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Original

Non-touch, Quick Removal of an Occluding Intratracheal Balloon Using High Intensity Focused Ultrasound and Limonene Emulsion

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Abstract: In recent years, fetal endoscopic tracheal occlusion (FETO) using a balloon has been clinically employed for promoting prenatal lung growth to ameliorate postnatal respiratory failure caused by severe *in utero* lung hypoplasia. After a successful FETO, in some limited fetal centers, planned or emergency balloon removal using another fetoscopy is performed to release the tracheal occlusion immediately before delivery. To overcome this additional fetoscopy, we previously reported an innovative simple procedure to remove the occluding balloon by bursting it with a pre-planned sequence of high intensity focused ultrasound (HIFU) irradiation. In that previous study, which used rabbits euthanized and submerged in degassed water, we inflated the balloon by injecting a mixture of perfluorocarbon and ultrasound contrast medium through a fetoscopically-guided catheter. The rate of successful balloon burst and deflation using HIFU irradiation was high enough (100%), but the mode and timing of tracheal reopening (i.e., sudden burst or slow shrinkage of the balloon) was rather varied and collateral damage to the dermal/tracheal tissue was identified in 72.7% of the experimental animals. Accordingly, to standardize the HIFU irradiation sequence and to achieve a reliable and secure balloon burst, we conducted another series of animal experiments in which the mixture of perfluorocarbon was replaced with "limonene emulsion" (D-limonene micelle emulsified in physiological saline) as the balloon injection fluid. In all 6 animals, we succeeded in reopening the airway by achieving an instantaneous and timely balloon burst without definite skin/tracheal damage. We conclude that HIFU irradiation together with injecting the balloon with "limonene emulsion" is an improved method for safe tracheal release from a balloon occlusion.

Key words: high intensity focused ultrasound (HIFU), limonene, fetal endoscopic tracheal occlusion (FETO), congenital diaphragmatic hernia

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Introduction

Several congenital anomalies, including fetal diaphragmatic hernia, are well known to be prenatally associated with devastating lung hypoplasia which potentially progresses *in utero* and is fatal postnatally[1, 2]. Fetal endoscopic tracheal occlusion (FETO) is a minimally invasive prenatal procedure for promoting fetal lung growth by totally occluding the trachea using a fetoscopically-inflated balloon[3, 4]. Although FETO has been widely adopted with an increase in successful cases, the most crucial current issue is the method of tracheal reopening before delivery[4, 5].

Several procedures have been reported for tracheal reopening including removal of the balloon by performing another fetoscopy. Every prenatal procedure, however, inevitably requires highly competent skills as well as expertise, especially in the case of an urgent preterm delivery. To overcome this clinical issue, using euthanized rabbits, we experimentally developed and previously reported a simple means of balloon deflation where an intratracheal balloon was injected with a perfluorocarbon mixture and then exposed to high intensity focused ultrasound (HIFU)[6]. Although our experiment showed a high success rate (100%) of tracheal reopening, the mode and timing of balloon deflation was rather irregular and unpredictable (i.e., an instantaneous burst or a slow shrinkage). Furthermore, collateral damage to the skin in the path of the HIFU beam occurred in as high as 72.7% of the animals.

The current report describes another series of experiments where the perfluorocarbon mixture was replaced with a "limonene emulsion" (i.e. limonene-encapsulated micelles dispersed in physiological saline). As a consequence, we succeeded in developing a much more reliable and safe HIFU procedure leading to a more regular mode and timing of balloon burst with definitely less incidence of collateral skin damage. Thus, in this report, we show our research outcomes which suggest the highly positive prospect of using HIFU for the successful completion of FETO without a second fetoscopy.

Materials and methods

Animal model

A total of six female Japanese White rabbits (weight : 1 kg) were used underwater following euthanasia as a model of an *in utero* human fetus within the amniotic fluid. FETO is clinically performed between 26 and 30 weeks gestation and we selected the body weight of the animals by referring to the human fetal growth curve. These animals were intramuscularly injected with 3 ml of 2% xylazine into the thigh and the neck and chest were shaved. Then, they were intravenously injected with 5 ml of 6% pentobarbital for euthanasia and were submerged within a tank filled with degassed water to mimic the pregnant uterus.

Balloon and specific fluid for inflation

To totally occlude the animal's trachea, we used a balloon (BALT Extrusion, Montmorency, France ; GOLDBAL2, 7.0×22 mm, full expansion volume of 1.0 ml) equipped with a special injection catheter and a check-valve at the proximal end. The balloon was fetoscopically

placed inside the trachea between the vocal cord and the manubrium. For this purpose, under ultrasound guidance, a fetoscope (Karl Storz, Tuttlingen, Germany; Model 11540AA, Miniature Straight Forward Telescope 0°, 1.3 mm) was inserted into the trachea to appropriately position and inflate the balloon. The balloon was inflated by injecting 0.5-0.6 ml of "limonene emulsion". The "limonene emulsion" consisted of 10 mg of D-limonene in 1 g of emulsion, with D-limonene contained in 0.1-1.0 μm diameter monolayer micelles consisting of lecithin, fatty acid and amino acids, and then dispersed and emulsified in physiological saline (dispersing medium). Before taking out the fetoscope, the balloon and its check-valve were confirmed to be appropriately positioned without any fluid leakage fetoscopically or sonographically.

HIFU irradiation setting with pre-planned pulse sequence

The HIFU transducer was held by a robotic arm to secure an appropriate operating position. An imaging probe (Microconvex, Hitachi-Aloka Medical, Tokyo; UST-9120) was set in the center of the HIFU transducer which had an aperture diameter of 120 mm, a focal depth of 120 mm, a focal range along the sound axis of 10 mm and a beam width of approximately 6 mm (Fig. 1). Based on a pre-planned pulse sequence, the wavelength of the regularly cycled irradiation wave was 1.0 MHz with an acoustic intensity of 7.05 kW/cm^2. Figure 2 shows the HIFU delivery sequence. Under ultrasound imaging guidance, each HIFU exposure time was set to 10 sec focusing on the back wall of the balloon. For preventing burns to the skin in the path of the HIFU beam, HIFU exposure was repeated a maximum of five times at regular intervals of 30 sec each. Figure 3 shows the balloon burst procedure using our novel "limonene emulsion" and HIFU irradiation sequence. After HIFU exposure, D-limonene leaks from the broken monolayer micelles and floats to the inside of the balloon surface. A part of the latex wall of the balloon is melted by the chemical reaction of D-limonene, resulting in the balloon burst.

Histological study

After a sonographic balloon burst by HIFU irradiation, the trachea was incised to observe the balloon, and specimens of dermal tissue in the path of the HIFU beam, along with the tracheal wall and perifocal tissues, were excised for histological study by hematoxylin-eosin staining.

Results

"Limonene emulsion" was used to inflate the balloons placed in the tracheas of a total of six animals (Table 1).

Balloon burst

All of the six balloons totally occluded the trachea, and all were ruptured in a timely fashion while the experimental animals were undergoing HIFU irradiation (Table 1).

Histological study

Following the balloon rupture, dermal tissue in the path of the HIFU beam and the tracheal

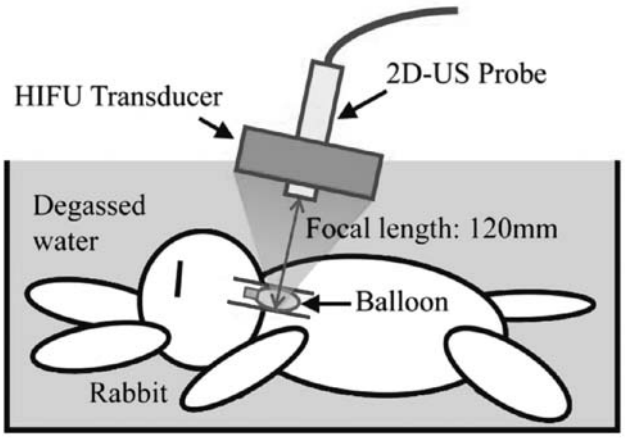

Fig. 1. A fetoscope was inserted in the trachea of each rabbit, through which a balloon was inflated and placed under 2-dimensional ultrasound guidance. High intensity focused ultrasound (HIFU) irradiation was applied focusing on the back wall of the balloon under ultrasound guidance.

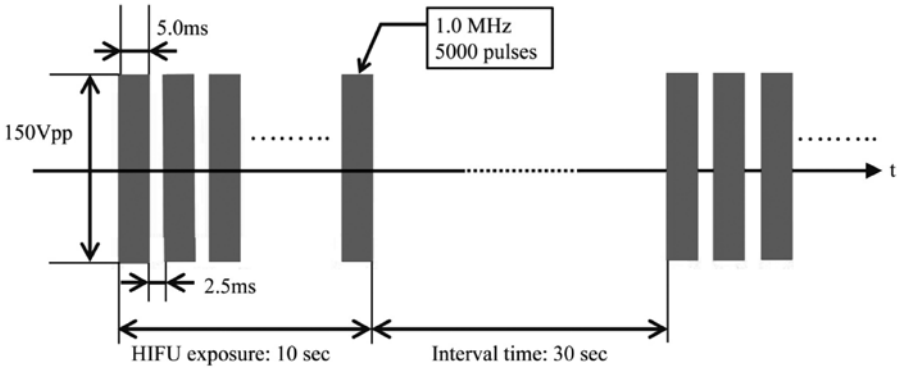

Fig. 2. A high intensity focused ultrasound (HIFU) irradiation sequence was used in the study, employing intermittent HIFU exposures (duty ratio of 67%) with 30 sec rest intervals.

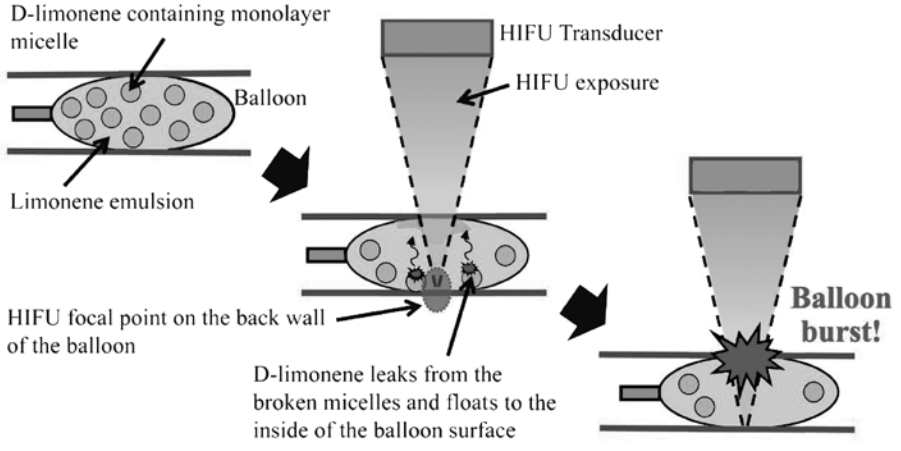

Fig. 3. The balloon burst procedure used our novel "limonene emulsion" and high intensity focused ultrasound (HIFU) irradiation sequence.

wall located around the HIFU focal point were studied grossly and histologically in all of the experimental animals.

As with the gross findings, hematoxylin-eosin staining revealed no significant microscopic thermal or mechanical damage to the tissue (Fig. 4). However, these findings only reflect the non-biological mechanical tissue response, as the experimental animals underwent HIFU irradiation after being euthanized.

Discussion

In this study, we demonstrated that a trachea occluded with a balloon can be re-opened on the spot by HIFU irradiation alone. This experimental outcome suggests the strong likelihood that a fetus who has had FETO for severe lung hypoplasia can undergo successful tracheal reopening before delivery by prenatal HIFU without a second fetoscopy. HIFU has already been clinically used for ablating diverse tumors including prostate and breast cancers[7, 8]. HIFU has also been employed for ablation of an acardiac twin to save the pump twin in cases of twin-reversed arterial perfusion sequence[9].

In our report, we adopted an emulsion of D-limonene microcapsules as an injection fluid ("limonene emulsion") to achieve a reliable and timely burst of the balloon by HIFU irradiation. D-limonene, a colorless chemically inactive monoterpene, is the major chemical found in the oil of citrus fruit peels and is, after being absorbed in the body, soon metabolized and

Table 1. Experimental results

Animal No.	Balloon location	'Limonene emulsion' injection volume (ml)	No. of irradiation cycles	Energy output of total HIFU dose (kW/cm^2)	Energy output of total HIFU dose (kJ)	Skin damage	Injury to tracheal membranes and wall	Result
1	Between vocal cord and manubrium	0.6	3	7.05	6.64	(−)	(−)	Deflated
2	Between vocal cord and manubrium	0.6	3	7.05	6.64	(−)	(−)	Deflated
3	Between vocal cord and manubrium	0.6	3	7.05	6.64	(−)	(−)	Deflated
4	Between vocal cord and manubrium	0.6	2	7.05	4.43	(−)	(−)	Deflated
5	Between vocal cord and manubrium	0.6	2	7.05	4.43	(−)	(−)	Deflated
6	Between vocal cord and manubrium	0.6	3	7.05	6.64	(−)	(−)	Deflated

HIFU, high intensity focused ultrasound.

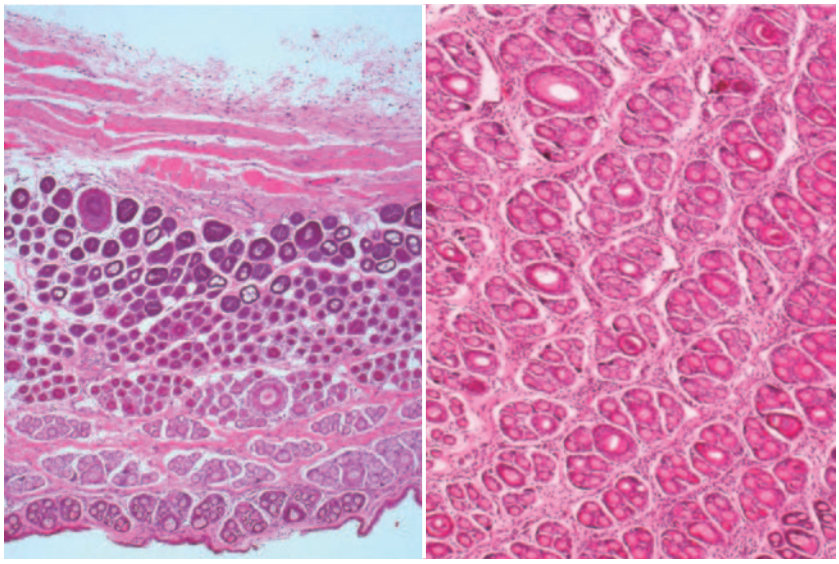

Fig. 4. Hematoxylin-eosin staining of the tracheal tissue after high intensity focused ultrasound (HIFU) irradiation. The left panel shows a coronal section between the skin and platysma fascia without any cell degeneration. The right panel shows a horizontal skin section without any degeneration of hair roots or peripheral connective tissue.

excreted in the urine (80%) and feces (20%). Furthermore, it is well-known to be totally safe for humans as it is biologically harmless to vital cells and tissues.

The reason why the "limonene emulsion", consisting of the dispersive medium (saline) and the limonene-encapsulated fine micelles (conc. 1 wt%), worked well for a reliable and timely balloon burst is thought to be as follows. Acoustic waves made by the HIFU irradiation first cause cavitation in the dispersive medium, generating innumerable minute bubbles which rapidly grow and densely cluster. Then, these microbubbles rapidly collapse producing destructive shock waves exerting pressure on the limonene-containing microcapsules. This results in the release of free limonene, which assembles together in the short-time intervals between the regularly repeated HIFU exposures. The enlarging collection of free limonene soon rises and comes in direct contact with the balloon wall and then chemically dissolves the latex rubber front wall of the balloon resulting in an instantaneous burst.

Along with the successful balloon bursts, the use of "limonene emulsion" led to a definite reduction in the mechanical and thermal collateral damage to the skin in the path of the HIFU beam, which we reported in our previous study[6]. This was probably because the D-limonene monolayer micelles were broken by the intermittent HIFU exposure (duty ratio of 67%) at time intervals of 30 sec allowing for successful balloon bursts, but producing less intradermal vibrations and heat energy than in our previous study.

One limitation of this study was the biological significance of our histological studies as all the experimental animals underwent HIFU irradiation after being euthanized; therefore, the microscopic findings reflect non-biological mechanical tissue effects. Accordingly, live animals should be studied in future to accurately investigate the histopathological damage caused by the HIFU

procedure.

In conclusion, this feasibility study demonstrated that if "limonene emulsion" is used with HIFU irradiation, an instantaneous release of balloon occlusion following FETO is likely to occur without the currently standard second fetoscopy. This development is also expected to enhance the safety and acceptability of FETO, leading to an improvement in the therapeutic outcome of fetal lung hypoplasia associated with congenital diaphragmatic hernia. Continued research is needed to further develop this promising HIFU-limonene technique.

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Conflict of interest disclosure

The authors declare there are no conflicts of interest regarding the subject or materials discussed in this manuscript.

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