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The Normal Porcine Ureter Retains Lumen Wall Integrity but not Patency Following Catheter Directed Irreversible Electroporation: Imaging and Histologic Assessment Over 28 Days

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Abstract

Purpose—To evaluate the effect of catheter-directed irreversible electroporation (IRE) on the integrity, patency and function of the normal porcine ureter.

Materials and Methods—A catheter-mounted electrode was used to perform fluoroscopy-guided IRE in 8 healthy pigs. Two unilateral ablations (90 pulses at 2000 V, 100 μ s) were performed in each animal in the proximal and distal ureter. Serum creatinine measurements and contrast-enhanced CT imaging was performed at 1, 7, 14, 21 and 28 days post-IRE, and findings were compared to baseline values with the Student's *t*-test. Two animals were sacrificed at 1, 7, 14 and 28 days post-IRE for histological assessment of treatment effects. Quantitative histology of regeneration and healing of the ureteral wall was graded using a five-point scale.

Results—IRE was successfully performed in all animals. Preservation of ureteral wall integrity was confirmed by the leakage free passage of contrast in the treated ureter of all animals through

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the observation period. Ureteral strictures and associated renal pelvicalyceal dilation were observed in all animals by study day 7 ($p=0.005$) and 14 ($p=0.007$), which did not resolve by day 28. Urothelial recovery was observed in tissue samples from day 7, with progressive replacement of the tunica muscularis with granulation tissue. Despite extensive scarring of the tunica muscularis, full recovery of the urothelium was observed by day 28.

Conclusions—The normal porcine ureter retains lumen wall integrity and function following catheter-directed IRE. Scarring of the tunica muscularis in the treated ureter results in stricture formation and reduction of lumen patency.

INTRODUCTION

Nephroureterectomy is considered a potentially curative option for patients with non-metastatic upper urinary tract cancers (UUTC) of urothelial or transitional cell origin¹. However, nephroureterectomy is associated with significant cardiovascular morbidity and reduction of renal function^{2–3}. Prior nephroureterectomy also limits treatment options for patients who subsequently develop contralateral disease⁴. More than 70% of patients diagnosed with UUTC are elderly (median age: 72.8 for females, 70.2 for males)^{5–6}. Surgical options are often limited in this population due to comorbidities, highlighting the need for nephron-sparing non-surgical treatment alternatives.

Ablation with laser or electrocautery is used to treat non-surgical candidates and patients with early-stage or low-grade disease^{7–9}. However, thermal ablation of UUTC is limited by efficacy and safety concerns. Penetrative delivery of thermal energy to achieve complete tumor control can disrupt lumen integrity and lead to perforation^{10–11}. At the same time, conservative energy delivery can negatively impact treatment efficacy; recurrence rates of 31–38% after laser or electrocautery thermal ablation have been reported, compared to surgery, with recurrence rates less than 12%^{7,8}. There is a clear unmet need for minimally invasive techniques that can safely improve local tumor control.

Irreversible electroporation (IRE) is a predominantly non-thermal ablation technique that has been applied in the kidney¹² and prostate¹³. IRE ablation can preserve the integrity of the urinary collecting system, and the urothelial lining in the renal pelvis has been shown to regenerate after ablation¹⁴. However, sharp applicator-based IRE devices are not well suited for endoscopic or focal image-guided ablation of UUTC. A catheter-mounted electrode has been developed for the purpose of performing intraluminal IRE¹⁵. This device can be used under ureteroscopic or fluoroscopic guidance for focal tumor ablation. Previous studies demonstrated acute preservation of normal porcine ureter lumen integrity, even at the highest energy setting evaluated experimentally. Longer-term functional outcomes after endoluminal IRE are unknown, therefore the objective of this study was to evaluate the function, integrity and patency of the normal porcine ureter over a four week period following treatment with IRE.

MATERIAL AND METHODS

Experimental Design

Yorkshire swine was chosen as the experimental model as their upper urinary tract is anatomically similar to humans. The study was designed to replicate findings, in duplicate, in the same animal and also between animals. Two IRE ablations were performed in each animal, and in two animals per time-point (4 ablations sites total per follow-up time point). Based on publications indicating that ablation related complications such as strictures typically manifest within 7–14 days following treatment,^{16–17} the final time point was set at 28 days. The contralateral urinary tract was not instrumented in order to preserve renal function in the event of obstructive uropathy in the IRE-treated ureter.

Animal Preparation and Ablation Protocol

Animal experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee. A total of 8 female swine (35–50 kg) were treated in this study. The animals were sedated with intravenous tiletamine hydrochloride and zolazepam hydrochloride (6 mg/kg; Telazol; Fort Dodge Animal Health, IA) and general anesthesia was maintained with inhaled isoflurane (2% Aerrane; Baxter Healthcare, Round Lake, IL). Following induction of anesthesia, intravenous blood samples were taken preoperatively, and at follow-up imaging time-points for analysis of serum creatinine. Animals were placed in prone position on the fluoroscopy table (Innova, GE Healthcare, Milwaukee, WI). The pelvicaliceal systems were visualized using ultrasound (Prosound, Hitachi-Aloka, Wallingford, CT) and fluoroscopy after intravenous injection of Omnipaque 350 (GE Healthcare, Milwaukee, WI) contrast medium. Following sterile preparation of the skin, unilateral posterior calyces (7 right, 1 left) were accessed percutaneously under either direct fluoroscopic or ultrasound guidance using a 21 G needle. A 9 French 10 cm length valved sheath (Terumo, Somerset, NJ) (Figure 1A) was inserted using Seldinger technique under fluoroscopic visualization to facilitate antegrade pyelography and electrode insertion. A catheter-mounted IRE electrode was then introduced through the sheath.

Irreversible Electroporation Ablation Parameters

Electric pulses (90 pulses at 2000 V, 100 μ s) were delivered between the monopolar electrode (15 mm exposure) and grounding pad affixed to the shaved flank following techniques previously reported by Srimathveeravalli *et al.*¹⁵. These pulse parameters were identified in prior work as the lowest energy setting for uniform, full thickness IRE of the normal porcine ureter¹⁵. A neuromuscular paralytic, rocuronium (0.7–0.12 mg/kg), was administered before delivering pulses. Distal ureter IRE was performed first (Figure 1B), and then the catheter was withdrawn to the more proximal ureter for the second treatment (Figure 1C). The untreated segment between the distal and proximal treatment locations, approximately 5cm in length, was preserved for analysis and served as the experimental control region. Preservation of luminal diameter and urine flow in the immediate post-IRE period was assessed by postoperative antegrade pyelography (Figure 1D). The nephrostomy sheath was then removed and a sterile dressing applied, and the animals were recovered. The nephrostomy tract was not embolized.

Post-Procedural Evaluation

The follow-up imaging protocol included intravenous contrast enhanced CT scans (Lightspeed A6, GE Healthcare, Princeton, NJ). Animals were sedated using techniques described for the ablation procedure. Nephrographic and delayed phase images were obtained by performing image acquisition 2 and 15 minutes after contrast injection. Images were acquired as 5 mm thick slices and reconstructed using a GE Advantage Workstation (ver4.4.). Renal pelvis diameter was assessed as an AP measurement on axial CT images. Hydronephrosis was graded as none, mild, moderate or severe using measures previously described by Malave *et al.*¹⁸. A total of 8 animals were imaged at 1 day, 6 at 7 days, 4 at 14 days and 2 at 21 days and 2 at 28 days post-IRE. Follow-up antegrade pyelography was performed after scheduled CT follow-up in 2 animals at 28 days following IRE using access and visualization techniques as previously described for catheter directed IRE. Safety and other outcomes were described with standard terminology¹⁹.

Two animals were sacrificed at each designated time-point (1, 7, 14 and 28 days post-IRE). Euthanasia was achieved with intravenous administration of pentobarbital sodium (100 mg/kg). During necropsy, both kidneys along with the ureters and bladder cuff were removed en bloc for analysis. The ureters were dissected longitudinally and the exposed mucosal surface was examined to identify the ablated regions. Each ablation zone was further sectioned longitudinally and axially, and preserved in 10% neutral buffered formalin. For each ablated site, samples incorporating the entire ablation zone and adjacent untreated tissue (control regions) were embedded in paraffin, sectioned at 4 μm thickness, and stained with hematoxylin-eosin and Masson's trichrome (for collagen).

Pathology Analysis

Independent interpretation of the immunohistochemical stains was performed by two individuals (S.M.; pathologist and G.S.; investigator). Discrepant interpretations were resolved via consensus review. The slides were evaluated for evidence of necrosis and cell death in samples from acute (1 day) and sub-acute time-points (7 days). Regeneration of the urothelium and tunica muscularis, scar formation and presence of inflammatory cells was evaluated in all samples. The longitudinal tissue sample from each ablation was photographed with an Olympus BX45 microscope using a 20x objective, a DP25 camera, and the cellSens Entry 1.9 software (Olympus Imaging America, Inc, Center Valley, PA). Measurements of maximum length and depth of treated regions were taken from the H&E stained slides. An average of 5 regions of interest, each measuring 567,162 μm^2 , was acquired from each sample. Semi-quantitative scoring followed techniques reported by Srimathveeravalli *et al.*¹⁵, where, on a scale 0– 4, 0 marked complete absence of a finding and 4 marked presence of a finding in the entire field being analyzed. Intermediary scores were graded as, 1 (<25%), 2 (<50%) and 3 (<75%), contingent on increased presence of findings.

Statistical Analysis

Mean, standard deviation and range calculations were performed on the imaging and histological measurements. Anatomic changes related to treatment were compared to

baseline anatomic measurements and statistical significance was evaluated using the Student's *t*-test.

RESULTS

Clinical Outcomes

No cardiac complications were encountered during pulse delivery and there was minimal bleeding from nephrostomy access sites. Overall procedure time was 73 ± 31 minutes, and pulse delivery was completed within 2 minutes at each treatment site. All animals survived through the intended period of observation and time frame (1 to 28 days post-ablation). Mild to moderate post-operative hematuria was observed in all animals, which resolved without intervention within 48 hours. It was not possible to distinguish whether the hematuria was from the ablation or from the nephrostomy access. Veterinary staff monitoring the animals did not observe signs of pain, morbidity or mobility limitations. There was no statistically significant elevation of serum creatinine in animals surviving longer than 7 days ($p > 0.05$). Renal function measured by serum creatinine remained within the normal range for swine (0.8–2.3 mg/dL) in all animals through the duration of the follow-up period (see Table 1).

Imaging Outcomes

Key treatment related imaging findings are summarized in Table 1. Peri-renal urinomas (Supplementary Figure 1), limited to the renal access site used for introducing the catheter, were observed in three animals at the 1 day time-point (animals 1, 2 and 3). There was no evidence of contrast extravasation in the vicinity of the ablation zones in any animal, indicating preservation of lumen integrity and absence of treatment related perforation of the ureteral wall. One urinoma associated with nephrostomy access resolved within 7 days without additional intervention in animal 3, though resolution was not recorded for animals 1 and 2 as they were sacrificed 1 day post-treatment.

Hydronephrosis was not observed in any of the animals on day 1 post-treatment imaging. Compared to baseline, mild to moderate hydronephrosis ($p = 0.005$) and ureteric strictures at the ablation site were observed at 7 days in all surviving animals. Compared to baseline measurements of the renal pelvis, moderate to severe hydronephrosis was observed at 14 days in 4/4 animals ($p = 0.007$) which persisted until euthanasia at designated endpoints. Ureteral and collecting system dilatation occurred above the location of the proximal ablation site where stricture of the ureter was visible in all cases. It was not possible to discern the location of distal ablation on follow-up imaging. Antegrade flow of contrast to the bladder could be observed even in animals with strictured ureters, but with substantial time delay when compared to the contralateral untreated ureter. Figure 2 shows delayed phase CT imaging of the urinary tract from animal 7 (sacrificed 28 days post-IRE). A summary of pooled renal pelvis measurements at different time points compared to baseline measurements is provided in Table 1.

Histopathologic Assessment

Gross examination of resected specimens allowed identification and isolation of all ablated regions and normal ureter adjacent to the treatment zone from all animals (16 ablations).

Ablated tissue obtained from the 1 day time-point revealed grossly darker mucosa when compared to the surrounding untreated mucosa. Dimensions of the treated region corresponded to the electrode length (~15mm) in ablation samples from this time point, with sharp visible boundaries between injured and uninjured mucosa. Ablated tissue obtained from the 14 and 28-day time points appeared pale in color relative to the adjacent normal ureter and with relative narrowing of the ureteral lumen. In gross examination, the ureter adjacent to the treated region did not appear visually different from uninstrumented contralateral ureter in all animals.

Histopathologic findings and sequential measurements of ablation zone size are summarized in Table 1. Histopathologic assessment of the samples obtained from the 1 day time point after ablation showed circumferential urothelial loss and full thickness (mucosa to adventitia) necrosis. Masson's trichrome stains revealed preservation of the collagenous stroma in necrotic regions, with separation of collagen fibers due to moderate edema and hemorrhage. There were no other observable differences in the appearance of collagenous material between treated and adjacent untreated ureter. The extent of scarring of the tunica muscularis within the ablative zone was quantified as grade 0 in all animals. Similarly, no urothelial regeneration (grade 0) within the ablation zone was observed. Although the ablated tissues showed hyperemia and neutrophilic inflammation, ureteral architecture was maintained across all layers and surrounding connective tissue.

From 7 to 28 days, except for one animal, the extent of urothelial regeneration within the ablation zone received a score of 3. Granulation tissue progressively replaced the normal tunica muscularis, and turned into scar tissue seen in samples between days 7 to 28 on Masson's trichrome stains. Scarring of the tunica muscularis was quantified as grade 4 in all animals sacrificed at 14 and 28 days post-treatment. Histologic evolution of urothelial regeneration and healing of the ureteral wall is summarized in Figure 3. Despite scarring, the ureteral wall's integrity appeared intact with no evidence of perforation or other pathology that would indicate disruption of its barrier function. Ureters from the untreated regions appeared histologically similar to uninstrumented normal ureters in all animals.

DISCUSSION

This study demonstrates that ablation of the normal porcine ureter with catheter-directed IRE does not affect integrity of the ureter lumen, and allows leakage-free transit of urine to the bladder. However, post-treatment scar formation at the ablation site reduces ureteral patency, obstructing urine flow and causing hydronephrosis. While the animals did not demonstrate statistically significant alterations in serum creatinine during the observation period, we are unable to draw conclusions about the longer-term functional impact due to the relatively short (28 day) follow-up period. Post-ablation placement of a urinary stent may have prevented this treatment related complication, but this was not verified as part of this study.

A potential advantage of an intraluminal catheter-directed approach compared to standard needle-based percutaneous IRE used in clinical practice today could be improved targeting of ureteral malignancies. The catheter device evaluated here is designed to be compatible for

ureteroscope guided placement and treatment, and this approach can aid device placement and direct visualization of the treatment site. Ureteroscope guided placement may also reduce the risk of urinoma formation, which was one of the complications associated with the percutaneous placement of the catheter into the ureter.

IRE caused cell necrosis in the entire lumen wall by 24 hours while sparing the collagenous acellular components of the treated ureter. This conclusion was based on the interpretation of Masson Trichrome stains where collagenous components of the IRE treated ureter were not histologically different from adjacent untreated ureter. Sparing of the extracellular matrix may have contributed to preservation of lumen integrity in the post-treatment setting.

Histology analysis suggested complete denudation of urothelium in treated regions by 1 day, followed by regeneration by 7 days post-treatment and the complete recovery of the normal urothelial layer by 28 days. Urothelial cells are believed to arise from basal cells that can divide and differentiate into different urothelial cell types such as intermediate, superficial and umbrella cells²⁰. The exact mechanism through which the basal cells repopulate the ablated region and subsequent replenishment of all layers of the urothelium is not clearly understood. Prior studies of urothelial eradication with laser ablation have reported similar findings; mucosa completely denuded of urothelium regenerated as long as contact with adjacent regions with undisturbed urothelium was maintained²¹. In contrast, the tunica muscularis layers of the ureteral wall did not regenerate completely and were replaced with granulation tissue or scar by 28 days post-treatment. Sparing of the extracellular matrix within the treated regions did not promote recovery of the cellular architecture of the ureteral wall or preclude scar formation. Scar formation is commonly observed following percutaneous ablation of tumors in other solid organs, which involutes over time. However, scar formation and involution of the treatment zone adjacent or within a hollow organ may be an important contributing factor in terms of loss of lumen patency. Stricture and genitourinary tract perforation have hindered efforts to advance focal therapy options for UUTC. These types of complications are also associated with non-oncologic interventions that use energy emitting devices, such as endoscopic lithotripsy for the management of kidney stones¹⁷. In other studies on transmural thermal ablation techniques, stricture was associated with scar tissue formation¹⁶, while superficially injured ureters did not stricture, and displayed only moderate inflammation confined to the lamina propria. Possible thermal effects of IRE were not investigated as part of this study²², which could have been another contributing factor to scar formation and subsequent stricture. Histologic analysis did not reveal hyalinization of the tissue samples or other overt signs indicative of thermal damage to the ureteral wall. In the absence of direct temperature measurements in the treatment region, it is not possible to conclude on the contribution of thermal injury to post-IRE cell death and the delayed effects reported in this work.

Our treatment parameters were selected based on previous work describing the minimum energy levels required to achieve IRE of the entire ureteral wall in a uniform fashion. Increasing the voltage may increase penetration of IRE for the treatment of larger tumors. Other treatment parameters such as the pulse width and pulse delivery interval can be optimized to limit the thermal effects of IRE²³. The IRE techniques described here warrant refinement before translation to patients.

This study is limited by its preclinical design and absence of an urothelial tumor model. Our assessment of renal functional preservation and tissue regeneration are relevant only for IRE treatment of the normal ureter. Tumors in the urinary tract often penetrate the ureteral wall, and the impact of ablation on lumen function and patency cannot be readily predicted. Urinary tract tumors themselves reduce the patency of the lumen, and therefore treatment may improve urinary function in some cases. Techniques to avoid or to treat urinary obstruction, such as peri-procedural ureteral stent placement, were not studied. Immediate postoperative imaging assessment following IRE is challenging in hollow organs such as the ureter. CT imaging allowed assessment of lumen patency but was not useful for evaluating the treatment zone. Techniques such as endoluminal ultrasound²⁴ may be better suited for monitoring and verifying treatment zones in patients.

Based on presented results, image guided intraluminal IRE has the potential to be developed as an alternative treatment to partial ureterectomy, in select patients with contraindications to surgery. Prior to clinical trial consideration, further studies are necessary to validate longer-term functional outcomes and to assess technical and catheter-mounted IRE device modifications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Fluoroscopy images of ureteral ablation with IRE, and assessment of immediate-post and mid-term patency of the urinary tract

A. Pre-treatment pyelography establishing baseline patency and anatomic dimensions of the upper urinary tract.

B. Distal treatment location, location of electrode indicated by arrowhead.

C. Proximal treatment location, location of electrode indicated by arrow.

D. Pyelography performed immediately after ablation following removal of the catheter-electrode from the ureter. Contrast extravasation is not observed at the treatment locations, confirming absence of perforation.

E. Pyelography performed 28 days following IRE. Proximal and distal locations of ablation are indicated marked with arrows. Patency of ureter is reduced at both treatment locations, along with dilation of the collecting system. Hydronephrosis of the kidney can be observed.

Figure 2. Longitudinal CT imaging of the renal pelvis and upper urinary tract following IRE
Imaging findings from longitudinal delayed phase CT follow-up of a single animal (animal 7) till 28 days post-IRE.

- A. Day 1: No hydronephrosis or urinary leakage was observed. Delayed passage of urine in the treated ureter can be observed when compared to untreated contralateral ureter.
- B. Day 7: Hydronephrosis (arrow) of the right kidney (treated side) was observed along with delayed contrast excretion.
- C. Day 14: Persistent hydronephrosis of the kidney on the treated side was observed.
- D. Day 21: Sagittal images show reduced patency of the ureter at both treatment locations (arrows). Ureter superior to the treatment sites appear dilated.
- E. Day 28: When compared to imaging from day 21, there is no apparent change in lumen patency, enlargement of the ureter or hydronephrosis in the urinary tract treated with IRE.

Figure 3. Comparative histology of tissue samples acquired at sequential timepoints following IRE. Stained with H&E and Masson's Trichrome stain

A. Cross sectional imaging of the normal porcine ureter.

B. Tissue samples 1 day post-IRE: Focally extensive necrosis observed in the full thickness of the ureter wall and surrounding connective tissue with complete urothelial ulceration, hyperemia, hemorrhage, edema, and neutrophilic inflammation.

C. Tissue samples 7 days post-IRE: Focally extensive granulation tissue was observed in the full thickness of the ureter wall and surrounding connective tissue, with urothelial hyperplasia, hemorrhage, and edema.

D. Tissue samples 14 days post-IRE: Focally extensive marked scarring in the full thickness of the ureter wall and surrounding connective tissue was observed with urothelial hyperplasia.

E. Tissue samples 28 days post-IRE: Marked focal scarring of the full thickness of the ureter wall and surrounding connective tissue observed with urothelial hyperplasia.

e: A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM03914

f: A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM03915

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Table 1

Summary of imaging findings, treatment related complications and histology analysis.

Animal Numbers	Follow-up (days)	Severity of Hydronephrosis	Stricture	Other Complications	Serum Creatinine (mg/dL) median (range)	Renal Pelvis Diameter (mm) median (range)	Average Length/Depth of Ablation Zone (mm)	Recovery of Urothelium Score (range)	Scarring of Muscularis Score (range)
8	0	None	No	–	0.97 (0.9–1)	6 (5–7)	NA	4 (normal)	0 (normal)
8	1	None	No	Urinoma	1.3 (1–1.8)	6.25 (5–8)	16.06±2.74/2.08±0.65	0	0
6	7	Mild-Moderate	Yes	–	1.6 (1.4–1.8)	14.7* (7–19)	12.25±3.18/3.21±1.42	3 (1–3)	4
4	14	Severe	Yes	–	1.4 (1.3–1.5)	19.25* (15–23)	11.45±4.31/1.79±0.43	3	4
2	21	Severe	Yes	–	1.3 (1.1–1.5)	14 (10–18)	NA	NA	NA
2	28	Severe	Yes	–	1.15 (0.9–1.2)	13.5 (8–19)	13.15±1.76/2.25±0.47	3	4

Note: Normal creatinine levels in pig vary in the range of 0.8–2.3 mg/dL; normal diameter of renal pelvis: <10mm.

* A significant difference was observed for the mean diameter of renal pelvis when comparing the baseline measurements with measurements performed at 7 days ($p=0.005$) and 14 days ($p=0.007$).