ORIGINAL PAPER

TRAUMA AND RECONSTRUCTIVE UROLOGY

Proximal ureteral reconstruction using renal capsule flap: a canine experimental model

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Introduction The aim of this article was to evaluate the effectiveness of using the renal capsule in ureteral reconstruction in a canine model.

Material and methods Ten clinically healthy male adult dogs were used in this study. Dogs underwent ureteral reconstruction using a tube-shaped flap of the renal capsule.

Results All but one animal (90%) survived till nephrectomy and thereafter. At 30 days after operation, the double-J stent was removed from the ureter, and at the 60th day, intravenous pyelography confirmed openness of the duct.

The internal surface of the tunneled flap was coated with thick, folded urothelium. Maturing granulation tissue and angiogenesis as well as fiber producing fibroblasts were observed in the lamina propria. The presence of smooth muscle cells beneath the lamina propria indicated complete reconstitution of the damaged ureter.

Conclusions The results showed that the autologous renal capsular flap provided a practical option for treating ureteral defects in dogs with an acceptable outcome. So, using the selfsame renal capsular tissue is a feasible method for restoration of the injured proximal ureter.

Key Words: canine ‹› fold ‹› reconstruction ‹› renal capsule ‹› ureter

INTRODUCTION

Ureteral injuries constitute approximately 1% of all urogenital system injuries [1]. Iatrogenic ureteral injuries are among the rare yet important complications that occur during abdominal operations such as gynecologic and vascular pelvic surgeries [2]. The ureters may also be damaged by penetrating or blunt abdominal trauma [3], recurrent calculi and retroperitoneal fibrosis [4]. When noted during surgery, primary attempt at healing of the damaged ureter is rather executable. However, the defect is not recognized in the majority of cases, leading to further complications such as ureteral stricture and necrosis, urinoma [5] and kidney loss [6]. The repair of the damaged ureter through inosculating of the interrupted ends of the tube is sometimes not feasible, needing additional efforts to fix the lesion. Based on the type of injury, different restoration techniques such as appendiceal substitution of the ureter [7], Boari bladder flap [8], ureteroenterostomy [9], transureteroureterostomy [5], psoas bladder hitch [10], ureteroneocystostomy [11], buccal mucosa graft [12], abdominal wall muscle flaps [3] and reinforced collagen scaffolds [13] have been developed over the years. Herein, we report a practicable technique aimed at the reconstruction of the damaged proximal ureter using an autologous flap of the renal capsule.

MATERIAL AND METHODS

Animals

Ten clinically normal mixed breed male adult dogs weighing 18–25 kg were used in the study. The dogs were acclimatized to the animal facility for 10 days prior to the operation during which, they were dewormed and vaccinated against rabies. The animals were kept separately, and fed twice a day with free access to water. Clinical signs including heart rate, respiratory rate, oral mucous membrane, rectal temperature, food and water intake and demeanor were observed and recorded every 12 h before and every 6 h during the first two weeks after the operation.
The experimental protocol followed the principles of the Helsinki Declaration and complied with the respective guidelines.

Surgical procedure

After sedation with acepromazine (0.02 mg/kg, IM), under local anesthesia, the right and left cephalic veins were cannulated aseptically with an 18 G IV catheter for general anesthetic administration (Propofol, 5 mg/kg, IV) and intraoperative fluid delivery (dextrose saline, 30 ml/kg/h), respectively. An endotracheal tube was inserted and the general anesthesia was maintained by isoflurane using an anesthetic machine with a rebreathing circuit. The vital signs and anesthetic depth were continuously monitored by an experienced veterinary surgeon during the operation. The animal was positioned in right lateral recumbency and the skin on the left side of the abdomen was shaved, scrubbed, and prepared for aseptic surgery. A 10 cm incision was made on the skin just beneath the last rib, followed by cutting of all abdominal muscular layers. After moving the parietal peritoneum aside, the left kidney was exposed, and its Gerota's fascia was incised longitudinally. The kidney was released out of surrounding connective tissue, moved to the skin level, and a piece of corresponding ureter (2 cm long) was transversely cut at about 7–10 cm from the renal pelvis. Then, a rectangular flap with 15 cm length and 2 cm width of renal capsule from the posterior part of the kidney was formed (Figure 1 A). A 2 mm wide hole was created at the proximal part of the flap, and the proximal ureter was crossed through it. A 4.8 Fr, 25 cm double-J (DJ) stent was positioned into the kidney and bladder for patency of the lumen. The proximal portion of the flap was subsequently sutured to the proximal end of the incised ureter using interrupted sutures of 6-0 polydioxanone. The sutures crossed all the layers of the ureter. The procedure succeeded in connection of the distal part of the flap to the

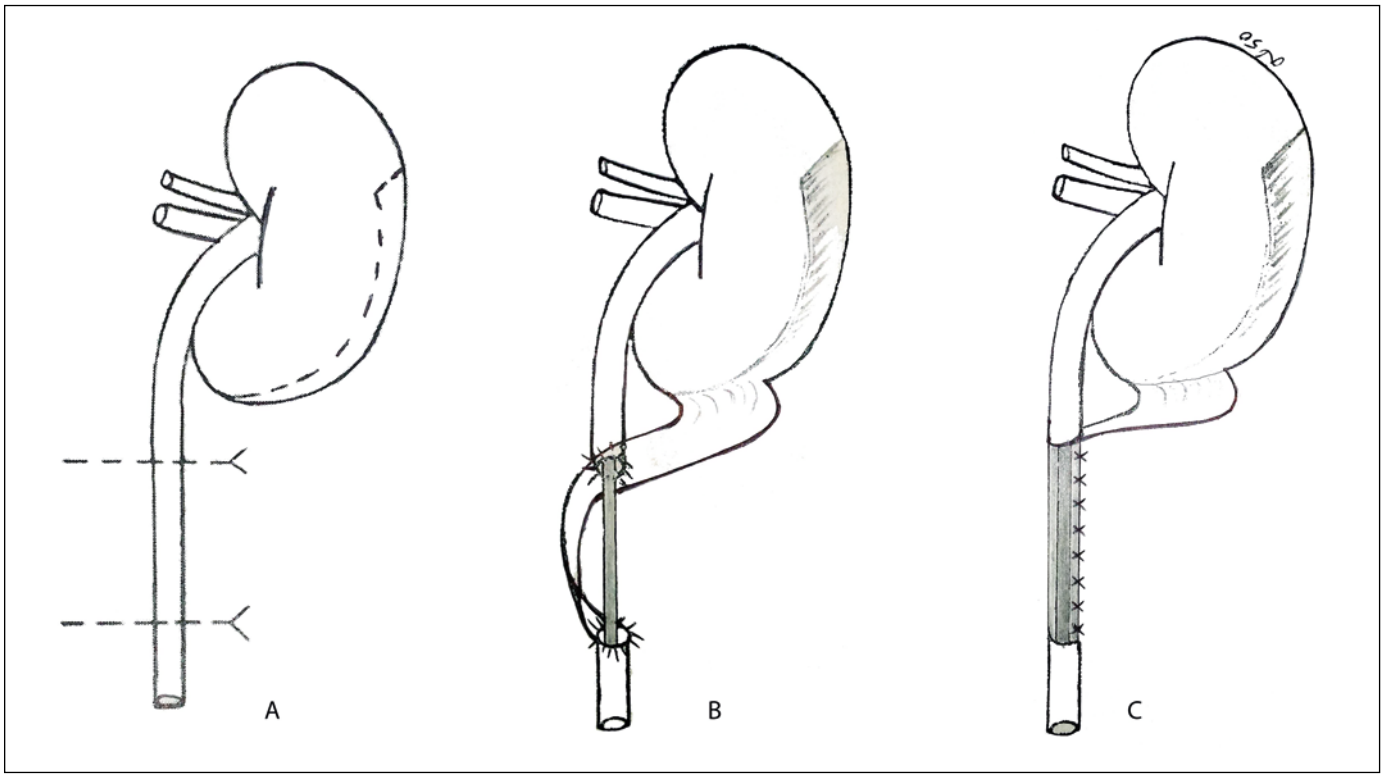

Figure 1. *Procedure of proximal ureteral reconstruction using renal capsule flap.*

distal end of the ureter (Figure 1B). The graft was then folded around the DJ stent and closed downward using continuous sutures of 6-0 polydioxanone (Figure 1C). Finally, the muscular layers and skin were sutured accordingly, and dressed with gauze. An Elizabethan collar was used to prevent the dog from attending to the operative site.

DJ stent removal

After 30 days, the animal was placed under general anesthesia and an incision was made in the prepubic region to make access to the urinary bladder. A longitudinal incision was made in the organ wall, the DJ stent was removed, and the bladder and all the abdominal layers were stitched up using 3-0 polydioxanone.

Radiography and pyelography

Thirty days after removal of the DJ stent, a simple kidney-ureter-bladder (KUB) radiograph was taken, followed by IV injection of 3 ml/kg contrast media (Visipaque) and intravenous pyelography (IVP) at supine position after 2 and 5 min.

Nephrectomy

After 10 weeks, the kidney and its repaired ureter were harvested under general anesthesia, examined macroscopically, placed in 10% neutral buffered formalin, and sent to the pathology lab. As the nephrectomy was carried out unilaterally, the animals were able to survive with the contralateral kidney.

Histopathologic examination

After fixation, the samples were dehydrated, cleared, paraffin embedded, sectioned at 5 μm thick, mounted on the glass slide, and stained with hematoxylin and eosin, and Masson's trichrome. The sections were assessed for epithelial growth and thickness, granulation tissue development, degree of inflammation (low, mild and high) and foreign body reaction. In addition, immunohistochemistry was performed by staining the sections of the newly formed portion of ureters for expression of cytoskeletal proteins α-smooth muscle actin (α-SMA), vimentin, and desmin.

RESULTS

Nine animals (90%) survived until nephrectomy at 10th week and afterward. On the 20th postoperative day, one animal showed progressive signs of discomfort, abdominal pain, elevated heart rate, fever, and anorexia, and was put to sleep by an IV overdose of barbiturate. Necropsy revealed ureteral leakage and stricture, hydroureteronephrosis and collection of urine in the retroperitoneal space. No signs of urinoma or other disturbances were noted in the other nine subjects.

Intravenous pyelography

Intravenous pyelography (IVP) taken at 60 days after the operation revealed patency of both ureters (Figure 2). None of the animals showed obstruction of the lumen.

Histopathology

Histopathological examination of the reconstructed part of the ureter in the sacrificed dog showed partial epithelization with 1–3 cellular layers along with significant edema and congestion of the subepithelial tissue as well as severe infiltration of mononuclear cells. In the other nine animals, the tunneled flap and suture sites were completely coated with thick, typical folded urothelium. Underneath the epithelium was the lamina propria in which, maturing granu-

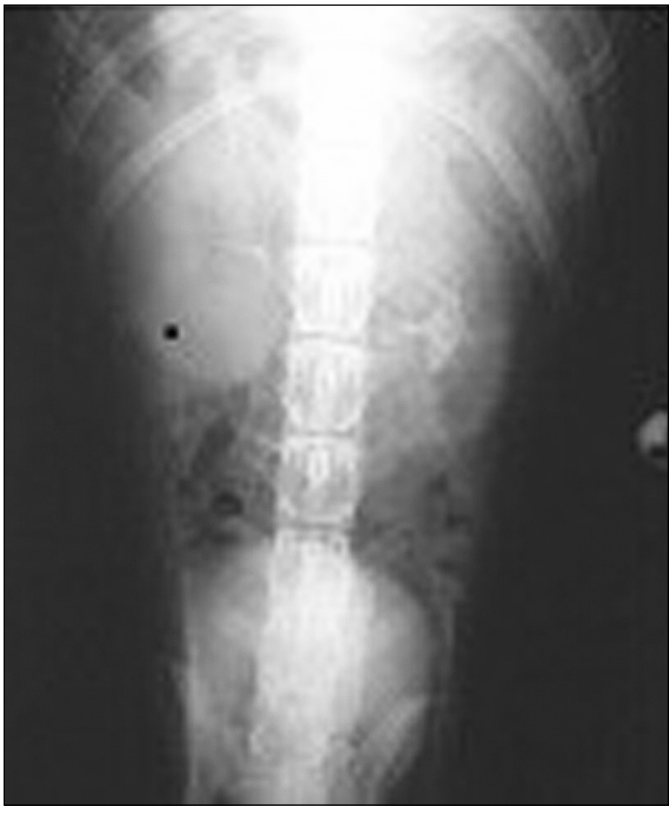

Figure 2. *Post-operative intravenous pyelogram (IVP). Ventro-dorsal radiographic view of IVP taken 60 days after operation with no sign of ureteral obstruction.*

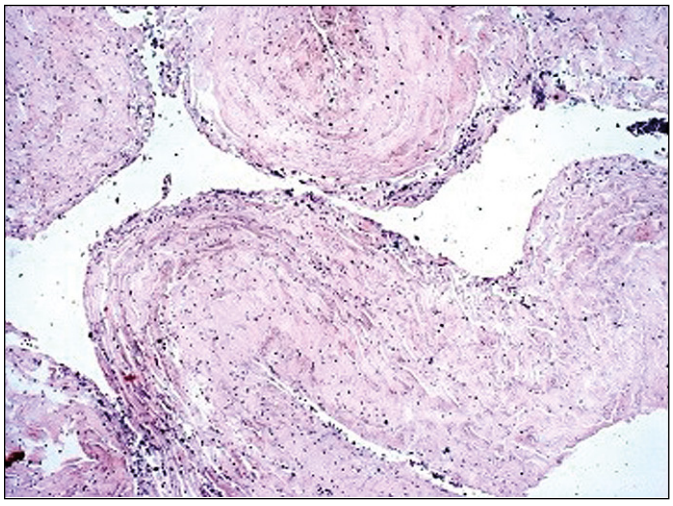

Figure 3. *Cross section through the reconstructed ureter. The stellate lumen is surrounded by the corrugated transitional epithelium. The lamina propria contains collagen fibers, fibroblasts, and axially running capillaries. Tapered smooth muscle cells are arranged longitudinally at the periphery of the lamina propria (H&E. x 100).*

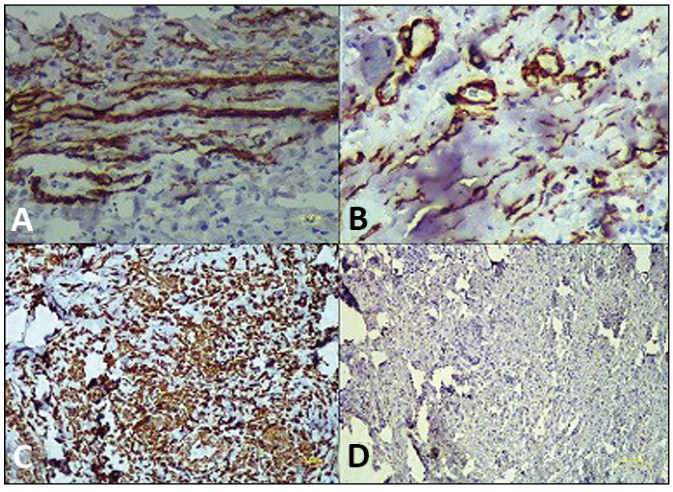

Figure 4. *Immunohistochemical micrographs of the repaired ureters resected at 10 weeks post operation.* **A, B.** *The healing area of the reconstructed ureter shows expression of contractile thin filaments of α-SMA indicating the presence of smooth muscle cells and myofibroblasts in the granulation tissue.* **C.** *Excess amount of vimentin stained cells corresponds the presence of mesenchymal cells in the developing connective tissue of lamina propria.* **D.** *The healing connective tissue was lacking of muscle specific filament desmin, representing the absence of smooth muscle cells in some areas.*

lation tissue along with angiogenesis and discrete fibroblasts were observed (Figure 3). In trichrome stained sections, collagen fibers could be detected in the newly forming lamina propria. Infiltration of the inflammatory cells in the connective tissue was minimal or not at all present. The ingrowth of smooth muscle cells could readily be seen, but otherwise, were not as abundant as in the normal ureter.
Immunohistochemical study of the healing area revealed the granulation tissue with a number of spindle-like cells positive for α-SMA, indicating the presence of stress fibers within the smooth muscle cells of the wall of newly formed vessels and myofibroblasts of the lamina propria, as well as cells of the muscular layer of the ureteral wall.
There was a significant number of cells positive for vimentin antibody indicating the presence of active fibroblasts in the newly forming tissue (Figure 4).

DISCUSSION

Ureteral injuries are associated with severe surgical complications [2]. Failure in the diagnosis of the lesion may result in further difficulties and even mortality [1]. The prompt repair or reconstruction of the organ is required after iatrogenic or external trauma. Although short defects may be managed by a straightforward operation, the repair of long ureteral lesions demands rebuilding of the damaged part. Prosthetic and synthetic materials are associated with an increased risk of infection and failure [14, 15]. Appendiceal interposition is suitable for repair of the right ureter due to ipsilateral position of the organ [16]. Other techniques such as Boari bladder flap and psoas hitch can be used in reconstruction of the distal ureter [17]. All the methods have some disadvantages, for example repair with small intestine submucosa or whole layers of the organ may result in complications such as urine leakage and peritonitis. In repair by buccal mucosa, a double step procedure including taking tissue from the oral cavity, extracorporeal manipulation and grafting it to the ureter may increase the risk of side effects of performing two operations.
The proximal third of the ureter is more prone to injury than its middle and distal third [1]. Renal capsule has been previously used in repair and or reconstruction of the Achilles tendon [18] and inferior vena cava [19]. To our knowledge, the first documented attempt of employing the renal capsular flap was done by Thompson et al. which dates back to 1963. They used a pedicle graft of renal capsule for reconstruction of the ureteropelvic junction [20]. In another study, Thompson and his team successfully used grafts out of dissected pieces of renal capsule to patch longitudinal defects, which was created by removing half the circumference of the ureters [21]. In the present study, however, we used a capsular flap with unbroken contact with the kidney to repair the transversely cut ureter at a distance

of 7–10 cm from the renal hilum. The main advantage of ureteral reconstruction with autologous tissue is avoiding graft rejection, developing complications and nephrectomy. The renal capsule has been shown as a tissue containing mesenchymal stem cells, which could potentially be used for the repair of urinary tract defects [22], especially of the upper part of the duct. This method requires neither external material nor additional incision, and involves minimal invasion of other organ systems and negligible bleeding. Also, in comparison with other suggested methods of repairing an obstructed ureteropelvic junction [23] while the renal pelvis is severely damaged, or there is no proper structure available, the renal capsule may be used for reconstruction of the proximal part of ureter.

The preliminary results showed feasibility of the method. The DJ stent was removed on the 30th postoperative day with no observed irregularity. On the excretory urogram taken 60 days postoperatively, the nephrogram and pyelogram phases revealed no evidence of blockade of the duct. Of the 10 animals, 9 (90%) survived until nephrectomy at the end of 10th week and afterward, with no clinical signs of abnormality and discomfort. Histopathology revealed urothelial regeneration on the surface of the fibrous sheath, as well as development of underneath connective tissue. The results of immunohistochemistry confirmed the presence of α-SMA, indicating existence of smooth muscle cells inside and under the lamina propria. Generally, smooth muscle cells express desmin and vimentin, while myofibroblasts express only vimentin [24]. The detection of cytoskeletal intermediate filament protein vimentin, which is normally expressed in mesenchymal cells, along with lack of desmin and α-SMA in most cells, could prove the presence of fibroblasts in the developing connective tissue, which are needed for sustainable healing. The results of current study is partly similar to of Hodjati et al. (2013), which showed the readiness of the renal capsule for being a platform for growth and proliferation of epithelial tissue [19]. Although it is not evolved to resist osmotic alterations of urine, the renal capsule flap in conjunction with a DJ stent can be used as a scaffold for movement and differentiation of proliferating cells. In addition, macroscopic observations and histopathologic results of our study indicate that when grafted, this tissue does not adhere to adjacent tissues/organs, and does not form neo-fibrous growth, and will be faded away completely at the end of the healing process. Its proximity to the proximal ureter is one of the advantages of the tissue, which makes it an appropriate candidate for the reconstructive purposes. Moreover, this leaf of irregular dense connective tissue bears a reasonable number of blood vessels, which are readily expandable when needed. Although the tissue exhibits unique tensile strength, the capsule shows little resistance to bending. These properties make the renal capsule a rather ideal internal resource for reconstruction of the lost parts and defects of the proximal ureter, and in maintaining renal function.

CONCLUSIONS

Ureteral defects remain an important challenge for urologists. The renal capsule is an available tissue source for the reconstruction of the upper part of the damaged ureter. The present study evaluated the usefulness of renal capsular fold in ureteral reconstruction and thus the preservation of the kidney. The autologous renal capsule provides a practical option for ureteral reconstruction in dogs. In order to gauge long-term results and a better conclusion, the study has to be continued for a period of longer than 10 weeks. The operation may be applicable in human cases. Further experiments for optimizing the method are warranted.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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