detection of focal liver lesions. For other applications, however, such as the characterization of those focal liver lesions, the bolus injection method would be preferable so that the lesions can be studied dynamically during the different phases (arterial, portal venous, and late) of contrast enhancement.

The principal limitation of this study is our limited number of patients; however, this number is probably sufficient for a pilot study. A further limitation is that we did not assess interreader and intrareader variability. A third limitation is that this study did not demonstrate the relationship between the peak enhancement and infusion rate, but this can be addressed in a study that has higher statistical power.

In conclusion, continuous infusion of a sulfur hexafluoride–filled microbubble contrast agent performed with an automatic power injector prolongs the period of contrast enhancement in the liver without significantly modifying the maximal enhancement over that at baseline, and the data from this pilot study can be used to design a larger study with adequate statistical power.

REFERENCES
