

# Adaptive Response Induced by Pre-Exposure to 915 MHz Radiofrequency: A Possible Role for Antioxidant Enzyme Activity

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## ABSTRACT

**Background:** Over the past few years, the rapid use of high frequency electromagnetic fields like mobile phones has raised global concerns about the negative health effects of its use. Adaptive response is the ability of a cell or tissue to better resist stress damage by prior exposure to a lesser amount of stress. This study aimed to assess whether radiofrequency radiation can induce adaptive response by changing the antioxidant balance.

**Materials and Methods:** In order to assess RF-induced adaptive response in tissues, we evaluated the level of GSH and the activity of GR in liver. 50 rats were divided into 5 groups. Three groups were pre-exposed to 915 MHz RF radiation, 4 hours per day for one week at different powers, as low, medium and high. 24 hours after the last exposure to radiation, they were exposed to 4 Gy sublethal dose of gamma radiation and then sacrificed after 5 hours. Their livers were removed, washed and were kept at -80° C until used.

**Results:** Our finding showed that pre-exposure to 915 MHz radiofrequency radiation with specific power could induce adaptive response in liver by inducing changes in the activity and level of antioxidant enzymes.

**Conclusion:** It can be concluded that pre-exposure to microwave radiation could increase the level of GSH and the activity of GR enzyme, although these increases were seen just in low power group, and the GR activity was indicated in medium power group. This increase protects tissue from oxidative damage induced by sublethal dose of gamma radiation.

## Keywords

Adaptive Response, RF Radiation, Antioxidant Enzymes, Glutathione Reductase (GR), Reduced Glutathione (GSH)

## Introduction

There has been an increasing concern about the possibility of negative health effects due to the exposure to radiofrequency radiations (RFR), like those radiations emitted by wireless communication devices [1]. The number of people who use wireless communication services (handheld mobile phones, as well as the newer personal communication devices that deliver voice, data and images) has been growing rapidly [2]. There are some conflicting reports about the ability of high frequency electromagnetic fields (EMFs), to induce oxidative stress because of the increasing level of Reactive oxygen spe-

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Received: 5 November 2016  
Accepted: 20 December 2016

cies, ROS, [3]. ROSEs are free radicals that are derived from oxygen metabolism [4]. Several research groups have found no effects on ROS production from RF radiation at specific absorption rate, SAR, values ranging between 0.3 and 2.0 W/kg [5-8]. Recently, some reports have shown that exposure to RF radiation enhanced intracellular ROS production at SAR values equal to or exceeding 2 W/Kg [9]. Growing production of ROS has also been reported in rat lymphocytes co-exposed to RF radiation and ferrous chloride (FeCl<sub>2</sub>) but not in RF radiation exposure alone [10]. Antioxidants are the first line of defense against free radical damage, and are essential to maintain perfect health and well-being [11, 12]. In the field of radiation biology, an adaptive response is classically expressed as the induction by a low dose of radiation of a protective effect against a high challenge dose [13]. The adaptive response, AR, to radiation was first described in 1984 by Olivieri *et al.* who reported that peripheral blood lymphocytes cultured in 3H-thymidine exhibited a reduced frequency of chromosome aberrations following a challenge with an acute moderate dose of X-rays [14]. This effect has been observed for many endpoints including chromosome aberration induction, mutation induction, neoplastic transformation, cell survival, fetal death, fetal abnormalities and tumor latency [13]. The phenomenon (AR) in animal and human cells or organs exposed to ionizing radiation is well documented in scientific literature [15]. On the contrary, there are a few articles that demonstrate this effect in non-ionizing radiation [16]. The first article about the non-ionizing radiation induced AR was published in 2009 by Anna Sannino. The authors revealed that pre-exposure of peripheral blood lymphocyte collected from human volunteers to non-ionizing RF radiation (900MHz, at peak SAR of 10 W/kg for 20 hours) increased their resistance to a challenging dose of Mitomycin C (100 ng/ml at 48 h) [17]. They conducted another research in 2011 and evaluated the ef-

fect of RF radiation-induced AR on cell cycle. This research showed that the cells revealed AR just in S phase [18]. Also in the same year, Mortazavi *et al.* did a comparative study on the increased radio resistance to lethal dose of gamma rays after exposure to microwave radiation and oral intake of flaxseed oil. Results demonstrated that pre-exposure to microwave radiation could increase the survival fraction to 100% [19]. There are some other research studies on this effect with different endpoints [20], like primary DNA damage [15], frequency of micronuclei in human lymphocyte [21]. To the best of our knowledge, the present study is the first investigation on the microwave-induced AR by evaluating the level of antioxidant enzyme.

## Material and Methods

### Animals

Fifty male adult Sprague Dawley rats (180-230 g, 2-month old) obtained from the animal house of Shiraz University of Medical Sciences (SUMS) were randomly divided into 5 groups of 10 animals. The animals were kept in special cages with controlled temperature, humidity and lightning in the animal house and fed with standard pellet and water. All laboratory animals used in this study, received human care in compliance with the SUMS regulation on animal care. The present study was approved by SUMS ethics committee.

### Grouping

Fifty animals were divided into 5 groups in which 3 adaptive groups that received microwave exposure with different powers, low power (0.25 W), medium power (0.79 W) and high power (1.58 W), 4 hours per day for one week. These groups were exposed to 4 Gy gamma radiation with the dose-rate of 23.5 cGy/min 24 hours later. Sham group was just placed in restrainers 4 hours per day for one week and then exposed to 4 Gy gamma irradiation. The fifth group was control in which

rats were placed in restrainers 4 hours per day for one week with no irradiation.

### RF Exposure

A Global System for Mobile communications, GSM, mobile simulator (made at the School of Engineering, Shiraz University) was used for microwave irradiation. The frequency of the simulator was adjustable between 800 to 1000 MHz. This research was done at frequency of 915 MHz and signal bandwidth of 200 KHz like GSM mobile phone channels. The power was adjustable from zero to over 2 Watts. All powers were measured at 915 MHz. The output microwave power generated by the signal generator, delivered to the dipole antenna. The antenna propagated the electromagnetic wave to the space omnidirectionally. We used Ferris wheel irradiation system to obtain uniform RF exposure to all ten rats in each group.

### Gamma Irradiation

Twenty four hours after a week of RF irradiation, on 8<sup>th</sup> day, animals were whole-body irradiated with 4 Gy sublethal dose of gamma radiation emitted by a therapeutic CO<sup>60</sup> source (100 cm SSD, 35×35 cm<sup>2</sup> field, 17 min irradiation time). All animals in each group were irradiated at one time.

### Tissue Preparation

Five hours after finishing gamma irradiation, rats were sacrificed. Liver tissue was taken out, washed with the Saline, homogenized and the supernatants were collected for biochemical studies.

### Biochemical Study

#### Determination of Reduced Glutathione

The assay of GSH with DTNB was performed followed by a standard Ellman's method [22]. Standard curves were made from 1mM to 0.1mM solutions of GSH. 200  $\lambda$  of sample supernatant, 2300  $\lambda$  of PBS and 500

$\lambda$  of DTNB were added, vortexed and put for 5 min in water-bath 37 °C. OD in 412 nm was measured spectrophotometrically.

#### Determination of Glutathione Reductase (GR) Activity

GR activity was estimated by oxidation of NADPH reduction in absorption in 340 nm by the procedure of Carlberg and Mannervik [23, 24]. GR assay was performed in a cuvette in a total volume of 640  $\mu$ l that contained 340  $\mu$ l buffer, 40  $\mu$ l albumin, 80  $\mu$ l GSSG, 100  $\mu$ l sample and 80  $\mu$ l NADPH. The decrease in absorbance, which reflects the oxidation of NADPH during the reduction of GSSG by GR present in the sample, was monitored spectrophotometrically at 340 nm. Results were based on a molar extinction coefficient for NADPH of  $6.22 \times 10^3 \text{M}^{-1} \text{cm}^{-1}$ . The GR enzyme activity in the liver supernatants was expressed as unit/gr tissue protein.

### Statistical Analysis

The difference between the level of GSH and the GR activity in the liver of 3 adaptive groups and sublethal group was assessed. All data were analyzed by Independent Samples T-Test, and then were expressed as mean  $\pm$  S.D (Table 1).  $P < 0.05$  was considered as significant (Table 2). SPSS 19 software was used for data analysis.

### Results

The level of GSH (nmol/ml) and the activity of GR (unit/gr protein) are shown in Table 1. Fifty animals in 5 groups (Low Power + Sublethal dose of Gamma, Medium Power + Sublethal dose of Gamma, High Power + Sublethal dose of Gamma, Sublethal dose of Gamma and the controls) were examined in which 3 adaptive groups receiving microwave exposure with different powers, low power (0.25 W), medium power (0.79 W) and high power (1.58 W), 4 hours per day for one week. These groups were exposed to 4 Gy gamma radiation with the dose-rate of 23.5 cGy/min 24 hours later. Sham group was just placed in restrain-

**Table 1:** Level of reduced glutathione, GSH, and the activity of glutathione reductase, GR, in rat liver tissues. Data are expressed as mean±SD. P<0.05 was considered as significant.

Groups	No.	GSH level in liver (nmol/ml)	GR activity in liver (Unit/gr protein)
Low Power + Sublethal Dose of Gamma	10	80.13±13.33	33.13±6.10
Medium Power + Sublethal Dose of Gamma	10	59.08±3.61	19.77±0.93
High Power + Sublethal Dose of Gamma	10	59.71±7.39	26.05±1.96
Control group	10	76.6±10.21	32.49±3.24
Sublethal Dose of Gamma	10	53.31±4.66	25.50±2.39

**Table 2:** The significance of the differences (P-value) in the GSH level and the GR activity in adaptive groups and sublethal group and the difference between control and sublethal group.

Groups	Significance (P-value)	
	GSH level in liver (nmol/ml)	GR activity in liver (Unit/gr protein)
Low Power + Sublethal Dose & Sublethal Dose of Gamma	0.003*	0.03*
Medium Power + Sublethal Dose & Sublethal Dose of Gamma	0.06	0.001*
High Power + Sublethal Dose & Sublethal Dose of Gamma	0.14	0.7
Control & Sublethal Dose of Gamma	0.004*	0.005*

ers 4 hours per day for one week and then exposed to 4 Gy gamma irradiation. The fifth group was control in which rats were placed in restrainers 4 hours per day for one week with no irradiation.

The significance of differences at GSH level and the GR activity in both adaptive and sublethal groups are shown in Table 2. Statistically significant differences at the level of GSH and the activity of GR in low power intensity group and in the GR activity of medium power group were seen. While these differences at the GSH level of medium power group and both at the level of GSH and the activity of GR in high power intensity group were not significant as indicated in Table 2.

## Discussion

The current study was designed to elucidate if pre-exposure to RF radiation could induce AR by analyzing two antioxidant enzymes

related to glutathione, GR and GSH in liver tissue of rats. The level of GSH and the activity of GR significantly decreased in sublethal group. These probably reflect the increase oxidative stress due to either an increase in the production of free radicals or a decrease in the antioxidant defense systems or both. Pre-exposure to RF radiations increase the level of GSH and the activity of GR in other groups except the activity of GR in medium power group. But this increase was just significant in low power group. This means that pre-exposure to low power high frequency radiations may induce AR in liver. It seems that the irradiation of radio waves results in activating the mechanisms or producing some factors in the tissue. It makes the tissue ready to encounter the challenge dose. For example, preliminary reduction in the level of GSH and the activity of GR in liver tissue leads to increase the immune system to encounter these decreases.

To combat oxidative conditions, the cells are provided with several antioxidants such as GSH which is a major and strong intracellular antioxidant. GSH acts as a donor of electron to free radicals, giving rise to GSSG which then reduces and recycles into the GSH through enzymatic reactions [25]. As a major component of the cellular antioxidant system, GSH has the following characteristics: (a) GSH in diet can be partly absorbed from the small intestine and can be synthesized de novo, so that GSH is an exogenous and endogenous antioxidant; (b) although GSH radical (GS $\cdot$ ) formed from the oxidation of GSH is a pro-oxidant radical, GS $\cdot$  combines with each other to yield GS-SG, which is then reduced to GSH by the NADPH-dependent GR; (c) GSH can react with a variety of xenobiotic electrophilic compounds in the catalytic reaction of glutathione-S-transferase; (d) GSH effectively scavenges ROS (e.g., lipid peroxy radical, peroxy nitrite and H<sub>2</sub>O<sub>2</sub>) directly and indirectly through enzymatic reactions [26, 27]. The first article about the RF-induced AR was published in 2009 by Anna Sannino. He used Mitomycin C as a challenge dose and could see AR in cells pre-exposed to RF radiation [17]. Sannino also conducted a study to indicate the effect of cell cycle on AR. The results showed that AR induced just in S phase of cell cycle [18]. In spite of these articles there are some studies that showed reverse results, as the research that was done on RF radiation induced AR by induction of primary DNA damages. The authors could not see any significant differences among groups [15]. In the present study, the AR was seen, although it was produced just by low power. There are some factors that may affect the results of the study, such as tissue types, frequency, power and SAR of RF radiation, the time interval between adaptive dose of RF radiation and challenge dose. The results of this study show that the alteration in antioxidant level and the activity made by microwave radiation could induce AR. Despite previous studies, our knowledge on RF-

induced AR is still inadequate and more investigations are needed to be done to eliminate the debates about this effect.

### Acknowledgment

This study, as a part of MSc student thesis Ms. Mehrdokht Daneshmand, was supported by grant No. 91-6234 from the office of Vice Chancellor for Research and the Center for Research on Protection against Ionizing and Non-Ionizing Radiation and Recombinant Protein Laboratory, School of Advanced Medical Sciences and Technologies for the financial assistance.

### Conflict of Interest

None Declared.

### References

1. Verschaeve L, Juutilainen J, Lagroye I, Miyakoshi J, Saunders R, de Seze R, et al. In vitro and in vivo genotoxicity of radiofrequency fields. *Mutat Res*. 2010;**705**(3):252-68. doi.org/10.1016/j.mrrev.2010.10.001. PubMed PMID: 20955816.
2. Komatsubara Y, Hirose H, Sakurai T, Koyama S, Suzuki Y, Taki M, et al. Effect of high-frequency electromagnetic fields with a wide range of SARs on chromosomal aberrations in murine m5S cells. *Mutat Res*. 2005;**587**(1-2):114-9. doi.org/10.1016/j.mrgentox.2005.08.010. PubMed PMID: 16202641.
3. Luukkonen J, Hakulinen P, Maki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872 MHz radiofrequency radiation. *Mutat Res*. 2009;**662**(1-2):54-8. doi.org/10.1016/j.mrfmm.2008.12.005. PubMed PMID: 19135463.
4. Kesari KK, Kumar S, Behari J. 900-MHz microwave radiation promotes oxidation in rat brain. *Electromagn Biol Med*. 2011;**30**(4):219-34. doi.org/10.3109/15368378.2011.587930. PubMed PMID: 22047460.
5. Lantow M, Schuderer J, Hartwig C, Simko M. Free radical release and HSP70 expression in two human immune-relevant cell lines after exposure to 1800 MHz radiofrequency radiation. *Radiat Res*. 2006;**165**(1):88-94. doi.org/10.1667/RR3476.1. PubMed PMID: 16392966.
6. Lantow M, Viergutz T, Weiss DG, Simko M. Comparative study of cell cycle kinetics and induction of apoptosis or necrosis after exposure of human Mono Mac 6 cells to radiofrequency radiation. *Radiat Res*. 2006;**166**(3):539-43. doi.org/10.1667/RR3601.1. PubMed PMID: 16953672.

7. Simko M, Hartwig C, Lantow M, Lupke M, Mattsson MO, Rahman Q, et al. Hsp70 expression and free radical release after exposure to non-thermal radio-frequency electromagnetic fields and ultra-fine particles in human Mono Mac 6 cells. *Toxicol Lett.* 2006;**161**(1):73-82. doi.org/10.1016/j.toxlet.2005.08.005. PubMed PMID: 16153791.
8. Zeni O, Di Pietro R, d'Ambrosio G, Massa R, Capri M, Naarala J, et al. Formation of reactive oxygen species in L929 cells after exposure to 900 MHz RF radiation with and without co-exposure to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. *Radiat Res.* 2007;**167**(3):306-11. doi.org/10.1667/RR0595.1. PubMed PMID: 17316071.
9. Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, et al. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. *Mol Vis.* 2008;**14**:964-9. PubMed PMID: 18509546. PubMed PMID: 2391079.
10. Zmyslony M, Politanski P, Rajkowska E, Szymczak W, Jajte J. Acute exposure to 930 MHz CW electromagnetic radiation in vitro affects reactive oxygen species level in rat lymphocytes treated by iron ions. *Bioelectromagnetics.* 2004;**25**(5):324-8. doi.org/10.1002/bem.10191. PubMed PMID: 15197754.
11. Percival M. Antioxidants clinical nutrition insights. New York: Advanced Nutrition Publications; 1998.
12. Thomas S, Senthilkumar GP, Sivaraman K, Bobby Z, Paneerselvam S, Harichandrakumar KT. Effect of s-methyl-L-cysteine on oxidative stress, inflammation and insulin resistance in male wistar rats fed with high fructose diet. *Iran J Med Sci.* 2015;**40**(1):45-50. PubMed PMID: 25650289. PubMed PMID: 4300480.
13. Ko M, Lao XY, Kapadia R, Elmore E, Redpath JL. Neoplastic transformation in vitro by low doses of ionizing radiation: role of adaptive response and bystander effects. *Mutat Res.* 2006;**597**(1-2):11-7. doi.org/10.1016/j.mrfmmm.2005.08.013. PubMed PMID: 16414089.
14. Olivieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science.* 1984;**223**(4636):594-7. doi.org/10.1126/science.6695170. PubMed PMID: 6695170.
15. Jiang B, Nie J, Zhou Z, Zhang J, Tong J, Cao Y. Adaptive response in mice exposed to 900 MHz radiofrequency fields: primary DNA damage. *PLoS One.* 2012;**7**(2):e32040. doi.org/10.1371/journal.pone.0032040. PubMed PMID: 22389679. PubMed PMID: 3289639.
16. Vijayalaxmi, Cao Y, Scarfi MR. Adaptive response in mammalian cells exposed to non-ionizing radio-frequency fields: A review and gaps in knowledge. *Mutat Res Rev Mutat Res.* 2014. doi.org/10.1016/j.mrrrev.2014.02.002. PubMed PMID: 24548818.
17. Sannino A, Sarti M, Reddy SB, Prihoda TJ, Vijayalaxmi, Scarfi MR. Induction of adaptive response in human blood lymphocytes exposed to radiofrequency radiation. *Radiat Res.* 2009;**171**(6):735-42. doi.org/10.1667/RR1687.1. PubMed PMID: 19580480.
18. Sannino A, Zeni O, Sarti M, Romeo S, Reddy SB, Belisario MA, et al. Induction of adaptive response in human blood lymphocytes exposed to 900 MHz radiofrequency fields: influence of cell cycle. *Int J Radiat Biol.* 2011;**87**(9):993-9. doi.org/10.3109/09553002.2011.574779. PubMed PMID: 21557704.
19. Mortazavi S, Mosleh-Shirazi M, Tavassoli A, Taheri M, Bagheri Z, Ghalandari R, et al. A comparative study on the increased radioresistance to lethal doses of gamma rays after exposure to microwave radiation and oral intake of flaxseed oil. *Iranian Journal of Radiation Research.* 2011;**9**(1):9-14.
20. Mortazavi S, Motamedifar M, Mehdizadeh A, Namdari G, Taheri M. The Effect of Pre-exposure to Radiofrequency Radiations Emitted from a GSM Mobile Phone on the Susceptibility of BALB/c Mice to Escherichia coli. *Journal of Biomedical Physics and Engineering.* 2012;**2**(4 Dec).
21. Jiang B, Zong C, Zhao H, Ji Y, Tong J, Cao Y. Induction of adaptive response in mice exposed to 900MHz radiofrequency fields: application of micronucleus assay. *Mutat Res.* 2013;**751**(2):127-9. doi.org/10.1016/j.mrgentox.2012.12.003. PubMed PMID: 23295244.
22. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959;**82**(1):70-7. doi.org/10.1016/0003-9861(59)90090-6. PubMed PMID: 13650640.
23. Shtukmaster S, Ljubuncic P, Bomzon A. The Effect of an Aqueous Extract of Teucrium polium on Glutathione Homeostasis In Vitro: A Possible Mechanism of Its Hepatoprotectant Action. *Adv Pharmacol Sci.* 2010;**2010**:938324. PubMed PMID: 21188245. PubMed PMID: 3005810.
24. Hajizadeh MR, Eftekhari E, Zal F, Jafarian A, Mostafavi-Pour Z. Mulberry leaf extract attenuates oxidative stress-mediated testosterone depletion in streptozotocin-induced diabetic rats. *Iran J Med Sci.* 2014;**39**(2):123-9. PubMed PMID: 24644381. PubMed PMID: 3957011.
25. Mashhoody T, Rastegar K, Zal F. Perindopril may improve the hippocampal reduced glutathione content in rats. *Adv Pharm Bull.* 2014;**4**(2):155-9. PubMed PMID: 24511479. PubMed PMID: 3915815.
26. Zal F, Mostafavi-Pour Z, Amini F, Heidari A. Effect of vitamin E and C supplements on lipid peroxidation and GSH-dependent antioxidant enzyme status in the blood of women consuming oral contraceptives. *Contraception.* 2012;**86**(1):62-6. doi.org/10.1016/j.contraception.2011.11.006. PubMed PMID: 22494786.
27. Ahmadi-Ashtiani HR, Allameh A, Rastegar H, Mortaz E, Saraf Z. Immunoregulatory effects of glutathione during mesenchymal stem cell differentiation to hepatocyte-like cells. *Iran J Immunol.* 2012;**9**(3):175-87. PubMed PMID: 23023382.