Original Contribution

PRECLINICAL IN VIVO EVALUATION OF AN EXTRACORPOREAL HIFU DEVICE FOR ABLATION OF PANCREATIC TUMORS

JOO HA HWANG,*† YAK-NAM WANG,† CINDERELLA WARREN,* MELISSA P. UPTON,† FRANK STARR,† YUFENG ZHOU,* and STUART B. MITCHELL‡

*Department of Medicine, Division of Gastroenterology; †Center for Industrial and Medical Ultrasound, Applied Physics Laboratory; and ‡Department of Pathology, University of Washington, Seattle, WA, USA

(Received 6 August 2008; revised 11 October 2008; in final form 10 December 2008)

Abstract—Extracorporeal high-intensity focused ultrasound (HIFU) can be used to ablate tissue noninvasively by delivering focused ultrasound energy from an external source. HIFU for clinical treatment of pancreatic cancer has been reported; however, systematic evaluation of the safety and efficacy of pancreatic ablation with HIFU has not been performed. The objectives of this in vivo study are as follows: (1) assess the safety and feasibility of targeting and ablating pancreatic tissue using the FEP-BY02 HIFU system (Yuande Bio-Medical Engineering, Beijing, China); (2) evaluate a method for estimating in situ acoustic treatment energy in an in vivo setting; and (3) identify the optimal treatment parameters that result in safe and effective ablation of the pancreas. The pancreata of 12 common swine were treated in vivo. Prior to therapy, blood was drawn for laboratory analysis. Animals were then treated with extracorporeal HIFU at three different acoustic treatment energies (750, 1000, and 1250 J). Endoscopy was performed prior to and immediately following HIFU therapy to assess for gastric injury. Blood was drawn after completion of the treatment and on days 2 and 7 following treatment to assess for biochemical evidence of pancreatitis. Animals were then euthanized 7 d following treatment and a necropsy was performed to assess for unintended injury and to obtain pancreatic tissue for histology to assess efficacy of HIFU ablation. Histologic scoring of pancreatic tissue changes was performed by a pathologist blinded to the treatment energy delivered. The degree of ablation identified on histology correlated with the treatment energy. No collateral tissue damage was seen at treatment energies of 750 and 1000 J. At 1250 J, thermal injury to the abdominal muscles and gastric ulcers were observed. There were no premature deaths, serious illnesses, skin burns or evidence of pancreatitis on biochemical analysis. HIFU treatment of the pancreas is feasible, safe and can be used to ablate tissue noninvasively. A clinical trial in humans examining the use of extracorporeal HIFU for palliation of pain related to pancreatic cancer is planned. (E-mail: jooha@u.washington.edu) © 2009 World Federation for Ultrasound in Medicine & Biology.

Key Words: Ultrasonic therapy, Pancreas, Animal study.

INTRODUCTION

Approximately 200,000 patients are diagnosed with pancreatic cancer annually worldwide with a 5-y survival rate following diagnosis less than 5% (Parkin et al. 1993). At the time of diagnosis, patients with pancreatic cancer often have advanced disease (stage III-IV) and are not considered to be operative candidates. In addition, 60% to 90% of patients will experience cancer associated pain (Reddy et al. 2005). Therefore, palliation of pain is important in the management of these patients. The application of HIFU in palliating patients who have symptoms of pain related to pancreatic cancer has been reported by several investigators from China where patients have been treated clinically since 1999 (He and Wang 2002; Wang et al. 2003; Wang and Sun 2002; Xie et al. 2003; Xiong et al. 2005; Xu et al. 2003; Yuan et al. 2003; Wu et al. 2004, 2005). These studies suggest that HIFU treatment can reduce the size of pancreatic tumors without causing pancreatitis, prolong survival and reduce pain. While high-intensity focused ultrasound (HIFU) is a noninvasive, nonsurgical treatment modality that has potential to eliminate or significantly reduce pain associated with pancreatic cancer, no prospective randomized controlled clinical trials have yet been conducted to
determine whether treatment of pancreatic tumors with HIFU will result in palliation of pain. Furthermore, there is little preclinical data to support the rationale for current treatment regimens.

Preclinical testing of an extracorporeal HIFU device, YDME FEP-BY02 (Yuande Bio-Medical Engineering, Beijing, China), was performed with the objectives of determining the appropriate acoustic treatment energy to damage targeted tissue consistently and to assess the safety and efficacy of HIFU therapy in a large animal model. The animal study presented here is a survival study that evaluates three different HIFU treatment energies for safety and efficacy. Prior to this survival study, several preliminary ex vivo and in vivo studies were performed to examine the effect of treating tissue with the FEP-BY02 using different treatment parameters. Based on these preliminary studies three in situ treatment energies (750, 1000 and 1250 J) were identified as candidate treatment energies for the in vivo survival studies.

The objectives of this in vivo study were as follows: (1) assess the safety and feasibility of targeting and ablating pancreatic tissue using the FEP-BY02 HIFU system; (2) evaluate a method of delivering an in situ acoustic treatment energy in an in vivo setting; and (3) identify the optimal treatment parameters that result in safe and effective ablation of the pancreas. The following hypotheses were tested: (1) ablation of pancreatic tissue with extracorporeal HIFU is feasible and safe; (2) the degree of ablation will be dependent on the energy delivered by the HIFU source; and (3) higher treatment energies will result in greater degrees of ablation but will also cause greater collateral damage.

MATERIALS AND METHODS

Animals

The pancreata of 12 domestic swine were treated in vivo following a protocol approved by the Institutional Animal Care and Use Committee at the University of Washington. Domestic swine was selected as the most appropriate animal model for this study due to their size and abdominal anatomy, which are similar to humans; however, unlike humans, the pig has more small and large intestine anterior to the pancreas that limits ultrasound visualization of the pancreas due to the presence of bowel gas. Animals were fasted for 24 h prior to the procedure with the exception of water. On the day of the procedure animals were sedated with an intramuscular (i.m.) injection of ketamine (22 mg/kg) and acetylpromazine (0.5–1.0 mg/kg) and intubated. Anesthesia was maintained using isoflurane (1.5%–2.5%) via endotracheal tube with controlled mechanical ventilation. The abdominal surface was washed, depilated, washed with degassed distilled water and wiped down with 70% isopropanol. Blood was drawn prior to treatment for biochemical analysis. Animals were administered intravenous (i.v.) pancuronium bromide (initial bolus of 0.04–0.15 mg/kg followed by incremental doses of 0.01–0.02 mg/kg every 30–40 min) as a paralytic to control respiratory movement during the delivery of HIFU therapy. Following HIFU therapy, the abdomen was examined for skin burns or induration. Animals were then administered a dose of atropine (0.04 mg/kg) followed by neostigmine (0.04 mg/kg) and blood was drawn. Animals were ventilated until spontaneous respirations were observed. Animals were then extubated and recovered. Animals were monitored on a daily basis for 7 d following the procedure for signs of decreased activity, lethargy, poor oral intake, vomiting, diarrhea, abdominal abnormalities or combativeness. Animals were sedated for blood draw on postprocedure day 2 with an i.m. injection of ketamine (22 mg/kg) and acetylpromazine (0.5–1.0 mg/kg). Animals were again sedated with i.m. ketamine/acetylpromazine and anesthetized with isofluorane (via endotracheal tube) on day 7 for endoscopy followed by blood draw and laparotomy. Following completion of the laparotomy, the animal was euthanized with i.v. sodium pentobarbital (90 mg/kg).

HIFU treatment

The FEP-BY02 HIFU system has two HIFU transducers; a lower transducer and an upper transducer providing flexibility in positioning of the patient. The two transducers cannot be used simultaneously. For this study, only the upper HIFU transducer was used. The HIFU transducer is made of 251 individual PZT (lead zirconate titanate) crystal elements that are driven in phase at 1 MHz and has an integrated B-mode ultrasound imaging probe (Logiq 5, General Electric Healthcare, Seongnam, Korea). The HIFU transducer has an aperture of 37 cm and a focal distance of 25.5 cm. The focus has a -6 dB beam width of 1.6 mm and axial length of 10 mm. Treatment is delivered using a computer program that allows the operator to identify the target region and specify the electrical power delivered to the HIFU transducer, pulse length, duty factor, interval spacing between treatment sites and number of pulses per treatment site. In addition, there is an electrical power system to drive the HIFU transducer and a water treatment system that vacuum degasses filtered water.

Pretreatment imaging

Animals were placed on the HIFU treatment bed in the supine position. Animals were imaged with B-mode ultrasound prior to each treatment. The purpose of ultrasound imaging was to confirm the presence of an adequate acoustic window for HIFU therapy. The pancreas must be well visualized on B-mode imaging with minimal pressure applied to the abdomen with the imaging transducer.
in order for treatment to proceed. If an adequate acoustic window could not be identified then the HIFU treatment was not administered. Imaging quality was rated as follows: good – the pancreas was well visualized with minimal bowel gas; fair – the pancreas was well visualized with some degradation of the image due to bowel gas; or poor - pancreas was visualized but regions are unable to be imaged due to shadowing from bowel gas.

Treatment planning and treatment energy

For animals that had an adequate acoustic window, the abdomen was coupled to the treatment transducer through a water-filled bladder. The water used for coupling was vacuum degassed prior to treatment. Ultrasound coupling gel was used to couple the water-filled bladder and the abdominal surface. The imaging transducer that is co-axial to the treatment transducer was then used to identify the target tissue for treatment planning. With the imaging transducer positioned just above the abdominal wall the following measurements were made: abdominal wall thickness (including skin, subcutaneous fat and abdominal wall muscles) and skin to target distance. These measurements were recorded and used to estimate total tissue attenuation (as described below).

A treatment plan was then programmed into the computer that controls the output of the HIFU transducer and positioning of the treatment table. A 2–3 cm diameter circular plane within the neck/body region of the pancreas was targeted. The porcine pancreas is only 0.7–1.5 cm in thickness, therefore, only one plane of tissue could be treated in a given animal. Because of the dimensions of the porcine pancreas, a 1 cm margin between the focus of the HIFU beam and surrounding structures, such as the portal vein and bowel wall, could not be maintained. Occasionally, adjacent structures (bowel wall and portal vein) were within the focus of the HIFU beam during treatment.

Treatment acoustic energy was calculated based on measurements made during treatment planning imaging (Fig. 1). The following equation was used to estimate the tissue attenuation:

\[
\alpha (dB) = 1.2 \, dB/cm \cdot Abd + 0.8 \, dB/cm \cdot (DSF - Abd).
\]

(eqns 1)

where \(\alpha\) is the tissue attenuation (in dB), Abd is the abdominal wall thickness (in cm) and DSF is the distance between the skin surface and the HIFU focus (in cm).

The attenuation values for the abdominal wall (1.2 dB/cm) and intra-abdominal tissue (0.8 dB/cm) were obtained experimentally on ex vivo tissue using the pulse-transmission method at 1 MHz. The estimated attenuation based on the above equation is for the operating frequency of 1 MHz. Nonlinear attenuation was not accounted for in this estimation since characterization studies of the prefocal beam demonstrated that the energy contribution from higher harmonics at the exposure intensities used in this study was minimal.

The in situ acoustic energies tested were 750, 1000 and 1250 J per site, which was determined based on preliminary ex vivo and acute in vivo studies. The input electrical power was then calculated based on the desired in situ acoustic energy (\(E_A\)), estimation of tissue attenuation (\(\alpha\)), efficiency of the transducer (\(\varepsilon_T\)) and total time of HIFU exposure per site calculated by multiplying the pulse length (\(\tau_p\)), duty factor (df) and number of pulses per site (n_p). The input electrical power (\(P_E\)) was calculated using the following equation:

\[
P_E = \frac{E_A}{\varepsilon_T \cdot 10^{\alpha/10} \cdot [\tau_p \cdot df \cdot n_p].}
\]

(eqns 2)

The treatment parameters were then input into the treatment planning software. The distance between treatment sites was kept constant at 3 mm for all treatments. The pulse length was 150–250 ms with a 50%–62.5% duty factor. The number of pulses was kept in the range of 60–80 per treatment spot. Treatment was then administered in an automated fashion based on the programmed treatment.

Biochemical analysis

Blood was drawn for analysis of serum amylase and lipase. Blood was drawn from the animal prior to HIFU
Endoscopy

Endoscopy (GIF-1T140 gastroscope, Olympus Corporation, Aizu City, Japan) was performed on all animals prior to HIFU treatment to suction any residual gastric contents and evaluate the gastric and duodenal mucosa prior to HIFU exposure. Simethicone was added to 100–500 cc of degassed water and used to lavage the stomach to remove any residual gas bubbles within the gastric lumen. Endoscopy was also performed immediately after HIFU treatment and on day 7 to assess for gastric or duodenal mucosal injury.

Gross examination

Gross examination of the abdomen was performed prior to HIFU treatment, immediately following HIFU treatment and on days 2 and 7 post-treatment to assess for skin burns and obvious abdominal muscle damage. Animals had endoscopic evaluation and laparotomy 7 d following treatment to perform a gross examination of the abdominal wall and cavity to evaluate treatment effects and unintended injury. Following harvesting of the pancreas the animals were euthanized. Skin burns, abdominal wall injury and bowel injury was assessed and, if present, was graded by severity (mild, moderate, or severe) based on a priori criteria listed in Table 1. These criteria were established based on preliminary in vivo studies.

Pancreatic lesion

The pancreas was inspected at the time of laparotomy, prior to euthanasia in order to assess the vascularity of the organ and to prevent autolysis of the tissue prior to harvesting for histology. The pancreas was visually inspected for evidence of local color changes suggestive of thermal injury (pale appearing parenchyma). The pancreas was then palpated for firm lesions. Pancreatic lesions were assessed as either present or absent. Tissue specimens from the targeted region were then harvested for histologic evaluation. Samples were carefully removed to minimize damage cause by handling. In addition, normal untreated pancreatic tissue was obtained as control samples for histology.

Histology

Treated and control samples were immediately embedded in optimum cutting temperature (O.C.T. Sakura Finetek, Torrance, CA, USA) medium by freezing in isopentane cooled on dry ice. Frozen sections were stained with hematoxylin-eosin (H&E) (Richard-Allan, Kalamazoo, MI). Briefly, 5–6 μm sections were cut from the center of the block and immediately subjected to alcohol fixation using 95% EtOH to preserve tissue structure. Two serial sections were placed on each slide. The alcohol fixed sections were then stained with H&E using standard procedures.

Slides (from treated and control samples) were randomized and reviewed by an independent experienced GI pathologist (M.P.U.), blind to the experimental condition. Both sections on each slide were evaluated before the sample was rated on a 0 (normal) to 4 (complete ablation) scale. The damage scale, established at a previous session with the pathologist, has four levels described in Table 2. To eliminate the influence of handling artifacts or processing artifacts, damage localized to the very edge of the sample was not counted as was other damage that was felt to be more consistent with freezing artifact.

Statistics

Due to the nature of the data, nonparametric statistics were carried out in all instances. Differences between medians of treated and control samples were compared using the Wilcoxon rank sum test for paired data. Differences were considered significant when \( p < 0.05 \). Data

### Table 1. Grading of injury severity

<table>
<thead>
<tr>
<th>Severity</th>
<th>Skin burns</th>
<th>Abdominal wall injury</th>
<th>Bowel injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Local erythema without skin breakdown.</td>
<td>Partial thickness thermal injury of the abdominal muscles.</td>
<td>Shallow gastric ulcers or bowel adhesions without obstruction.</td>
</tr>
<tr>
<td>Moderate</td>
<td>Superficial blistering without breakdown.</td>
<td>Full thickness injury to the abdominal muscle without involvement of the subcutaneous fat.</td>
<td>Large gastric ulcer without hemorrhage or perforation, bowel adhesion with partial obstruction.</td>
</tr>
<tr>
<td>Severe</td>
<td>Ulceration or skin breakdown.</td>
<td>Full thickness injury involving the abdominal muscles and subcutaneous fat.</td>
<td>Small bowel or colonic necrosis, bowel perforation, or bowel adhesion with complete obstruction.</td>
</tr>
</tbody>
</table>

### Table 2. Histology damage score criteria

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Localized or patchy areas with reactive/swollen cells or with subtle structural differences</td>
</tr>
<tr>
<td>2</td>
<td>Large areas with reactive/swollen cells with subtle structural differences and/or Localized or patchy areas of cell damage or major structural changes</td>
</tr>
<tr>
<td>3</td>
<td>Large areas of cell damage or major structural changes. Significant damage to tissue.</td>
</tr>
<tr>
<td>4</td>
<td>Complete ablation</td>
</tr>
</tbody>
</table>
were not further separated by treatment level as the low sample size did not allow useful statistical analysis to be carried out.

RESULTS

All 12 animals tolerated the HIFU treatment without significant clinical sequelae. All 12 animals demonstrated normal activity without any evidence of distress, discomfort, or abnormal oral intake over the 7-d observation period following HIFU treatment.

Gross examination

A summary of the findings on gross examination are given in Table 3. At an in situ treatment energy of 750 J, no gross abnormalities were identified including evidence of injury to the target tissue. At 1000 J, one of four animals had an obvious lesion in the targeted tissue with a palpable firm nodule, decreased local vascularity and adherent small bowel without evidence of obstruction (upstream bowel was not dilated). In one animal, there was a minor skin burn noted immediately following treatment that was felt to be due to debris within the water bladder (size and dimension of the skin lesion was identical to the piece of debris). This skin burn completely healed by day 7 and did not require any specific treatment. No other skin burns were noted in this group. In two animals in this group, mild thermal injury to the abdominal wall was noted. These animals did not exhibit any abnormal behavior during the 7-d observation period. The abdominal image quality was rated as “fair” in both these animals, suggesting that reflection of ultrasound (US) energy due to bowel gas may be a reason for thermal injury to the abdominal wall. Animals treated with 1250 J had several significant findings on gross examination including two of four animals with identifiable lesions in the targeted region of the pancreas. Two of four animals had minor skin burns that did not require any specific treatment. Three of four animals had injury to the abdominal wall (one with minor injury and two with moderate injury). The animal that was noted to have good US image quality at the time of treatment did not sustain injury to the abdominal wall, again suggesting that reflection of US energy from the abdominal wall is a mechanism for this type of injury. Three of four animals had injury to the bowel including a gastric antral ulcer with full thickness ablation of the gastric wall but no evidence of perforation. There were no animals that experienced small bowel or colon necrosis. Two animals had small bowel adhesions to the treated region of pancreas without evidence of obstruction.

Histology

All control samples had a damage score of 0 or 1. Two of the animals (pigs 1 and 2, 750 J) did not show any significant damage in the test compared with the control samples.

Figures 2A shows an example of a control sample with a damage score of 0. The structure is typical of normal pancreatic tissue with pancreatic acini displaying bizonal staining, pancreatic islet cells and stromal cells with small thin nuclei. In comparison, Fig. 2B shows an example of a damage score 1. Subtle structural differences within the pancreas can be observed in areas with the acini appearing to be “swollen”. Some acini and stromal cells have enlarged reactive nuclei. However, no significant damage is obvious. Figure 2C is an example of a sample with a damage score of 2. The damage is localized to distinct regions and undamaged acini can be found in surrounding regions. For larger areas of damage, a score of 3 was given (Fig. 2D). There is a large area of damage (in this case ablation) that spans over more than one lobule. However, an acinar structure that is relatively normal can be observed outside of the damage zone. In comparison, Fig. 2E shows an example of complete ablation. The pancreatic structure is completely disrupted and hardly recognizable except for the suggestion of pancreatic ducts and a faint outline of the lobules. Complete ablation was only observed in two animals, one treated at 1000 J and the other at 1250 J. These animals were two of three that had palpable firm nodules in the pancreas (Table 3).

Table 3. Treatment parameters and results from gross examination

<table>
<thead>
<tr>
<th>Pig</th>
<th>Acoustic Energie in situ (J)</th>
<th>Exposure duration per spot (s)</th>
<th>Acoustic Intensity in situ (I_{RAPA} – W/cm²)</th>
<th>US image quality</th>
<th>Pancreas lesion</th>
<th>Skin burn</th>
<th>Abdominal wall injury</th>
<th>Bowel injury</th>
<th>Bowel adhesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>750</td>
<td>9</td>
<td>2348</td>
<td>good</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>750</td>
<td>9</td>
<td>2348</td>
<td>good</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>750</td>
<td>9</td>
<td>2348</td>
<td>good</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>750</td>
<td>12</td>
<td>1761</td>
<td>good</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>9</td>
<td>3131</td>
<td>fair</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1000</td>
<td>9</td>
<td>3131</td>
<td>good</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>1000</td>
<td>12</td>
<td>2348</td>
<td>fair</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>12</td>
<td>2348</td>
<td>good</td>
<td>yes</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>1250</td>
<td>12</td>
<td>2935</td>
<td>good</td>
<td>yes</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>1250</td>
<td>12</td>
<td>2935</td>
<td>fair</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>1250</td>
<td>20</td>
<td>1761</td>
<td>poor</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>1250</td>
<td>20</td>
<td>1761</td>
<td>fair</td>
<td>yes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Grading scale: - absent; + mild; ++ moderate; +++ severe.
Aside from complete ablation (Fig. 2E) resulting in the complete destruction of the tissue, other methods of cell damage/death were observed. These include liquefactive necrosis, coagulative necrosis and apoptosis. Cell death was generally due to a mixture of the three with liquefactive necrosis being the least common. No correlation between treatment energy and mechanism of damage/death was observed from the H&E stained samples. In the more severely damaged samples, an inflammatory response was often observed surrounding the damaged area.

When comparing the treated to control tissue, the amount of damage, denoted by the damage score, in the treated pancreas (Median = 2.5) was significantly greater than in the nontreated control samples (Median = 1), $T = 57.5$, $p < 0.005$, $r = 0.60$. Figure 3 demonstrates

Fig. 2. Examples of histology damage scores. (A) Damage score of 0. Slide shows the pancreatic acini (black arrowheads), pancreatic islet cells (long white arrow) and stromal cells (white arrowheads). Scale bar represents 50 μm. (B) Damage score of 1. Pancreas showing subtle structural changes (S), swollen stromal cells (black arrows) and cells with large reactive nuclei (white arrows). Scale bar represents 50 μm. (C) Damage score of 2. Section shows localized areas of cell damage (black arrows). Scale bar represents 50 μm. (D) Damage score of 3. There is a large area of cell damage (long black arrow) that spans over a number of lobules. Some areas of tissue still have acini structure present (black arrowhead). Scale bar represents 200 μm. (E) Damage score of 4 with complete ablation. Scale bar represents 200 μm. H&E staining.
that at each acoustic energy, there is an increase in severity of damage in the treated pancreas compared to control tissue. However, due to the small sample size, no statistical analysis could be done to determine if the increase at each treatment energy was significant. In addition, no statistical analysis could be done between test samples exposed to different energies.

**Biochemical analysis**

In one animal (pig 8, 1000 J) there was a rise in the lipase level from a baseline of 28 U/L up to 70 U/L on day 2 post-treatment. However, the amylase was minimally changed from baseline and the animal did not exhibit any abnormal behavior during the 7-d observation period. The serum lipase concentration decreased to 9 U/L by day 7. This animal did have an identifiable lesion in the pancreas on gross examination and histologic evidence of ablation. The serum amylase and lipase levels remained within the range of baseline values at all other time points for all other animals.

**DISCUSSION**

Three hypotheses were tested in this study to examine the feasibility, safety and efficacy of the YDME FEP-BY02 HIFU tumor therapy system in a large animal model for treatment of the pancreas.

**Hypothesis 1: Ablation of pancreatic tissue with extracorporeal HIFU is feasible and safe**

Extracorporeal ablation of HIFU is feasible as demonstrated by both gross and histologic examination following HIFU therapy. The safety of extracorporeal HIFU is dependent on the treatment parameters used for delivering therapy as well as an individual subject’s characteristics such as the amount of bowel gas, size of the target and proximity of the target to other structures such as bowel or vascular structures. For tumor therapy in humans, proximity of other structures will be less of an issue since the target size will be substantially larger. The porcine pancreas is only 0.7–1.5 cm in thickness along the axis of treatment, which is similar to the length of the HIFU focus. In the treatment of the porcine pancreas, targeting of the bowel wall was unavoidable. However, in treatment of advanced pancreatic tumors in humans (typically over 3 cm in diameter), a 1 cm margin between structures such as the bowel wall or large vessels can be obtained. Therefore, the incidence of gastric ulcers and bowel adhesions is likely to be much lower that what was observed in this study. In addition, there were no significant clinical sequelae from the gross findings of bowel injury. The main concern from a gastric ulcer is bleeding, which was not observed in any of the animals that developed a gastric ulcer; however, due to the mechanism by which the gastric ulcer is created (thermal injury), these gastric ulcers are not likely to bleed since the blood vessels in this region have been ablated.

The most important aspect of the treatment appears to be the quality of the ultrasound image at the time of treatment planning. This appears to be the strength of using ultrasound image guidance for HIFU therapy since the form of energy used for imaging is the same as for treatment. Therefore, if the image of the target is highly attenuated or there is shadowing, it is likely that the HIFU energy will also be attenuated or reflected prior to reaching the target leading to increased deposition of energy in pre-focal tissue. This appears to be the mechanism for thermal injury to the abdominal wall. In animals who had sustained thermal injury to the abdominal wall the injury pattern on gross examination appeared as if the source of the energy was coming from within the abdomen and only occurred when the image quality was either “fair” or “poor”. Abdominal wall injury was not seen in treatments where the image quality was rated “good”. In addition, the efficacy of treatment is improved with good image quality since the HIFU energy is able to reach the focus with less attenuation. Therefore, the key to safe and effective therapy is good US image quality during treatment planning and having a sufficient treatment margin (~1 cm) between the HIFU focus and nontumor tissue.

**Hypothesis 2: The degree of ablation will be dependent on the energy delivered by the HIFU source**

The treatment parameter space for this specific device is large. Variables that are adjustable by the operator of the device include input electrical power to the HIFU transducer, pulse length, duty factor, number of pulses per treatment site and distance between treatment...
in situ performed acoustic field mapping. Values for acoustic energy/exposure duration) and the -6 dB beam from the in situ calculated since the power and the higher attenuation values in these animals, be increased to 20 s because of limitations on the electrical animals (pigs 11 and 12) the exposure duration had to the exposure duration between 9–12 s; however, for two the acoustic intensity within a narrow range by keeping an estimated in situ acoustic energy to the target.

The ability to achieve ablation of targeted tissue is dependent on the energy delivered since only microscopic ablation lesions were identified at an in situ treatment energy of 750 J and macroscopic ablation was noted at both 1000 and 1250 J. The ability to achieve ablation also appears to be dependent on the quality of the US image since ablation was not achieved consistently at 1000 and 1250 J but was observed when the image quality was rated as “good” at 1000 J and as “good” or “fair” at 1250 J. This is most likely due to the effect of the actual attenuation of the tissue. When the image quality of the target is suboptimal this suggests increased attenuation in the tissue which will decrease the acoustic power delivered to the focus. The estimated attenuation does not account for increased attenuation due to the presence of gas or other attenuating structures in the path of the HIFU beam. Therefore, it is recommended that HIFU treatment only be attempted if the US image quality of the target is “good”.

It should also be noted that the acoustic energy values (i.e., 750, 1000 or 1250 J) using this method for determining treatment energy is only applicable to this particular transducer since this method only accounts for tissue attenuation and does not account for focal characteristics of the transducer. The in situ focal intensity can be calculated since the in situ acoustic power can be derived from the in situ acoustic energy (acoustic power = acoustic energy/exposure duration) and the -6 dB beam width at the focus is known (1.6 mm) from previously performed acoustic field mapping. Values for in situ spatial average, pulse average intensity (ISAPA) ranged from 1761 to 3131 W/cm² and are provided in Table 3. During treatment, planning attempts were made to keep the acoustic intensity within a narrow range by keeping the exposure duration between 9–12 s; however, for two animals (pigs 11 and 12) the exposure duration had to be increased to 20 s because of limitations on the electrical power and the higher attenuation values in these animals, which resulted in lower acoustic intensities (ISAPA 1761 W/cm²) despite relatively high treatment energies (1250 J). The greatest damage (damage score of 4) was actually in pig 12, which was exposed to the lowest acoustic intensity but highest acoustic energy. This method could be used with other transducers; however, a calibration study would be required to determine the value for the optimal energy that would result in lesion formation for a given transducer. If the acoustic field at the focus were linear for HIFU transducers then it might be possible to determine an energy “dose” per mm² based on the full-width-half-maximum contour of the focus; however, given the high intensities at the focus, the acoustic field is likely to be highly nonlinear and will be dependent on the focusing characteristics of the transducer and the acoustic properties of the tissue that the ultrasound propagates through. Furthermore, the absorbed energy will be dependent on nonlinear absorption characteristics of the tissue (Khokhlova et al. 2006). This illustrates the challenge in determining a standardized “HIFU dose.”

Hypothesis 3: Higher treatment energies will result in greater degrees of ablation but will also cause greater collateral damage

Higher treatment energies did not demonstrate greater degrees of ablation in this study; however, the group that was treated with 1250 J had poorer imaging quality than the 1000 J group (Table 3). Theoretically, poorer imaging quality translates to poorer delivery of acoustic energy to the target due to greater attenuation of ultrasound energy. Additional studies would need to be performed where both groups had similar US image quality to evaluate this hypothesis. Furthermore, in two cases (pigs 11 and 12), the acoustic intensities that were used to deliver 1250 J were actually lower than for 1000 J (Table 3). The delivery of lower acoustic intensities was achieved by increasing the exposure time. Lower acoustic intensity potentially decreases the magnitude of nonlinear effects at the focus, which is another possible explanation for the lack of differences in histologic damage scores between 1000 J and 1250 J; although the greatest damage to the pancreas was seen in pig 12. However, at higher energies, greater collateral damage did occur. At a treatment energy of 750 J, there was no evidence of collateral damage; however, there was also no ablation of tissue, although there was histologic evidence of tissue damage. At 1000 J, ablation of targeted pancreatic tissue was observed; however, there was also evidence of collateral tissue damage with injury to the abdominal wall, a mild skin burn and bowel adhesions. At 1250 J ablation was also observed; however, there were more findings of collateral injury than at 1000 J.

A limitation of this study in translating these results to treatment in humans is the size of the target. The targeted region of the porcine pancreas was typically 2–3 cm in diameter and 0.7–1.5 cm in thickness with no margin between adjacent structures (e.g., portal vein or bowel wall). In humans, there will be a 1 cm margin between adjacent normal tissue and the targeted region.
Therefore, this study is likely to over-estimate the incidence of unintended injury to adjacent tissue.

In regards to the unintended injury observed in this study, none resulted in clinically significant sequela. None of the findings of collateral injury had an obvious clinical impact on the overall health of the pig and there was no clinical or biochemical evidence of pancreatitis. It appears that the reason that pancreatitis does not occur from these energies is that these energies cause gradual tissue heating to approximately 70–80°C over 18–24 s. No hyperecho was observed at the HIFU focus during treatment. Previous studies have demonstrated that the hyperecho observed at the focus of a HIFU treatment is due to tissue boiling (Khokhlova et al. 2006). Therefore, cell lysis from rapid boiling of cellular contents, as is seen in electrosurgery such as RFA, does not occur with these HIFU acoustic energies. The bowel injury pattern that was observed was primarily bowel adhesions that did not result in any form of bowel obstruction.

CONCLUSIONS

Extracorporeal HIFU treatment of the porcine pancreas using the FEP-BY02 HIFU tumor therapy system appears to be safe and effective as long as certain treatment conditions are maintained. In terms of patient factors, the most essential condition that must be met is the ability to obtain good ultrasound imaging of the target during treatment planning. There should be minimal bowel gas in the path of the HIFU beam. Based on this study, estimated in situ acoustic energies between 750–1000 J are likely to be safe and effective resulting in apoptosis, coagulative necrosis, liquifactive necrosis and/or thermal ablation in the targeted tissue. No serious adverse events were observed in this study. Most importantly, there was no evidence of pancreatitis from HIFU treatment directed at normal pancreatic tissue. These findings provide encouraging results that support evaluating the use of this device in palliation of pain related to pancreatic cancer in humans.

Acknowledgments—Research support was provided by Yuande Bio-Medical Engineering, Ltd., Beijing, China. The authors acknowledge Marla Paun for her assistance with ultrasound imaging.

REFERENCES


