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Sonographic shift of hypervascular liver tumor on blood pool harmonic images with definity: Time-related changes of contrast-enhanced appearance in rabbit VX2 tumor under extra-low acoustic power

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Abstract

We elucidated the features of the time-related contrast-enhanced ultrasound appearance of hypervascular liver tumor using Definity, which has no accumulation activity in the liver. Ten rabbits with VX2 tumors broadcast into the liver were used. Changes in contrast-enhanced sonograms were evaluated by real-time observation (FR 15 Hz) of harmonic imaging under extra-low MI (MI 0.065) with Definity, and their intensity changes were analyzed. Hepatic angiography (4/10) and histopathological examination (10/10) were performed to investigate the tumor vascularity. VX2 tumors were hypervascular on angiogram (4/10) and histology (10/10). They showed time-related sonographic appearance changes from hyperechoic to hypoechoic, which were confirmed by quantitative intensity analysis. Hypervascular VX2 tumors showed characteristic time-related shift on contrast-enhanced sonograms in real-time and extra-low MI harmonic images with Definity. These findings may be useful for the ultrasound diagnosis of human hypervascular liver tumor like hepatocellular carcinoma with blood-pool contrast agent.

Keywords: Ultrasound; Contrast agent; Definity; Low MI (mechanical index); Liver; VX2 tumor

1. Introduction

Recent advances in sonographic technologies and sonographic contrast agents have made it possible to demonstrate peripheral blood flow in the liver on ultrasound (US) images [1–5]. For an efficient echo enhancement of microbubbles, harmonic imaging has gained considerable acceptance as a method for observing contrast-enhanced sonograms with high resolution and less artifacts [6–15]. Furthermore, images of interest such as blood vessels or tissue perfusion are available by changing acoustic power levels according to diagnostic purpose [11].

Definity, a perfluoropropane gas-filled microbubble, provides real-time harmonic images of peripheral blood flow in the liver under a very low acoustic power level [11,14,15]. There are high expectations regarding the real-time observation of contrast-enhanced sonograms using this agent for the diagnosis of liver tumors in clinical practice. The acoustic and biological behaviors of Definity and Levovist, a galactose-based popular US contrast agent, have displayed some differences. The most profound difference is that Definity, in contrast to Levovist, does not accumulate in the liver [16–18]. Therefore, contrast-enhanced sonograms with Definity may be quite different from those using Levovist. However, the time-related sonographic appearance of liver tumors during the course of echo enhancement has not been fully discussed in contrast-enhanced harmonic

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images with Definity under extra-low mechanical index (MI).

In this study, we examined the time-related changes of contrast-enhanced sonograms of VX2 tumor, as a model for hypervascular liver tumor, in rabbit liver by real-time observation of harmonic imaging under extra-low MI with Definity. The aim of this study was to elucidate the features of contrast-enhanced US appearance of hypervascular liver tumor with Definity, a contrast agent without accumulation activity in the liver.

2. Materials and methods

2.1. Animals

2.1.1. Preparation of rabbits with VX2 tumor

VX2 tumor cells were inoculated in a left lateral lobe of the liver under laparotomy, with a concentration of 1×10^8 cells/ml. The resection of the tumor from donor rabbits was performed 2 weeks after the inoculation. VX2 tumor tissue removed from a donor rabbit was crushed into fine pieces (about 0.5 mm) that were then suspended in Hank's balanced salt solution (HBSS) after filtering through a stainless steel mesh. Ten recipient rabbits, New Zealand White male rabbits, underwent laparotomy under intravenous pentobarbital sodium (30 mg/kg). The 1.0 ml of HBSS containing 10 pieces of VX2 tumor tissue was inoculated directly into the superior mesenteric vein with needle (20G) under laparotomy to produce liver metastases [19]. We did not have any surgical complications except for a minor bleeding after the removal of the needle. The present study was conducted 3–4 weeks after the inoculation. These 10 rabbits weighing 1.8–2.3 kg (2.09 ± 0.15 kg, mean $\pm$ S.D.) carrying VX2 tumors were used in this study. The diameter of the largest VX2 tumors averaged 9.2 ± 2.2 mm (4.9–13.2 mm) as observed by tissue harmonic grey-scale sonography.

2.1.2. Preparation of rabbits for US examination

The rabbits with VX2 tumors were anesthetized with pentobarbital at a dose of 30 mg/kg by bolus injection followed by continuous infusion at 3.0 mg/(kg h) into the auricular vein. The skin of the abdomen was shaved after the animals were stabilized, and US examination was performed at the body surface in a supine position. A 4-Fr 1.0 mm plastic tube was inserted into the femoral artery to monitor and record arterial pressure and pulse rate during the examination. Then, a 3-Fr 1.35 mm catheter was inserted into the right femoral vein and a three-way stopcock was applied at the end of the catheter, with one branch being used for administration of the contrast agent and another for the saline flush after the administration. All studies were conducted in compliance with the regulations of the Animal Ethics Committee of Yamanouchi Pharmaceutical Co. Ltd.

2.2. Equipment

Contrast-enhanced harmonic images (pulse inversion harmonic mode) were obtained with SSA-770A (APLIO, Toshiba, Tokyo, Japan) by using a linear probe that transmitted and received center frequencies of 3.5 and 7.0 MHz. All the images were taken by continuous scan at a frame rate of 15 Hz, and the dynamic range was set at 50 dB during the entire examination. Sensitivity time control (STC) was set at optimal levels and the focal point was set at the level of the tumor in pre-enhancement tissue harmonic images. The contrast-enhanced study was performed under extra-low acoustic power levels (MI 0.065) according to the previous reports [14,15].

2.3. Contrast agent

The contrast-enhanced US examination was performed with Definity^{TM} (a perfluoropropane gas-filled lipid-stabilized microbubble, Bristol-Myers Squibb, North Billerica, MA, USA), at a dose of 30 μl/kg [11,14]. The agent was prepared by shaking the vial with an agitation machine for 45 s. After then, Definity was manually injected into the rabbits via a cannula inserted into the femoral vein at a rate of about 0.1 ml/s, followed by a 1.0 ml of saline solution flush.

2.4. Contrast-enhanced sonography

Two operators examined all 10 rabbits (5 rabbits by H.M. and the other 5 by S.M.). Before enhancement, tissue harmonic imaging was performed to find the largest tumor nodule among multiple liver tumors. Following this, Definity was injected into each rabbit. All the movies were recorded digitally from the time of injection of the contrast agent to the disappearance of contrast enhancement, and blinded readers (H.M. or S.M., who were not operators for the images) were used to analyze the images.

2.5. Intensity analysis for contrast-enhanced images

A specialized program for APLIO was used for the analysis of contrast-enhanced sonograms. All the images, still images digitally stored, were recorded every second from the injection of Definity for 90 s and then every 30 s after the 90 s. A circular region of interest (ROI), with the size being dependent on the tumor diameter, was set on the tumor, and the circular ROI was also set on the liver parenchyma at the same depth from the skin surface as the control ROI. The average of the pixel intensities over the ROI was measured on each image.

2.6. Hepatic angiography

After the US examination, selective hepatic angiography was performed to evaluate the vascularity of VX2 tumors using X-ray system specialized for animals (VPX-100A,