Effect of Tadalafil on Prevention of Urethral Stricture After Urethral Injury: An Experimental Study

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OBJECTIVE To evaluate the preventive effect of phosphodiesterase type 5 inhibitor (tadalafil) on the formation of urethral stricture after urethral injury.

MATERIALS AND METHODS A total of 28, 4-month-old male New Zealand rabbits were included and divided into 3 groups. Group 1 was a sham group with 8 rabbits that underwent only urethroscopy. Group 2 was a nontreatment group with 10 rabbits that underwent urethral electrocoagulation without any treatment. Group 3 was the treatment group with 10 rabbits that underwent urethral electrocoagulation with systemic tadalafil treatment. After 30 days of follow-up, urethroscopy and retrograde urethrography were performed to evaluate the morphological changes in the urethra. The urethra tissues were examined with standard light microscopy by a histologist, and apoptosis was evaluated by the terminal dUTP nick end-labeling assay.

RESULTS Urethral diameters in group 1, group 2, and group 3 were 9.14 ± 0.73 mm, 3.52 ± 1.2 mm, and 7.68 ± 1.14 mm, respectively. The differences in urethral diameters were statistically significant between groups ($P < .01$). Collagen deposition in submucosal connective tissue was significantly less in the tadalafil group vs the nontreatment group. The numbers of apoptotic cells in submucosal connective tissue were also quantitatively higher in urethral stricture groups compared to the sham group.

CONCLUSION Tadalafil treatment had a protective effect against the formation of urethral stricture in rabbit model. This treatment can be a promising opportunity for urethral stricture and must be supported by clinical studies. UROLOGY 91: 243.e1–243.e6, 2016. © 2016 Elsevier Inc.

Urethral stricture is characterized by a narrowing of the urethra with formation of scar tissue. Male urethral stricture disease is a prevalent condition with an incidence of 200-1200/100,000 individuals. It may have a profound effect on the quality of life resulting in infection, bladder calculi, fistulas, sepsis, and ultimately renal failure.[1] Management of urethral stricture is a complex situation and depends on the characteristics of the stricture. Three major forms of treatment exist including urethral dilatation, visual internal urethrotomy, and urethroplasty. Although urethral dilation and visual internal urethrotomy are not routinely recommended, they are the most commonly used treatment methods. The only procedure that cures urethral stricture is urethroplasty. Data show that there is no difference between urethral dilation and internal urethrotomy in terms of long-term outcomes, with success rates ranging between 8% and 80%.[2] Because of these low success rates, several techniques have been used to treat wound contraction and to prevent stricture recurrence. These include the indwelling Foley catheter, home self-catheterization, and urethral stents. Unfortunately, these methods have several complications and often the stricture inevitably recurs unless the procedure is continued indefinitely.[3] Urethroplasty is the gold standard technique and has better cure rates, but it requires certain expertise.

Tissue fibrosis is the main etiopathological factor in urethral stricture development. Enlightened by the treatment of organ fibrosis, a few antifibrotic drugs have been used in trials to limit urethral stricture formation including docetaxel, halofuginone, mitomycin C, bitoxin A, somatostatin analogue, and triamcinolone.[4-8] However, these studies are limited in number and further studies are needed to evaluate the effectiveness and safety of antifibrotic medications before clinical testing. Therefore, there is an urgent

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need to identify new agents that can be used to treat urethral stricture. Phosphodiesterase type 5 (PDE-5) inhibitors, which are commonly used for erectile dysfunction, have both antioxidant and antifibrotic effects and reduced the formation of tissue fibrosis after injury. Their antifibrotic effect might also work on urethral strictures. The aim of this experimental animal study is to evaluate the preventive effect of PDE-5 inhibitor (tadalafil) on the formation of urethral stricture after urethral injury.

MATERIALS AND METHODS

With the approval of Local Animal Ethics Committee, 28 4-month-old male New Zealand rabbits weighing 2.50 ± 0.30 kg were used for the study. They were kept in special cages, in a specific pathogen-free environment with a natural solar day-night cycles. They were allowed free movement and access to food and drink.

The rabbits were anesthetized with intramuscular ketamine hydrochloride (15 mg/kg) and intramuscular xylazine (6 mg/kg). They were placed in supine position, and the genitalia were scrubbed with povidone-iodine solution. An 11F pediatric resectoscope (Karl Storz Endoskope, Tuttlingen, Germany) was used for endoscopic procedures. The rabbits were randomly allocated into 3 groups. Group 1 was a sham group with 8 rabbits that underwent only urethroscopy. Group 2 was a nontreatment group with 10 rabbits that underwent urethral electrocoagulation without any treatment. Group 3 was the treatment group with 10 rabbits that underwent urethral electrocoagulation with tadalafil (Cialis, Lilly, Basingstoke, Hampshire, UK) treatment. In group 3, daily tadalafil (2 mg/kg dissolved in 2 mL saline) treatment was given via a nasogastric tube for 30 days, beginning on the first day of urethral electrocoagulation. The dosage was based on experimental animal models.[9,10]

A standard 10-mm long circumferential electrocoagulation of the bulbar urethra was induced in group 2 and group 3. This procedure was performed distal to the verumontanum and away from the external sphincter with a hook-shaped electrode at a power of 40 W.[11] Electrocoagulation was continued until the mucosa blanched and ulcerated. All procedures were performed blindly by the same urologist (O.K.) who aimed to form the same length and depth of tissue defect for all animals. Urine was not diverted, and no antibiotics were given.

After 30 days of follow-up, urethroscopy and retrograde urethrography were performed to evaluate urethral gross morphology. The urethrography was performed blindly by the same radiologist (O.O.). Contrast medium was injected slowly and directly into the urethra by the same researcher, with X-ray direction used to visualize the configuration of the lumen. To estimate the percentage of urethral constriction, urethrograms were used to measure the diameter of the stricture at its narrowest area. The diameter of the urethra in the control group was measured at the same point. Urethral diameters and lumen reduction rates were compared between groups. Strictures were considered significant if the urethral lumen diameter decreased by more than 50% of the control group.[11]

All rabbits were killed by Pentothal overdose on the 30th day after surgery, and the urethras were removed en bloc with the penis. The urethra specimens were individually immersed in Bouin's solution, dehydrated in alcohol, and embedded in paraffin. Sections (5 μm) were deparaffinized and stained with Masson's trichrome, which was used to investigate fibrotic degree. The urethra tissues were examined and evaluated in random order under blinded conditions (C.A.) with standard light microscopy by a histologist. A score of 0 to 3 was assigned as follows based on the degree of staining and fibrosis: negative—absence of staining and fibrosis (0 points); mildly positive—slight staining and fibrosis (1 point); moderately positive—moderate staining and fibrosis (2 points); and strongly positive—strong staining and severe fibrosis (3 points).[12]

Apoptosis was evaluated by the terminal dUTP nick end-labeling (TUNEL) assay. The TUNEL method, detects fragmentation of deoxyribonucleic acid (DNA) in the nucleus during apoptotic cell death and was employed using an apoptosis detection kit (ApopTag Peroxidase In Situ Apoptosis Detection Kit, Cat. No. S7100, Millipore). The number of TUNEL-positive cells was evaluated semi-quantitatively: "0" means no positive cells, "1" means less than 10% positive cells, "2" means 10-50%, and "3" means >50% positive cells.

Statistical Analysis

All data were analyzed with the Statistical Package for the Social Sciences for Windows software (SPSS Version 17.0, Chicago, IL). Data were presented as the mean and standard deviation or percentage. Data in independent groups were analyzed for normalcy with the Kolmogorov-Smirnov test and further evaluated with an independent *t* test or Mann-Whitney *U* test.

RESULTS

None of the rabbits died during the study period. Mild urethrorrhagia that resolved in 24 hours was observed in all rabbits by inspection after electrocoagulation. One of the rabbits in the tadalafil group was excluded from the study because of excessive water consumption. Thus, the treatment effect was evaluated on 9 rabbits in the tadalafil group. There was no obvious abnormal reaction during the follow-up period, and none of the rabbits developed total urethral occlusion.

Urethral diameters in group 1, group, 2 and group 3 were: 9.14 ± 0.73 mm, 3.52 ± 1.2 mm, and 7.68 ± 1.14 mm, respectively (Fig. 1). The differences in urethral diameters were statistically significant between groups (Table 1) ($P < .01$). According to the urethral lumen reduction rates, the rabbits in group 2 had significant urethral stricture formation (lumen reduction >50%) vs group 3. There was no lumen size reduction in the sham group. The rate of decrease in the lumen cross-section area was 84.9% ± 11.5% (73.4%-89.5%) in group 2 and 29.3% ± 22.4% (6.9%-45.5%) in group 3 ($P < .01$).

Based on the urethroscopy findings, varying degrees of bulbar urethra narrowing was observed and the injured urethral surface had been covered by epithelium in all electrocoagulated rabbits (Fig. 1). The healed mucosa had a rugous surface with edema and slight hyperemia. In histopathological evaluation, the sham group showed normal collagen fiber configuration (Fig. 2A) whereas extensive collagen deposition in the submucosal connective tissue was found at urethral stricture groups (Fig. 2B,C). Collagen deposition in the submucosal connective tissue was significantly less in the tadalafil group vs the nontreatment group (Fig. 2C, Table 2). The numbers of apoptotic cells