900 MHz Radiofrequency Radiation Has Potential to Increase the Expression of rno-miR-145-5p in Brain

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Abstract

Interaction between radiofrequency radiation (RFR) and miRNA which plays paramount role in growth, differentiation, proliferation, and cell death by suppressing one or more target genes, is still unknown. Therefore, the purpose of this study is to investigate the effects of long term 900 MHz mobile phone exposure on some of the miRNA in brain tissues (sham: 7; Exposure : 7)., which were kept in appropriate laboratory conditions for the second phase of our previous study [5]. It is remembered that rats in the exposure group were exposed to 900 MHz RFR for 3 h per day (7 days a week) for one year. For the aim of this study, expression of miRNAs such as rno-miR-22-3p, rno-miR-24-1-3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p and rno-miR-222-3p were investigated. Results indicated that long-term exposure of 900 MHz RF radiation increased only expression of rno-miR-145-5p (adj *P* * = 0.047) value where 1g average SAR value in brain was 0.198 W/kg. Our results indicated that chronic exposure of 900 MHz RFR has potential to increase expression of rno-miR-145-5p. Therefore, further studies are necessary to understand the relation between 900 MHz mobile phone exposure and diseases related to the expression of rno-miR-145-5p.

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Introduction

Very little is known about molecular mechanism of effects happening at non-thermal level of radiofrequency radiation (RFR). However, the International Agency for Research on Cancer in 2011 classified RFRs as possible carcinogen (2B) to humans, opening this field for further investigation.[1]

The discovery of microRNAs has revealed an unexpected and spectacular additional level of fine tuning of the genome, and how genes are used again and again in different combinations to generate the complexity that forms, for instance, the brain. Although, microRNAs are abundantly expressed in the brain, relatively little is known about the multiple functions of these RNA molecules in the nervous system. MicroRNA pathways play major roles in the proliferation, differentiation, function, and maintenance of neuronal cells. Several intriguing studies have linked microRNAs as major regulators of the neuronal phenotype, and have implicated specific microRNAs in the regulation of synapse formation and plasticity. Dysfunction of microRNA pathways is also slowly emerging as a potential important contributor to the pathogenesis of major neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease.[2,3] It is also stated that miRNA research has mainly focused on cancer.[3]

Based on very few studies, the interaction between non ionizing radiation and miRNAs has been usually focused on the effects of ultraviolet and RFR radiation. For instance, Zhou et al studied to assess the effects of ultraviolet B (UVB)

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irradiation on microRNA (miRNA) expression in normal human keratinocytes. They indicated that upon 30 or 60 mJ/cm2 of UVB radiation, the expression of 44 miRNAs was up or down regulated more than two fold compared with non-irradiated keratinocytes.[4] On the other hand, Dasdag et al reported that long term and excessive use of 900 MHz and 2.4 GHz RFR radiofrequency radiation altered microRNA expression in brain.[5,6] In one of Dasdag's previous works, which were a part of a project, the effects of 900 MHz RFR and 2.4 GHz RFR were studied for some miRNAs namely, rno-miR-9-5p, rno-miR-29a-3p, rnomiR-106b-5p, rno-miR-107 and rno-miR-125a-3p in brain. It is reported that one year of excessive 900 MHz RFR exposure only decreased rno-miR107 expression ($p < 0.05$).[5] In another study, the effects of one-year exposure of 2.4 GHz Wi-Fi on the same miRNAs were investigated.[6] The study also indicated that one-year exposure of 2.4 GHz Wi-Fi radiation altered expression of some miRNAs such as miR-106b-5p ($p = 0.010$) and miR-107 ($p = 0.005$). The results showed that miR-107 expression was 3.3 times and miR-106b-5p expression was 3.65 times lower in the exposure group than in the control group. Therefore, the aim of this study, which is a second phase of our previous study is to investigate the effects of long term exposure of 900 MHz mobile phone exposure on new miRNAs such as rno-miR-22-3p, rno-miR-24-1-3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p, rno-miR-222-3p.

Materials and Methods

Subjects

The brain tissues of fourteen Wistar Albino adult rats (Sham: 7; exposure: 7), which were kept in appropriate laboratory conditions for the second phase of our previous study [5] were studied to investigate the effects of 900 MHz RFR on ten miRNAs such as rno-miR-22-3p, rno-miR-24-1-3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p, rno-miR-222-3p. All procedures were in agreement with the Principles of Laboratory Animal Care and the rules of Scientific and Ethics Committee of Dicle University Health Research Center.

Exposure and field measurements

It is remembered that the rats in the experimental group were exposed to RF radiation 3 h per day (7 days a week) for 12 months. For the sham group, the rats were placed in the carousel and the same procedure was applied to the rats (3 h per day, 7 days a week for 12 months), except that the generator was turned off, i.e., no RF signal was present. The experimental set-up is illustrated in Figure 1. Detailed information on exposure conditions and measurements can be find in the study performed by Dasdag et al.[5]

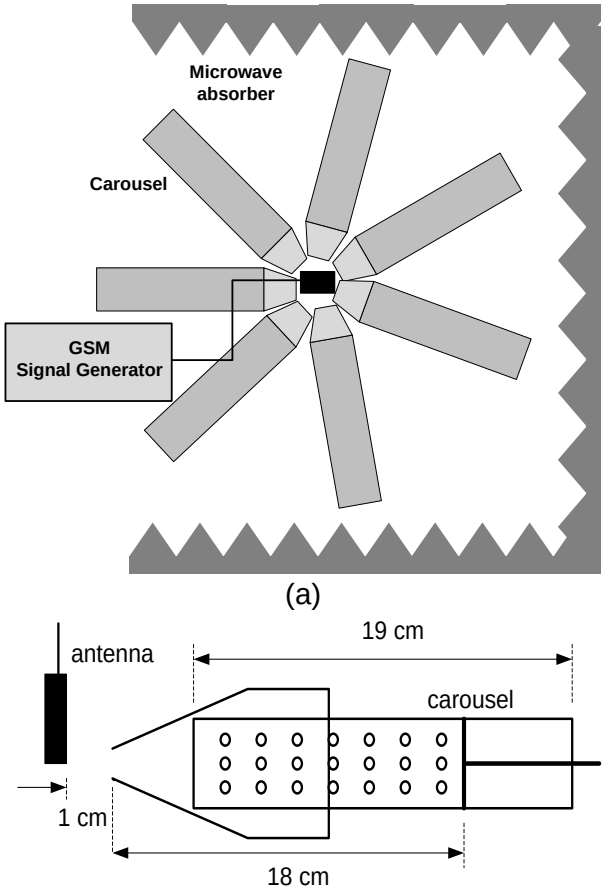

Figure 1. Experimental Setup, a) Top View, b) Side View for One Carousel.

Statistical Analysis

The data were processed and analyzed using the statistical package STATISTICA 13 for Windows. Normality assumption of $2^{-\Delta\Delta CT}$ values was checked by Shapiro Wilk test. Since the assumption of normality was met $2^{-\Delta\Delta CT}$ values were expressed as mean and standard deviation, and the comparisons between groups were

performed using independent t -test and the significance values were adjusted for multiplicity using Benjamini-Hochberg adjustment [7, 8]. Error-bar graph was used to represent data distribution of rno-miR-22-3p, rno-miR-24-1-3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p, rno-miR-222-3p variables according to the groups. Significant differences (two-tailed *P*) less than 0.05 were regarded as significant.

Results

The results of this study showed that the long term and excessive exposure of 900 MHz RF exposure (3 h per day, 7 days a week for one year) altered expression of some of the miRNA such as rno-miR-145-5p (adj *P** = 0.047). The results revealed that long term and excessive exposure of 900 MHz RF radiation only increased expression of rno-miR-145-5p. The results are summarized in Table I and Figure 2. Point, 1 g and 10 g average SAR level of brain were found as 0.169 W/kg, 0.0845 W/kg and 0.0543 W/kg, respectively (Figure 3). However, whole body SAR was found as 0.132 W/kg and 0.0719 W/kg for 1 g and 10 g, respectively. Average SAR values are fairly below the maximum values set by the regulations

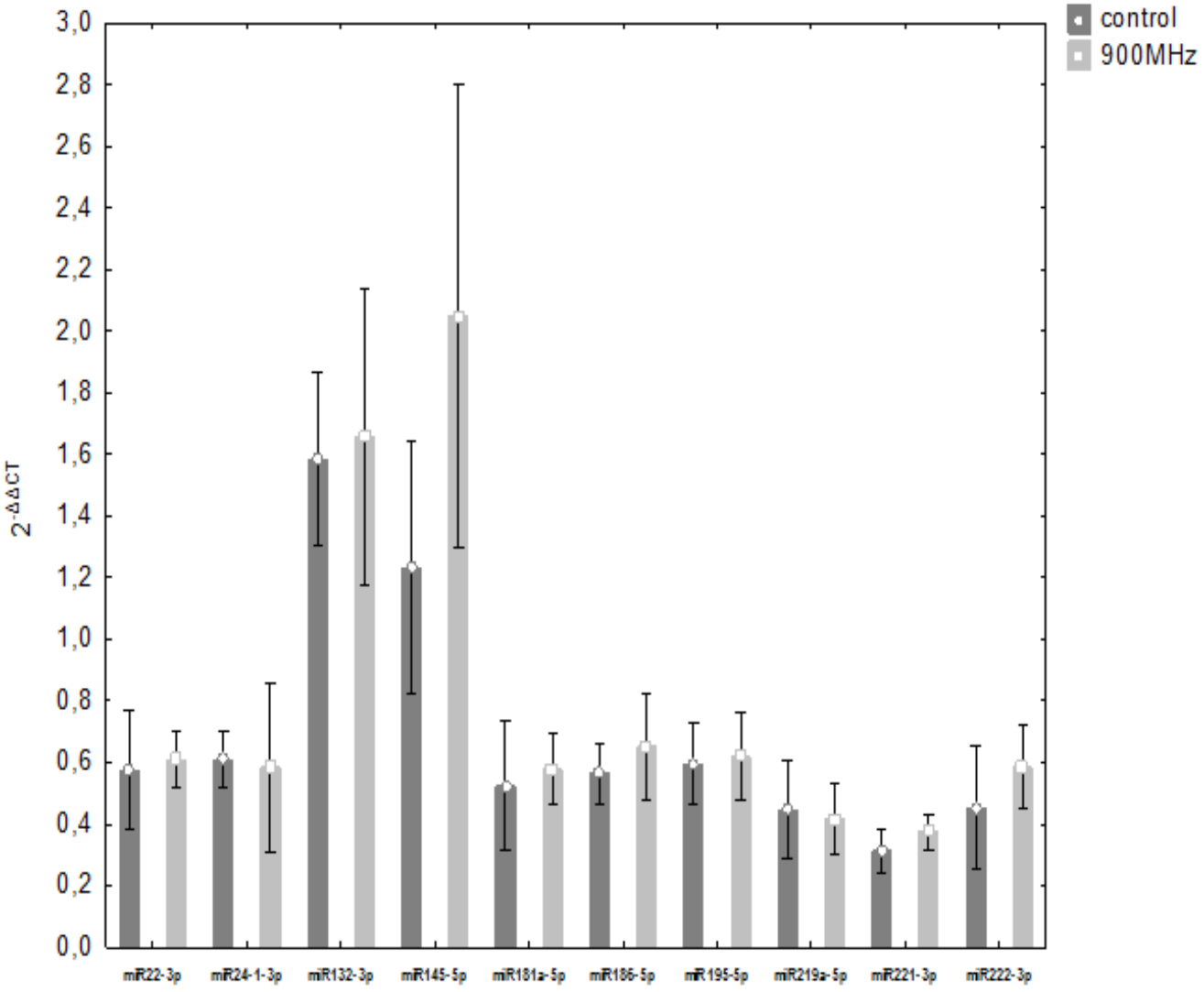

Figure 1. Experimental Setup, a) Top View, b) Side View for One Carousel.

miRNAs	Sham Exposed Group		900 MHz RF Exposed Group		*Adj* P*
	Mean	SD ±	Mean	SD ±	
rno-miR-22-3p	0,575314	0,185424	0,608482	0,097996	0,687742
rno-miR-24-1-3p	0,608638	0,087402	0,584419	0,296952	0,851475
rno-miR-132-3p	1,584311	0,269291	1,656696	0,518132	0,764442
rno-miR-145-5p	1,233200	0,392203	2,048840	0,812279	**0,047012**
rno-miR-181a-5p	0,525017	0,197910	0,577761	0,124870	0,570681
rno-miR-186-5p	0,564174	0,095195	0,651243	0,188452	0,329137
rno-miR-195-5p	0,594765	0,126773	0,617798	0,153168	0,775714
rno-miR-219a-5p	0,447435	0,151160	0,415886	0,125865	0,688888
rno-miR-221-3p	0,312749	0,070305	0,375875	0,061985	0,113057
rno-miR-222-3p	0,454360	0,193445	0,584528	0,146230	0,194477

Table 1. Statistical comparison of miRNA levels between the sham and exposure groups. *Benjamini-Hochberg adjusted p values.

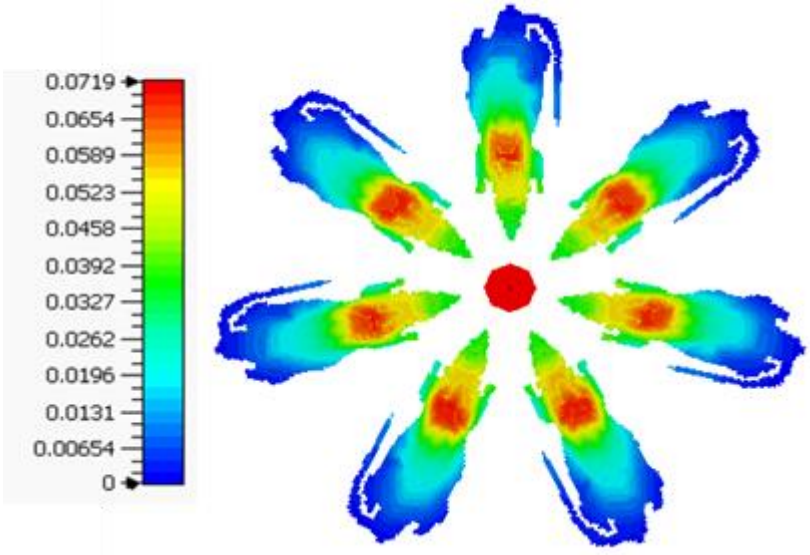

Figure 3. SAR Distribution (10 g average, W/kg) at 900 MHz (top view).

Discussion

Although many of the contradictive studies on health effects of mobile phone exposure exist, studies on the side-effects of long term and excessive use of mobile phones are still not available to date.[5,6,9-11] Due to concerns about possible health effects of radiofrequency radiation, International Agency for Research on Cancer (IARC) classified mobile phones as 2B at the end of contradictive discussions.[1] There is an unmet need to illuminate the health effects of long term and excessive use of mobile phones. Earlier, it was reported that 900 MHz radiation emitted from mobile phones had potential to alter some biomolecules in brain.[5,9,10,12] Taking this as the starting point, the effects of 900 MHz RF radiation on some microRNAs in the rats' brain, which is exposed 3 h per day (7 days/week) for one year are investigated in this study. For this purpose, a GSM signal generator, which produces 900 MHz band RF signal identical to the one in mobile phones was used for the exposure standardization in the study.

MicroRNAs are important regulators of gene expression that control both physiological

and pathological processes such as cancer development and incidence. However, it is reported a new relation between p53 (tumor suppressor gene) and miR-145 (suppressor of cell growth) up regulation.[13] However, Sachdeva and Mo defined microRNAs as a large group of negative gene regulators through a post-transcriptional suppression mechanism and stated that microRNAs play an important role in proliferation, differentiation, and apoptosis [14]. Qi et al. stated that miRNAs can regulate gene expression through either directly binding to or affect the translation process of some specific mRNAs and miRNAs, as an endogenous regulatory factor, is related to various diseases of ischemic injury, including miR-145-5p. MiR-145-5p abnormally expressed in heart tissues of myocardial infarction in rats.[5] They additionally reported that miR-145-5p has been found to be associated with endometriosis miR-145-5p which may serve as tumor suppressor to suppress epithelial-mesenchymal transition and invasiveness, and miR-145-5p down-regulation contributed to brain metastasis. They also informed that miR- 145 facilitated proliferation and migration of endothelial progenitor cells and recanalization of arterial thrombosis in cerebral infarction mice.[15]

In this study, long term effects of 900 MHz mobile phone exposure were investigated on ten different miRNAs; rno-miR-22-3p, rno-miR-24-1-3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p, rno-miR-222-3p, and it was observed that a statistically difference was only on the expression of rno-miR-145-5p. Therefore, our discussion will be focused on miR 145 especially on the rno-miR-145-5p. Foroutan et al. reported that "Human *miR-145* is broadly expressed in germ line and mesoderm-derived tissues such as the breast, ovaries, testes, uterus, prostate, heart, and spleen".[13] According to the statement of Foroutan et al., we can speculate that 900 MHz mobile phone exposure has the potential to affect the expression of rno-miR-145-5p in the germ line and mesoderm-derived tissues.[13] Sachdeva and Mo have also reported *miR- 145* mediated suppression of cell growth, invasion, and metastasis. Based on these findings, they proposed that as a tumor suppressor, *miR-145* might be a valuable biomarker for cancer diagnosis.[14] Starczynowski et al. reported that in various cancers, *miR-145* prevented tumor angiogenesis and metastasis by targeting c-Myc[16]. Galani et al. indicated that miRNA expression profiling of meningioma exhibited down regulation of miR-29c-3p, miR-200a, miR-145, and miR- 219-5p, and up regulation of miR-21, miR-335 and miR-190a levels[17].

In our study we also determined expression of rno-miR-219a-5p and rno-miR-145-5p and found that 900 MHz RFR increased the expression of rno-miR-145-5p only. Therefore, 900 MHz RFR should be accounted as a factor in certain cases. Gusar et al. stated that "the level of miRNA expression tended to increase 48 h after the onset of ischemia in brain tissue and leukocytes.[18] However, they also indicated that miRNAs were differentially expressed in the brain tissue and blood plasma of rats 24 h after exposure, among which miR-145-3p and miR-375-3p were down regulated and miR-19a-3p, miR-92a-3p, miR-188-5p, and miR-532-5p were up regulated.[18]

In our study, only rno-miR-145-5p expression tended to increase after one year of 900 MHz RFR exposure (3 h per day; 7 days/week). Xie et al. used a miRNA screening approach and identified miR-145-5p as a putative regulator of Nurr1, which is a member of the nuclear receptor 4 family of orphan nuclear receptors that is decreased in inflammatory responses and leads to neurons death in Parkinson's disease.[19] They also stated that miR-145-5p has been shown to be up-regulated in the pathological process of vascular neointimal lesion formation, cardiomyocyte survival, and H_2O_2-induced neuronal injury and their study identified and confirmed a novel regulation of Nurr1 by miR-145-5p in both in vitro and in vivo cerebral ischemic conditions.[19] According to the results of our study, it can be stated that long-term and excessive exposure of 900 MHz can result alteration of miR-145-5p expression. Therefore, increase of miR-145-5p expression by 900 MHz would be important for the problem indicated by Xie et al., Donzeli et al. stated that "Brain metastasis is a major cause of morbidity and mortality of lung cancer patients and restored expression of miR-145-5p and selective depletion of individual targets markedly reduced *in vitro* and *in vivo* cancer cell migration".[19,20] They also concluded that their results, attributed to miR-145-5p and its direct targets, play pivotal roles in malignancy progression and in

metastasis.[20] The results of Donzelli et al. indicated very important role of miR-145-5p in terms of metastasis [20]. Therefore, more attention should be paid to the results of 900 MHz exposure on miR-145-5p. Thuringer et al. recently reported that miR-145-5p is anti-tumor and reduces glioma growth.[21] Therefore, our result, which indicates a relation between 900 MHz RFR and miR-145-5p, is important in this regard. It was also shown that 900 MHz RFR only decreased rno-miR107 in brain and this result could lead to adverse effects.[5] In the another study, it was observed that rats exposed to 2.4 GHz RFR emitted from Wi-Fi for 24 hours a day for one year led to mir 107 expression of 3.3 times and miR- 106b-5p expression of 3.65 times lower in the exposure group than in the sham group. However, it was concluded that long-term exposure of 2.4 GHz RF could lead to adverse effects such as neurodegenerative diseases originated from the alteration of some miRNA expression and more studies should be devoted to the effects of RF radiation on miRNA expression levels.[6] The results of these two earlier studies support the results of this study where we observed that 900 MHz RFR affected the expression of miR-145-5p. According to the results of the previous studies performed by us and other research groups, we can speculate that 900 MHz RFR has the potential to alter expression of some of miRNAs.

Conclusion

The results of this study indicated that long term and excessive exposure of 900 MHz RFR increased rno-miR-145-5p. The studies performed in this field also indicated that 900 MHz RFR decreased rno-miR107, and 2.4 GHz RFR altered the expression of some of the miRNAs such as rno-miR-106b-and rno-miR-107. Therefore, it can be stated that long term and excessive exposure of RF radiation emitted from mobile phones can be associated with prognoses of some diseases. Further investigation on the effects of RFR and biological aspects of miRNAs in brain can provide an explanation to understand the pathogenesis of some diseases.

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Declaration of Interest

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

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