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ENDOSCOPIC HIGH-INTENSITY FOCUSED US: TECHNICAL ASPECTS AND STUDIES IN AN IN VIVO PORCINE MODEL

Tong Li,

Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington, Seattle WA 98105

Tatiana Khokhlova, Ph.D.,

Division of Gastroenterology, Department of Medicine, Applied Physics Laboratory, University of Washington, Seattle WA

Yak-Nam Wang, Ph.D.,

Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington, Seattle WA

Ezekiel Maloney, M.D.,

Department of Radiology, University of Washington, Seattle WA

Samantha D'Andrea,

Division of Gastroenterology, Department of Medicine, University of Washington, Seattle WA

Frank Starr,

Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington, Seattle WA

Navid Farr,

Department of Bioengineering, University of Washington, Seattle WA

Kyle Morrison,

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Joo Ha Hwang, M.D., Ph.D., jooha@u.washington.edu, Tel.: 206-744-7050, Address: Gastroenterology Section, Harborview Medical Center, 325 Ninth Ave, Seattle WA 98104, Box 359825.

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Tong Li: Contributed to the design of animal experiment, research and characterization of the endoscopic device, drafting and revising the article.

Tatiana Khokhlova: Contributed to the design of animal experiment, research and characterization of the device, drafting and revising the article.

Yak-Nam Wang: Contributed to operation of animal experiment, research and experiment of the device

Ezekiel Maloney: Contributed to operation of animal experiment, drafting and revising the article.

Samantha D'Andrea: Contributed to design and preparation of animal experiment

Frank Starr: Contributed to design and preparation of animal experiment

Navid Farr: Contributed to research and development of the device

Kyle Morrison: Contributed to design and development of the endoscopic device

George Keilman: Contributed to design and development of the endoscopic device

Joo Ha Hwang: Contributed to operation of animal experiment, research and experiment of the device, drafting and revising the article.

Sonic Concepts, Bothell WA

George Keilman, and
Sonic Concepts, Bothell WA

Joo Ha Hwang, M.D., Ph.D.

Division of Gastroenterology, Department of Medicine, University of Washington, Seattle WA

Abstract

Background—High-intensity focused ultrasound (HIFU) is becoming more widely used for non-invasive and minimally invasive ablation of benign and malignant tumors. Recent studies suggest that HIFU can also enhance targeted drug delivery and stimulate an anti-tumor immune response in many tumors. However, targeting of pancreatic and liver tumors using an extracorporeal source is challenging due to the lack of an adequate acoustic window. The development of an endoscopic ultrasound (EUS)-guided HIFU transducer has many potential benefits including improved targeting, decreased energy requirements and decreased potential for injury to intervening structures.

Objective—To design, develop and test an EUS-guided HIFU transducer for endoscopic applications.

Design—A pre-clinical, pilot characterization and feasibility study.

Setting—Academic research center.

Patients—Studies were performed in an *in vivo* porcine model.

Intervention—Thermal ablation of *in vivo* porcine pancreas and liver was performed with EUS guided focused ultrasound through the gastric tract.

Results—The transducer successfully created lesions in gel phantoms and *ex vivo* bovine livers. *In vivo* studies demonstrated that targeting and creating lesions in the porcine pancreas and liver are feasible.

Limitations—This is a pre-clinical, single-center feasibility study with a limited number of subjects.

Conclusion—An EUS-guided HIFU transducer was successfully designed and developed with dimensions that are appropriate for endoscopic use. Feasibility of performing EUS-guided HIFU ablation *in vivo* has been demonstrated in an *in vivo* porcine model. Further development of this technology will allow endoscopists to perform precise therapeutic ablation of peri-luminal lesions without breaching the wall of the gastric tract.

INTRODUCTION

High-intensity focused ultrasound (HIFU) is a promising non-invasive technology, in which high amplitude US waves are focused inside the human body to thermally ablate or mechanically disrupt the tissue at the focus without affecting surrounding tissues. The thermal ablation effect of HIFU has been used to coagulatively necrose or cauterize tissue¹ and applied to treatment of benign and malignant tumors including pancreatic cancer²⁻⁴ and

liver cancer^{5,6}. Recent studies also found that mechanical effects of HIFU, e.g. acoustic cavitation, can enhance targeted drug delivery⁷⁻⁹ and stimulate systemic anti-tumor immune response^{10,11} in cancer patients with liver¹² and pancreatic tumors¹³, among others. However, targeting of these tumors using an extracorporeal source can be challenging due to the lack of an acoustic window. Bowel gas, intestinal loops and ribs are major obstacles that reflect and/or absorb HIFU energy. As a result, the ultrasound waves are redistributed or absorbed before they reach the target, and may not cause the desired bioeffects, and may result in adverse effects in the intervening tissues. Burning of the abdominal wall and necrosis of the ribs are also common adverse effects of extracorporeal HIFU ablation¹⁴.

It has been long acknowledged that an endoscopic device that would integrate a HIFU transducer and an endoscopic ultrasound (EUS) imaging probe would be a safer and more efficient alternative to extracorporeal HIFU in the ablation of peri-luminal lesions^{1,15}. Such an EUS-guided HIFU device would bring many potential benefits including improved targeting, decreased energy requirements, and decreased potential for injury to intervening structures. The aim of this study was to demonstrate the feasibility of precise ablation of the pancreas and liver by a prototype EUS guided HIFU device in tissue-mimicking gel phantoms, *ex vivo* tissues and *in vivo* porcine model.

METHODS

EUS-HIFU setup

The EUS guided HIFU apparatus consisted of a custom-built spherically curved HIFU transducer (12 mm diameter with a 35 mm radius of curvature) integrated with a 10 MHz EUS imaging probe (BF-Y0044; Olympus Medical System Corp, Tokyo, Japan). The HIFU transducer (SU121; Sonic Concepts, Bothell, WA) operated at 3.73 MHz. (Fig. 1A). The dimensions of the focal area at the -6 dB level were 28 mm X 1.6 mm. The HIFU transducer and the imaging probe were tightly fitted into a metal housing and were aligned so that the focus of the HIFU transducer appeared at the depth of 2.4 cm from the EUS probe and was centered laterally. The metal housing was covered by a thin latex sheath that was sealed to be filled with degassed water for US coupling (Fig. 1B). The water balloon was contained inside a specially designed plastic cover and could be inflated and deflated using 2 water channels on the side of the metal housing. The plastic cover ensured the frontal distension of the water balloon to control the depth of the HIFU focus in tissue. The full bending capability of the distal end of the endoscope was maintained (Fig. 1C).

The HIFU transducer was powered by a function generator (AFG 3022B; Tektronix, Beaverton, Oregon) and a 400W power amplifier (ENI 400B; ENI, Rochester, NY), and the peak electric power used in all experiments was 150 Watts. This power level resulted in the focal acoustic waveform shown in Fig. 2, as measured by a fiber optic probe hydrophone (FOPH 2000; RP Acoustics, Leutenbach, Germany) in water. The peak-compressional and peak-rarefactional pressures were 17 MPa and 9 MPa, respectively. Most importantly, the focal waveform contained a shock front – a sharp rise in the pressure resulting from nonlinear propagation effects of the acoustic waves. The shock fronts are known to result in much more rapid and localized heat deposition as compared to linear ultrasound waves, so that the boiling temperature of water (100°C) can be reached in a matter of milliseconds¹⁶.

As demonstrated in both *ex vivo* and *in vivo* tissues, the resulting vapor bubble is clearly visible as a hyperechoic region on B-mode ultrasound, and can be efficiently used for therapy monitoring. The waveform shown in Fig. 2, would allow reaching the boiling temperature at the focus within 134 milliseconds, according to theoretical estimations¹⁷. Therefore, the following HIFU pulsing protocol was chosen: 0.1 s pulse duration, 0.2 s pulse repetition period, and total exposure duration of 30 s.

Gel phantoms and *ex vivo* tissues

Visual observation of thermal lesion formation was first performed using tissue-mimicking transparent gel phantoms. The gel phantoms were composed of 5 % weight/volume (w/v) polyacrylamide (PA) hydrogels and 7 % w/v bovine serum albumin (BSA) that becomes optically opaque when thermally denatured¹⁸. To characterize the lesion formation in *ex vivo* tissue, *ex vivo* bovine liver tissue was obtained from an abattoir on the same day as experiments. The tissue was cut into samples to fit in a custom-designed tissue holder and degassed in a desiccant chamber before experiments. During the experiment, the endoscopic device was placed so that the HIFU focus was 15 mm deep below the surface for both gel phantoms and *ex vivo* tissue. The tissue samples were then bisected and photographed to observe gross damage to the tissue.

In vivo porcine model

To test the device in an *in vivo* setting, a pig model was chosen because of its similarity to the human digestive anatomy. The *in vivo* studies were performed in 5 pigs to demonstrate the feasibility of targeting and creating lesions. The animal protocol was approved by the University of Washington Institutional Animal Care and Use Committee. Before each experiment, the animal was anesthetised with Telazol premedication, intubated and maintained in a surgical plane of anesthesia with isoflurane. The gastrointestinal tract was imaged, thoroughly flushed and cleaned with degassed saline solution using an upper gastroscope (GIF-1T140, Olympus Medical System Corp, Tokyo, Japan). A solution of acetylcysteine (Mucomyst; Bristol-Myers Squibb Company, New York, NY) was then sprayed onto the mucosa in the stomach and the esophagus to remove the excess mucous which is known to harbor air bubbles that can reflect and absorb the ultrasound waves¹⁹. The EUS guided HIFU device was then inserted through the esophagus and pressed against the stomach wall as shown in Fig. 3. The US image was used to identify the treatment locations in the pancreas and liver. After a treatment location was chosen, a short-term apnea was induced by a mechanical ventilator, and HIFU treatment was delivered. In each pig, HIFU exposure was applied to 4 to 5 locations in liver and pancreas by moving the endoscopic device along the stomach and esophageal wall. After the treatment, the gastrointestinal tract was imaged again by videoscope to identify any surface lesions, and the animals were sacrificed immediately afterwards. The liver and pancreas tissues containing lesions were collected for histological analysis to evaluate the damage. The tissue samples were snap-frozen, sectioned longitudinally to the lesion and stained with hematoxylin and eosin (H&E) to visualize tissue structure and mechanical disruption, as well as nicotinamide adenine dinucleotide diaphorase (NADH-d) to evaluate the extent of thermal damage.

RESULTS

Lesion formation in the gel phantoms and *ex vivo* liver tissue

Pulsed HIFU exposure of gel phantoms and *ex vivo* bovine liver samples resulted in well-defined tadpole-shaped thermal lesions with small vacuoles in the middle of the prefocal region (Fig. 4A). The vacuoles corresponded to the location of the boiling bubbles, as indicated by the hyperechoic region that appeared in the B-mode image 2 seconds after the start of HIFU exposure (Fig. 4B). The hyperechoic region expanded in direction towards the transducer throughout the exposure, demonstrating preferential energy deposition in the prefocal region due to the ultrasound scattering by the boiling bubbles (Video 1). The size and shape of the hyperechoic region corresponded well to these of the resulting lesion, thus demonstrating the capability of B-mode ultrasound to monitor the treatment.

Lesion formation in the *in vivo* porcine liver and pancreas

HIFU ablation of the porcine liver and pancreas *in vivo* caused the appearance of the area of slightly enhanced tissue echogenicity as observed on the B-mode images (Figs. 5A,B,D,E). However, this change in echogenicity was far less pronounced and localized than that observed *ex vivo*, and it formed closer to the HIFU transducer surface (Video 1). Immediately after the ablation, the endoscopic observations showed thermal injury to the gastric mucosa corresponding to the treatment locations. The solid thermal lesions found in the excised liver and pancreas (Figs. 5C,F) were larger in diameter than in the *ex vivo* case and involved a large area of the parenchymal surface, which corresponded to the prefocal area of the HIFU transducer. This preferential heating of the pre-focal area was likely due to the increased attenuation of the intervening tissues - gastric wall and mucus - that could still harbor residual bubbles. The presence of the bubbles is also suggested by the increased brightness of the stomach wall surface on the B-mode image after the HIFU exposure. The partial blocking of the HIFU beam by the bubbles prevented the tissue at the focus from reaching the boiling temperature.

Histological analysis

H&E stained sections showed the evidence of coagulative necrosis, but the cellular structure in the treated area appeared intact in both pancreas and liver (Figs. 6A and 6C). The corresponding sequential NADH-d stained sections confirmed a shallow subsurface area of thermal damage in porcine pancreatic tissue (Fig. 6B), in which the thermally denatured tissue appeared more white than intact tissue, and a larger area of thermal damage in the porcine liver tissue (Fig. 6D), where the lesion boundaries were clearly demarcated.

Lesion formation and histological analysis in an open abdomen porcine model

To evaluate the influence of the mucous deposits in the pig's stomach on the outcome of HIFU treatment, thermal lesions were induced in porcine liver through the gastric wall immediately post euthanasia, in an open abdomen. The stomach was opened, and the stomach wall was thoroughly rinsed with degassed saline solution before HIFU exposures. B-mode images (Fig. 7A) acquired during HIFU exposures and the subsequent gross observations (Fig. 7B) showed that the lesions were successfully induced in depth of the

liver, without damage to the liver surface or gastric wall. The histological analysis of the lesion confirmed that both structural damage and thermal effect were contained in the liver tissue (Figs. 7C,D), which was consistent with the previous findings from our group²⁰.

In an additional set of experiments, the attenuation of the stomach wall *ex vivo* was measured directly by placing it in between the hydrophone and the HIFU transducer, and comparing the measurements to these in water. The measurements showed that the attenuation is largely due to the mucous deposits on the stomach wall: the US pressure is attenuated 1.64-fold by freshly excised stomach wall, and only 1.15-fold by the stomach wall thoroughly rinsed with saline solution, to remove the mucous deposits.

DISCUSSION AND CONCLUSIONS

In this study, we have demonstrated the feasibility of using an endoscopic HIFU device for transgastric thermal ablation under EUS image guidance. Thirty-second pulsed HIFU exposures of the transparent tissue-mimicking gel phantoms and *ex vivo* bovine liver have resulted in repeatable tadpole-shaped thermal lesions, at depths of 2-3 centimeters in tissue. Boiling bubbles, which are essential for treatment monitoring and guidance, were consistently formed in 0.1-2 seconds at the HIFU focus, and corresponded to the bright hyperechoic region on the B-mode image. Due to the high reflectivity of the boiling bubbles, the thermal lesion formed prefocally in all cases, and its size and location corresponded well to the size of the hyperechoic region.

In the *in vivo* studies, the device successfully induced thermal lesions in pancreas and liver through gastric wall with no immediate adverse effects to surrounding organs. Histological analysis using H&E and NADH-d staining confirmed gross observations of the HIFU lesions as the areas of thermally fixed tissue. In the current study, the observations were limited to the acute effects only; the delayed damage to liver or pancreatic tissue surrounding the HIFU lesion was studied previously by our group and others^{1,21-23}. Within 5 to 7 days post treatment, a border area of granulation tissue forms around the ablated region, and increased infiltration of inflammatory cells in that area is observed. Within 10-14 days after the treatment the boundary area is replaced by fibrous tissue.

The lesions from the *in vivo* studies appeared to be larger in the lateral dimension and formed shallower in tissue than in the *ex vivo* case. In addition, associated thermal damage was induced in the gastric wall. This effect is likely due to the high attenuation and reflectivity of the stomach wall, which is covered by mucosa, harboring a lot of air bubbles and food debris. These properties lead to the broadening of the HIFU beam, hence the broadening of the lesions, and to additional heat deposition at the stomach wall and liver or pancreas surface.

Thus, to reduce the chance of surface damage, special attention should be paid to cleaning of the stomach before HIFU treatment, and removing mucous deposits. A HIFU transducer with higher focusing gain would allow further eliminating the attenuation and reflection effects in the acoustic pathway and make the ablation safer, more predictable and more rapid. Given the required depth of ablation, this would imply using a transducer with a larger

aperture, which may not be acceptable for endoscopic use. Foldable or expandable HIFU transducers may represent a viable solution, and are currently under development. Potential applications of this technology include ablation of peritumoral tumors in the liver (hepatocellular carcinoma, metastatic liver lesions), pancreas (adenocarcinoma, neuroendocrine) and bile ducts. In addition, EUS-guided HIFU could be used to perform ablation of the celiac plexus for palliation of pain associated with pancreatic cancer. Furthermore, it could be used for enhancing targeted drug delivery of chemotherapy by using mechanical effect of HIFU to enhance tissue permeability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Acronym

HIFU	high intensity focused ultrasound
US	ultrasound
EUS	endoscopic ultrasound
PA	polyacrylamide
BSA	bovine serum albumin
H&E	hematoxylin and eosin
NADH-d	nicotinamide adenine dinucleotide diaphorase

Figure 1.

(A) Photograph of the prototype of the EUS guided HIFU apparatus. The additional lines attached to the endoscope head were a pair of water tubes for inflating and deflating the water balloon (red arrow), a power line for the HIFU transducer (yellow arrow) and a pair of connecting cables for miniature passive cavitation detectors. (B) The front view of the EUS imaging probe (pink) integrated with the HIFU transducer (black) with inflated water balloon for US coupling. The white plastic cover capping the device ensures the frontal inflation of the balloon for the adjustment of the depth of HIFU focus in tissue. (C) The side

view of the distal end of the endoscope demonstrated the bending capability for improved *in vivo* maneuvering.

Figure 2.

Focal pressure waveforms produced by HIFU at 3.73 MHz operating frequency, 144 Watt electric power and measured in water by fiber optic probe hydrophone. The peak-compressional and rarefactional focal pressure levels were 17 MPa and 9 MPa, correspondingly. The waveform contained a sharp rise in pressure – a shock front (red arrows) formed due to nonlinear ultrasound propagation effects. Shock waves allowed depositing ultrasound energy in a very localized fashion and very rapidly, so that the boiling temperature of water was reached in a matter of milliseconds. The resulting boiling bubble activity was readily seen on B-mode ultrasound as bright hyperecho, and was used for therapy monitoring and guidance.

Figure 3.

Illustration of the EUS guided HIFU apparatus placement in the stomach during HIFU ablation of the pancreas. The device was coupled to the stomach wall by the distended water balloon. The HIFU transducer and the EUS imaging probe were aligned so that the HIFU focus was at the center of the B-mode image that allowed monitoring lesion formation in real time.

Figure 4.

(A) Two separate thermal lesions in bovine liver, seen as regions of whitening, were created with the same HIFU exposure protocol as the lesions in the gel phantom. Small cavities in the middle of the thermal lesions (white arrows) resulted from HIFU-induced bubble activity. (B) Formation of the hyperechoic region during the HIFU exposure was observed by B-mode ultrasound. The size and location of the hyperechoic region corresponded well with the thermal lesion. The red arrow shows the direction of incidence of the HIFU wave, and the red cross marks the HIFU focus location.

Figure 5.

The B-mode EUS images were acquired before and after HIFU ablation of liver (A), (B) and pancreas (D), (E), correspondingly, in an *in vivo* porcine model. The red arrow shows the direction of incidence of the HIFU wave, and the red cross marks the HIFU focus location. The increase in brightness corresponding to the region of ablation (red dashed lines in A, E), was considerably lower than that observed in the experiments in gel phantoms and *ex vivo* tissue, suggesting that the boiling temperature was not reached at the focus. This is likely due to enhanced attenuation of the intervening tissues – gastric wall and mucosa. The corresponding area of the interior surface of the gastric wall (yellow arrows) has an

increased brightness after HIFU exposure, suggesting the presence of bubbles in the mucosa, that are reflective to ultrasound and partially block the HIFU penetration to the focal area. After the animal was euthanized, the liver (C) and pancreas (F) were excised to photograph the thermal lesions.

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Figure 6.

H&E stained histological sections of HIFU lesions in both the pancreas (A) and liver (C) revealed no structural disruption in either pancreas or liver, but evidence of coagulative necrosis was seen in the treated region (red dashed line). The consecutive sections of the samples, which were stained by NADH-d in the pancreas (B) and liver (D) for thermal damage, showed whitening in the treated area (red dashed line), indicating complete thermal necrosis of tissue. The direction of incidence of the HIFU wave is from the right of the images (red arrows).

Figure 7.

(A) B-mode EUS image acquired during ablation of liver through the gastric wall in an open pig. Hyperechoic region identified the region of ablation, which started around HIFU geometrical focus (red cross) and grew in direction towards HIFU transducer. The red arrow shows the direction of incidence of the HIFU wave, and the red cross marks the HIFU focus location. (B) The thermal lesion was induced in depth of tissue in this case, without damage to the tissue surface. NADH-d (C) stained slices showed white region, which indicated thermal effects were resulted in treated regions (red dashed line). H&E (D) stained slices showed clusters of vacuoles (yellow arrows) in the treated region (red dashed line), which

were evidence of mechanical disruption from boiling. The pulsing scheme and histology results were consistent with previous findings from our group²⁰.

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