Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2007 February 8; 6440: 644006–.

Correlation of contrast-enhanced MR images with the histopathology of minimally invasive thermal and cryoablation cancer treatments in normal dog prostates

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Abstract

Magnetic Resonance Imaging (MRI) is a promising tool for visualizing the delivery of minimally invasive cancer treatments such as high intensity ultrasound (HUS) and cryoablation. We use an acute dog prostate model to correlate lesion histopathology with contrast-enhanced (CE) T1 weighted MR images, to aid the radiologists in real time interpretation of *in vivo* lesion boundaries and pre-existing lesions. Following thermal or cryo treatments, prostate glands are removed, sliced, stained with the vital dye triphenyl tetrazolium chloride, photographed, fixed and processed in oversized blocks for routine microscopy. Slides are scanned by Trestle Corporation at .32 microns/pixel resolution, the various lesions traced using annotation software, and digital images compared to CE MR images. Histologically, HUS results in discrete lesions characterized by a “heat-fixed” zone, in which glands subjected to the highest temperatures are minimally altered, surrounded by a rim or “transition zone” composed of severely fragmented, necrotic glands, interstitial edema and vascular congestion. The “heat-fixed” zone is non-enhancing on CE MRI while the “transition zone” appears as a bright, enhancing rim. Likewise, the CE MR images for cryo lesions appear similar to thermally induced lesions, yet the histopathology is significantly different. Glands subjected to prolonged freezing appear totally disrupted, coagulated and hemorrhagic, while less intensely frozen glands along the lesion edge are partially fragmented and contain apoptotic cells. In conclusion, thermal and cryo-induced lesions, as well as certain pre-existing lesions (cystic hyperplasia – non-enhancing, chronic prostatitis - enhancing) have particular MRI profiles, useful for treatment and diagnostic purposes.

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D. M. Bouley; B. Daniel; K. Butts Pauly; E. Liu; A. Kinsey; W. Nau; C. J. Diederich; G. Sommer, Correlation of contrast-enhanced MR images with the histopathology of minimally invasive thermal and cryoablation cancer treatments in normal dog prostates, *Proc. SPIE*. 6440, Thermal Treatment of Tissue: Energy Delivery and Assessment IV, 644006. (February 08, 2007) doi: 10.1117/12.701049 One print or electronic copy may be made for personal use only. Systematic reproduction and distribution, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper are prohibited. DOI abstract link: <http://dx.doi.org/10.1117/12.701049>

Keywords

Prostate; high intensity ultrasound; cryoablation; histopathology; canine model

1. INTRODUCTION

Prostate cancer is the 3rd leading cause of death in men, striking 1 in 6, and benign prostatic hyperplasia (BPH) ultimately affects most men at some point after the age of 50 (1-4). These statistics make the need for reliable treatments paramount. In recent years, cryotherapy and thermotherapies which include high intensity ultrasound (HUS - focused, slightly focused or unfocused), laser ablation, radiowave and microwave treatments, have gained popularity as means of treating cancer in various organs or BPH (1, 5, 6). Treatments for prostate cancer and BPH, such as surgical resection, radiation or hormone treatments may have detrimental side effects such as long term post surgical recovery times, nerve damage, and impotence (7). Both prostate cancer and BPH are ideal candidates for treatments with these various minimally invasive techniques.

Clinical reports of human prostate cancer patients address the long term effects of thermal treatments and their cure rates, yet the acute pathology resulting from thermal treatment is unique, and few reports in the literature provide detailed descriptions of the lesions. Using Magnetic Resonance Imaging (MRI) and post treatment contrast enhanced T1 weighted images (T1-CE), our radiologists are able to visualize and monitor the location and extent of the thermal damage within the target tissue. Improved interpretation of these images based on understanding the underlying histopathology will greatly improve real time interpretation of *in vivo* lesion boundaries as they appear on MRI. Our group works to develop MRI compatible delivery systems for HUS and cryoablation by creating lesions in normal dog prostates (8-13). In order to evaluate and compare the area and severity of the tissue damaged with the MRI T1-CE images obtained *in vivo*, we remove, examine, and perform histology on the prostate glands. This is an ideal animal model to use because the dogs fit well inside the magnet and the canine prostate is of similar size and shape to the human prostate gland. Although these dogs have “normal” prostates, they often have pre-existing lesions such as chronic prostatitis or benign cystic hyperplasia, similar to conditions encountered in older men. Correlation of MR images of experimentally induced lesions with actual histopathology, and recognition of pre-existing conditions by T1-CE profiles may further aid the radiologists in their ability to accurately deliver treatment to specific target areas.

2. METHODOLOGY

2.1 Animal studies

All animal studies were approved by the Stanford University Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals. Adult male, non-castrated mixed breed or Beagle dogs weighing 10-20 kg were used for all studies. The dogs were fasted for 24 hrs, administered enemas prior to the MRI studies, and catheterized using a 6.0 Fr. urinary catheter. The dogs were pre-anesthetized with Ketamine

(10 mg/kg) and valium (5 mg/kg) and maintained on Isoflorane gas anesthesia throughout the procedures as previously described (9). Following treatments and imaging, all dogs were euthanized with an IV injection of B-euthanasia D (1 ml/4 kg).

2.2 Prostate thermal treatments

Two kinds of applicators were used to administer HUS, interstitial or transurethral. The interstitial ultrasound applicators (previously described) (11) consisted of arrays of two 1.5 mm OD $\times$ 10 mm tubular radiators sectorized to produce an approximately 180° heating pattern that operated at 7-8 MHz. They were employed in a catheter-cooled configuration within 2.4 mm OD plastic implant catheters. The MR compatible transurethral ultrasound applicators (90° sectorized tubular, planar and lightly focused curvilinear devices) (9, 13) consisted of two transducer sections (3.5 mm $\times$ 10 mm long, 6.5-8 MHz), mounted on a 4 mm delivery catheter with an inflatable 10 mm urethral cooling balloon. A water-cooling jacket on the endorectal MR imaging coil provided cooling of the rectum. The temperatures reached in these ablation studies were in excess of 60°C and heating took place for at least 15 minutes.

2.3 Prostate cryoablation treatments

Bilateral prostate cryoablations were performed using an Argon-powered commercial MRI-compatible cryosurgery system (Cryohit™, Galil Medical, Yokneam, Israel, Sold in US by Oncura, Boston, MA). Dogs were positioned in dorsal recumbency in the magnet, with a prototype rigid endorectal receive coil (General Electric Global Research Center, Schenectady, NY) and a custom 10 cm diameter loop coil positioned over the ventral pelvic wall immediately cranial to the symphysis pubis. Using rapid fast spin echo imaging, a 7 Fr MRI-compatible sheath was inserted through the ventral pelvic skin, through the rectus muscle, impaling the left lateral aspect of the prostate. Similarly, a second sheath was then inserted to abut the right lateral aspect of the prostate. In addition, an 18g sheath was inserted parallel, and medial to each 7 Fr sheath to the same depth, terminating in the mid-prostate. A two mm diameter MRI-compatible Galil cryoprobe was then inserted through each 7 Fr sheath, and 0.5mm diameter MRI-compatible Fluoroptic thermometers were then inserted through each 18g sheath, completing the experimental set-up (12).

Cryoablation was performed using various freeze/thaw regimes, using MRI to monitor the size of the developing ice ball to an end where the internal probe temperature of -118°C was reached, and the iceball was 2 cm in diameter. Freezing was then discontinued, and the tissue was allowed to warm passively to body temperature. Post-cryoimaging was performed, the cryoprobes removed, and sheaths were filled with heparinized saline to displace any air bubbles.

2.4 In vivo imaging

Imaging for both thermal and cryoablation studies was performed in an open, interventional MR system (0.5Tesla Signa-SP, GE Medical Systems, Milwaukee, WI). Likewise, following either treatment, T1-CE images were acquired approximately 90 s after IV injection of Gadolinium contrast agent (Magnevist, Gadopentetate dimeglumine, Berlex Laboratories, Wayne, NJ).

2.5 Veterinary vs. human anatomic terminology

The terminology that appears in the text, figure legends, and figures in this paper is standard for veterinary quadruped anatomy. For clarification purposes, a list of veterinary terms followed by the corresponding bipedal human anatomic terms are provided: Ventral = anterior, dorsal = posterior, cranial = superior, caudal = posterior, dorsal recumbency = supine position. In MR images, and in the corresponding photos of gross and macroscopic histology images, ventral is always at the top of the picture as the dogs are placed in the magnet and imaged in dorsal recumbency.

2.6 Prostate histopathology

Immediately following euthanasia the dogs' prostates were removed, measured, and marked on the ventral right surface with green tissue dye (Bradley Products, Inc, Bloomington, MN). Using a custom designed Plexiglas slicing platform (Figure 1) that facilitates obtaining ~ 5 mm thick uniform slices, prostates treated with HUS were sliced on the axial plane, and prostates treated with cryoablation were sliced on the coronal plane. Both surfaces of each prostate slice were photographed. Prostate slices were immersed for 20 min into 37°C 1% triphenyl tetrazolium chloride (TTC), an oxidizing compound that upon reduction by enzymes in the mitochondria of viable cells produces the red precipitate, formazan (14). Following incubation in TTC, prostate slices were again photographed, fixed in 10% buffered neutral formalin (BNF), processed for paraffin embedding in standard or oversized blocks, sectioned at 4 microns, placed on glass slides and stained with hematoxylin and eosin (H&E). Slides were scanned by Trestle Corporation at .32 microns/pixel resolution, the various lesions traced using annotation software, and the gross and digital images compared to T1-CE MR images.

3. RESULTS

3.1 HUS treatment of the prostate gland

3.1.1 Gross pathology—Gross lesions were similar in all dog prostates treated with HUS. Within 3 hrs of treatment delivered via transurethral or interstitial applicators, the dogs were euthanized and the prostate glands were removed and sliced into ~5 mm thick axial slices. Figure 2 depicts one representative gland following removal. The gland appeared normal. Following slicing, HUS lesions consisted of relatively discrete regions of dull tan, grey or black parenchyma, while the unheated tissues remained pale beige (Figure 3, top row). Following staining with the vital dye, TTC, the areas subjected to the highest degree of thermal treatment remained pale grey in color, were often bordered by a variably thin zone of pale pink tissue, while the surrounding untreated, normal tissue stained bright red (Figure 3, bottom row).

3.1.2 Microscopic analysis—The TTC stained sections of prostate were routinely processed for histopathology and stained with H&E. The histopathological results from our experiments were similar in all dog prostates treated with HUS. Referring to the T1-CE MRI in Figure 4, we first examined the center of the non-enhancing region (#1). At low magnification, glandular parenchyma and stroma appeared relatively normal. At higher magnification, however, the tissues directly targeted by the ultrasound applicators were very

abnormal. This tissue appeared “heat fixed”, ie, although the glands maintained normal histoarchitecture, epithelial cells had an increased affinity for eosin staining, and most cell nuclei appeared smaller and more hyperchromatic than normal glands. Similar nuclear changes were noted in the interstitial vascular endothelium and smooth muscle cell nuclei. Within the “heat fixed zone” (HFZ) (15), subtle changes consistent with cellular thermal damage/coagulation included the following: loss of cytoplasmic “granularity” typical of normal canine prostatic epithelium, thin, elongated nuclei in slightly elongated epithelial cells, minimal, discontinuous separations between epithelial cells and basement membranes, minimal pyknotic debris, and mild interstitial edema. A total lack of TTC staining on fresh prostate slices correlated with these heat-fixed regions. Furthermore, many blood vessel walls had a glassy appearance (hyalinization), consistent with severe smooth muscle cell damage (Figure 5).

Next we examined areas within or just outside the brightly enhancing ring (Figure 6A, #2 and Figure 7A, #3). At the lowest magnification, this tissue formed the outer edge of the ablated tissue area and was characterized by dramatic disruption of the normal histoarchitecture. Fragmentation of the glands in these areas was severe. Most glands were devoid of any attached epithelial lining cells and had lumina filled with sloughed epithelial cells and granular eosinophilic debris (Figure 6C). We referred to these areas as the fragmented or transition zone (TZ) (15). The interstitial connective tissue in these regions contained congested capillaries and small venules, and collagen fibers separated by clear spaces, consistent with acute interstitial edema. Pale pink TTC staining on fresh prostate slices correlated with the TZ.

3.1.3 Comparison of T1-CE MR images with gross and microscopic pathology

—CE MR images of ablated prostate correlated well visually with TTC stained fresh tissues and with lesions examined histologically (Figures 4, 6 and 7). The non-enhanced regions represented the most severely damaged prostate tissue – the HFZ, while enhancing tissue outside of the HFZ, corresponded to the TZ. In this area, vascular permeability and congestion promoted contrast agent uptake.

3.2 Cryoablation of the prostate gland

3.2.1 Gross pathology—A representative prostate treated with cryoablation is depicted in Figure 8. Hemorrhage was noted on the capsule because the lesions were created using periprostatic placement of the cryoprobes, and all dogs treated in this manner had similar areas of capsular hemorrhage. Coronal slicing of the prostate revealed the depth of the diffuse hemorrhage extending from the lateral capsule medially to mid-gland (Figure 9, top row). In all dogs, these large hemorrhagic regions consistently corresponded to the frozen/thawed tissue. Post-TTC staining, the hemorrhagic regions turn black, surrounded by red viable tissue. A very thin outer rim of pink staining could be noted in some dogs (Figure 9, bottom row).

3.2.2 Microscopic analysis—Referring to the T1-CE MRI in Figure 10, we first examined the center of the non-enhancing region (#1). There was obvious loss of the normal histoarchitecture and severe coagulative necrosis of all the glands in this area. Even

scaffold-like remnants of basement membranes were not apparent. Hemorrhage was present throughout and few intact capillaries or blood vessels were detected in the most frozen tissues (Figure 10C).

In the bright CE rim (Figure 11, #2), gland epithelium was recognizable, but still severely damaged. Basement membranes appeared intact, and a few basal cells were present. Just outside the bright CE rim (Figure 12, #3), the glands remained disrupted, but most displayed sub lethal damage. Many epithelial cells in this region exhibited morphologic changes consistent with apoptosis (shrunken, rounded nuclei and cytoplasmic hypereosinophilia).

3.2.3 Comparison of T1-CE MR images with gross and microscopic pathology in cryoablated prostates—T1-CE MR images of cryoablated prostate correlated well visually with TTC stained fresh tissues and with lesions examined histologically (Figures 10-12). The non-enhanced regions represented severely damaged prostate tissue, diffuse hemorrhage, coagulative necrosis, and destruction of blood vessels, while the enhancing bright ring contained sub lethally damaged glands and intact capillaries.

3.3 T1-CE MR images of pre-existing prostate pathology

Certain common pathological conditions or underlying structural differences in the prostates of these dogs could be detected on T1-CE images. For example, in Figure 13 A, the particularly bright area of T1-CE MRI represented an area primarily composed of connective tissue and smooth muscle. Several thin smooth muscle trabeculae, outlined in blue on the histological section, also enhanced on the T1-CE image. In Figure 13 B, a non-enhancing focus (surrounded in red) represented an area of benign cystic prostatic hyperplasia.

4. DISCUSSION

MRI has proven to be a useful tool to visualize probe placement and delivery of temperature-based therapy. Various methods such as Gadolinium contrast enhancement, diffusion weighted images and thermometry are all useful to determine the size of the lesions created, but none can accurately depict the severity and extent of damage done at the cellular level. We have used the dog model to optimize conditions for the minimally invasive cancer treatments, HUS and cryoablation, and to better understand the acute tissue damage that occurs in the normal dog prostate subjected to these treatments through the use of T1-CE MRI and histopathology.

The ability to predict the degree of irreversible tissue damage that results using these treatments can only be understood by examining the tissues grossly and microscopically. For immediate evaluation of gross lesions on freshly sliced prostate, we find that TTC staining can be a useful tool. In HUS treated prostates, the gross lesions are dry, depressed, and tan/grey in color surrounded by a hyperemic rim. Staining with TTC enhances visualization of viable (red) from non-viable (no stain) tissues, and provides an immediate correlation between these fresh tissue slices and the T1-CE MR images. This method is sensitive, easy to use and gives reliable results. We have found, however, that TTC is less useful when used on cryoablated tissues due to the hemorrhagic nature of the freeze/thaw lesions. In addition,

because TTC only works on fresh tissues, the prostate must be sliced immediately after removal. Accurately cutting fresh prostate into slices of uniform thickness can be challenging. The prostate gland is surrounded by a dense connective tissue and smooth muscle capsule – and even when a completely normal, unfixed dog prostate is sliced, the soft glandular parenchyma bulges outward, distorting the cut surface. In cryoablated prostates, the freeze/thaw technique creates lesions that are very viscous and soft, making slicing even more difficult. The use of our custom designed prostate slicing device has greatly improved our ability to obtain relatively even, ~ 5 mm thick fresh prostate slices, and provides better samples for histopathology and better correlation with T1-CE images.

Microscopic findings in thermally ablated tissues in animals and humans have been described, but many lack mention of subtle cellular changes described in these studies (8, 10, 16-19). In HUS treated prostates, the heated tissue appears “normal” at low power, and is generally surrounded by a more prominently affected zone in which the histoarchitecture is severely disrupted (15, 20, 21). In the center of the lesions (or those areas subjected to the highest thermal dose), the high temperatures coagulate cellular structural proteins and proteinaceous secretory granules, and the extracellular matrices undergo hyalinization. In addition, functional intracellular enzymes are denatured preventing dissolution of the injured cells by endogenous cellular lysosomes, hence, preventing the appearance of classic “coagulative” necrosis (22). High temperatures are also responsible for peracute vascular damage and subsequent thrombosis (23, 24). This obstructs the afferent blood flow to the HFZ, limits edema within the central-most area of the targeted region, and prevents the influx of inflammatory cells that participate in resolution of tissues that have undergone more typical coagulative necrosis. The maintenance of “normal” prostate histoarchitecture in the HFZ can be deceiving. In one report in which patients with liver tumors were treated with thermal ablation followed by liver transplants, lesions in the removed liver tissue were described as areas of “thermal fixation” while surrounding tissues were replaced by fibrous connective tissue (scar tissue) (21). Furthermore, in these chronic studies, multinucleated giant cells were often found surrounding the HFZs in what resembled a foreign body response, or a variant of the inflammatory response used to remove substances resistant to normal degradation. Because we examined the prostates during the acute phase of tissue damage, however, inflammatory cells were not seen, even in the TZ where blood flow remains in tact.

Acute lesions caused by cryoablation have a dramatically different appearance from the HUS lesions, both grossly and histopathologically. Acute cryo lesions are moist, soft, gelatinous, dark red, and easily distinguished from adjacent normal gland. At the microscopic level, the normal gland histoarchitecture was completely lost and replaced by diffusely necrotic cells and ruptured capillaries. At the outer edge of the necrotic lesions, gland outlines and some intact epithelial cells remained admixed with epithelial cells that had undergone apoptosis. Under physiological freezing, cell death occurs by several mechanisms at temperatures below -40°C including intracellular ice crystal formation (25). However rapid tissue freezing to temperatures below -15°C also results in intracellular ice crystal formation, lethal damage to epithelial and endothelial cells, and acute coagulation necrosis, ischemia and hemorrhage. At temperatures above -15°C , apoptosis may contribute

to cell death. *In vitro* studies support a role for apoptosis as an additional means of cell death (26), but warmer temperatures that result in apoptosis, may not be adequate to destroy basement membranes or basal cells, hence allowing regeneration of epithelial cells (27).

Finally, we have shown that pre-existing pathologies can be detected on T1-CE images in our dog model. Normal structures such as smooth muscle trabeculae enhance, as do areas of chronic prostatitis where interstitial fibrosis and inflammation displace glands. On the contrary, areas of benign cystic hyperplasia composed of dilated viable glands do not enhance. Both chronic prostatitis and BPH are common conditions in man, and familiarity with the T1-CE MRI profiles of these pre-existing conditions can facilitate interpretation of treatment images.

5. SUMMARY

In both the HUS and cryoablated prostates, T1-CE images depict large non-enhancing lesions surrounded by rims of brightly enhancing tissue. In general, the non-enhancing regions correspond histologically to regions of extensive cell death and severe vascular damage. Furthermore, the enhancing rim seen by T1-CE MRI correlates with the TZ in both treatment types, and histologically represents variability in the degree of cell damage. Chronic studies are currently underway to better understand the ultimate fate of damaged prostate glands in the T1-CE bright rim and to understand how lesion characteristics and size change over time. The combination of T1-CE MRI, TTC staining of fresh tissue, and histopathology provide the ideal combination of methods to answer these questions and to evaluate the effectiveness of these cutting edge ablation technologies.

ACKNOWLEDGMENTS

We would like to thank our animal health technicians, Diane Howard and Wendy Baumgardner, and our histotechnologist, Pauline Chu.

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Figure 1.

Custom designed Plexiglas slicing platform. The L-shaped platform contains a $4.5\text{ cm} \times 4\text{ cm}$ square depression that is 5 mm deep. The prostates fit into this area and can be held securely for slicing into ~5 mm thick sections.

Figure 2.
Dog prostate following HUS treatment.

**Fresh
Tissue**

2,3,5 triphenyltetrazolium chloride (TTC)

Figure 3.

Top row – freshly sliced prostate gland with well demarcated HUS thermal lesions. Bottom row – same sections following TTC staining.

Figure 4.

Comparison of CE MRI, gross and microscopic images of HUS treated prostate. A) T1-CE, #1 non-enhancing region corresponds histopathologically to the HFZ (B and C). Normal dog prostate gland histology for comparison (D).

Figure 5.
Heat damaged blood vessels have a hyalin or glassy appearance.

Figure 6. Comparison of CE MRI, gross and microscopic images of HUS treated prostate. A) T1-CE, #2 bright enhancing regions correspond histopathologically to severely fragmented glands, capillary congestion and interstitial edema (B and C).

Figure 7. Comparison of CE MRI, gross and microscopic images of HUS treated prostate. A) T1-CE, #3 outer edge of bright enhancing region corresponds histopathologically to less severely fragmented glands, capillary congestion and interstitial edema (B and C).

Figure 8.
Prostate treated with cryoablation.

Figure 9.

Top row – freshly sliced prostate gland with hemorrhagic cryoablation lesions. Bottom row – same sections following TTC staining.

Figure 10.

Comparison of CE MRI, gross and microscopic images of cryoablated prostate. A) T1-CE, #1 non-enhancing region corresponds histopathologically to coagulation necrosis and zone of cell death (B and C).

Figure 11.

Comparison of CE MRI, gross and microscopic images of cryoablated prostate. A) T1-CE, #2 bright enhancing regions correspond histopathologically to markedly damaged epithelial cells with persistence of gland outlines (B and C).

Figure 12.

Comparison of CE MRI, gross and microscopic images of cryoablated prostate. A) T1-CE, #3 outer edge of bright enhancing regions correspond histopathologically to less damaged epithelial cells, persistence of gland outlines, and a few apoptotic cells (arrow) (B and C).

Figure 13. Comparison of CE MRI and microscopic images of normal structures or pre-existing pathologies. A) Enhancing areas correspond histologically to areas of dense connective tissue and smooth muscle. B) Non-enhancing area (outlined in red) corresponds histologically to areas of benign cystic hyperplasia.

Figure 14.

Comparisons of T1-CE MR images (A), TTC stained prostates (B), subgross (C), and microscopic (D) images of dog prostates subjected to cryoablation (top row) or HUS (bottom row). TZ – transition zone, HF – heat fixed zone, Coag – coagulation necrosis.