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The Effects of Vancomycin on Sperm Motility and Testicular Inflammation in Rats

Vankomisin'in Ratlarda Sperm Motilitesi ve Testiküler Yangı Üzerine Etkileri

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ABSTRACT

This study aimed to investigate the effect of vancomycin on sperm motility and testicular inflammation in male rats. In the study, 12 adult Sprague Dawley rats were used. They were divided into 2 equal groups. First group (n=6) was called as control group. Control group received physiological saline via intraperitoneal injection. Second group was called as vancomycin group and vancomycin group received vancomycin at the dose of 200 mg/kg via intraperitoneal injection for consecutive 7 days. At the end of study, the rats were sacrificed under sevoflurane anesthesia. Testes and cauda epididymis were collected. Mean testes weight, mean cauda epididymis weight, and sperm motility were evaluated. Also, tumor necrosis factor-alpha and nuclear factor kappa B levels were evaluated in testis tissues. According to our results, vancomycin treatment significantly decreased sperm motility while did not change mean testis weight and mean cauda epididymis weight when compared to control group. Besides, tumor necrosis factor-alpha and nuclear factor kappa B levels were significantly higher in vancomycin group than in the control group. In conclusion, vancomycin treatment (200 mg/kg BW, consecutive 7 days) decreases sperm motility significantly and increases the levels of testicular inflammation biomarkers (tumor necrosis factor-alpha and nuclear factor kappa B). Further studies are needed for protection from the side effects of vancomycin in male reproductive system.

Keywords: NFK B, sperm motility, testicular inflammation, TNFα, vancomycin

ÖZ

Bu çalışma, erkek sıçanlarda vankomisinin sperm motilitesi ve testis yangısı üzerindeki etkisini araştırmayı amaçlamıştır. Çalışmada on iki yetişkin Sprague Dawley sıçanı kullanıldı. Ratlar iki eşit gruba ayrıldılar. Birinci grup (n=6) kontrol grubu olarak adlandırıldı. Kontrol grubuna intraperitoneal enjeksiyon yoluyla ardışık 7 gün boyunca serum fizyolojik verildi. İkinci gruba Vankomisin grubu adı verildi ve vankomisin grubuna ardışık 7 gün boyunca intraperitoneal enjeksiyon yoluyla 200 mg/kg dozunda vankomisin verildi. Çalışmanın sonunda ratlar sevofluran anestezisi altında sakrifiye edildi. Testisler ve kauda epididimisleri toplandı. Ortalama Testis Ağırlığı (OTA), Ortalama kauda epididimis ağırlığı (OKEA) ve sperm motilitesi değerlendirildi. Ayrıca testis dokularında TNFα ve NF KB düzeyleri değerlendirildi. Sonuçlarımıza göre Vankomisin tedavisi, kontrol grubuna göre OTA ve OKEA değiştirmezken sperm motilitesini önemli ölçüde (P <,05) azalttı. Vankomisin grubunda ise TNFα ve NF KB düzeyleri kontrol grubundan önemli ölçüde (P <,001) yüksek bulundu. Sonuç olarak Vankomisin tedavisi (200 mg/kg vücut ağırlığı, ardışık 7 gün) sperm motilitesini önemli ölçüde azaltmakta ve testiküler inflamasyon biyobelirteçlerinin (TNFα ve NF KB) düzeylerini artırmaktadır. Vankomisinin erkek üreme sisteminde yan etkilerinden korunmak için yeni çalışmalara ihtiyaç olduğu kanaatine varılmıştır.

Anahtar Kelimeler: NF KB, sperm motilitesi, testis yangısı, TNFα, vankomisin.

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INTRODUCTION

Vancomycin is an antibiotic drug used in treatments against methicillin-resistant Gram-positive bacterial infections like *Staphylococcus aureus*.[1] Although this drug has been used since 1958, the side

effects of vancomycin have been reported in many studies including hepatotoxicity[2] and nephrotoxicity.[3] In a study conducted by Kandemir et al.[3] it is reported that vancomycin treatment increased the levels of inflammatory biomarkers including B-cell lymphoma-3 (Bcl-3), tumor necrosis factor alpha (TNFα), interleukin-33 (IL-33), nuclear factor kappa B (NFK B), interleukin-1β (IL-1β), prostaglandin E2 (PGE2), cyclooxygenase-2 (COX-2), myeloperoxidase, and inducible nitric oxide synthase (iNOS) in kidney tissue. In another study conducted by Kucukler et al.[2] it is suggested that vancomycin treatment increased inflammatory (IL-1β, NF-κB, iNOS, TNF-α, COX-2), apoptotic (P53, caspase-3 caspase-8), and oxidative stress (SOD, CAT, and GSH-px) biomarkers in liver tissue. Naidu et al[4] reported that solid lipid nanoparticle vancomycin treatment (20 mg/kg BW for 4 weeks) decreased sperm count significantly in Sprague Dawley rats. They also reported that testosterone level decreased numerically but not statistically.

The inflammation is initiated by various mechanisms such as chemical, mechanical, or thermal stress. In inflammatory situations, somatic cells release various cytokines, like TNF-α, and NFK B, etc., that activates to neutrophils to produce a host of cytotoxic substances.[5] The mechanism of vancomycin-induced toxicity may be related to increased inflammation. Inflammation in the testis increases the heat in the tissues and stimulates the immune system cells.[6] So, inflammatory situations induce reproductive damage and decrease sperm quality due to increased heat stress. The current study aimed to investigate the effects of vancomycin on sperm motility and testicular inflammatory biomarkers in male rats.

MATERIALS AND METHODS

Chemicals

Vancomycin and physiological saline were purchased from a pharmacy. All other chemicals used in the study have analytical purity.

Animals and Experimental Designs

Twelve adult male Sprague Dawley rats (12 weeks old, 200-250 g) were used in the study. The rats were kept under the standard laboratory conditions (40%-50% humidity, 12 h light/12 h dark period, 20-24°C). The rats were fed with commercial pellet feed and given daily fresh tap water. The rats were divided into 2 groups of 6 rats each. Control group rats received intraperitoneal physiological saline injection for consecutive 7 days. Vancomycin group rats received intraperitoneal vancomycin injection at the dose of 200 mg/kg daily for consecutive 7 days. Following the day of the last applications, the rats were euthanized under sevoflurane anesthesia. Both testes were randomly selected and 1 of cauda epididymis was collected. The testes were kept −20°C until biochemical examinations were performed. One of the cauda epididymis was trimmed in a petri dish including 5 mL Physiological saline and waited five minutes for sperm cells to migrate to the fluid. The approval number of the study has been given before the study has been started from Kastamonu University Local Ethic Board of Animal Experiments (05/2022). The dose of vancomycin was selected according to study of Kucukler et al[2] has been referred.

Reproductive Parameters

Mean cauda epididymis weights (MCEW) and mean testis weights (MTW) were detected according to Aksu et al.[7] Briefly, testes were removed following the euthanization process mentioned before, both left and right testes were weighed by using a precision scale, and arithmetic mean values were calculated as MTW (grams) for each rat in same group. Similarly, both left and right cauda epididymis were weighed by using a precision scale and arithmetic mean values were calculated as MCEW (grams) for each rat in same group.

Sperm motility estimation was performed according to our previous method.[7] Briefly, 20 μL of semen sample was released on previously warmed (34 °C) slide and a lamella covered on the semen sample. The motility estimations were performed under light microscopy at ×400 magnification, at least 2 different fields were analyzed by visual examination, and arithmetic mean value of 2 fields was calculated as final score. Motility score was expressed as the percentage (%).

Biochemical Evaluations

Testes were homogenized via an automatic homogenization device. TNF-α in the testicular homogenate was evaluated by a commercial TNF detection ELISA kit available for rats. Nuclear factor kappa B in the testicular homogenate was evaluated by a commercial ELISA kit available for rats. For the evaluation process, manufacturer's instructions were followed.[2] Both NF KB and TNFα levels were expressed as ng/g tissue.

Statistical Analyses

All values were stated as mean value ± standard error of mean. Data were analyzed using statistical analysis program and independent *t* test was used for the comparison of values. The difference between the groups at $P < .05$ was accepted as statistically important.

RESULTS

Reproductive Parameters

Mean testis weights, MCEW, and sperm motility values are presented in Table 1. According to Table 1, statistical difference has not been detected among the groups regarding MTW and MCEW. Also, sperm motility in vancomycin group was significantly ($P < .05$) lower than in the control group.

Testicular Inflammatory Biomarkers

Tumor necrosis factor-alpha values in testicular tissues are presented in Figure 1. According to Figure 1, TNFα values were 1.20 ± 0.01 ng/g tissue in the control group and 2.36 ± 0.02 ng/g tissue in the vancomycin group. The difference between control group and vancomycin group was statistically important (p<0.001).

Nuclear factor kappa B values in testicular tissues are presented in Figure 2. According to Figure 2, NFK B were 26.10 ± 0.51 ng/g tissue in the control group and 38.46 ± 0.46 ng/g tissue in vancomycin group. Both TNFα and NF KB values were significantly ($P < .001$) higher in vancomycin group than the control group. These results indicated that vancomycin treatment increased the inflammation in testes.

Table 1. Reproductive Parameters of the Groups

	Control	Vancomycin
MTW (g)	0.171 ± 0.011	0.186 ± 0.005
MCEW (g)	1.320 ± 0.030	1.338 ± 0.038
Motility (%)	62.50 ± 3.00[a]	49.00 ± 3.3[b]

[a,b]Statistical difference at $P < .05$.
MTW, mean testis weights; MCEW, mean cauda epididymis weights.

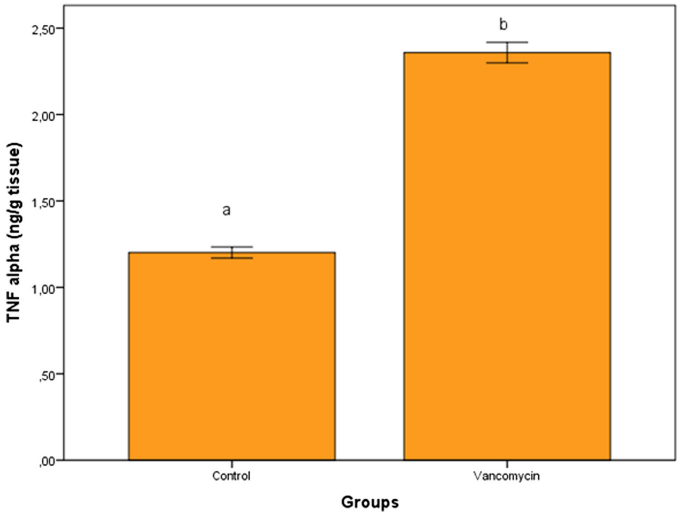

Figure 1. TNF alpha (α) (ng/g tissue) levels of the groups. a-b, statistical difference at $P < .001$.

DISCUSSION

Previous studies reported the side effects of vancomycin treatment like hepatotoxicity, nephrotoxicity, and reprotoxicity. These researchers suggested that the main mechanism of vancomycin-induced organ toxicity could have been attributed to inflammation and oxidative stress in the various organs like liver, kidney, and testis.[2-4] Many factors that cause infertility in men reveal their effects by activating the common pathophysiological pathways of inflammation and oxidative stress mechanisms.[8] The inflammation in reproductive tract stimulates immune cells and attracts them to the site of inflammation. Also, the inflammation activates the production of overactive substances and exacerbates oxidative stress. Reactive oxygen species can stimulate intracellular signaling cascade that induces the activation of genes relating to pro-inflammatory response.[6,9,10]

Cytokines are produced physiologically in the testes and are required for proper function of the male gonads.[11] Tumor necrosis factor-alpha is a pro-inflammatory cytokine. This cytokine is a pleiotropic polypeptide that has an important role in inflammatory activities. Tumor necrosis factor-alpha regulates the inflammation by encouragement of capillary endothelial cell pro-inflammatory reactions.[12] Tumor necrosis factor-alpha also promotes the migration of leukocytes into inflammation field of tissues. Moreover, TNF-α stimulates the apoptotic process in the cells[13]

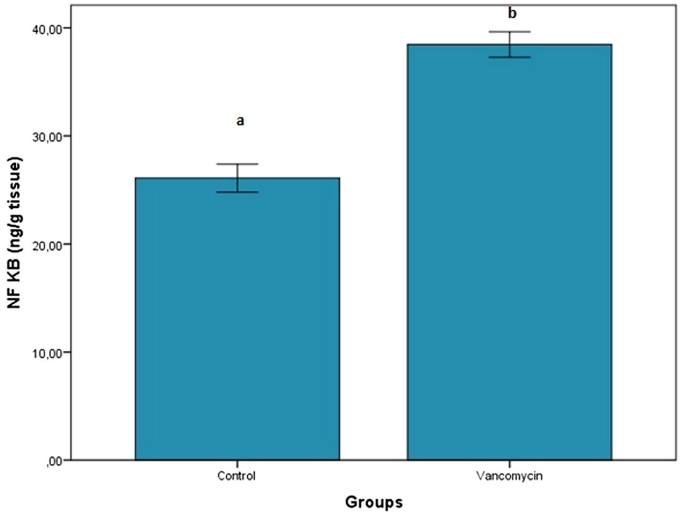

Figure 2. NFK B (ng/g tissue) levels of the groups. a-b, statistical difference at $P < .001$.

Nuclear factor kappa B is the omnipresent transcription factor, and also, it is a pleiotropic regulator of various genes interested in the inflammatory situations.[14] In inflammatory situations as a result of inflammation, the heat is increased in the tissue. As well known, increase in heat in testes causes the degeneration of testicular cells. Also, increased heat stress induces the basal metabolism of the sperm cells.

In the current study, the mechanism of decrease in sperm motility can be explained by inflammation induced by vancomycin. Increased inflammation in testes decreases mitochondrial oxidative phosphorylation process and Adenosine triphosphate production. As well-known, ATP is required for the movement of flagella (tail of sperm) and sperm motility. So, if ATP production decreases, sperm cells cannot move since ATP pools were exhausted.[7,15-19] Besides, increased heat as a result of inflammation in the testicles can accelerate sperm metabolism and cause rapid consumption of ATP.

In the current study, it is found that vancomycin treatment significantly increased TNFα and NF KB levels compared to the control group. Our results support the fact that vancomycin can increase inflammation in testes,[4] as well as liver[2,20,21] and kidney.[3,22] Naidu et al[4] suggested that 10 mg/kg vancomycin dissolved in linoleic acid solution did not affect sperm motility. However, in the current study, there is an significant decrease in sperm motility in vancomycin group when compared to control group. On the other hand, the decrease in sperm motility in our study can be explained due to the inflammation of testes induced by vancomycin.

In conclusion, vancomycin treatment decreased sperm motility significantly and increased the levels of testicular inflammation biomarkers (TNFα and NF KB). This drug can contribute to male infertility. More studies are required to minimize the side effect of vancomycin on male reproductive parameters.

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REFERENCES

1. Álvarez R, López Cortés LEL, Molina J, Cisneros JM, Pachón J. Optimizing the clinical use of vancomycin. *Antimicrob Agents Chemother*. 2016;60(5):2601-2609. [CrossRef]
2. Kucukler S, Darendelioğlu E, Caglayan C, Ayna A, Yıldırım S, Kandemir FM. Zingerone attenuates vancomycin-induced hepatotoxicity in rats through regulation of oxidative stress, inflammation and apoptosis. *Life Sci*. 2020;259:118382. [CrossRef]
3. Kandemir FM, Yildirim S, Kucukler S, Caglayan C, Mahamadu A, Dortbudak MB. Therapeutic efficacy of zingerone against vancomycin-induced oxidative stress, inflammation, apoptosis and aquaporin 1 permeability in rat kidney. *Biomed Pharmacother*. 2018;105:981-991. [CrossRef]
4. Naidu ECS, Olojede SO, Lawal SK, Peter AI, Akang EA, Azu OO. Effects of vancomycin linoleic acid nanoparticles on male reproductive indices of Sprague–Dawley rats. *Artif Cells Nanomed Biotechnol*. 2021;49(1):587-595. [CrossRef]
5. Butterfield TA, Best TM, Merrick MA. The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair. *J Athl Train*. 2006;41(4):457-465.
6. Zhang P, Zheng Y, Lv Y, et al. Melatonin protects the mouse testis against heat-induced damage. *Mol Hum Reprod*. 2020;26(2):65-79. [CrossRef]
7. Aksu EH, Akman O, Ömür AD, et al. 3, 3 diindolylmethane leads to apoptosis, decreases sperm quality, affects blood estradiol 17 β and testosterone, oestrogen (α and β) and androgen receptor levels in the reproductive system in male rats. *Andrologia*. 2016;48(10):1155-1165. [CrossRef]
8. Dutta S, Sengupta P, Slama P, Roychoudhury S. Oxidative stress, testicular inflammatory pathways, and male reproduction. *Int J Mol Sci*. 2021;22(18):10043. [CrossRef]
9. Yang D, Elner SG, Bian ZM, Till GO, Petty HR, Elner VM. Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells. *Exp Eye Res*. 2007;85(4):462-472. [CrossRef]
10. Anderson MT, Staal FJ, Gitler C, Herzenberg LA, Herzenberg LA. Separation of oxidant-initiated and redox-regulated steps in the NF-kappa B signal transduction pathway. *Proc Natl Acad Sci U S A*. 1994;91(24):11527-11531. [CrossRef]
11. Hales DB, Diemer T, Hales KH. Role of cytokines in testicular function. *Endocrine*. 1999;10(3):201-217. [CrossRef]
12. Feuerstein GZ, Liu T, Barone FC. Cytokines, inflammation, and brain injury: role of tumor necrosis factor-alpha. *Cerebrovasc Brain Metab Rev*. 1994;6(4):341-360.
13. Cudicini C, Lejeune H, Gomez E, et al. Human Leydig cells and Sertoli cells are producers of interleukins-1 and -6. *J Clin Endocrinol Metab*. 1997;82(5):1426-1433. [CrossRef]
14. Zhang T, Ma C, Zhang Z, Zhang H, Hu H. NF-κB signalling in inflammation and cancer. MedComm. 2021;2(4):618-653.
15. Aksu EH, Kandemir FM, Yıldırım S, et al. Palliative effect of curcumin on doxorubicin-induced testicular damage in male rats. *J Biochem Mol Toxicol*. 2019;33(10):e22384. [CrossRef]
16. Aksu EH, Kandemir FM, Özkaraca M, Ömür AD, Küçükler S, Çomaklı S. Rutin ameliorates cisplatin-induced reproductive damage via suppression of oxidative stress and apoptosis in adult male rats. *Andrologia*. 2017;49(1):e12593. [CrossRef]
17. Aksu EH, Kandemir FM, Altun S, Küçükler S, Çomaklı S, Ömür AD. Ameliorative effect of carvacrol on cisplatin-Induced reproductive damage in male rats. *J Biochem Mol Toxicol*. 2016;30(10):513-520. [CrossRef]
18. Aksu EH, Kandemir FM, Küçükler S. Effects of hesperidin on colistin-induced reproductive damage, autophagy, apoptosis by reducing oxidative stress. *Andrologia*. 2021;53(2):e13900. [CrossRef]
19. Ömür AD, Kandemir FM, Yıldırım BA, et al. Protective effect of dandelion (Taraxacum officinale) extract Against gentamicin-induced reproductive damage in male rats. *Kafkas Üniv Vet Fak Derg*. 2016;22(6):929-936.
20. Sahin M, Cam H, Olgar S, et al. Protective role of erdosteine on vancomycin-induced oxidative stress in rat liver. *Mol Cell Biochem*. 2006;291(1-2):155-160. [CrossRef]
21. Güzel S, Şahinoğullari ZU, Canacankatan N, Antmen ŞE, Kibar D, Bayrak G. The ameliorating effect of silymarin against vancomycin-induced apoptosis and inflammation in rat liver. *J Res Pharm*. 2019;23(4):719-728.
22. Elyasi S, Khalili H, Dashti-Khavidaki S, Mohammadpour A. Vancomycin-induced nephrotoxicity: mechanism, incidence, risk factors and special populations. A literature review. *Eur J Clin Pharmacol*. 2012;68(9):1243-1255. [CrossRef]