

The gene expression level of p53 and p21 in mouse brain exposed to radiofrequency field

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ABSTRACT

Background: Widespread and growing sources of electromagnetic radiation raised concerns attributed to the potential adverse health risk of radiofrequency fields. Given the functional importance of the hippocampus, this study aimed to investigate the effects of electromagnetic waves radiated by mobile jammer on hippocampal expression of p21 and p53 genes as regulators of cellular apoptosis. **Materials and Methods:** Forty-eight male BALB/c mice were randomly divided into six groups (n=8 each). Animals in the experimental groups were radiated at the frequencies of 900 and 1800 megahertz for a period of 30 consecutive days, while the control group remains constant during the experiment. The hippocampal expression of p21 and p53 mRNAs were evaluated using Real-Time PCR. **Results:** There were not differences between the mean expression level of p53 and p21 genes of the exposure groups compared to those of the control group ($P>0.05$). The ratio expression of p53 and p21 genes was increased to greater than one ($p53/p21>1$) in almost all experimental groups compared to controls. However, there was not significant differences between the expression level of p53 and p21 genes among the experimental groups using paired t test ($p>0.05$). **Conclusion:** Taken together, our findings demonstrate changes in hippocampal expression level of p53 and p21 after mobile jammer radiation. However, cell condition expected to remain relatively stable over the exposure period due to parallel changes of both pro- and anti-apoptotic genes at the same time.

Keywords: Radio frequency, gene expression, hippocampus, p53, p21.

INTRODUCTION

Increasing sources of non-ionizing radiofrequency electromagnetic radiation in the modern environment aroused concerns about the potential human health hazard. Mobile phones and their base stations are among the most widespread RF (radio frequency) sources and medical researchers are concerned that any associated health risks, even small ones, could cause significant public health problems^(1, 2).

Contradictory scientific evidence, often reported in the popular media, has further fueled public concern. Many changes in cellular functions by RF radiation have been proposed including; altered RNA DNA replication and gene transcription, cell cycle progression and its metabolism, and changes in membrane integrity⁽³⁾. In 1998, guidelines on reference values, exposure limits, and restrictions for exposure to RF radiation were established by the International Commission on Non-Ionizing

Radiation Protection (ICNIRP). The aim is to disseminate information and advice on the potential health hazards of exposure to non-ionizing radiation in order to protect citizens against the possible harmful effects of RF exposure. Given the importance of the problem, WHO also emphasized research on electromagnetic fields to make better health risk assessments^(4,5).

There is a particular focus of concern about the health effects of Phone radiofrequency signal exposure on brain cells because of the close proximity of mobile phone to the head in talk mode^(1,6). Many studies have been conducted to determine whether, there is a causal relationship between RF energy emitted from cell phone and harmful effects including cancer induction, headache, intracellular calcium increase, DNA damage, and increased apoptosis^(7,8). While epidemiological studies were not able to demonstrate convincing evidence of adverse health effects from the established levels of RF exposure, the existence of harmful effects particularly with regard to long-term exposure remains a possibility^(4,9). In the case of genomic aberrations in vicinity of mutagen or genotoxic factors, self-regulatory mechanisms of the cell interfere with cell cycle progression and survival. This occurs largely through induction of p53 response upon stress while it is tightly controlled under the cell's normal condition^(10,11).

Alterations in the p53 transcriptional activation and protein induction gives rise to a variety of cellular outcomes such as cell malignancy. The transcription of several genes that mediate cell-cycle arrest or apoptosis in response to cellular stress is under the control of p53⁽¹²⁾. Cell cycle arrest is imposed by p53, mainly through p21 induction, preventing cells from entering S phase. Such a pause allows the cells enough time to repair DNA damage and proceed with the cell cycle in case of successful repair of DNA. If DNA repair fails, p53 protein promotes apoptosis in order to eliminate the damaged cell^(13,14).

Currently, laboratory studies especially on gene expression level are very commonplace and useful for identifying how cells and

organisms adapt to changes in the external environment such as, harmful effects of mobile phone radiations⁽¹⁵⁾. Yilmaz et al. studied the effects of mobile phones on apoptosis in rats' cerebral tissue in terms of gene expression level. In their study radiation leads to changes in p53 and bcl-2 genes expression level and apoptotic and anti-apoptotic characteristics of the cells⁽¹⁶⁾. The albumin release into neural cells was reported in another study by Nittby H et al. In the mentioned study differentially expressed gene of the exposed animal versus control group were observed when both cortex and hippocampus of rats' brain were exposed to GSM 1800 MHz radiation⁽¹⁷⁾. The above mentioned studies results show a change in gene expression level relevant to cell performance. However, many investigations report a lack of cytotoxic effects in the cells of exposed animals⁽¹⁸⁾. These contradictory views concerning the possible biological effects of RF radiation on animal cells and tissues, making any judgment subordinate to more research⁽¹⁹⁾.

The aim of the present study was to determine effects of mobile jammer radiation on gene expression in mice Balb/C cerebellum. Although, the involvement of p53 and p21 genes in cell cycle arrest and cell death was addressed in many studies but the general reaction of these genes to RF exposure in vivo remains obscure and was subject of the present study. In this study mice brain expression of p53 and p21 transcript level was measured, using Real-Time PCR (as a high throughputs technique), if it could be altered due to RF- radiation energy.

MATERIALS AND METHODS

Forty eight adult BALB/c male mice were divided randomly into five experimental and one control groups, comprising of 8 animals in each. Animals were given standard diet and kept at standard temperature $21 \pm 2^\circ\text{C}$ and animal room was maintained on 12 hour light/dark cycle.

Microwave Radiation exposure

Experimental animals were housed in the standard plastic cage located at a distance of 2

meters from the radiofrequency wave generator. A commercially available wireless Cell Phone Jammer was used as the RF generator base, which constantly emits radiation at a bandwidth of 1880–1900 MHz, very close to the GSM1800 band, scanning all 10 allocated RF channels. The device was programmed to operate twice a day, at 12-hour intervals for 30 consecutive days (table 1). The sham group was kept in a similar room as the exposed groups, under the same conditions of living without phone jammer.

After the last irradiation mice were euthanized, followed by rapid brain tissue removal. The sample taken from the hippocampus was put into a micro tube filled with the RNA preservative reagent (RNAlater, Qiagen, Germany) and stored at -80°C until sample processing for further analysis.

RNA extraction and cDNA synthesis

Next, TriPure Isolation Reagent (Roche) was used for RNA extraction from the hippocampus according to the manufacture's procedure. RNA sediments were dissolved in DEPC water and were assessed by electrophoresis on agarose gel. The presence of 18s and 28s ribosomal RNA bands proved accuracy of RNA extraction. 1 µg of total RNA was used to synthesize the first strand cDNA using the oligo(dT)18 primers

following the instructions of the RevertAid First Strand cDNA synthesis kit (Fermentas Life Science, Vilnius, Lithuania). cDNA samples were stored at -20°C.

Real-Time reverse transcription polymerase chain reaction (RT-PCR)

Real-Time (RT)-PCR was performed to analyze the changes in hippocampal expression of p21 and p53 genes. The primer sets were designed based on the sequences from the NCBI database. The sequences of primers used in the quantitative RT-PCR assay are listed in table 2.

Each RT-PCR reaction mixture containing 1 µL of cDNA, 7.5 µL SYBR green, 0.3 µL Rox, 0.3 µL related primers, and the final volume was topped up to 15 µL by adding 5.6 µL of distilled water. The assay was performed with SYBR Premix Ex. TaqTM Kit (TaKaRa, Biotechnology Co., LTD, Dalian, China) under the following condition: initial denaturation stage at 95°C for 30 sec, followed by 45 cycles with denaturation at 95°C for 10 sec, annealing at 60°C for 30 sec, and extension at 72°C for 15 sec. The real-time detection of emission intensity of SYBR Green bound to double-stranded DNAs was performed using the Applied Biosystems (ABI) Prism 7000 Sequence Detection System. GAPDH mRNA was used as an internal control to measure the relative expression quantity of the target genes.

Table 1. Characteristics of the study groups and duration of a month-long radiation.

Groups	Duration of radiation (hour)	Times of radiation in 24 hours	Time of radiation in the morning	Time of radiation in the evening
Control (0h)	-	-	-	-
0.5 h of radiation twice a day (0.5h×2h)	0.5	twice	4:00-4:30	16:00-16:30
1 h of radiation twice a day (1h×2)	1	twice	4:00-5:00	16:00-17:00
2 h of radiation twice a day (2h×2)	2	twice	4:00-6:00	16:00-18:00
4 h of radiation once a day (4h×2)	4	once	4:00-8:00	-
4 h of radiation twice a day (4h×2)	4	twice	4:00-8:00	16:00-20:00

Table 2. Oligonucleotide sequences used in SYBR Green real-time PCR.

Gene name	Sequence (5'-3')	Length
P53 Forward	GTATTCACCCCTCAAGATCC	83 bp
P53 Reverse	TGGGCATCCTTAACTCTA	
P21 Forward	CTTGCACCTGGTGTCTG	106 bp
P21 Reverse	CTTGGAGTGATAGAAATCTGTCA	
GAPDH Forward	GAGAAACCTGCCAAGTATG	123 bp
GAPDH Reverse	GGAGTTGCTGTTGAAGTC	

The relative analysis of gene expression was made through the standard curve method by the Applied Biosystems SYBR green I fluorescence. Standard curve: Obtained by plotting Ct values against log-transformed concentrations of ten-fold serial dilutions of a reference cDNA pool.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package, version 15.0 (SPSS). The Kolmogorov-Smirnov test demonstrated a normal distribution for the quantitative variables. Significant differences ($P < 0.05$) between groups were determined using an independent sample t test and one-way analysis of variance (ANOVA) followed by Tukey's test. A P value of less than 0.05 was accepted as statistically significant.

RESULTS

The Kolmogorov-Smirnov test demonstrated a normal distribution for the quantitative variables expression levels of p53 and p21 genes. The absence of gene expression levels of p53 and p21 genes among experimental groups was revealed according to one-way ANOVA analysis. A significant difference in p53 expression level mean ($P=0.001$) and p21 expression level mean ($P=0.049$) between the $2h \times 2$ and 4h radiation groups were found, using Tukey test.

The graph in picture 1 shows the mean level expression of p53 and p21 in mice study groups. The dash line is showing the basal expression

level of p53 and p21 gene in the control group. In four experimental groups the level of p53 and p21 gene expression is close to the basal expression level of control group except for the 4 h radiation group which is almost twice higher than the normal expression level. While, there is a fluctuation in the expression level of p53 and p21 genes but these expression shifts were generally highly parallel (figure 1).

The ratio expression of p53 and p21 genes was increased to greater than one ($p53/p21 > 1$) in all experimental groups compared to controls, except for the group with consecutive 2 hours exposure twice a day. However, there was not significant differences between the expression level of p53 and p21 genes among the experimental groups using paired t test ($p > 0.05$) (table 3).

DISCUSSION

This study generally indicated that mobile phone waves with a variety of radiation durations changed the expression levels of p53 and p21 genes in the hippocampus of the examined mice. The changes mostly were observed among the cases that had been radiated for four hours once a day and the lowest gene expression responses were observed among the cases radiated for two hours twice a day. However, p53 and p21 expression levels did not show statistically significant difference between the cases and control group.

Specific alterations of transcript levels of

Table 3. The table shows mean and standard deviation of p53 and p21 expression levels, p53/p21 ratio, and the related Paired t test result among six study groups.

Group	Gene	Mean	SD	p53/ p21	Paired t test
Control	P 53	1	0	1	0
	p21	1	0		
0.5 h of radiation twice a day (0.5h×2)	p53	0.8	0.73	1.6	0.368
	p21	0.5	0.40		
1 h of radiation twice a day (1h×2)	p53	1.1	0.17	1.6	0.587
	p21	0.7	0.24		
2 h of radiation twice a day (2h×2)	p53	0.3	0.22	0.8	0.122
	p21	0.4	0.92		
4 h of radiation once a day (4h)	p53	2.0	1.28	1.3	0.079
	p21	1.5	0.99		
4 h of radiation twice a day (4h×2)	p53	1.2	0.47	1.2	0.685
	p21	1.0	0.74		

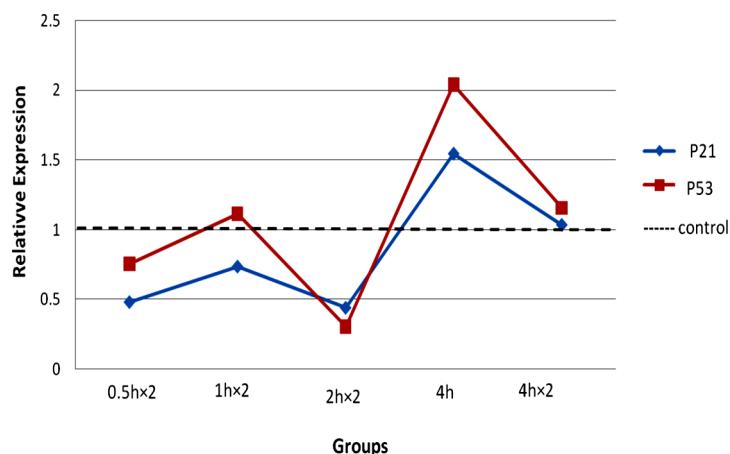


Figure 1. The graph shows the mean level expression of p53 and p21 in mice study groups.

various cell cycle regulatory and apoptosis-related genes have been shown in other Studies (20). In these studies the exposure duration was the primary reasons for the differences in regulatory gene expression level among study groups. However, the details of duration exposure effects were not fully explored yet (21).

The comparison between the groups in the current study indicated that consecutive 4 hours exposure once a day and successive 2 hours exposure twice a day with a time interval of 12 hours had different effects on mean expression level of p53 and p21 genes in hippocampus of Balb/c mice. This shows that when the total dose was split in two equal fractions with a time intervals of several hours the less enhancement of gene expression occurred. Also, expression of p53 and p21 genes increases in consecutive 4h and 4h×2 exposure and the peak expression of these genes for 4h successive exposure once a day has been more on the rise. In the main, fractionated radiation showed less responsiveness to electromagnetic radiation than the successive dose of radiation which can be explained in terms of affectability of cellular viability in response to the damage induced by increased duration of radiation time. Apparently, target repair mechanism occurs between two consecutive dose fractions of the radiation schedules.

The result show that bcl-2 expression is correlated with the expression of p53 but the basal level of p53 expression is higher than p21.

The ratio expression of p53 and p21 genes was increased to greater than one ($p53/p21 > 1$) in all experimental groups compared to control, except for the group with 2 hours twice a day exposure. Therefore, there is a relation between p53 level and expression of p21. It can be hypothesized that RF irradiation can induce p53, which results in enhanced p21 mRNA level and leading cells into cell cycle arrest. This allowing time for DNA repair and preventing serious damage to the cell and eventually its apoptosis by establishing balance between the expression of these two genes (12). However, more research is needed to justify the underlying stress-induced cellular behaviors.

Also, the expression of p53 and p21 genes shows variations according to the radiation time. For example, expression of these genes in 0.5h×2, 1h×2, and 2h×2 groups was unaffected or lower compared with normal control (figure 1). This observation can be related to the maturation of post-mitotic neurons and slow reactivity of neural cells to short duration of radiation (22) or the induction of adaptive response phenomena (23, 24). Although, the level of p53 and p21 mRNA expression is altered in exposed animals when compared with normal controls but the ratio of p53 and p21 expression levels has remained relatively constant in the five experimental groups. Given the ability of the cells to maintain p53 and p21 genes expression levels' ratio can decrease these harmful effects on the cells.

It appears that the duration and intensity of radiation, as used in this study, do not show an irreversible harmful effect on the cells but have had a cellular response against the radiation. Another point to be mentioned is that single successive exposure and fractionated radiations have different effects on cells. Most current published research findings did not indicate an increased risk of perceptible adverse biological effects related to short-term mobile phone use. However, some results indicate that using a cell phone for more than 10 years increased the risk of being diagnosed with a brain tumor^(25, 26).

In sum, it can be concluded that radiofrequency radiation of mobile jammer result in relative changes in the expression level of p53 and p21 genes in the hippocampus of mouse brain. Since, the possibility of p53 and p21 expression level imbalance could not be observed across sample groups, it is not likely that apoptosis occurs in the hippocampus of mouse brain. In all, there are limitations at any rate because gene expression measurements through real-time PCR are relative quantitative rather than absolute⁽²⁷⁾. Thus, it is necessary to repeat or continue the experiments in order to make more certain of the findings.

The potential ability of RF radiation to modify gene transcription and protein levels has been investigated in a variety of cellular and animal models. There have been conflicting results with respect to RF exposure and gene expression levels^(17, 28). However, the majority of research does not suggest a role for RF radiation in altered gene or protein expression but a number of studies exist, including current study, where RF field induced response on p53 and p21 regulatory gene, while conditions seem well controlled⁽³⁾. More studies are required to evaluate these observations further.

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