Histopathological Effects of 2.45 Gigahertz Electromagnetic Radiation on the Rat Kidney, and Protective Effects of Vitamin C

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Özet

Amaç: Elektromanyetik radyasyonun sıçan böbreğinde yol açtığı histopatolojik hasarı ve C vitamininin (askorbik asit) oluşturulan bu renal hasara karşı iyileştirici etkilerini araştırmak.

Materyal-Metot: On sekiz dişi Sprague-Dawley sıçanı, her biri altı sıçan olmak üzere üç gruba ayrıldı. First group was the control group; these rats were kept in their cages without stress or electromagnetic radiation exposure. Second group was exposed to 2.45 gigahertz (GHz) electromagnetic radiation for 30 days. Third group was also exposed to 2.45 GHz electromagnetic radiation for 30 days (1 h / day) but received vitamin C 250 mg / kg – 5 cc daily orally 24 hours prior to the first exposure and daily throughout the experiment. All rats underwent nephrectomy for histopathological examination.

Bulgular: Kontrol grubunda herhangi birpatolojik değişiklik olmadı. EMR grubunda tübüler ve glomerüler hasarı içeren önemlipatolojik değişiklikler izlendi (p<0.05). Interstitiyanın ve vaskülerin hasar açısından EMR ve EMR + C vitamini grubunda önemli bir değişiklik izlenmedi (p>0.05). Tüberler ve glomerüler hasar EMR + C vitamini grubunda EMR grubuna göre daha az şiddetliydi (p<0.05). Kontrol grubu ve EMR + C vitamini grubu arasında tüberler, glomerüler, interstisyal ve vasküler hasar açısından önemli bir farklılık izlenmedi (p>0.05).

Sonuç: Elektromanyetik radyasyonun sıçan böbreğinde yol açtığı tüberler ve glomerüler hasar, elektromanyetik radyasyon maruziyeti öncesinde alınan C vitamini ile neredeyse tamamen normale dönüştü.

Anahtar kelimeler: Antioksidanlar, Ascorbic Acid, Electromagnetic Radiation, Kidney, Oxidative Stress
Introduction

Technological progress has increased the spread of electromagnetic radiation (EMR) due to common daily usage of various electronic devices. EMR affects all organisms, especially children and pregnant women, so it is an important public health concern (1). EMR sources, including wireless networks, microwave ovens, mobile phones, and base stations have been proven to influence biological systems (2, 3). EMR is a non-ionizing type of radiation that has thermal and non-thermal effects on biological systems (4). Although it is known that the thermal effects are not sufficient to cause harm to the human body, the mechanisms of the non-thermal effects are not exactly known (5).

It is very easily affected by oxidants such as EMR and also antioxidants, because the blood flow in the kidney is too high. Some studies have shown that EMR causes oxidative stress and tissue damage (6). When there is an excess of oxidant agents relative to antioxidant agents oxidative stress occurs. Oxidative stress plays a role in the pathophysiology of many diseases, including atherosclerotic, neurological, and inflammatory conditions (7,8). The oxidative stress parameters of lipid peroxidase and reactive oxygen species (ROS) both increase in tissues after exposure to 2.45 GHz EMR (9).

Vitamin C is one of the most important antioxidants in tissues (10,11), and its absence can lead to serious problems. In addition, it has been used to decrease the harmful effects of EMR on tissues, as it functions as a hydrophilic free-radical scavenger in extracellular fluids and protects biomembranes from peroxidative damage (12).

The aim of this study was to investigate whether 2.45 GHz radiofrequency (RF) emissions induce renal damage and whether co-administration of vitamin C decreases this damage.

Material and Methods

Animal Model

All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health, and were approved by the Committee on Animal Research at Suleyman Demirel University, Isparta. The animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, prepared by Suleyman Demirel University.

Eighteen female Sprague-Dawley rats were used, with an average weight of 250–300 g. The rats were housed individually in stainless-steel cages in pathogen-free conditions in our laboratory at a temperature of 24 ± 3 °C, with light between 08:00 a.m. and 08:00 p.m. and free access to water and food. They received a commercial chow diet (Korkuteli Yem, Antalya -Turkey). Each rat had 40 cc / day water for drinking not to effect inequality of hydration. Environmental average light intensity was 4000 lux and humidity was 40±10%.

Study Groups

After a one-week adaptation period, the animals were randomly divided into three groups of six rats each, as follows:

- The control group (n=6); the EMR group (n=6), exposed to 2.45 GHz EMR (1 h / day for 30 days between 9:00 a.m. and 12:00 p.m.); and the EMR + vitamin C group (n=6), exposed to 2.45 GHz EMR (1 h / day for 30 days between 9:00 a.m. and 12:00 p.m.) while receiving vitamin C 250 mg / kg – 5 cc daily orally (4). The control group received oral gavage of isotonic saline solution in the same volume as the vitamin C.

The welfare of the rats was observed to be normal during the exposure period. The first dose of vitamin C was given 24 h prior to the first exposure. The rats were not anesthetized during the radiation exposure. The control group rats were kept in their cages under the same environmental conditions, without stress or EMR exposure (Fig. 1).

Exposure System and Design

The EMR source was a radiofrequency (RF) test generator SET ELECO (Set Electronic Co., Istanbul, Turkey) with 2.45 GHz RF emissions, pulsed with 217 Hz by its monopole antenna system (13). This device produces an electric field with a strength of 0.1–45.5 V / m. All of the exposure systems were located in a screened room with a shielding effectiveness of >80 db in the frequency range of 2.0–2.5 GHz. The overall performance of the exposure device was tested and checked in the laboratory of the Department of Electronics and Communication Engineering (Suleyman Demirel University, Isparta, Turkey) (14).

The carousel (or Ferris wheel) setup accommodates 12 rats at once. Each rat was placed in a cylindrical plastic restrainer, as shown in Fig. 1. Each restrainer provided appropriate and equal exposure conditions for the physical size of one rat (length: 15 cm, diameter: 5 cm). The rats’ noses were positioned close to the monopole antenna and the restrained animals were ventilated from head to tail to decrease their distress while in the tube. The health status of the rats during the exposure was normal for both experimental groups. All reflection and exposure measurements were carried out utilizing the Portable RF Survey System (HOLADAY, HI-4417, Minnesota, USA) with its standard probe. The repetition time, frequency, and amplitude on the RF spectrum.
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were observed and verified by the satellite level meter (PROMAX, MC-877C, Barcelona / Spain). The specific absorption rate (SAR) was calculated by the electromagnetic dosimeter using measured electric field intensity (V / m) and digital anatomical models based on the FDTD (Finite Difference Time Domain) numerical code. SAR values were predicted for the same conditions, orientation, and antenna power, based on 2.63 mW / kg for the kidney tissue. Groups exposure's did not affect the other groups.

Histological Analysis

The left kidney of each animal was removed and immediately placed in neutral-buffered formalin solution (10%). After fixation, the specimens were washed in tap water, dehydrated in a graded series of ethanol, and cleared in xylene. After being embedded in paraffin, the tissue blocks were cut into 3–4 μm sections with a sliding microtome (SM2000R, Leica, Germany). Slides were obtained from each group and stained with hematoxylin-eosin for histopathological examination. Histopathological changes were evaluated for four types of damage: tubular damage (vacuole degeneration, atrophy, dilatation, desquamation, pyknotic nucleus), glomerular damage (granular degeneration, hemorrhage), interstitial damage (mononuclear cell infiltration), and vascular damage (cortical / medullar hemorrhage / congestion). A modified semi-quantitative scale (0–3) was used for classification: 0=no damage, 1=mild damage, 2=moderate damage, and 3=severe damage. The samples were imaged with a binocular light microscope (DM500, Leica, Germany).

Statistical Analysis

The Kruskal-Wallis test, a non-parametric test, and the Mann-Whitney U test were used to compare two measurements. The lowest-difference test was used to compare mean values between the groups. p<0.05 was considered significant and all data were expressed as mean ± standard deviation (x±s).

Ethics Statement

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Suleyman Demirel University Hospital (No: 21438139-324).

Results

Vitamin C provided a protective effect against EMR-induced kidney damage in rats. Histopathological changes were observed that were statistically and significantly different among groups. In the control group, renal histology was found to be normal (Fig. 2G-H). In the EMR group, renal slides revealed significant pathological changes, including tubular damage, glomerular damage, interstitial damage, and vascular damage. We evaluate vacuolar degeneration, atrophy, dilatation, desquamation and pyknotic nucleus in tubular damage, granular degeneration and hemorrhage in glomerular damage, mononuclear cell infiltration in interstitial damage, cortical - medullar hemorrhage and congestion in vascular damage (Fig. 2A-D). However, vitamin C administered before EMR decreased the tubular and glomerular damage (Fig. 2E-F).

Compared to the control group, tubular and glomerular damage was significantly higher in the EMR group (p<0.05), but the interstitial and vascular damage was not different (p>0.05). Compared to the EMR group, tubular and glomerular damage was significantly less in the EMR + vitamin C group (p<0.05). There was no difference in levels of tubular and glomerular damage between the control group and the EMR + vitamin C group (p>0.05). EMR-induced tubular and glomerular

Figure 2. Rat kidney tissue section. A, B, C, D
Tubular vacuole degeneration (black arrow), tubular dilatation (brown arrow), tubular desquamation (yellow arrow), tubular pyknotic nucleus (green arrow), glomerular granular degeneration (purple arrow), interstitial mononuclear cell infiltration (blue arrow), vascular cortical / medullar congestion (red arrow) in EMR group. E, F) Mild histopathological changes with restoration of the normal histological structures of the kidney tissue in EMR + C vitamin group. G, H ) Normal histology of the kidney tissue in the control group. Scale bar 100 μm, H&E, X200
damage was almost completely reversed with administration of vitamin C before exposure (Table 1 and Table 2).

Discussion

Exposure to 2.45 GHz EMR can lead to acute or chronic renal failure due to atrophic glomeruli and renal tubules with cytoplasmic vacuolation and pyknotic nuclei (9). There are varying grades of tubular damage in all cases of acute renal failure. Tubular atrophy, interstitial fibrosis, degeneration in tubular cells, separation and thickening of the glomerular basement membrane, thickening of the Bowman capsule, and glomerular capillary thrombosis are pathological findings of renal failure that can be observed under microscopy. Irregularities in the endothelium can be seen as a sign of glomerular damage (15). In the present study, EMR led to tubular and glomerular damage in the kidney after 2.45 GHz EMR exposure. We defined tubular damage as the presence of at least one of the following: vacuolar degeneration, atrophy, dilatation, desquamation, and pyknotic nucleus. Glomerular damage was defined as the presence of granular degeneration and hemorrhage. In some published studies, interstitial damage and vascular damage were observed in the kidney after EMR exposure (15,16).

Vitamin C is an electron donor, which explains its roles in hydrophilic free-radical scavenging and glutathione-mediated cellular redox processes (12). Vitamin C functions in the elimination of free radicals from cell membranes and is involved in altering the amount of these oxidative parameters (17). It also reduces tissue levels of malondialdehyde (MDA) and nitric oxide (NO) by eliminating oxidants (18). Thus, vitamin C can have a protective effect against EMR nephropathy. In contrast, Devrim et al. reported that oxidative MDA levels and catalase (CAT) activity increased much more in the EMR + vitamin C group (4). Vitamin C intake might create additional sensitivity to radiation, so it might also play a pro-oxidant role. In the present study, the protective effect of vitamin C was observed, but the mechanism responsible for this effect was not investigated.

In comparing the EMR group with the EMR + vitamin C group in the present study, tubular damage and glomerular damage were significantly less severe in the EMR + vitamin C group. We did not see any differences between the control group and the EMR + vitamin C group with regard to tubular and glomerular damage.

It has been shown that EMR causes cells to produce stress proteins, leads to DNA damage, and has neurological impacts (19,20). EMR increases free-radical activity in cells and ROS production, while decreasing antioxidant enzyme activity (21). EMR also leads to increases in oxidative parameters, including glutathione (GSH), cellular glutathione peroxidase (c-GPx), conjugated dienes (CDs), hydroperoxides, MDA, NO, and CAT in blood and tissues (4,21). The effects of EMR on the kidneys could be determined not only pathologically but also by detecting blood and tissue levels of biochemical markers. Variable parameters such as NO, H2O2, GSH, c-GPx, CDs, hydroperoxides, MDA, and CAT may fluctuate after EMR exposure. The present study did not evaluate the levels of these components in blood or tissue after 2.45 GHz EMR exposure to the kidney. The severity of damage can also be assessed by measuring oxidant and antioxidant markers; however, we did not employ this method. Measurements of these biochemical values after vitamin C intake would confirm the protective effect of vitamin C against EMR-induced nephropathy.

Conclusion

EMR-induced tubular and glomerular damage in the kidney is almost completely reversed with administration of vitamin C before exposure. A diet rich in vitamin C has protective effects against EMR nephropathy, and for individuals exposed to EMR, it may be advisable consume vitamin C to reduce nephrotoxicity.

Table 1. Histopathological changes in renal samples in the experiment groups (control group, EMR group, EMR + vitamin C group) and p values according to the Kruskall-Wallis Test

<table>
<thead>
<tr>
<th>Parameters / Score</th>
<th>Control Group (n: 6)</th>
<th>EMR Group (n: 6)</th>
<th>EMR + vitamin C Group (n: 6)</th>
<th>Kruskall-Wallis P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular damage</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Glomerular damage</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Intersitial damage</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Vascular damage</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Score explanations: “-”: no differences, “+”: weak differences, “++”: mild differences, “+++”: severe differences

Table 2. Histopathological changes in renal samples in the experiment groups and p values according to the Mann - Whitney U Test

<table>
<thead>
<tr>
<th>Control Group –EMR Group</th>
<th>Tubular damage</th>
<th>Glomerular damage</th>
<th>Intersitial damage</th>
<th>Vascular damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMR Group - EMR + vitamin C Group</td>
<td>P&lt;0,05</td>
<td>P&lt;0,05</td>
<td>P&lt;0,05</td>
<td>P&lt;0,05</td>
</tr>
</tbody>
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Conflict of Interests
The authors declare that there are no conflicts of interest.

This study was carried out in Suleyman Demirel University laboratory of experimental animals. The data were collected and analyzed in Suleyman Demirel University library.

Purchase and maintenance of rats used in this study were jointly funded by all researchers themselves. The radiofrequency generator, biochemical tests, histological examinations and stainings used in this study are already available in Suleyman Demirel University laboratory.

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