P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• LIVER CANCER •

Assessment of hepatic VX2 tumors of rabbits with second harmonic imaging under high and low acoustic pressures

Wen-Hua Du, Wei-Xiao Yang, Xiang Wang, Xiu-Qin Xiong, Yi Zhou, Tao Li

Wen-Hua Du, Wei-Xiao Yang, Xiang Wang, Xiu-Qin Xiong, Yi Zhou, Tao Li, Department of Ultrasonography, Daping Hospital and Research Institute of Surgery, the Third Military Medical University, Chongqing 400042, China
Supported by the National Natural Science Foundation of China, No. 30070227
Correspondence to: Wen-Hua Du, Department of Ultrasonography, Daping Hospital and Research Institute of Surgery, the Third Military Medical University, Chongqing 400042, China. duwenhua001@163.com
Telephone: +86-23-68757441
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Abstract

AIM: To investigate the possible clinical application value of second harmonic imaging under low acoustic pressure.

METHODS: Six New Zealand rabbits, averaging 2.7±0.4 kg, were selected and operated upon to construct hepatic VX2 tumor carrier model. Hepatic VX2 tumors were imaged with B mode Ultrasonography (US), and second harmonic imaging (SHI) under high mechanic index (1.6) and low mechanic index (0.1). Echo agent was intravenously injected through ear vein at a dose of 0.01 mL/kg under B mode US and high MI SHI, and 0.05 mL/kg under low MI SHI, and then the venous channel was cleaned with sterilized saline. All the images were recorded by magnetic optics (MO), and they were analyzed further by at least two independent experienced sonographers.

RESULTS: Totally 6 hypoechoic and 3 hyperechoic lesions were found in the six carrier rabbits with a mean size about 2.1±0.4 under B mode ultrasound, they were oval or round in shape with a clear outline or a hypoechoic halo at the margin of the lesions. Contrast agent could not change the echogenicity of the lesions under B mode US and SHI under high acoustic pressure. However, it could greatly increase the real time visualization sensitivity of the lesions with SHI under low acoustic pressure.

CONCLUSION: Our results suggest that contrast enhanced SHI with low MI and a bubble non-destructive method would be much more helpful than conventional SHI in our future clinical applications.

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INTRODUCTION

Second harmonic imaging (SHI) technique has been shown more valuable than conventional B mode ultrasonography (US). SHI involves transmitting at frequency *f* and receiving at frequency *2f*, and the contrast enhanced echoes can therefore be obtained at second harmonic frequency because of the non-linear motion of the gas bubbles when destroyed by high acoustic pressures[1,2]. However, the use of second harmonic technique under high acoustic pressure (mechanical indexes 0.6-1.2) does not usually provide ideal images because of the short duration of enhancement and no quantitative evaluation and other technical difficulties. To avoid this, new dedicated software operating at low acoustic pressure (mechanical index <0.4) has been demonstrated to produce significant harmonic answer together with a real time imaging based on the maintenance of microbubbles. Only very small amount of microbubbles would allow the evaluation of blood flow volume and blood flow in normal and pathologic tissues. Liver image has been recently proven to be the first area where microbubbles manifest similarly to that of computed tomography or magnetic resonance imaging contrast media. In particular, this technique, applied to the evaluation of perfusional pattern of hepatic mass lesions, provides a significant contribution to their detection and characteristics. In this study, we aimed to find out the features of these two ultrasonographic methods in the depiction of hepatic metastasis.

MATERIALS AND METHODS

Preparation of animal models

Six New Zealand rabbits weighing 2.6-3.2 kg, average 2.7±0.4 kg, were anaesthetized by Sumianxin (a product of the Changchun Argo-Pastoral University) at 0.2 mL/kg through intramuscular injection. Hairs over the abdominal region were moulted by 8 % sodium sulfide, then the region was cleaned by saline water. Median incision right beneath the metasternum was made to expose the right lobe of liver. A tunnel about 3 cm deep at the lobe was constructed with an ophthalmic nipper. Viable VX2 tumor masses about 2-3 mm^3 were implanted into the tunnel, locally stanched and then each layer of the abdominal wall was sutured accordingly. 2 or 3 weeks later, these rabbits were ready for use. VX2 tumor is a kind of skin squamous cancer induced by Shope virus, viable VX2 tumor could be transplanted and underwent passages in the New Zealand rabbits, and therefore was used as mimicking metastatic hepatic tumor models.

Preparation of echo contrast agent

Self made echo contrast agent was made from 5 % (g/L) human albumin and 40 % (g/L) Dextran at a ratio of 1:3 (v/v), the mixture was then undergone electromechanical sonication (Sonication machine JY92-2D was manufactured by Ninbo Xinzhi Research Institute) for 90 seconds under mechanical energy of 280 W. During the sonication process, perfluropropane gas was mixed into the mixture. Microbubbles manufactured in this way were counted by a Coulter counter, at a concentration of 1.6×10^9 bubbles/L averaging 4.3±2.1 μm.

Equipment

A transducer S8 connected to HP-5500 ultrasound system was used, second harmonic imaging was transmitted at frequency 3MHz receiving at frequency 6 MHz, conventional mechanic index was tuned to 1.6, while low mechanic index was tuned

to 0.1. During the whole process of experiment, the image depth, compensation and TGC should be kept constant.

Methods

Hepatic VX2 tumors were imaged with conventional B mode US, and second harmonic imaging (SHI) under conventional mechanic index (1.6) and low mechanic index (0.1). An intravenous bolus echo agent was injected through ear vein at a dose of 0.01 mL/kg under conventional B mode US and high acoustic pressure SHI, and 0.05 mL/kg under low acoustic pressure SHI, and then the venous channel was cleaned with sterilized saline. All the images were recorded real-timely by magnetic optics (MO), and they were analyzed further by at least two independent but experienced sonographers.

RESULTS

Features of VX2 tumor under conventional and harmonic B mode US

A total of 6 hypoechoic and 3 hyperechoic lesions were found in the six carrier rabbits with a mean size of 2.1±0.4 cm under conventional B mode ultrasound, no hyperechoic or iso echoic lesions were found. They were oval or round in shape with a clear outline or a hypoechoic halo at the margin of the lesions. Contrast images under conventional B mode US also showed no improvement at all (Figure 1).

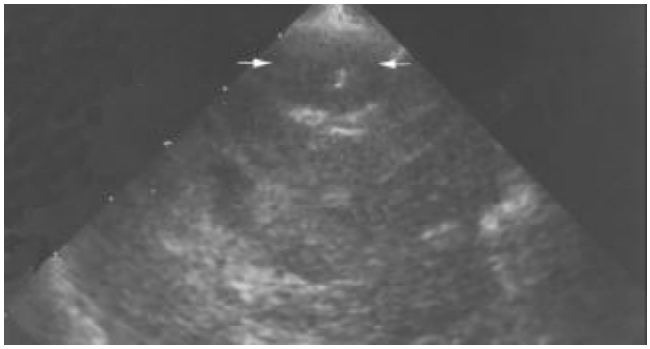

Figure 1 Image of VX2 tumor lesion under conventional B mode US. Arrow indicated the VX2 tumor lesion at the anterior part of the right lobe. It was oval and hypoechoic with a small hyperechoic scar at the center of the lesion.

VX2 tumor lesions assessed by SHI under high and low acoustic pressures

SHI under high acoustic pressure (Mechanic index 1.6) could reveal a short duration enhancement of the hepatic arteries and tumor lesions at the early phase, and an enhancement of the liver parenchyma and a decreased echo at the later phase. A pronounced arterial enhancement was also found at one side of the lesion, which might be considered as the nutrient artery of the tumor. In addition, branches of the afferent artery were seen at the same time. Interestingly, a branch vessel was also found coming from the same artery going to the other side of the lesion as shown in Figure 2a. The whole process of contrast enhanced SHI under high acoustic pressure lasted only for a few seconds.

Visualization by second harmonic imaging under low acoustic pressure was quite different from that by SHI under low acoustic pressure. The echogenicity of liver parenchyma and hepatic VX2 tumor lesions was extremely low, and no structures were observable at first. About five seconds after injection of contrast agent, image of the inferior vena cava could be observed. Again about fifteen seconds later, the contrast agent could be observed within the tumor lesion, as time went by, the afferent artery and its branches of the lesion could gradually and clearly be visualized. A suspicious branch artery was also observed going to the other side of the lesion near the afferent vessels, as what we observed by second harmonic imaging under high acoustic pressure (Figure 2b). As the contrast agent accumulated in the lesion, VX2 tumor appeared as a hyperechoic contour and even satellite lesions could also be observable just beside the original lesion (Figure 3). About 40 seconds later, arteries in the liver parenchyma gradually appeared markedly, and about 20 to 30 seconds afterwards the portal venous system could be visualized. At this stage, the tumor lesion was revealed as hypoechoic. During the last stage period, about 2 minutes after injection of contrast agent, the echogenicity of the liver parenchyma became hyperechoic, while the tumor lesion and its satellite lesion became typically hypoechoic. The whole process of visualization by second harmonic imaging under low acoustic pressure lasted for almost four minutes.

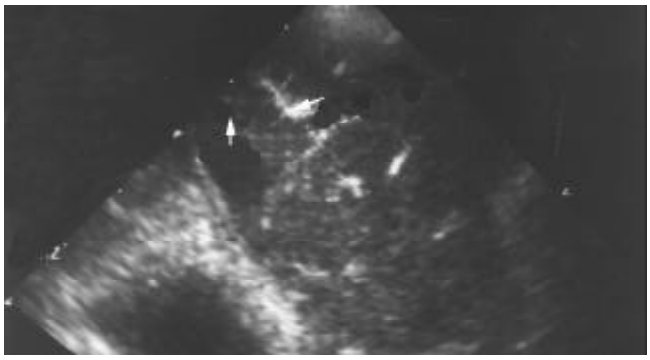

Figure 2a Image of VX2 tumor lesion under high MI second harmonic imaging. Arrow indicated the nutrient artery of same VX2 tumor lesion, and the suspicious artery of a satellite lesion.

Figure 2b Contrast enhanced second harmonic image under low MI revealed the enhancement of nutrient arteries of VX2 tumor lesion and the nutritive artery of satellite lesion.

Figure 3 Image of second harmonic under low MI. It showed the clear tumor and its satellite lesion.

DISCUSSION

The accurate recognition or exclusion of focal liver lesions was a primary objective of diagnostic imaging in patients who were suspected to have a tumor, and the detection of tumor lesions should include their number, location and size. Detection of small liver lesions may be difficult when the acoustic properties of the lesions were similar to those of the surrounding liver parenchyma[3]. The overall accuracy of US imaging in the detection of liver lesions has been shown to range from 53-77 %, but the sensitivity of milimeter nodules has been found to be as low as 20 %[4-6]. US contrast agents were originally developed and used to overcome some of the shortage of US in the assessment of lesional and parenchymal microcirculation by increasing the linear backscattering from the microvascular blood pool. The latest generation of US contrast agents prepared from perflurocarbon gases has been shown to be highly effective in enhancing Doppler signals within the macrovasculature and the microvasculature for several minutes following an intravenous bolus injection[7-9]. However, US contrast agents do not enhance the tumor lesion or parenchyma microvasculature on the fundamental gray-scale image, since the echoes from the tissues are too strong compared to the small volume of microbubbles in the microcirculation[10]. Thus these agents do not significantly improve the detectability of liver lesions when used in association with fundamental imaging, as what we have found in this study. It is necessary to take advantage of their nonlinear characteristics and selectively detect their emission of harmonics in order to increase the US sensitivity to contrast.

A dramatic improvement of contrast enhanced US was the discovery that the bursting of air-based microbubbles caused by high mechanic acoustic pressure US generated large quantities of harmonic frequencies (non-linear response). By decreasing the fundamental frequency, the contrast between highly vascularized lesions containing microbubbles and poorly vascularized tissues were increased[11-14]. Therefore, both macrovasculature and microvasculature of the liver tumor lesions could be well visualized. Some significant limitations about this technique have also been found. Due to the need of breaking air based microbubbles in order to achieve harmonics, the scans have to be started only 2-3 minutes following contrast agent bolus injection, and thus would miss completely the arterial and early portal phases. Furthermore, since all malignancies appear as hypoechoic and all benign tumors isoechoic in late phase[15], discrimination between hepatocellular carcinoma and metastasis, hemangiomas and focal nodular hyperplasia cannot be achieved. In our experiment, although contrast enhanced second harmonic imaging under high acoustic pressure could reveal an increased visualization of the tumor lesions and its surrounding liver parenchymal arteries, the major defect of this method was the short duration of visualization. A suitable method for the detection of hypervascular and hypovascular focal liver lesions should be a bubble non-destruction method with very low MI and high sensitivity for harmonics, allowing continuous real-time imaging of the whole liver and the visualization at the arterial, portal, and late phase during the same examination period with a single contrast agent administration only[16-21]. Furthermore, it should allow us to carry out perfusion and reperfusion studies following the planned bubble destruction and characterization of either hyper- or hypovascular lesions simultaneously[21-25]. We herewith reported our experimental results using this new technique, using a low MI SHI, a nearly complete cancellation of signals from stationary tissues was achieved. Prior to the injection of self-made albumin contrast agent, only high amplitude signals were visualized, such as large vessel walls and the diaphragm. After albumin contrast agent injection, a true subtraction effect was obtained due to the high level harmonic signals coming from the bubbles and the dynamic threshold suppressing low amplitude signals moving toward the transducer. In all rabbits, the whole vasculatures could be observed and studied, including an arterial phase about 15-40 seconds, an early portal phase about 40 to 50 seconds, and a complete portal phase about one and a half minutes. This result is in agreement with what has been described by Solbiati, and this "portal phase" is suggested to be "hepatic sinusoidal phases"[26-31]. The scans could be performed to study the changes of enhancement in these areas by moving the transducer throughout the liver visualizing not only the vascular phases in real time, but also any peculiar region of interest. Using this technique of second harmonic imaging under low acoustic pressure, we could therefore achieve the best visualization of macrocirculation and microcirculation simultaneously. Most interestingly, some satellite lesions, which were not found by high MI SHI, were now clearly revealed by low MI SHI as shown in Figures 2b and 3. It can be concluded that contrast enhanced ultrasonography with second harmonic imaging under low acoustic pressure is currently more sensitive than that with second harmonic imaging under high acoustic pressure in the detection of metastatic lesions as VX2 tumors. This study suggests that this new technique of low MI and microbubble non-destructive method would be much more helpful in our future clinical applications.

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