The effects of wireless on carbonic anhydrase and acetylcholinesterase enzyme activities in some tissues related to respiratory, excretory and reproductive systems of rats

Wireless'in (wi-fi) boşaltım ve üreme sistemleri ile ilgili bazı dokularda karbonik anhidraz ve asetilkolinesteraz enzim aktivitelerine etkileri

Summary

The aim of our studies is to understand the effects of wireless (Wi-Fi) waves on carbonic anhydrase (CA) and acetylcholinesterase (AChE) enzyme activities in the different tissues related to respiratory, excretory and reproductive systems of the wistar albino rats. For this respect, 2 different groups each of which contains 6 rats (n = 6) were formed as control group and Wi-Fi wave administered groups. The rats were necropsied and the different tissues of the rats were extracted. Activities of CA and AChE enzymes were measured for each tissue by using the hydratase, esterase and acetylcholineiodide methods. All the experimental results were provided in mean ± standard deviation (±SD). Statistical significance was identified to be p<0.05. It was observed that there were significant decreases of enzyme activities in wireless administered group in brain, spleen, kidney, and trachea tissues according to CA and AChE levels.

Keywords: Wireless electromagnetic fields; acetylcholinesterase; carbonic anhydrase; enzyme activity; rat

Introduction

There are epidemiological studies in the literature regarding the effects of magnetic fields on cancer, reproduction, and neurobehavior. Lately, newer studies linking the effects of electromagnetic fields to neurological, degenerative, and cardiac diseases have been presented. Moreover, the global increase in the use of smartphones operating in exceeding radio frequencies (RF)
mustered a significant focus on the possible health effects of electromagnetic radiation (Ann. 2001). Extensive use of wireless fidelity communication devices and their networks significantly increased the exposure of humans to RF fields. Both national and international agencies and organizations have established regulations for the safety of RF fields, yet public anxiety remains on the possibly hazardous effects of such fields to human health. Even though the recent studies were indicated no concise adverse health effects in human health caused from RF exposure, further and detailed studies are recommended to define the health effects caused by RF electromagnetic wave (RF-EMW) exposure to humans more clearly (Habash et al. 2009).

Epigenetic investigations of Radio frequency–Electro magnetic waves (RF-EMW) exposure in some studies were indicated that heat shock proteins (HSPs), ornithine decarboxylase (ODC), p38 mitogen-activated protein kinase (MAPK), c-jun, c-myc, p21, Bax, GADD45, Nur1, etc. are possible reactive proteins and genes to such exposures (Cotgreave IA 2005; Czyz JI et al. 2004; Nikolova T, et al. 2005). Cells exposed to electromagnetic fields (EMF) were analyzed by using quantitative RT-PCR method for transcription level changes in cell cycle regulations, apoptosis and proliferation of cells, cytogenic changes, and in genes responsible for apoptosis, neural genes, and proteins. However, these analyses were revealed no pronounced effects of RF-EMW in mitochondrial functions, nuclear apoptosis, cell proliferation, and chromosomal aberrations (Nikolava T et al. 2005; Bartsch H et al. 2002; Avendaño C. et al. 2012; Pouletier de Gannes F. et al. 2012, Skakkebaek NE. et al. 2006; Deepinder F, 2007; Kundi M. 2004).

Wireless fidelity RF-EMF (Wi-Fi RF-EMF) has found itself a great application in telecommunications with a wide array of devices which has become a standard in modern communications. Therefore, Wi-Fi RF-EMFs are now an everyday part of people using these tools which results in an increasingly high exposure to such fields and absorbing the transmitted energy and thus having increased body temperatures (Pourlis AF 2009; La Vignera S. et al. 2012 a-b; Imai N et al. 2011; Ribeiro EP et al. 2007; Lee HJ et al. 2010; Dasdag S et al. 2008).

Carbonic anhydrase (CA) family of enzymes are known to be associated with various physiological and pathological processes such as respiratory functions, pH balance, CO₂ hemostasis, electrolyte secretion in tissues, gluconeogenesis, lipogenesis, urea synthesis, bone absorption, calcification, tumorigenesis, etc. Enzymatic inhibitions on these enzymes can be facilitated for the treatments of different diseases like glaucoma, osteoporosis, cancer, obesity, and epilepsy. CA family is among the metalloenzymes, which have Zn^{2+} ion in their active sites, and catalyzes the slow acting reaction of transition of CO₂ to bicarbonate and proton. It’s one of the most common enzymes in nature, and first identified in erythrocytes and found to be present in almost all tissues later on (Dasdağ S et al. 1999; Berg JM 2014; Gocer H et al. 2016; Kocyigit UM et al. 2016; Gul H et al, 2016;).

Acetylcholinesterase (AChE, E.C.3.1.1.7) is the principal enzyme for the proper functioning of neural transmission and works to prevent aggregation of active neurotransmitters during the synaptic transmission of nerves by hydrolyzing the acetylcholine (Kocyigit UM et al, 2018; Bhatt, A et al, 2017; Akincioglu A et al. 2017; Göçer H et al. 2014). For Alzheimer’s Disease (AD), cholinergic therapeutical agents have acetylcholinesterase inhibitors (AChEI), acetylcholine precursors (ACh), ACh expression facilitators, post-synaptic muscarinic M1 receptor agonists Kose LP et al. 2015; Özbey F et al. 2016; Taslimi P et al. 2016).
In the present study, it was aimed to investigate exposure effects of Wi-Fi RF-EMW in CA (I and II) and AChE enzyme activities from tissues of respiratory, excretory, and reproductive systems of rats that were not studied before. Obtained data would provide a scientific contribution to the understanding the effects of Wi-Fi RF-EMW exposure to organisms.

EXPERIMENTAL AND METHODS

Environmental Conditions of Animal Experiments

The study was granted approval from the Ethics Committee of the Cumhuriyet University on Experimental Animals (Issue: 06.08.2015/75). Total of 12 adult Wistar albino rats (180 ± 10 g body weight) were used in the study. Rats were housed in standart cages at temperature 24 ± 2 °C in Animal Experimentation Unit of Cumhuriyet University Faculty of Medicine in 2018 year. Animal experimentations were conducted in accordance with the Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals according to article writing by Durna Daştan S. et al. 2018. Rats were randomly selected, and divided into two groups (n=6) as Group 1 for control and, Group 2 for Wireless electromagnetic waves exposure (Scozzafava A. et al. 2015; Gülçin I. et al. 2004). A common wireless internet router (Zyxel NBG-418N V2 wireless Access Point/Router) was used for Wi-Fi RF-EMW exposure. The router was able to generate RF-EMW field at 2.4 GHz and was working in 300 Mbps transmission. External electromagnetic field pollution was eliminated by placing the router and rat cages inside a Faraday Cage. Rats in Group 2 were exposed to RF-EMW field of 2.4 GHz for 12 hours/day for 21 days.

Calculations of Carbonic Anhydrase Activity

Hydratase Activity

A modified method by Rickli et al. for the measurement for CA enzyme activity was used by measuring hydratase activity (Aksu K et al. 2016). This modified method has the principle of having the pH change caused by the H+ ion from CO2 hydration is identified by the bromine thymol blue indicator during the time plot. To this end, 1 mL vernal buffer (0.025 M, pH: 8.2), 0.1 mL 0.04% bromine thymol blue, 0.6 mL purified water and 2.5 mL CO2 solution were added into a blind tube. Thus, the total volume in the activity measurement medium was 4.2 mL. Then, the time elapsed for the blue color of the indicator to turn into the yellowish green (pH: 6.3), starting from the moment the CO2 solution was added, was measured via a chronometer. 0.05 mL water was removed and instead, enzyme solution was added into the sample tube, and finally, the time elapsed for the blue color of the indicator to turn into the yellowish green (pH: 6.3) as soon as CO2 solution was added (t_c) was measured via a chronometer.

Homogenisation of Tissues

Rats were sacrificed 60 minutes after the IP administration of chemicals. Extracted samples were washed three times with using NaCl (0.9%) to clear away the blood and other contaminants. For the preparation of homogenates, samples were first dispersed by the ultra-turrax device which turned samples into extremely small pieces. They were dispersed again in liquid nitrogen, and were homogenized in 3mL/g with 25 mM Tris HCl - 0.1 M Na2SO4 (pH: 8.7) buffer solution which was centrifuged two times at 20000 rpm for 60 minutes, and subjected to supernatant analyses (Scozzafava A. et al. 2015; Gülçin I. et al. 2004).
According to this relative activity method, an enzyme unit (EU) was defined as the amount of enzyme that was reduced in time plot compared to CO₂ hydration without the enzyme.

**Esterase activity**

The principle of method is to hydrolyze the p-nitrophenylacetate (PNA), used as a carbonic anhydrase enzyme substrate p-nitrophenolate, providing absorption at 348 nm. Both p-nitrophenol and p-nitrophenolate display the same absorbance at 348 nm (Kucukoglu K. et al. 2016). Since PNA has very little absorption at this wavelength, it is used as blind (Gül HI. et al. 2016). The procedure was observed for determination of activity measured by using a 1 mL quartz vessel, per sequence of involvement of the substances forming the reaction mixture into the treatments. Esterase activity was measured by preparing sample tubes containing Tris-SO₄ (0.05 M; pH: 7.4), PNA, pure water and additional enzyme solution. After preparing the reaction mixture above, the amount of absorbance was checked at 15 seconds intervals and the absorbance difference was calculated by using the absorbance at 348 nm at 25°C after 3 minutes. PNA substrate solution is 3 mM, and it is not possible to prepare more concentrated version due to the limited solubility of ester (Gülçin I. et al. 2004).

**Measurement of Acetylcholinesterase Activity**

The basis of this method can be described as follows; cholinesterases catalyze the reaction decomposing acetylcholine into thiocolchine and acetaldehyde. Yellow colored 5-thio-2-nitrobenzoic acid is formed by the reaction of thiocolchine and DTNB which are obtained as product. Resulting color is measured at 412 nm (Gülçin I. et al. 2004) The absorbance of the sample and blind vessels was measured at 412 nm wavelength for 5 minutes. Vessel content was formed with Tris-HCl, Pure water, DTNB, Enzyme solution, and acetylcholinethioiodide for the control tube.

**Statistical Analyses**

Results are provided with mean ± SD. Normal distribution analysis was conducted by the Shapiro-Wilk test. Enzyme data were analyzed by conducting the unpaired t test. Statistical significance was at a p-value of less than 0.05. SPSS v.22 software (IBM, Chicago, IL, USA) was used for the conduction of all statistical analyses.

**RESULTS**

Table 1 presents the levels of CA and AchE enzymes in the selected tissues being relevant with the respiratory, excretory and reproductive systems of rats. There was significant difference between control group and WiFi-RF EMF groups with regard to the CA enzyme levels of trachea, spleen and kidney tissues (p<0.05). There was no significant difference in the other seven different tissues between control and experimental groups with regard to the CA enzyme levels (p>0.05). In the trachea, spleen and kidney tissues, the CA enzyme level of WiFi-RF EMF groups were significantly lower than that of control groups (p<0.05). In the study, the activity of CA enzyme was measured via both esterase and hydratase methods and consistent results were obtained (Fig. 2).

![Figure 2. The results of carbonic anhydrase (CA) enzyme in different tissues related to respiratory, excretory and reproductive systems of wistar albino rats.](image-url)
Table 1. The results of carbonic anhydrase (CA) and, acetylcholinesterase (AChE) enzymes in different tissues related to respiratory, excretory and reproductive systems of wistar albino rats.

<table>
<thead>
<tr>
<th>Selected Tissues of Rat</th>
<th>Carbonic Anhydrase Enzyme</th>
<th>AChE Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Group (n=6)</td>
<td>Wireless Group (n=6)</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Trachea</td>
<td>0.95725 ±0.33504</td>
<td>0.56880 ± 0.07052</td>
</tr>
<tr>
<td>Lung</td>
<td>3.18138 ±0.91297</td>
<td>2.87962 ± 11.37202</td>
</tr>
<tr>
<td>Heart</td>
<td>2.84163 ±0.79514</td>
<td>2.07800 ± 0.79023</td>
</tr>
<tr>
<td>Brain</td>
<td>1.48863 ±0.43270</td>
<td>1.43375 ± 0.61966</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.34343 ±0.84250</td>
<td>2.85550 ± 0.51127</td>
</tr>
<tr>
<td>Eye</td>
<td>1.18800 ±0.22524</td>
<td>1.19029 ± 10.63645</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.20300 ±0.18214</td>
<td>0.72833 ± 0.05322</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.61417 ± 0.06714</td>
<td>0.69771 ± 0.35959</td>
</tr>
<tr>
<td>Tuba uterina</td>
<td>0.60590 ± 0.13130</td>
<td>0.55875 ± 0.13833</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.62357 ± 0.11844</td>
<td>0.58150 ± 0.17441</td>
</tr>
</tbody>
</table>

When the activities of acetylcholinesterase enzyme in these selected tissues are evaluated. It was observed that there was a statistically significant difference (p<0.05) between the groups just for the brain tissue. There were no significant differences in the other nine different tissues between control and experimental groups with regard to the AChE enzyme levels (Fig. 3).

Agents and factors that are affecting the functions of CA and AChE in different organisms and tissues are interesting research area for the scientist. Limited information is available for the exposure effects of wireless fidelity as a kind of RF-EMW especially in regards to headaches and cancer. Consequently, improving our understanding in this field is a necessity. The association of parameters used in our study with tissue damage is not certain. Even so, recent studies have demonstrated the relationship
between oxidative DNA damage (Tunc O., Tremellen K., 2009). We suspected that the continuous exposure to Wi-Fi devices may have more pronounced effects compared with the shorter and intermittent exposure to Wi-Fi. One may suspect that this does not reflect real life conditions, where the duration and intensity of exposure may be more variable.

Limited data are available in the literature for the health risks posed by wireless fidelity waves, and long-term exposure remains a focal point for the human health. Despite the presence of regulations on the safety of RF operated devices, ongoing concerns for public health are still evident especially for the long-term health effects caused by wireless fidelity technology. Hence, the World Health Organization is promoting research on this matter. Notable topics for the investigation of RF-EMW exposure are their supposed relation with carcinogenesis, oxidative DNA damage, and tissue level differentiations (Ribeiro EP. et al. 2007).

**DISCUSSION**

As the wireless telecommunication technology is growing at a rapid rate and devices operating in such fields are becoming more common, their potential health risks to human health should be taken into account as the occurrences and progressions of many kinds of diseases are depending on the exposure of organisms to hazardous compounds and factors. We see the very little study about the effects of WiFi radio frequency wave exposure according to the literature (Atasoy HI. et al. 2013). In this area, more rigorous studies are needed. Various studies have investigated the effects on the human body of RF EMR from mobile phones, within conclusive results, but there have been no studies as yet on the health effects of Wi Fi effects, which are associated with higher frequency radio waves and longer exposure times than the other EMW resource. The out put power of Wi-Fi devices ranges from 20 mW to 4 W, and is similar to or greater than that of mobile phones, which range from 1 mW to 2 W (Lönn S. et al. 2004). Furthermore, they commonly expose the whole body to RF EMR, which generally cause a more uneven and irradiation of all parts of the body. Previous animal studies have not found any significant effect of RF EMR on testicular histology, sperm count and morphology. The researchers studied the effects of RF in the mouse testis and found no significant differences in sperm count and no increase in the percentage of morphologically abnormal sperm in microwave-exposed animals (Cairnie A. B. and Harding. R. K. 1981). Ono T. et al. (2004) could not find any mutagenic effect of 2.45 GHz intermittent whole body RF exposure in the testes of mouse embryos (Ono T. et al. 2004).

Results of the present study demonstrate similarity with some literatures (Kilgallon SJ, Simmons LW. 2005; Makker K, Agarwal A, Sharma R.2009; Tunc O, Tremellen K. 2009). In this study in several tissues of rats, the decreased CA and AChE activities, though in significant, could imply a partially RF-induced differentiation in enzyme activities. As CA level in trachea, spleen, and kidney tissues and AChE level in brain tissue differ between groups, we can suggest just for these tissues that Wi-Fi can induce oxidative stress, change the CA and AChE enzyme activity and disturb the function and structure of these tissues in rats exposed Wi-Fi waves. In the study, the activity of CA enzyme was measured via both methods and consistent results were obtained. It was observed that there was a statistically significant difference (p<0.05) between the control group and the Wi-Fi administered group just for three tissue (trachea, spleen, and kidney tissues) and that there was no statistically significant difference for the other seven tissues. When the activities of acetylcholine esterase enzyme in these tissues are evaluated, it was observed that there was a statistically significant difference (p<0.05) between the control group and the experimental group for just brain tissue. But the other hand, enzyme levels mostly did not differ between our control and Wi-Fi RF groups, in this case we suggest that RF EMR from Wi-Fi did not cause oxidative stress, and didn’t change the CA and AChE enzyme activity between control and experimental groups in lung, heath, eye, ovary, tumba uterina, and uterus tissues in rats exposed to the Wi-Fi devices.

**CONCLUSION**

CA activity was found to be decreased in trachea, spleen, and kidney tissues collected from Wi-Fi RF-EMW field exposed rats. Significant changes in other tissues and in control group were not detected. AChE enzyme activity was also found to be lower in brain tissues collected from exposed rats as compared to control. In the present study, young rats in their growing periods were used as it was believed that developing organs are more likely to be affected by the RF-EMW exposures as in everyday life, young children are spending much of their time inside such fields both at home
and in school. The present study was provided with the Wi-Fi exposure effects on enzyme inhibitions, and we believe that further research is necessary to conduct in this subject as wireless fidelity communication is now a significant part of everyday life. It should be noted that, however, exposure to electromagnetic waves fields should be minimized in every possible opportunity.

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REFERENCES


