



Changes in Sperm Parameters Following Administration of Theophylline, a Competitive Antagonist of Adenosine in Rats Exposed to Bleomycin, a Chemotherapeutic Agent

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Abstract: The aim of the present study is to determine the effects of theophylline (THP), a methylxanthine derivative, on the sperm parameters of rats exposed to bleomycin (BLM) as a chemotherapeutic agent. The study was performed on 32 male rats. The rats in Group 1 were administered only with sterile saline intratracheally. The rats in Group 2 were administered a single dose of 7.5 mg/kg of BLM intratracheally. The rats in Group 3 received a single dose of 7.5 mg/kg of BLM intratracheally. In addition, THP was administered to rats in Group 3 intraperitoneally at a dose of 75 mg/kg/day for 14 days. The rats in group 4 were administered an intraperitoneal injection of THP at a dose of 75 mg/kg/day for 14 days. Motility, sperm density, abnormal sperm and dead sperm rates were examined. Significant deteriorations were observed in sperm parameters of rats that were administered BLM. In sperm parameters of rats that were administered THP, it was determined that it only increased motility, but did not significantly change sperm density, abnormal sperm and dead sperm count. In this study, THP only increased motility. There was no positive effect on sperm parameters except motility.

Keywords: Bleomycin, Rat, Sperm Parameters, Theophylline.

Bir Kemoterapotik Ajan Olan Bleomisine Maruz Kalan Ratlarda Adenozinin Kompetitif Antagonisti Olan Teofilinin Uygulanması Sonrasında Sperm Parametrelerindeki Değişimler

Öz: Bu çalışmanın yapılmasındaki amaç, bir kemoterapotik ajan olan bleomisine (BLM) maruz bırakılan ratlarda metilksantin türevi olan teofilinin (THP) sperm parametreleri üzerinde ne gibi etkiler göstereceğini belirlemektir. Çalışma 32 adet erkek rat üzerinde yapıldı. Grup 1'deki ratlara sadece steril serum fizyolojik intratrakeal yoldan uygulandı. Grup 2'deki ratlara, 7.5 mg/kg BLM intratrakeal olarak tek doz uygulandı. Grup 3'deki ratlara, 7.5 mg/kg BLM intratrakeal olarak tek doz uygulandı. İlave olarak 14 gün boyunca 75 mg/kg/gün dozunda THP intraperitoneal yoldan uygulandı. Grup 4'deki ratlara, 14 gün boyunca 75 mg/kg/gün dozunda THP intraperitoneal yoldan uygulandı. Motilite, sperm yoğunluğu, anormal sperm ve ölü sperm oranları incelendi. BLM uygulanan ratların sperm parametrelerinde ciddi bozulmalar tespit edildi. THP uygulanan ratların sperm parametrelerinde sadece motilitede anlamlı artışlar oluşturduğu, ancak sperm yoğunluğu, anormal sperm ve ölü sperm sayısı üzerinde anlamlı olarak değişiklikler yapmadığı tespit edildi. Bu çalışmada THP sadece motiliteyi arttırmıştır. Motilite dışında sperm parametreleri üzerinde olumlu bir etkisi yoktu.

Anahtar Kelimeler: Bleomisin, Rat, Sperm Parametreleri, Teofilin.

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INTRODUCTION

Bleomycin (BLM) is an antibiotic with a chemotherapeutic characteristic and is produced by *Streptomyces verticillus* bacteria. This antibiotic is predominantly used in the treatment of cancers such as lymphoma, carcinoma and testicular cancer (1). Despite its healing effects, it is acknowledged that BLM causes several undesirable side effects on the respiratory system, gastrointestinal tract and the reproductive system (2,3). Earlier studies reported that BLM reduced sperm motility and adversely affected sperm morphology through affecting the gonadal cells on male reproductive system (4,5,6). It is known that the application of chemotherapeutics generally results with a serious damage on the seminiferous epithelium of the testis (7). It was reported that approximately 50-70% of BLM was eliminated through the kidneys (8).

Theophylline (1,3-dimethylxanthine; THP), a competitive antagonist of adenosine induced via ATP hydrolysis, is a member of the xanthine family and is naturally present in tea. THP has a vasodilatory effect on the renal vascular bed and also affects smooth muscle cells in the bronchi of asthma patients (9-15). Furthermore, an additional acknowledged characteristic of THP is the inhibition of the phosphodiesterase enzyme that inactivates cyclic adenosine mono phosphate (cAMP). Phosphodiesterase enzyme inhibitors are key regulators of intracellular cAMP levels. It is known that spermatogenesis is regulated by the cAMP-dependent mechanism (16).

It was reported that high concentrations of THP adversely affected spermatogenesis and caused infertility through inhibiting the support functions of sertoli cells (15). Previous studies also reported that high doses of THP caused decreases in testis weight and increased abnormal sperm count (14,17).

The conducted literature review indicated that several methylxanthines such as THP were added to sperm suspensions with the intent to improve sperm functions, however high dose administrations

resulted with a reprotoxic effect. Therefore, in this study, we investigated whether THP had a curative effect on the negative effects of BLM on sperm parameters.

MATERIALS and METHODS

Chemicals

A commercial preparate of BLM (Bleocin; bleomycin hydrochloride; Nippon Kayaku Co., Ltd., Tokyo, Japan) was obtained from a pharmacy. THP (Theophylline, Product code: T-1633) was purchased from Sigma-Aldrich (Sigma Louis Aldrich St. Louis, Mo., USA). Other materials used in the study were present in the Department of Reproduction and Artificial Insemination in the Faculty of Veterinary Medicine in Van Yüzüncü Yil University, Van, Turkey.

Animals

The study was performed on 32 male rats. Animals were obtained from the Experimental Animals Center of the Van Yuzuncu Yil University. Standard temperature, humidity and light/dark conditions were provided for the rats. The rooms were cleaned and ventilated daily and an appropriate environment was provided. The rats were provided with clean and fresh feed and water.

Experimental Design

Necessary permissions were obtained from the Local Ethics Committee of Animal Experiments of Van Yuzuncu Yil University (Authorization number: 2019/05). Ethical rules were complied with in all applications. 32 male rats were divided into 4 groups.

1. Control Group (n=8): Sterile saline was administered intratracheally.

2. BLM Group (n=8): BLM was administered as a single dose of 7.5 mg/kg intratracheally.

3. BLM + THP Group (n=8): BLM was administered as a single dose of 7.5 mg/kg intratracheally. Additionally, THP was administered

intraperitoneally at a dose of 75 mg/kg/ day for 14 days.

4. THP Group (n=8): THP was administered intraperitoneally at a dose of 75 mg/kg/ day for 14 days.

At the end of the study, each rat was anesthetized via ketamine hydrochloride 75 mg/kg and xylazine hydrochloride 10 mg/kg administration. Subsequent to the formation of a full anesthesia, testicles were rapidly removed before they lost temperature, the cauda epididymis was retrieved and the spermological evaluation was performed. Especially the motility examination was performed very quickly, before the tissue lost its temperature.

Obtaining Semen and Evaluation of Spermological Traits

Immediately after the testes were removed before losing their temperature, the motility measurement of the sperm, obtained from the cauda epididymis section via small incisions with the help of a scalpel, was carried out by means of a heated plate microscope (plate temperature was adjusted to 37 °C). During motility examination, the sperm was diluted with isotonic saline on the heated plate. Subsequently, the cauda epididymis was divided into small pieces with a scalpel in a petri dish (0.9% w/v NaCl) containing 2 ml of physiological saline, for evaluation of other spermological parameters. The samples were kept at the room temperature for 10 minutes in order to ensure the maximal sperm passage. Density, abnormal sperm and dead sperm examinations were performed with this suspended mixture (18). Previously described methods were

Table 1. Sperm parameters of all groups.

Tablo 1. Tüm grupların sperm parametreleri.

Groups	Motility Rate (%)	Density (x10 ⁶)	Dead Sperm Rate (%)	Abnormal Sperm Rate (%)		
				Head	Tail	Total
Control (n=8)	67.50±2.67 ^b	81.75±1.75 ^a	14.62±0.74 ^b	8.25±0.70 ^b	4.62±0.91 ^b	12.87±0.83 ^b
BLM (n=8)	43.12±2.58 ^d	46.50±16.81 ^b	35.75±0.88 ^a	16.50±1.19 ^a	12.37±1.30 ^a	28.87±0.83 ^a
BLM+THP (n=8)	49.37±1.76 ^c	51.75±1.66 ^b	34.87±0.35 ^a	16.12±1.35 ^a	12.00±1.30 ^a	28.12±0.83 ^a
THP (n=8)	71.25±2.31 ^a	83.25±1.16 ^a	13.75±0.46 ^b	7.87±0.99 ^b	4.25±0.88 ^b	12.12±0.99 ^b

BLM: Bleomycin, THP: Theophylline

Note: Different lettering (a, b, c, d, e) on the same columns indicate significant differences among groups (P <0.001).

used for the evaluation of sperm density and morphology (18,19,20).

Statistical Analysis

SPSS software was used to evaluate the data statistically. Differences between groups were determined via the Post hoc Tukey-HSD test and one-way analysis of variance (ANOVA). The obtained values were presented as mean ± standard deviation.

RESULTS

Sperm Parameters

The detailed results of motility, density and abnormal sperm and dead sperm ratios for all groups were presented in Table 1 and Figure 1. It was determined that the motility and density values of the BLM group were significantly lower than the control group (P<0.001). The examination among groups indicated that the most significant increase in the number of abnormal sperm and dead sperm was detected in the BLM group. Sperm density of the THP group was found to be increased when compared to the control group, however, such increase was not statistically significant (P>0.05). In the THP + BLM group, THP had no effect on morphological disorders. In the present study, it was determined that THP administration resulted with an increase, especially in motility, when compared to the control and other treatment groups, and such finding was statistically significant.

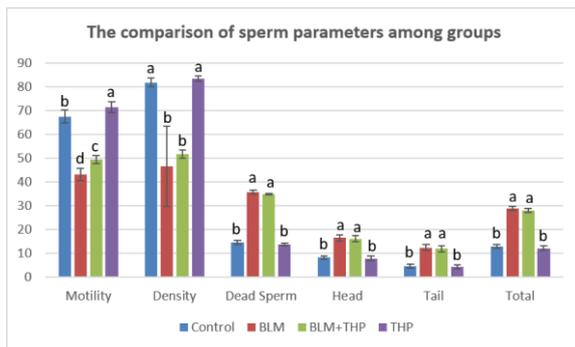


Figure 1. The comparison of sperm parameters among groups.

Şekil 1. Gruplardaki sperm parametrelerinin karşılaştırılması.

DISCUSSION and CONCLUSION

Mammalian sperm is an excellent marker for monitoring the potential effects of chemical agents on the male reproductive system. BLM is used in the treatment of several cancers. However, several concerns are present regarding the continuity of its use in treatment due to its side effects. In the present study, it was determined that sperm density and motility in rats significantly decreased compared to the control group due to BLM treatment, and our findings were similar with the deteriorations in the sperm parameters caused by BLM, reported in previous studies (4-6). Furthermore, increase in abnormal sperm and dead sperm ratios was consistent with the outcomes of previous studies (4,5).

Due to their known phosphodiesterase inhibitory effects, methyl xanthines positively affected sperm motility, capacitance and acrosome reaction due to an increase in sperm cAMP levels (21-24). It was reported that methyl xanthines such as pentoxifylline, caffeine and THP, which were added to sperm suspensions to improve sperm characteristics, improved sperm function (25-27).

The administration dose and duration of phosphodiesterase inhibitors were found to be influential in terms of their efficiency (28). Caffeine, pentoxifylline and THP, which are non-selective phosphodiesterase enzyme inhibitors were specified to increase sperm motility, however such effect was

not obvious in vivo and even high-dose caffeine was reported to have adverse effects on fertility (29). A conducted study reported that the effects of THP administered at 800 ppm dose for 18, 30 and 42 days varied depending on the duration of administration, especially abnormal sperm count increased in rats, administered THP for 30 and 42 days, and no adverse effects were observed in sperm parameters due to 18 days of application. In a study conducted by Tengowski et al. (14), it was determined that longer the duration of administration caused higher the deterioration, thus, was reported to have a reprotoxic effect at high concentration and prolonged exposure. The results of the present study were similar to the results of the 18-day administration. In the present study, THP was administered at a dose of 75 mg/kg for only 14 days and no problems were detected. Decreases in testis weight and abnormal sperm count were detected in another study, which focused on THP administration for 13 weeks (17). Of the rats, which were treated with the methylxanthines caffeine, theobromine or THP between the 14th to 75th weeks of age, aspermatogenesis or oligospermatogenesis were reported in 85-100% of those treated with caffeine or theobromine, whereas THP caused severe testicular atrophy in 14% of rats and moderate atrophy in 71% (15). The findings of the present study were not consistent with the results of that study due to the short duration of administration. In the present study, there was no statistically significant change in abnormal sperm count due to THP administration.

In conclusion, the present study established that THP did not have a statistically significant effect on the sperm density, morphology and dead sperm ratio, in cases where the destructive effects of BLM on sperm parameters were present. However, we would like to draw attention to the significant increase in sperm motility due to THP, a non-specific phosphodiesterase enzyme inhibitors, in the present study, given the fact that weak sperm motility is an important factor in male infertility and there is a

strong correlation between sperm motility and fertility. Considering such effect of THP, it is possible that several further studies could facilitate its use in assistive reproductive technology, serving as an auxiliary therapeutic agent in alleviating male infertility.

Conflict of interest

The authors declare that they have no conflict of interest.

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