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A microbubble agent improves the therapeutic efficiency of high intensity focused ultrasound: a rabbit kidney study

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Abstract Eighty kidneys (40 left and 40 right kidneys) of New Zealand rabbits were ablated using high intensity focused ultrasound (HIFU), (14,300 W/cm^2, 1.0 MHz). Kidneys were randomly divided into two groups. HIFU was performed in the manner of linear scan in both groups. Prior to HIFU, normal saline solution and isovolumetric microbubble agent were administrated intravenously in groups I and II, respectively. HIFU was finished in all left kidneys and in 26/40 right ones. The therapeutic efficiency was reflected using necrosis rate (cubic centimeters per second), which was the tissue volume of coagulative necrosis per 1 s HIFU exposure. In both groups, predetermined volumes were damaged without harming overlying tissues. Necrosis rates were increased in group II both in left (0.0089 ± 0.0107 vs. 0.0493 ± 0.0777, $P = 0.0323$) and in right (0.0039 ± 0.0055 vs. 0.0162 ± 0.0168, $P = 0.0248$) kidneys. Pathological examinations confirmed that there were no intact tissue focuses within exposed regions in either group. These findings suggested that the microbubble agent improved the therapeutic efficiency of HIFU. Hemorrhage and hyperemia were also detected on the margin of the ablated tissues (both in cortex and medulla) in both groups.

Keywords High intensity focused ultrasound · Microbubble agent · Kidney · Therapeutic efficiency · Acoustic characteristics of tissue

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Introduction

High intensity focused ultrasound (HIFU) destroys tissues mainly via heat and cavitation mechanisms [1, 2]. It has been used to non-invasively ablate kidney tumors [3]. Clinical investigations have shown that this technique is both effective and safe. Especially in some patients with inoperable masses and/or bilateral renal cancers, this approach makes it possible to destroy lesions, whilst preserving organ functions as far as possible [4].

Blood perfusion reduces the tissue ablation induced by HIFU [5]. Kidney is an organ with high blood flow rate; maybe this is one of the reasons why ablating kidney with HIFU is more difficult than destroying others tissues. Compared with other organs such as liver and muscle, HIFU led to a smaller volume occurring coagulative necrosis in kidney despite identical ultrasound intensity, frequency, exposure duration and focal depth in tissue [6]. This demonstrated that the therapeutic efficiency in kidney was lower than that in other tissue type. Theoretically, this shortcoming can be overcome by elevating acoustic intensity or prolonging sonication duration. Elevating intensity concurrently increases the incidence of side effects, and the technical difficulty of treatment as well. That a local temperature-rise above 65°C should be reached in a few seconds during HIFU suggests that ablating tissues via prolonging sonication duration is not an efficient approach [7]. Another potential solution is to change the acoustic property of tissues, making energy deposit more efficiently; this will of necessity improve the therapeutic efficiency.

Microbubble agents could potentiate cell rupture resulting from insonation in vitro [8]. Furthermore, microbubbles could be administrated intravenously [9]. Bioeffects of ultrasound both in vitro and in vivo result from heat, cavitation and mechanical effects [10, 11], and this makes us believe that the microbubble agent could perhaps enhance HIFU. In the present study, we

investigate the effect of intravenous administration of a microbubble agent on kidney ablation using HIFU.

Materials and methods

Animals

Forty New Zealand white rabbits, weighing 1.5–2.0 kg, were supplied by Laboratory Animals Center of Chongqing Medical University. Animals were fasted 24 h before experiments and abdomen and back were shaved.

The experiment was approved ethically and scientifically by our university and complied with Practice for Laboratory Animals in China.

HIFU treatment system

JC HIFU tumor therapy system (Chongqing Haifu Technology, Chongqing, China) was used. This device, which was designed and manufactured for clinical tumor therapy, comprised an ultrasonic therapeutic unit and an ultrasonic diagnostic unit under the control of a central processing unit. The built-up of this system has been described in detail in previous papers [12, 13]. A therapeutic transducer with a diameter of 150 mm and a focal length of 150 mm was fixed at the bottom of a tank filled with degassed water. Ultrasound waves were in continuous waves. The therapeutic transducer could move six-dimensionally (Fig. 1). A diagnostic transducer was localized in the center of the therapeutic transducer; thus, tissues in the path of therapeutic ultrasound waves could be viewed in diagnostic ultrasonic images. Ultrasonography was used to guide HIFU treatment and monitor therapeutic effects in real time. The focal intensity (I_{SATA}) of the therapeutic transducer, which was calibrated by a radiation force assay in degassed water, was 14,300 W/cm^2. The frequency of ultrasound wave was 1.0 MHz. The focal region (–3dB) of the therapeutic transducer, which was calibrated by a PVDF needle hydrophone with spot diameter of 0.5 mm (Shanghai Jiaotong University, Shanghai, China) in a tank filled with degassed water, was an ellipsoid with dimensions of 14 mm along the beam axis and 1.4 mm in the transverse direction.

Renal tissue ablation

After anesthesia, animals were positioned laterally or semi-laterally to make the target kidneys viewed clearly with ultrasonography. Skin in the path of therapeutic ultrasound was immersed into degassed water (Fig. 2). Ultrasonography-guided renal tissue ablation was performed. If the target volume and its adjacent tissues could not be clearly identified in the ultrasonic images or there were bones/air-containing organs in the path of therapeutic ultrasound, the therapy was not carried out; this phenomenon occurred while ablating right kidneys in some animals, because (1) they lay in pelvis or behind the ribs, or (2) bowels lay in the path of therapeutic ultrasound. HIFU was performed in the manner of linear scan. The focus was set 0.5 cm below the renal capsula. A 1-cm-long "line" was predetermined for ablation. Therapeutic ultrasound swept across the target tissue at a velocity of 3 mm/s, and scan was repeated in the same direction until achieving therapeutic effects. Therapeutic effects were determined in real time using ultrasonography based upon changes of echoes (gray scale) of the focal region. The indicator of effective treatment was a hyperechoic region that appeared in the target volume immediately after HIFU and lasted at least 2 min (Fig. 3). In order to judge echoic changes objectively, pre- and post-therapy echoes (gray scale) of the target region were automatically evaluated and compared using software GrayVal 1.0 (Chongqing Haifu Technology, Chongqing, China; JC HIFU system was equipped with the software). If hyperecho occurred, gray scale value increased. This approach was very helpful while it was difficult to determine echoic changes visually. Once the therapeutic effect was realized, HIFU was finished. The distance from skin to the target tissue (focal depth in tissue, FD, cm) and the exposure duration (t, second) were measured.

Forty left and 40 right kidneys were randomly divided into groups I and II, respectively. HIFU was performed in both groups. Group II were administrated 0.2 ml lipid-coated perflupropane microbubble agent (with a diameter of 2–4 μm), Haifu-SUT (Chongqing Haifu Technology, Chongqing, China), via ear vein, and group I received isovolumetric normal saline solution.

The behavior of the agent in kidney was investigated using ultrasonography. Both echoes and blood flow signals in renal parenchyma were intensified 20–40 s after 0.2 ml were bolus-injected intravenously (Fig. 4). The elimination time of the agent in kidney was 4–5 min. Therefore, HIFU was carried out 1.5 min after agent administration.

If both kidneys were candidates for HIFU in a rabbit, a 30-min interval was set.

Inspection

Animals were recovered and fed 24 h after HIFU and were then sacrificed and kidneys were removed. The coagulative necrosis was white and the demarcation between viable and non-viable tissue

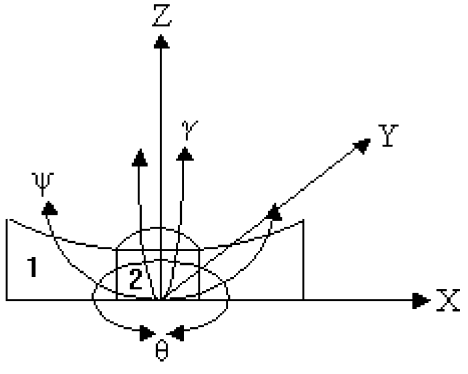

Fig. 1 Six-dimension movement of transducer. *1* therapeutic transducer; *2* diagnostic transducer

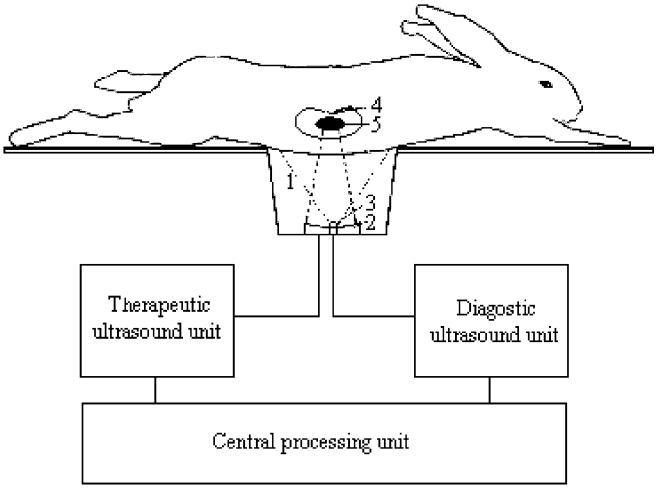

Fig. 2 Illustration of HIFU exposure. *1* tank containing degassed water, *2* therapeutic ultrasonic transducer, *3* diagnostic ultrasonic transducer, *4* kidney, *5* target volume

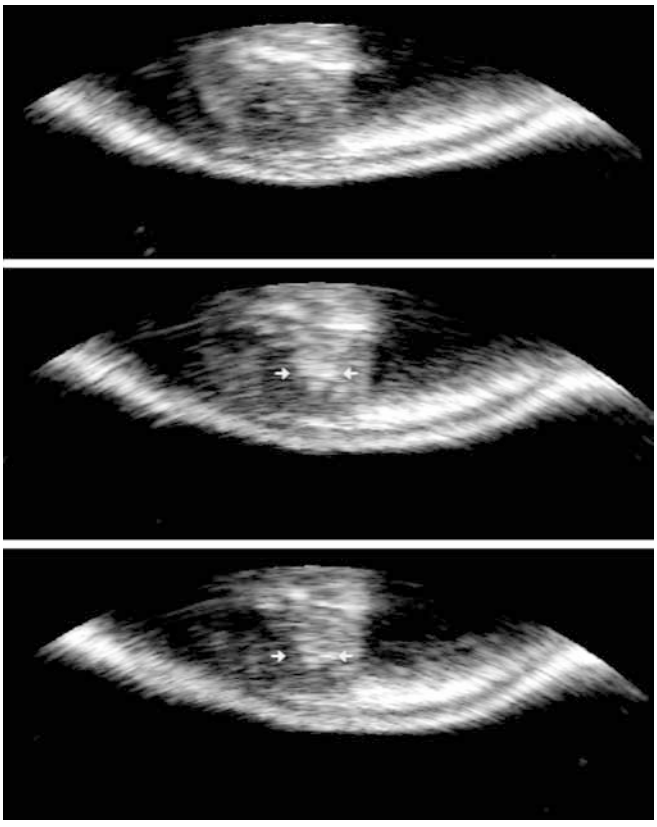

Fig. 3 Diagnostic ultrasonic images of kidney before (*upper*), immediately (*middle*) and 2 min (*down*) after exposure to HIFU. A hyperechoic region occurred within the target area (*arrows*) immediately after HIFU, and the hyperecho existed after 2 min. Such a change indicated the occurrence of coagulative necrosis

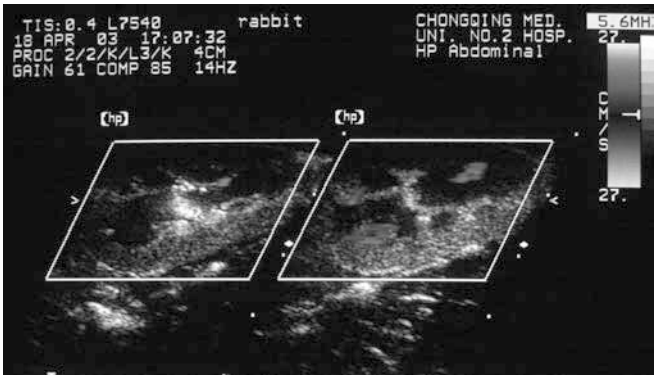

Fig. 4 Ultrasonic images of kidney before (*left*) and after (*right*) injecting 0.2 ml Haifu-SUT. Both echoes and doppler blood flow signals were intensified after agent administration

was sharp, making it easy to identify. The length (L, centimeters), width (W, centimeters) and depth (D, centimeters) of the deactivated tissue were measured and its volume (V, cubic centimeters) was calculated using the following formula [14].

$$V = \frac{\pi}{6} \times L \times W \times D \quad (1)$$

Necrosis rate (NR, cubic centimeters per second) was adopted to reflect the therapeutic efficiency. NR was the volume of necrosis produced per 1 s HIFU exposure and it was calculated using: $NR = V/t$.

Pathological examinations were performed to determine whether there were any obvious regions of cells within the exposed region which had been spared from damage.

Statistics

All data were processed by the statistics software SAS 8.0 and the exact probability was calculated. t test was used if variances were equal; otherwise, t' test was adopted. The difference was significant if the P value was < 0.05.

Results

Therapeutic efficiency

HIFU was finished in 40 (100%) left kidneys and 26 (65%) right ones. Necrosis did occur if ultrasonic images suggested the realization of the effective treatment, and pre-selected regions were ablated without harming overlying tissues (skin and subcutaneous tissues). The length of the necrosis volume is longer than the predetermined scan line in some animals in both groups. Both in left and right kidneys there was no difference in FD between two groups and the therapeutic efficiency in group II was improved, compared with that in group I (Tables 1 and 2).

Pathological examination

There were no intact tissue focuses within exposed tissues in either group. The demarcation between dead and viable tissues was sharp. There were hemorrhage and hyperemia (both in cortex and medulla) on the margin of the necrosis tissues in both groups (Figs. 5, 6 and 7).

Discussion

NR was increased while the microbubble agent was administrated intravenously prior to HIFU. This finding demonstrated that a bigger volume could be ablated in a shorter duration if the microbubble agent was used, resulting in an improved therapeutic efficiency.

The length of the necrosis volume was longer than the predetermined scan line in some rabbits. This was due to the shift of kidney attributable to respiratory motion during HIFU. Theoretically, the movement may lead to damaging tissues outside the focus. A definite area surrounding the malignant volume must be resected in a radical cancer surgery; this was one of the most important reasons why the shift did not hamper implementing HIFU in most patients.

HIFU damaged tissues via heat and cavitation [1, 2]. Microbubbles rapidly distributed in kidney after intravenous injection; thus, acoustic characteristics of renal

Table 1 Focal depth (*FD*), exposure duration (*t*), volume of necrosis tissue (*V*) and necrosis rate (*NR*) in groups I and II in left kidney (range listed in parenthesis)

	Group I (n = 20)	Group II (n = 20)	*P* value
FD (cm)	1.74 ± 0.27 (1.4–2.4)	1.74 ± 0.23 (1.3–2.0)	0.9499
t (s)	29.85 ± 7.88 (16–48)	21.10 ± 7.39 (10–39)	0.0009
V (cm^3)	0.2830 ± 0.3663 (0.0090–1.3214)	0.9753 ± 1.6252 (0.0252–5.7671)	0.0772
NR (cm^3/s)	0.0089 ± 0.0107 (0.0005–0.0378)	0.0493 ± 0.0777 (0.0009–0.2551)	0.0323

Table 2 Focal depth (*FD*), exposure duration (*t*), volume of necrosis tissue (*V*) and necrosis rate (*NR*) in groups I and II in right kidney (range listed in parenthesis)

	Group I (n = 13)	Group II (n = 13)	*P* value
FD (cm)	1.79 ± 0.28 (1.5–2.3)	2.05 ± 0.52 (1.2–3.0)	0.1256
t (s)	29.62 ± 8.44 (11–43)	22.08 ± 4.19 (14–27)	0.0101
V (cm^3)	0.1250 ± 0.1781 (0.0039–0.6045)	0.3830 ± 0.4163 (0.0183–1.5072)	0.0564
NR (cm^3/s)	0.0039 ± 0.0055 (0.0001–0.0183)	0.0162 ± 0.0168 (0.0012–0.0603)	0.0248

tissues were changed. It was demonstrated that microbubbles enhanced ultrasonic absorption in tissues, resulting in a higher temperature rise [15]. Microbubbles also benefited the occurrence of cavitation [8, 10, 16]. Both heat and cavitation were intensified if the microbubble agent was adopted. Perhaps these mechanisms led to an improved therapeutic efficiency. The interaction between heat and lipid resulted in free radical generation and free radicals had the potential of extending the volume of necrosis attributable to hyperthermia [17]. This mechanism maybe was involved in the potentiation due to Haifu-SUT.

It was reported that microbubbles (mostly resulted from the boiling of water in tissue) led to damaging tissues in the near field in previous investigations in which single exposure was adopted [18, 19, 20]. Investigators believed it was ascribed to thermal buildup and an intersonication delay was recommended to avoid this side effect [21, 22]. Linear scan was adopted in the present study with no harm to overlying tissues (skin and subcutaneous tissues). The manner of scan made each ''focus'' insonated for very a limited number of seconds. This just played the role of ''intersonication delay,'' making tissues within the near field protected

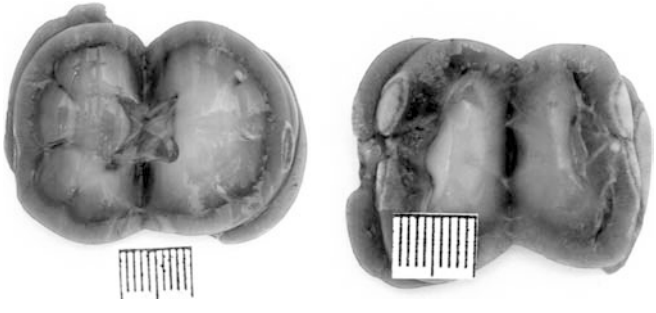

Fig. 5 Macroscopic inspection of kidneys in groups I (*left*) and II (*right*). The necrosis tissues were white and the demarcation between dead and viable tissues was sharp. The scale was 10 mm

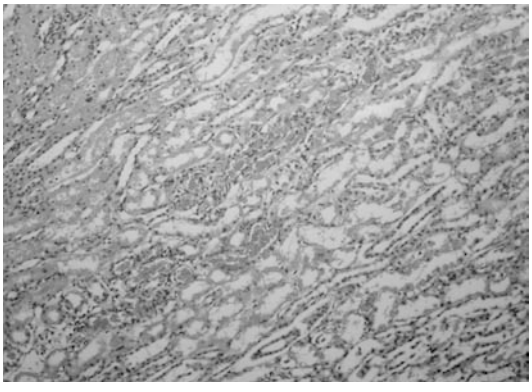

Fig. 7 Medullary hemorrhage occurred on the margin of the ablated tissue (×100)

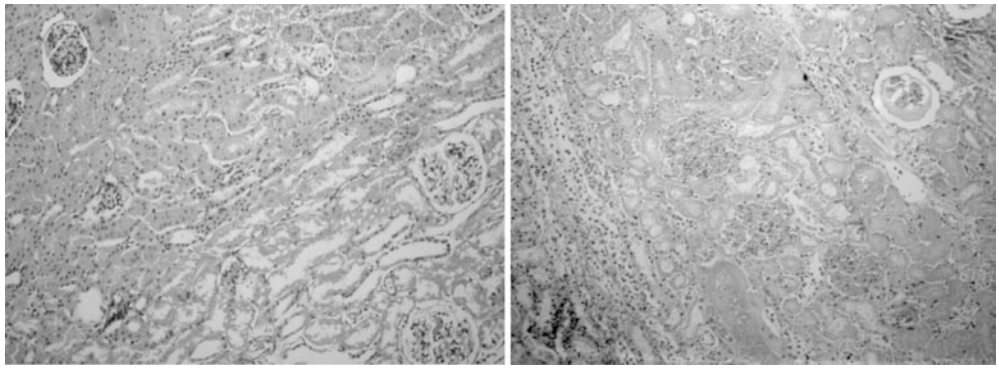

Fig. 6 The demarcation between dead and viable tissues was sharp in both groups I (*left*) and II (*right*).Complete necrosis occurred within the focal region. On the margin of ablated tissues, hemorrhage and hyperemia were detected (×100)

against being damaged. Only skin and subcutaneous tissue could be included in the near field in rabbits; thus, effects of the introduction of Haifu-SUT during HIFU on overlying kidney tissues could not be determined. Similar investigations should be carried out in big animals.

HIFU could not be performed in all right kidneys in the present study. This was because the location of right kidney in rabbits varied, ruling out some right kidneys as candidates for HIFU (no appropriately acoustic window). Fortunately, this was not a problem when ablating human kidney tumors [4]. This study also showed the necrosis rate in left kidneys was not identical to that in right ones. The exact mechanism remained unclear and further investigations were needed.

Pathological examinations demonstrated that there were no spared tissues within exposed regions in both groups. This manifested that the introduction of the microbubble agent did not lead to residual intact focuses, which must be avoided during HIFU [7, 22]. The demarcation between viable and necrosis tissues was still sharp, suggesting the precision of HIFU was preserved. Hemorrhage and hyperemia were detected on the margin of the ablated tissues (both in cortex and medulla) in some animals in both groups. Köhrmann et al. ablated human renal cell carcinoma using HIFU. Profound streaky bleeding occurred in the medulla while the focus was set on the corticomedullary border [23]. Paterson et al reported that there was congestion at the edge of the lesion [24]. It was reported that even diagnostic ultrasound could induce capillary rupture in mice while a microbubble agent, Optison, was used and the exposure duration was long enough [25]. This demonstrated that ultrasound had the potential of damaging vessel and microbubbles could enhance this effect. If this technique could be used to damage the predetermined vessels (such as the vessel mainly supplying the cancer), another therapy for malignancies could be developed.

The necrosis did occur if ultrasonic images suggested the realization of ablation, demonstrating that ultrasonography could be used to monitor the therapeutic effects during HIFU.

The microbubble agent could improve diagnostic ultrasonic images, benefiting the detection of lesions [9]. Ultrasonography was usually used to guide and monitor therapy during HIFU; it also played an important role in the follow-up. It was demonstrated that the microbubble agent could also be used to improve the therapeutic effect in the present study. These results suggest that the microbubble agent is a very useful tool for HIFU.

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