The Effect of Pre-exposure to Radiofrequency Radiations Emitted from a GSM Mobile Phone on the Suseptibility of BALB/c Mice to *Escherichia coli*

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Abstract

Background: Pre-exposure to radiofrequency radiations of mobile phones would significantly increase the survival rate of exposed animals compared to those exposed to a lethal dose of gamma radiation alone. Stimulation of the immune system is believed to be a key mechanism for the induction of this phenomenon, the so-called "adaptive response." The immune system protects organisms against infection with multiple lines of defense of increasing specificity.

Objective: In this animal study, the effect of pre-exposure to radiofrequency on the survival adaptive response of a group of BALB/c mice which received intraperitoneal injections of *Escherichia coli* was investigated.

Methods: Groups of BALB/c mice (exposure groups) were exposed to radiofrequency radiations emitted from a GSM mobile phone for 2, 4, 8 or 12 hours a day for 3 days. Other groups (sham exposed groups) were treated as exposure groups but the mobile phone was switched off during the experiment. On day 4, animals received intraperitoneal injections of *E. coli*. Survival of the animals was carefully monitored by an expert scientist.

Results: 15 days after exposure to the bacteria, the survival rate of the animals exposed to mobile radiations for 12 h/day was significantly ($p=0.021$) higher than those which only exposed to the bacteria (no pre-exposure to radiofrequency).

Conclusion: Pre-exposure of BALB/c mice to radiofrequency radiations emitted from a GSM mobile phone increases their resistance to *E. coli* infection. This finding may have important clinical implications in treating bacterial infections.

Keywords

Radiofrequency (RF); Adaptive response; Nonionizing radiation; Survival; GSM mobile phone; *Escherichia coli*

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Introduction

Adaptive response is the acquisition of resistance against detrimental effects of high doses of physical or chemical agents in cultured cells or organisms that had been pre-treated with a priming low dose radiation (LDR). The priming LDR is usually called "adapting dose" or "conditioning dose" while the high dose is called "challenge dose." The induction of adaptive response was first reported by Olivieri, *et al*., [1] who showed that the frequency of chromatid aberrations was 50% lower than the expected value after exposure of cells to 1.5 Gy of x-ray. Although there is substantial evidence about the induction of adaptive response with low doses of ionizing radiation, there is

still doubt about whether it is possible to induce such a response after exposure to adapting doses of non-ionizing radiations such as microwaves.

Mobile phones, as wireless communication devices with drastically widespread use and increased popularity, use electromagnetic radiation in the microwave range. Although mobile phones and base stations (towers) produce the same type of radiation, phones play a much more significant role in human exposures than towers [2]. Although the output power of towers is two to three times greater than that of mobile phones, as phones are held just a few centimeters from some sensitive parts of the body such as the brain and the eyes, their health effects are of much more concerns. It is estimated that approximately 50% to 70% of the power output of a phone is absorbed by the user [2].

In 2009, it was shown that irradiation of cells in culture medium with radiofrequency (RF) would induce an adaptive response that increases the resistance of these cells to mytomycin C [3]. Over the past several years, our laboratory has been investigating various aspects of adaptive response [4-10]. Mortazavi, *et al*., recently showed that rats pre-exposed to RF radiation were less susceptible to subsequent lethal effects of high doses of ionizing radiation [11]. Other scientists also showed that compared with animals exposed to gamma radiation alone, mice pre-exposed to RF at 120 W/cm^2 and then subjected to gamma-irradiation had a significantly higher survival time and lower damage to hematopoietic tissues [12]. In a more recent study, it was shown that the pre-exposure for more than four hours a day is necessary to induce the adaptive response [13].

It is believed that stimulation of the immune system is a key mechanism for the induction of radiation hormesis and adaptive response [14]. On the other hand, the immune system protects organisms against infection with multiple lines of defense of increasing specificity. The immune system not only provides protection against infection through natural barriers, but also induces acquired immunity following exposure to specific micro-organisms. Plews, *et al*., in 2010 reported that the adaptive response induced by low-dose whole-body radiation prolonged the survival of prion-infected mice through reducing oxidative stress [15]. It is worth noting that in this report the ionizing radiation used was gamma ray at adapting dose.

In this study, a BALB/c mice model was used to investigate the possibility of the induction of RF-induced survival adaptive response through mechanisms such as stimulation of the immune system after intraperitoneal injection of *Escherichia coli*.

Materials and Methods

Animals

In the first phase of the study, 80 male BALB/c mice weighing 20–25 g were randomly divided into 12 groups of 5 or 10 animals. Grouping of the animals and interventions (adapting and challenge doses) in each group are presented in Table 1. The animals were kept in special cages with controlled temperature, humidity and lighting. All animal experiments were considered and approved by the Animal Experimentation Ethics Committee of Shiraz University of Medical Sciences prior to commencing work. In the second phase of the study, to investigate if exposure of animals for 12 h/day for three days can induce the same survival adaptive response, 30 male BALB/c mice were randomly divided into two groups of 15 animals.

Adapting Dose (RF Radiation)

A Nokia E71 GSM mobile phone (SAR=1.4 W/kg) in talk mode was used as the source of microwave radiation. During the microwave exposure, the animals were immobilized by placing their body through plastic restrainers. The distance between the antenna of the mo-

Table 1: Grouping of the animals and interventions (adapting and challenge doses) in each group

Groups	Number of animals	Treatment		Comment
		Adapting dose (RF Exposure)	Challenge dose (Exposure to bacteria)	
Group 1	10	RF 2 h/day for 3 days	ip injection of *E. coli*	
Group 2	5	RF 2 h/day for 3 days	No bacteria (ip injection of normal saline)	
Group 3	5	Sham exposure (No RF)	ip injection of *E. coli*	
Group 4	10	RF 4 h/day for 3 days	ip injection of *E. coli*	
Group 5	5	RF 4 h/day for 3 days	No bacteria (ip injection of normal saline)	
Group 6	5	Sham exposure (No RF)	ip injection of *E. coli*	
Group 7	10	RF 8 h/day for 3 days	ip injection of *E. coli*	
Group 8	5	RF 8 h/day for 3 days	No bacteria (ip injection of normal saline)	
Group 9	5	Sham exposure (No RF)	ip injection of *E. coli*	
Group 10	10	RF 12 h/day for 3 days	ip injection of E. coli	
Group 11	5	RF 12 h/day for 3 days	No bacteria (ip injection of normal saline)	
Group 12	5	Sham exposure (No RF)	ip injection of *E. coli*	
Group 13 (Repeating Group 10)	15	RF 12 h/day for 3 days	ip injection of *E. coli*	As previous 12 h/day irradiation protocols showed the highest magnitude of the survival adaptive response, these protocols were repeated to verify whether the results are reproducible
Group 14 (Repeating Group 12)	15	Sham exposure (No RF)	ip injection of *E. coli*	
Total Number	110			

bile phone and animal's head was 5 cm.

Challenge Lethal Dose (Exposure to Bacteria)

A confirmed isolate of *E. coli* (PTCC No. 1789) isolated from a patient hospitalized in Namazi Hospital, Shiraz, Iran, was used in this study. The mean intraperitoneal lethal dose of this germ for BALB/c mice was previously determined in a series of experiments. Before use, the germ was cultured at 37 °C in nutrient broth (Merck, Germany) for 24 h. Subsequently, the bacteria were harvested by centrifugation at 1,800 × g for 10 min, washed twice with sterile isotonic saline, and resuspended in saline at a final concentration of 10^9 CFU/mL. On day four, animals were exposed to a lethal dose of 0.3 mL of the above-mentioned bacteria suspension via an intraperitoneal injection.

Assessment of Survival Adaptive Response

After exposure to bacteria, animals were returned to their cages; survival of the animals was carefully monitored every 12 h by an expert scientist.

Statistical Analysis

Kaplan-Meier's survival analysis was used for assessing the survival rate in each group.

Table 2: Survival rates in different groups of BALB/c mice 15 days after exposure to *E. coli*.

Intervention (Exposure to RF for 3 days)	Survival rate after 15 days		p value Log-Rank (Mantel-Cox)
	RF → Bacteria	0 → Bacteria	
2 h/day (20 animals)	20%	0%	0.186
4 h/day (20 animals)	10%	60%	0.046
8 h/day (20 animals)	20%	20%	1.000
12 h/day (20 animals)	50%	20%	0.280
Repeated 12 h/day (30 animals)	60%	20%	0.021
Pooled 12 h/day (45 animals)	56%	20%	0.018

A dead animal was counted as 0, whereas alive animals were defined as 1. The difference among the survival rates of the groups was evaluated by the log-rank (Mantel-Cox) test. Log-rank test was considered statistically significant if p value obtained from χ^2 test was less than 0.05.

Results

The survival rate of animals after 15 days of mice in different treatment groupsare shown in Table 2. Irradiation for 12 h/day provided the highest magnitude of survival adaptive response. Therefore, we repeated this protocol to verify whether these results are reproducible.

When the 12 h/day RF exposure protocol was repeated, the survival rates in animals that received both adapting (RF) and challenge dose (bacteria) and the animals received only the challenge dose (bacteria) were 60% and 20%, respectively (p=0.021). After pooling the results of two phases of 12 h/day exposures, the survival rates in 25 animals that received both adapting dose (RF) and challenge dose (bacteria) and the 20 animals received only the challenge dose (bacteria) were 56% and 20%, respectively (p=0.018).

Kaplan-Meier survival plots of the mice pre-exposed/sham-exposed to microwave, low dose rate gamma irradiation or both of these adapting doses before receiving a lethal dose (LD) of gamma radiation are shown in Figure 1.

Discussion

Two repeated phases of 12 h/day exposure to RF radiation showed that the survival rate in animals that received both adapting dose (RF) and challenge dose (bacteria) was significantly higher that that of animals that received only the challenge dose. These findings generally confirm that an earlier exposure of cells to a small dose of ionizing or non-ionizing radiation can increase their resistance to toxicity caused by high doses of physical or chemical agents, a phenomenon the so-called "adaptive response." Although there is one report by Plews, *et al*., indicating that induction of adaptive response induced by low-dose whole-body radiation treatments prolonged the survival of prion-infected mice by reducing oxidative stress [15], to the best of our knowledge, this is the first study showing that induction of adaptive response would prolonge the survival of *E. coli*-infected BALB/c mice by pre-exposure to RF radiation (non-ionizing radiation). In spite of the fact that there were some basic differences between our study and the above-mentioned investigation, both studies confirmed the potential of adapting doses of ionizing and non-ionizing radiation for induction of adaptive response with resultant increase in the resistance to a subsequent

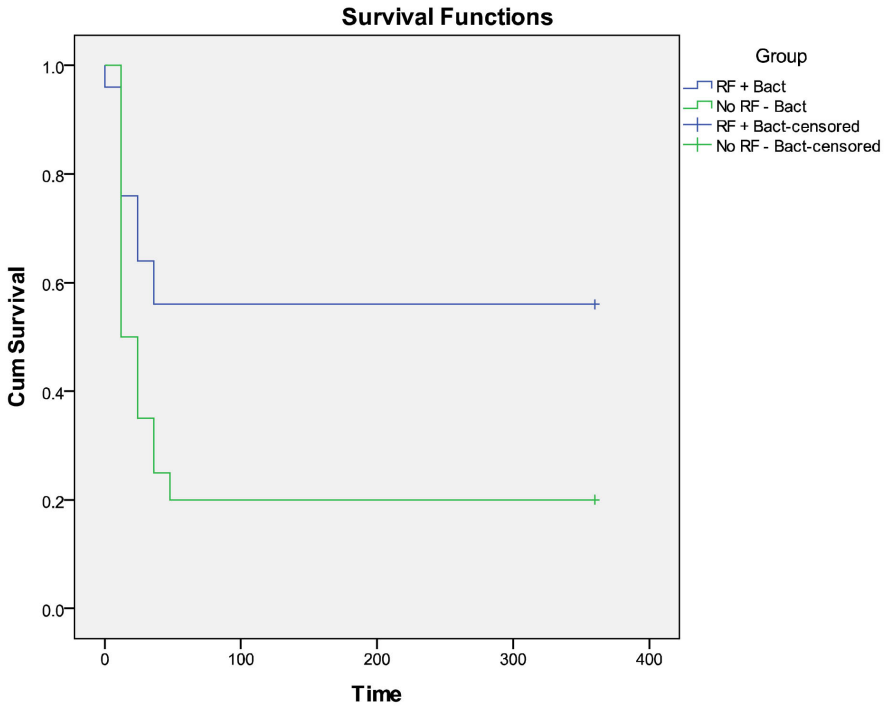

Figure 1: Kaplan-Meier survival curves of the BALB/c mice pre-exposed/sham-exposed to microwave (MW), before exposure to bacteria. The results of two repeated phases of 12 h/day exposure are pooled. The survival rate in 25 animals that received both adapting (RF) and challenge dose (bacteria) was significantly higher than those 20 animals received only the challenge dose of bacteria (p=0.018).

infection. The most important difference between our study and that conducted by Plews, *et al*., is the type of the adapting dose—RF as a non-ionizing radiation in our study *vs* gamma radiations emitted from a ^{60}Co source in Plews'study.

These findings are in line with our previous reports [10, 11] as well as the very limited recently published studies that indicated the possibility of the induction of adaptive response after pre-treatment with microwave radiation [12, 13, 16-18]. Sannino, *et al*., have previously reported that pre-exposure of peripheral blood lymphocytes collected from human volunteers to RF radiation (900 MHz, at a peak specific absorption rate of 10 W/kg for 20 h) would increase their resistance to a challenge dose of mitomycin C (100 ng/mL at 48 h) [16]. Later, they confirmed their previous results and showed that the timing of exposure to the adapting dose plays an important role in the process of the induction of adaptive response [17]. On the other hand, Chinese researchers have recently shown that pre-exposure of mice to 900 MHz RF would induce adaptive response and thus reduce the hematopoietic tissue damage from a subsequent exposure to a challenge dose of ionizing radiation [12].

Our results are in line with those reported recently by Zeni, *et al*., who showed that when lymphocytes were pre-exposed to RF at 0.3 W/kg SAR and then treated with mitomycin C, they showed a significant reduction in the frequency of micronuclei compared with the cells treated with MMC alone [18]. Jiang, *et al*., also recently used a relatively similar method as we did previously (using gamma radiation as the challenge dose) and showed that mice pre-exposed to RF for 3, 5, 7 and 14 days showed progressively less damage than those exposed to gamma-radiation alone [13].

United Nations Scientific Committee on the

Effects of Atomic Radiation (UNSCEAR) in its 1994 report to the United Nations (UN) General Assembly, recognized the adaptive response induced by low-dose radiation for the first time [19]. In this document, stimulation of the immune system by low-dose radiation was reported. Although high doses of ionizing radiation are immunosuppressive, the stimulatory effect of low-dose radiation on the immune system has been widely documented [20-24]. Therefore, stimulation of the immune system may be a key mechanism for the induction of adaptive response. On the other hand, some recent studies showed that RF radiation causes oxidative injury in different tissues mediated by lipid peroxidation, increased level of nitric oxide (NO) and suppression of antioxidant defense mechanism [25, 26]. Feinendegen, *et al*., in their previous studies, proposed that adaptive response could be induced by reactive oxygen species (ROS) [27, 28]. Increased levels of ROS or NO have been usually observed in the adapted cells [29]. The ROS refers to a group of molecules including peroxides and free radicals that are derived from oxygen and are highly reactive toward biomolecules [30]. ROS react with critical biomolecules such as DNA and induce oxidative stress (imbalance of pro-oxidants *vs* antioxidants) and damage to macromolecules including multiple localized lesions such as base damage, single strand breaks (SSBs) and double strand breaks (DSBs), DNA-DNA cross links and DNA-protein cross links [31-34]. As indicated by some investigators [35-37], we believe that induction of adaptive response by pre-exposure to ionizing and non-ionizing radiation needs a minimum level of damage that triggers this phenomenon with resultant increase in the resistance of living organisms (*in vivo*) or cells (*in vitro*) to higher levels of the same or other sources of stress. In our experiments, only the animals exposed to mobile radiations for 12 h/day showed a significantly higher survival rate compared to those only exposed to bacteria (no pre-exposure to RF radiation). These findings are in line with the results of a recent study of Jiang, *et al*., who showed that pre-exposure for more than 4 h/day is necessary to induce the adaptive response [13]. These findings can be explained by the fact that the lower dose threshold for adaptation depends on the presence of a minimum number of lesions per unit of time.

In conclusion, we found that exposure of laboratory animals to RF radiations emitted from a common mobile phone can induce a survival adaptive response and resultant increased survival rate after exposure to *E. coli*. Our results also lead us to assume that the induction of adaptive response by pre-exposure to ionizing or non-ionizing radiation needs a minimum level of damage that triggers this phenomenon. These findings may have important clinical implications in treating some bacterial diseases.

Conflicts of Interests: None declared

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