



Effects of mobile phone prolonged radiation on kidney cells; an in-vitro study

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ABSTRACT

Introduction: Mobile phones have been shown to have hazardous effects on the human renal system, reproduction and development, central nervous system and immune function.

Objectives: This study aimed to investigate whether the mobile phone prolonged radiofrequency (RF) radiation could affect healthy kidney cells in an in-vitro study.

Materials and Methods: Human kidney healthy cells in three groups were placed into three batches. The first and second groups (exposed groups) were exposed to a mobile phone simulator for 1 and 2 h/d, respectively. The irradiation was performed for 8 consecutive days. For the third group (control group), no radiation was applied to the samples and they completed the assay cycle under identical conditions. Cells' survival was evaluated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Statistical significance of the differences between control and exposed groups were assessed using the repeated measure ANOVA method ($P < 0.05$).

Results: Results showed that radiation exposure from mobile phones simulator decreased the kidney cell survival in the exposed groups (up to 40%). ANOVA test revealed that a significant decrease in cell survival in the exposed group compared to the control group ($P = 0.014$). No significant differences between the irradiated groups were found ($P > 0.05$).

Conclusion: Mobile phone exposure affects kidney cells survival during an in vitro condition. To study the effects of mobile phone radiation on kidney, further in vivo studies on mammals are needed.

Implication for health policy/practice/research/medical education:

Nowadays, mobile phones are widely used not only for making calls but also for many other applications, such as banking transactions and web browsing. Histological and physiological studies have shown the hazardous effects of mobile phones on the human central nervous system, cardiovascular system, immune function, reproduction and development. It should be noted that mobile telephone radiation may be absorbed by kidney more than other internal organs because mobile phones are often carried in belts. The present study investigated that the mobile phone prolonged radiofrequency radiation could affect healthy kidney cells in an in vitro study.

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Introduction

The increased application of radiofrequency (RF) radiation, for domestic and industrial purposes, generated from various electrical devices such as mobile phone base transceiver stations (BTSs) and power transmission lines, proves that RF plays a critical role in human daily life

(1). In this regard, the emergence of telecommunication services using electromagnetic field (EMF) principles greatly enhance the ability of individuals and groups to communicate with each other (2,3).

Nowadays, mobile phones are used not only for making and receiving calls but also for many other applications,

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such as banking transactions and web browsing. According to International Telecommunication Union (ITU), about 4.6 billion mobile phones subscribers in the world was estimated in 2009, and it was expected to reach 8 billion by 2015, which represent one of the fast growing environmental influences (1).

In recent years many histological and physiological studies have been performed in the evaluation of the hazardous effects of EMF on human health regarding cancer, renal system, reproduction and development, central nervous system and immune function (4-10).

The emitted RF radiation from mobile phones have been shown to have many effects upon the sperm motility and morphology (11,12), immune functions (10), mammalian brain (3), and neurological pathologies syndrome (13). However, many potential harmful effects of cell phone exposure remain controversial (10,12). Hinrikus et al have shown that the EEG beta rhythms of human brain were significantly increased by exposure to 450 MHz RF radiation (14). Lai et al studied the effects of microwave radiation on cholinergic systems of rat brain (7,15,16). They showed that a dose-response relationship for each brain region with respect to different microwave power densities was existed (7,15,16). Nittby et al investigated that RF radiation affected the mammalian blood-brain barrier permeability 7 days after exposure to the radiation from a 900 MHz mobile phone radiation (17). Moreover, other experimental studies in mammals have shown that exposure to mobile phone radiation led to considerable testicular germ cell morphological changes and apoptosis (18-20). Lu et al found that air conditioners, which generate significant amounts of RF radiation, are associated with low semen quality (21). In addition, it is now generally accepted that this kind of radiation can activate DNA to synthesize proteins (22). Furthermore, recent reports have revealed that long-term exposure to EMF decreases thyroid tri-iodothyronine-thyroxin (T3) levels and stimulating hormone (9,23).

The mobile telephone RF radiation may be absorbed by pelvic organs, especially the kidney, more than other internal organs because mobile phones are often carried in belts. According to the best of our knowledge, no study on the effects of cell phone prolonged RF radiation on the kidney cells has been published.

Objectives

The aim of this study was to investigate whether the mobile phone prolonged RF radiation could affect healthy kidney cells in an in-vitro study.

Materials and Methods

Cell culture and mobile phone radiation

Human kidney healthy cells, provided from Isfahan University of Medical Science Laboratory (Isfahan, Iran) were cultured in 25 mL culture flasks in Dulbecco's modified Eagle's medium (DMEM Gibco Laboratories, Cergy Pontoise, France) including 10% fetal bovine

serum (FBS Gibco Laboratories, Cergy Pontoise, France), 100 U/mL penicillin, 2 mM glutamine, and 100 mg/mL streptomycin (Gibco Laboratories, Cergy Pontoise, France). A humidified cell incubator, with 5% CO₂ atmosphere and 95% air, was used for the cells growth at 37°C.

Before the experiment, cells were trypsinized by trypsin-EDTA (Gibco, Invitrogen, Carlsbad, CA, USA) and plated at a density of 10⁴ cells/well in 96-well culture plates.

The cells were divided into three groups and were placed into three batches. The first and second groups (exposed groups) were exposed to a mobile phone simulator for 1 and 2 h/d, respectively, for 8 days. The simulator produced 900 MHz RF radiation, to represent the exposure of GSM. The distance between the simulator antenna and each specimen was kept at 2.5 cm. The cells in the third group (control group) were completed the assay cycle with no radiation.

Cells' survival was evaluated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay at different days during and post-irradiation period.

Mobile phone simulator

A mobile phone simulator (designed and produced at the Medical Physics Department, School of Medicine, Isfahan University of Medical Sciences) was used for RF irradiation. In this experiment, similar to mobile phone, the frequency of 900 MHz radiation (a wavelength of about 33.4 cm) was used. The simulator power was adjusted to 1.0 W for the exposure.

MTT assay

The MTT assay involves the conversion of the MTT in the form of water-soluble to an insoluble formazan. The MTTs was dissolved in sterile phosphate-buffered saline (PBS) at 5 mg/mL, the solution was filtered and stored in dark condition at 4°C for less than 3 weeks.

Five wells of a 96-well plate were used for every experimental condition. The medium was renewed, without making any damage to the adhesive cells, every three days to avoid possible medium product inaccuracy. For the MTT assay, according to our basic protocol, the solution with proper concentration (10 µL MTT solution in each 100 µL media) was added to each well and the plates were then incubated at 37°C for 4 hours. Following the incubation, the remaining MTT solution was removed and 100 µL of dimethyl sulphoxide (DMSO) was added to each well to dissolve the formazan crystals. In order to ensure adequate solubility, the plates containing the cells were shaken for 5 minutes using a plate shaker. Absorbance readings of each well were performed at 570 nm (single wavelength) using an ELISA reader (Stat Fax 2100, Awareness Technology Inc., USA). It should be noted that the amount of the formed purple formazan crystals is directly correlated with the number of viable cells. The study was repeated for three independent trials to avoid the intrinsic variability of the assay used.

Ethical issues

The research followed the tenets of the Declaration of Helsinki, and the study was approved by the ethical committee of Sabzevar University of Medical Sciences, Iran (grant #1394-43).

Statistical analysis

Mean values and standard deviations of the cell's survival were calculated. Statistical significance of the differences, between control and exposed groups, was evaluated using ANOVA method in SPSS version 16.0 (Chicago, IL, USA). A significant level of 0.05 was considered to the tests.

Results

Results showed that radiation exposure from mobile phones simulator decreased the kidney cell survival in the exposed groups (Table 1). Table 1 gives the variance analyses of the effects of day, group and the interaction between these two parameters. Using the ANOVA test, a significant decrease in cell survival in the exposed group compared to the third group (control one) was seen ($P = 0.014$) (Figure 1). In other words, the kidney cell survival in the assay was affected by exposure from 900 MHz mobile phone simulator, following 1 to 2 h/d irradiation for 8 consecutive days. No significant differences were investigated between the irradiated groups ($P > 0.05$). It means that a significant difference between 1 and 2 h/d irradiated groups was detected. Therefore, although the level of cell death in various days was different (the effect of day) in all groups these differences were the same.

Discussion

Currently, the use of mobile phones has become one of the fastest growing technological developments worldwide (12). Due to close proximity of the mobile phone antenna to the pelvic organs, the emitted radiation from such phones has raised concerns about the biological effects of RF radiation on the kidney (24). The 900-MHz mobile phones are widely used in Iran and in many other countries and they usually carried in belts close to kidney. According to our knowledge, direct biological effects of kidney due to exposure to mobile phone RF radiation have not been studied extensively. This study aimed to investigate the biological effects of mobile phone prolonged RF radiation on kidney cell.

In this work, kidney cells were irradiated to RF radiation of a mobile phone simulator for 8 days and 1 to 2 hour/day. It should be noted that to study the hazardous effects, the

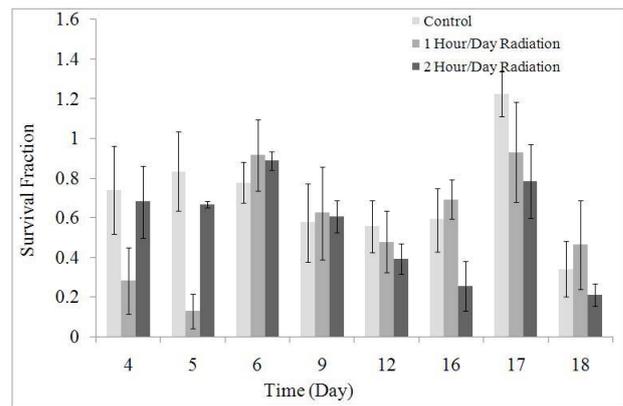


Figure 1. A comparison of average \pm SD kidney cell survival among control and the mobile phone exposed groups in different days.

duration period for mobile phone use and its frequency are main factors, but the exact duration varies from individual to individual (10,12). Therefore, the estimated average time of mobile phone use was considered for RF radiation.

Results of our study showed that kidney cell survival decreased due to mobile phone RF radiation ($P = 0.014$). However, differences were not significant between the irradiated groups ($P > 0.05$). In other words, there was not a significant difference between 1 and 2 h/d irradiated groups.

Recently, studies on kidney reported that melatonin plays a regulatory role in renal system and affects urine output (8,25). Moreover, localizations of specific melatonin receptors have been found in renal tissue (26). This finding is highly suggestive of a direct critical role for melatonin in the renal function. It should be noted that specific receptors of melatonin are found in renal tissue and they have strong biological inference which indicates the effects on cellular actions throughout intracellular signaling and transduction of the membrane (24).

Oktem et al have reported that exposure to mobile phone RF radiation imposed a significant effect on rat kidney (24). However, their results showed that melatonin, as an efficient free radical scavenger, may exhibit a protective effect on mobile phone-induced renal impairment in rats (24). Moreover, it has been reported that RF radiation also resulted in the expansion of free radicals and decrease in the levels of serum melatonin (27).

Al-Glaib et al reported that the exposed mice kidney to electromagnetic radiation from a mobile phone resulted

Table 1. Variance analyses of the studied groups of kidney cells

Source	Type III Sum of Squares	df	Mean Square	F	P
Day	2.420	1.000	2.420	11.565	0.014
Group	0.315	2.000	0.158	1.423	0.312
Day * Group	1.631	2.000	0.816	3.897	0.082
Error (day)	1.256	6.000	0.209		
Error (group)	0.664	6.000	0.111		

in more mononuclear leukocyte infiltration between the renal tubules, compared to the sham exposed group (28). In addition, they have stated that following the radiation, some glomeruli were atrophied and some kidney tubules were vacuolated (28).

The results of this study were obtained by using a commercial 1.0 W at 900 MHz mobile phone simulator to reproduce the reality of the human exposition. Due to limitations and difficulties associated with *in vivo* studies, in this work an *in vitro* experiment was performed to investigate the effects of exposure to the cell phone on the kidney cells. Nevertheless, *in vivo* studies, on either animals or human beings, may provide more convincing evidence of the EMF effects.

Conclusion

In this work, the biological effects of mobile phone prolonged RF radiation on kidney cell were studied. Results showed that mobile phone exposure affects kidney cells survival during an *in vitro* condition. To study the effects of mobile phone radiation on kidney, further *in vivo* studies on mammals are needed. However, the evaluation of the possible effects of mobile phones radiation on the living organism is a complex process that needs the combined contributions of many scientific regulations.

Limitations of the study

More accurate follow up studies on mammalian kidney are needed for the evaluation of the effects of the mobile phone. The results here should be more evaluated in larger series, employing repeated *in vivo* exposure-dose related effect design and providing a detailed assessment of the radiation dose produced by the phones.

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Authors' contribution

GM was the principal investigators of the study. GM, SN and MBGh participated in preparing the concept and design. MM and AD revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revisited the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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