ÖNSÖZ

Üretra darlığı, üretranın skar formasyonu sonucu daralmasıyla oluşan bir durumdur.Erkeklerde üretra darlığı 200–1,200/100,000 sıklıkla yaygın bir durumdur.üretra darlığı tekrarlayan sistitler, mesane taşı, sepsis ve böbrek yetmezliğine sebep olması nedeniyle hayat kalitesini bozmaktadır. Çeşitli tedavi modelitesi olmasına ragmen birçoğunun başarı oranı düşüktür.En iyi sonuçlar üretroplasti operasyonunda elde edilmesine karşın bu cerrahi ciddi tecrübe gerektiren bir durumdur.Bu yüzden üretra darlığını oluşumunu engellemek daha uygun bir seçenek hailne alıyor. Bu amaçla çeşitli maddeler(docetaxel, halofuginone, mitomycin C, bitoxin A, somatostatin analogue ve triamcinolone) denenmesine ragmen klinik olarak güvenlik ve etkinlik açısında çalışmalara ihtiyaç duruyor. Bu yüzden üretra darlığının oluşmasını engelleyecek yeni bir ajana ihtiyaç vardır.Phosphodiesterase type 5 (PDE-5) inhibitörleri erektil disfonksiyon hastalarında yaygın kullanılmasının yanında dokularda yaralanma sonrası fibrosis oluşumunu engellediği ve antioksidan özelliği olduğu biliniyor.Biz Namık Kemal Üniversitesi Bilimsel Araştırma Proje birimin desteğiyle tadalafil'in üretra darlığı oluşumunu engellemede etkinliği konusunda çalışmayı amaçladık.

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ÜRETRA YARALANMASI SONRASI ÜRETRA DARLIĞINI ÖNLEMEDE TADALAFİL'İN ETKİSİ. BİR DENEYSEL ÇALIŞMA

Özet

Amaç: Üretra yaralanmasına bağlı üretra darlığı oluşmasını PDE-5 inhibitorünün(Tadalafil) önleyici etkisini araştırmayı amaçladık.

Materyal, Metod: Toplam 28 tane 4 aylık erkek New Zealand tavşan 3 gruba bölündü. Sham olan grup1'deki 8 tavşana sadece üretroskopi yapıldı. Tedavisiz grup olan grup2'deki 10 tavşanın üretrasına elektrokoterizasyon yapıldı ve herhangi bir tedavi verilmedi.Tedavi grubu olan grup 3'deki 10 tavşanın üretrasına elektrokoterizasyon yapıldı ve sistemik tadafil tedavisi verildi. 30 gün takip sonra, üretradaki morfolojik değişiklikleri değerlendirmek için üretroskopi ve retrograde üretrografi yapıldı. Üretra dokusu tekbir histolok tarafından standart ışık mikroskopuyla değerlendirildi. Ayrıca apoptosis terminal dUTP nick end-labeling (TUNEL) ile değerlendirildi.

Sonuçlar: Grup 1, grup2 ve grup3'ün üretra çapı sırasıyla 9.14 ± 0.73 mm, 3.52 ± 1.2 mm ve 7.68 ± 1.14 mm bulundu. Gruplar arasında üretra çapı istatistiksel olarak farklıydı($p<0.01$). Submukozal konnektif dokuda kollejen depolanması tadalafil grubunda tedavisiz gruba göre belirgin azdı. Submukozal konnektif dokudaki apoptotic hücre sayısı üretral darlığı gruplarında, sham grupbundan daha yüksektir.

Sonuç: Tadalafil tedavisi, tavşan modelinde üretra darlığı oluşmasına karşı koruyucu etkiye sahiptir. Bu tedavi üretra darlığına karşı umut veren bir seçenek olabilir ve klinik çalışmalarla desteklenmelidir.

Key Words: Urethra, Stricture, Tadalafil, Treatment, Rabbit

EFFECT OF TADALAFIL ON PREVENTION OF URETHRAL STRICTURE AFTER URETHRAL INJURY. AN EXPERIMENTAL STUDY

Abstract

Objective: To evaluate the preventive effect of PDE-5 inhibitor (Tadalafil) on the formation of urethral stricture after urethral injury.

Material and Method: A total of 28, four-month-old male New Zealand rabbits were included and divided into three groups. Group 1 was a sham group with 8 rabbits that underwent only urethroscopy. Group 2 was a non-treatment group with 10 rabbits that underwent urethral electro-coagulation without any treatment. Group 3 was the treatment group with 10 rabbits that underwent urethral electro-coagulation with systemic tadalafil treatment. After 30 days of follow-up, urethroscopy and retrograde urethrography was 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 (TUNEL) 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.($p<0.01$) Collagen deposition in submucosal connective tissue was significantly less in the tadalafil group versus the non-treatment group. The numbers of apoptotic cells in submucosal connective tissuewere 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.

Key Words: Urethra, Stricture, Tadalafil, Treatment, Rabbit

INTRODUCTION

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 200–1,200/100,000 individuals. It may have a profound effect on the quality of life resulting in infection, bladder calculi, fistulas, sepsis, and ultimately renal failure.(Santucci, 2007)[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 shows that there is no difference between urethral dilation and internal urethrotomy in terms of long-term outcomes with success rates ranging between 8–80%.(Santucci 2001)[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.(Jordan 2002)[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 anti-fibrotic drugs have been used in trials to limit urethral stricture formation including docetaxel, halofuginone, mitomycin C, bitoxin A, somatostatin analogue and triamcinolone.(Fu 2014, Nagler 2000, Mazdak 2007, Andersen 2003, Mazdak 2010)[4-8] However, these studies are limited in number and further studies are needed to evaluate the effectiveness and safety of anti-fibrotic medications before clinical testing. Therefore, there is an urgent 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 anti-fibrotic 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 METHOD

With the approval of Local Animal Ethics Committee, 28 four-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 povidine-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 non-treatment group with 10 rabbits that underwent urethral electro-coagulation without any treatment. Group 3 was the treatment group with 10 rabbits that underwent urethral electro-coagulation with tadalafil (Cialis, Lilly, Basingstoke, Hampshire, UK) treatment. In group 3, daily tadalafil (2 mg/kg solved in 2 ml saline) treatment was given via a nasogastric tube for 30 days, beginning on the 1st day of urethral electro-coagulation. The dosage was based on experimental animal models.(Vignozzi 2014, Guzeloglu 2013)[9,10]
A standard 10-mm-long circumferential electro-coagulation 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.(Jaidane 2003)[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.(Jaidane 2003)[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 de-paraffinized 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).(Sahinkanat 2009)[12]

Apoptosis was evaluated by the terminal dUTP nick end-labeling (TUNEL) assay. The TUNEL method, detects fragmentation of 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, USA). The number of TUNEL-positive cells were 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 (Version 17.0 SPSS, 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 (Figure 1). The differences in urethral diameters were statistically significant between groups (Table 1) ($p<0.01$). According to the urethral lumen reduction rates, the rabbits in group 2 had significant urethral stricture formation (lumen reduction >50%) versus 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 ($p<0.01$).

Table 1: Urethral diameters and rate of urethral constriction of study groups at the 30th day of urethral electro-coagulation. Statistical analysis was performed with Mann-Whitney U test after the evaluation of data normalcy.

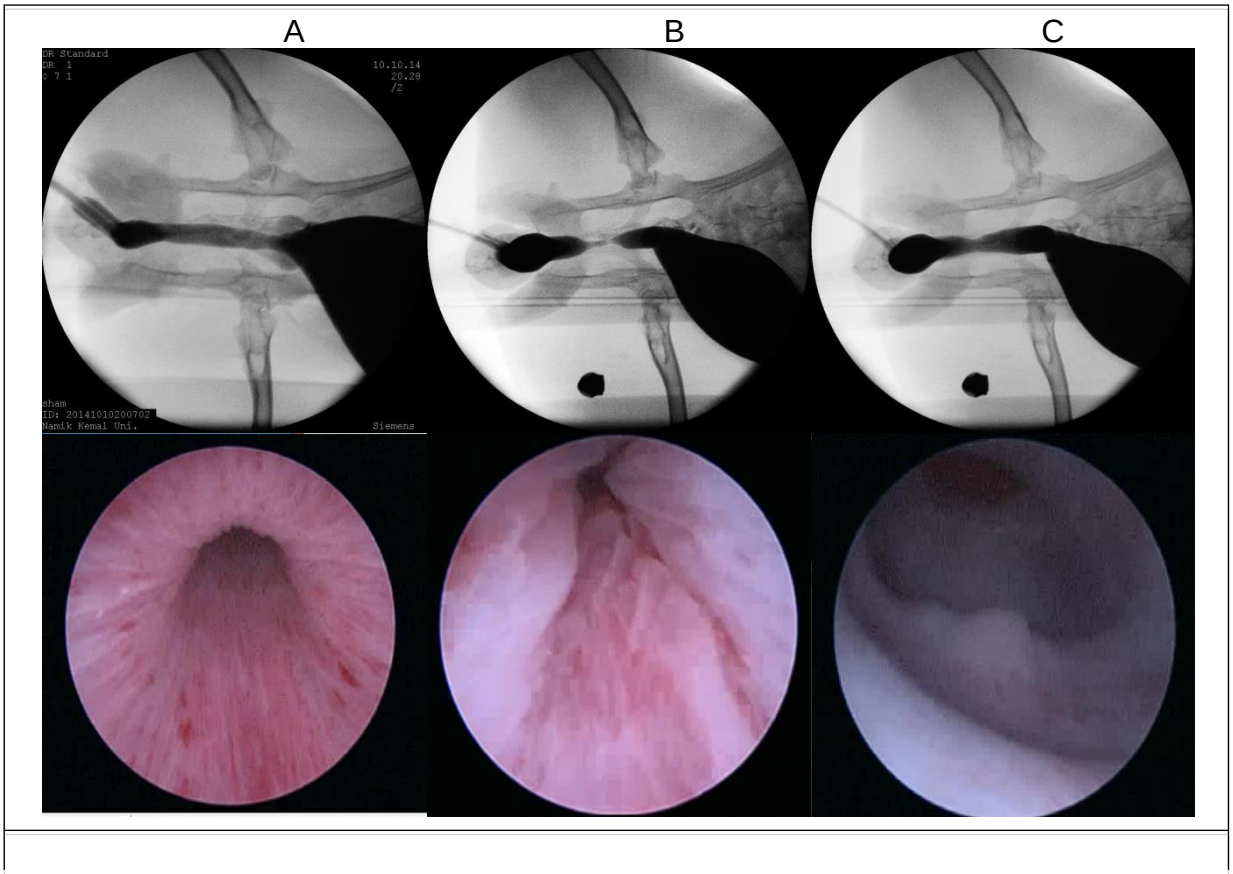
	Study Group	**Mean± SD**	**"p" value**
Urethral Diameter (mm.)	Sham (Group 1)	9.14 ± 0.73	< 0,001
	Electro-coagulation (Group 2)	3.52 ± 1.2 [a1]	
	Electro-coagulation plus tadanafil (Group 3)	7.68 ± 1.14[b1,c1]	
Urethral constriction rate (%)	Electro-coagulation (Group 2)	84.9 ± 11,5	<0,001
	Electro-coagulation plus tadanafil (Group 3)	29,3± 22,4	

[(a1)]Analysis between Group 1 and Group 2 (p<0,001)
[(b1)]Analysis between Group 1 and Group 3 (p<0,001)
[(c1)]Analysis between Group 2 and Group 3 (p<0,001)

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 electro-coagulated rabbits (Figure 1). The healed mucosa had a rugous surface with edema and slight hyperemia.

Figure 1: Retrograde urethrography and urethroscopy of study groups.

(a): Normal retrograde urethrography and urethroscopy appereance in Sham group (Group 1).
(b): Retrograde urethrography and urethroscopy demonstrating severe constriction in urethral lumen in electro-coagulation group (Group 2).
(c): Retrograde urethrography and urethroscopy demonstrating mild constriction in urethral lumen in electro-coagulation plus tadalafil group (Group 3).



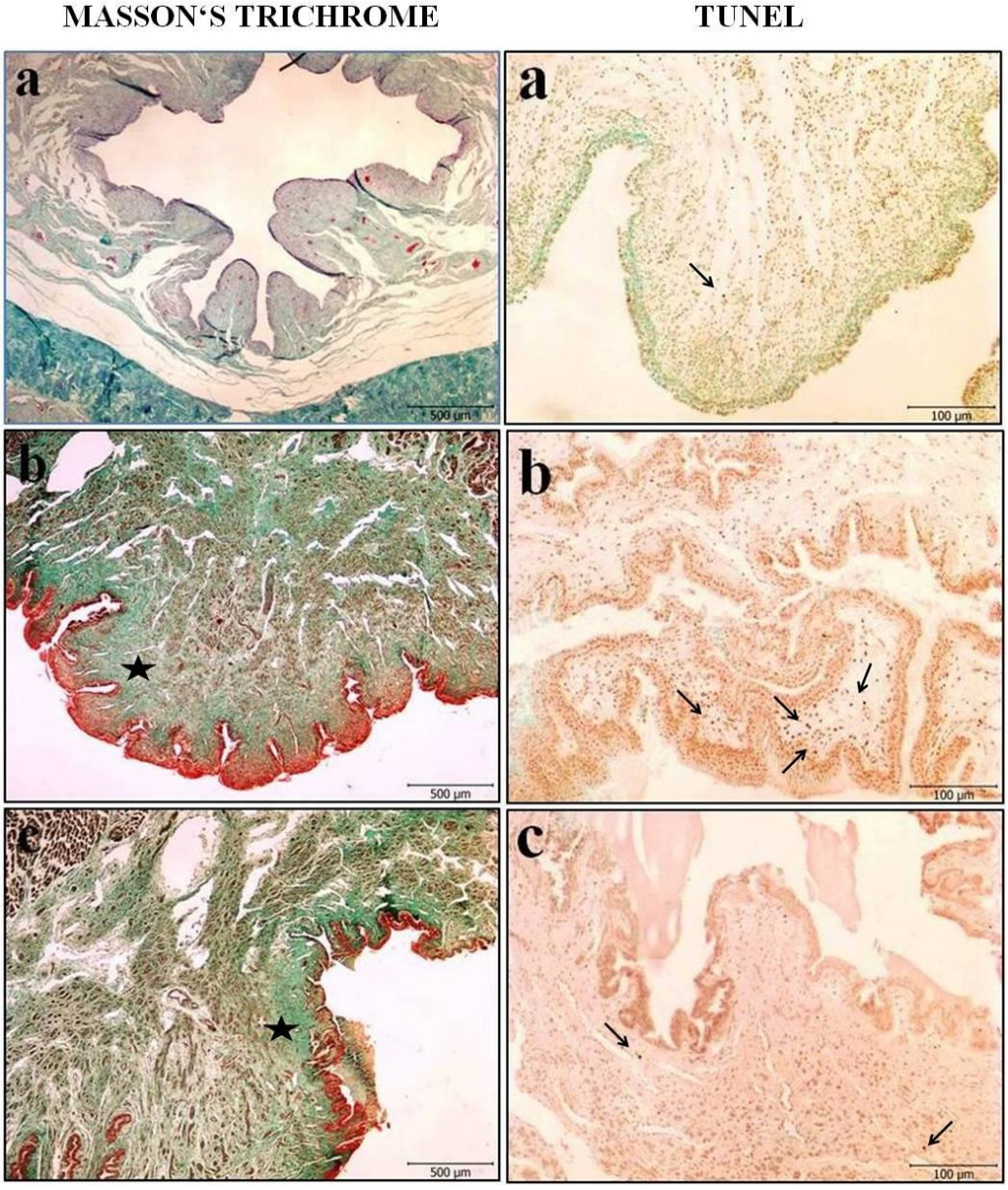
In histopathological evaluation, the sham group showed normal collagen fiber configuration (Figure 2a) whereas extensive collagen deposition in the submucosal connective tissue was found at urethral stricture groups (Figure 2b, 2c). Collagen deposition in the submucosal connective tissue was significantly less in the tadalafil group versus the non-treatment group (Figure 2c, Table 2). The numbers of apoptotic cells in the submucosal connective tissue were also quantitatively higher in urethral stricture groups compared to sham group (Figure 2a, b). Treatment of tadalafil markedly decreased the number of apoptotic cells (Figure 2c, Table 2).

Table 2: The degree of fibrosis and the number of TUNEL-positive cells of study groups at the 30th day of urethral electro-coagulation.

	Study Group	**Mean± SD**	**"p" value**
Fibrosis score(MASON)	Sham (Group 1)	0.25 ± 0.46	< 0,001
	Electro-coagulation (Group 2)	2.7 ± 0.48	
	Electro-coagulation plus tadanafil (Group 3)	1.33 ± 0.50	
Apoptosis score (TUNNEL)	Sham (Group 1)	1 ± 0.0	< 0,001
	Electro-coagulation (Group 2)	2.5 ± 0.52	
	Electro-coagulation plus tadanafil (Group 3)	1.66 ± 0.50	

Subgroup analysis of MASON scores were significant between each groups. ($p<0,001$)
Subgroup analysis of TUNNEL scores were significant between each groups.
$p<0.001$ between group 1and 2 and group 2 and 3.
$p=0.011$ between group 1 and 3.

Figure 2: Representative photographs of urethral tissue (Masson's trichrome and TUNEL staining).
Masson's trichrome; (a): Sham group (Group 1) showed normal collagen fiber distribution, (b): Electro-coagulation group showed extensive collagen deposition which are recognized as green in the *submucosal connective tissue,* (c):Electro-coagulation plus tadalafil group showed significant less collagen deposition in the urethra. Asteriks: collagen fibers (Masson's trichrome, scale bar: 500 μm).
TUNEL; The number of apoptotic cells in the *submucosal connective tissue* were quantitatively higher in urethral stricture groups than control groups (Tunnel Figure 1a,b). Treatment of tadalafil markedly reduced the number of apoptotic cells (Tunnel Figure 1c). Arrow: TUNEL positive cells (TUNEL staining, scale bar: 100 μm).



COMMENT

Urethral stricture is defined as a process of fibrosis caused by extensive collagen synthesis and ill-defined compositional changes of the extracellular matrix. This process results in scar formation, which decreases the urethral lumen caliber. In this pathology, dense interspersed fibers with fibroblasts replace normal connective tissue. There is a decrease in the type III to type I collagen ratio.(Baskin 1993)[13] These changes are accompanied by a decrease in smooth muscle to collagen ratio and a significant change in the synthesis of nitric oxide (Cavalcanti 2004, Da-Silva 2002)[14,15]

This study was designed to evaluate the protective effect of tadalafil treatment in urethral stricture. Lacono et al showed the protective effect of sildenafil in the fibrotic process of corpora cavernosa after radical prostatectomy. They demonstrated that 50 mg sildenafil, 3 days a week significantly decreased the fibrosis in corpora cavernosa.(Iacono 2008)[16] Similar to this finding, Kovanecz et al reported a protective effect of tadalafil treatment on cavernosal tissue fibrosis of rats after cavernous nerve denervation.(Kovanecz 2008)[17] We also demonstrated a protective effect of tadalafil treatment (2mg/kg) on formation of urethral stricture. Standard electrocoagulation of the urethra caused scar formation with a distinct collagen deposition, which was significantly lower in the tadalafil treatment group. In this study, we demonstrated a protective effect of tadalafil on fibrotic process and urethral stricture formation.

Other antifibrotic agents such as mitomycin C, triamcinolone and docataxel have also been studied for the treatment of urethral stricture. In a clinical study, Mazdak et al reported that intraurethral infusion of mitomycin C and triamcinolone decreased the recurrence rate of urethral stricture from 50% to 10% and 21.7%, respectively.(Mazdak 2007, Mazdak 2010)[6,8] In another study, Fu et al evaluated the effect of docataxel treatment on rabbits after urethral trauma. (Fu 2014)[4] They demonstrated a significant dose dependent decrease in urethral lumen stricture rates in a treatment group versus non-treatment group. We also demonstrated a protective effect of tadalafil treatment on a rabbit urethral stricture model. In our study group, the rate of luminal narrowing was 83% in the non-treatment coagulation group, but this was 30.3% in the tadalafil group. We used a sham group to differentiate the pathological changes, and we performed histological evaluation of both sham and study groups, which may be regarded as an important issue to determine pathology-related changes and compare them to a non-pathological process.

Urethral stricture is the end result of an inflammatory process that occurs with urethral injury. This process induces fibrotic changes in tissue and causes constriction in urethral lumen. Nitric oxide (NO) is important in this process.(Witte 2002)[18] During inflammation, expression of inducible nitric oxidesynthatase (iNOS) increases. Macrophages are the main sources of iNOS during the early phase of inflammation. This enzyme forms NO, which causes irreversible cellular injury and collagen synthesis. These mechanisms are the main components of tissue repair.(Witte 2002, Thornton 1998)[18,19] Inhibition of iNOS results in a decrease in collagen deposition of scar tissue. (Witte 2002)[18] Several studies have shown that this relation and production of iNOS related NO could be inhibited by PDE-5 inhibitors.(Santucci 2001, Abdellatif 2013, Jeong 2009, Toward 2004)[2,20-22] This inhibition could be related to the suppression of pro-inflammatory mediators and/or negative effects of increased cGMP on the production of iNOS-related NO.(Abdellatif 2013, Jeong 2009, Toward 2004)[20-22] Inducible nitric oxidesynthatase activity had also been shown in human urethral stricture tissue. Cavalcanti et al documented iNOS activity in human urethral stricture tissue and proposed that this activity may be related to collagen accumulation.(Cavalcanti 2004)[14]

Phosphodiesterase type 5 inhibitors acts on PDE-5, which is the responsible enzyme of cGMP degradation. This inhibition accumulates cGMP and activates the protein kinase G pathway. This inhibition also decreased collagen synthesis and cellular apoptosis.(Piazza 2001, Thompson 2000)[23,24] Beside these pathways, cellular differentiation of fibroblasts to myoblasts could affect fibrotic changes and induce fibrotic tissue remodelling.(Chipev 2000)[25] This differentiation could be modified via 8Br- cGMP, which is an exogenous cGMP agonist.(Shimizu 1999, Lee 1997)[26,27] This cGMP agonist could decrease collagen synthesis and tissue fibrosis in a cardiac tissue model.(Redondo 1998)[28] These experimental findings indicate the importance of cGMP on the formation of tissue fibrosis. The importance of cGMP on the formation of tissue fibrosis was also determined in penile tissue. Lysiak et al documented that tadalafil treatment decreased the level of tissue fibrosis and apoptosis in penile corpus cavernosum in a cavernous nerve denervation model. In their model, tadalafil increased the levels of 2 cell survival associated kinases; Akt and ERK1/2.(Lysiak 2008)[29] We observed similar results. Versus the non-treatment group, tadalafil treatment could significantly decrease apoptotic index and fibrosis in urethral stricture model. This finding may indicate a protective effect for tadalafil on the formation of urethral stricture.

Our study had some limitations. The biochemical analysis of some mediators such as NO, iNOS, TNF and TGFβ_1 ,was not performed. The tissue antioxidant enzymes superoxide dismutase, glutathion peroxidase and catalase were not measured. This biochemical evaluation might give an idea about the pathways by which tadalafil acts. Another limitation was the use of the TUNEL assay, which does not directly document scar formation. This technique showed that tissue viability and apoptosis could indirectly document scar formation. On the other hand, we used Masson's technique to determine scar formation and combined these techniques for direct evaluation of scar formation. Thus, we could not discriminate how tadalafil treatment prevented progressive fibrosis and decreased apoptotic index. This will be the subject of future researches identifying the specific pathways that are responsible for this protective effect.

Conclusion

In spite of technological developments in endourology, urethral stricture is still a challenging pathology in the lower urinary tract with high recurrence rates. Any treatment opportunity that can decrease both the incidence and recurrence of urethral stricture can be promising for clinicians. We found that 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.

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