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Histotripsy Fractionation of Prostate Tissue: Local Effects and Systemic Response in a Canine Model

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Abstract

Purpose—Histotripsy is an extracorporeal ultrasound (US) technology that utilizes cavitation mechanisms to produce non-thermal tissue destruction. Previously, we demonstrated the feasibility of histotripsy for fractionation and immediate debulking of prostate tissue. The purpose of this study is to characterize the local effects and systemic response after histotripsy treatment of prostate tissue in an in-vivo canine model.

Materials and Methods—Histotripsy was applied transabdominally to the prostate in eighteen intact male canine subjects under general anesthesia. Acoustic bursts (4 microseconds) were delivered at 300 Hz pulse repetition rate from a highly focused 750 kHz piezoelectric US transducer (15 cm aperture, 3×3×8 mm focal volume). The prostate and surrounding structures were harvested at prescribed time points (0, 7, 28, or 56 days) following histotripsy. Blood and urine parameters were assessed periodically while clinical evaluation incorporating a validated veterinary pain scale was performed daily.

Results—Conventional transrectal US imaging facilitated targeting of the focal volume and provided real-time assessment of cavitation activity. Fractionation of the targeted volume and clearance of the resultant debris with urination produced a treatment cavity within each prostate. No acoustic collateral damage was seen and urothelialization of the treatment cavity occurred within 28 days of treatment. Only transient lab abnormalities and minimal hematuria were noted after treatment. Pain scores revealed only mild post treatment discomfort.

Conclusions—Histotripsy produced consistent tissue fractionation and prostate debulking without collateral acoustic injury or clinical side effects and was well tolerated in the canine model.

Keywords

Benign Prostatic Hyperplasia; Prostate; Histotripsy; Cavitation; Ablation

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Introduction

Successful definitive treatment of BPH is predicated on the concept of debulking expansile adenomatous prostate tissue that impedes urinary drainage. For decades, the gold standard has been transurethral resection of the prostate (TURP). More recently, encouraging results have been seen with newer technologies (PVP, HoLEP) where laser energy is used to cut or vaporize prostate tissue.^{1,2} Although these treatments are effective and recent advances have reduced blood loss and metabolic imbalance, they remain invasive transurethral procedures with associated surgical risks.^{3,5}

Minimally invasive therapies (TUNA, microwave) were developed as less morbid, less invasive alternatives that thermally coagulate adenomatous prostate tissue while sparing the urethra from damage.⁶ Unfortunately these modalities failed to achieve significant tissue debulking which is likely the reason that objective improvement, symptom relief, and durability fall short of the results seen with TURP.^{7,8} As a result, many men now present for definitive treatment of BPH later in life (having failed pharmacologic management or minimally invasive treatments) with additional co-morbidities, and greater risk of surgical and perioperative complications.^{9,10} Thus there remains an unmet need for a less invasive, less morbid technology capable of “TURP-like” tissue debulking.

Histotripsy is an experimental extracorporeal ultrasound (US) technology that utilizes cavitation mechanisms to produce non-thermal tissue fractionation. Delivery of intense, short (< 10 microsecond) bursts of US energy generate extreme pressures within the targeted focal volume leading to microbubble formation. Subsequent oscillation, coalescence, and collapse of these bubbles mechanically fractionates targeted tissues reducing architectural and cellular structures to a fine slurry of acellular debris.^{11–15} This histotripsy process is markedly different than high intensity focused ultrasound (HIFU), where less intense 3–5 second pulses of acoustic energy are used to heat tissues and produce thermal coagulation. This distinction was demonstrated in an in-vitro porcine kidney model where acoustic parameters that produced desiccated HIFU lesions resulted in an average tissue temperatures of 67.5 degrees C, while mechanical tissue disruption (characteristic of histotripsy lesions) appeared to be temperature independent.¹⁶

In-vivo canine studies have demonstrated that histotripsy fractionation of prostate tissue is feasible with rapid urethral drainage of the thin slurry of resultant debris.¹⁵ The purpose of this study is to characterize the local effects and systemic response after transabdominal histotripsy is applied to prescribed volumes of prostate tissue in an in-vivo canine model.

Materials and Methods

Eighteen intact male canine subjects weighing 19.5 to 40.7 kg were used in this study. Following approval from the University Committee for Use and Care of Animals. Subjects were prepared for histotripsy by intramuscular administration of Durapen (40,000 IU/kg) and tap water enema with digital rectal disimpaction following intubation. The lower abdomen and suprapubic region were shaved prior to positioning supine on the procedural table. A conventional transrectal biplane US probe (Brüel & Kjær Diagnostic US System Model 3535) was used to measure prostate volume ($\text{Volume} = \pi/6[\text{height} \times \text{width} \times \text{length}]$). Subjects were maintained on inhalational anesthesia during the course of treatment.

Histotripsy consisted of transabdominally delivery of 3-cycle acoustic bursts (4 microsecond) at a repetition rate of 300 Hz. from a 750 kHz 18-element piezo-composite transducer (14 cm aperture, 10 cm focal distance, 3×3×8 mm focal volume; Imasonic, Besancon, France). Coupling was achieved by placing the therapy apparatus in a bath of degassed water contained in a plastic membrane in direct contact with the shaved abdomen

(Figure 1). A diagnostic 8-MHz imaging probe (Model E8C, General Electric Medical Systems, Milwaukee, WI) placed through the center of the therapeutic transducer and aligned with the geometric focus confirmed location of the therapy volume within the prostate. A transrectal imaging probe (Model I739, General Electric Medical Systems, Milwaukee, WI) was subsequently used to monitor cavitation activity during treatment. Using this real-time US feedback, the therapeutic transducer focus was manually scanned through the target volume (central prostate tissue encompassing urethra) in the first three subjects. In subsequent subjects an automated 3-axis positioning system was used to uniformly scan through a predefined rectangular volume grid of 2 mm spaced focal locations. Histotripsy doses up to 270,000 pulses/cm³ were applied to preplanned treatment volumes of 4 cm³ (n=11), 2 cm³ (n=3), or 0.75 cm³ (n=1) to ensure the treatment did not extend outside the prostate.

Once histotripsy was complete subjects were euthanized immediately (5), at 7 days (5), 28 days (6), or 56 days (2). Subjects were assessed at baseline and daily after treatment for hematuria, pain, and urinary incontinence. Pain scores were calculated (0–18) using the University of Melbourne Pain Scale.¹⁷ Incontinence was assessed by direct observation of the dogs and location and size of urine stains within the cages. Bloodwork including complete blood count, and comprehensive metabolic panel was assessed at baseline, one day after treatment, and at euthanasia for each subject. Likewise, urine was obtained for analysis and culture before treatment and at euthanasia. A 12-Fr urinary catheter was placed following histotripsy and maintained for 24–72 hours. Immediately after euthanasia the prostate, bladder, rectum, and adjacent tissue were closely inspected for signs of hemorrhage or tissue damage. The prostate, bladder and rectum were then removed en-block, inspected grossly, then fixed in 10% buffered formalin. Prostate and rectal tissues were step sectioned transversely, stained, and examined histologically. In each subject, measurements of treatment cavity height and width were recorded from an haematoxylin and eosin slide through the center of the treated volume.

Results

US visualization of the bubble cloud, indicative of the presence of cavitation, confirmed accurate targeting within the prostate in all cases (Figure 2). In general, the bubble cloud first appeared elongated, however after 15–30 seconds of sonication it decreased in axial dimension and took on a more spherical shape. Movement of the bubble cloud through the prescribed target volume produced a hypoechoic appearance of the region previously treated. This appearance persisted for 1–2 minutes before a speckle pattern gradually re-emerged. Subsequent delayed passes through the treated area produced an immediate reversion to a hypoechoic US appearance. Transrectal US imaging 7, 28, or 56 days after treatment (just prior to euthanasia) revealed an obvious well demarcated hypoechoic volume within the prostate (Figure 3).

In 2 of 5 prostates harvested immediately after histotripsy, residual fractionated material was present within the treatment cavity. In the 3 other acute cases most of the fractionated debris had already drained. Prostates harvested at delayed time points exhibited a vacuous cavity (Figure 4) with sharp boundaries and corners consistent with the applied pattern of ablation (Figure 5). Communication of the cavity with urethra was apparent and in some cases the prostate had partially collapsed into the cavity distorting its cross-sectional contour. In the 13 subjects treated with planned ablation patterns of 1×2 cm cross sectional dimension, the measured cross sectional cavity area (height x width) was 1.95 cm² (s.d. 0.4) [range 1.4–2.9]. The remnants of a typical acute inflammatory response, seen in prostates harvested 7 days after histotripsy, were replaced by normal histologic evidence of wound healing with

minimal fibrosis and urothelialization of the treatment cavity at 28 and 56 day time points (Figure 6).

Superficial rectal mucosal irritation and edema without associated hematoma were seen on gross inspection in subjects harvested immediately after histotripsy. This was histologically confirmed to be confined to the mucosa and was not present in specimens harvested at delayed time points. No injury to other adjacent structures, periprostatic hematoma, fluid collection, urine leak, or fistula was apparent on transrectal US evaluation, gross inspection, or histologic assessment of tissue sections.

In most subjects, laboratory evaluation one day following histotripsy demonstrated elevation of aspartate amino transferase (AST), creatine kinase (CK), and mild leukocytosis. These abnormalities had resolved when blood parameters were next assessed (at time of harvest). Average hematocrit also remained stable (pre-procedural 36.6, one day post treatment 36.8, and harvest 39.3).

Mild hematuria without blood clots occurred in all cases but cleared within 24–48 hours of urinary catheter removal. Urinary catheter irrigation was not required nor did any of the subjects develop urinary retention or incontinence after catheter removal. Two subjects had positive urine cultures prior to treatment and were treated with antibiotics. One other subject had a positive urine culture prior to harvest but had been asymptomatic.

All subjects tolerated histotripsy treatments without incident. Observation of vital signs did not reveal any episodes of acute oxygen desaturation, pronounced tachycardia, or hypo/hypertensive episodes. The overall average elevation in pain score (scale 0–18) was 0.7 points (s.d. 0.5). An increase in pain score greater than 0.5 points was seen in 6 subjects, and returned to within 0.5 points of baseline one day after urinary catheter removal in all but two cases. One case of anorexia, fever with diarrhea, and traumatic foreleg bursitis occurred during follow-up but were not felt to be related to histotripsy. These subjects improved with antibiotics, hydration, and surgical drainage respectively.

Discussion

The most effective treatments for BPH are those that directly debulk the prostate by resection or laser vaporization of tissue. A number of less invasive technologies (e.g. TUNA, microwave) have been developed to treat BPH by delivering energy to induce thermal coagulative necrosis within the prostate. However, these technologies have not approached the efficacy or durability of TURP, probably a result of poor resorption of the thermally desiccated tissue and lack of true tissue debulking.

Histotripsy is a form of focused US therapy that specifically utilizes mechanical (cavitational) processes to finely fractionate tissue with negligible thermal contribution.¹⁶ Furthermore, the mechanical process is the result of a cloud of microbubbles that are readily identifiable on transrectal US, thus providing immediate feedback on the location of the acoustic focus and the adequacy of cavitation activity. Progressive subdivision of tissue produces a slurry of cytoplasmic and subcellular debris. Once tissue is fractionated, US scatterers within the treated volume are destroyed, hence producing a hypoechoic appearance. We postulate that the gradual re-emergence of speckle may be a result of admixture of the fractionated material with blood which alters the US appearance and re-introduces speckle.

Evidence of immediate tissue debulking and creation of a treatment cavity is reminiscent of the appearance seen after TURP and other transurethral resection/vaporization modalities (PVP and HoLEP). Rapid urothelialization and minimal scarring of the treatment margins is

also an encouraging finding and is perhaps a result of the nonthermal mechanical nature of histotripsy fractionation which does not leave a terminally damaged zone of tissue at the margin susceptible to delayed sloughing.

The only collateral damage apparent in this study was superficial rectal irritation seen in specimens harvested immediately after treatment. This likely resulted from several rough edges on the custom transrectal US probe housing and was likely clinically insignificant as it was not observed in specimens harvested at 7 days and later time points. Similar superficial rectal injuries have not been seen in subsequent studies with a modified transrectal US imaging probe. The tight focus of the bubble cloud, lack of energy spread beyond the focal volume, and central prostatic targeting are all factors that likely contributed to the absence of any periprostatic or adjacent structure injuries. None of the subjects developed incontinence following histotripsy treatment suggesting functional preservation of the urinary sphincter.

Concerns that tissue cavitation may release microbubbles into the bloodstream that could migrate to the lungs, coalesce, and create a pulmonary gas emboli have been raised. However, this theoretical concern is unlikely when one considers that microbubbles collapse within 1 ms of formation; a much shorter time period than would be necessary for transit to the lungs.¹⁴ Experimentally, no episodes of acute intraoperative oxygen desaturation or cardiopulmonary instability were observed during or after histotripsy in the canine subjects in this study.

A truly relevant measure of pain and discomfort can really only be assessed in human clinical trials. This canine study revealed little significant discomfort following histotripsy treatment, however the question of pain experienced during histotripsy remains unanswered as it was necessary to maintain all subjects under general anesthesia during these experiments. It is interesting to draw a parallel between histotripsy and piezoelectric lithotripsy which utilizes a similar acoustic delivery method and also induces cavitation activity as part of the process for urinary stone fragmentation. Most patients undergo piezoelectric lithotripsy require only a low level of sedation and report minimal discomfort.

Aside from a transient elevation in white blood cell count, creatine kinase, and aspartate amino transferase (probably secondary to inhalational anesthetics), no other systemic abnormalities were noted up to 56 days after histotripsy. Catheterization time was decreased during the study to only one day without occurrence of urinary or clot retention. Furthermore, the mild hematuria and minimal blood loss observed during and after prostate tissue fractionation has been confirmed in a study of histotripsy prostate fractionation in anticoagulated canines, where on average no change in hemoglobin concentration was observed and the greatest individual decrease in hemoglobin after treatment was 2.5 g/dl.¹⁸ This suggests a possible hemostatic component to cavitation and will be investigated further.

Although it was not the main focus of this study, we did attempt to compare the volume of the tissue cavity created by histotripsy to the planned treatment volume. Unfortunately, several factors precluded an accurate measurement of volume determination. Cavity volumes were often positioned at an angle with respect to the transrectal imaging transducer which confounded measurement of US dimensions (as we did not have the capability to perform step-section reconstructions). Attempts to reconstruct the volumes from histologic slides were further confounded by our inability to accurately measure distance along the apex-base axis since a variable amount of material was shaved off the blocks and discarded in order to create whole mount prostate slides. Despite these limitations, we were able to measure cross sectional cavity dimensions and calculate the cross sectional area of the histotripsy cavity compared to the planned 2 cm² area in 13 of the subjects. Although imperfect, the relatively modest range of cross sectional areas about the planned volume

coupled with the histologic appearance of treated volumes with sharp margins and right angle contours is encouraging preliminary data and will lead to future studies designed to more accurately measure cavity volume and dimensions with respect to planned treatment volumes.

Although the canine prostate is the best practical pre-clinical model in which to study BPH technologies, it introduces several limitations that must be understood prior to translation for human use. Firstly, differences in pelvic anatomy (longer path length and intervening bones) may limit use of an abdominal approach for acoustic targeting the prostate. Analysis of human cross-sectional images and reconstructions suggests a perineal approach provides sufficient acoustic access to the prostate.¹⁹ Additionally, transrectal access may become practical when engineering advances lead to development of small transducer elements sufficiently powerful to initiate cavitation. Secondly, differences in anatomic structure and tissue composition between canine and human may alter histotripsy treatment effect. A similar issue was identified when using canine prostate as a model for development of laser technology.²⁰ Thirdly, although a validated canine pain metric was utilized and revealed encouraging results, relevant perception of pain associated with histotripsy can only be assessed in human studies.

Conclusions

Histotripsy produced consistent mechanical tissue fractionation and urethral drainage of the thin slurry of resultant debris when applied extracorporeally in a canine model. Debulking of the prostate was confirmed by presence of a cavity with sharp margins and corners on prostate cross-sections corresponding to the planned histotripsy target volume. No subjects exhibited incontinence, urinary fistula, or retention; nor was there any evidence of acoustic collateral injury on direct and microscopic examination. Minimal hematuria, mild elevation in pain score, and overall stability of laboratory parameters are encouraging findings that have prompted further evaluation and development of histotripsy.

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Abbreviations

BPH	benign prostate hyperplasia
HoLEP	holmium enucleation of prostate
PVP	photoselective vaporization of prostate
TUNA	transurethral needle ablation
TURP	transurethral resection of prostate
US	ultrasound

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Figure 1.

Therapeutic US energy is delivered transabdominally from a transducer suspended within a degassed water bath coupled to the shaved abdomen of a canine subject in supine position. A separate 10 MHz transrectal US imaging probe provides high resolution feedback of the cavitation activity during histotripsy treatment.

Figure 2.

A sagittal transrectal US image through the left lobe of a canine prostate during in-vivo histotripsy demonstrates cavitation. The hyperechoic (bright) approximately 3×8 mm histotripsy bubble cloud is easily seen within the middle of the prostate tissue and represents the geometric focus of the therapeutic histotripsy transducer which is located above the top of the image and delivers acoustic energy transabdominally. The treated area immediately surrounding the bubble cloud has been fractionated and appears hypoechoic (dark) with respect to untreated tissue.

Figure 3. Transverse US appearance of canine prostate before and 28 days after histotripsy therapy guided manually. The large hypoechoic cavity on the post-treatment image represents a treatment cavity corresponding to the histotripsy targeted volume.

Figure 4. Gross appearance of fixed specimen harvested 28 days after histotripsy therapy with manual guidance. Prostate is same specimen imaged in Figure 3.

Figure 5. Representative histologic prostate cross sections at 0, 7, and 28 days following histotripsy treatment. In each case the cross-sectional area of the targeted volume was 20×10 mm and corresponds to the cavities seen on histologic sections. In some cases a portion of the targeted volume on the right side of the prostate was shielded by the os penis and was not fractionated.

Figure 6.

By 28 days after histotripsy treatment, urothelialization of the urethra and lining of the treated cavity is apparent. The central prostate and urethra were treated with manual (joystick) control using US feedback to assess adequacy of treatment.