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Histotripsy Focal Ablation of Implanted Prostate Tumor in an ACE-1 Canine Cancer Model

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Abstract

Purpose—Histotripsy is a nonthermal ablative focused ultrasound technology with possible future applications for prostate cancer focal therapy. We used the ACE-1 prostate tumor model and evaluated the feasibility of treating prostate tumors with histotripsy.

Materials and Methods—A total of 10 immunosuppressed (cyclosporine treated) canine subjects received transrectal ultrasound guided percutaneous intraprostatic injection of ACE-1 canine prostate cancer cells. Prostates were serially imaged with transrectal ultrasound to monitor tumor growth. Subjects were sham treated (3) or underwent transabdominal histotripsy of the prostate, which targeted implanted tumor and adjacent parenchyma using a 750 kHz piezoelectric ultrasound therapy transducer. Prostates were examined histologically to confirm tumor and the histotripsy treatment effect.

Results—ACE-1 tumors were visualized on transrectal ultrasound in all 10 subjects within 2 weeks of tumor injection. Lesions demonstrated growth in the prostatic capsule, glandular lobules, fibrous septa and periurethral stroma with significant desmoplastic reaction and areas of central necrosis on histology. Lymph node and/or pulmonary metastases developed in 4 subjects. Ultrasound tumor localization and initiation of cavitation during histotripsy therapy were feasible in all treated subjects. Histologically there was evidence of homogenization of tumor and prostatic parenchyma in all 4 acute subjects with necrosis and hemorrhage in the 3 chronic subjects.

Conclusions—This study shows the feasibility of histotripsy destruction of prostate tumors in a canine ACE-1 model. It suggests a potential role for histotripsy based focal therapy for prostate cancer. Further studies are needed to better characterize the effects of histotripsy on malignant tissues.

Keywords

prostate; prostatic neoplasms; ultrasonography; necrosis; dogs

While PCa remains the most common noncutaneous cancer and the second most lethal cancer among American men,¹ the advent of the PSA era and widespread screening has resulted in stage migration with most patients now presenting with lower PSA and lower stage disease.² For those who seek treatment the standard therapeutic options for localized PCa (radical prostatectomy, brachytherapy and external beam radiation) continue to be associated with significant morbidity and decreased health related quality of life.^{3,4} Such adverse effects, combined with the desire to minimize overtreatment of low stage disease, have led to the development of focal therapies such as cryotherapy and HIFU. They potentially allow for targeted treatment of a specific site of cancer, while minimizing injury to uninvolved tissue, thereby decreasing morbidity in patients with low grade cancer.

Histotripsy, an experimental, noninvasive, extracorporeal focused ultrasound technology, is capable of fractionating prostate tissue in an in vivo canine model.⁵ Histotripsy involves the delivery of high amplitude, short (4- μ second) pulses of ultrasound energy to a cigar-shaped geometric focal volume. The resultant compression and rarefaction of sound waves result in the development of microbubbles. Oscillation, coalescence and collapse of these bubbles produce nonthermal mechanical fractionation of targeted tissue, transforming targeted tissue and cellular structures to a homogenate of acellular debris. Volumetric ablation is accomplished by moving the transducer focal volume throughout the targeted region. As the tissue is fractionated, the ultrasound appearance becomes progressively hypoechoic.⁶⁻⁹ Previous studies demonstrating the safety and efficacy of transabdominal histotripsy of the prostate in an in vivo canine benign prostatic hypertrophy model¹⁰ led us to hypothesize that prostate histotripsy could potentially have a role in the focal management of PCa.

The ACE-1 canine PCa cell line, originating from the Labrador Retriever, was previously used as an invasive prostate cancer xenograft in mouse models, in which pulmonary, bone and lymph node metastases developed.^{11,12} We used the ACE-1 cell line in a canine model to examine the feasibility of histotripsy as a modality to focally target and disrupt in situ prostate tumors.

Materials and Methods

Tumor Implantation

After receiving approval from the university animal use and care committee, 10 intact male canine subjects weighing 12 to 13 kg were obtained. Subjects were given oral cyclosporine at a starting dose of 200 mg daily (about 15 mg/kg per day). Doses were titrated to achieve therapeutic cyclosporine trough levels between 400 and 600 ng/dl with biweekly blood draws. After this window was achieved, each subject was maintained in the therapeutic range throughout the remainder of the study.

After achieving therapeutic cyclosporine levels for 1 week, subjects were anesthetized with subcutaneous acepromazine (0.1 mg/kg) and intravenous propofol (2 to 8 mg/kg), and intubated. They were prepared with a tap water enema with digital rectal disimpaction after intubation. Inhalational anesthesia (isoflurane 1% to 2%) was maintained throughout the procedure. All subjects received intramuscular penicillin G benzathine/procaine (40,000 IU/kg) for prophylaxis before the procedure. The lower abdomen was shaved before

positioning each subject supine on the procedural table. The lower abdomen was prepared with povidone-iodine and draped in sterile fashion.

TRUS imaging was performed using a Logiq™ 6 ultrasound scanner with an ERB probe (GE Healthcare, Piscataway, New Jersey) positioned manually. Prostate volume was calculated using the ellipsoid estimation, $\text{height} \times \text{width} \times \text{length} \times \pi/6$. A 22 gauge spinal needle was introduced through the abdominal skin to the left of the penis and passed into the left lobe of the prostate under sagittal and transverse TRUS guidance. After the needle tip was positioned approximately 1 cm lateral to the urethra, 0.5 cc of a cellular suspension of 6×10^7 ACE-1 cells per cc were injected in the prostate in 0.1 cc intervals (total of 5 boluses) with slight repositioning of the needle between boluses.

Tumor Surveillance

After tumor cell injection, subjects were monitored for signs of disseminated tumor with daily physical examination. All subjects underwent TRUS weekly under anesthesia using the same anesthetic protocol as described to assess for tumor growth and changes in prostate volume using the ellipsoid method. Beginning 2 weeks after injection, we performed flexible cystourethroscopy weekly with an 8.2Fr Dur™-8 flexible ureteroscope at TRUS using anesthesia to assess for intraurethral tumors. After documenting successful tumor implantation, subjects were allocated to sham (3), or acute (4) or chronic histotripsy (3) treatment. Subjects underwent histotripsy when tumors appeared approximately 1 cm or larger on TRUS to facilitate adequate targeting but not before 2 weeks after injection.

Experimental Histotripsy Setup and Procedure

After administering anesthesia as described, TRUS imaging was performed, including estimation of prostate volume using the ellipsoid method. The therapeutic histotripsy system (Imasonic, Voray sur l'Ognon, France) consisted of a 16-element piezoceramic composite array (750 kHz, 11×14 cm diameter oval shape, focal length 10 cm and focal volume $3 \times 3 \times 8$ mm) on a 3-axis computer controlled positioning system using MATLAB (Math-Works®). Coupling was achieved by placing the therapy transducer in a bath of degassed water contained in a plastic membrane in direct contact with the shaved abdomen (fig. 1).

Histotripsy pulses consisted of 5 cycle bursts of acoustic energy at 750 kHz with a duty cycle of less than 1% and a pulse repetition frequency of 500 Hz, which were targeted at no more than half of the tumor as well as a portion of the periphery of the hypoechoic tumor and adjacent normal prostatic parenchyma. Treatment of the entire tumor was not attempted to allow for the identification of viable tumor in the untreated portion at harvest. Treatment was performed until hypoechoic changes were apparent in the targeted portion of the tumor and adjacent parenchyma. Sham treated subjects underwent TRUS imaging under anesthesia and were then recovered.

Post-Procedure Care and Endoscopic Evaluation

After completing histotripsy treatment, chronic subjects were recovered from anesthesia and monitored for treatment related adverse events. Chronic subjects received oral carprofen (37.5 mg daily) before the procedure and for 24 hours thereafter for analgesia. TRUS and

cystourethroscopy under anesthesia were repeated weekly until sacrifice. Sacrifice was planned 3 weeks after histotripsy in chronic subjects but it was performed 1 week early in 1 due to enlargement of a large periurethral tumor, which was concerning due to pending urinary retention, and a palpable subcutaneous tumor nodule.

Sacrifice and Specimen Processing

Subjects treated with histotripsy were sacrificed with pentobarbital sodium (140 to 160 mg/kg intravenously) immediately after histotripsy (acute at 2 to 3 weeks after injection) or 2 to 3 weeks after histotripsy (chronic at 5 to 6 weeks after injection). Sham treated subjects were sacrificed 5 to 6 weeks after ACE-1 cell injection. At sacrifice TRUS imaging was performed, followed by laparotomy via a midline incision. A cystotomy was created, through which antegrade cystourethroscopy was done using a 16Fr Cy2™ flexible cystoscope to assess for urethral/periurethral tumors and treatment related changes.

The prostate and bladder were surgically removed en bloc along with extraprostatic tissues. Bilateral pelvic lymph node dissection was performed, including the obturator, and internal, external and common iliac lymph nodes. If additional enlarged retroperitoneal lymph nodes were noted, they were also harvested. The adrenal glands and a portion of the liver were then harvested, followed by thoracotomy and bilateral pneumonectomy with excision of any macroscopic or palpable lung nodules.

Harvested tissues were fixed in formalin for 1 week, cut into 5 mm slabs, dehydrated in 25% ethanol, paraffin embedded, cut using a microtome in 5 μ m sections at 1 mm increments, mounted and stained with hematoxylin and eosin for histological assessment. All specimens were reviewed and interpreted by a veterinary pathologist (TJR).

Results

Before tumor cell injection, mean $\pm$ SD prostate size was 12.4 ± 2.7 cc. Tumor growth was evident in 9 of 10 subjects by 1 week after injection and in all by 2 weeks after injection. On TRUS intraprostatic tumors appeared as discrete hypoechoic lesions in normal appearing parenchyma (fig. 2). Tumors arising in and extending from the prostatic capsule were evident with clear delineation between tumor and periprostatic fat in some cases. On gross examination tumors were well demarcated, white and firm to rubbery in consistency compared to normal parenchyma (fig. 3). Histologically, tumors demonstrated growth in the prostatic capsule, interstitial fibrous septa and glandular lobes, and periurethral fibrous stroma. Some tumor cells invaded the stroma and glands of the prostatic lobules. Tumor cells were larger than normal prostate epithelial cells and had prominent basophilic atypical nuclei. Tumors arising in the prostatic capsule tended to have a desmo-plastic capsule containing small nests of invasive cancer cells with central regions of coagulative necrosis (fig. 4). Asymptomatic, histologically confirmed, macroscopic pulmonary and lymph node metastases developed in 2 of 3 and 3 of 3 sham treated subjects, respectively, which were survived 5 to 6 weeks after injection.

Histotripsy Treatment

Transabdominal histotripsy therapy was performed in 4 acute and 3 chronic subjects when tumors were about 1 cm or larger in greatest dimension. Under TRUS guidance, initiation of intraprostatic cavitation and successful tumor targeting with the histotripsy bubble cloud were feasible in all subjects. Treatment was preferentially targeted at a portion of the border between capsular tumors and normal parenchyma due to ease of visualization on TRUS and to preserve a portion of the tumor to document viable tumor cells. Cavitation occurred readily in the normal parenchyma and central regions of the tumor with decreased cavitation activity seen at the tumor periphery. Histotripsy treatment resulted in progressive loss of acoustic echoes in the targeted volume, creating hypoechoic cavities in the central regions of the tumors and adjacent normal parenchyma with relative sparing of the desmoplastic tumor periphery (fig. 5).

Immediately following the completion of histotripsy therapy, acute subjects were sacrificed. Gross examination of the prostate revealed focal hemorrhage involving the targeted portion of normal parenchyma and tumor (fig. 6), corresponding to the region targeted on TRUS. Histological examination of acutely treated prostatic parenchyma revealed normal glandular structures with focal areas of hemorrhage adjacent to regions of homogenized cellular debris, indicating destruction of targeted normal parenchyma with sharp transition to untreated normal parenchyma. In all acute subjects examination of targeted ACE-1 tumors revealed areas of punctate hemorrhage with small focal areas of architectural disruption of tumor cells in the desmoplastic periphery of the tumor, correlating with relative resistance of the tumor periphery to the hypoechoic changes seen on TRUS during treatment. Centrally, viable tumor cells were separated by a sharp demarcation from regions of adjacent homogenized debris with loss of tumor architecture, consistent with a histotripsy treatment effect, which was distinct from areas of central necrosis. A single acute subject showed asymptomatic microscopic pulmonary and lymph node metastases at necropsy, which was 3 weeks after injection.

After histotripsy, 2 of 3 chronic subjects were survived for 3 weeks (total of 6 weeks after tumor implantation). The other chronic subject was survived for only 2 weeks (total of 5 weeks after tumor implantation) due to concern for potential urinary retention secondary to a large, intraprostatic periurethral tumor that was evident on TRUS and confirmed by cystoscopic examination. Histological examination of the prostate revealed multifocal lymphoplasmacytic inflammation in all subjects, which was interpreted as a background lesion in all. The targeted volume of the prostate had extensive areas of treatment induced necrosis with focal persistent hemorrhage and squamous metaplasia of adjacent viable prostatic glands, consistent with an evolving histotripsy treatment effect. Targeted tumors revealed similar extensive areas of necrosis with focal hemorrhage (fig. 7). There was a minimal transition zone between homogenized and untreated tissue.

No chronic subjects had apparent metastases despite survival to 5 and 6 weeks after injection in 1 and 2, respectively. There were no observed complications following histotripsy treatment in any subject.

Discussion

We used the ACE-1 in situ canine prostate cancer model and applied it to evaluate the feasibility of histotripsy as a potential therapy for prostate cancer. TRUS guidance during histotripsy treatment demonstrated successful targeting and initiation of intratumor cavitation in all cases. While the entire tumor was not targeted to document viable tumor at treatment, we anticipate that histotripsy ablation of the entire tumor would be feasible by guiding the histotripsy bubble cloud throughout the entire region of the lesion. Post-procedure histology confirmed successful targeting and revealed histological evidence of homogenization of viable tumor cells and stroma without evidence of a tissue effect in untargeted regions. Histotripsy effects were distinct histologically from areas of central necrosis. These findings suggest that transabdominal histotripsy is capable of mechanically ablating an in situ prostate tumor in a canine model. It also suggests the feasibility of prostate cancer focal therapy with histotripsy.

During histotripsy treatment, cavitation appeared to occur preferentially in the normal peritumor prostatic parenchyma and central region of the tumors with less effect in the desmoplastic periphery of tumors in the prostatic capsule. This suggests that regions of desmoplasia may be relatively resistant to histotripsy treatment and may indicate a potential shortcoming of the ACE-1 model to evaluate histotripsy since PCa in men does not typically demonstrate a significant desmoplastic reaction.¹³ However, more centrally located tumors growing in the prostatic lobules or periurethral stroma had less of a desmoplastic response, more closely simulating native PCa. While these central tumors were visible on TRUS, they tended to be smaller and less apparent than tumors implanted in the prostatic capsule. As a result, although previous canine prostate histotripsy studies have shown that targeting any portion of the prostate is feasible,^{5,10,14,15} due to the ease of identifying capsular ACE-1 tumors on TRUS we preferentially targeted tumors at this location as opposed to more centrally located tumors.

This finding mimics to some extent the difficulty of identifying multifocal PCa clinically with current imaging technologies, including ultrasound, underscoring the need for better localization techniques to facilitate optimal focal therapy for PCa clinically. To this end imaging modalities, such as TRUS elastography, magnetic resonance imaging spectroscopy and diffusion weighted magnetic resonance imaging, have shown improved prostate cancer detection.^{16,17} If further refined, they may become useful clinical adjuncts to assist in image guided prostate cancer focal therapies, including histotripsy.

Histotripsy has potential benefits over HIFU and cryotherapy, the only 2 energy sources clinically available for prostate cancer focal therapy, of which only cryotherapy is approved by the Food and Drug Administration in the United States. Whereas the histotripsy induced loss of ultrasound backscatter intensity during treatment correlates to the degree of tissue homogenization observed histologically, allowing for real-time ultrasound assessment of the adequacy of targeting and the treatment effect,¹⁸ HIFU does not produce readily appreciable feedback on standard TRUS imaging.¹⁹ Instead, it relies on mathematical models of heat diffusion to determine the adequacy of targeted tissue dosing.²⁰

Similarly, real-time TRUS monitoring of cryotherapy is limited by reflection of up to 99% of the acoustic waves from the surface of the ice ball,²¹ limiting assessment of the adequacy of freezing during treatment. TRUS also does not provide reliable temperature feedback to determine adequate dosing to the targeted volume,²² which has resulted in the use of invasive thermocouples to determine adequate dosing. As a result, the real-time TRUS feedback (bubble cloud and transition from normal to hypoechoic tissue architecture) seen with histotripsy would potentially allow real-time image guided determination of the adequacy of treatment, which is currently not available with existing technologies.

An additional potential benefit of histotripsy is the nonthermal treatment effect. Unlike histotripsy, HIFU and cryotherapy rely on thermal tissue effects to precipitate coagulative necrosis.^{23–25} This is in contrast to histotripsy, which results in nonthermal mechanical homogenization of targeted tissue with resultant, dose dependent tissue debulking and rapid tissue resorption.^{5,15} Furthermore, the small focal nature of the histotripsy bubble cloud allows for precise targeting and possible tissue sculpting. The combination of its focal effect and lack of thermal effects could potentially minimize collateral damage to adjacent nontargeted tissues. Finally, data suggest differential targeted tissue sensitivity to the effects of histotripsy. In particular, nerves, the urinary sphincter and the prostatic urethra appear more resistant than prostatic parenchyma in the canine model.²⁶ If true in humans, such observations may further aid efforts to minimize complications seen with other focal therapy modalities, such as erectile dysfunction, urinary incontinence and irritative voiding symptoms.^{27,28}

An important hurdle that must be overcome before human oncological applications of histotripsy can be evaluated is the theoretical potential for metastatic potentiation, resulting from the showering of malignant cells from mechanically disrupted tissue. Although the current study was not designed to assess malignant potential, the finding of no pulmonary or lymph node metastases in chronic subjects following histotripsy compared to metastases in all 3 sham treated subjects that were survived to the same time point after injection is intriguing. Studies designed to specifically address metastatic potential are clearly needed before performing a human clinical trial to evaluate histotripsy for prostate cancer.

The initial results seen with the ACE-1 prostate tumor model are encouraging and suggest the feasibility of histotripsy treatment for PCa. Further study is needed to better define the usefulness of the ACE-1 model for application in future histotripsy studies. Similarly, further studies are planned to continue to evaluate the effectiveness and safety of histotripsy as a potential modality for focal therapy for prostate cancer.

Conclusions

Noninvasive transabdominal histotripsy produced homogenization of malignant prostate tissue in the canine ACE-1 PCa model. These results support the further investigation and development of histotripsy for applications related to PCa focal therapy.

Acknowledgments

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Abbreviations and Acronyms

HIFU	high intensity focused ultrasound
PCa	prostate cancer
TRUS	transrectal ultrasound

Figure 1. Histotripsy experimental setup with degassed water bath on abdominal wall with TRUS probe in place to monitor treatment (A). Therapy transducer is lowered into water bath to deliver acoustic energy to targeted volume (B).

Figure 2.
TRUS appearance of ACE-1 tumors. Note hypoechoic appearance of central (white arrowhead) and subcapsular (blue arrowhead) tumors.

Figure 3. Gross formalin fixed histological appearance of untreated tumors reveals well circumscribed white lesions (A). Locations were confirmed on histology (inset). Note prostate appearance (inset) at different magnifications (B).

Figure 4.
Histological appearance of subcapsular tumor with central necrosis (asterisk).

Figure 5.

TRUS appearance of histotripsy treatment. ACE-1 tumor extends from prostatic capsule (white arrowhead) and before treatment tumor was well circumscribed (A). During treatment tumor and adjacent parenchyma became progressively more hypoechoic with bubble cloud visible (blue arrowhead) (B). At treatment conclusion hypoechoic cavity was seen in tumor (plus sign) and normal parenchyma (asterisk) with relative sparing of tumor periphery (C).

Figure 6.

Gross formalin fixed histological appearance of acute treatment effect. Tumor extends from subcapsular position out from prostate with focal hemorrhage from histotripsy treatment effect (A). Prostate and tumor (inset) appearance at different magnifications reveals focal hemorrhage peripherally with homogenization of viable tumor centrally (asterisk) and focal homogenization peripherally (blue arrow) in areas of desmoplasia (B).

Figure 7. Histological appearance of ACE-1 tumor and prostate in chronic subjects 3 weeks after histotripsy at different magnifications. Note necrotic homogenized tissue (asterisk) and hemorrhage with viable tumor (plus sign) at bottom.