Initial Evaluation of a Novel Modulated Radiofrequency-based Bladder Denervation Device

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OBJECTIVE To determine if targeted and modulated radiofrequency ablation (RFA) of the urinary bladder using our novel ablation device (Denerblate) reduces bladder nerve density, potentially leading to a novel strategy for the management of overactive bladder.

METHODS Fifteen pigs were divided into 4 groups: control ($n = 3$), 1-week ($n = 4$), 4-week ($n = 4$) and 12-week ($n = 4$) survival times. Denerblate was deployed on the trigone area of the bladder. Three 240-second cycles of modulated RFA were applied with 30 seconds between cycles. At the end of each survival term, urinary bladders were harvested for histopathologic evaluation. Nerve count and density were manually calculated.

RESULTS All procedures were successfully completed, and all animals survived to the desired time points. Mean nerve density (nerves/mm^2) was highest in the control and 1-week survival group compared to the 4-week and 12-week groups, both of which demonstrated significant diminishment. Nerve density in the bladder neck at control, 1 week, 4 weeks, and 12 weeks were 1.8, 1.35, 0.87, and 0.12, respectively ($P < .001$). Nerve density in the bladder trigone area at control, 1 week, 4 weeks, and 12 weeks were 1.5, 0.98, 0.65, and 0.112, respectively ($P < .001$). Epithelial heat injury was observed in 14.3% at 1 week, 10.7% at 4 weeks, but completely resolved by 12 weeks.

CONCLUSION In the porcine model, modulated RFA delivered by our novel device reduced nerve density in the bladder neck and trigone by 88.6% and 88.9% at 12 weeks without evidence of lasting epithelial injury. UROLOGY 134: 237–242, 2019. Published by Elsevier Inc.

In the United States and in European countries approximately 16% of the population suffer from symptoms of overactive bladder (OAB), with prevalence increasing with age.[1,2] This translates to approximately 50 million patients annually in the US who need treatment for OAB.

There is no uniform treatment strategy for the management of symptoms associated with OAB.[3] A variety of treatment modalities are available, including behavioral modifications (first-line) and pharmacotherapy such as anticholinergic or beta-adrenergic agents (second-line). In addition to the significant systemic side effects of anticholinergic medications, in many cases these medications are not effective, which often results in the termination of treatment and deterioration of patient quality of life. In patients with symptoms refractory to behavioral and medical therapy, third-line therapies such as sacral neuromodulation (SNM), intradetrusor injection of Botulinum toxin A (Botox), or percutaneous tibial nerve stimulation may be considered. Although Botox injection, SNM and percutaneous tibial nerve stimulation have been shown to improve symptoms, they are associated with significant cost and often have only short term effects and require repeat treatment sessions.

Radiofrequency ablation (RFA) utilizes electrodes to generate a high-frequency, alternating current that flows from the electrodes to tissues. RFA has numerous clinical applications, which range from complete localized tissue destruction to more modulated applications for selective ablation of tissues.[4,5] Specifically, RFA is used in the treatment of arrhythmias, a variety of tumors, and nerve ganglion ablation.[6-8] Moreover, modulated RFA has been used for denervation of the sympathetic nervous supply around the renal arteries in patients with treatment-resistant reno-vascular hypertension.[9]

In order to assess RFA as a potential treatment modality for OAB, we developed a novel device, Denerblate, and an energy-based algorithm to safely and effectively deliver modulated radiofrequency (RF) energy transurethrally to the bladder trigone. The primary goal of this study was to evaluate the feasibility and safety profile of the Denerblate

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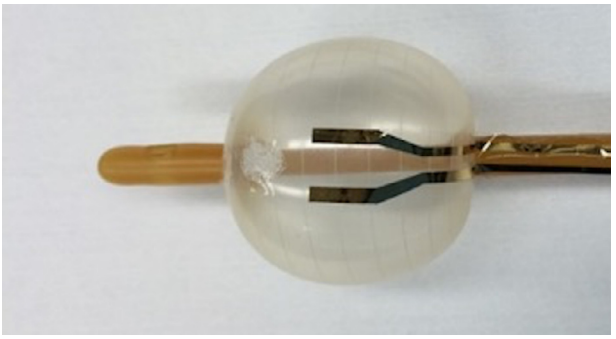

Figure 1. The prototype device with the radiofrequency antenna fabricated on an ultrathin Teflon film (<20 um in thickness) and mounted on the balloon. (Color version available online.)

device. In addition, we sought to determine the efficacy of Denerblate in regards to diminishing nerve density in the trigone area of the urinary bladder, which is the area of highest nerve concentration.[10] To the best of our knowledge, our novel concept of intravesical bladder denervation using modulated RF energy has not been previously evaluated.

METHODS

Development stage: Our primary goal was to develop and test an expandable, intravesical balloon for selective bladder denervation. In collaboration with a team of electrical engineers (RM, YZ, and GPL), we designed a series of micro antennae for targeted delivery of RF energy. A conventional Foley catheter balloon was used to carry out our initial design tests. The balloon was selectively covered with flexible electrodes capable of targeted RF application to the bladder trigone (Fig. 1). The ultraflexible antennae allowed for seamless contact between the antenna and the bladder wall. The electrodes can be multiplexed to address specific nerve structures for minimal collateral injury to healthy tissues. The electrical impedance of the bladder surface can be measured and calibrated for the most effective RF setting specific to the individual patient.

Experimental design: Approval from the Institutional Animal Care and Use Committee at the University of California, Irvine was obtained in order to conduct the study experiments. Two phase animal studies were carried out for the evaluation of the Denerblate: a nonsurvival and a survival animal test. A total of 15 domestic Yorkshire pigs were obtained for the nonsurvival (3 pigs) and survival (12 pigs) phases of the study.

In-vivo nonsurvival experiments: The purpose of the nonsurvival animal test was to develop an energy algorithm to optimize the modulated RF settings with the goal of maximizing diminution of nerve tissue while minimizing injury (eg, inflammation, hemorrhage, and scarring) to surrounding bladder tissue. We used 3 Yorkshire pigs (25 kg) in order to test these variables. After induction of general anesthesia, open laparotomy was performed to expose the urinary bladder, and the anterior bladder wall was exposed and dissected. The RFA balloon was positioned onto the bladder trigone area, and energy was applied at different settings. We used Valleylab Force and FX electrosurgical generators (Force FX; Valleylab, Tyco Healthcare Group LP, Boulder, CO) at power levels 1, 2, 3, 5, and 10. A thermal camera (FLIR E5, FLIR Systems, Boston, MA) was utilized to monitor the temperature during modulated RFA testing procedures. Multiple energy and deployment time settings were tested. After testing, the bladders were harvested, and the nerve density was evaluated histopathologically. These data were used to establish the algorithm for optimal energy settings, deployment time, tissue temperature/impedance, and number of RFA cycles; this algorithm was used for the subsequent survival studies. Histopathologic slides obtained from the intact trigone area of these 3 animals served as the control group for the study.

In-vivo survival experiments: For the survival phase of the study, a total of 12 domestic Yorkshire pigs were obtained. The animals were divided into 3 groups. Groups 1, 2, and 3 each consisted of 4 animals and were assigned to survival periods of 1 week, 4 weeks, and 12 weeks, respectively. None had a history of urinary tract intervention, urinary tract malignancy, urinary tract infection, or any genitourinary condition. All animals were monitored in the vivarium for potential postoperative complications including bleeding, urinary retention, or signs of urinary tract infections.

Surgical procedure: After animal sedation, the Denerblate prototypes were deployed as a standard catheter into the bladder, and the balloon was inflated with 30 mL of sterile water. In groups 2, 3, and 4 there was no open exploration. Following complete drainage via the catheter, the bladder was filled with 350 mL of normal saline; this was standardized to all experimental animals in order to sufficiently distend the bladder for optimal catheter placement. Gentle traction was placed in order to position the balloon on the trigone area of the bladder. The electrical impedance of the bladder surface was measured and calibrated for the most effective RFA specific to each individual animal. After balloon deployment, the Denerblate was activated with three 240-second treatment cycles, with 30-second rest intervals between each cycle. After completing 3 cycles, the balloon was deflated and removed.

Group 1: Evaluation of acute tissue reaction in nonsurvival group: Four animals in this group underwent transurethral RFA of the urinary bladder as described. Animals were euthanized 1 week after the procedure and urinary bladders were collected for histopathologic evaluation of RFA lesions.

Group 2: Evaluation of short-term tissue reaction to RFA in survival group: Four animals in this group underwent transurethral RFA of the urinary bladder as described. Following RFA of the urinary bladder, animals were recovered from the procedure and kept *in vivarium* for 4 weeks. Four weeks after the procedure, animals were euthanized, and urinary bladders were collected for histopathologic evaluation of RFA lesions.

Group 3: Evaluation of long-term tissue reaction to RFA in survival group: Four animals in this group underwent transurethral RFA of the urinary bladder as described. Following RFA of the urinary bladder, animals were recovered from the procedure and kept *in vivarium* for 12 weeks. Twelve weeks after the procedure, animals were euthanized, and the urinary bladders were collected for histopathologic evaluation of RFA lesions.

Anatomic dissection and gross tissue preparation: An experienced anatomist harvested the bladders and procured intact urethra and bladder specimens. Transverse sections were made at 2 mm intervals starting at the distal urethra and extending to the dome of the bladder. Specimens were placed in standard cassettes with 10% formalin buffered solution for paraffin embedding. A total of 10 slides were obtained from each bladder including the ablation zone.

Histologic evaluation: Histologic sections were prepared by baking at 60°C for 30 minutes. We performed endoperoxidase

block in 70% methanol/50% hydrogen peroxide solution for 10 minutes. After a thorough rinsing with purified water, we pretreated the slides using citrate buffer (pH = 6.0) for 25 minutes at 90°C in a vegetable steamer. We then incubated the slides for 45 minutes at room temperature with rabbit S100 antibody. This was followed by rinsing with Phosphate Buffered Saline with Tween 20 (PBST) and incubation with antirabbit secondary antibody for 30 minutes at room temperature. We again rinsed the slides with PBST, and the antirabbit antibodies were detected with a Betazoid DAB Chromogen Kit. Finally, the sections were rinsed with water, counterstained with hematoxylin, dehydrated through graded ethanol, cleared with xylene, and cover-slipped.

All images were subjectively and objectively reviewed by a fellowship-trained genitourinary pathologist (TL), who was blinded to the animal groups. Nerves were marked and manually counted for each slide. The pathologist subjectively assessed and characterized epithelial injury, hemorrhage, acute inflammation, chronic inflammation, edema of the muscularis propria and lamina propria as none, mild, moderate, or severe.

Statistical analysis: We used analysis of variance (*F* test, Kruskal-Wallis Test) to test for the differences among the groups. We used SYSTAT statistical software, version 13.0 to analyze the data (Systat Software, Inc., San Jose, CA). Statistical significance was defined as *P* value <.05.

RESULTS

In vivo nonsurvival experiments: All 3 animals completed the study and there were no intraoperative complications related to use of Denerblate. The main purpose of the in vivo nonsurvival study was to identify the optimal engineering parameters including energy level and duration. The experimental setup is shown in Fig. 2. The initial deployments and temperature measurements from group 1 demonstrated that the safest energy threshold was RF power level 1, which maintained the local temperature below 50°C during three 240-second cycles of treatments. This power level caused the least amount of coagulation necrosis while still achieving the depth of penetration required to affect the nerves (Fig. 3). The mean width and length of the treatment area was 4 mm (±0.9 mm) and 12 mm (±1.0 mm), respectively.

In vivo survival experiments: All 12 animals completed their survival terms. There were no intraoperative or postoperative complications related to the procedure or the application of RFA.

Nerve density changes: Density of nerves per mm^2 in the bladder neck in the control, 1-week, 4-week, and 12-week groups was 1.8, 1.35, 0.87, and 0.12, respectively (*P* <.001). Nerve density in the bladder trigone area in the control, 1-week, 4-week, and 12-week groups was 1.5, 0.98, 0.65, and 0.112, respectively (*P* <.001).

Distance of nerves to urothelium: In the control group, there was no statistically significant difference in nerve distance-to-urothelium in the bladder trigone compared to the bladder neck (2.2 vs 2.1 mm, *P* = 3.14). Mean nerve distance-to-urothelium in the bladder trigone area in the control, 1-week, 4-week, and 12-week groups was 2.2, 2.2, 2.6, and 3.6 mm, respectively (*P* = .03). In the bladder neck, mean nerve distance-to-urothelium in the control, 1-week, 4-week, and 12-week groups was 2.1, 2.1, 2.4, and 3.2 mm, respectively (*P* = .01).

Subjective assessment of histopathologic slides is demonstrated in Table 1.

COMMENT

OAB symptoms result from overactivity of the detrusor muscle. Given that the bladder is a smooth muscle organ innervated by the central nervous system, the pathophysiology of OAB is neurogenic and/or myogenic in origin. Common neurologic etiologies of OAB include injury to axons in the spinal cord, increased afferent nerve activity, loss of peripheral inhibition, and enhancement of excitatory neurotransmission in the micturition reflex pathway. The myogenic theory is mostly applicable to patients with bladder outlet obstruction; an increase in intravesical pressure causes partial neurologic dysfunction of the bladder smooth muscle. Thus, rather than synchronized detrusor contraction that empties the bladder, spontaneous action

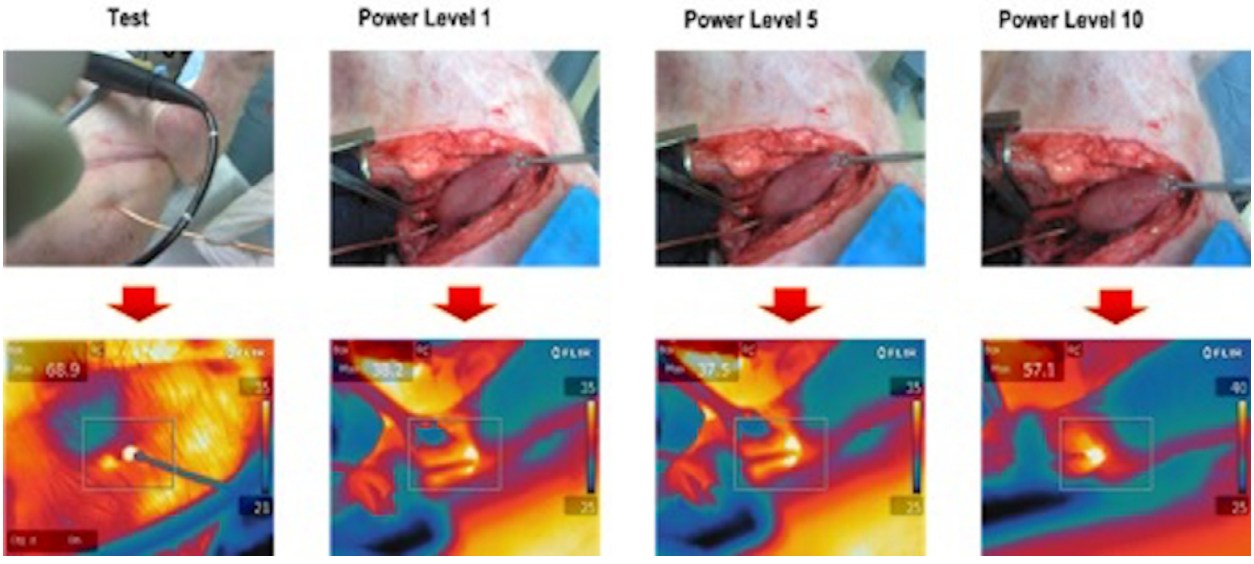

Figure 2. Experimental in vivo nonsurvival setting for testing different levels of radiofrequency settings (power levels 1, 5, and 10). (Color version available online.)

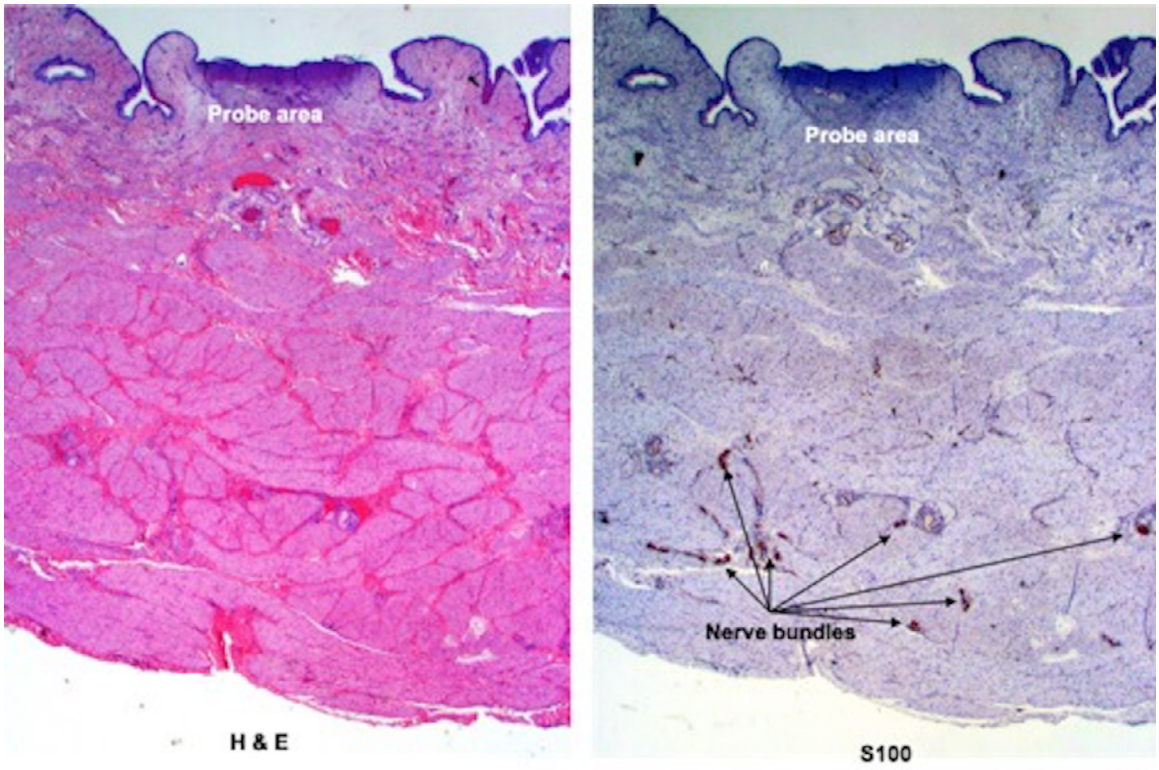

Figure 3. Histopathologic images. Location of the probe on bladder epithelium. Nerve bundles are labeled in the immunostained (S100) slide. The nerves are ~2.0-3.00 mm from the mucosa. (Color version available online.)

Table 1. Subjective histologic assessment of epithelial injury at 1 week, 4 weeks, and 12 weeks

Epithelial Injury	*1 Wk (*n*-40) %	4 Wk (*n*-40) %	12 Wk (*n*-40) %
None	19.0	66.7	77.7
Mild	19.0	27.3	22.3
Moderate	47.6	3.6	0.0
Severe	14.3	2.4	0.0
Total	100	100	100
Epithelial hemorrhage	1 wk %	4 wk %	12 wk %
Absent	66.7	85.7	100.0
Present	33.3	14.3	0.0
Total	100	100	100
Nerve density change	1 wk %	4 wk %	12 wk %
No	69.5	0.0	0.0
Yes	30.5	100.0	100.0
Total	100	100	100
Nerve distribution change	1 wk %	4 wk %	12 wk %
Normal	9.5	0.0	0.0
Mild loss	71.4	100.0	100.0
Severe loss	19.0	0.0	0.0
Total	100	100	100

* 4 animals in each group; 10 histologic slides per animal.

potentials are generated, causing "micromotions" of the detrusor smooth muscle. These micromotions increase intravesical pressure and stimulation of afferent receptors in the detrusor smooth muscle, resulting in the symptomology associated with OAB.

Currently, the standard of care for OAB consists of oral medications such as anticholinergics. These medications have potentially harmful systemic side effects that may worsen rather than ameliorate the physical burden faced by patients with OAB. Additionally, a significant number

of patients do not experience improvement with first- and second-line therapies, as approximately 5% of all OAB patients progress to third-line therapy.[11] Intradetrusor injection of Botox reduces OAB symptoms for approximately 6 months, and repeat injections have shorter effect durations, with the majority of patients discontinuing injections due to lack of efficacy after 6-7 treatment cycles. Botox injections are also associated with urinary tract infections and urinary retention. SNM consists of surgical placement of a lead and generator, which must be periodically exchanged and may be associated with pain, electric shock, infection, and relocation of the lead. Due to the limitations of the existing third-line treatment modalities, a significant number of patients with OAB receive suboptimal treatment. An alternative minimally invasive treatment with tolerable and durable effects would be an asset to OAB management.

The aim of the current study was to test the feasibility and safety of a novel denervation device. Based on the results of our animal study, the Denerblate is a safe and effective device for minimally invasive, selective bladder denervation using modulated RFA. The optimal energy settings demonstrated in this study was RFA level 1 applied for 3 cycles (240 seconds per cycle with 30 seconds off time in between cycles) at a temperature of 40°C-50°C. Regarding the safety of the Denerblate, the greatest risk was the potential for injury to the urinary tract epithelium. However, we observed that local RFA of the bladder caused minimal epithelial changes. At 12 weeks postablation, histologic examination revealed that the epithelium had recovered in all 12 animals, with no epithelial hemorrhage, inflammation, or edema, and only mild epithelial injury remained in a quarter of specimens. There were no indications of permanent injury to the epithelium, and there were no adverse events nor complications associated with the RFA or the procedures. These experiments demonstrated that modulated RFA can be applied to the bladder without significant injury to the bladder epithelium and muscle tissue.

As for the efficacy of the Denerblate, nerve density in the affected area had diminished in all of the specimens by 4 weeks postablation, with an 89% reduction in nerve density at the 12-week time point. Specifically, there was a gradual and statistically significant decline in nerve density that was positively associated with time from ablation. Additionally, an abnormal nerve distribution was observed in all specimens after 4 weeks. Normal nerve density was observed in approximately 30% of specimens at 1 week postablation and in no specimens at 4 weeks postablation, indicating that the denervation effect of RFA takes longer than 1 week to fully manifest.

Modulated RFA has previously been described for nerve ablation in the renal arteries.[12] Localized RF energy allows for denervation without collateral injury to surrounding tissue. In this study, we examined the feasibility of using RFA as a treatment modality for OAB. The minimally invasive nature of RFA may potentially allow for an outpatient, office-based treatment option without systemic side effects. Fugett et al tested RFA using a transurethral device with needle deployment in the trigone of ovine bladders.[13] The RFA caused denervation and some fibrosis with a tissue healing response observed at 12 weeks. However, the investigators did not assess the impact of RFA on nerve density nor distribution. In contrast, our experiments were performed in porcine models with active deployment of a RFA device and histopathologic assessment of postablation changes in nerve density and distribution. Tu et al reported their initial clinical experience with selective bladder denervation of the trigone area in women with refractory OAB. The authors used a cystoscopic device that allowed delivery of RF energy to the trigone area. The probes were placed 3 mm below the urothelium, which was intended to avoid urothelial injury. Postoperative follow-up at 12 weeks demonstrated significant improvement of all OAB symptoms, with a significant improvement of urge incontinence episodes in 79% of patients. Six months following treatment, 70% of patients experienced a 50% or greater reduction in urgency urinary incontinence, 27% of patients reported no incontinence, and 17% had experienced a device-related complication.[14]

Our study is unique in several ways. First and foremost, in order to identify the most appropriate area to target during RFA, we collaborated with clinical anatomists and pathologists and precisely mapped the urinary bladder innervation using a 3-dimensional virtual model.[15] Our model demonstrated that, in cadaveric human tissue, the innervation of the bladder is highly focused in the posterior aspect of the proximal urethra and in the bladder neck/trigone area in both males and females. This allowed us to precisely target the most highly innervated areas in the bladder. Second, we performed an in vivo experiment to evaluate the safety thresholds for modulated RFA in swine urinary bladder. We identified safe energy thresholds, duration, and the number of RFA cycles required to impact the nervous tissue while avoiding significant collateral tissue injury. These initial experiments allowed us to modify the RFA antenna to optimize the energy distribution in the tissue. Furthermore, we objectively quantified the nerve density and distribution by manual marking and histopathologic characterization. To the best of our knowledge, this is the first study to histopathologically assess the effect of modulated RFA on nerve density of live bladder tissue.

There are several limitations to this study. First, we used porcine models that did not have a history of OAB or urinary tract diseases, as we sought to characterize the feasibility of the device and its effect on the epithelial and nervous tissue. Second, ideally, urodynamic studies would be used to observe functional changes before and after modulated RFA application in the bladder. However, measuring intravesical pressures using urodynamic methods was not feasible in swine models. Third, longer term nerve changes were not examined. As previous studies with nervous tissue have shown, injured nerves may regrow, potentially leading to recurrence of OAB symptoms. Future studies are needed in order to characterize the long-term effect of modulated

RFA on nervous tissue and subsequent regrowth of nerves innervating the bladder.

CONCLUSION

We developed a novel modulated RFA device intended for intravesical application at the trigone area. Uroepithelial injury completely resolved by 3 months postablation. Trigone nerve distribution and density decreased significantly after RFA. Our data demonstrate that deployment of the Denerblate RFA device in living swine is feasible and safe.

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