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The Effects of Statins on Testicular Ischemia Reperfusion-Induced Histopathologic Injury*

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Objective: Testicular torsion is a common syndrome that could lead to infertility. This study was designed to evaluate the histopathological effects of single dose of statins which have different pharmacological properties, in testicular ischemia/ reperfusion (IR) injury model that resembles testicular torsion/detorsion.

Material and Methods: Rats were anesthetized with intraperitoneal (i.p.) thiopental sodium (50 mg/kg) before the operation. Left testicular artery and vein of rats were occluded for 1 hour and the organ was allowed to reperfuse 24 hour before the orchiectomy for histologic examination. Simvastatin (5 mg/kg, i.p.), pravastatin (5 mg/kg, i.p.), atorvastatin (10 mg/kg, i.p.) or vehicle (10 % dimethylsulfoxide) was administrated before reperfusion.

Results: IR caused a significant histopathologic damage of left testis. The mean Johnsen score in IR group was lower than sham group (p=0.001). The score of testes treated with simvastatin was lower than sham (p=0.001) but higher than IR group (p=0.004). Atorvastatin and pravastatin treatments did not alter the mean Johnsen score.

Conclusion: The present study showed that simvastatin treatment reduced IR-induced histopathologic injury, while atorvastatin and pravastatin did not alter. In conclusion, we thought that these different effects of statins may be dependent on their different chemical and pharmacological properties.

Key Words: *Simvastatin, pravastatin, atorvastatin, ischemia-reperfusion injury, testis.*

Statinlerin Testiküler İskemi Reperfüzyon ile Oluşturulan Histopatolojik Hasar Üzerine Etkileri

Amaç: Testiküler torsiyon infertiliteye neden olabilen yaygın bir sendromdur. Bu çalışma farklı farmakolojik özelliklere sahip statinlerin, testiküler torsiyon/detorsiyonu yansıtan bir model olan testiküler iskemi/reperfüzyon (IR) hasarı modelinde, tek dozlarının histopatolojik etkilerini değerlendirmek için planlandı.

Gereç ve Yöntem: Sıçanlar operasyondan önce intraperitonal (i.p.) tiyopental sodyum (50 mg/kg) ile anestezi edildi. Sıçanların sol testiküler arter ve venleri 1 saat süreyle oklüze edildikten sonra, 24 saat reperfüze edildi. Histolojik değerlendirme için orşiektomi yapıldı. Simvastatin (5 mg/kg, i.p.), pravastatin (5 mg/kg, i.p.), atorvastatin (10 mg/kg, i.p.) veya çözücü (% 10 dimethilsülfoksit) reperfüzyondan önce uygulanıldı.

Bulgular: IR sol testislerde belirgin histopatolojik hasara neden oldu. IR grubunun ortalama Johnsen skoru sham grubundan daha düşüktü (p=0.001). Simvastatinle tedavi edilmiş testislerin skoru sham grubundan daha düşük (p=0.001), fakat IR grubundan daha yüksek bulundu (p=0.004). Atorvastatin ve pravastatin tedavileri ortalama Johnsen skorunu değiştirmedi.

Sonuç: Bu çalışma, IR ile oluşturulan histopatolojik hasarı, tek doz uygulanan simvastatin tedavisinin azalttığını; tek doz atorvastatin ve pravastatin tedavilerinin ise değiştirmediğini göstermektedir. Sonuç olarak, statinlerin sergilemiş olduğu bu farklı etkilerin, onların farklı kimyasal ve farmakolojik özelliklerine bağlı olabileceğini düşünmekteyiz.

Anahtar Kelimeler: *Simvastatin, pravastatin, atorvastatin, iskemi-reperfüzyon hasarı, testis.*

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Introduction

Testicular torsion is a common urologic emergency among infants and adolescents. The main pathophysiology of testicular torsion/detorsion appears to be ischemia/reperfusion (IR) injury of the testis (1,2).

Statins, competitive inhibitors of 3-hydroxy-3-methyglutaryl coenzyme (HMG-CoA) reductase, inhibit cholesterol biosynthesis. It has been suggested that statins have anti-oxidant (3, 4) and anti-inflammatory (5, 6) properties that may be beneficial in the treatment of IR-induced heart (7), kidney (8), intestines (9) and brain (10) injuries. Recently, in the experimental testicular torsion models, Karakaya et al. (11) suggested

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that rosuvastatin can protect tissue perfusion and Yang et al. (12) reported that simvastatin protects testes in a dose dependent manner. Although all statins share a common mechanism of action, they differ in terms of their chemical structures and pharmacokinetic profiles (13). The aim of this study is to investigate the effect of using a single dose of statins which have different pharmacological properties on testicular IR injury. The effects of simvastatin, pravastatin and atorvastatin on IR-induced histopathological changes are evaluated.

Material and Methods

Animals, Male Wistar albino rats weighing 200-250 g were placed in a temperature (21±2°C) and humidity (60±5%) controlled room in which a 12 h-12 h light-dark cycle was maintained. All experiments in this study were performed in accordance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1984) and were approved by the Committee on Animal Research at Zonguldak Karaelmas University, Zonguldak.

Surgery and experimental protocol, Under anesthesia induced by intraperitoneal (i.p.) injection 50 mg/kg sodium thiopental (Pental®, İE Ulugay İlaç Sanayi AŞ), an abdominal incision was made. The testicular artery and vein of the left testis were occluded with a vascular clamp for 1 h, after which time the clamp was removed and the organ was allowed to reperfuse for 24 h. The control group underwent a sham operation of the left testis. Sham operations were performed in a similar fashion, except the vessels were not clamped (14, 15). The rats were treated with simvastatin (5 mg/kg; i.p.), pravastatin (5 mg/kg; i.p.), atorvastatin (10 mg/kg; i.p.) and vehicle. Simvastatin, a prodrug, was given 30 min prior to reperfusion. Pravastatin, atorvastatin and vehicle were given 10 min prior to reperfusion. Simvastatin (Nobel İlaç Sanayi ve Ticaret AŞ., Istanbul, Turkey), pravastatin (Sigma St. Louis, MO, USA), atorvastatin (Fako İlaçları AŞ., Istanbul, Turkey) were dissolved in 10 % dimethylsulfoxide (DMSO).

Histological Analysis, The rats were sacrificed at 24 h of reperfusion for histopathologic evaluation. Removed testes were fixed in 10% neutral buffered formalin, processed routinely by automatic tissue processor and embedded in paraffin wax blocks. Four μm sections were obtained and stained with hematoxylin-eosin (H-E) before investigation under light microscope (Zeiss Axio Imager A1, Carl Zeiss Microimaging, GmbH 37081, Göttingen-Germany). Histological findings in seminiferous tubuli were evaluated according to the Johnsen's scoring system (16). Spermatogenesis of tubuli in 10 consequtive 400 X field areas were scored and mean values were determined. The Johnsen's score is based on the premise that with testicular damage there is successive disappearance of the most mature cell type, with progressive degeneration of germinal epithelium, with the disappearance of sperm and spermatids, then spermatocytes and finally Sertoli cells, in that order. A score of 1 indicates no seminiferous epithelial cells and tubuler sclerosis; score 2, no germ cells, only Sertoli cells; score 3, spermatogonia only; score 4, no spematids, since spermatocytes and arrrest of spermatogenesis at the primary spermatocyte stage; score 5, no spermatids and many spermatocytes; score 6, no late spaermatids with few early spermatids, arrest of spermatogenesis at the spermatid stage, and disturbance of spermatid differentiation; score 7, no late spermatids and many early spermatids; score 8, few late spermatids; score 9, late spermatids and disorganised tubuler epithelium; and score 10, full spermatogenesis.

Statistics, All data were expressed as the arithmetic mean ± S.E.M. P-value <0.05 was considered statistically significant. Distribution of the samples in the groups was analyzed with one sample of Kolmogorov–Smirnov test. The results were statistically analyzed by the Kruskal–Wallis H test. The differences between the groups were evaluated by the Mann–Whitney U test.

Results

The mean Johnsen's score of testes are shown in Table 1. In sham group, normal testicular morphology and orderly spermatogenesis with abundant mature spermatids in seminiferous tubuli were observed (Fig 1A). The Johnsen score of this group varied between 7 and 9 (mean; 8.2±0.10). The greatest significant histopathologic scores were observed in IR group's testes (Table 1). General disorganisation and sloughing of cells into tubular lumen as the result of loss of cohesion; maturation arrest in orderly spermatogenesis, nuclear pyknosis and cellular necrosis of germ cells were observed (Fig 1B). The mean Johnsen score in this group was 6.0 ± 0.19 and pretty low when compared with sham group (p=0.001). The mean Johnsen score of testes treated with simvastatin (6.8 ±0.12) was lower than sham (p=0.001) but higher than IR group (p=0.004). Simvastatin treatment significantly showed positive effects on sperm maturation, but it did not reverse the scores back to normal levels. Regenerative epithelial changes and onset of spermatogenic maturation was observed in some tubuli (Fig 1C). Scores of groups treated with pravastatin and atorvastatin were similar to IR group and these treatments did not alter IR-induced histopathologic injury (Table 1, Fig 1D and E).

Table 1. Semiquantitative estimates of testicular injury at 24 h reperfusion.

Groups	Score
Sham (n=9)	8.2 ±0.10[b]
IR (n=8)	6.0 ±0.19[a]
S+IR (n=7)	6.8 ±0.12[ab]
P+IR (n=9)	6.5 ±0.13[a]
A+IR (n=9)	6.1 ±0.03[a]

Data shown as mean values ± SEM. IR= Ischemia/reperfusion; S=Simvastatin; P=Pravastatin; A=Atorvastatin

[a]: p<0.05 vs Sham,[b]: p<0.05 vs IR.

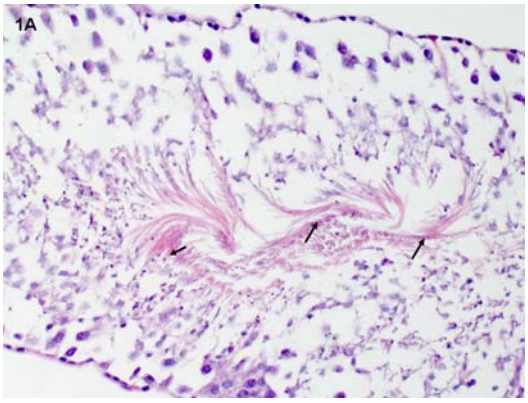

Figure 1A. Normal spermatogenesis with fully mature spermatids (arrows) at the lumen in sham group (H&E, 400X).

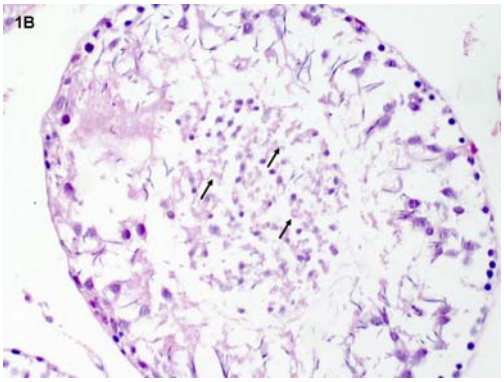

Figure 1B. Necrotic cellular debris at the lumen, maturation arrest, loss of cohesion (arrows) in IR group (H&E, 400X).

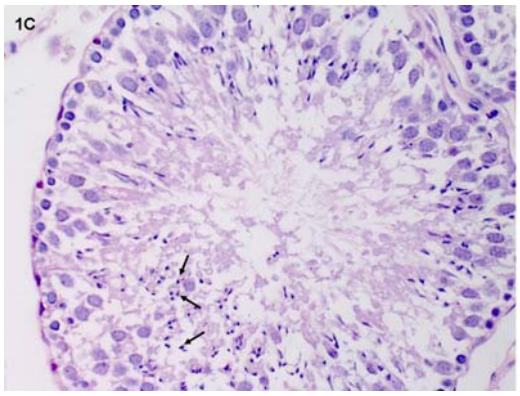

Figure 1C. Onset of spermatogenic maturation (arrows) in simvastatin group (H&E, 400X) .

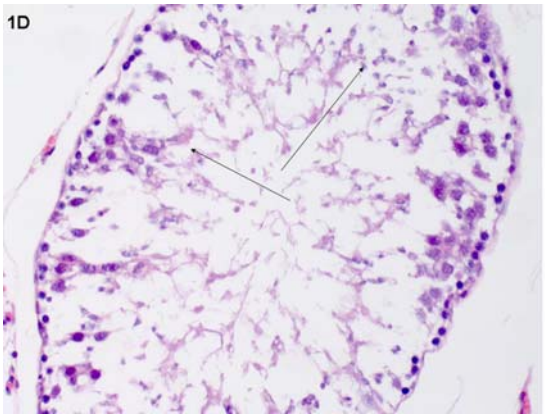

Figure 1D. Sloughed off germ cells and maturation arrest (arrows) in pravastatin group (H&E, 400X).

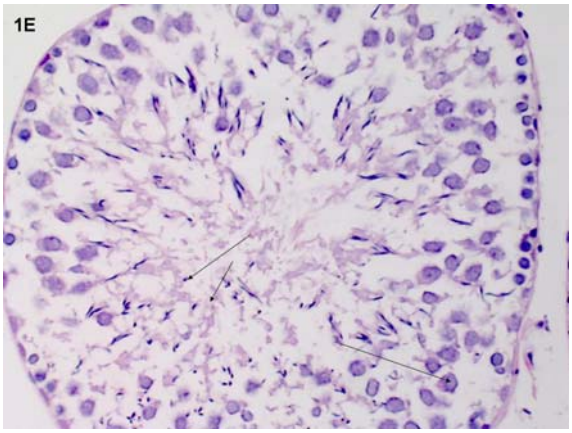

Figure 1E. Loss of cohesion and incomplete maturation (arrows) in atorvastatin group (H&E, 400X).

Discussion

Testicular IR involves multiple pathophysiologic mechanisms, such as reactive oxygen species production (17, 18), production-release of inflammatory mediators (15, 19) and neutrophil recruitment (20). Oxidative stress plays an important role in testicular IR injury (3, 4) and antioxidant agents such as melatonin (14, 21), allopurinol (22), lycopene (23), could limit testicular IR injury. It has been reported that statins have additional antioxidant, anti-inflammatory, immunomodulatory, antithrombotic, vascular protective and neuroprotective pleiotropic effects beyond their lipid lowering effects lowering effects (24- 27). In various

experimental models of IR injury, it has been notified that the protective effects of statins were shown after prolonged pretreatment lasting a few days up to four weeks (7- 10).Recently, it has also been reported that acute pretreatment with one or two doses administrations of statins may be protective to IR-induced organ damages, such as heart (28), kidney (8), brain (29) and testis (11, 12). The researchers have suggested that these protective effects of statins are related to upregulation of endothelial nitric oxide synthase (eNOS), increasing the production/release of NO, reducing inducible NOS (iNOS), and inhibition of NADPH oxidase-dependent superoxide anion production (28, 30, 31)

In our study, we histologically evaluated the testicular injury by the Johnsen's score, and simvastatin (5 mg/kg), atorvastatin (10 mg/kg) and pravastatin (10 mg/kg) were administrated by single dose. Among these drugs, only simvastatin provided significant improvement in IR testes. Similar to our study, Yang et al. (12) reported that simvastatin protected testes from torsion-detorsion injury in a dose dependent manner and histological findings revealed severe injury in testes of the torsion-detorsion and torsion-detorsion-simvastatin (1mg/kg) groups while testes in the torsion-detorsion-simvastatin (5mg/kg) group showed moderate injury. They suggested that mechanisms of protective effect of simvastatin may involve attenuating nuclear factor-kappaB activation and decreasing oxidative stress induced by torsion-detorsion. Karakaya et al. (11) measured the blood flow of the testis with laser doppler flowmeter in the experimental testicular torsion model and observed that rosuvastatin could protect the tissue perfusion.

Statins display remarkable chemical and pharmacokinetic differences that are crucial for their potential protective effects on IR-induced tissue damages. Lovastatin, simvastatin and pravastatin are fungal derived inhibitors of HMG-CoA reductase, while atorvastatin, pravastatin, cerivastatin, fluvastatin, pitavastatin and rosuvastatin are fully synthetic compounds (32). When the lipophilicity of statins that are used in our study are compared; pravastatin is more hydrophilic as a result of a polar hydroxyl group; and although simvastatin and atorvastatin have similar lipophilic properties, simvastatin is more lipophilic than atorvastatin (33, 34). Lipophilic statins, such as simvastatin and lovastatin, easily cross blood-brain barrier by simple diffusion, whereas hydrophilic statins, such as pravastatin and rosuvastatin, do not (35). In our study, the protective effect which is presented by only simvastatin may be related to its more lipophilic property than pravastatin and atorvastatin, thus simvastatin may easily across the blood-testis barrier, be compared to others. Simvastatin is a prodrug and converts to its active metabolites by phase I metabolism. Pravastatin and atorvastatin are not prodrugs. Pravastatin is metabolized to inactive metabolites while atorvastatin has active metabolites (36). In the present study, while atorvastatin and pravastatin were administered 10 min before the reperfusion, simvastatin, as a prodrug, was administered 30 min before. While simvastatin and pravastatin has a short peripheral plasma elimination half-life (2-3 h and 1.3–2.8 h, respectively) (37), atorvastatin has a longer peripheral plasma elimination half-life (7 h), with a prolonged inhibitory effect (20–30 h) resulting from the contribution of its active metabolites (38). In our study, simvastatin presented protective effect, but it did not reverse the scores back to normal levels. This condition is related to its short elimination half-life. If it had been administered twice instead of single dose, it would have presented a more protective effect. Although atorvastatin has similar lipophilicity with simvastatin, it did not present the protective effect like simvastatin. In our study, since atorvastatin was administrated 10 min before the reperfusion, we thougt that atorvastatin might not be sufficiently metabolized to its active metabolites which have been responsible for most of its main effect.

In conclusion, in this study, while single dose administration of atorvastatin and pravastatin did not present the protective effect, simvastatin caused a reduction of testicular damage during IR injury via mechanisms independent of lipid lowering activity. These results show that different statins present different effects in testicular IR model, and this difference may be related to remarkable chemical structures and pharmacokinetic properties of these drugs.

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Conflict of Interest

No conflict of interest was declared by the authors.

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