

Effect of 2100 MHz Radio Frequency Radiation on Oxidative Stress on Testicular Tissue of Hypertensive and Non-Hypertensive Rats

Hipertansif Ve Hipertansif Olmayan Sıçanlarda 2100 MHz Radyo Frekans Radyasyonun Testis Dokusunda Oksidan Stres Üzerine Etkisi

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Abstract

INTRODUCTION: Nowadays exposure to radiation has been increasing with the advancement of technology. Although considerable research has focused on the effects of radiation exposure on biological systems, its effect on hypertensives has previously never been addressed. In this study, the effect of 2100 MHz radio frequency radiation (RFR) on oxidative stress on testicular tissue of hypertensive and non-hypertensive rats is aimed to be investigated. **METHODS:** Twenty-four male Wistar Albino rats were allocated into four groups: 1) Control (C), 2) Control +Hypertension (C+H), 3) Radiation (R), 4) Hypertension+ Radiation (H+R). In order to induce hypertension in rats, 60 mg/kg L-NAME dissolved in 1 ml tap water was orally administered to rats for one month. Rats were exposed to 2100 MHz RFR for 60 min /day, 5 days/ week for 8 weeks. Following the experiments, the rats were decapitated under anesthesia and malondialdehyde (MDA), Nitrite+nitrate (NOx) and glutathione (GSH) levels were examined on testicular tissue. Results were compared with One-way ANOVA followed by post hoc Tukey tests. Values of $p < 0.05$ were accepted as significant.

RESULTS: When testicular tissues of R and H+R groups were compared with those of C and H groups, MDA and NOx levels were seen to increase while GSH levels decreased ($p < 0.01$).

DISCUSSION AND CONCLUSION: Radiation exposure led to an equal amount of increase of oxidative stress on testicular tissues of hypertensive and non-hypertensive rats. Oxidative stress caused by radiation exposure may negatively affect male reproductive system in both hypertensives and non-hypertensives.

Keywords: NG-nitroarginine methyl ester, radiation, rats, oxidative stress, testis

Öz

GİRİŞ ve AMAÇ: Günümüzde ilerleyen teknoloji ile birlikte daha fazla radyasyona maruz kalmaktayız. Radyasyon maruziyetinin biyolojik sistemlerdeki etkilerini inceleyen çok sayıda çalışma olmasına rağmen hipertansiflerdeki etkilerini inceleyen çalışma bulunmamaktadır. Bu çalışmada hipertansif ve hipertansif olmayan sıçanların testis dokusunda 2100 MHz RFR nin oksidatif strese etkilerini incelemeyi amaçladık.

YÖNTEM ve GEREÇLER: 24 adet, erkek Wistar Albino sıçan 4 gruba ayrıldı; 1) Kontrol (K), 2) Kontrol+Hipertansiyon (K+H), 3) Radyasyon (R), 4) Radyasyon+Hipertansiyon (R+H). Sıçanlarda hipertansiyonu indüklemek için 1 ay süre ile 1 ml çeşme suyunda çözünen 60 mg/kg L-NAME oral gavaj ile verildi. Sıçanlar 2100 MHz RFR'ye günde 60 dakika/5 gün/8 hafta boyunca maruz bırakıldı. Uygulamaların bitiminde sıçanlar anestezi altında dekapite edilerek testis dokusunda göstergesi malondialdehit (MDA), Nitrit+nitrat (NOx) ve glutatyon (GSH) düzeyleri çalışıldı. Sonuçlar One-way ANOVA followed by post hoc Tukey tests ile karşılaştırıldı. $p < 0.05$ olanlar anlamlı kabul edildi.

BULGULAR: R ve H+R gruplarının testis dokusunda hem C grubu hemde H grubu ile karşılaştırıldığında MDA ve NOx düzeylerinde artış, GSH düzeylerinde ise azalma olduğu belirlendi ($p < 0.01$).

TARTIŞMA ve SONUÇ: Hipertansif olan ve hipertansif olmayan sıçanların testis dokusunda radyasyon maruziyeti oksidatif streste aynı oranda artışa neden oldu. Hem hipertansiflerde hemde hipertansif olmayanlarda radyasyon maruziyetine bağlı artan oksidatif stres erkek üreme sistemini olumsuz yönde etkileyebilir.

Anahtar Kelimeler: NG-nitroarjinin metil ester, radyasyon, oksidatif stres, testis

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INTRODUCTION

Hypertension is a chronic disease that increase the blood pressure (BP) in the arteries. It is a risky element of sexual function for males as it harms the testicular tissue and entails the risk of reproductive function. However, the effect of hypertension on male fertility has not been examined in depth (1). Hypertension leads to an increase in reactive oxygen species (ROS) and a decrease in antioxidant enzyme activity. Previous studies have reported that increased oxidative stress triggers lipid peroxidation in spermatozoa, decline in sperm count, and DNA sperm damage (2-4).

Nitric oxide (NO), generated from L-arginine by nitric oxide synthase (NOS), has a crucial role in regulating blood pressure and arterial resistance. Chronic application of N-nitro-L-arginine methyl ester (L-NAME), which is a NOS inhibitor, is widely preferred with the aim of inducing chronic NO deficiency and therefore hypertension in experimental animals (5).

Potential harmful effects of radiofrequency (RF), which has a wide application in telecommunications, have received considerable attention lately (6). The effects of radiofrequency radiation (RFR) on reproductive system is a cause for concern. Several studies have been conducted regarding this issue so far. Some studies reported that RFR decreases spermatozoa motility, induces DNA damage, and decreases leydig cell number. It was also reported that RFR has harmful effects on male reproductive system as it increases oxidative stress and decreases antioxidant capacity (7-9). Contrary to these studies, some researchers reported that RFR did not have an effect on the structure and function of the tissue, sperm motility and concentration, and spermatogenic cycle (10-12). Literature on the effect of radiation on reproductive system has remained unclear. Moreover, no prior studies have examined the effect of RFR on oxidative stress on testicular tissues in the presence of hypertension.

In this study, the aim is to examine the effect of 2100 MHz radio frequency radiation (RFR) on oxidative stress on testicular tissues of hypertensive and non-hypertensive rats.

MATERIALS AND METHODS

Experimental Design

Twenty-four male Wistar Albino rats (250±20 g) were grouped into four experimental groups on a random basis as six rats in each group. First group "Control (C)" was administered tap water while second group "Hypertension (H)" was administered L-NAME. Third group "Radiation (R)" was exposed to RFR while fourth group "Hypertension+Radiation (H+R)" was both administered L-NAME and exposed to RFR. In order to induce hypertension in rats, 60 mg/kg L-NAME dissolved in 1 ml tap water was orally administered to rats for one month. Rats were exposed to 2100 MHz RFR for 60 min /day, 5 days/ week for 8 weeks. Systolic and diastolic blood pressure of the rats was recorded on a weekly basis by using non-invasive indirect blood

pressure system. Rats with over 140 mmHg systolic blood pressure and over 90 mmHg diastolic blood pressure were determined as hypertensive (13). (The systolic and diastolic blood pressure results of all groups are presented in Table 1).

Table 1: The systolic and diastolic blood pressure results of all groups

	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
C	112±6	79±5
H	148±4	96±3
R	119±7	82±3
H+R	150±5	99±6

The values are means ± SD; n = 6.

C.Control, H.Hypertension, R.RFR, H+R.RFR+Hypertension.

2100 MHz RF radiation was calculated with an electric field probe. In RF fields, the root mean square value for electric field was determined as 17.25 V/m (14, 15).

The rats were housed in polyethylene cages and kept in a well-ventilated room at 27 °C. Animals were given free access to water and food throughout the study. A 12-h light-dark cycle were maintained. At the end of three months, all the rats were anesthetized with intramuscular Rompun (5mg/kg) + Ketamine (45mg/kg) and sacrificed after blood samples were taken from their hearts. All procedures applied on animals and the study design were approved ethical and research committee of the university (Approval No: G.Ü.ET-16.006).

Biochemical studies on testes tissue

With the measurement of thiobarbituric acid reactive substances (TBARS) formation, quantification of the Lipid peroxidation of the tissue was achieved. Samples were homogenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer (Heidolph Diax 900, Germany). Following centrifugation of the homogenate at 3000 × g for 10 min (Hermle Z 323 K, Germany), 750 µl of supernatant was added to an equal volume of 0.67% (m/v) thiobarbituric acid and heated at 100°C for 15 min. The absorbance of the samples was measured at 535 nm. Lipid peroxide levels are expressed in terms of MDA equivalents using an extinction coefficient of 1.56 × 10⁵ l·mol⁻¹·cm⁻¹ (16).

By utilizing Elisa reader by vanadium chloride (VCl₃)/Griess assay total nitric oxide (NO_x) levels of the tissue were attained. Tissues were homogenized in five volumes of phosphate buffer saline (pH = 7) and centrifuged at 2000 × g for 5 min. After centrifugation, 0.25 ml of 0.3 M NaOH was added to 0.5 ml supernatant. The incubation of the samples for 5 min at room temperature was followed by addition of 0.25 ml of 5% (w/v) ZnSO₄ for deproteinization. This mixture was then centrifuged at 3000 × g for 20 min and supernatants were used for the assays. Nitrate standard solution was serially diluted and the plates were loaded with samples (100 µl). Then Vanadium III chloride

(VC13) (100 µl) and Griess reagents sulphanilamide (SULF) (50 µl) and N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) (50 µl) were added to each well. After incubation in 37°C for 45 min, samples were measured at 540 nm using ELISA reader (17).

Reduced glutathione (GSH) levels in the tissue were obtained by Ellman method through some modifications. Samples were homogenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer. After centrifugation of the homogenates at $3\,000 \times g$ for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 M $\text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O}$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and after mixing, the absorbance at 412 nm was measured using a spectrophotometer (UV 1208, Shimadzu, Japan) at room temperature immediately. The GSH levels were calculated using an extinction coefficient of $13\,600 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ (18).

Statistical analysis

Results are given as mean \pm standard deviation (SD) and analyzed by One-way ANOVA followed by *post hoc* Tukey tests. Based on the reference studies, the G-power program was used and it was decided to experiment with a total of 24 rats with 80% power and 5% margin of error. Results which were found to be significant when $p < 0.05$ with respect to the control group.

RESULTS

Results of the study indicated that there was no statistically significant difference in NOx, MDA, and GSH levels of H Group when compared with those of C group ($p < 0.01$). As for R and H+R groups, when compared with C and H groups, an increase in MDA and NOx levels and a decrease in GSH was detected ($p < 0.01$). When R and H+R groups were compared, no statistically significant difference was found in NOx, MDA, and GSH levels ($p > 0.05$) (Results are presented in Table 2)

DISCUSSION

In the present study, 60 mg/kg L-NAME dissolved in 1 ml tap water that was orally administered to rats for one month did

not cause a change in NOx, MDA, and GSH levels on testicular tissue. Contrary to our findings, previous studies in literature reported that L-NAME increased oxidant stress on testicular tissue while decreasing antioxidant capacity.

In a study by Gulhan, 40 mg/kg L-NAME was administered for 28 days. The study concluded that TOS and MDA levels increased on testicular tissue at the end of 28 days while NO, CAT, and TAS levels decreased. It was reported that the rate of unsaturated fatty acid of testicular tissue cells was high; therefore, it was highly affected by the free radicals caused by L-NAME induction and one of the most important indicators of this was MDA. Parallel to increasing antioxidant status, intracellular and extracellular antioxidant enzyme systems were reported to decrease (19). Akinyemi et al. found out that there was an increase in TBARS and ROS levels and a decrease in GSH and NO levels on the testicular tissue of rats which were orally administered 40 mg/kg L-NAME for 10 days. It was reported that L-NAME decreased NO levels by inhibiting endothelium nitric oxide synthase (eNOS) and thus increased superoxide anion production. Also, NO was reported to prevent and inactivate the production of free radical scavenger and superoxide anion (20). In another study, 40 mg/kg L-NAME administered for four weeks caused a decrease in SOD (Superoxide dismutase), CAT (catalase), GPx (Glutathione peroxidase), GSH and NO levels while causing an increase in MPO (myeloperoxidase), LPO (Lipid Peroxidation) and H_2O_2 (Hydrogen peroxide) levels on testicular tissue (21). Contrary to the findings in literature, results of this study may have stemmed from the use of higher dose of L-NAME which led to an early stimulation of antioxidant system on the tissue and its protective feature against lipid peroxidation.

In this study, 2100 MHz RFR exposure for 60 min /day, 5 days/ week for 8 weeks caused an increase in MDA and NOx levels and a decrease in GSH levels on testicular tissue. This finding is consistent with what has been found in previous studies in the literature. In a study, 900 MHz RFR exposure for 4 hours /day for 40 days resulted in an increase in MDA levels and a decrease in catalase levels on testicular tissue (22). In another study, 900 MHz RFR exposure for 3 h twice/day for 35 days led to an increase in TBARS levels while decreasing SOD activity. Researchers reported that RFR exposure decreased

Table 2: The results of testes MDA, NOx and GSH levels

	MDA Levels (nmol/per mg of protein)	NOx Levels (µmol/per mg of protein)	GSH Levels (nmol/per mg of protein)
C	5,48±0,67	8,83±0,76	3,01±0,19
H	5,77±0,59	10,55±0,68	2,89±0,31
R	7,79±0,5 * #	13,29±1,83 * #	2,42±0,12 * #
H+R	8,05±0,47 * #	13,89±0,83 * #	2,26±0,13 * #

The values are means \pm SD; n = 6.

* $p < 0.01$ versus C; # $p < 0.01$ versus H

C.Control, H.Hypertension, R.RFR, H+R.RFR+Hypertension.

antioxidant level and antioxidant enzyme activity and this led to an increase in lipid peroxidation (TBARS) (23). 2.45 GHz RFR exposure for 3 h/day for 30 days led to an increase in MDA and TOS levels while decreasing TAS levels in rats (24). 8 Gy γ -Radiation exposure caused an increase in MDA and NOx levels of rats while decreasing GSH levels (25). Özorak et al. applied 900 MHz, 1800 MHz, and 2.45 GHz radiation exposure on rats for 60 min/day, 5 days/week for up to the 6th week after birth. They found an increase in lipid peroxidation level on testicular tissue. However, unlike our findings, no significant difference was observed in GSH levels (26). In light of these studies, it is possible to say that radiation exposure may lead to an increase in $O^{\cdot -2}$, H_2O_2 , $\cdot OH$, and $ONOO^-$ on the tissue. Subsequently, these radicals may be causing an increase in MDA levels by interacting with polyunsaturated fatty acids of cell membrane phospholipids. In addition, these radicals may be increasing NOx levels in the cell with inducible nitric oxide synthase (iNOS) activation. The decrease of GSH levels on tissues may result from the use of GSH in H_2O_2 detoxification or its reaction with $ONOO^-$ to form S-nitrosoglutathione.

In this study, radiation exposure resulted in an increase in MDA and NOx levels and a decrease in GSH levels of hypertensive rats. No previous studies have investigated the effect of radiation exposure on hypertensive rats. Therefore, the effect of radiation exposure on oxidative stress of hypertensives was not discussed in this section.

CONCLUSION

In the present study, 2100 MHz radiation exposure increased oxidative stress and decreased antioxidant capacity on testicular tissues of both hypertensive and non-hypertensive rats evenly. Oxidative stress caused by radiation exposure on testicular tissues may negatively affect male reproductive system in both hypertensives and non-hypertensives. However, the limitation of our study is that sperm analysis was not performed and a histopathological image was not obtained in this study. That's why, further research is needed to comprehensively examine the effects of radiation directly on reproductive system.

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