Innovative Treatment of a Fetal Lung Mass Model Using High-intensity Focused Ultrasound (HIFU)

Noriyoshi Nakayama¹, ², Keri Kim², Akihiko Ishiyama², Tetsuko Ishii², Hiromasa Yamashita², Toshio Chiba², and Akira Toki¹

Abstract: Current therapy for space-occupying fetal lung mass lesions (fetal pulmonary lobectomy) is invasive and technically demanding. Accordingly, new therapeutic procedures are required which are much less invasive and more efficient. The purpose of this study was to investigate the feasibility of high-intensity focused ultrasound (HIFU) as a new therapeutic modality for fetal lung mass lesions, using an experimental animal model. We created a solid fetal lung model by differential lung ventilation using anesthetized adult rabbits. In this model, experimental animals with a unilateral independent (fluid-filled) lung were maintained by single lung ventilation of the other dependent lung. Within the independent lung, target blood vessels depicted by color flow Doppler were repeatedly irradiated with HIFU energy beams (n=19). Occlusion of these blood vessels in vivo was confirmed by evaluation of the flow using color flow Doppler. After the procedure, the animals were sacrificed and their harvested lungs were assessed grossly and microscopically. Pulmonary blood vessels (artery and/or vein) were effectively occluded with 2 to 5 cycles of HIFU energy delivery (10.5 seconds each) with a success rate of 62.5% (arteries) and 72.7% (veins). No clear changes including tissue perforations were observed grossly on the surface of the lungs. Ultrasound-guided HIFU energy delivery seems promising for occlusion of the pulmonary blood vessels within a fluid-filled independent lung (fetal lung model). Thus in the future, HIFU irradiation could be used as a less invasive technique to occlude the feeding vessels of fetal lung mass lesions in utero.

Key words: feeding vessel occlusion, fetal lung mass lesion, high-intensity focused ultrasound (HIFU)

Introduction

Fetal lung mass lesions are known to be predominantly congenital cystic adenomatoid malformations (CCAM) or bronchopulmonary sequestrations (BPS) or hybrid-type masses. Occasionally, the mass shrinks spontaneously in utero giving an improved postnatal prognosis.

¹ Pediatric Surgical Services, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan.
² Department of Clinical Research and Development, National Center for Child Health and Development.
On the other hand, although infrequently, these masses become enlarge before birth and displace the mediastinum and normal lung tissues leading to fetal hydrops (heart failure), pulmonary hypoplasia and/or polyhydramnios. In these cases, the perinatal prognosis is quite poor1-3).

Prenatal intervention in fatal cases of fetal lung mass lesions by ultrasound-guided cyst aspiration and/or fetal lobectomy has been reported to save these fetuses 4). These procedures, however, are technically demanding and invasive to both the mother and fetus.

High-intensity focused ultrasound (HIFU) irradiation employs highly focused ultrasound energy with a low energy density except for the focal point. Therapeutic HIFU delivery could potentially allow much less invasive surgeries with efficient tissue coagulation and/or perforation, and with little thermal damage to the underlying or overlying tissues5-8). Currently, further investigation of HIFU treatment, including its use for fetal surgical interventions, is underway9,10).

The purpose of this study was to investigate the in vivo feasibility of this new modality to coagulate and occlude the blood vessels within a fluid-filled fetal lung model created by experimental differential lung ventilation in the rabbit.

Materials and Methods

We developed an image-guided HIFU delivery system11, consisting of a HIFU transducer combined with an ultrasound diagnostic imaging probe on the side (Fig. 1). The focal length was 40 mm, and the focal point was elliptical in shape (0.6 mm wide and 5.0 mm long). The HIFU-operating sinusoidal wave was 3.30 4.01 MHz in frequency, and the temporal average intensity of the continuous-wave spatial peak was 6.5 kW/cm².

The focal point of the HIFU transducer was calibrated in advance on a 2-dimensional ultrasound imaging monitor. Then, the HIFU transducer was positioned using a 3-axis mechanical manipulator so that the focal point was accurately adjusted to the target vessels. Because the lungs and other organs can change position during the procedure, to avoid inadvertent damage to the adjacent tissues, the HIFU delivery was controlled by a computer-aided program which automatically tracked the target vessels, keeping the HIFU focal point consistently adjusted.

Animal model of the fetal lung mass lesion

Unlike postnatal lungs, fetal lungs are filled with lung fluid so that the ultrasound energy emitted by the HIFU transducer can reach the lung tissue without being attenuated, allowing coagulation of the focal area. The unilateral independent (fluid-filled) lung used as the fetal lung model was created by single lung ventilation of the other dependent lung (differential ventilation) as follows. A thoracotomy was performed on a Japanese white rabbit (2.8 kg, male) which had undergone endotracheal intubation on intramuscular xylazine (5 mg/kg) and was under general anesthesia with inhalational isoflurane (ventilator settings:
tidal volume = 140 ml, I : E ratio = 1 : 2, respiratory rate = 20 breaths/min). The animal’s oxygen saturation and electrocardiogram were monitored throughout the experiment. Then, a median sternotomy was performed on the animal in the supine position and the heart and both lungs were exposed.

Single lung ventilation (differential lung ventilation) was achieved by inserting a bronchial occluder through the intubation catheter. Through the bronchus effectively occluded by the bronchial occluder, the remaining intrapulmonary gas was aspirated from this independent lung. Then, the separated independent lung was gradually filled with 20 ml saline, resulting in the creation of a model of fetal lung or fetal lung mass lesion (Fig. 2).

An acrylic water tank having a sheet of silicon-film on the bottom was placed on the surface of the independent lung of the supine-positioned animal. The tank was filled with degassed water (Fig. 3a) and the space between the film and the thoracic cavity was filled with ultrasound gel to eliminate ultrasound attenuation by intervening gas. Using the HIFU transducer equipped with a diagnostic imaging probe on the side (Fig. 3b), color flow Doppler images were obtained depicting the intrapulmonary blood vessels within the independent lung.

**Experimental protocol**

After focusing on the target vessels depicted on the color flow Doppler images, the HIFU beam (3.3 MHz) was delivered to heat and coagulate the vessels. The artery and vein, distinguished by flow waveform analysis, were treated with HIFU irradiation separately.

The ventilation rate was set mechanically at 20 breaths/min. To synchronize with the respiratory movement of the target vessels, the HIFU delivery was controlled by a newly-developed computer program so that the HIFU beam could be emitted when the target movement was minimal. All animal experiments were performed according to the institutional animal ethics guidelines, based on that of the National Institute of Health (USA).

The experiment was conducted as follows:

1. Search for the vessels within the lung filled with saline using color flow Doppler.
2. Focus the HIFU beam onto the target vessels.
3. Delivery of HIFU; the HIFU beam (300 mV) was emitted for 1.5 seconds each with an interval of 1.5 seconds, and the total time for a single delivery cycle was 10.5 seconds.
5. Start another cycle of HIFU delivery with a 30-second intermission until confirmation of blood flow occlusion.
6. The animal was euthanized with intravenous pentobarbital and both lungs were harvested.
7. Harvested organs were fixed with formalin and sections of tissue were paraffin-embedded for hematoxylin-eosin staining for pathological work-up.
Results

During the entire procedure, our image-guided HIFU delivery system could identify the focal point, automatically track the target, and control the HIFU energy delivery according to the instructions from our computer program.

We used 10 animals for conduction of 19 experiments of vessel occlusion (8 arterial and 11 venous targets; Table 1). Arteries could be occluded with 2 (n=1), 3 (n=2), or 5 (n=2) cycles of HIFU delivery. Venous occlusion was accomplished by 2 (n=2), 3 (n=5),
or 4 (n=1) cycles of HIFU irradiation, resulting in a success rate of 62.5% for arteries and 72.7% for veins. We failed to occlude the blood vessel in 3 experiments due to misfocusing of the HIFU beam resulting in inadvertent complications such as epidermal burns and vessel perforation.

After the HIFU treatment, no clear tissue alterations (perforation etc.) were observed on the surface of the lungs (Fig. 4). In the hematoxylin-eosin-stained tissues (Fig. 5), vascular diameter was mostly unaffected, but intravascular blood clots were observed in some areas, suggesting some inadvertent thrombogenesis due to thermal effects of HIFU delivery. In addition, some vacuolization was identified in the vascular wall. Thermal necrotic degeneration was also observed in perivascular tissue areas.

**Discussion**

Using HIFU irradiation, we could successfully achieve minimally-invasive occlusion of the blood vessels within animal lungs filled with saline (fetal lung model).

The difference in success rate of occlusion, 62.5% for arteries and 72.7% for veins, appeared vessel-dependent, being attributed to the vascular size, vascular wall thickness, and flow rate. Failure in vessel occlusion was mainly due to misfocusing. Throughout the experiment, HIFU delivery was controlled by a computer program so that the HIFU energy was emitted when the target was most static in position, although focus-fixing failures

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Artery or Vein</th>
<th>HIFU irradiation</th>
<th>Occlusion</th>
<th>Remark</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>V</td>
<td>3 cycles</td>
<td>o</td>
<td></td>
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<tr>
<td>2</td>
<td>V</td>
<td>5 cycles</td>
<td>x</td>
<td>Epidermal burn</td>
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<td>3 cycles</td>
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<td>3</td>
<td>V</td>
<td>5 cycles</td>
<td>x</td>
<td>Focus fixing failure</td>
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<td></td>
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<td>4 cycles</td>
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<td>7</td>
<td>A</td>
<td>10 cycles</td>
<td>x</td>
<td>Pulmonary perforation</td>
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<td>V</td>
<td>10 cycles</td>
<td>x</td>
<td>Vascular perforation</td>
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<td>9</td>
<td>A</td>
<td>3 cycles</td>
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<td>Focus fixing failure</td>
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were possible due to an unavoidable time-lag in our system. Further studies to refine the computer program are currently being conducted. In other cases, 5 cycles of HIFU delivery caused epidermal burns, and 10 cycles of HIFU delivery caused lung tissue damage or vascular perforation. Further investigation is needed to reveal the actual cause of these complications.

With technological advances in ultrasonographic evaluation, pathologic features of the fetal lung mass become clearer. In general, fetal abnormalities, even if they start as a relatively simple anomaly in the early developmental stages, might have the potential to progress into more complex and serious conditions before birth. The likelihood of the occurrence of these specific developmental abnormalities might depend on the location and severity, as well as the timing of the development of the lesion in utero.

Adzick reviewed a series of fetal cases having CCAM or BPS, reporting that these lesions are likely to be associated with a prenatal mortality of 100% if compromised by fetal hydrops and managed solely in an expectant way, whereas all the fetuses not associ-
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ated with fetal hydrops could survive\(^2\). This result indicates that fetuses that have CCAM or BPS and are associated with hydrops are highly likely to need some form of prenatal intervention. In a fetal patient with BPS, ultrasound-guided laser coagulation of the feeding vessels substantially reduced the size of the mass with an amelioration of the pleural effusion and fetal hydrops\(^2\). However, these procedures are technically quite demanding and frequently invasive to both the mother and fetus\(^2\). HIFU has been increasingly employed for minimally- or non-invasive treatment in several surgical fields and could be used for compromised fetal lung lesions.

However, the efficacy of the HIFU procedure, which is probably dependent on the vascular flow of the feeding vessels of the fetal lung mass lesion, needs further investigation because the vascular inflow of CCAM originates from the pulmonary circulation, unlike BPS which has an arterial blood supply arising from the systemic circulation. Further understanding about the blood supply in CCAM is also essential\(^9\). Additionally, it is important to consider that the fetal lung mass lesions occasionally occur as mixed lesions of CCAM and BPS. Therefore future development in diagnostic imaging technology is likely to define the blood supply more accurately allowing much more focused treatment of these fetal lesions.

In this study, the long-term effect on the lung tissue following HIFU treatment is still unknown as the animals were euthanized for pathological assessment immediately after the procedure. In addition, the possibility of vessel recanalization or lung crush injury cannot be excluded in the long-term. Accordingly, continuing follow-up is needed following the procedure. Further studies are also needed in pregnant animals to determine whether the vessel occlusion is due to thrombogenesis or degeneration of the vessel walls and surrounding tissues.

HIFU is a promising new therapeutic modality for occluding the feeding vessels in animal fetal lung models as well as in fetal lung mass lesions. The advent of this technology might indicate that a minimally-invasive intervention for the treatment of human fetal lung masses is likely in the near future.

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References


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