Effects of 2600 Mhz Radiofrequency Radiation and Melatonin on Skin and Liver Tissue Hydroxyprolin Levels of Male Rats

2600 Mhz Radyofrekans Radyasyonun ve Melatoninin Deri ve Karaciğer Dokusu Hidroksiprolin Düzeyleri Üzerine Etkileri

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ABSTRACT

Objective: In the present study, the effect of 2600 MHz radiofrequency radiation (RFR) exposure, and melatonin treatment on skin and liver tissue hydroxyproline (Hyp) levels were evaluated.
Methods: In the study, 2600 MHz RFR application was performed by Rohde & Schwartz Radiofrequency signal generator via ETS-Lindgren horn antenna. 36 Wistar albino male rats were randomly divided into 6 groups (n = 6); Control, sham, RFR, control + Melatonin, Sham + Melatonin, RFR + Melatonin. RFR was applied to the related groups for 30 days (5 days/week) for 30 minutes a day. Average electric field value is 21.74 V / m. Melatonin groups were subcutaneously injected with melatonin (10 mg/kg) for 30 days. At the end of the experiment, Hyp levels were measured in the skin and liver tissues.
Results: Hyp levels in skin tissue decreased significantly in RFR treated groups compared to the control and sham groups ($p < 0.05$). Also, Hyp levels in skin tissue decreased significantly in RFR + melatonin treated group compared to the control, sham and sham+melatonin groups. No changes in Hyp levels in liver tissue were determined.
Conclusion: In this study, RFR exposure leads to significant changes in skin Hyp levels but not liver tissue. This effect is thought to be due to the closer the skin tissue to the surface.

Keywords: Radiofrequency radiation, melatonin, hydroxyproline, liver tissue, skin tissue, rat.

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ÖZET

Amaç: Bu çalışmada 2600 MHz radyofrekans radyasyon (RFR) maruziyeti ve melatonin uygulamasının deri ve karaciğer dokusu Hyp düzeyleri üzerine etkisi değerlendirildi.
Yöntem: Çalışmada 2600 MHz RFR uygulaması ETS-Lindgren horn anten kullanılarak Rohde & Schwartz Radyofrekans sinyal jeneratörü ile yapılmıştır. 36 wistar albino erkek sıçan rastgele 6 gruba ayrıldı (n = 6); Kontrol, Sham, RFR, kontrol + Melatonin, Sham + Melatonin, RFR + Melatonin. RFR gruplarına 30 gün (5 gün / hafta) boyunca günde 30 dakika RFR uygulandı. Ortalama elektrik alan ± 21.74 V / m ölçüldü. Melatonin gruplarına ise 30 gün boyunca subkutan melatonin enjeksiyonu (10 mg/kg) yapılmıştır. Deney sonunda deri ve karaciğer dokularında Hyp seviyeleri ölçülmüştür.
Bulgular: Deri dokusundaki Hyp seviyeleri kontrol ve sham grubuna kıyasla RFR grubunda önemli ölçüde azalmıştır ($p < 0.05$). Ayrıca, kontrol, sham ve sham + melatonin gruplarına kıyasla RFR + melatonin grubunda deri dokusundaki Hyp düzeyleri anlamlı olarak azalmıştır ($p<0.05$). Karaciğer dokusu Hyp seviyelerinde değişiklik tespit edilmemiştir.
Sonuç: Bu çalışmada RFR maruziyeti deri Hyp seviyelerini değiştirirken karaciğer dokusunda bir değişikliğe neden olmadığı gösterilmiştir. Bu etkinin, deri dokusunun yüzeye daha yakın olmasından kaynaklandığını düşünüyoruz.

Anahtar Sözcükler: Radyofrekans radyasyon, melatonin, hidroksiprolin, karaciğer dokusu, deri dokusu, sıçan.

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INTRODUCTION

Radiation is defined as the type of energy that originates from a source and travels in space. RFR is known as a type of non-ionizing electromagnetic radiation, a combination of electric and magnetic fields that move together in space in waves. The best known source of RFR is mobile phones and related base stations, which are constantly used in daily life. Because of the rapid growth of communication technology, especially in the last 30 years, the debate over whether RFR emitted by mobile phones harmful to people is increasing day by day. There is also evidence that exposure to RF fields from cell phones or base stations can seriously affect people's health (1). Cellular responses of tissues to RFR are different and emerge as a function of the metabolic state of the cell could be called radiosensitivity. The sensitivity of each cell to radiation is different (2). The human body contains a complex extracellular matrix in the content of organs and tissues that act as an integral part due to their structural integrity and functions. Collagen is the most abundant of these extracellular matrix proteins in the body (3). Collagen contains approximately one-third of the amount of protein in the body, and hydroxyproline (Hyp) is known as the main component of collagen containing amino acid contents (4). Hyp is an indicator of collagen synthesis in skin, liver, lung and kidney tissues. In animal tissues, Hyp and hydroxylysine are mainly found only in collagen (4). It is known that the most important protein in tissues that controls the degradation processes of the matrix structure is collagen. Therefore, the imbalance between these processes affects the accumulation rate of collagen in tissues and organs, especially lung, liver, kidneys, and heart, and results in fibrogenesis, a mechanism of wound healing and repair. (5-7). Irregularities in collagen synthesis and degradation can also be seen in the liver, which has a very rapid regeneration capacity. This can cause fibrotic changes in liver tissue. Liver fibrosis or increased collagen content of the liver can lead to hemodynamic and functional abnormalities when it is common, resulting in liver dysfunction (8-10). The skin has an important potential for the first absorption of dangerous substances encountered in the environment (11). For this reason, the skin is expressed as the organ most exposed to RFR in cell phone use (12, 13). Collagen has an important role in wound healing. During collagen wound healing, it provides repair by pulling fibroblasts and encouraging the accumulation of new collagen in the wound. There are studies demonstrating different effects of RFR on the skin (14, 4, 15).

The pineal gland is the organ where the melatonin hormone is secreted. Melatonin is involved in the regulation of sleep patterns, it has an important role in many physiological processes, such as immunomodulatory role. In addition, it is the most important endogenous hormone with its antioxidant properties for free radical defense (16, 17, 10). Melatonin has also been found to stimulate the synthesis of prostaglandin E1, an inhibitor of collagen production (18, 19). Studies are reporting that collagen levels in healthy skin are replaced by the hormone melatonin (10) Studies have reported that Melatonin has a protective effect by reducing the effects of radiofrequency radiation in various tissues such as brain, heart, eye, and kidney (20-23). It has been shown that the negative effect of high-dose radiofrequency radiation on spermatogenesis is reduced by the protective effect of melatonin (24). The aim of present study is to evaluate the possible effects of 2600 MHz RFR on skin and liver tissue Hyp levels and possible healing effect of melatonin.

MATERIALS and METHODS

A total of 36 adult male Wistar albino rats (200-250 g) were used. All applications were approved by Gazi University Animal Experiments Local Ethics Committee (G. Ü. ET - 22.045 code number) in the study. Rats were taken from GUDAM (Gazi University Experimental Animal Center) and randomly divided into 6 groups of 6 animals each: 1) Control, 2) Sham, 3) RFR, 4) Control + Melatonin, 5) Sham + Melatonin, 6) RFR + Melatonin. Sham groups took same procedure with groups RFR groups, but the exposure system was kept off. Melatonin groups were subjected to subcutaneous melatonin (TCI, M1105, UK) injection (10 mg / kg) for 30 days (14). At the end of 30 days, animals were decapitated and Hyp levels were measured in skin and liver tissues. During the experiment, all animals were placed in plexiglass cages in a room with controlled temperature (22 ° C), humidity (50-55%) and a 12-hour light-dark cycle. Rats were allowed to drink water during the study and the animals were fed with standard pellet feed.

RFR Exposure

2600 MHz RFR exposure was performed via Rohde & Schwartz RFR signal generator (Model SMBV100A, Rohde&Schwarz, Munich, Germany) and ETS-Lindgren horn antenna (ETS Lindgren, Model 3164-03, USA). RFR exposure was applied to RFR groups for 30 days (5 days / week) for 30 minutes daily, RFR levels were measured with an EMR 300 (Narda, Germany) with an electric field probe type 8.3. RF field average electric field value is 21.74 V / m. E field values were measured in the middle and corners of the application box. After the rats were placed in plastic cages (34x24x13 cm), exposure was applied and the horn antenna was placed on the plastic cage. In groups exposed to melatonin + RFR, firstly RFR exposure performed then Melatonin was injected subcutaneously.

Skın and Liver Hydroxyproline Determination

Hyp levels were measured in skin and liver tissues. Skin samples were taken from the upper part of the abdominal ribs. After removing the skin and liver tissues from the freezer, they were kept constant at 2-8 ° C and weighed 0.1 g and homogenized with 1 ml PBS (ph 7.4). It was centrifuged at 3000 rpm for 20 minutes. Skin and liver tissue samples were prepared from homogenates prepared in accordance with the manufacturer's (Sunredbio / China) protocol using BMG LABTECH SPECTROstar Nano / Germany Eliza reader.

Statistics

SPSS v20 (SPSS Corporation, Chicago, IL, USA) statistics program was used for data analysis. One-way ANOVA analysis was used to compare the data between the groups and Tukey post hoc test was used to determine the difference between the groups. Data were given as mean ± standard deviation (SD) and $p < 0.05$ was considered statistically significant.

RESULTS

Skin Hydroxyproline Results

The effects of RFR exposure and melatonin administration on Hyp levels in skin homogenates are shown in figures 1. Hyp levels in skin tissue decreased significantly in RFR treated groups compared to the control and sham groups ($p < 0.05$). Hyp levels in skin tissue decreased significantly in RFR + melatonin treated group compared to the control, sham and sham+melatonin groups There was no difference between the control, sham and control+melatonin in the skin tissue Hyp analysis.

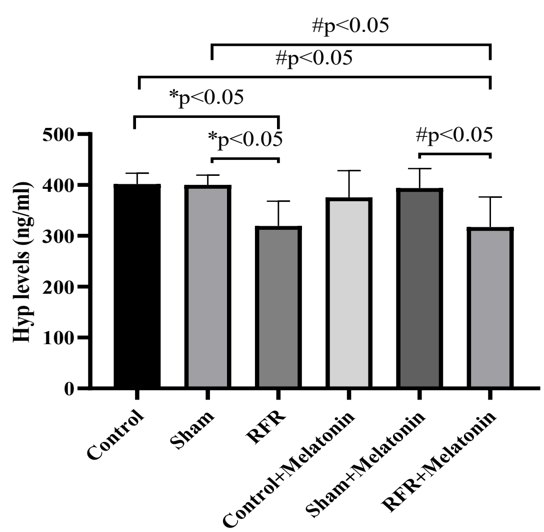

Figure 1: Hyp levels of skin tissue. Data were presented as mean ± SD (n = 6). *p <0.05 control and sham groups versus RFR group and #p <0.05 control, sham and sham + melatonin groups versus RFR + melatonin group.

Liver Hydroxyproline Results

The effects of RFR exposure and melatonin administration on Hyp levels in liver homogenates are shown in figures 2. No change in Hyp levels were detected in liver tissue (p> 0.05). While effects were observed in the skin tissue groups, no effects were found in the liver.

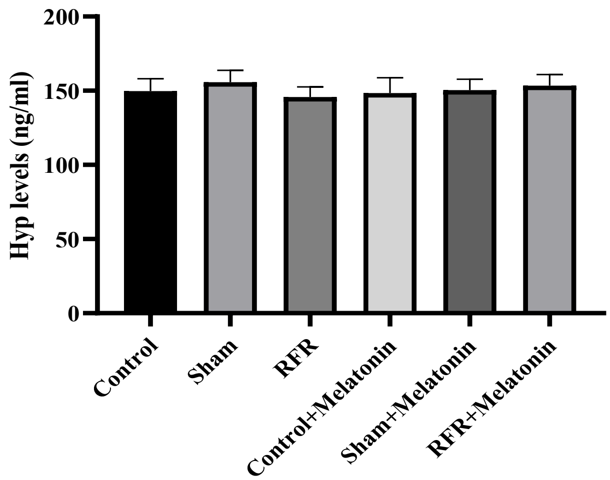

Figure 2: Hyp levels of liver tissue. Data were presented as mean ± SD (n = 6). No change in Hyp levels was detected in liver tissue (p >0.05).

DISCUSSION

The possible health effects of mobile phones, which are so popular in our daily lives in last years, are classified as main source of RFR, and on human health these effects are one of the most frequently discussed title in scientific community. Today, 4G technology is frequently used and 2600 MHz is one of the spectrum bands of 4G. Therefore, the effects of 2600 MHz RFR exposure, one of frequencies of mobile phones, on Hyp levels of male rat skin and liver tissues, and the role of melatonin, on these levels were investigated. In present study, We have shown that RFR exposure affects skin Hyp levels. Hyp; is found only in the collagen in the human body and has a large number of functions such as preserving bone integrity in the collagen, maintaining the skin's elasticity, maintaining the integrity of the skin, and cleansing the body from harmful toxins. So, it is very important to investigate the effectiveness of a protein that has such a wide range of functions for the body on the skin and liver possible effect of 2600 MHz radiation skin and liver Hyp levels is again important due to rapidly increasing mobile phone usage with developing technology. When evaluating RFR effects, we also evaluated the effectiveness of melatonin, a powerful antioxidant molecule. In our study, RF radiation absorption is mostly superficial and deep tissues suggest that RF radiation effects may be less. While 2600 MHz radiation decreased Hyp levels in skin tissue, it did not have a significant effect on liver tissue. In the literature, there are few studies evaluating the exposure of radiation at different frequencies to Hyp levels in skin and liver tissue (25-27).

Collagen and elastic fibers, which are important for the mechanical structure of the skin, have many important roles in the preservation of homeostatic balance, genetic expression, regulation of various signal paths and ion channel activity (28). For this reason, changes in the collagen structure can be seen due to the deterioration of the homeostatic balance and skin structure and ROS levels in the skin after exposure to RF radiation (25). However, there are studies that do not show a significant change in collagen level after exposure to RFR (26, 27, 29).

In a study on rodents, it was shown that there were no significant histological changes in their skin after GSM-900/1800 exposure to hairless rats. Sanchez et al. (27) showed that a 48-hour GSM-1800 exposure on human primary skin cells had no effect on keratinocytes or fibroblasts. However; in studies with different RF frequencies, RFR exposure has been reported to increase oxidative stress in the skin tissue (25) and cause some structural changes (22). It has been reported that these changes in the skin can be alleviated with melatonin treatment (22).

As is known, conductivity of living tissues increases with frequency in the same environment (30). For this reason, the amount of energy absorbed in the tissue increases at the same rate. Mobile phones are located close to the abdomen and surrounding organs, and therefore cause concern in terms of electromagnetic radiation and biological interactions between internal organs (31). The liver is an easier target for the effects of the electromagnetic field due to its high iron content (32). There are many studies investigating effects of RFR on liver, but there are conflicting results. Postacı et al. reported that chronic 2600 MHz exposure did not affect MDA, SOD and catalase levels in liver tissue (30). In the study of Çetin et al. (33) 900 and 1800 MHz RFR exposure during the pregnancy period to 6th week has been shown to decreased glutathione peroxidase activity in the liver tissues of rats. However, in our study, 2600 MHz RFR exposure did not cause a change in Hyp level in liver. (34) RFR field in the high frequency range have an effect on the superficial organs than the low frequency electromagnetic fields. Also, differences in the exposure area may have caused different results.

2600 MHz RFR applied in our study is significantly higher than the electromagnetic field frequencies used in previous studies. Therefore, while it was stated that there were changes in skin and liver Hyp levels at lower frequencies (900 and 1800 MHz), we observed that it did not cause any change in the liver tissue in our study. In addition, it is known that the effect and amount of damage caused by radiation is positively correlated with exposure time (35). While it is noteworthy that 30 minutes RFR application causes changes in the skin Hyp level, it can be stated that it has no effect on the liver.

CONCLUSION

In this study, it was shown that RFR exposure affects skin Hyp levels. These effects observed as a result of short-term RFR exposure for 30 minutes indicate that the effects of mobile phones and other RFR sources on human health and possible protective measures require further investigation.

Conflict of interest
No conflict of interest was declared by the authors.

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