Effect of Echo-Guided High-Intensity Focused Ultrasound Ablation on Localized Experimental Tumors

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ABSTRACT. We fabricated a prototype 3.25-MHz split-focus therapeutic transducer combined with a small 6.5-MHz imaging ultrasonic probe for transrectal treatment of prostate cancer and evaluated the feasibility of using split-focus high-intensity focused ultrasound (HIFU) to ablate localized tumor tissue without injuring the surrounding organs. We therefore established a localized tumor model by inoculating VX2 tumor into rabbit livers. The effects of split-focus HIFU ablation on the VX2 tumors and the surrounding tissue were evaluated. The six shots of split-focus HIFU destroyed the VX2 tumors without injuring the liver surfaces or the surrounding organs. These results suggest that split-focus HIFU ablation could be an effective treatment of localized tumors.

KEY WORDS: HIFU, liver, rabbit, split-focus, VX2.

As in humans, prostate cancer in dogs can cause problems for older animals. The most frequent type of prostatic neoplasm observed in dogs is malignant adenocarcinoma [11]. This type of prostatic neoplasm is prone to metastasis to surrounding tissues such as the pelvic lymph nodes, bladder neck, and ureters and to the vertebral bodies and lungs [4]. Surgical removal of the prostate gland is the most common treatment for prostate cancer in dogs, but a dog’s quality of life (QOL) after radical prostatectomy is compromised by urinary incontinence. Localized prostate cancer would therefore be better treated by using a minimally invasive, nonsurgical procedure.

Transrectal ultrasonography is often used in human urology for imaging the prostate through the rectum wall [12] and has also been used for diagnosing ovarian cysts in goats [10]. As a guide in obtaining biopsy samples, it is useful in the differential diagnosis of prostatic disorders such as prostatic hypertrophy and benign prostatic hypertrophy. The technique of transrectal ultrasonography allows early diagnosis of localized prostate cancer. Early identification and management of prostate cancer in dogs is becoming more important in veterinary medicine.

High-intensity focused ultrasound (HIFU) surgery was first used to create focal lesions deep in liver tissue [8] and was further developed by a group headed by William Fly in Illinois in the 1950s. At the point where the ultrasound waves are focused (focal point), sudden and intense absorption of the ultrasound beam can create a rapid elevation in temperature, and this destroys the cells located at the targeted area without damaging tissue elsewhere in the path of the beams. HIFU has been used to treat glaucoma in human patients [19] and for ablation of prostatic tissue in dogs [7]. Clinical studies with human patients have also explored the use of HIFU for transrectal treatment of benign prostatic hyperplasia and prostate cancer [2, 9, 18].

HIFU works by focusing a short pulse (several seconds) of high-energy ultrasound waves on a volume of tissue about the size of a grain of rice. Its main disadvantage is that only a small amount of tissue is ablated in a single exposure. When larger amounts of tissue are to be ablated, as in tumor therapy, it is necessary to scan the focused beam over the targeted region. Mechanical scanning methods result in long treatment periods and thereby adversely affect the patient’s QOL.

The split-focus approach was developed about a decade ago to create a broad heating pattern without forming unwanted secondary foci in front of or behind the focal plane [3, 14, 15]. This approach has also been reported to have the potential to substantially improve the throughput of HIFU thermal ablation (that is, to reduce treatment time) because it heats a volume of tissue larger than that heated in the conventional single-spot scanning approach [5, 6, 13].

We developed a prototype split-focus transducer combined with a small imaging ultrasonic probe for transrectal treatment of the prostate cancer and demonstrated that the split-focus approach produced a larger coagulation volume in normal tissues such as the canine prostate [17] and pig liver [13] without injuring the surrounding tissues.

The purpose of this study was to evaluate the feasibility of using a prototype transducer to ablate localized tumor tissue. We therefore established a localized tumor model by inoculating VX2 tumors into rabbit livers. The effects of split-focus HIFU ablation on the VX2 tumors and the surrounding regions under the guidance of real-time echo
imaging were then examined.

MATERIALS AND METHODS

 Prototype ultrasonic transducer: A prototype split-focus power transducer with 2 elements, constructed for transrectal treatment of the prostatic cancer, was used in the exposure experiments. The dual element PZT transducer (Fuji Ceramics, Shizuoka, Japan) had a resonant frequency of 3.25 MHz, a spherical curvature radius of 35 mm, and an aperture of 40 mm × 20 mm. The area of each element was 40 mm × 10 mm. As shown in Fig. 1, the elements were mounted in an aluminum housing that also contained a small imaging probe (EUP-F331, Hitachi Medical Co., Ltd., Tokyo, Japan) operating at 6.5 MHz and having a convex curvature radius of 10 mm. The position and angle of the imaging probe relative to the power transducer were adjusted in calibration experiments before the animal experiments. The whole assembly was contained in a polymer housing with a 30-mm outer diameter and was covered with a latex water bag (Echo probe cover B, Utsunomiya Seisakusho, Osaka, Japan) when used in experiments. To prevent cavitation bubbles from forming in the bag, we filled it with degassed water just before each use. Each element of the transducer was driven with an RF amplifier (2100L, ENI, Rochester, NY, U.S.A.). The input signal to the amplifier, the phase of which needed to be controlled, was generated by an arbitrary waveform generator (HP33120A, Hewlett Packard, Palo Alto, CA, U.S.A.).

When the two elements of the power transducer are driven at opposite phases, the focused beam is split into two. This dual split-focus beam was used instead of the conventional single-spot focal beam to produce a larger coagulation volume [17]. Schlieren images of the focal field from the prototype transducer are shown in Fig. 2. The side image and front images of both the single-spot and split-focus fields are shown. In split-focus mode, the two focal beams were separated from each other by about one millimeter.

The total acoustic power output from the transducer was calibrated against the drive voltage by measuring the radiation force on a hollow aluminum plate in degassed water [16]. The acoustic intensity distribution pattern on the focal plane was measured in degassed water with a 0.5-mm diameter needle-type hydrophone (Imotec, Coesfeld, Germany) at a low drive voltage. From these results, the spatial peak acoustic intensity was obtained as a function of drive voltage assuming that the intensity distribution pattern does not significantly change as the drive voltage increases. This peak acoustic intensity in water was used to describe the exposure condition in the animal experiments, ignoring ultrasonic attenuation while traveling a short distance in tissue.

Animal experiments: A female Japanese White rabbit inoculated with VX2 by intramuscular injection was supplied by Sankyo Labo Service Corporation (Tokyo, Japan) as a tumor source. Nine female Japanese White rabbits, weighing 2–2.5 kg, were used as recipient animals and were anesthetized with xylazine (1.0 mg/kg, i.m.) and ketamine (5.0 mg/kg, i.m.) for all surgical procedures. For tumor implantation into the liver, a small midline incision was made in the recipient animals through which the exterior left lobe of the liver was delivered. Approximately 1-mm³ pieces of fresh tumor block were gently injected into the middle of the liver lobe using a trocar. The tumor-bearing liver was returned to the abdominal cavity, and the abdomen was sutured closed. HIFU exposure was begun on the 7th day after tumor implantation. Animal experiments were conducted in accordance with guidelines for the care and use of laboratory animals, Central Research Laboratory, Hitachi, Ltd.

VX2-bearing rabbits were fasted for 24 hr prior to HIFU experiments and were anesthetized with a mixture of ketamine and medetomidine. The hair on the skin over the liver was shaved and cleaned to facilitate good acoustic coupling. During the experiments, anesthesia was maintained by ketamine injection. Before HIFU exposure, the liver of each rabbit was carefully examined through the skin using a small 6.5-MHz diagnostic imaging probe (EUP-F334, Hitachi Medical Co., Ltd., Tokyo, Japan) operated by an ultrasonic scanner (EUB-6000, Hitachi Medical Co., Ltd., Tokyo, Japan).

After diagnosis, the prototype split-focus transducer was positioned on the skin of the rabbit. To facilitate transfer of the ultrasound beam, the water bag of the transducer was filled with degassed water and placed onto the rabbit abdomen, as shown in Fig. 3a. VX2 tumors were transdermally observed in livers by the imaging probe attached to the power transducer. Real-time pulse-echo images obtained with the probe were used to position the focus of the power transducer at the VX2 tumor in the liver tissue as shown in Fig. 3b. The VX2 was insolated for 4 s at a peak intensity (in the water) of 6.0 kW/
The treatment cycle consisted of 4-s period of split-focus HIFU insonation followed by a 20-s period (off) for heat dissipation. Each VX2 tumor was treated with six of these cycles.

The liver lobes were excised a day after insonation, fixed in 10% formaline, and later stained with hematoxylin and eosin (H & E) for histological examination.

RESULTS

As shown in Fig. 3 (b), the power transducer was transdermally focused on the VX2 tumor in the rabbit liver using real-time pulse-echo images obtained with the probe. The VX2 tumor was seen as a hyperechoic region before HIFU exposure as shown in Fig. 4 (a). Just after HIFU exposure, tissue surrounding the tumor appeared to be slightly more hypoechoic than before exposure [Fig. 4 (b)]. As shown in Fig. 4 (c), the VX2 tumor was observed to be hyperechoic and was similar to the image before exposure.

All rabbits recovered from the anesthesia within an hour after the experiments without any clinical disorders. All nine rabbits were euthanatized a day after HIFU ablation. Two of the nine rabbits showed mild skin burns on the abdomen just after HIFU exposure. The subcutaneous tissue under the skin burns of the rabbits, euthanatized a day after HIFU ablation, were slightly reddened but the abdominal muscle appeared grossly normal. The surface of the livers excised a day after HIFU ablation showed no visible damage. No gross lesions of the gastrointestinal tracts were observed.

Figure 4 (d) shows a typical lesion a day after the HIFU exposure. The ablated zone of the liver tissue seemed to be a contiguous lesion (13 mm × 7 mm × 7 mm) that contained the VX2 tumor. No gross lesions were observed on the surface of the liver.

A large volume of tissue was coagulated as shown in the histological section (Fig. 5). The VX2 tumors were completely coagulated and were surrounded by ablated liver tissue. The coagulated liver tissue took up less stain than the normal intact liver cells, as shown in Fig. 5 (b).

The photographs in Fig. 6 show the typical histological changes of the lesions. The treated VX2 tumor cells showed complete coagulation necrosis (a) in comparison with the intact VX2 tumor cells (b).

DISCUSSION

VX2 tumor inoculated into liver tissue was used as a localized tumor model in this study to examine the effect on the tumor of split-focus HIFU ablation fabricated for localized prostate cancer treatment. Six shots of split-focus HIFU ablation were used to destroy the localized experi-
Fig. 3. (a) To facilitate transfer of the ultrasound beam, the water bag of the transducer was filled with degassed water and placed on the rabbit abdomen. (b) Transdermal sonographic image of a tumor-bearing rabbit liver just before exposure. The power transducer was focused on VX2 tumor in the liver using real-time pulse-echo images obtained with the probe. S: skin.

Fig. 4. (a) Transdermal sonographic image of a tumor-bearing rabbit liver just before HIFU exposure. (b) Just after HIFU exposure. (c) A day after HIFU exposure. (d) Cross section of liver a day after HIFU exposure.
mental tumor. We found that HIFU exposure caused contiguous thermal coagulation in the rabbit liver as well as the VX2 tumor [Fig. 4 (d)]. We have previously demonstrated that split-focus HIFU created a contiguous thermal coagulation in pig liver tissue within a few seconds [13]. There were no intact tumor cells in the VX2 tumor region or in the surrounding region as shown in Fig. 5 (c). These results indicate that the VX2 tumor and the surrounding liver tissue
exposed to split-focus HIFU were heated enough to create an area of coagulation.

Just after HIFU ablation, the surrounding tissue of the tumor was seen as slightly more hypoechoic than before exposure as shown in Fig. 4 (b). The HIFU ablation of the liver caused thermal coagulation of liver tissues [Fig. 4 (d)]. This focal coagulation has most likely led to a decrease in echogenicity due to protein degeneration in the cells. An intriguing aspect of HIFU is the potential for imaging damaged tissue in real time during lesioning exposure. It is difficult to examine the region to be ablated by conventional ultrasonography. Magnetic resonance imaging (MRI) is also useful in demonstrating the extent of damaged tissue [1]. T2-weighted images show regions of tissue necrosis clearly. MRI is a useful technique for following the progress of tissue destruction in vivo following ultrasound exposure. It has also been shown that tissue volumes in which the temperature is elevated can be visualized using MRI [1]. HIFU in combination with an MRI system may be used in the future in veterinary medicine.

All rabbits recovered from anesthesia normally within an hour after the experiments, and their appetites became normal within a day after exposure. Two of the nine rabbits showed mild skin burns on the abdomen just after HIFU exposure. It is important for the safe and effective use of HIFU that the energy level at the skin surface be kept below a level that will lead to burns. For shallow target volumes, wide aperture beams are preferable to narrow ones. Use of cold water in the water bag of the transducer is effective in preventing HIFU exposure from heating the skin.

Necropsy a day after treatment found the surface of the livers and the gastrointestinal tracts to be grossly normal. The six shots of split-focus HIFU destroyed the VX2 tumor without injuring the surface of the liver or the surrounding organs. These results suggest that split-focus HIFU ablation can be used for effective treatment of localized tumors.

Although HIFU can be used for most sites that can be readily visualized by transcutaneous diagnostic ultrasound, there are some limitations to its use. To ablate tissue volumes at depth, an adequate acoustic window at the skin must be available. Therefore, localized tumors such as those in the brain and lungs are difficult to treat with HIFU because it is essential that neither bone nor gas lies in the beam path. Localized kidney and liver tumors may be good clinical targets for HIFU treatment in the future.

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REFERENCES