The Influence of Blood Supply on High Intensity Focused Ultrasound:

A Preliminary Study on Rabbit Hepatic VX2 Tumors of Different Ages

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Rationale and Objectives: The aim of this study was to explore the effects of blood supply on high-intensity focused ultrasound (HIFU) applied to rabbit hepatic VX2 tumors of different ages.

Materials and Methods: Eighteen rabbits with VX2 hepatic tumors were randomly divided into three groups according to the time of sacrifice after tumor implantation: 10, 15, or 20 days. Contrast-enhanced ultrasound was performed immediately before HIFU ablation. The same settings for HIFU dose parameters were used to ablate the central tumor area in each group, and the real-time temperature of the targeted site of the tumor was measured. After HIFU, the coagulation necrosis volumes of tumor tissue and the microvascular density of residual tumor tissue were determined.

Results: Histopathologic analysis showed that the extent of a tumor's blood supply followed the order 10-day group > 15-day group > 20-day group ($P < .01$). Contrast-enhanced ultrasound showed the same results. There was no statistically significant difference among the three groups in terms of temperature-increase parameters during HIFU treatment ($P > .05$). However, there were statistically significant differences between the groups in terms of temperature-decrease parameters during HIFU treatment and in terms of necrosis volumes after HIFU treatment ($P < .05$). Necrosis volume was inversely related to absolute enhanced intensity ($r = -0.823$, $P < .001$).

Conclusions: The extent of a tumor's blood supply had a significant effect on the temperature-decrease phase but not on the temperature-increase phase during HIFU treatment. The longer the temperature-decrease phase, the more slowly heat dissipated after HIFU, resulting in larger coagulation necrosis volumes.

Key Words: Rabbit; VX2 tumor; liver tumor; high-intensity focused ultrasound.

High-intensity focused ultrasound (HIFU) is a rapidly developing noninvasive technology for thermal treatment. HIFU ablation of tumors is based on the absorption of ultrasound energy in a focal zone located at a distance from the therapeutic ultrasound transducer. In recent years, the technique has been used clinically to treat a variety of solid tumors, with promising prospects (1–4). Nevertheless, many studies of HIFU ablation have shown that tissue heating and lesion coagulation are affected by many factors, such as overlying ribs, abdominal gas, and the extent of blood perfusion within tumors (5–7). Tumor blood supply has been identified as one of the most important predictive factors. Early studies reported that HIFU ablation was less effective in the presence of abundant tumor blood perfusion, which was able to carry part of the focused ultrasound energy away. This was especially true in malignant tumors with high neovascularity and vascular invasion (7–9). Thus, the extent of blood perfusion within the focused region can limit ablation size and alter ablation shape by lowering the maximal temperature induced with HIFU (10).

The splanchnic circulation in rabbits is similar to that in humans (11). Rabbit hepatic VX2 tumors are highly vascularized by the hepatic artery because of the induction of neovascularity in the host animals, thereby resembling human hepatic carcinoma (12). This tumor model has thus been widely used for research on interventional therapy and imaging evaluation. Lorelius and Stridbeck (13) found age-related changes in VX2 tumor vascularity of the rabbit hind leg. We propose that rabbit hepatic VX2 tumors of different ages may also possess age-related changes in blood supply.

Real-time contrast-enhanced ultrasound (CEUS) is the most convenient imaging modality for demonstrating this phenomenon. The method been shown to be highly sensitive for detecting microbubble contrast media in tumor vasculature (14,15) and is a valuable imaging choice for assessing the extent of blood supply of rabbit hepatic VX2 tumors.

To our knowledge, there has been little research on the quantitative evaluation of real-time temperature phase on

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targeted areas of highly aggressive rabbit hepatic VX2 tumors of different ages ablated by HIFU. The purpose of this study was to further investigate how variability in blood perfusion influences HIFU's effects on tissue by detailed analysis of temperature increase and decrease during HIFU treatment.

MATERIALS AND METHODS

Experimental Setup and Animal Handling

The experiment was conducted in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals. The study was approved by the local animal ethics committee.

Eighteen healthy New Zealand White rabbits weighing 2.5 to 3 kg were supplied by the laboratory animal center of our university.

The rabbit hepatic VX2 tumor model was adopted according to previous reports (16,17). The VX2 tumor tissue in the thigh of one tumor-bearing rabbit was dissected and cut into pieces 1 to 2 mm^3 in volume under sterile conditions. The 18 recipient animals were anesthetized by ear marginal vein injection of 3% pentobarbital solution (1 mL/kg), and their upper abdomens were shaved and depilated with 8% sodium sulfide solution. The rabbits were fixed in supine position and disinfected. A median incision was made below the xiphoid process to expose the left lobe of liver, where a hole 1 to 2 cm deep was made using ophthalmologic forceps. The two VX2 tumor tissue pieces were implanted into each hole. Bleeding points were stopped with gelatin sponge, the abdominal wall was sutured, and the incision was disinfected. After tumor inoculation, the rabbits were allowed to recover and were randomly divided into three groups of six rabbits each according to the time allowed for survival after tumor implantation: 10, 15, or 20 days. Ultrasound was used to monitor the tumor growth consecutively, and the size of the tumor was measured before HIFU ablation.

CEUS Examinations

On the 10th, 15th, and 20th days after tumor implantation, rabbits anesthetized with 3% pentobarbital sodium (1.0–1.5 mL/kg) intravenously were fixed on supine boards, maintaining access to the ear veins.

The Aloka α10 scanner (Aloka, Tokyo, Japan) was used, with a 7-MHz to 10-MHz linear-array broadband probe. The Aloka α10 scanner is equipped with software for contrast media imaging and time-intensity curve (TIC) analysis. Fundamental ultrasound was performed to verify the presence of VX2 liver tumor and to measure the diameter. SonoVue (Bracco, Milan, Italy), a second-generation ultrasound contrast agent containing sulfur hexafluoride phospholipid-stabilized microbubbles, was used for this study. A white, milky suspension of sulfur hexafluoride microbubbles was obtained by adding 5 mL of physiologic saline (0.9% sodium chloride) to the powder (25 mg), using standard aseptic techniques, followed by hand agitation. After entering CEUS mode (mechanical index, 0.11), SonoVue (0.1mL/kg) was administered as a bolus into the ear vein using a 21-gauge intravenous catheter, immediately followed by a 5-mL flush of 0.9% saline. Data acquisition was started after the injection of contrast agent. Technical settings such as mechanical index, frame rate, and focal zone were optimized to obtain images of the best quality. Perfusion of contrast agent in the tumor was observed, and all sonographic examinations were recorded on digital disks.

All imaging examinations were performed by a single ultrasound physician with animal imaging experience. The peak intensities of tumor and surrounding normal liver tissue were acquired automatically by means of TIC software. Absolute enhanced intensity (AEI) was calculated as the peak intensity of tumor minus the peak intensity of surrounding normal liver tissue at the same depth.

HIFU Treatments

HIFU was performed using an extracorporeal, ultrasound-guided focused tumor therapy system (FEP-BY02; Yuande Biomedical Engineering Co, Ltd, Beijing, China). This system consisted of a therapeutic transducer and a real-time imaging transducer. The transducers were mounted in a water reservoir with the beam axis directed upward, and the rabbits were positioned above the transducers in a prone or right-down decubitus position with the skin overlying the lesion inside the water. The therapeutic transducers focused ultrasound beams into small, clinically relevant volumes, which induced locally high temperatures at the points of focus. The area of destroyed tissue was referred to as the ablation zone.

Immediately before HIFU, a thermocouple probe (Yamari Industries Ltd, Osaka, Japan) was inserted under ultrasound guidance to position the needle in the tumor, and temperature measurements were made by an intelligent digital thermometer controlled by a computer (18).

The body temperature of the rabbits was regarded as the initial temperature (T_0) of tumor, which was normally about 39°C (19). The HIFU focus was accurately positioned at the tumor target site by adjusting the position of the treatment couch. HIFU ablation was performed by a single exposure, causing ellipsoidal-shaped focal ablation zones. Single exposure was radiated with identical treatment parameters, including a continuous sonication time of 5 seconds, acoustic power of 300 W, frequency of 1.1 MHz, mean diameter of the focal field of 3.0 mm, length of the focal field of 8.0 mm, and focal length of 150 mm.

Temperature variations in the tumor target site were measured by the thermocouple probe and recorded simultaneously and continuously every second for a total of 30 seconds after beginning treatment to create time-temperature curves. The following data were measured and calculated: peak temperature ($T_{\max}$), peak temperature time (t_1), temperature decrease time (t_2), temperature increase slope (k_1), and temperature decrease slope (k_2), where $k_1 = (T_{\max} - T_0)/t_1$, and $k_2 = (T_{\max} - T_0)/(t_2 - t_1)$.