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Original

Evaluation of Second-generation HIFU Systems : Less-invasive Fetal Therapy for TRAP Sequence

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Abstract : In this report, the second-generation high-intensity focused ultrasound (HIFU) systems were clinically evaluated for human fetal therapy in two cases of twin reversed arterial perfusion sequence. The HIFU systems comprised an improved lead zirconate-titanate transducer with an imaging phase array sector probe, a Sonachill cooling system, and three phases of HIFU exposure : a trigger pulse, a continuous heating wave, and an idle period to obtain images with the imaging probe set on the transducer. To evaluate skin surface temperature, a thermal camera was used. To evaluate vessel occlusions, blood flow was measured at fixed timings after exposures. Target vessel occlusion was achieved with HIFU in only one of the cases, but recanalization occurred the following day. Both cases were finally treated with radiofrequency ablation and one infant was successfully delivered without any complications. This case highlighted three advantages with the change to second-generation HIFU systems in human fetal therapy : the simplicity of maneuvers by reduced range of motion disturbance ; the ability to observe in real time during the exposure ; and a decrease in total ultrasonic output. Treatment interruption due to burns or complaints of heat sensation represented an obstruction to treatment completion. This remains an issue to be addressed in the future.

Key words : HIFU, TRAP sequence, ultrasound, less invasive, fetal therapy

Introduction

High-intensity focused ultrasound (HIFU) is a non-invasive therapeutic tool that causes biological reactions (such as thermal denaturation) at the target. HIFU has two main effects, thermal and non-thermal. Thermal effects are due to target absorption of the ultrasound radiation, while the non-thermal effects derive from cavitation, micro-streaming, and acoustic streaming.

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Specifically, the cavitational effect describes physical forces generated by the ultrasound waves. These physical effects manifest as characteristic compression and rarefaction resulting in micro-gas bubbles that contract and expand the tissue. It is generally thought that the rapid changes in pressure associated with cavitation, both in and around the cell, can cause cellular damage[1, 2]. Capitalizing on these effects, HIFU therapy has been already applied in the clinical setting for the treatment of prostate cancer[3], breast cancer[4], and uterine myoma[5].

Twin reversed arterial perfusion sequence (TRAPs) is a relatively rare complication, affecting 1 in 35,000 monochorionic twin pregnancies[6]. If TRAPs is left untreated, the mortality rate of the pump fetus is approximately 55%[7], with spontaneous resolution of up to 21%[8]. Various treatment methods have been reported, such as cord occlusion[9–12], anastomotic vessel ablation[13, 14], and intra-acardiac fetal ablation[15, 16]. Of these, radiofrequency ablation (RFA) provides good results due to its relatively low invasiveness and ease of treatment[12, 17–21]. Such minimally invasive techniques reduce, but do not remove the risks, without changing survival rates. Although TRAPs may be diagnosed from 11 to 13 weeks gestation, treatment is often delayed until after 16 weeks gestation to allow time for fusion of the amnion and chorion, by which time intrauterine death of the pump twin still occurs in up to one-third of cases[8].

HIFU applied from outside the mother's abdominal wall is thought to be less invasive and provide good acoustic efficiency due to the presence of amniotic fluid in the uterus. Therefore, the fetus is likely to be a suitable target for HIFU treatment. We have reported several preclinical studies of the clinical applications of HIFU in prenatal treatment[22–25], and in 2013, we described the first successful application of HIFU as a non-invasive in utero treatment for TRAPs[26]. This successful case revealed improvements that can contribute to safer and less invasive treatment. First, we could not obtain real-time images during the exposure, because HIFU and imaging signals interfere with each other. Second, there were motion range disturbances during scanning (because a large biaxial device was used, and because the imaging probe was located lateral to the HIFU transducer). Finally, the exposure was insufficient in some cases because the patient complained of a burning sensation during exposure. To overcome these challenges second-generation HIFU systems have been developed (Fig. 1)[25, 26]. The present study evaluated a second-generation HIFU system to not only clinically evaluate its efficacy, but also investigate the adverse effects of such systems.

Material and methods

The HIFU transducer and systems

A lead zirconate-titanate (PZT) transducer with a resonant frequency of 1.1 MHz and a focal distance of 60 mm was used with a phased array sector probe for imaging (Hitachi Aloka Medical Ltd., Tokyo, Japan) at an operating frequency of 1–5 MHz. A Sonachill cooling system was used (Sonablate 500; SonaCare Medical, LLC., Charlotte, NC, USA), supplied with circulating, degassed, cooled water. The first-generation HIFU units were large biaxial units. In contrast, in second-generation HIFU units, the transducer has a hole in the center and the imaging probe is arranged coaxially within the hole (contained within aluminum housing) (Fig. 1)[25, 26]. There

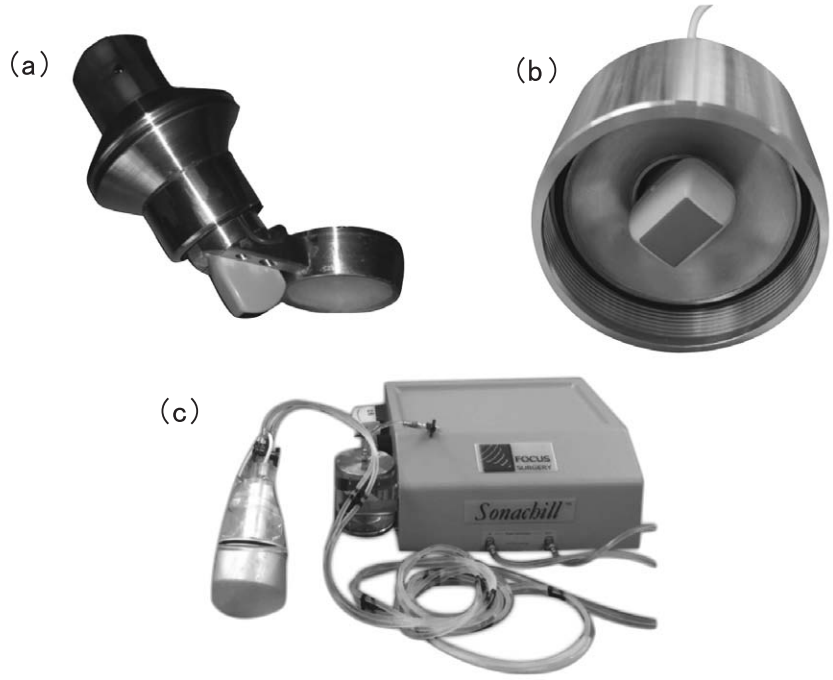

Fig. 1. HIFU systems
(a) In a first-generation HIFU unit, the imaging probe is located lateral to the HIFU transducer. Therefore the device used in this study was large and biaxial.
(b) In a second-generation HIFU unit, the HIFU transducer has a hole in the center, and the imaging probe is arranged coaxially within the hole (contained within aluminum housing).
(c) There are two holes in the upper part of the aluminum housing in the second-generation units that are connected to the SONACHILL cooling system. They are sealed with an ultrasound probe cover. Circulatory de-aeration coolant water is contained within the aluminum housing and the probe cover.

are two holes in the upper part of the aluminum housing in the second-generation units that are connected to the Sonachill cooling system. They are sealed with a latex-free ultrasound probe cover (sterile 17.8 × 147 cm telescopically-folded cover Civ-Flex, Civco, Kalona, IA, USA). Circulating, degassed, cooled water is contained within the aluminum housing and the probe cover (Fig. 1). In this study, the HIFU was driven by a radiofrequency (RF) amplifier (RF Power Amplifier Model 1040L; Electronics and Innovation, Ltd., Rochester, NY, USA), and the input signal to the amplifier was transferred by a function generator (DS5614, Iwatsu Test Instruments Corp., Tokyo, Japan). The skin surface temperature was measured using a thermal camera (FLIR CPA-T620; CHINO Corp., Tokyo, Japan). The error of the camera is ±2% or ±2°C.

HIFU exposures

A successful first-generation HIFU exposure uses a continuous exposure pattern (focal length, 60 mm; frequency, 1.71 MHz; ISATA, 2.3 kW / cm^2, 4.6 kW / cm^2; duration, 10 sec). In second-generation units, the HIFU exposure consists of three phases. The first phase is a trigger pulse

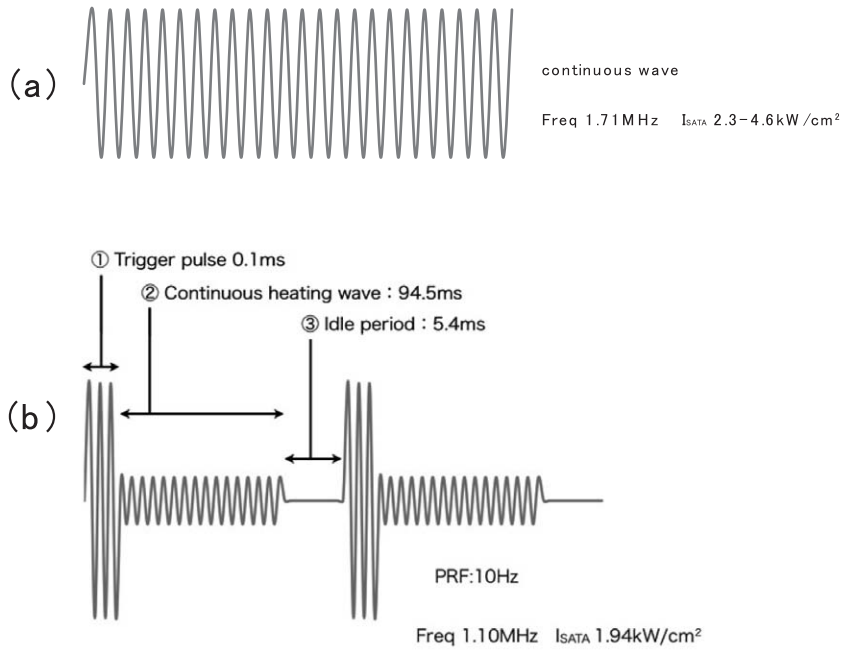

Fig. 2. Schematic drawings of the irradiation sequence
Vessel stenosis and reduced blood flow were confirmed, but occlusion was not completely confirmed. Similar to the following day of irradiation, vessel stenosis and reduced blood flow were detected post-irradiation, but occlusion was not confirmed.

to produce the cavitation (frequency, 1.10 MHz ; ISATA, 5.8 kW / cm^2 ; duration, 0.1 ms). The second phase is a continuous heating wave to oscillate the bubbles generated by cavitation (frequency, 1.10 MHz ; ISATA, 1.5 kW / cm^2 ; duration, 94.5 ms). The third phase is an idle period (duration, 5.4 ms) that is used to obtain images used an ultrasound probe during the HIFU exposure. A single HIFU exposure generally lasts for approximately 100 ms (10 repeats per second, 10 Hz), and is represented schematically in Fig. 2. The trigger HIFU sequence was developed as a cavitation-assisted ultrasonic heating method[27, 28]. By using a high-speed camera[27] and ultrasonic imaging[28], the occurrence of inertial cavitation was observed. Ablation performance and total ultrasonic output reduction was reported by a previous study that was carried out similarly[29].

Subjects of the study

Case 1 : A 40-year-old primigravida woman who was pregnant with twins was referred to our hospital at 14 weeks of gestation due to the absence of a heartbeat in one fetus. TRAPs was diagnosed based on the ultrasonographic findings. HIFU therapy was performed at 14 weeks and 6 days of gestation. The maternal abdominal thickness and myometrium was approximately 30 mm. Case 2 : A 32-year-old primigravida woman who was pregnant with twins was referred to our hospital at 14 weeks of gestation due to the absence of a heart beat in one fetus. TRAPs was diagnosed based on the ultrasonographic findings, and HIFU therapy was performed

at 14 weeks and 5 days of gestation. The maternal abdominal thickness and myometrium was 25 mm, and the placenta thickness was 20 mm.

Clinical settings of HIFU exposures

The patients were treated in the supine position as for a normal ultrasound scan. Umbilical cord insertion into the body of the acardiac fetus was evaluated by color Doppler imaging. The chosen HIFU protocol involved exposure repeated 15 times in an 8-second cycle, even if interruption of the blood flow was seen during the exposure protocol. Exposure was performed with the aim of blocking arteries and veins simultaneously. This protocol was determined from the findings obtained in basic studies[22–26, 29].

Just after exposure, the bag was released from the maternal abdominal wall and the temperature of the skin surface was measured using a highly precise thermal camera (during exposure, just after exposure, 30 seconds later, and 60 seconds later). Exposed blood vessels were observed by color Doppler and pulse wave Doppler imaging (just after exposure, 1 hour later, 3 hours later, and the next morning). Measurements were made with a Doppler incidence angle correction of $\pm 10^{\circ}$, and the results of 5 measurements were averaged.

The methods were carried out in accordance with relevant guidelines and regulations of the "Ethical Guidelines for Medical and Health Research Involving Human Subjects" (Japan, 2015). All experimental protocols were approved by Showa University School of Medicine Ethics Committee (Permission number : 700), and written informed consent was obtained from each patient before the study.

Results

Clinical courses

Case 1 : With a distance to the focal point of 55 mm, matching the focus to the HIFU target required circulating, degassed, cooled water adjusted to a distance of 5 mm from transducer to abdominal wall. Eight cycles of HIFU exposure were administered. Although 15 courses were planned, HIFU exposure was stopped in this case because the patient complained of a burning sensation during the 8th exposure. Vessel stenosis and reduced blood flow were confirmed, but complete occlusion was not confirmed. The vessel stenosis and reduced blood flow were still apparent the next day, and a high level of brightness was observed at the site of exposure ; occlusion was still not confirmed (Fig. 3). After the HIFU therapy, this patient visited her previous hospital at 15 weeks and 5 days of gestation and an ultrasound scan was performed. The acardiac twin's umbilical artery resistance index (UmA-RI) was 0.63, and peak systolic velocity (PSV) was 23.1 cm^2 / s. There were no changes compared with the pre-exposure parameters, she underwent a RFA at 16 weeks and 5 days of gestation. Combined spinal epidural anesthesia (CSEA) was administered, and the operation time was 27 min (5 mm 30 W, 20 s ; 10 mm 30 W, 47 s ; 15 mm 30–40 W, 90 s ; 40 W, 102 s). She was discharged 2 days after the operation, and her prenatal course after the RFA treatment was uncomplicated. Cesarean section was performed at 37 weeks of gestation due to placenta previa, and a 2246-g male infant was delivered

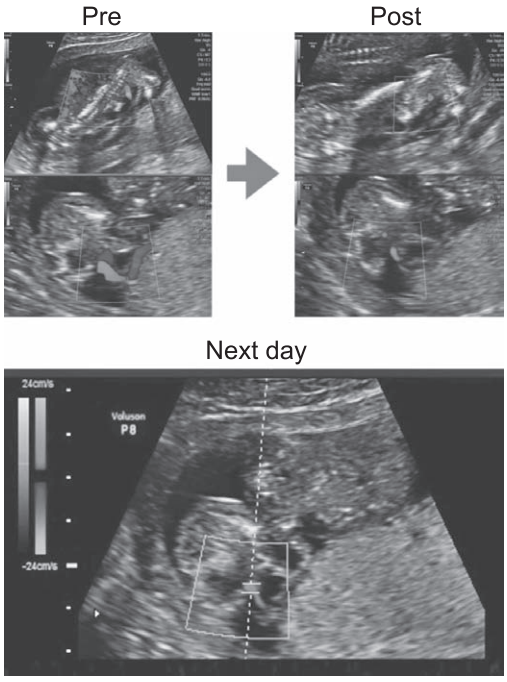

Fig. 3. Ultrasound *images pre- and post-Irradiation* (Case 1)
The blood flow disappeared after the 7th cycle. Vessel stenosis and reduced blood flow were detected; however, the occluded vessels had recanalized the following day.

with Apgar scores of 8 at 1 minute and 9 at 5 minutes.

Case 2: With a distance to the focal point of 45 mm and in order to match the focus to the target, the amount of circulating, degassed, cooled water was adjusted to provide a distance of 10 mm between the transducer and abdominal wall. Eight cycles of HIFU exposure were administered. The blood flow disappeared after the 7th cycle. As with Case 1, this case ended with insufficient exposure, because a burn was detected on the mother's abdominal wall during the 8th exposure and the planned 15 exposures were not completed. The next day, vessel stenosis, reduced blood flow, and high brightness on the ultrasound image were confirmed at the site of exposure; however, the occluded vessels had recanalized (Fig. 4). After HIFU therapy, the woman visited her previous hospital at 15 weeks and 3 days of gestation. An ultrasound scan showed that the acardiac twin's UmA-RI was 0.63, and the PSV was 29.9 cm^2 / s. There were no changes compared with the pre-exposure parameters. The patient underwent RFA treatment at 16 weeks and 4 days of gestation; she received CSEA anesthesia, and the operation time was 12 min (10 mm 30 W, 30 s; 15 mm 30 W, 50 s). She was discharged one day after the operation, and her prenatal course after the RFA treatment was uncomplicated. The woman gave birth by normal spontaneous vaginal delivery at 39 weeks and 4 days of gestation. A 2870-g male infant was born with Apgar scores of 8 at 1 minute and 8 at 5 minutes.

Neither case involved complications to the pump fetus. In terms of maternal complications, Case 1 had redness and a first-degree burn, while Case 2 showed blistering and was diagnosed with a second-degree burn. The condition of Case 1 resolved with follow-up alone, while the condition of Case 2 resolved with steroid ointment treatment for 2 weeks. No lasting discoloration of the skin or scarring was apparent.

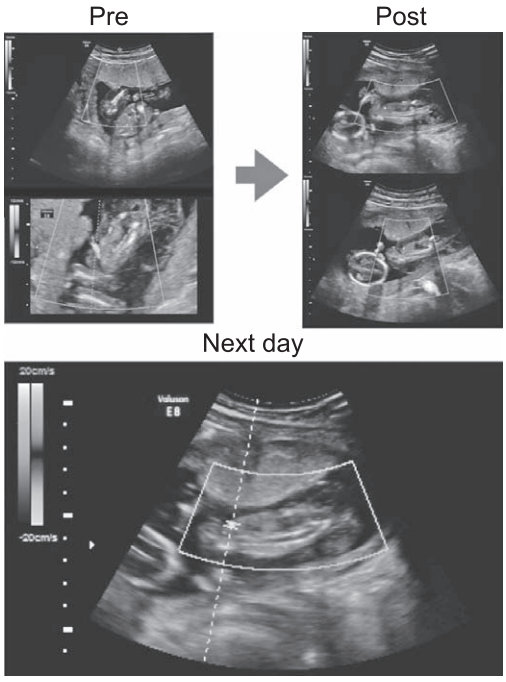

Fig. 4. Ultrasound *images pre- and post-irradiation* (Case 2)
(a) A first-generation HIFU system used a continuous irradiation pattern, (b) In contrast, second-generation HIFU systems used sequential irradiation. ① A trigger pulse to produce the cavitation (frequency, 1.10 MHz ; ISATA, 5.8 kW / cm^2 ; duration, 0.1 ms). ② A continuous heating wave to oscillate the bubbles generated by cavitation (frequency, 1.10 MHz ; ISATA, 1.5 kW / cm^2 ; duration, 94.5 ms). ③ An idle period (duration, 5.4 ms), used to obtain an image using an imaging ultrasound probe during the HIFU irradiation. The pulse repetition frequency (PRF) during the three phases was set at 10 Hz. Total HIFU intensity was set at 1.94 kW / cm^2.

Evaluation of clinical usage for HIFU

First-generation HIFU systems suffered from a wide range of motion range disturbances, and coherence could not be achieved between the apparatus and the abdominal wall because the system itself was a large, biaxial unit[25, 26]. In second-generation HIFU devices, the imaging probe is arranged in the center of the HIFU transducer and is a small, coaxial device thus it is subjected to less motion range disturbance. Coherence between the apparatus and the abdominal wall is achieved, and the device is more maneuverable. In the first-generation systems, the ultrasound and imaging signals interfered with each other due to the continuous exposure, but the newer devices have adopted a sequential exposure system, allowing the system to capture real-time ultrasound images in the short downtime period (Fig. 2). This helps adjust for misalignment that can occur due to the patient's respiratory fluctuations, fetal movements, or tremor of the operator's hand. In addition, it allows observation of the process of tissue denaturation during exposure (Fig. 5), and increases in surface temperature are controlled using a circulating, degassed, cooled water unit (Fig. 6).

Discussion

In Case 1, the target vessels were constricted, but occlusion was not achieved just after therapy. The patient complained of a burning sensation during the 8th exposure. On the next day, vessel stenosis, reduced blood flow, and high brightness at the site of exposures were observed on the ultrasound image, but occlusion was not confirmed. It was thought that the spinal bones of the acardiac fetus had prevented sufficient acoustic energy from reaching the target, because it was in a prone position within the uterus. Indeed, no better condition for exposure could be found ;

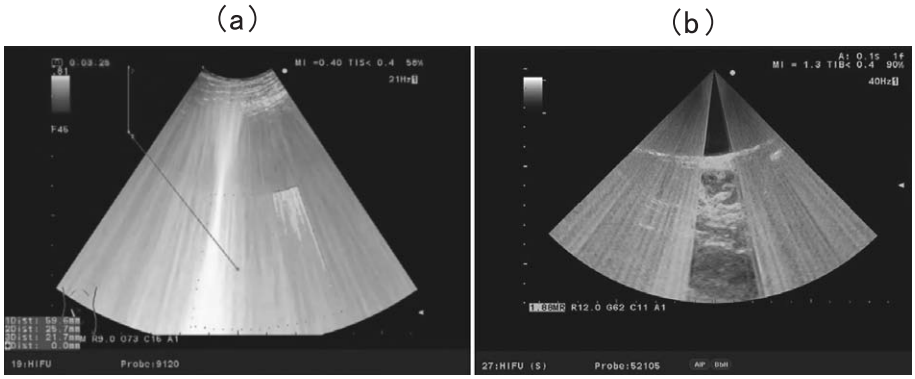

Fig. 5. Images during irradiation
Using a first-generation HIFU system, we could not obtain real-time images during irradiation, because the HIFU and imaging signals interfere with each other. (b) For the new device, a sequential irradiation system was adopted, allowing real-time ultrasound images to be captured in the short downtime period. It allows observation of the process of tissue denaturation during irradiation.

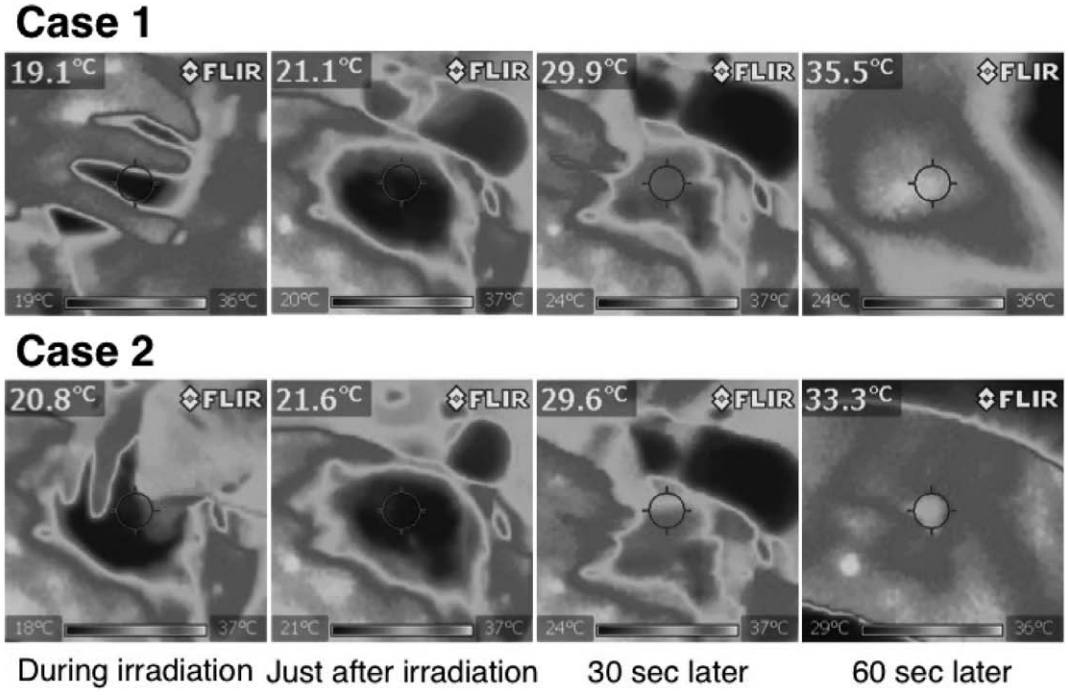

Fig. 6. Changes in skin surface temperature after HIFU exposure in both cases (thermal camera findings)
Increases in surface temperature were controlled using a circulatory de-aeration coolant water unit in both cases.

however, the patient then wished to change to RFA when the HIFU treatment could not be accomplished within a single day of hospitalization. If the treatment could have been repeated without time restrictions, occlusion of blood flow may have been achieved.

In Case 2, vessel occlusion was achieved at the 7th exposure ; however, the case ended with

insufficient exposure because a burn was detected on the mother's abdominal wall during the 8th exposure and the plan of 15 exposures was aborted. On the next day, the occluded vessels had recanalized, although vessel stenosis, reduced blood flow, and high brightness on the ultrasound image were confirmed at the site of exposures. In past reports, high echogenicity in tissue also meant histological thermal degeneration[22–24]. Similar thermal degeneration seemed to occur in the current two patients, although not to the point of permanently occluding the blood vessels. Of note, ultrasonic measurements were performed in this study; however, they were acquired using color Doppler ultrasonography, PSV, and RI. As the blood vessel narrows, the changes that the PSV increases presented herein mirror findings from a previous preclinical study[22]. Although 15 cycles of exposures had been planned, based on other preclinical studies, treatment was stopped due to a slight burn on the mother's abdominal wall. The distance between the target and the abdominal wall was close in Case 2. Since the focal length is fixed at 60 mm by the device's shape, the distance between the abdominal wall and the device was therefore adjusted by the amount of degassed cooled water in the probe cover. It was therefore thought that the distance between the device and the skin caused the burn. In both cases, poor conditions were partially responsible for the treatment failure, and flexibility is essential to successfully perform the treatment under good conditions. To achieve this, informed consent from the patient for conditions such as a fixed hospitalization period may be needed.

The second-generation HIFU systems incorporate three significant improvements. First, the physical entity was transformed from a large biaxial unit to a small coaxial one, resulting in decreased motion range disturbance, coherence between the apparatus and the abdominal wall, and a more maneuverable device. This overall improvement seems to have expanded the possibilities for HIFU in fetal treatment. Second, the conversion from a blind view to real-time imaging is a key improvement that enables direct observation of the target during the HIFU exposure and allows the operator to adjust any misalignment. This feature seems to have improved the safety of HIFU in fetal treatment. Third, the systems were upgraded from a non-circulating, degassed, cooled water bag to a circulating, degassed, cooled water system. The Sonachill cooling system, which reduces the increase in skin surface temperature, was added to mediate this third improvement, although a mild burn still occurred in both cases presented herein. Thermal and non-thermal effects are present, and the information obtained by the thermal camera showed the result that skin surface temperature was low. This result suggests prevention of the thermal effect, at least on the skin surface. However, we were not actually able to prevent burns, probably due to the influence of non-thermal effects (e.g., cavitation between the probe cover and the abdominal skin surface).

In both cases, poor conditions were partly responsible for treatment failure, but they were not the sole cause. The problem of burning has not been resolved, and attempts to identify recanalization and the formation of collateral circulation were insufficient. These problems need to be resolved for the reliable application of HIFU in such cases. The cause of the burn was thought to be cavitation between the probe cover and the abdominal skin surface, thus a method to prevent this effect must be established. In addition, the present study showed that recanalization

remains possible in cases that seem to be occluded, and HIFU treatment might only cause temporary thrombus formation or vessel spasm just after exposure. It is therefore necessary to investigate exposure intensity and the number of exposures cycles needed to achieve complete occlusion.

There are three main advantages attributed to second-generation HIFU systems in the human clinical setting : the simplicity in maneuverability and reduced disturbance to the range of motion ; the ability to image in real time during exposures ; and the decrease in total ultrasonic output. In the two cases presented herein, complete occlusion was not achieved, but a reduction was obtained in one patient and temporary occlusion was seen in the other. In both cases, no long-term adverse events were apparent for either the mother or the fetus. Treatment interruption due to burns or complaints of heat sensation obstructed the treatment completion, and such an outcome remains an issue to be addressed in the future.

Acknowledgements

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Conflict of interest disclosure

The authors declare no conflict of interest associated with this manuscript.

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