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The Prophylactic Effect of Vitamin C on Oxidative Stress Indexes Following Exposure to Radio Frequency Wave Generated by a BTS Antenna Model in Rat Liver and Kidney

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Abstract

***Background*:** Radio frequency wave (RFW) generated by base transceiver station (BTS) has been reported to make deleterious effects on liver and kidney, possibly through oxidative stress. This study was conducted to evaluate the effect of radiofrequency wave (RFW)-induced oxidative stress in the liver and kidney and the prophylactic effect of vitamin C on this organs by measuring the antioxidant enzymes activity including: glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and malondialdehyde (MDA).

***Materials and Methods*:** In this experimental study, thirty-two adult male Sprague-Dawley rats were randomly divided into four experimental groups and treated daily for 45 days as follows: control, vitamin C (L-ascorbic acid 200 mg/kg of body weight/day by gavage), test (exposed to 900MHz RFW) and the treated group (received vitamin C in addition to exposure to RFW). At the end of the experiment all animals were sacrificed and their liver and kidney were removed and were used for measurement of antioxidant enzymes and MDA activity.

***Results*:** The results indicate that exposure to RFW in the test group decreased antioxidant enzymes activity and increased MDA compared with the control groups ($p<0.05$). In the treated group vitamin C improved antioxidant enzymes activity and reduced MDA compared to the test group ($p<0.05$).

***Conclusion*:** It can be concluded that RFW causes oxidative stress in liver and kidney, and vitamin C improves the antioxidant enzymes activity and decreases MDA.

Introduction

High-frequency electromagnetic fields (EMF) are produced by a variety of different sources such as radar installations, radio and television transmitters, medical microwave diathermy devices, and domestic use of microwave ovens. However, expanding telecommunication technologies have become by far the most important source of non-ionizing high-frequency EMF. A base transceiver station (BTS) is a technical apparatus which includes the antennas and their transmitter/receiver blocks, and manages the area corresponding to a geographical cell. Exposure to radio frequency fields emitted by mobile telephones (which operate between 400 and 2,000 MHz frequency bands) and their base stations has caused public concern regarding the possible adverse effect on human health. In previous investigations it was shown that non-thermal microwave exposure is able to induce several changes at the level of DNA and protein molecules [1], alter heat shock proteins [2], and increase permeability in the blood-brain barrier [3]. Reports of potential adverse effects of RFW on the brain [4], eye [5], testis [6], epididymal sperm [7] hematological parameters and bone marrow [8] and endocrine system [9] in human and animals are widely documented in the literature. Liver and kidney damage have been observed as well [10-13]. The liver and kidney are specific organs because of their continuous exposure to chemicals, drugs, and other metabolites and responsible for clearance those. It is also known that oxidative stress accelerate the aging process in human tissue, and promote adult chronic diseases, cancer, diabetes and age-related macular degeneration [14, 15]. L-ascorbic acid is a six-carbon lactone ring structure with 2, 3-enediol moiety. The antioxidant activity of ascorbic acid comes from 2, 3-enediol [16]. Vitamin C is a powerful antioxidant, acts outside and within the cell, and provides a protective effect against several diseases including oxidative imbalances arising from various causes in the kidney and other tissues [5, 6, 11, 17]. Vitamin C also acts as a pro-oxidant, depending on the environment in which the molecule is present. Intracellular vitamin C concentrations in the low millimolar range (much higher than that in plasma) seem to be necessary to support its role as an antioxidant. The aim of the present study was to investigate the effects of 900 MHz radiofrequency waves on antioxidant enzymes activity and malondialdehyde (MDA) levels in the rat liver and kidney, and to evaluate the protective effects of vitamin C in theses tissues.

Materials and Methods

Animal Experiments: All investigations were conducted in accordance with the "Guiding Principles for the Care and Use of Research Animals" approved by Shiraz University. Thirty-two adult male Sprague-Dawley rats (220±15 g) colony-bred in the Animal House Center, Shiraz, Iran were housed (eight rats per cage) in the animal room under controlled lighting (12 h light: 12 h darkness) and temperature (20±2ºC) conditions and had free access to a pelleted food (formulated and made by Javaneh Khorasan Company, Iran) and tap water. All of the experimental procedures were carried out between 09:00 am and 13:00 pm.

Radio frequency signal generator: The signal generator for producing a 900 MHz signal was made in the Department of Electrical Engineering of Shiraz University, and the output was monitored by a spectrum analyzer (FSH6, from Rohde and Schwarz, Germany) to ensure the correct forward power from the custom-designed mobile base stations on the animals exposed. The power of the BTS antenna at minimum distance from their installation site to a citizen's residence (17 m) was measured via a probe connected to the spectrum analyzer. The power level reading was 75 db. Based on our calculation and evaluation, the signal generator could radiate the same power level at a 5m distance. Hence, the test group and treated group were placed 5m from the signal generator.

Experimental design: The effect of radiofrequency wave (900 MHz) (Power density 0.6789 mW/cm^2) on the oxidative stress biomarkers in the liver and kidney of male rat and the role of vitamin C were studied by dividing the animals into four groups, each cage included eight animals and was treated orally as follows:

Group 1: The control group; Group 2: The control-vitamin C group, received vitamin C (200 mg/kg body weight/day) orally by gavage [5, 6]; Group 3: The test group was exposed to radiofrequency wave of 900 MHz; Group 4: As the treated group, received vitamin C orally by gavage (200 mg/kg body weight/day) before exposure to RFW of 900 MHz.

Animals were exposed to the RFW 4 h/day (between 9:00 am and 13:00 pm) during a period of 45 consecutive days. The controls rats were placed in the same conditions without applying the RFW.

Sampling and tissue preparation for enzyme assay: The animals were sacrificed after duration of treatment. Liver and right kidney were quickly removed and carefully dissected from the surrounding fat and tissue and immediately rinsed in ice saline. The tissue was rapidly homogenized manually in cold phosphate buffer (pH=7.4) and debris removed by centrifugation at 2750 *g* for 15 min. The upper clear supernatants were then recovered and stored at -70ºC for later enzymes activity and protein assays.

Measurement of superoxide dismutase (SOD) activity: Total SOD activity was evaluated with SOD detection kit (Ransod kit produced by Randox Co.) according to the manufacturer's instructions. The role of SOD is to accelerate the dismutation of the toxic superoxide produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes 50% inhibition of the rate of reduction of INT under the conditions of the assay. SOD levels were recorded at 505 nm and through a standard curve, and expressed as unit per mg of tissue protein (U/mg protein).

Measurement of glutathione peroxidase (GPx) activity: The activity of GPx was evaluated with GPx detection kit (Ransel kit produced of Randox Co.) according to the manufacturer's instructions. GPx catalyze the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm against blank was measured spectrophotometrically. One unit (U) of GPx activity was defined as the amount of enzyme that converts 1 µmol of NADPH to NADP+ per minute. The GPx activity was expressed as unit per mg of tissue protein (U/mg protein).

Measurement of Catalase (CAT) activity: Tissue catalase activity was assayed spectrophotometrically by monitoring the decomposition of H_2O_2 using the procedure of [18] Aebi (1984). Briefly, 0.5 mL of 30 mmol/l H_2O_2 solution in 50 mmol/l phosphate buffer (pH=7.0), 1 ml of 1:10 diluted tissue supernatant was added and the consumption of H_2O_2 was followed spectrophotometrically at 240 nm for 2 min at 25ºC. The molar extinction coefficient was 43.6 l/mol per cm for H_2O_2. Catalase activity was expressed as the unit that is defined as µmol H_2O_2 consumed/min per mg tissue protein.

Measurement of lipid peroxidation (MDA): To evaluate lipid peroxidation in liver and kidney a modified HPLC method was used which is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a coloured MDA-TBA adduct [19]. Briefly, 0.5 mL tissue supernatant was added to 2 mL TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25 mol/L HCl the mixture was immediately heated (60 min at 95ºC) and cooled with running water and thereafter butanol-pyridine (15:1, v/v) (1 ml) was added and the final volume was adjusted to 2 mL with distilled water. After vigorous mixing, the organic layer was separated by centrifugation (16000 g, 3 minutes, at room temperature). The supernatant was analyzed on a UV-visible spectrophotometer fitted with an 80 µL flow cell [20, 21]. The absorbance was measured at 532 nm (the mobile phase consisted of 300 ml/L methanol in 50 mM KH_2PO_4, pH=7.0). 1, 1, 3, 3-tetraethoxypropane was used as a standard, and MDA-TBA reactive substances values were expressed as nmol per milligram of tissue

protein (nmol/mg protein). The HPLC system consisted of a solvent delivery pump (Jasco 980-PU, Tokyo, Japan), a reversed-phase column (Luna C18, 250 mm × 4.6 mm, Phenomenex, CA, USA), and a UV–Vis detector (Jasco, UV-975, Tokyo, Japan) operated at 532 nm.

Protein content: The total protein concentration of tissue homogenates was determined according to [22].

Statistical analysis: The results were expressed as means±standard error of mean (Mean±SEM). All data were recorded with the Statistical Package for Social Sciences (SPSS-16.0). The results were analyzed using one-way analysis of variance (ANOVA) followed by post Hoc multiple comparisons Tukey test for comparison between different treatment groups. Statistical significance was set at $p<0.05$.

Results

The mean values (±SEM) of GPx, SOD, CAT and MDA activity (as the biomarker for lipid peroxidation) in the rat liver and kidney are presented in figures 1-4. Exposure to RFW significantly decreased the activity of SOD in the test group compared to other groups, while administration of vitamin C could significantly increase the activity of this enzyme and bring it to normal level with no significant difference compared to the control group ($p<0.05$) (Fig. 1). Exposure to RFW also significantly decreased the activity of GPx compare to control group and administrated vitamin C raised its activity to the normal level with no significant difference compared to the control group. The control-vitamin C group receiving vitamin C also no significant difference the activity of this enzyme compared to control group (Fig. 2). CAT activity was significantly lower in the group exposed to RFW compared to the other groups (Fig. 3) and pretreatment by vitamin C could have prevented this effect. The activity of this enzyme no significant difference in the treated group compared to control group. Exposure of rats to RFW significantly increased lipid peroxidation products (as shown by MDA concentration) as compared to control groups, while pretreatment of rats with vitamin C suppressed MDA concentration significantly (Fig. 4).

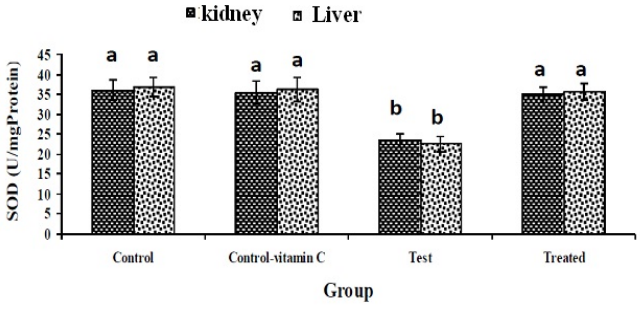

Figure 1. Comparison of superoxide dismutase (SOD) activity among the control and treated rats (N=8). Values represent mean±SEM of enzyme activity (U/mg protein of tissue). Different alphabet shows significant difference with other group in liver and kidney ($p<0.05$)

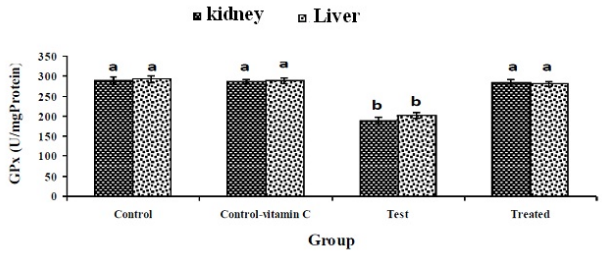

Figure 2. Comparison of glutathione peroxidase (GPx) activity among the control and treated rats (N=8). Values represent mean±SEM of enzyme activity (U/mg protein of tissue). Different alphabet shows significant difference with other group in lliver and kidney ($p<0.05$)

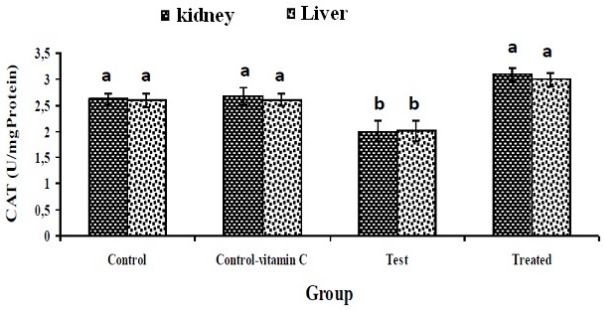

Figure 3. Comparison of catalase (CAT) activity among the control and treated rats (N=8). Values represent mean±SEM of enzyme activity (U/mg protein of tissue). Different alphabet shows significant difference with other group in liver and kidney ($p<0.05$)

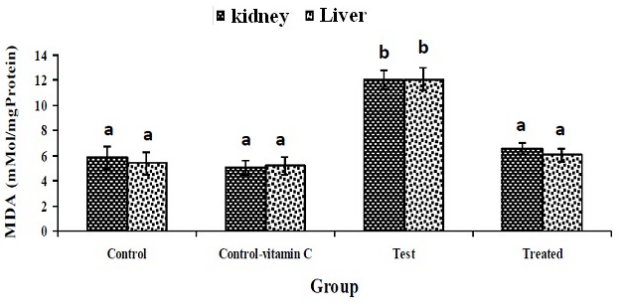

Figure 4. Comparison of malondialdehide (MDA) activity among the control and treated rats (N=8). Values represent mean ±SEM of enzyme activity (u/mg protein of tissue). Different alphabet shows significant difference with other group in liver and kidney ($p<0.05$)

Discussion

In the present study, significant changes in indexes of oxidative stress was observed in liver and kidney following exposure of animals to RFW, which is in agree with previous reports [5, 6, 13, 23, 24]. Administration of vitamin C to exposed group could significantly reduce effects of oxidative stress. Vitamin C was reported to improve antioxidant enzymes activity and lipid proxidation in testis [6, 13, 25], brain [26] and eye [5]. Various studies have indicated that radiofrequency waves emitted from mobile phone (900 MHz) affect biological and biochemical responses, including cell proliferation [27], cell surface properties [28], apoptosis [29], and DNA damage [30]. It is possible that non-thermal effects

of radiofrequency waves emitted from mobile phone increase the ROS in tissues and cells. It is well known that ROS lead to oxidative damage in major cell macromolecules, such as lipids, proteins and nucleic acids, and proposed to be the caused in tissue injury. ROS are scavenged by SOD, GSH-PX, and CAT. MDA is the byproduct of the major chain reactions leading to the oxidation of polyunsaturated fatty acids and, thus, serves as a biomarker of oxidative stress-mediated lipid peroxidation [5, 6]. The disruption of the oxidant/antioxidant balance in the liver, kidney and other tissues exposed to radiofrequency waves emitted from mobile phone has been shown in experimental studies [5, 6, 31-33].

In previous studies [34, 35], an increase was observed in the activation of NADPH cytochrome, P450 reductase, and cytochrome P450 in rat liver tissue irradiated with microwaves, and the eventual peroxidative damage reduced vitamin E, an antioxidative substance [35]. Faruk et al, showed that exposure to 900 MHz-emitted mobile phone decreased renal antioxidant enzymes activity and increased lipid peroxidation level in the rat kidney [11]. Meral et al. showed that exposure to radiofrequency waves emitted from mobile phone (12 h/day, for 30 days) increased production of free radicals and decreased the antioxidant enzymes activity and increased lipid peroxidation in the brain and blood [23]. Agarwal et al. reported that chronic exposure to electromagnetic radiation reduced the enzymes activity of SOD, GPx and catalase and increased lipid peroxidation [32]. Jelodar et al. showed that exposure to radiofrequency waves emitted from BTS (4 h/day, for 45 days) decreased the antioxidant enzymes activity and increased lipid peroxidation in the eye and testis. Also they showed that administration vitamin C improves antioxidant enzymes activity and decreased lipid peroxidation in these tissues [5, 6]. Vitamin C has a potent physiological role, also it has a very low standard reduction potential (282 mV) and is capable to regenerate intracellular compounds such as glutathione (GSH), NADH and NADPH [16]. GSH is a predominant endogenous antioxidant and used as a cofactor to remove hydrogen peroxide and lipoperoxides by the GPx family during which GSH is converted into oxidized form of glutathione (GSSG). Oxidized glutathione is converted back into GSH by another rate controlling enzyme the glutathione reductase (GSR) thereby maintain the intracellular GSH levels. This optimum level of GSH is an utmost criterion in maintaining the structural integrity and physiology of cell membranes [36]. Vitamin C also removes hydrogen peroxidase and other free radicals thereby adjust the activity of glutathione peroxidase and catalase [37]. In addition, vitamin C is capable to regenerate α-tocopherol from tocopherol radical species [16], thereby decreases lipid peroxidation levels.

The results of this study suggest that RFW leads to oxidative stress in liver and kidney and vitamin C via pro-oxidant and antioxidant role improved antioxidant enzymes and decreased lipid peroxidation. There is a need for further study with different frequencies and exposure periods in order to discover the effects of radiofrequency wave-induced oxidative stress in these tissues.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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