

Use of Bosentan, Theophylline and Vardenafil in Treatment of Priapism

Priapizm Tedavisinde Bosentan, Teofilin ve Vardenafil Kullanımı

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ABSTRACT

Objective: To evaluate the early therapeutic alternatives such as Bosentan an Endothelin receptor blocker, Theophylline and Adenosin receptor blocker and Vardenafil a non-selective Phosphodiesterase 5 enzyme inhibitor for the therapy of ischemic priapism in the rat models.

Methods: Twenty-four Sprague-Dawley rats were randomly divided into 4 equal groups. Control group, Vardenafil group, Bosentan group and Theophylline group. Erection was provided by vacuum constriction method and it was maintained for 4 hours for achieving the priapism in all groups. Then, Six rats from each group were sacrificed by cervical dislocation. Consequently, cavernous tissue samples were collected and placed in the tissue bath. Tissue samples were prepared as 0.5-0.2 cm. strips and put into heat jacketed double walled organ bath containing 37°C krebs solution which is constantly bubbled with 95% O₂ and 5 % CO₂ and mounted at a resting tension of 1000 mg. After taking the 1 hour record of the three groups except the control group, Bosentan, Theophylline and Vardenafil were admitted in increasing doses. Consequently the alterations of the contractions in the strips due to the drugs and their increasing doses are observed.

Results: In this study we detected that Bosentan increased the frequency and amplitude of the contractions of the cavernous tissue in the Priapism status in a statistically significant manner, Theophylline decreased the frequency and the amplitude significantly and Vardenafil had statistically no effect on the frequency and amplitude.

Conclusion: Inhibition of priapism induced apoptosis with Bosentan, seems promising on preserving erectile function.

Key words: Bosentan, ischemic priapism, theophylline, vardenafil

ÖZET

Amaç: Endotelin reseptör blokleri olan Bosentan, Adenosin reseptör blokleri olan Teofilin ve non selektif Fosfodiesteraz 5 enzimi inhibitörü olan Vardenafil'in iskemik Priapizm oluşturulmuş rat modelinde terapötik etkinliğinin araştırılması hedeflendi.

Yöntemler: Randomize olarak 24 Sprague-Dawley rat kontrol grubu, Vardenafil grubu, Bosentan grubu ve Teofilin grubu olmak üzere 4 eşit gruba dağıtıldı. Ereksiyon her grupta vakum konstriksiyon metodu ile sağlandı ve priapizm sağlanması için 4 saat sürdürüldü. Daha sonra her gruptaki 6 rata Servikal dislokasyon yöntemi ile dekapitasyon uygulandı. Kavernöz dokular ayrıldı ve organ banyosuna bırakıldı. Doku örnekleri 0,5-0,2 cm. şeritler şeklinde ısı çeperli, çift duvarlı içinde 37°C krebs çözeltisi bulunan ve devamlı 95% O₂ ve 5% CO₂ ile gazlandırılan organ banyolarına istirahat gerimi 1000 mg. olacak şekilde asıldılar. Kontrol grubu dışında kalan grupların 1 saatlik kasılma kayıtları alındıktan sonra grubuna göre artan dozlarda Bosentan, Teofilin ve Vardenafil uygulandı ve şeritlerdeki ilaç ve doz bağımlı kontraksiyon değişiklikleri gözlemlendi.

Bulgular: Bu çalışmada Bosentanın priapizm oluşturulmuş Kavernöz doku kasılmalarında frekans ve amplitüdüleri istatistiksel olarak anlamlı şekilde artırıp Teofilinin ise yine anlamlı bir şekilde azalttığı gözlemlendi. Vardenafilin ise frekans ve amplitüd üzerinde herhangi bir etkisi gözlemlenmedi.

Sonuç: Priapizm ile indüklenmiş apoptozisin Bosentan ile inhibe edilmesi erektil fonksiyonun korunması açısından umut vaat etmektedir.

Anahtar kelimeler: Bosentan, iskemik priapizm, teofilin, vardenafil

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INTRODUCTION

Priapism is a full or partial erection that continues more than 4 hours beyond sexual stimulation and orgasm or is unrelated to sexual stimulation. There are three kinds of priapism: ischemic priapism (veno-occlusive, low flow), stuttering priapism (intermittent), and non-ischemic priapism (arterial, high flow) [1]. Typically, only the corpora cavernosa are affected [2].

Ischemic priapism (veno-occlusive, low flow) is a persistent erection marked by rigidity of the corpora cavernosa, and little or no cavernous arterial inflow. In ischemic priapism, there are time-dependent changes in the corporal metabolic environment with progressive hypoxia, hypercarbia, and acidosis. The patient typically complains of penile pain and the examination reveals a rigid erection. The condition is analogous to a muscle compartment syndrome, with well-documented histological changes occurring to the corporal smooth muscle by 12 hours. Interventions beyond 48-72 hours of onset may help relieve erection and pain, but have little benefit in preserving potency. Histologically, by 12 hours, corporal specimens show interstitial edema, progressing to destruction of sinusoidal endothelium, exposure of the basement membrane, and thrombocyte adherence at 24 hours. At 48 hours, thrombi can be found in the sinusoidal spaces and smooth muscle necrosis with fibroblast-like cell transformation is evident [3].

The tone of arterial and cavernous smooth muscle cells is a key regulator of tumescence and detumescence, and is determined by the balance of relaxants and constrictors [3]. Compared to normal erection, the turning point for ischemic priapism is the disruption of the smooth muscle cells (SMC) tone control system, which is induced by obviously cause-effect pharmaceutical agents, inexplicit Sick Cell Disease (SCD), and other hematological dyscrasias, and obscure causes [4].

Ischemic priapism (including stuttering priapism) thus becomes the research focus, because it is more common and associated with poorer outcomes as compared with non ischemic priapism [5]. The causes of ischemic priapism include hematologic dyscrasias and medication used in treatment of erectile dysfunction (ED) and psychosis treatment,

although over 46% of the cases are elusive [4, 6]. In addition, mediators such as adenosine, noradrenaline, endothelin and rho-kinase also play major roles in the regulation of penile smooth muscle tone. Recently, it was shown in experimental priapism models that adenosine and endothelin are effective in the development of recurrent priapism attacks. Moreover, it was reported that dysregulated phosphodiesterase type 5 (PDE-5) activity, decreased NE response and altered endothelin receptor activity may be effective in the pathophysiology of priapism.

Two aims were described for this experimental ischemic priapism study. First the effect of Endothelin-1 (ET-1), Adenosine Deaminase (ADA), PDE-5 enzymes on the cavernosal smooth muscle tissue were assessed. Secondly; the possible preventive effect of Endothelin receptor blockers (Bosentan), Adenosine receptor blockers, non selective phosphodiesterase inhibitors (Theophylline) and vardenafil a PDE-5 inhibitor on the observed cavernosal smooth muscle in ischemic priapism.

METHODS

This study was approved by Ethical Committee of Experimental Research on Animals. Twenty-four adult, Sprague Dawley male rats with a mean weight of 245-300 gr were used. They were divided into four equal groups; all were housed under constant environmental conditions and were fed on standard laboratory rat chow and distilled water. The rats were randomly divided into 4 groups, each containing 6 rats: the control group, Vardenafil group, Bosentan group, Theophylline group. Erection was provided by vacuum constriction method and it was maintained for 4 hours for achieving the priapism in all groups. Then, six rats from each group were sacrificed by cervical dislocation.

In organ bath experiments the paired corpora cavernosa had the tunica albuginea removed, were divided into a total of 10-14 strips, 2-5 mm in length, and tied between thin threads. Cavernous tissue samples were collected and placed in the tissue bath. The reparations were suspended in 10 ml organ baths containing Krebs solution at 20°C, and after 20 min were heated to 37°C during continuous aeration with 5% CO₂ in O₂ and are mounted at a resting tension of 1000 mg. The muscle prepa-

rations received trans-mural electrical field stimulation (EFS). Mechanical muscular activity was recorded isometrically by Grass force-displacement transducers (FT03; Grass Products, warwick, USA). After taking the 1 hour record of the three groups except the control group. Vardenafil (Levitra; Bayer, İstanbul, Turkey) ,Bosentan(Bosentan; Sigmaaldrich, St. Louis ,USA); and Theophylline (Aminocardol; Novartis, İstanbul, Turkey) were admitted in increasing doses(10µmol, 100µmol ve 1000µmol). Bosentan and vardenafil were dissolved in distilled water and Theophylline were dissolved in dimethyl sulfoxide. Consequently the alterations of the contractions in the striped due to the drugs and their increasing doses are observed.

Experimental data are expressed as mean ± s.e.m. All statistical calculations were done by using a computer program (Statistical Package for Social Sciences 21,0). Mann-Whitney U test was used for comparison with the control group. Significant difference between treatment groups was accepted for $p < 0,05$.

RESULTS

In this study we detected that Theophylline decreased the frequency and amplitude of the contractions of the cavernous tissue in the priapism status in a statistically significant manner compared to control group. Furthermore, application of Theophylline with increasing doses(10µmol, 100µmol ve 1000µmol) lowered the tone of the tissue (Table 1).

Table 1. Effect of Theophylline administration to the frequency and amplitude of the contractions (mean ± s.e.m).

	Control (n=6)	10µM (n=6)	100µM (n=6)	1000µM (n=6)
Frequency	100.0±0.0	69.1±18.0**	40.4±29.7**	14.3±12.1**
Amplitude	100.0±0.0	77.7±16.9**	44.3±13.8**	19.5±10.8**

*: $p < 0,05$; **: $p < 0,01$

We observed that in the priapic cavernous tissue Bosentan increased the frequency and amplitude of the contractions of the cavernous tissue in a statistically significant manner compared to control group and also we observed that in the priapic cavernous tissue increasing concentrations of Bosentan induce increasing cavernosal smooth muscle tone (Table 2).

Table 2. Effect of Bosentan administration to the frequency and amplitude of the contractions (mean ± s.e.m).

	Control (n=6)	10µM (n=6)	100µM (n=6)	1000µM (n=6)
Frequency	100.0±0.0	114.9±2.2**	130.2±6.8**	167.4±24.6**
Amplitude	100.0±0.0	102.3±4.1	107.8±4.0*	125.6±17.5*

**: $p < 0,01$; *: $p < 0,05$

Vardenafil has statistically no effect on the frequency and amplitude in the priapic ischemic cavernous tissue compared to control group (Table 3).

Table 3. Effect of Vardenafil administration to the frequency and amplitude of the contractions (mean ± s.e.m).

	Control (n=6)	10µM (n=6)	100µM (n=6)	1000µM (n=6)
Frequency	100.0±0.0	94.2±11.5*	92.4±19.7*	103.3±21.3*
Amplitude	100.0±0.0	97.7±16.8*	96.5±15.3*	93.1±16.5*

*: $P > 0,05$

DISCUSSION

In recent years, many studies that have been made on the physiology of penile erection and pathophysiology of erectile dysfunction revealed the cavernosal smooth muscle tone regulation mechanisms. Especially in the treatment of ischemic priapism, using the mediators that active on the regulation of penile cavernosal smooth muscle is the new research topics.

Adenosine signaling plays important roles in regulating homeostasis in a number of physiological systems including the cardiovascular, nervous, renal, immune systems and erectile function. Adenosine is a signaling nucleoside that elicits its effect on target cells by engaging specific G-protein-coupled receptors [7]. Four such receptors have been described: A1 adenosine receptor (A1R), A2A adenosine receptor (A2AR), A2B adenosine receptor (A2BR), and A3 adenosine receptor (A3R). Each receptor has a unique affinity for adenosine and a distinct cellular and tissue distribution. Most evidence suggests that the A1 and A3 adenosine receptors are coupled to adenylyl cyclase by the inhibitory G-protein subunit (G α i) and hence serve to lower intracellular levels of the second messenger cyclic adenosine monophosphate (cAMP) [8,11]. The A2A and A2B adenosine receptors are commonly coupled to adenylyl cyclase by the stimula-

tory G-protein subunit (Gas) and serve to increase intracellular cAMP [11,12].

Adenosine shares multiple features with nitric oxide (NO), making it an excellent candidate for contributing to normal and abnormal penile erection. Adenosine-mediated cAMP induction and NO-mediated cyclic guanosine monophosphate (cGMP) induction are capable of activating protein kinase A and protein kinase G, respectively, resulting in decreased calcium calmodulin-dependent myosin light chain phosphorylation and enhanced smooth muscle relaxation [13]. Dai et al. [14]. Reported that the increasing amount of Adenosine in the cavernosal tissue is prolonging the erection through A2B receptor. Also in the same study it is reported that the erection is terminated with the polyethylene glycol-modified (PEG-ADA) therapy which is the modified form of the enzyme ADA This effect occurs in the cavernous tissues, destruction of adenosine by the deaminase enzyme. These experimental data suggest that increased levels of adenosine in the cavernosal tissue, may be effective in priapism. In pathogenesis of priapism, adenosine is likely to be effective via A2B receptor. There is no study about blocked A2B receptors and the result of this condition.

In our study, the non-selective adenosine receptor antagonist theophylline was used. As well as, nonselective adenosine receptor blocker theophylline, is thought to act through mechanisms such as PDE inhibition, stimulation of catecholamine secretion, inhibition of mediators (prostaglandins, TNF-alpha), intracellular calcium release, inhibition of histone deacetylase activity increased (increasing the effectiveness of corticosteroids effect). Chiang et al. [15]. reported that, Theophylline, an adenosine receptor antagonist, inhibited adenosine-induced penile tumescence. Tiejuan et al. [16]. reported that treatment with theophylline significantly inhibited adenosine-mediated corpora cavernosal strips (CCS) relaxation in a dose-dependent manner. We observed that theophylline decreased the frequency and amplitude of the contractions of the cavernous tissue in the priapism status in a statistically significant manner. And according to the control group at concentration of 10 μ M, 100 μ M, 1000 μ M theophylline increased smooth muscle relaxation in a statistically significant manner.

ET-1 is expressed by endothelial and stromal cells of the human penis since the earliest stages of external genitalia development [17]. And is considered the most potent stimulator of trabecular SMC [18].

In normal organ systems ET1 constitute the vasoconstrictor effect via ETA receptors. However, under pathological conditions, such as hypoxia, ET-1 acting as a defense mechanism, reveals vasodilation via ETB receptor. Prolonged hypoxia in penile SMCs activated other counter-regulatory mechanisms, besides an up-regulation of ETB receptors. Endothelin plays an active role in the regulation of penile smooth muscle tone and cause dilation of the hypoxic corpus cavernosum smooth muscle [19]. Filippi et al observed that in preparations of human fetal smooth muscle cells, prolonged hypoxia (more than 24 h) dramatically increased ETB receptor expression and responsiveness, as well as ET-1 gene and protein expression. During hypoxia, contractile effect of ET-1 is mediated by increased NO production and/or activity in penile cells, most probably mediated by the decreased previously described ETB receptor up-regulation [19].

Bosentan, is a nonselective endothelin receptor blocker and possesses the effect of vasodilation and antiproliferation [20]. Karakeci et al. have reported that inhibition of priapism induced apoptosis with bosentan, preserving erectile function [21]. By utilizing the mentioned effects of Bosentan we aimed to decrease the rate of ETB receptors in the cavernosal smooth muscle cells of the ischemic cavernosal tissue. Our study results have shown that Bosentan increased the frequency and amplitude of the contractions of the cavernous tissue in the priapism status in a statistically significant manner by blocking endothelin B receptors.

PDE-5 inhibitors selectively on the erectile tissue causing penile smooth muscle relaxation and vasodilatation leading to penile erection. Bialecki et al. [22]. first reported on sildenafil having a paradoxical effect in controlling stuttering priapism in three patients with SCD. Although this proposal would immediately seem illogical based on the knowledge that PDE5 inhibitors exert erectogenic effects, scientific basis for using these agents to treat priapism. In a small case series, Burnett et al. [23]. Have shown that daily sildenafil or tadalafil

therapy reduces ischemic priapism episodes in men with stuttering priapism. Accordingly, when used in long-term dosing regimen unassociated with erection stimulatory conditions, PDE5 inhibitor therapy alleviates recurrent priapism episodes in men with SCD- associated priapism and idiopathic priapism without affecting normal erectile capacity [24,25]. The working theory is that surges of cGMP go unchecked because of downregulated levels of PDE5; this results in stimuli-like nocturnal erection resulting in unchecked corporal smooth muscle relaxation. PDE5 inhibitors should be started under conditions of complete penile flaccidity, not during a stuttering episode. Efficacy is seen after a week or more of dosing [26]. Addition, transgenic sickle mice, which also display a priapic phenotype [27,28] also has lowered PDE5 activity in the penis transfection of endothelial Nitric Oxide Synthase (eNOS) into the penis of eNOS knockout mice restored both the expression of phosphorylated PDE5 and erectile response to near normal levels [29]. Together, these data suggest that PDE5 dysregulation is a fundamental mechanism for priapism. Interestingly, it has been shown that chronic PDE5 inhibitor administration alleviated or resolved priapism recurrences in six of seven patients who have sickle cell disease-associated “stuttering” priapism or idiopathic recurrent priapism [23]. Chronic PDE5 inhibitor administration prevents recurrent priapism by reconditioning PDE5 regulatory function.

In our study, we used vardenafil which is phosphodiesterase type 5 inhibitors. We observed that Vardenafil had no effect in the priapic ischemic cavernous tissue. Therefore, acute administration of phosphodiesterase type 5 inhibitors can not resolve priapism, chronic PDE5 inhibitor administration can solve priapism.

The mechanism of nonischemic priapism is documented, along with its treatment; whereas the mechanism of ischemic priapism is still somewhat unclear [4]. Especially in the treatment of ischemic priapism, using the mediators that active on the regulation of penile cavernosal smooth muscle is the new research topics. In our study it is aimed to discover new therapy alternatives. In this study we detected that Bosentan increased the frequency and amplitude of the contractions of the cavernous tis-

sue in the priapism status in a statistically. Bosentan promising on treating of ischemic priapism.

Conflict of Interest: No conflict of interest was declared by the authors.

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