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● *Original Contribution*

IN VITRO MITRAL CHORDAL CUTTING BY HIGH INTENSITY FOCUSED ULTRASOUND

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Abstract—Mitral regurgitation, when it arises from functional restriction of mitral leaflet closure, can be relieved by surgical cutting of the mitral tendineae chordae. We hypothesized that high intensity focused ultrasound (HIFU) might be useful as a noninvasive extracorporeal technique for cutting mitral chordae. As a pilot study to test this hypothesis, we examined the *in vitro* feasibility of using HIFU to cut calf mitral chordae with diameters from 0.2 to 1.6 mm. Sixty-seven percent of chordae were completely cut with HIFU, operated at 4.67 MHz and 45 W acoustic power, with up to 120 pulses of 0.3-s duration at 2-s intervals. Forty-five percent were completely cut when the pulse duration was reduced to 0.2 s. The average diameter of those chordae, which were completely cut, was significantly smaller than that of incompletely cut chordae (0.59 ± 0.30 versus 1.14 ± 0.30 mm with a pulse duration of 0.2 s, $p < 0.0001$; 0.68 ± 0.29 versus 1.32 ± 0.20 mm with a pulse duration of 0.3 s, $p < 0.0001$). For each pulse duration, the number of pulses required for complete cutting exhibited a strong positive correlation with the chordae diameter. In conclusion, *in vitro* feasibility of mitral chordal cutting by HIFU depended on the diameter of chordae but was controllable by HIFU settings. (E-mail: abeyukio@aol.com) **© 2008 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **High intensity focused ultrasound, Tendineae chordae, Therapeutic ultrasound, Ultrasound surgery, Mitral regurgitation.**

INTRODUCTION

High intensity focused ultrasound (HIFU) is a noninvasive extracorporeal technique capable of thermally ablating subsurface structures without injuring interventing tissues (Fry et al. 1955). Ultrasonic energy can be applied in a target volume to induce molecular agitation, absorptive heating and ultimately thermal coagulative tissue necrosis. Several studies have confirmed the histologic changes related to HIFU ablations in the liver, kidney, prostate, breast, brain and cardiac tissues (Chen et al. 1999; Damianou 2003; Engel et al. 2006; Fujikura et al. 2006; Lee et al. 2000; Otsuka et al. 2007; Strickberger et al. 1999; Susani et al. 1993; Vykhodtseva et al. 1994; Wu et al. 2003). We previously reported that HIFU can perforate cardiac valves by its thermal ablative effect and might prove useful for an ultrasound-based valvuloplasty (Otsuka et al. 2005). However, the effect of HIFU on the subvalvular structure has never been studied.

Mitral tendineae chordae are string-like subvalvular structures that attach the ventricular surface or the free edge of the mitral leaflets to the papillary muscles. Since mitral chordae support mitral leaflets, chordal rupture leads to loss of support and accompanying geometric changes in the mitral apparatus. When chordal rupture occurs in subjects with normal mitral geometry, mitral valve prolapse and the resultant mitral regurgitation (MR) can be frequently generated. In contrast, artificial cutting of mitral chordae is feasible as a therapeutic technique for functional MR with incomplete mitral leaflet closure, when the incomplete closure is due to restriction by the chordal attachments (Messas et al. 2001, 2003).

We hypothesized that HIFU might be useful as a noninvasive extracorporeal technique for cutting mitral chordae. To test this hypothesis, we conducted a pilot

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study in which we examined the feasibility of using HIFU to cut calf mitral chordae *in vitro*.

MATERIALS AND METHODS

Materials

A previous study showed that the range of diameters of mitral chordae in 50 normal human hearts was 0.2 to 1.6 mm (Rusted et al. 1952). We accordingly used 84 mitral leaflet chordae with diameters ranging from 0.2 to 1.6 mm. The chordae were dissected from six calf hearts, vacuum-packed soon after slaughter and obtained from a butcher. The diameters of the chordae tendineae were measured at the chordae midpoints with a digital caliper graduated to 0.01 mm. Three chordae in each 0.1 mm diameter range were selected for one of two HIFU pulse durations. Each chorda was manually stretched by the least force required to keep the chorda straight and the ends of the chorda were affixed to a rubber pad with steel pins. The rubber pad with chordae was placed in a polyethylene container filled with normal phosphate buffered saline solution, which was degassed by a deaerator and a dry vacuum pump. The container was immersed in a 37°C water bath; the temperature was maintained with an electric heater (Fig. 1).

High intensity focused ultrasound

The HIFU energy was supplied with a modified Sonocare model CST-100 therapeutic ultrasound system (Sonocare Inc., Upper Saddle River, NJ, USA), originally designed for clinical glaucoma treatment (Muratore 2006). This system consisted of a signal generator, a power amplifier and a spherical cap HIFU transducer made from piezoelectric ceramic (PZT-4). The transducer had a diameter of 80 mm, a focal length of 90 mm and a central 23-mm hole that housed a 7.5-MHz diagnostic A-mode transducer (model MD 3657; Panametrics; Waltham, MA, USA). The diagnostic transducer

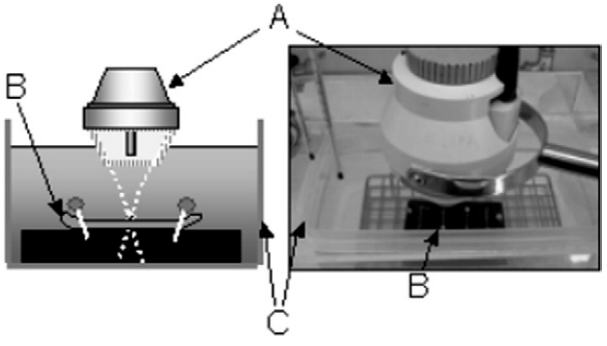

Fig. 1. Experimental set-up with HIFU transducer (A). Each chorda (B) was mounted on a rubber pad and placed in a plastic container (C) filled with degassed phosphate buffered saline at 37°C.

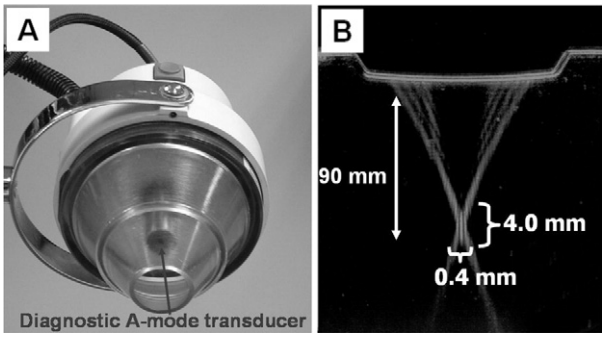

Fig. 2. (A) HIFU transducer with a centrally attached diagnostic A-mode transducer. (B) Schlieren image of the HIFU beam; the ellipsoidal focal zone was approximately 4 mm axially and 0.4 mm transversely at the half-power points.

was aligned to be coaxial and confocal with the HIFU transducer. The operating frequency of the HIFU transducer was 4.67 MHz. The acoustic output power of HIFU was 45 W as determined by the radiation force on an absorber. This corresponds to a nominal spatial-peak temporal-average intensity of 26 kW/cm^2. The focal zone beam shape was measured using a pulse-echo reciprocity technique with a point target; at the half-power points, the focal zone was approximately 4 mm axially and 0.4 mm transversely (Fig. 2). The transducer assembly was attached to an acrylic resin coupling cone with a 25-mm diameter exit hole. The cone was filled with degassed water and the exit hole was covered with a polyvinylchloride membrane. The ultrasound beam focus was positioned at the midpoint of each chorda with guidance from the diagnostic A-mode transducer.

We planned the timing of exposures based on the consideration that this method would eventually be used for a beating heart. For *in vivo* studies, the exposure timing should be synchronized with the cardiac cycle and HIFU exposures should be made during systole when the mitral valve is closed and the motion of mitral chordae is minimal. When the heart rate ranges from 60 to 100 beats per min, the duration of systole is approximately 0.2 to 0.3 s. Thus, the HIFU transducer was operated at a pulse duration of 0.2 s (0.2s-PD) for 42 chordae and 0.3 s (0.3s-PD) for an additional 42 chordae. The pulse repetition frequency was 0.5 Hz.

As soon as complete cutting of a chorda was visually affirmed on the target site, HIFU exposures were terminated and the number of HIFU pulses required for complete cutting was recorded. We considered incomplete cutting to occur when a chorda was not fully severed within 120 pulses. After HIFU exposures, each chorda was fixed in 10% formalin, embedded in paraffin and sectioned at 5 μm. Slide preparations were stained with Masson's trichrome and reviewed to assess degeneration and injury of the chordae's fibrous tissue.

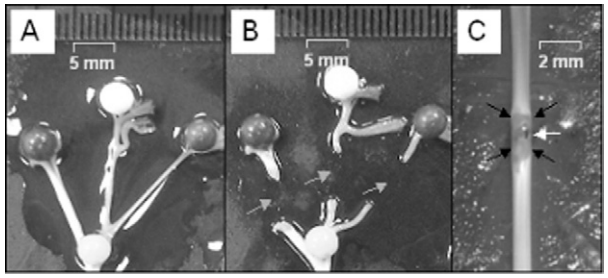

Fig. 3. (A) Before and (B) after complete cutting of mitral chordae by HIFU (gray arrows). (C) Thinning (black arrows) and partial perforation (white arrow) of a chorda with incomplete cutting.

Statistical analysis

Data are presented as absolute values (percentages), or mean values ± one SD. Differences in the proportion of chordae with complete cutting between two HIFU settings were assessed by the χ^2 test. Differences in diameters between chordae with complete cutting and those with incomplete cutting were assessed by Mann Whitney's *U* test. Linear regression analysis was applied to study the correlation between the diameter of a chorda and the number of pulses required for complete cutting for each pulse duration. A value of $p < 0.05$ was considered significant.

RESULTS

Our HIFU system completely cut 19 (45%) and 28 (67%) chordae with 0.2s-PD and 0.3s-PD, respectively (0.2s-PD versus 0.3s-PD, $p = 0.048$). All chordae with incomplete cutting also showed some visual changes, such as thinning or partial perforation of chordae after HIFU exposures (Fig. 3). For each pulse duration setting, the diameter of chordae with complete cutting was smaller than the diameter of chordae with incomplete cutting (0.2s-PD: 0.59 ± 0.30 versus 1.14 ± 0.30 mm, $p < 0.0001$; 0.3s-PD: 0.68 ± 0.29 versus 1.32 ± 0.20 mm, $p < 0.0001$) (Fig. 4). The number of pulses required for complete cutting (y) strongly correlated with the diameter of a chorda (x, mm) for both pulse durations used (0.2s-PD: $y = 7.0 + 90x$, $r = 0.88$, $p < 0.0001$; 0.3s-PD: $y = -21 + 100x$, $r = 0.91$, $p < 0.0001$) (Fig. 5).

The degeneration of fibrous tissue adjacent to the chordal edge cut by HIFU was distinct in histologic slides (Fig. 6). The

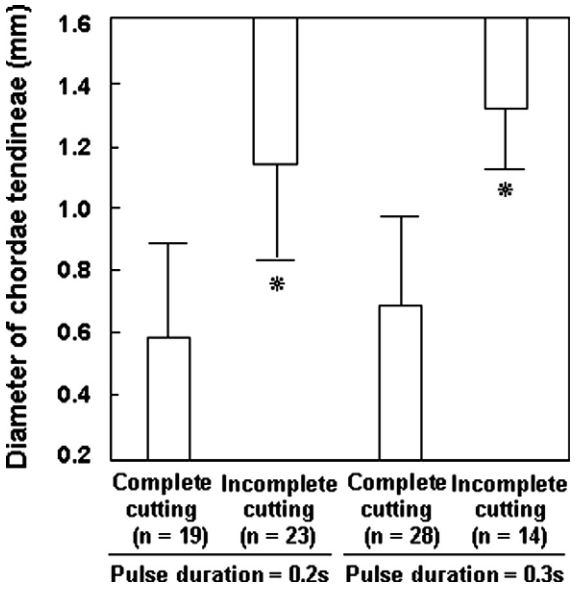

Fig. 4. Differences in diameters between chordae with complete cutting and those with incomplete cutting. *$p < 0.0001$ vs. complete cutting.

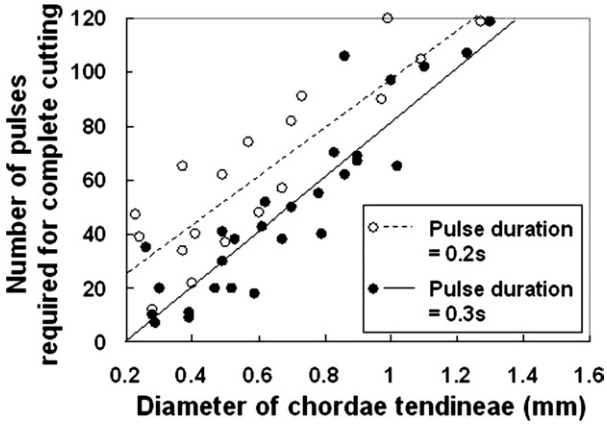

Fig. 5. Relationship between the diameter of a chorda and the number of pulses required for complete cutting.

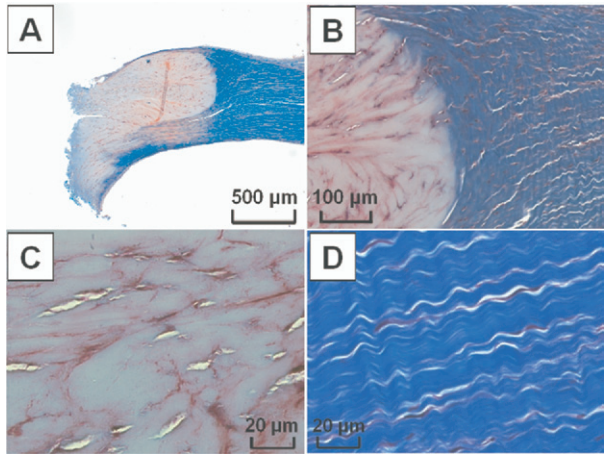

Fig. 6. All, Masson's trichrome stain. Original magnification was × 4 in (A), × 10 in (B) and × 40 in (C) and (D). (A) The degeneraton of fibrous tissue around the chordal edge cut by HIFU. (B) Borderline between degenerate fibrous tissue (left side) and normal fibrous tissue (right side). (C) Degenerate fibrous tissue. (D) Normal fibrous tissue.

regular parallel alignment of fibrous tissue and fibroblasts seen in normal fibrous tissue was destroyed around the chordal edge cut by HIFU. The affected connective tissue was pale and disorganized.

DISCUSSION AND SUMMARY

In the present study, we found that HIFU could cut mitral chordae *in vitro*. The degeneration of fibrous tissue was the prominent histologic finding around the chordal edge cut by HIFU. This result suggests that the primary factor that allows HIFU to cut chordae is its heating effect. We previously reported that the thermal ablative effect of HIFU on cardiac valve tissue is affected by the acoustic power, the pulse duration and the total exposure time (product of number of pulses and pulse duration) (Otsuka et al. 2005). This is consistent with the findings presented here, that chordal cutting by HIFU depended on the pulse duration and the number of pulses. In addition, the present study demonstrated that the feasibility also depended on the diameter of chordae. We anticipate that thicker chordae would be severable with higher acoustic power, longer pulse duration or more pulses.

Comparison with other thermal ablative techniques

There are several other thermal ablative techniques, such as carbon dioxide laser, Er:YSGG laser, Ho:YAG laser and radio-frequency (RF) ablation (Huang et al. 1987; Isner et al. 1985a, 1985b; Lindsay et al. 1993; Williamson et al. 1993). However, laser techniques can only be performed when the target is directly visible. RF is not focused and the generated energy is absorbed in proportion to the distance between the tissue and the RF catheter. In contrast, HIFU beam can be focused and transmitted through solid tissues to create small thermal lesions in subsurface structures, without surgical exposure or insertion of catheters or other instruments. Thus, HIFU ablation can be a less invasive alternative ablation technique.

Experimental and clinical implications

MR seen in patients with left ventricular dysfunction but without organic abnormalities in the mitral valvular complex is defined as functional MR. The presence of functional MR almost doubles mortality and is an independent predictor for a worsened prognosis (Blondheim et al. 1991; Grigioni et al. 2001; Lamas et al. 1997). Thus, the management of functional MR is an important responsibility for physicians and surgeons. Numerous clinical and experimental studies have demonstrated the role of papillary muscle displacement, secondary to remodeling and distortion of the impaired left ventricle, in the development of functional MR (Otsuji et al. 1997; Tibayan et al. 2003; Yiu et al. 2000). Such papillary muscle displacement results in leaflet tethering, apical displacement of leaflets' coaptation (*i.e.*, mitral tenting) and incomplete mitral leaflet closure *via* the chordal attachments. Messas et al. (2001, 2003) proposed cutting second-order chordae tendineae as a therapeutic option for patients with functional MR to improve leaflet coaptation and decrease MR by reducing leaflet tethering at the insertion sites of the second-order chordae on the leaflet's belly. This emerged technique of chordal cutting is performed only surgically in the current clinical setting (Fayad et al. 2005; Yamamoto et al. 2005). Our results suggest that HIFU could be applied to ultrasound-based chordal cutting, using either a transesophageal or a thoracoscopic probe. Furthermore, it might be possible to use HIFU as a noninvasive extracorporeal technique for chordal cutting.

Chordal separation (also known as chordal rupture) can also occur spontaneously or as a result of infectious or degenerative disease (Hickey et al. 1985; Oliveira et al. 1983; Scott-Jupp et al. 1981). When chordal rupture occurs in mitral valves without geometric abnormalities such as mitral tenting, it has no therapeutic significance but is more likely to generate MR with an etiology different from that of functional MR. Secondary to chordal rupture, mitral valve prolapse and the resultant MR can be frequently generated by the loss of chordal support to mitral leaflets. The degree of MR depends on the number of chordae involved in rupture and the rate at which rupture occurs. A previous study demonstrated that an animal model with controlled degrees of MR due to chordal rupture can be created using transvalvular needle or scissors under echocardiographic guidance (Kunzelman et al. 1999). This previous study suggests that catheter chordal cutting in an animal could improve the understanding of MR due to chordal rupture, the effects of such MR on hemodynamics and the time course of such MR. Chordal cutting by HIFU also might be useful as a less invasive technique to create an animal model with MR due to chordal rupture.

Limitations and further investigations

In future studies, we plan to examine the feasibility of *in vivo* mitral chordal cutting by HIFU. Here we discuss limitations of the current technique and further investigations that will be required to apply the HIFU to *in vivo* mitral chordal cutting.

The present study did not account for the effects of mechanical strain, generated by left ventricular contraction, on chordal cutting. The tension of the individual mitral chorda during the cardiac cycle differs by location and chordal type (Lomholt et al. 2002; Nielsen et al. 2004). The elastic properties of the individual mitral chorda differ by location and chordal type and also by

chordal diameter (Barber et al. 2001; Sedransk et al. 2002). Therefore, the association between the feasibility of chordal cutting by HIFU and the diameter of chordae presented in our *in vitro* study may be altered by these factors in the actual *in vivo* setting.

For the application of this technique to *in vivo* beating hearts, it will be necessary to precisely target a chorda and to pulse the HIFU during the desired phase of the cardiac cycle. The current system software already incorporates EKG gating to synchronize exposures with the cardiac cycle. We also have developed a HIFU transducer capable of attaching a confocal B-mode ultrasound imaging transducer, which should allow us to more precisely aim and to monitor the process of cutting. (For our present *in vitro* study, the available integrated A-mode transducer provided the necessary positioning information, *viz.*, the distance from the therapeutic transducer to the tissue surface.) The targets of chordal cutting as the treatment for functional MR are centrally attached, thick, second-order chordae (strut chordae). In human beings, strut chordae range in diameter from 0.6 to 1.6 mm, with an average diameter of 0.9 mm (Messas et al. 2001; Rusted et al. 1952). These chordae are easily visualized with transthoracic B-mode ultrasonography (Kornbluth et al. 1998). Therefore, transthoracic HIFU chordal cutting is feasible with B-mode aiming and EKG gating, despite cardiac and respiratory motions.

In vivo, blood cooling (Kolios et al. 1995, 1996, Weinbaum and Jiji 1985) and cardiac motion will reduce the thermal effect of HIFU. Ultrasonic motion-tracking (Lizzi et al. 2005) of the strut chordae can potentially reduce the periodic cooling that accompanies EKG gating of HIFU, thus, partially compensating for the cooling that accompanies blood flow.

In an extracorporeal noninvasive *in vivo* application of HIFU, acoustic power will be attenuated by the chest wall and the left ventricular wall. In addition, uneven attenuation of the therapeutic ultrasound, produced by different transmission characteristics through costae and through the intercostal space, could influence the ultrasound shape and the ability to focus on thin structures might be disrupted. Thus, there is a need to develop a HIFU system capable of producing higher focal region intensity following transmission through the intercostal spaces without injury to intervening tissues.

CONCLUSIONS

Our results demonstrate that HIFU can cut mitral chordae *in vitro*. This ability depends on the chordal diameter but is controllable by the HIFU settings, *e.g.*, the pulse duration and the number of pulses. This technique may lead to ultrasound-based chordal cutting as a less invasive treatment for functional MR, which is surgically performed at present and as a less invasive method to create animal models with MR *via* chordal rupture.

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