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● *Original Contribution*

GREY-SCALE CONTRAST ENHANCEMENT IN RABBIT LIVER WITH SONOVUE™ AT DIFFERENT DOSES

JIE LI,* BAOWEI DONG,† XIAOLING YU,† XINGHUA WANG† and CHUANFU LI*

*Department of Ultrasound, Qilu Hospital, Shandong University, Jinan, China; and †Department of Ultrasound, The General PLA Hospital, Beijing, China

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Abstract—To evaluate the dose of ultrasound (US) contrast agent (UCA) in relation to the contrast-enhancement effect, an *in vivo* model of perfusion was studied using SonoVue™, a second-generation UCA, and low mechanical index (MI) grey-scale harmonic imaging. SonoVue™, at eight different doses (0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14 and 0.16 mL/kg BW), was applied in five normal rabbits. Flow-related parameters obtained from time-intensity curves were calculated and plotted over the contrast agent doses, and nonlinear curve fitting was performed. Results showed that, along with an increase of the administrated contrast agent dose, the enhancement duration (ED) and the area under the curve (AUC) increased logarithmically, and the time to enhancement (ET) decreased logarithmically. There was a progressive increase of the peak signal intensity (PSI) following an increase of SonoVue™ dose only in the dose range of 0.02 up to 0.10 mL/kg body weight (BW) in the portal vein and in the dose range of 0.02 up to 0.12 mL/kg BW in the liver parenchyma. Moreover, a good correlation was observed between the parameters obtained from liver parenchyma and those obtained from the portal vein. The results indicated that SonoVue™ in conjunction with continuous harmonic low-MI grey-scale imaging has the capability of flow quantification on both vessels and parenchyma. The parameters of time-intensity curve were influenced intensely by different contrast agent doses. (E-mail: jieli301@hotmail.com)

Key Words: **Ultrasound, Contrast agents, Harmonic imaging, Liver, *In vivo*, Dose.**

INTRODUCTION

The development of more stable contrast microbubbles (second-generation agents), as well as harmonic imaging, has greatly improved the tissue delineation for difficult patients (Dill-Macky et al. 2002). Recent studies have focused on the capability of contrast ultrasonography to quantify *in vitro* and *in vivo* flow by means of time-intensity curves, using grey-scale imaging (Heidenreich et al. 1993; Kono et al. 1997; Sugimoto et al. 2002). Different noninvasive imaging modalities can be used to study tissue perfusion in various parenchymatous organs, including computed tomography (CT), single photon emission computed tomography (SPECT), positron emission tomography (PET) and, recently, magnetic resonance imaging (MRI) (Blomley et al. 1995; Chen et al. 2002; Yamada et al. 1999). High costs, reduced availability and long examination time still limit these techniques (Schlosser et al. 2001).

Contrast-enhanced ultrasonography is a widely available and relatively inexpensive method with a high potential for tissue perfusion measurements (Blomley et al. 1998; Hirokawa et al. 2002; Kaul 1995; Pohl et al. 2000; Spiezia et al. 2001). However, a lot of factors have effects on the parameters of the time-intensity curve. Sirlin et al. (1999) found that liver enhancement progressively increased as the frame rate was reduced; peak, duration, and area under the curve (AUC) were all greater at the lower frame rate than at the higher frame rate. Ugolini et al. (2000) reported that a good correlation for the tissue model was observed between absolute flow and onset time of enhancement, time to maximal enhancement, peak intensity, AUC and maximal ascending slope parameters. Maruyama et al. (2002) showed that the contrast enhancement depended on the contrast agent dose and the acoustic power. Previous experiments *in vitro* (Claassen et al. 2001) and *in vivo* (Bos et al. 1999; Forsberg et al. 1999; Seidel et al. 2001) have confirmed a good correlation between some flow parameters and different contrast agent doses. However, as far as we know, the actual relationship between contrast media

Address correspondence to: Jie Li, M.D., Department of Ultrasound, Qilu Hospital, Shandong University, Jinan 250012 China. E-mail: jieli301@hotmail.com

dose and parameters derived from the time-intensity curve has not yet been demonstrated in detail. The purpose of this study was to find *in vivo* the dose of the contrast agent in relation to the contrast-enhancement effect of grey-scale imaging, and to demonstrate the relationship between the time-intensity data obtained form the liver parenchyma and those obtained from the portal vein. It was conducted using transit-time analysis, simultaneously in rabbit liver parenchyma and portal vein, with grey-scale contrast imaging.

MATERIALS AND METHODS

Animal model

Five male New Zealand White rabbits, weighing 2.4 to 2.7 kg (mean body weight: 2.5 kg) with healthy livers, were anesthetized with sumianxin (Changchun Argo-Pastoral University, Jilin, China), an anesthetic compound without influence on the cardiovascular system, at a dose of 1.5 mg/kg body weight (BW) by IM injection. The rabbits were placed in a supine position after the skin of the abdomen was shaved with 8% Na_2S solutions to minimize the difference in acoustic impedance between the ultrasound (US) probe and the skin. Then a 26-gauge catheter was placed into a marginal ear vein, and a three-way stopcock was applied at the end of the catheter, with one branch being used for administration of the contrast agents and another for a physiological saline flush after contrast agent administration. The studies were conducted in compliance with the regulations of the Animal Ethics Committee of PLA General Hospital.

Equipment

Fundamental grey-scale imaging and contrast tuned imaging (CnTi), a nonlinear imaging modality optimized for harmonic signal display, were performed using a Technos^{MPS} DU8 US system (Esaote Biomedica, Genoa, Italy). A broadband linear-array transducer (8 MHz for fundamental imaging and 1.56 MHz for CnTi) was used in this study. All the images were taken by continuous scan at a frame rate of 21 Hz. Scanning setting for the optimal visualization, including the gain, scanning depth, field of view and time gain control (TGC), were determined from test experiments in several rabbits and remained unchanged throughout this study, thus ensuring maximum objectivity. After an optimal imaging plane showing the main branch of left portal vein and surrounding liver was determined, the transducer was held in a fixed position during examination. With a low mechanical index (MI) mode of 0.077, a nearly complete cancellation of signals from stationary tissue was achieved. Before the contrast agent administration, only high-amplitude signals, such as diaphragm, large vessels, etc., were somewhat visualized. After each contrast agent injection, 6-min digitized grey-scale images were recorded on a built-in hard disk for off-line analysis.

Contrast agent

The contrast agent used in this study was SonoVue™ (Bracco, Milan, Italy). It contains sulphur hexafluoride (SF6), a highly echogenic, poorly soluble and totally innocuous gas that is isotonic in human plasma and has the same viscosity as blood. A white, milky suspension of microbubbles was obtained by adding 5 mL physiological saline (0.9% sodium chloride) to the powder (25 mg), using standard clinical aseptic techniques, followed by hand agitation. The suspension was then left to settle for at least 2 min. After reconstitution, the bubble concentration was in the range of (1–5) $\times$ 10^8 microbubbles/mL, with 90% of microbubbles less than 8 μm in diameter. SonoVue™, at eight different doses (0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14 and 0.16 mL/kg BW), was applied in randomized order into a marginal ear vein as a bolus followed by a 1.5-mL saline flush. The time interval between two injections was 10 min.

Data collection and analysis

For the analysis of harmonic grey-scale parameters, the digitally stored data of the liver parenchyma and the portal vein were measured off-line using wash-in/wash-out software built-in Technos^{MPS} DU8 US system. This software was used to obtain time-intensity curves of the portal vein and the liver parenchyma. The sample rate used to record the time intensity curves was 0.18 s. The regions-of-interest (ROIs) of same shape and sizes were set in the main branch of left portal vein and its ventral liver parenchyma, respectively. The curve represents the sum of the signals of the acquisition sample volume present on the chosen scanning plane. The examination could be reviewed by moving back, point by point, along the curve and, at the same time, the corresponding contrast grey-scale image was observed for a simultaneous graphic and morphological evaluation of the transit of the contrast agent, moment by moment. Corresponding parameters of the time-intensity curve: the time to enhancement (ET), time to peak intensity (PIT), peak signal intensity (PSI), enhancement duration (ED) and area under the curve (AUC) were measured. The ET was defined as the delay from injection till the first echogenic bubbles of contrast agent could be seen. The PIT was defined as the time interval from the beginning of the injection to the peak of the time-intensity curve. The PSI was expressed in arbitrary units. The ED was defined as the time interval from the time to enhancement until the echogenic bubbles of contrast agent could not be seen.

Statistical analysis and nonlinear curve fitting were performed with Excel® (Microsoft Corporation,