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Original research

The effects of microwave radiation on rabbit's retina

Mohammad R. Talebnejad [a], Ali Sadeghi-Sarvestani [a], M. Hossein Nowroozzadeh [a], Seyed Mohammad J. Mortazavi [b], Abbas Alighanbari [c], Mohammad R. Khalili [a,*]

[a] *Poostchi Ophthalmology Research Center, Department of Ophthalmology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[b] *The Ionizing and Non-Ionizing Radiation Protection Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[c] *School of Electrical and Computer Engineering, Shiraz University, Shiraz, Iran*

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Abstract

Purpose: Mobile cell phones are used extensively these days, and their microwave (MW) radiation has been shown to affect the eye. The purpose of the present study was to evaluate the effects of MW radiation on rabbit retina.

Methods: This experimental study (concluded in 2015) was conducted on 40 adult white New Zealand rabbits. A Global System for Mobile Communications (GSM) cell phone simulator was used for MW irradiation. The rabbits were randomized into five groups (8 in each) and treated as follows: Group 1: no irradiation (sham); Group 2: irradiation at 10 cm for 1 day; Group 3: irradiation at 30 cm for 1 day; Group 4: irradiation at 10 cm for 3 days; and Group 5: irradiation at 30 cm for 3 days. Scotopic and photopic electroretinography (ERG) responses were obtained at baseline and 7 days after the last exposure. Then all the rabbits were euthanized, and their eyes were enucleated and sent for pathology examination. Kruskal–Wallis and Chi-Square tests were used to evaluate intergroup differences in ERG parameters and histological findings, respectively.

Results: ERG responses obtained 7 days after irradiation did not show any statistically significant difference between the groups ($P > 0.1$, for all tested parameters). There were statistically non-significant trends toward greater changes in the MW irradiated eyes. In pathological examination, retina was normal with no sign of degeneration or infiltration. Ciliary body congestion was observed in greater fraction of those who received higher MW doses. ($P = 0.005$).

Conclusions: Histopathologically, cell phone simulated MW irradiation had no significant detrimental effect on the retina. However, ciliary body congestion was observed in greater fraction of those who received higher MW doses. Although there was no significant difference between post-treatment mean ERG values, there were statistically non-significant trends toward greater changes in the MW irradiated eyes.

Keywords: Ciliary body; Electroretinography; Irradiation; Microwave; Retina

Introduction

Microwaves (MWs) are a subgroup of electromagnetic waves with frequencies between 300 MHz and 300 GHz.[1] Many modern devices, such as cellular phone transmitters and receivers, radars, radio and television transmitters, and video display terminals emit MWs.[2,3] Recent dramatic increase in the application of these devices has raised public concern about their possible detrimental effects on human health. Indeed, it has been well established that MWs affect the biological functions of living organisms at both cellular and molecular levels.[4,5] However, the underlying mechanisms are not fully understood.[6,7] In general, two main mechanisms have been proposed: thermal, and non-thermal.[2,8] MWs are capable of generating heat within living tissue with subsequent

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* Corresponding author.
E-mail address: khalilimr57@gmail.com (M.R. Khalili).
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health implications. They may also affect cell growth, cell cycle progression, and DNA synthesis through unknown non-thermal mechanisms.[8,9]

Because of its natural sensitivity to radiation, the eye has been evaluated for possible complications after experimental MW irradiation.[2,9,10] Actually, cataract has been described as the most frequent complication of MW exposure in men.[11] Previous studies have shown the role of thermal effect of MWs on their cataractogenesis.[12] However, recent studies have underscored the possible role of non-thermal effects of MWs in cataract formation as well.[1] Despite the extensive research regarding MWs and cataract, the possible impact of MWs on the retina has not been evaluated in detail.

To date, with the widespread use of cell phones, there is a strong rationale for determining the detrimental effect of MWs emitted from these devices on health. Because of the way they are used, cell phones are usually kept in the close vicinity of the eye for up to several hours a day. Considering its delicate structure, the eye may be the primary site of injury from these devices. We aimed to evaluate the possible side effects of cell phone simulated MWs on the retina of rabbits.

Methods

Animals

In this experimental study (concluded in 2015, Shiraz, Iran), forty healthy male New Zealand white rabbits (weighing 2–3 kg) were included. With an estimated power of 0.8 and 2-sided P value of 0.05, 8 rabbits were required in each group to detect a 25% change from the sham group in the ERG combined b-wave amplitude. Rabbits with any health issues were excluded from the study. The rabbits were kept in a controlled environment with suitable temperature (23–25 °C) and ventilation and a 12-h on/off light cycle. Food and water were provided as needed. The Animal Care and Use Committee of Shiraz University of Medical Sciences approved all aspects of this study, and the research protocol adhered to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

Microwave irradiation

A Global System for Mobile Communications (GSM) cell phone simulator designed at the (School of Engineering, Shiraz University, Shiraz, Iran) was used for MW irradiation. The frequency of the device was set at 915 MHz, and the emitted power (circular space distribution) of the generator was fixed at 2 W during exposure. Before study, all devices had been checked and controlled in the lab to ensure that they produced constant wavelength for study duration. After baseline electroretinography (ERG), the rabbits were randomly assigned into five groups (8 in each), and treated as follows: Group 1: no irradiation (sham); Group 2: irradiation at 10 cm for 1 day; Group 3: at 30 cm for 1 day; Group 4: at 10 cm for 3 days; and Group 5: at 30 cm for 3 days. To make sure that the rabbits would receive MW irradiation as per protocol, each rabbit was confined within a restrainer during the irradiation period, and the device was placed in front of the animals' head (the above-mentioned distances were measured from the animals' eyes after putting into the restrainer). The dosage of irradiation was tried to set similar to routine GSM cell phones. The distance was set according to usual distance between the device and the human eye when the device is used on the ear as a voice call (10 cm) or as a video calls (30 cm). The selected durations of exposure (1 vs. 3 days) were chosen to imitate an exaggerated exposure, which had the potential to yield positive results.

Examinations

After anesthesia and pupillary dilation, standard scotopic (dark-adapted) and photopic (light-adapted) ERGs were done by masked trained operators at baseline and 7 days after the last exposure. The rabbits were dark-adapted for at least 1 h and were anesthetized about 10 min before ERG recordings. ERG waveforms were obtained using the RETI-port (®) system (Roland, Wiesbaden, Germany). The ERG recordings were obtained according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards. ERG has been validated as a safety measure for experimental investigation in several previous studies on rabbits.[13–15] All recordings were performed while the rabbits were under general anesthesia induced by intramuscular injections of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). Pupils were dilated using topical tropicamide 0.1% and phenylephrine hydrochloride 2.5%. The following ERG parameters were recorded and analyzed: scotopic b-wave amplitude, combined a-wave amplitude, combined b-wave amplitude, photopic b-wave amplitude, 30-Hz flicker n1p1 amplitude, and 30-Hz flicker p1 implicit time.

Immediately after the 1-week ERG, the animals were euthanized with intracardiac pentobarbital overdose (200 mg). Then the eyes were enucleated and fixed in 10% formalin for 24 h. All histologic sections were evaluated by an expert pathologist. Following gross examination, semi-thin sections through optic nerve head and macula were provided and stained with hematoxylin and eosin for light microscopic evaluation.

Data analysis

Only data from the right eyes of the animals were used for statistical analyses. Data were analyzed using IBM SPSS software version 21 (SPSS Inc., Chicago, IL, USA) and MedCalc version 12.2.1 (MedCalc Software, Mariakerke, Belgium). Kruskal–Wallis and Chi-Square tests were used to evaluate intergroup differences in ERG parameters, and histological findings, respectively. P values < 0.05 were considered statistically significant.

Results

Figure. 1 summarizes ERG responses at baseline and after treatment for all groups. The baseline mean ERG parameters

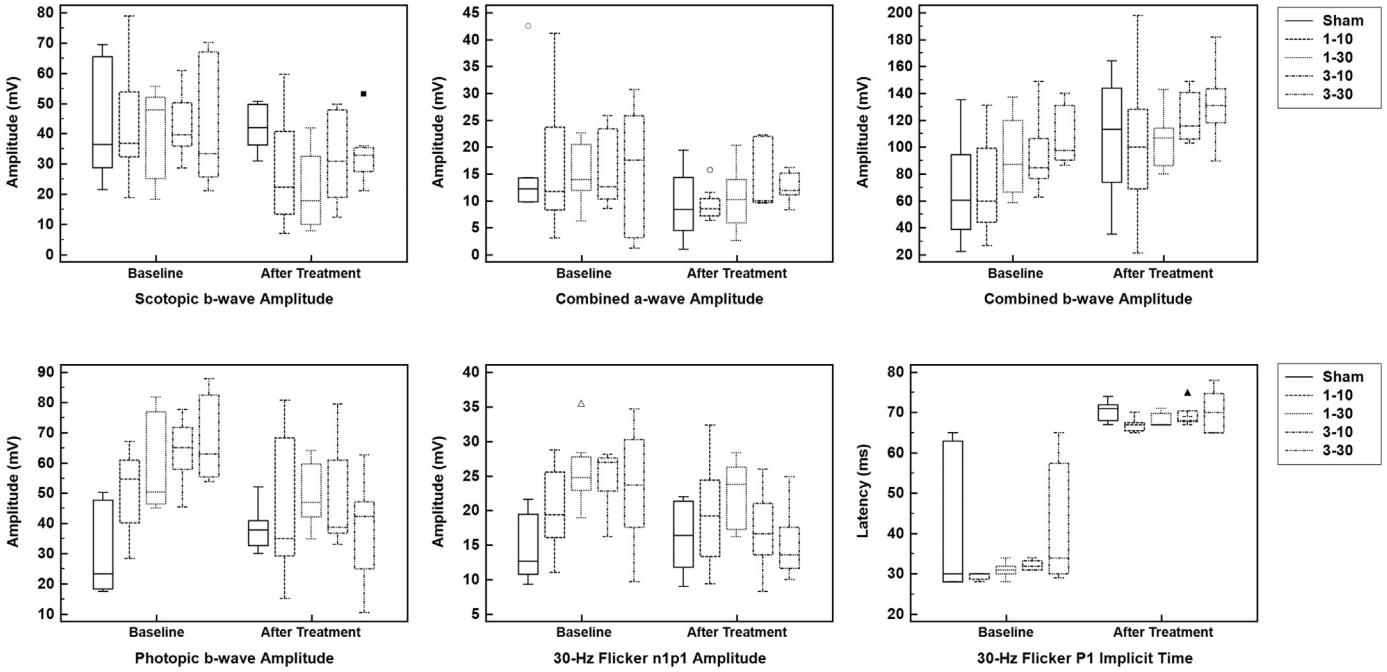

Fig. 1. This figure shows pre- and post-treatment electroretinography (ERG) parameters compared between different groups: 1–10, treatment for 1 day at 10 cm distance; 1–30, treatment for 1 day at 30 cm distance; 3–10, treatment for 3 days at 10 cm distance; 3–30, treatment for 3 days at 30 cm distance.

were statistically similar in all groups ($P > 0.1$ for all parameters; Kruskal–Wallis test). The corresponding P values from statistical analyses are given in Tables 1 and 2. Overall, photopic and scotopic ERG responses obtained 7 days after irradiation did not show any statistically significant difference between the groups ($P > 0.1$, for all tested parameters; Fig. 1 and Table 2). Because of high variations in the ERG recordings in normal population, (in addition to mean differences which are given in Figures and Tables), we also compared each recording with the baseline to explore if there were any change of more than 50%; and the Chi-square test revealed no statistically significant difference between the groups ($P > 0.2$, for all tested parameters). The rate of eyes with $\geq$50% change in ERG parameters after treatment categorized by each subgroup are summarized in Table 3. Although not significant, the rate of eyes with >50% change in ERG recordings was higher in the treatment group.

In pathological examination, the retina was normal with no sign of degeneration or infiltration. However, the histological cut through ciliary bodies showed ciliary body congestion in eyes treated with MW radiation, as follows: 0% in Group 1, 50% in Group 2, 12.5% in Group 3, 87.5% in Group 4, and 87.5% in Group 5; $P = 0.005$; Figs. 2 and 3). The ciliary body contains the ciliary muscle, vessels, and fibrous connective tissue. In normal eye, few vessels in ciliary body contain scattered red blood cells (RBC's). Congestion means excess of blood. In congestion, the blood vessels are easily seen, resulting from impaired outflow from a tissue.

Discussion

In this study, we could not find a significant effect of MW radiation on histologic sections of the retina; however, the ERG findings were not consistent. In spite of the absence of

Table 1
Statistical analysis of changes in mean electroretinographic parameters (between baseline and post-treatment values) in each subgroup.

ERG parameter	*P* value*				
	Sham	1–10	1–30	3–10	3–30
Scotopic b-wave Amplitude	0.600	0.069	0.091	0.500	0.398
Combined a-wave Amplitude	0.500	0.161	0.091	0.345	0.499
Combined b-wave Amplitude	0.028	0.139	0.237	0.225	0.063
Photopic b-wave Amplitude	0.116	0.263	0.128	0.225	0.046
30-Hz Flicker n1p1 Amplitude	0.345	0.327	0.063	0.080	0.128
30-Hz Flicker p1 Implicit Time	0.027	0.011	0.018	0.039	0.018

*Calculated using Wilcoxon Signed Rank test.
1-10, treatment for 1 day at 10 cm distance; 1–30, treatment for 1 day at 30 cm distance; 3–10, treatment for 3 days at 10 cm distance; 3–30, treatment for 3 days at 30 cm distance.
ERG: Electroretinography.

Table 2
Statistical analysis of comparison of post-treatment mean electroretinographic values between different subgroups.

ERG parameter	P value*
Scotopic b-wave Amplitude	0.105
Combined a-wave Amplitude	0.262
Combined b-wave Amplitude	0.384
Photopic b-wave Amplitude	0.545
30-Hz Flicker n1p1 Amplitude	0.200
30-Hz Flicker p1 Implicit Time	0.174

*Calculated using Kruskal–Wallis test.
ERG: Electroretinography.

Table 3
Rate of eyes with ≥50% change in electroretinography (ERG) parameters after treatment categorized by each subgroup.

ERG parameter	Number of eyes with ≥50% change*					
	Sham	MW (Total)	1–10	1–30	3–10	3–30
Scotopoic b-wave Amplitude	0/6 (0)	11/28 (39.3)	4/9 (44.4)	4/7 (57.1)	1/5 (20)	2/7 (28.6)
Combined a-wave Amplitude	1/6 (16.7)	9/28 (32.1)	3/9 (33.3)	2/7 (28.6)	1/5 (20)	3/7 (42.9)
Combined b-wave Amplitude	0/6 (0)	1/28 (3.6)	1/9 (11.1)	0/7 (0)	0/5 (0)	0/7 (0)
Photopic b-wave Amplitude	0/6 (0)	6/28 (21.4)	2/9 (22.2)	0/7 (0)	1/5 (20)	3/7 (42.9)
30-Hz Flicker n1p1 Amplitude	1/6 (16.7)	5/28 (17.9)	1/9 (11.1)	0/7 (0)	1/5 (20)	3/7 (42.9)
30-Hz Flicker p1 Implicit Time	4/6 (66.7)	25/28 (89.3)	8/9 (88.9)	7/7 (100)	5/5 (100)	5/7 (71.4)

*Data are presented as: number of eyes with ≥50% change/total number of eyes in each subgroup (percent).
1-10, treatment for 1 day at 10 cm distance; 1–30, treatment for 1 day at 30 cm distance; 3–10, treatment for 3 days at 10 cm distance; 3–30, treatment for 3 days at 30 cm distance; MW, total microwave-treated group.
ERG: Electroretinography.
MW: Microwave.

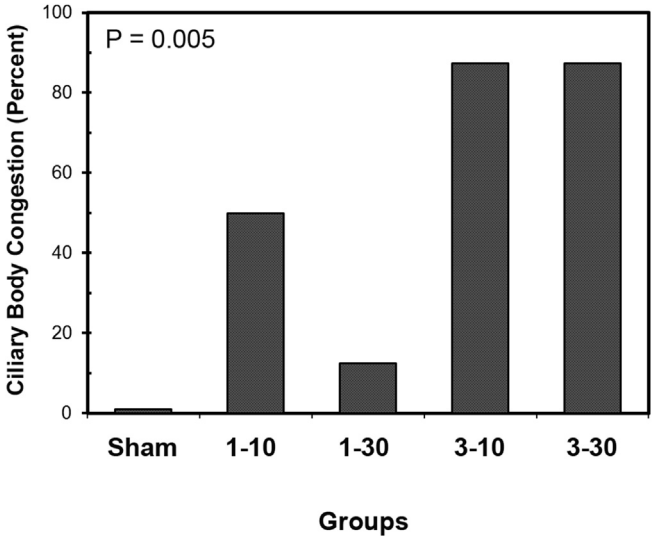

Fig. 2. This diagram presents percent of eyes with ciliary body infiltration after treatment with microwave (MW) for different groups: 1–10, treatment for 1 day at 10 cm distance; 1–30, treatment for 1 day at 30 cm distance; 3–10, treatment for 3 days at 10 cm distance; 3–30, treatment for 3 days at 30 cm distance.

any significant post-treatment difference in ERG parameters between the groups, there were statistically non-significant trends toward greater changes in the MW irradiated eyes compared to the sham group. Some ERG responses (even in the sham group) were statistically different between the baseline and post-treatment recordings, however, they had no pattern, and seemed to be the product of device interobserver repeatability limitations (Table 1).

So far, several experimental studies have been done regarding the effect of MW radiation on retina. In 1979, Paulsson and colleagues,[11] evaluated the impact of higher doses of MW radiation (550 W/m^2; 3100 MHz; up to about 53 h of exposure in 100 days) on rabbit retina in vivo, and reported degenerative changes in electron microscopic images of the retina. They did not find any notable alterations in funduscopy or light microscopy. Recently, several experimental studies have reported on detrimental effects of high-dose MW radiation on the retinal cells and functions.[16–22] Both outer and inner retina involved in the MW toxicity.[16,21] Wei et al.[21] evaluated the effects of MW radiation on the rat retina. They reported a statistically significant reduction in the amplitude of ERG b-wave and Flash visual evoked potentials on the 3rd day and 7th day after MW exposure. They also

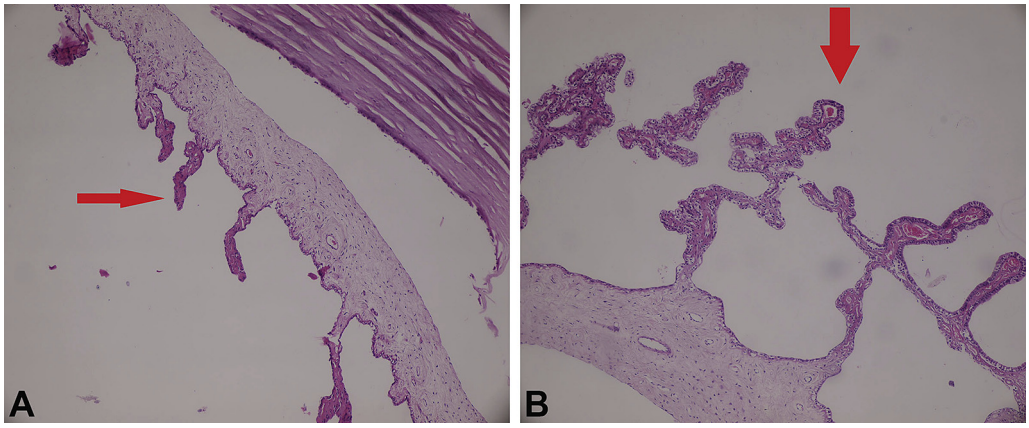

Fig. 3. This figure compares a normal ciliary body (A) in the sham group with a typical congested one (B) in the group received microwave (MW) treatment for 3 days at 10 cm distance.

found that the apoptotic rate of retinal ganglion cells increased from 2.85% to 6.73% after only 12 h of MW exposure.[21] Alterations in the retinal levels of cholinergic neurotrophic factors has been implicated in retinal injury after high-dose MW irradiation.[19,20] MW could also induce up-regulation of several stress and apoptosis-related genes transcriptions in human retina pigment epithelial cells in vitro.[16]

Overall, the above-mentioned investigations suggest the possibility of retinal injury after MW irradiation. However, they all used MW intensities much higher than that of the GSM cell phones, as the one simulated in the present work. There are some studies on human eyes assessing the detrimental functional effects of MW. To investigate the potential effects of radiofrequency (RF) exposure on the human eye, Irlenbusch and others applied a GSM signal of 902.4 MHz (pulsed with 217 Hz) to the subjects.[23] They used visual discrimination threshold as a functional parameter to evaluate the influence of a GSM signal. Comparing the data obtained from the eyes with RF exposure with those data obtained from the eyes with sham exposure, no statistically significant differences in the visual discrimination threshold was found. Although they assessed only a functional effect of a GSM signal, their applied frequency was similar to our study (902.4 MHz, and 915 MHz, respectively), and their results support our findings.[23] In another study, Schmid and colleagues assessed the influence of 1970 MHz Universal Mobile Telecommunications System (UMTS)-like exposure on parameters of human visual perception and compared the data between the exposure conditions and sham exposure. Their results revealed no statistically significant differences between the groups.[24] This clinical study is consistent with the results of our experimental study.

Regarding the histopathological examination, we found that after MW exposure the retina was normal with no sign of degeneration or infiltration. Previously, possible histopathological detrimental effects of MW have been studied. It has been demonstrated that MW-induced hyperthermia (2.45 GHz) can create retinal and retinal pigment epithelium destruction and scarring without significant damage to the sclera and choriocapillaris.[25] In another study, in vitro effects of 2450 MHz MW on retinal ganglion cells was assessed and a dose-dependent damage to these cells was demonstrated.[26] In both of the mentioned histopathological studies, MW with a 2450 MHz frequency was applied.[25,26] Compared with the mentioned studies, we used MW frequencies and intensities similar to the GSM cell phones, and we found no significant detrimental effect on the retina on histopathological examinations.

Another important finding in our study was the dose-dependent rate of ciliary body congestions in eyes treated with MW radiation. Ciliary body is involved in several important functions of the eye such as accommodation and aqueous secretion. Moreover, ciliary body inflammation or spasm is an important source of ocular pain and headache in conditions such as traumatic iritis, uveitis, and untreated presbyopia. Although the clinical relevance of our finding is unclear at this time, future studies may evaluate ciliary body congestion as a possible explanation for some cell phone-related headaches.[23]

The present work had several strengths. It was the first study that evaluated GSM cell phone simulated MW radiation with distances similar to that of real life on the retina in vivo. Using light microscopy and ERG, our study aimed to find a possible clinically relevant change after MW irradiation. The study had several limitations. The time of exposure and the follow-up time were relatively short. Therefore, this investigation did not evaluate the long-term impact of MW radiation on the retina which should be tested through advanced histological studies such as electron microscopy and immunohistochemistry. It also did not address the possible subcellular or ultra-structural alterations, which should be tested through advanced histological studies such as electron microscopy and immunohistochemistry. ERG findings were not consistent. Although there was no significant post-treatment difference in ERG parameters between the groups, there were statistically non-significant trends toward greater changes in the MW irradiated eyes compared to the sham group.

In conclusion, our results revealed that cell phone simulated MW radiation with the distances, durations, and doses used in this study had no notable detrimental effect on the histologic sections of retina. However, ciliary body congestion was observed in greater fraction of those who received more doses of MW radiation. Regarding ERG findings, although there was no significant difference between post-treatment mean electroretinographic values, there were statistically non-significant trends toward greater changes in the MW irradiated eyes compared to the sham group. Therefore, future studies with larger sample size are needed to further evaluate the effect of MW radiation on the eye. Further studies are also warranted to elucidate long-term implications of such exposures.

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