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Growth inhibition of high-intensity focused ultrasound on hepatic cancer *in vivo*

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Abstract

AIM: To investigate the damaging effect of high-intensity focused ultrasound (HIFU) on cancer cells and the inhibitory effect on tumor growth.

METHODS: Murine H_{22} hepatic cancer cells were treated with HIFU at the same intensity for different lengths of time and at different intensities for the same length of time *in vitro*, the dead cancer cells were determined by trypan blue staining. Two groups of cancer cells treated with HIFU at the lowest and highest intensity were inoculated into mice. Tumor masses were removed and weighed after 2 wk, tumor growth in each group was confirmed pathologically.

RESULTS: The death rate of cancer cells treated with HIFU at 1 000 W/cm^2 for 0.5, 1, 2, 4, 8, and 12 s was 3.11±1.21%, 13.37±2.56%, 38.84±3.68%, 47.22±5.76%, 87.55±7.32%, and 94.33±8.11%, respectively. A positive relationship between the death rates of cancer cells and the length of HIFU treatment time was found (r = 0.96, P<0.01). The death rate of cancer cells treated with HIFU at the intensity of 100, 200, 400, 600, 800, and 1 000 W/cm^2 for 8 s was 26.31±3.26%, 31.00±3.87%, 41.97±5.86%, 72.23±8.12%, 94.90±8.67%, and 99.30±9.18%, respectively. A positive relationship between the death rates of cancer cells and the intensities of HIFU treatment was confirmed (r = 0.98, P<0.01). The cancer cells treated with HIFU at 1 000 W/cm^2 for 8 s were inoculated into mice *ex vivo*. The tumor inhibitory rate was 90.35% compared to the control (P<0.01). In the experimental group inoculated with the cancer cells treated with HIFU at 1 000 W/cm^2 for 0.5 s, the tumor inhibitory rate was 22.9% (P<0.01). By pathological examination, tumor growth was confirmed in 8 out of 14 mice (57.14%, 8/14) inoculated with the cancer cells treated with HIFU at 1 000 W/cm^2 for 8 s, which was significantly lower than that in the control (100%, 15/15, P<0.05).

CONCLUSION: HIFU is effective on killing or damage of H_{22} hepatic cancer cells *in vitro* and on inhibiting tumor growth in mice *ex vivo*.

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Key words: HIFU; Liver cancer; Growth inhibition

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INTRODUCTION

High-intensity focused ultrasound (HIFU) consists of focused ultrasound (ULS) waves emitted from a transducer and is capable of inducing tissue damage. By means of this thermal effect and other mechanisms, HIFU-treated tumor tissues result in direct thermal cytotoxic necrosis and fibrosis, thus leading to inhibition of tumor growth. Therefore, HIFU is a new-sophisticated high-technology based minimally invasive treatment option for some cancers, which allows radiation-free treatment. Until now there are many kinds of tumors, such as tumors of prostate, liver, kidney, bladder, breast, and brain, that were treated with HIFU clinically and experimentally, some cancers were effectively controlled after HIFU treatment. As one of the minimally invasive surgical techniques for cancer treatment, HIFU is of great interest today[1,2].

Malignant cells are sensitive to therapeutic ULS treatment, which leads to a transient decrease in cell proliferation[3] through inducing a complex signaling cascade with upregulation of proapoptotic genes and downregulation of cellular survival genes[4]. In *in vitro* study, it was confirmed that CZ901 HIFU inhibits proliferation and induces apoptosis of cancer cells[5]. This study aimed to investigate the effects of HIFU on cancer cell damage *in vitro* and tumor growth inhibition *ex vivo*.

MATERIALS AND METHODS

Experimental materials

Cancer cell line in mouse Murine hepatoma H_{22} cell line

was kept in liquid nitrogen for regular use in our laboratory[6].

Experimental animals Female Balb/C mice, weighing 18-22 g, were purchased from Beijing Biological Products Research Institute under Ministry of Public Health (approval number: 013072). The procedures involving animals and their care were conducted in accordance with institutional guidelines for Laboratory Animal Care of Experimental Animal Center, Sichuan University.

Experimental device CZ901 HIFU device for cancer treatment was designed and supplied by Mianyang Electronic Equipment Factory.

Experimental methods

Experiment *in vitro* Ascites taken from H_{22} hepatic cancer bearing mouse on d 8 or 9 was diluted with normal saline at 1∶5 (2.5×10^7 cells/mL) and distributed into 14 PVC tubes, 7 tubes in each test, each containing 1.8 mL. Twelve tubes were treated with HIFU, and two were used as controls. H_{22} hepatic cancer cells were treated with HIFU at the frequency of 1.048 MHz and at the intensity of 1 000 W/cm^2 for 0.5, 1, 2, 4, 8, and 12 s, respectively, and for 8 s at intensity of 100, 200, 400, 600, 800, and 1 000 W/cm^2, respectively. After HIFU treatment, the cells were incubated in a humidified atmosphere of 50 μg/mL CO_2 at 37 ℃ for 6 h, and then the viability of cancer cells was determined by exclusion of trypan blue staining. The viable cells were not stained, the dead cells were stained blue. The viable cells and dead cells were counted with an erythrocytometer under microscope, respectively (total cell number counted >1 000). The death rate was determined by dead cell number÷(the dead cell number+the viable cell number)×100%. Each experiment was performed in triplicate.

Inoculation of HIFU-treated cancer cells *ex vivo* Cancer cells including viable and dead cells treated with HIFU at 1 000 W/cm^2 for 8 s were inoculated into 14 mice, 2×10^6 cells/0.2 mL per mouse. The same number of untreated cancer cells was inoculated into 20 mice as control. In addition, cancer cells treated with HIFU at 1 000 W/cm^2 for 0.5 s were inoculated into 18 mice, 2×10^6 cells/0.2 mL per mouse. The same number of untreated cancer cells was inoculated into 20 mice as control.

Examining the tumor growth *ex vivo* The animals inoculated with cancer cells were raised routinely, with free access to food and water and weighed every 2 d. After 2 wk of inoculation, the animals were killed, the tumor masses were removed and weighed, and the tumor inhibitory rate was calculated[6].

Histopathological examination Tumor masses were fixed with 4% paraformaldehyde, embedded with paraffin, sectioned and stained with HE. The tumor growth inhibition was confirmed by microscopy.

Statistical analysis

The experimental data were expressed as mean±SD and analyzed with χ^2 test. $P<0.05$ was considered statistically significant.

RESULTS

Cell damage effect of HIFU in vitro

The death rate of cancer cells in controls was 3-5% (Figure 1A), and increased significantly after HIFU treatment (Figure 1B). The death rate of cancer cells treated with HIFU at 1 000 W/cm^2 for 0.5, 1, 2, 4, 8, and 12 s were 3.11±1.21%, 13.37±2.56%, 38.84±3.68%, 47.22±5.76%, 87.55±7.32%, and 94.33±8.11%, respectively (Figure 1C). A positive

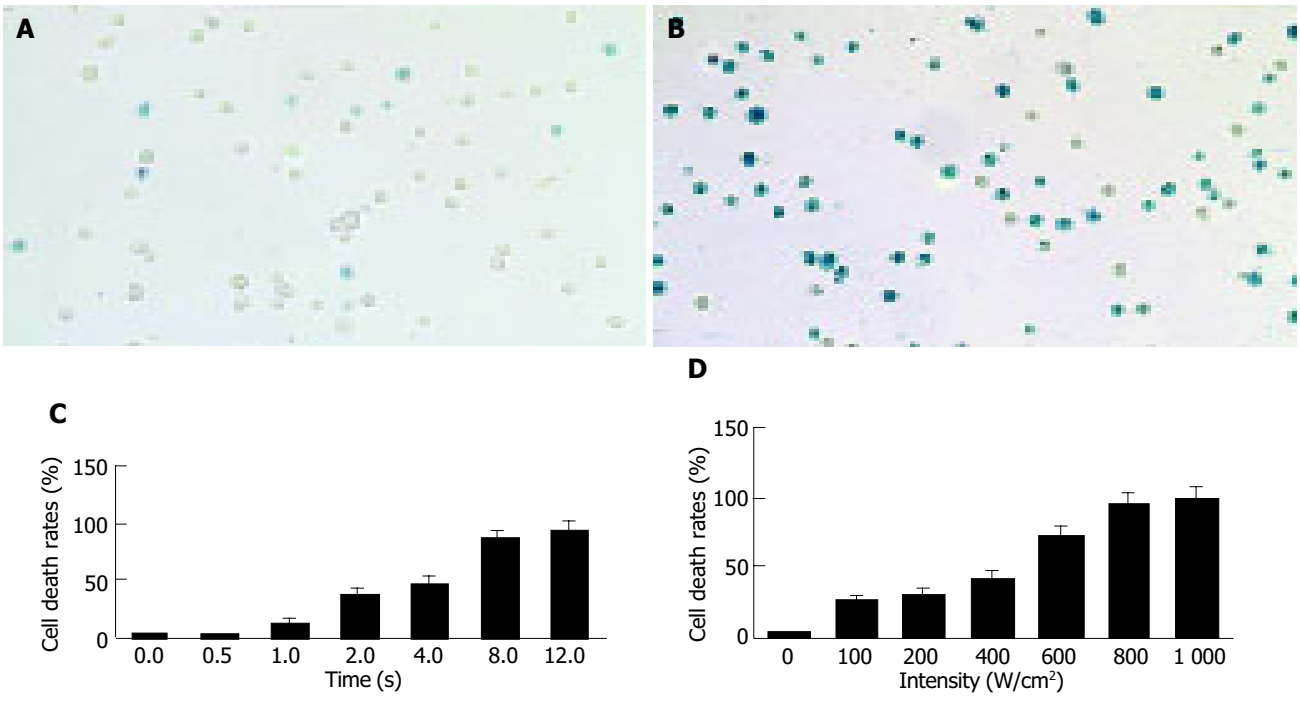

Figure 1 Cell damage effect of HIFI *in vitro*. **A**: Murine hepatic cancer cells before HIFU treatment; **B**: murine hepatic cancer cells treated with HIFU at 1 000 W/cm^2 for 8 s; **C**: significant difference between cell death rate and time of HIFU treatment; **D**: significant difference between the cell death rate and intensity of HIFU treatment.

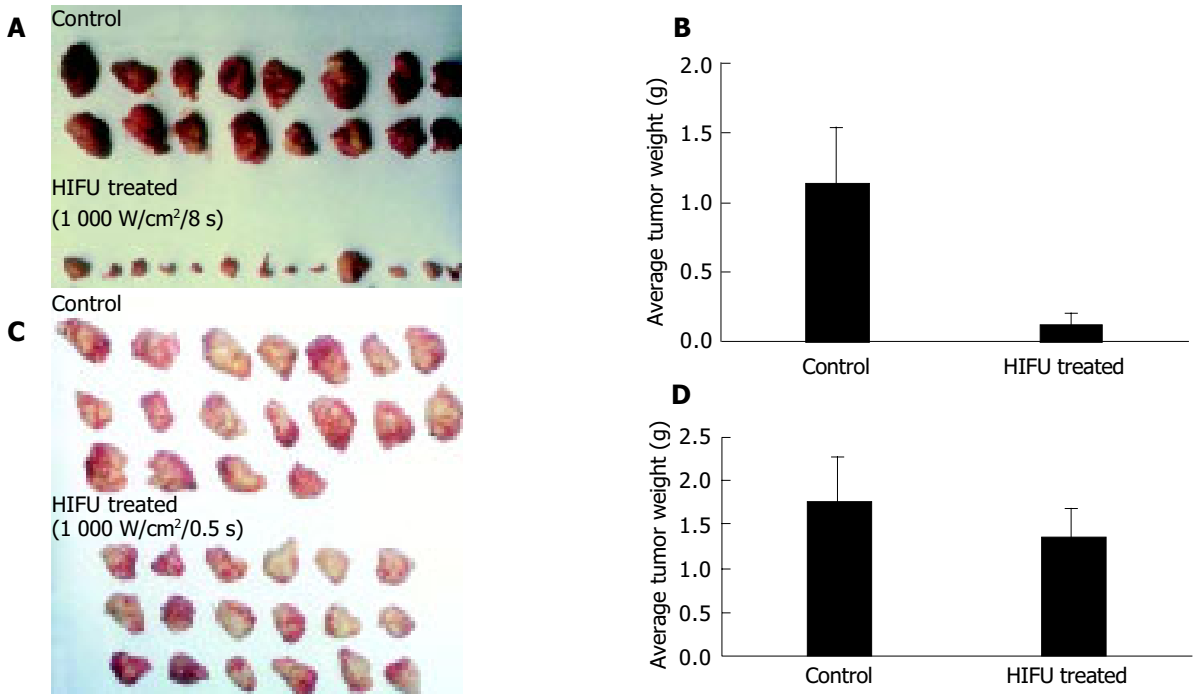

Figure 2 Tumor growth inhibition of HIFU *ex vivo*. **A**: Tumor growth inhibition of HIFU at 1 000 W/cm^2 for 8 s; **B**: significant difference in average tumor weight between the control and HIFU-treated mice; **C**: tumor growth inhibition of HIFU at 1 000 W/cm^2 for 0.5 s; **D**: significant difference in average tumor weight between the control and HIFU-treated mice.

relationship was found between the death rate of cancer cells and the time of HIFU treatment (r = 0.96, P<0.01).

The death rate of cancer cells treated with HIFU at the intensity of 100, 200, 400, 600, 800, and 1 000 W/cm^2 for 8 s was 26.31±3.26%, 31.00±3.87%, 41.97±5.86%, 72.23±8.12%, 94.90±8.67%, and 99.30±9.18%, respectively (Figure 1D). A positive relationship was confirmed between the death rates of cancer cells and the intensities of HIFU treatment (r = 0.98, P<0.01).

Tumor growth inhibition of HIFU ex vivo

Tumor growth inhibition of cancer cells treated with HIFU *ex vivo* is listed in Table 1. In experiment 1, six mice in the control group died of tumor burden spontaneously, none died in the HIFU-treated group. There was no significant difference in body weight increase between two groups of animals. In the group of animals inoculated with cancer cells treated with HIFU at 1 000 W/cm^2 for 8 s, the average tumor weight was 0.11±0.16 g, and the average tumor weight in control group was 1.14±0.4 g (Figures 2A and B), the tumor inhibition rate was 90.35% compared to the control (P<0.01). In experiment 2, cancer cells treated with HIFU at 1 000 W/cm^2 for 0.5 s were inoculated. The average tumor weight in HIFU-treated group and control was 1.36±0.33 and 1.75±0.53 g, respectively (Figures 2C and D), the tumor inhibitory rate was 22.90% (P<0.01).

By pathological examination, tumor growth was confirmed in 8 out of 14 mice (57.14%, 8/14) inoculated with cancer cells treated with HIFU at 1 000 W/cm^2 for 8 s, which was significantly lower than that of the control (100%, 15/15, P<0.05).

DISCUSSION

HIFU consists of focused ULS waves emitted from a transducer and is capable of inducing tissue damage. By means of this thermal effect and other mechanisms, HIFU-treated tumor tissues resulted in direct thermal cytotoxic necrosis and fibrosis, thus leading to inhibition of tumor growth. Therefore, HIFU is a new-sophisticated high-technology based, minimally invasive treatment option for some cancers[1,4,5]. But, there are many factors affecting its therapeutic effect, such as, intensity of the transmitted pulse, the exposure time, the signal frequency, the time interval

Table 1 Inhibitory effect of HIFU on growth of hepatic cancer *ex vivo* (mean±SD)

Experiment group		HIFU treatment (1 000 W/cm^2)	Animals (n)		Average BW (g)		Tumor wt (g)	TIR (%)
			d_1	d_{14}	d_1	d_{14}		
1	Control	0	20	15	19.05±2.03	19.62±2.13	1.14±0.40	
	HIFU	8	14	14	19.69±1.34	21.45±0.99	0.11±0.16^b	90.35
2	Control	0	20	19	21.03±2.14	28.39±3.83	1.75±0.53	
	HIFU	0.5	18	18	22.68±2.73	27.46±4.03	1.36±0.33^b	22.29

BW: Body weight; wt: weight; TIR: tumor inhibitory rate. $^b P$<0.01 *vs* control.

between two firing bursts, and biological medium, *etc.*[1,7-9].

The HIFU device used in this experimental study was designed and manufactured in Mianyang Electronic Equipment Factory. Its signal frequency emitted is 1.048 MHz, the intensity of the transmitted pulse and exposure time can be manipulated[5]. In this experimental study, H_{22} hepatic cancer cells were treated with HIFU at the same intensity for different lengths of time and for the same length of time at different intensities *in vitro*. It showed a intensity and time-dependent damaging effect on cancer cells (Figures 1B-D), suggesting that HIFU has a damaging or killing effect on cancer cells *in vitro*. The effective parameters are: an intensity of 1 000 W/cm^2 and an exposure time of 8 s.

There are many experimental and clinical studies on treatment of tumors with HIFU[1,10-14], especially hepatic cancers[15-19]. The results of these studies *in vitro* and *in vivo* indicate that HIFU has damaging or killing effect on cancer cells *in vitro* and inhibitory effect on tumor growth *in vivo*. However, to our best knowledge, there is no study on the growth potential of cancer cells after HIFU treatment. The findings in this study indicate that most cancer cells treated with HIFU at 1 000 W/cm^2 would die and lose the proliferating potential, but few cells may survive and form tumors.

Although minimally invasive methods for the treatment of cancer, such as HIFU, and high-energy shock waves, have been proposed recently, their feasibility for treatment of human cancers needs to be confirmed[1,20]. This experimental study has confirmed that HIFU has effects on killing or damage of H_{22} hepatic cancer cells *in vitro* and on the inhibiting tumor growth in mice *ex vivo*. Its inhibitory and therapeutic effects on other cancers, and mechanisms of action need to be studied and confirmed further.

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