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• *BRIEF REPORTS* •

Contrast-enhanced ultrasonographic imaging diagnosis on assessment of vascularity in liver metastatic lesions

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

Wen-Hua Du, Wei-Xiao Yang, Xiu-Qin Xiong, Xiang Wang, Yi Zhou, Hui Wang, Department of Ultrasonography, Daping Hospital and Research Institute of Surgery, the Third Military Medical University, Chongqing 400042, China
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Correspondence to: Wen-Hua Du, Department of Ultrasound, 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 vasculature of rabbit liver metastatic lesions by color Doppler imaging and power Doppler imaging (PDI) techniques.

METHODS: Eight New Zealand rabbits with implanted VX2 liver tumors were used. All ultrasound examinations were performed with a HP 5500 color Doppler ultrasound scanner. Before and after the injection of contrast agent, the changes of gray scale and the periphery and intralesional blood flow of the liver metastatic lesion were carefully observed by B mode ultrasound, color Doppler flow imaging (CDFI) and PDI.

RESULTS: Twelve lesions were found in the eight rabbits with implanted VX2 liver tumors, whose diameter ranged from 1.6 to 4.8 cm. Echoes of these lesions were not characterized and has lack of specificity. After the injection of contrast agent, the numbers of dot or strip-like flow messages increased both at the periphery and inside of these lesions under the mode of CDFI and PDI, and were more pronounced under PDI. Morphology of intralesional vessels extended, even branched and some signals were clearly found encircling the lesion. And some vessels were found penetrating into the center of the lesion.

CONCLUSION: PDI after injection of self-made echo contrast agent can show a pronounced sensitivity than that of B mode ultrasound and CDFI in diagnosis of vascularity of a metastatic lesion.

Key words: Doppler imaging; Vasculature; Color Doppler imaging

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INTRODUCTION

Diagnostic imaging, such as CT and MRI depict tumoral vascularity mainly through angiography of the tumoral vessels, and the differential diagnosis of tumors is achieved based on these results. Contrast-enhanced ultrasonography is a recently and rapidly developed technique. Color Doppler flow imaging (CDFI) and power Doppler imaging (PDI) have been proved to have higher sensitivity and specificity in detecting tumor vessels, especially when combined with echo contrast agent[1,2], and therefore they have greatly increased the accuracy of diagnosis and may provide more reliable basis for treatment of tumors.

MATERIALS AND METHODS

Preparation of animal models

Eight New Zealand rabbits weighing 2.6-3.2 kg on an average 2.7±0.4 kg were anesthetized by Sumianxin (a product of the Changchun Argo-Pastoral University) at 0.2 mL/kg through intramuscular injection. Hairs in the abdominal region were molted by 80 g/L sodium sulfide, and 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 established with an ophthalmic nipper. Viable VX2 tumor tissue masses about 2-3 mm^3 were implanted into the tunnel, locally stanched and then each layer of abdominal wall was sutured accordingly. Two or three weeks later, these rabbits were ready for the experiment. VX2 tumor is a kind of dermatological squamous cancer induced by Shope virus, viable VX2 tumor that can be transplanted and generated through New Zealand rabbits, and therefore is used to simulate metastatic hepatic tumor models.

Preparation of echo contrast agent

Self made echo contrast agent was made from 50 g/L human albumin and 400 g/L Dextran in a ratio of 1:3 (v/v), the mixture was then made to undergo electromechanical sonication (Sonication machine JY92-2D was manufactured by Ninbo Xinzhi Research Institute) for 90 s 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,

which concentration was about 1.6×10^9 bubbles/L with an average size 4.3±2.1 μm.

Equipments

A transducer L11-3 connected to HP-5 500 ultrasound system was used, whose fundamental wave frequency was 5-13 MHz. During the whole process of experiment, the image depth, color gain and depth gain compensation were kept constant.

Methods

Hepatic VX2 tumors were imaged with conventional B mode US, CDFI, and PDI. Echo agent was intravenously injected at a dose of 0.01 mL/kg through ear vein, and then the venous passage was cleaned with sterilized saline. All images were recorded real-timely by magnetic optics (MO), and they were analyzed further by at least two independent experienced sonographers.

In order to compare and analyze, scanning of CDFI and PDI were recorded when the depiction of blood flow sections was the richest. The vessel numbers of the same section before and after echo-contrast injection was compared and semi-quantified (spot-like, short bar-like and strip-like blood flow signals were all considered as one vessel). Standards for grading were as follows: Grade 0, no blood signals; Grade I, less than three vessels inside the tumor; Grade II, three to five vessels inside the tumor; Grade III, more than five vessels inside the tumor.

RESULTS

Features of VX2 tumor under conventional B mode US

Total 12 lesions were found in the eight carrier rabbits, the size of these lesions ranged from 1.6 to 4.8 cm respectively. Among these lesions, seven lesions were even echoic, three were hypoechoic, one was slightly hyperechoic and one was of echogenecity mixed with hyperechoic and cystic areas. They were oval or round in shape with a clear outline or a hypoechoic halo at the margin of the lesions.

Changes of intralesional blood flow under CDFI and PDI

At the periphery of all these lesions, spot-like or short bar-like flow signals could be revealed by CDFI, while at the inside of these lesions, only spot-like flow signals could be seen. And there were seven lesions which could be graded as grade I, another five lesions as grade II. PDI could reveal a clear bar-like flow signal at one side of the lesion, and relatively more signals around and inside the lesions as compared with CDFI. There were three lesions, which could be graded as grade I and another nine lesions as grade II. After the injection of contrast agent, it was found that eight lesions were depicted with remarkable increased flow signals under CDFI, four lesions without any obvious increase of flow signals, and that two lesions could be graded as grade I and 10 lesions as grade II. While comparing with CDFI, it was found that 11 lesions were depicted with remarkable increased flow signals under PDI, only one lesion without any obvious increase of flow signals. After grading, no lesion was graded as grade I, one lesion as grade II, 11 lesion as grade III. The results of semi quantification on vessel numbers were listed in Table 1.

Table 1 Changes of depicted intratumoral vessel numbers

Grade	CDFI (number of vessels)		PDI (number of vessels)	
	Before CA	After CA	Before CA	After CA
0	0	0	0	0
I	7	2	3	0
II	5	10	9	1
III	0	0	0	12

CA: contrast agent.

Changes of the morphology of intralesional blood flow under CDFI and PDI

After injection of contrast agent, the length of vessels elongated and the numbers of vessels increased under both CDFI and PDI, especially under the mode of PDI. Tumoral vessels depicted by PDI were as elongated as a strip, and some of them were even branched. At the periphery of the lesions, some vessels were found encircling and even penetrating into the center of the lesion (Table 2).

Table 2 Changes of vessel morphology inside or at periphery of lesions

Blood flow	CDFI (number of vessels)		PDI (number of vessels)	
	Before CA	After CA	Before CA	After CA
No flow signals	1	0	0	0
Spot-like flow signals	9	14	17	21
Short bar-like flow signals	10	15	13	16
Strip-like flow signals	2	4	5	7
Penetrating flow signals	0	0	0	2

CA: contrast agent.

DISCUSSION

It was of great importance for both ultrasonographic diagnosis and differential diagnosis to detect out the blood flow in small hepatocellular carcinoma lesions, especially that in the small metastatic liver lesions[3]. The growth and development of microvascular net of tumor are absolutely necessary for the growth of tumor cells. Malignant tumors usually grow invasively and are not restricted by the development of tumor vessels[1]. Tumor vessels own some biological and morphological characteristics, and the growth of newly formed vessels manifests as follows: (1) grows unrestrictedly by itself; (2) the newly formed vascular net distributes around the tumor margin twisted and disorderly, and trespassing into the mass of the tumor radially; (3) the number of vessels in the tumor is usually increased, therefore, malignant tumors are often hypervascularized; (4) the patterns of these vessels are usually prototype with a thin wall and lack of the layer of smooth muscles, their inner layers are usually discontinuous or only have some connective tissues and even covered directly by tumor cells; (5) at the periphery of the tumor nodules anastomosis of interfacing artery and vein could be often seen[2-6]. The aforementioned features of the tumor

vessels thus determine some of the hemodynamic characters of CDFI and PDI during scanning. That is to say, we could detect a very low flow velocity inside the tumors and a relatively high flow velocity around the tumors, and a turbulent flow signals at the site of arteriovenous anastomosis.

It has been already demonstrated that the imaging diagnosis of metastatic liver lesions with B mode ultrasonography has lack of specificity[7-9]. In those very small metastatic lesions, both CDFI and PDI could not detect the Doppler flow signals due to the relatively low sensitivity of machine update[9]. However, to those lager lesions, CDFI and PDI may find out arterial flow signals[7]. Some researchers reported that PDI could reveal flow signals as low as 0.8 cm/s in a diameter of only 0.7 mm, while CDFI could reveal flow signals as low as 1.5 cm/s only[10-14]. Within the changing variety of acoustic beam angles from 33° to 52°, the sensitivity of PDI is not influenced, but the sensitivity of CDFI is lowered at least four folds. Thus an effective differentiation, between metastatic lesion and a benign lesion (such as hemangioma) could not be made by a simple scanning of B mode ultrasound, or CDFI or PDI[14]. Under these situations, the application of contrast enhanced PDI might provide us a new diagnostic way, which is not only convenient to detect out lesions, but also helpful for the qualitative diagnosis. Contrast bubbles may increase the reflection of acoustic waves, and thus remarkably increase the ultrasound signals for both B mode and Doppler ultrasound. Using conventional imaging under fundamental waves, tissues may bring out intensive scattering signals. Receiving scattering signals from both contrast bubbles and tissues after the application of contrast agent, conventional B mode ultrasound could not tell the difference of the scattering signals whether from contrast bubbles or from tissues. However, contrast bubbles could help us realize the diagnosis and differential diagnosis of tumor lesions through the increase of Doppler effects. Second harmonic imaging US technique involves transmitting at frequency *f* and receiving at frequency *2f*, thus it filters out the fundamental signals from the liver parenchyma and extracts especially the non-linear harmonic signals in the acoustic field to realize a remarkable contrast effects[15-17]. It was demonstrated in this study that both CDFI and PDI could reveal a marked enhancement of Doppler flow signals at the periphery or inside the metastatic lesions after the application of the self-made contrast agent, which manifested as the clearly visioned vessel numbers increased obviously, and especially the focal lesions with vessels characterized as grade II and III significantly increased. The morphologic features of flow signals also changed obviously, shown as an increase of spot-like and strip-like flow signals. All these changes were shown most remarkably under PDI. PDI showed that both the increase of vessel numbers and changes of morphological features were remarkable, which were shown as the increase of spot-like or strip-like vessels, sometimes a branched vessel or a surrounding flow signal, or even a long strip-like flow penetrating into the tumor center might be observed, and these flow signals were complete and clear[18-23]. Within the specific sample, CDFI could show whether there is a flow signal, the flow velocity, its directions and its spatial distribution, *etc.* Comparing with CDFI, PDI has obvious priority in the detection of tiny vessels, however, it could not provide information such as flow velocity, flow directions or turbulent signals. But it was very sensitive to low velocity flow signals. This might be the main mechanism of why PDI had some priority to CDFI in the detection of tumor vessels after application of contrast agent[24,25].

PDI and CDFI could reveal characters of tumor vascularity, such as the number, the distribution and the directions of tumor vessels, but the changes of tumor vessel numbers and the morphological changes of flow signals may have higher diagnostic values especially after the use of echo-contrast agents[26-31]. Power Doppler contrast imaging technique may also provide more reliable information for the diagnosis and differential diagnosis of tumor lesions[32,33].

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