Effects of Sildenafil Citrate on Ipsilateral Testis Damage Following Experimental Testicular Torsion in Rats

Ratlarda Deneysel Testis Torsiyonunu Takiben Tek Taraflı Testis Hasarı Üzerine Sildenafil Sitratın Etkileri

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ABSTRACT

Objective: Testicular torsion results in damage of the gonad and represents surgical emergency. Testicular torsion induces sterility as a result of ischemia. The aim of this study was to determine the effects of a vasodilator agent sildenafil on the testicular damage following testicular torsion.

Methods: Forty-two Wistar-albino male albino rats were divided into seven groups, each containing six rats. C: Control, S: Sham (operative procedure without torsion), T: Torsion (detorsion was performed 2 hours after 720° left testis torsion and orchiectomy was done at the 4th hour.), Sildenafil (Viagra; Pfizer) was given without torsion to V_1 group and V_2 group (respectively 1 mg/kg and 2 mg/kg). Sildenafil was given to V_1T group and V_2T group respectively 1mg/kg and 2mg/kg at the first hour following torsion then detorsion was performed 2 hours after torsion and orchiectomy was done at the 4th hour. At the end of the study blood and testes tissue samples were obtained for malonyldialdehyde (MDA), nitric oxide (NO), Johnsen tubuler biopsy score (JTBS) and diameter of seminiferous tubule (STD) analysis.

Results: JTBS and STD levels were highest in the C group and the lowest T Group and torsion reduced spermatogenesis significantly compared to the C and S groups ($p<0.05$). Sildenafil administration without torsion (V_1 and V_2) did not change JTBS scores. JTBS levels of the V_2T group were increased significantly when compared to V_1T ($p<0,05$).

C compared to study groups showed statistically different changes in terms of STD ($p<0,05$). Torsion reduced STD significantly compared to the C and S group ($p<0,05$). Tissue and plazma MDA levels were the highest in the V_2T group and the lowest C Group. Tissue NO levels were the highest in the V_1T group and lowest in C Group. Sildenafil administration (1 mg/kg and 2 mg/kg) without torsion group compared to torsion groups in terms of plasma and testis tissue MDA and NO levels showed significantly statistically different changes.

Conclusion: Sildenafil showed a protective effect against tubular damage histologicaly after unilateral torsion and detorsion.

Keywords: Testicular torsion, sildenafil

Received: 10.08.2022 **Accepted:** 10.23.2022

ÖZET

Amaç: Testis torsiyonu gonad hasarı ile sonuçlanır ve cerrahi aciller arasında yer alır. Testis torsiyonu iskemi oluşturarak steriliteye yol açar. Bu çalışmanın amacı testis torsiyonunu bağlı gelişen testis hasarı üzerine vazodilatör ajan olan sildenafilin etkilerini belirlemekti.

Yöntem: Toplam 42 erkek Wistar-albino rat herbir grup 6 rattan oluşacak şekilde 7 gruba ayrıldı. C: Kontol, S: Sham (torsiyon yapılmadan operasyon prosedürleri uygulandı), T:Torsiyon (720º derece dönecek şekilde sol testis torsiyonundan 2 saat sonra detorsiyon yapıldı. 4.saatte orşektomi uygulandı). V_1 ve V_2 gruba tosyion yapılmadan sildenafil (Viagra; Phizer) sırasıyla 1mg/kg ve 2 mg/kg dozunda verildi. V_1T grup ve V_2T gruba torsiyonu takiben 1. saatte sırasıyla 1mg/kg ve 2 mg/kg dozunda sildenafil verildi. Bunun ardından torsiyondan 2 saat sonra detorsiyon ve torsiyonun 4. saatinde orşektomi yapıldı. Çalışmanın sonunda malondialdehit (MDA), nitrik oksit (NO), Johnsen tübüler biopsi skoru (JTBS) ve seminifer tübül çapı (STD) analizleri için kan ve testis doku örnekleri alındı.

Bulgular: JTBS ve STD düzeyleri en yüksek C grubunda, en düşük T grubunda idi ve torsiyon C ve S gruplarına göre spermatogenezi anlamlı olarak azalttı ($p<0.05$). Torsiyonsuz sildenafil uygulaması (V_1 ve V_2) JTBS skorlarını değiştirmedi. V_2T grubunun JTBS düzeyleri V_1T'ye göre anlamlı olarak yüksekti ($p<0,05$).

C gruplarına kıyasla diğer çalışma grupları STD açısından istatistiksel olarak farklı değişiklikler gösterdi ($p<0,05$). Torsiyon, STD'yi C ve S grubuna göre anlamlı olarak azalttı ($p<0,05$). Doku ve plazma MDA düzeyleri en yüksek V_2T grubunda, en düşük C grubundaydı. Doku NO seviyeleri V_1T grubunda en yüksek, C grubunda en düşüktü. Torsiyonsuz sildenafil uygulaması (1 mg/kg ve 2 mg/kg) torsiyonlu gruplara göre plazma ve testis dokusu MDA ve NO düzeylerinde istatistiksel olarak anlamlı farklılık gösterdi.

Sonuç: Tek taraflı torsiyon ve detorsiyon uygulamasından sonra sildenafil histolojik olarak tübüler hasara karşı koruyucu bir etki göstermiştir.

Anahtar Sözcükler: Testis torsiyonu, sildenafil

Geliş Tarihi: 08.10.2022 **Kabul Tarihi:** 23.10.2022

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doi:http://dx.doi.org/10.12996/gmj.2023.37

INTRODUCTION

Testicular torsion has been implicated in testicular injury and infertility. Testicular injury is proportional to the duration and degree of torsion, and detorsion is one of the most important factors in further injury (1). During the detorsion process, with the resumption of blood flow, a huge amount of molecular oxygen is supplied to the tissues and abundant amounts of free oxygen radicals, which are responsible for reperfusion injury, are produced (2).
Sildenafil is an inhibitor of cGMP-specific phosphodiesterase type 5 (PDE_5). Sildenafil causes increase in cGMP by inhibiting PDE_5 and this increase in cGMP results in vasodilation in vascular smooth muscle (2,3). The aim of this study is to determine the effects of a vasodilator agent sildenafil on testicular damage following testicular torsion.

MATERIALS and METHODS

Gazi University Ethical Committee approval was obtained prior to the study. The study was performed in the Animal Research Laboratory of Gazi University Medical Faculty. Forty-two pre- adult wistar-albino male rats weighing 200-250 g. were divided into seven groups. All surgical procedures were performed while the rats were under intramuscular ketamine (50 mg/kg) anesthesia. Torsion, detorsion, and sham operations were performed through mid-scrotal incisions. Torsion was created by rotating the left testis 720° clockwise and maintained by fixing the testis to the scrotum with a 6/0 polypropilen suture placed through the tunica albuginea. After 2 hours of torsion, the testis was counter-rotated back to the natural position and reinserted into the scrotum. A total of seven groups, consisting of six animals in each group, underwent the following procedure:

C: Control, S: Sham (operative procedure, without torsion), T: Torsion (detersion was performed 2 hours after 720° extravaginally left testis torsion and orchiectomy was done at the 4th hour), V_1: Sildenafil (Viagra; Pfizer) (1 mg/kg) was administered oragastrically without torsion, V_2: Sildenafil (2 mg/kg) was given oragastrically without torsion,V_1T: Torsion, detorsion and sildenafil (1 mg/kg) was given oragastirically at the first hour following torsion, detorsion was performed 2 hours after torsion and orchiectomy was performed at the 4th hour and V_2T: torsion, detorsion and sildenafil 2mg/kg was given oragastirically at the first hour following torsion, detorsion was performed 2 hours after torsion and orchiectomy was performed at the 4th hour. At the end of the study blood and testes tissue samples were obtained for MDA, NO, JTBS and STD analysis.

Testes were fixed in Bouin's solution, paraffin embedded, sectioned at 5 micron, stained with Hemotoxylin-Eosin and examined under light microscopy. All specimens were evaluated in a blind fashion by two pediatric pathologists, who were not informed of the study groups. Plasma and tissue MDA ve NO levels were evaluated by one physiologist. All organs were washed twice with cold saline solution, placed into glass bottles, labeled and stored in a deep freeze (-30º C) until processing.

To determine the Johnsen tubuler score, 60 seperate transversely cut seminiferous tubules were examined under 400 X magnification. The mean seminiferous tubuler diameter measurements were done on 10 seperate transversely cut seminiferous tubules in each case with on ocular micrometer under 400 X magnification. The modified Johnsen Testicular Biopsy Score was utilized (4,5).

NO and MDA levels measurements

The levels of plasma MDA were analyzed using spectrophotometric methods. These results were expressed in nanomoles per mililiter plasma. We collected tissue samples to determine the tissue MDA levels. Tissue MDA levels were determined spectrophotometrically and they were expressed in nanomole per gram tissue and micromole per gram tissue, respectively (6-8).

The NOx levels were obtained using an enzyme-linked immunosorbent assay reader by vanadium chloride (VCl3) / Griess assay. Plasma specimens were deproteinized by incubation with equal volumes of NaOH and ZnSO4. The supernatants were centrifuged at 14.000 rpm at 4 ^{0}C. Prior to NOx determination tissues were homogenized in five volumes of phosphate buffered saline (pH=7.5) and centrifuged at 2000 g for 5min. Then 0.25 ml of 0.3M NaOH was added to 0.5 ml supernatant. After incubation for 5min at room temperature, 0.25ml of 5%(w/v) ZnSO4 was added for deproteinization. This mixture was then centrifuged at 3000 g for 20 min and supernatants of tissues and plasma were used for the assays. The NO3 levels were determined spectophometrically by comparing the absorbency at a wavelength of 540 nm against standard solutions of NaNO3 diluted to a range of 0–100 µmol and. NaNO2 diluted to a range of 0–10 µmol. The determinations carried out were expressed as NOx in the final outcome. The results were expressed in micromoles per liter for plasma and nanomoles per gram tissue for tissues (9-11).

The results were expressed in nanomoles per deciliter. Data were expressed as mean ± standard deviations (SD). All statistical analyses were carried out using SPSS statistical software for Windows. Nonparametric analyses with the Mann-Whitney U-test were performed on the biochemical variables data. Correlation analyes in each group were tested with Spearman's test P values of less than 0.05 were considered to be significant.

RESULTS

The results obtained from all groups are summarized in table 1. JTBS and STD levels were highest in the C group and lowest in the T group(Figure 1 and 2). Comparison of C and study groups showed statistically different spermatogenic activity in terms of JTBS ($p<0,05$), and torsion reduced spermatogenesis significatly compared to the C and S group(Figure 2 and 3) ($p<0,05$). Sildenafil administration without torsion(V_1 and V_2) did not change JTBS scores significantly when compared to the C and S groups ($p>0,05$). In the sildenafil (2 mg/kg) following torsion group (V_2T), JTBS was increased significantly when compared to torsion and sildenafil (1 mg/kg) following torsion group (V_1T) ($p<0,05$), JTBS scores were significantly higher in V_1 and V_2 groups compared to the V_1T and V_2T groups ($p<0,05$). Compared to controls, study groups showed statistically different changes in terms of STD ($p<0,05$). Torsion reduced seminiferous tubuler diameters significantly compared to the C and S group ($p<0,05$) and sildenafil administration without torsion did not change STD significantly when compared to the C and S groups ($p>0,05$). In the sildenafil (2 mg/kg) following torsion group (V_2T), STD was increased significantly when compared to T and sildenafil (1 mg/kg) following torsion group (V_1T) ($P<0,05$). STD were significantly higher in V_1 and V_2, compared to the T and V_1T and V_2T ($p<0,05$).

Tissue and plazma MDA levels were highest in the V_2T group and the lowest C Group. Ipsilateral testicular torsion and after detorsion; plasma and testis tissue MDA levels were increased. Plazma NO levels were the highest in the V_2T group and the lowest in the C Group(table 2). Tissue NO levels were the highest in the V_1T group and the lowest in the C Group(table 2). Ipsilateral testicular torsion and after detorsion; plasma and testis tissue NO levels were increased. In the sildenafil (1 mg/kg and 2 mg/kg) following torsion groups to plasma and testis tissue MDA and NO levels are not statistically different changes. Sildenafil administration (1 mg/kg and 2 mg/kg) without torsion groups to torsion groups plasma and testis tissue MDA and NO levels showed significant statistically different changes.

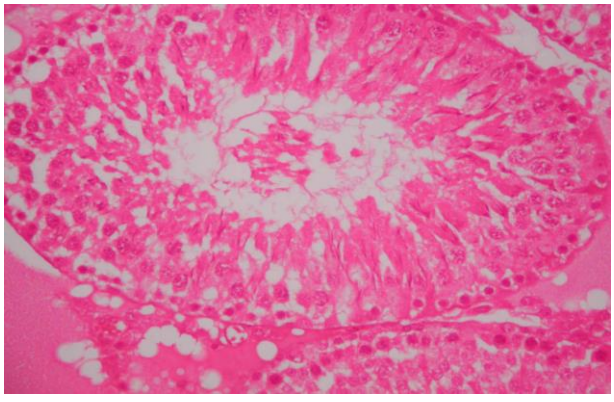

Figure 1: Normal spermatogenic activity with numerous spermatozoa in group C (HE,200 X)

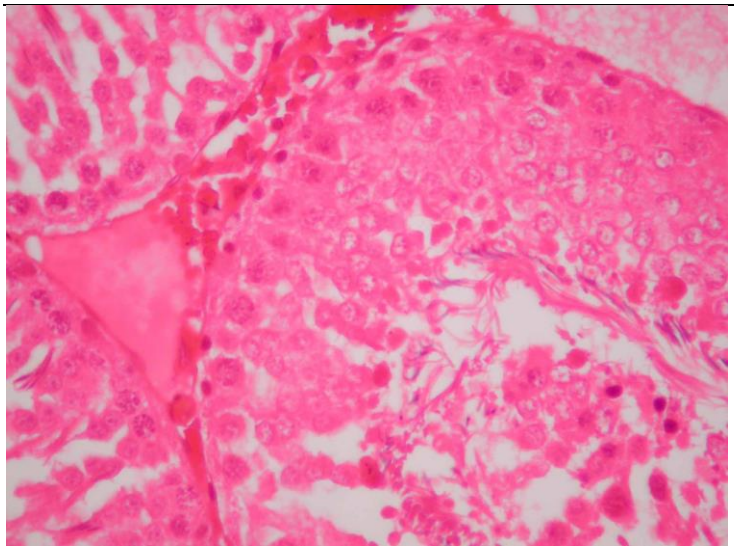

Figure 2: The seminiferous tubule showing hipospermatogenesis and a few number of spermatozoa in group T (HE, 200 X)

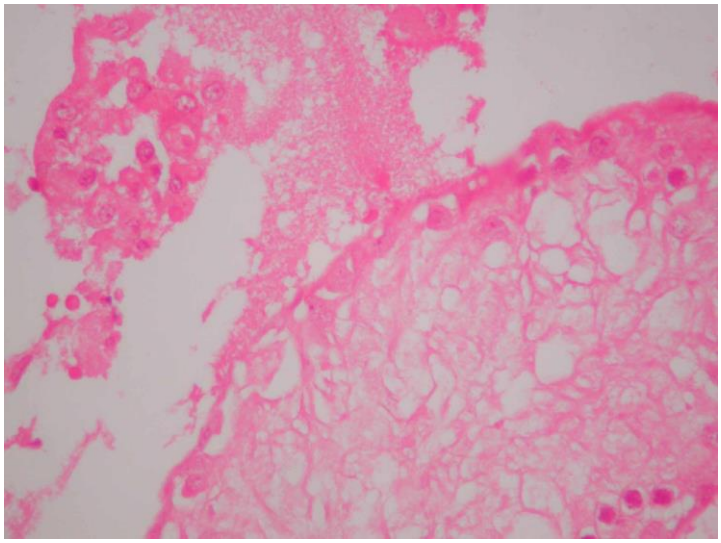

Figure 3: Part of the affected seminiferous tubule in which only the Sertoli cell is seen in the torsion group. (HE, 400 X).

Table 1: Mean ± SD of Johnsen Tubuler Biopsy Score (JTBS) and Diameter of Seminiferous Tubule(µM) (STD) levels of groups.

Groups(n=6)	JTBS (mean ±SD)	STD (mean ±SD)
C	9.70±0.12	292.38±1.24
S	9.48±0.07	278.91±3.27
T	*6.80±0.17	*182.18±2.28
V_1	9.60±0.14	287.45±2.14
V_2	9.58±0.15	287.83±2.42
V_1T	#8.88±0.11	#257.90±1.94
V_2T	9.35±0.16	273.76±3.21

*p<0.05 group T versus C,S, V_1,V_2, V_1T,V_2T for JTBS and STD
# p<0.05 group V_iT versus V_2T for JTBS and STD

Table 2: Mean ± SD of levels of tissue and plasma levels of MDA and NO for all groups.

Groups(n=6)	Plasma NO µmol/lt	Tissue NO nmol/gr.tissue	Plasma MDA nmol/dl	Tissue MDA nmol/gr.tissue
C	#6.35 ±0.11	#6.73±0.10	#4.30±0.09	#1.36±0.04
S	6.56±0.13	8.93±0.12	6.12±0.11	2.83±0.08
T	*28.09±0.21¥	*20.10±0.25¥	*27.36±0.29¥	*5.63±0.11¥
V_1	7.57 ± 0.14	8.60±0.14	6.40±0.13	2.47±0.07
V_2	6.44 ± 0.12	8.39±0.13	7.33±0.16	2.20±0.06
V_1T	16.45±0.18	20.61±0.26	27.24±0.28	6.72±0.15
V_2T	28.56±0.22	19.12±0.20	36.18±0.30	7.40±0.18

* p>0.05 group T versus V_1T and V_2T , # p>0.05 group C versus V_1, V_2 and S and ¥ p<0.05 group T versus C, S,V_1 and V_2 for plasma and tissue NO levels and plasma and tissue MDA levels.

DISCUSSION

This experimental study on the effects of sildenafil in testicular torsion was done in the rat testis with its very well known responses to such an injury (12-14). Previous studies with a rat model of testicular torsion have demonstrated that a 2-hour, 720° rotation of the testis followed by reperfusion causes a significant increase in testicular lipid peroxidation products and nitric oxide content. The events result in the permanent loss of spermatogenesis (15). The lesions in our study are characterized by a decrease in JTBS, STD and increse in NO and MDA for group T.

Various mechanisms responsible for tissue damage after testicular ischemia–reperfusion (I-R) have been proposed, ranging from an immune reaction, a sympathetic mechanism, to the consequence of I-R injury (16). There are two components of posttorsion testicular injury: hypoxic injury and reperfusion injury. During ischemia, ATP is degraded to hypoxanthine, and xanthine dehydrogenase is converted to xanthine oxidase, thus resulting in decreased tissue ATP levels (17). During reperfusion, oxygen becomes abundant, and superoxide anions are generated by XO and the mitochondrial electron transport chain. I-R injury initiates a pathophysiologic cascade, including an activation of neutrophils, inflammatory cytokines, and adhesion molecules with increased thrombogenicity, release of massive intracellular Ca^2, and generation of oxygen-derived free radicals. Reactive oxygen species, including superoxide anions, hydrogen peroxide or hydroxyl radicals, and nitric oxide or peroxynitrite, cause DNA damage, endothelial damage, and germinal cell necrosis (18-20).

To date various drugs, chemical substances, and physical methods have been used to protect testes against I-R injury in experimental animals, and some of these have been found to be effective in preventing testicular damage, such as, allopurinol, vitamins, nitro-L-arginine methyl ester (a precursor of NO), polyethylene-glycol-superoxide dismutase, caffeic acid phenethyl ester (active component of honeybee propolis), and hypothermia (19). To our knowledge, Sildenafil has never been used in experimental testis I-R injury. A new drug sildenafil citrate, the specific inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE $_5$) was studied as a potential antianginal drug at the late 1980s (21). The physiological mechanism is initiated by the liberation of nitric oxide. The activation of the enzyme guanilate cyclase by NO occurs then, and the increased levels of guanylyl cyclase increase the levels of cGMP, which relaxes the smooth muscles of the vein and increase the blood flow. Sildenafil citrate may cause dilatation of peripheral arteries and veins and the inhibition of the thrombus-forming capabilities of platelets in vivo (21). These doses of sildenafil was chosen in an effort to mimic peak plasma concentrations of sildenafil produced after the administration of a 50-mg oral dose to a 70-kg human being. In addition previous studies sildenafil was administered by oral route so we prefered oral intake in this study (21,22). After oral administration, sildenafil was absorbed rapidly from rat gastrointestinal tract; the drug was detected in plasma from the first blood sampling time (5 min) and rapidly reached Tmax values (22).

Ipsilateral testicular injury resulting from testicular torsion and detorsion resembles the phenomenon of ischemia and reperfusion. It has been demonstrated that testicular torsion in the rat causes permanent aspermatogenesis (239. This loss of spermatogenesis has been shown to be due to JTBS. In this study, the statistically lower JTBS (6.80) and STD (182.18) levels in the T group ($p<0.05$) is in accordance with current literature (5,13). In the present study sildenafil prevented testicular injury, that is reflected in the significantly higher JTBS and STD values obtained in V_1T and V_2T groups compared to the T group ($P<0.05$).

Furthermore JTBS and STD levels obtained by 2mg/kg of sildenafil administration were higher than those obtained by 1 mg/kg of sildenafil in testicular I-R, these were statistically significant ($p<0.05$, when JTBS and STD levels were compared between V_1T and V_2T groups). As can be seen in the present study, sildenafil exerts this effect in a dose-dependent manner for the given doses.

MDA is the end product of lipid peroxidation and is a well-known parameter for determining the increased free radical formation in post-ischemic tissue (20). In the present study, we were also able to obtain significantly increased levels of tissue and plasma MDA in the T group compared with the S and C groups (Table2, $p<0.05$). With sildenafil administration in the V_1T and V_2T groups, statistically similar MDA levels were obtained compared with the T group. In fact, MDA levels obtained by 2 mg/kg of sildenafil administration (V_2T) were higher than the T group which was not statistically significant (Table 2, $p>0.05$, when MDA levels were compared between V_2T and T groups).

Analysis of NO levels in both testes after unilateral torsion showed that only after 24 h of reperfusion, and irrespective of the ischemic period, NO levels were elevated in the ipsilateral testis. Nitric oxide rapidly react with other radicals, especially with O^2, yielding the deleterious and very reactive peroxinitrite and promoting further damage to reperfused tissues. A testicular torsion of 3 h was found sufficient to enhance the NO levels to a point in which they are able to initiate apoptosis in germinal cells (18).

In the present study, we were also able to obtain significantly increased levels of tissue and plasma NO in the T group compared with the S and C groups (Table 2, $p<0.05$). These results are consistent with the literature (2).

With sildenafil administration in the V_1T and V_2T groups, statistically similar tissue NO levels were obtained compared with the T group ($p>0,05$). 2 mg/kg of sildenafil administration (group V_2T) was higher than T group and these were not statistically significant (Table 2, $p>0.05$, when tissue NO levels were compared between V2T and T groups).

Plazma NO levels of the T group were higher than V_1T and lower than the V_2T group, but these results were not statistically significant ($p>0,05$). MDA and NO levels of V_1 and V_2 groups were lower than the T group ($p<0.05$) and statistically smilar to C and S groups. This shows the fact that sildenafil that is adminestered in the pre-torsion period has no effect on the NO and MDA levels. But the persisting high levels of plasma and tissue NO and MDA in torsionated and treated groups can be attributed to following mechanisms; sildenafil induced regulation of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) and the vasodilation induced by sildenafil can mediate this effect of ischemic preconditioning: release of adenosine/ bradykinin triggers a signaling cascade, leading to the phosphorylation of nitric oxide synthases (eNOS/iNOS), with subsequent release of NO [24]. When compared with the torsion group, JTBS and STD levels in the V_1T and V_2T groups were found to be significantly higher which indicates a protective effect of sildenafil which is an inhibitor of cGMP-specific phosphodiesterase type 5. This effect comprises with a rise in cGMP in the testis, cGMP signal transduction pathways are involved in a variety of local functions, based on autocrine or paracrine effects. In particular, cGMP has been suggested to influence motility in spermatozoa, development of testicular germ cells, relaxation of peritubular lamina propria cells, testosterone synthesis in Leydig cells and dilatation of testicular blood vessels (25). Additionally the protective effects of sildenafil on testiculer torsion can be explained by the following mechanisms. In a study antiaggregant and vasodilating effects of sildenafil led to the idea that it may improve the viability of flaps (21). Multiple lines of evidence have emphasized the importance of the vascular endothelium in regulating vasomotor, thrombotic, and inflammatory mechanisms that are critical in the pathophysiology of tissue injury induced by ischemia and reperfusion.

Endothelial cells appear to be sensitive to IR during ischemia, a state of reduced endothelial responsiveness to specific stimuli ("endothelial dysfunction") temporally precedes (and contributes to) the appearance of I-R induced tissue necrosis (3). Interestingly, although the majority of research in this area has involved multiple lines of evidence suggest that stimuli leading to K_{ATP} channel opening can induce a potent protective effect against I-R in different cell types, and recent studies have confirmed that similar mechanisms can also modulate the endothelial response to I-R (3). A study demonstrates that sildenafil increased coronary artery patency, reduced ex vivo platelet aggregability, and stopped cyclic flow reduction in the majority of animals. The PDE_5 inhibitors have been shown to reduce platelet aggregation in an animal model of endothelial injury, and reduce human ex vivo platelet aggregability. PDE_5 inhibitors also augment the ability of NO-donor compounds to decrease platelet aggregation in vitro and platelet-mediated thrombosis in vivo. In humans, oral sildenafil induces potent protection against I-R induced endothelial dysfunction through opening of K_{ATP} channels (26,27). The vasodilatory effect of nitric oxide is prolonged by the PDE 5 inhibitor sildenafil, which may be limiting the harmful diminished perfusion, free radical-induced tissue damage, venous insufficiency.

Sildenafil has a protective effect when administered after unilateral testicular torsion and detorsion in testis. This effect could only be documented patologically. This effect of sildenafil can be explained by the following mechanism; phosphodiesterase type 5 enzyme is inhibited selectively which leads to a rise of cGMP followed by the positive effects of cGMP mentioned above and additionally by vasodilatation and inhibition of platelet aggregation which altogether salvage ischemic tissue.

Conflict of interest

No conflict of interest was declared by the authors.

Acknowledgment

This study was sponsored by the Scientific Research Foundation of Gazi University (01/2002-119).

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