Evaluation of Subacute and Chronic Cryotherapy Lesions Using Histopathology and Contrast Enhanced MR Images in the Dog Prostate Model

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Introduction

Minimally invasive thermal-based treatments, such as cryosurgery, are options for the treatment of Benign Prostatic Hyperplasia (BPH) and prostate cancer. We have used MRI to monitor delivery of this treatment in a canine prostate model and use contrast enhanced (CE) T1-weighted images compared with the histopathology of acute lesions in order to better predict the extent of target tissue ablation in vivo. In this study, we created cryolesions using two different treatment protocols and analyzed the lesions at three different post-treatment intervals with CE MR images and histopathology in order to understand the MRI appearance of subacute to chronic lesions and the potential for tissue regeneration in the prostate.

Materials and Methods

Three adult male beagles were anesthetized and placed in the 0.5T Signa open MRI system. Cryolesions were created in each prostate using 17 g cryoprobes, and two treatment protocols, protocol A (PA) and B (PB):

- **PA**: Dog’s left side, 2 freeze-thaw cycles, 2 probes, “Hard” freeze – 40°C
- **PB**: Dog’s right side, 1 freeze-thaw cycle, 1 probe, “Soft” freeze – 10°C

A total of 6 cryolesions were created bilaterally in the 3 canine prostates. The sizes of the acute cryolesions were measured with CE T1-weighted images after administration of gadolinium. All dogs were recovered from anesthesia with no peri-procedural complications. At the end of each different post-treatment interval, the prostate lesions were re-evaluated with CE T1-weighted images, and the dogs were euthanized. Dog 1 was sacrificed after 4 days, dog 2 after 14 days, and dog 3 after 53 days. The prostates were excised, sliced, measured and photographed, then transferred to 10% buffered neutral formalin, processed for routine paraffin embedding, and stained with hematoxylin and eosin (H&E) and a Trichrome stain. Most slides were scanned by CLARiENT, Inc. or Biolmage Inc. for digital analysis.

Results

Post-treatment CE images of the acute cryolesions demonstrated large non-enhancing areas surrounded by an enhancing rim (Fig. 1 A). Lesions created with PA were consistently larger and less enhanced than the lesions created with PB. At 4 days post-treatment, pre-euthanasia CE images revealed marked shrinkage in lesion size and gradual reduction in non-enhanced areas (Fig. 1 B). At necropsy, peri-prostatic fat was adhered to the capsule and displayed evidence of fat necrosis and mild inflammation. On cut surface, parenchymal lesions were dark red-purple with sharply demarcated borders (Fig. 1 C). Histology of an entire slice through the 4 day prostate corresponds with the level of the MR images and demonstrates 2 discrete lesions (Fig. 1 D). The PA lesion has a large central area of hemorrhage and gland necrosis surrounded by a narrow rim of regenerative glands, mild mixed inflammation, neocapillary and fibroblast proliferation (Fig. 1 E), while the PB lesion has minimal central necrosis and a wide rim of regeneration (Fig. 1 F).

Discussion and Conclusions

MRI provides the tool to monitor not only the delivery of minimally invasive cancer treatments to particular target tissues, but also the ability to monitor the progression of in vivo tissue changes post-treatment. The canine prostate gland has tremendous regenerative capacity, and the extent to which the normal glands regenerate after treatment may serve as an indicator of the initial success of the ablation. In this study, two cryoablation protocols were used, PA using two freeze-thaw cycles and a minimum temperature of -40°C, and PB using just one freeze-thaw cycle and minimum temperature of -10°C. Gland regeneration within a hemorrhagic interstitium was evident diffusely in the PB protocol treated lesion as early as day 4 while hemorrhagic necrosis persisted in the PA treated lesion. Significant gland regeneration and extracellular matrix remodeling were noted in the PB lesion by day 14, while hemorrhagic necrosis persisted in the PA lesion. By 53 days post treatment, lesions created by both protocols were contracted, consisting of fibrosis, smooth muscle proliferation, and varying degrees of gland regeneration. Noticeably, however, the center of the PA lesion was predominantly devoid of any viable glands, and regeneration was present only at the lesion edges.

In conclusion, comparison of CE MR images with histopathology in the canine prostate cryoablation model provides reliable documentation of cell killing and overall treatment success. Evaluation of acute, subacute and chronic lesions delivered by two different cryoablation protocols has demonstrated that even lesions created with -40°C and two freeze-thaw cycles may regenerate. Therefore, temperatures less than -40°C and increased numbers of freeze-thaw cycles should be used when treating cancer in the prostate in order to guarantee irreversible cell damage and more complete ablation of the target tissue.

References and grant support