Study of Change in Enzymatic Reaction under Radiowaves/Microwaves on Lactic Acid Dehydrogenase and Catalase at 2.1, 2.3 and 2.6 GHz

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Abstract— There is an increasing need to study and evaluate the impact of weak radiofrequency (RF) radiation at the cellular and molecular levels because of the worldwide increase in use of wireless telecommunication devices, mainly mobile phones, which has resulted in increased human exposure to RF fields. This study aims to improve our understanding of impact of low power RF/MW radiation (4G mobile network frequencies) on enzymatic reactions. The selected enzymes play crucial roles in the biological processes. L-Lactic dehydrogenase (LDH) is extensively present in blood cells and heart muscles, and is a marker of common injuries and disease. Catalase enzyme can be found in all living organisms, it is important for protecting a cell from oxidative damage by reactive oxygen species (ROS). This *in vitro* study evaluates the effects of low power RF/MW on kinetics of LDH and Catalase enzymes irradiated at the frequencies of 2.1, 2.3, and 2.6 GHz and two powers of 17 and −10 dBm using the commercial Transverse Electro-Magnetic (TEM) cell. Furthermore, changes in biological activity of Catalase enzyme irradiated at the particular frequency and different powers were also evaluated. The comparative analysis of experimental findings reveal that MW exposures at the particular studied parameters can induce changes in the enzymes' kinetics, which in turn lead to modulation of rate of change in corresponding reactions these enzymes catalyse.

1. INTRODUCTION

In modern life, global population is continuously exposed to electromagnetic radiation emitted by mobile phones, computers, radars, smart meters and medical technology equipment etc.. In the last 10 years, use of mobile phone worldwide has increased by almost 200% resulting in 4.65 billion users in 2014. This rate is further increasing at approximately 5% annually [1]. Because mobile phone use is so widespread, public concerns about the possible health effects of RF exposures receive a lot of coverage in the media. Recent reports confirm that even weak RF/MW radiation can induce modulating effects on various biological systems [2, 4]. Intensive international research has found no conclusive or convincing evidence that mobile phones are damaging to health in the short or long term. However, in May 2011, IARC, which is a specialised agency of the World Health Organization, classified radiofrequency electromagnetic fields emitted from mobile phones, wireless devices, radio, television and radar as Group 2B or possibly carcinogenic to humans, based on an increased risk for glioma, a type of brain cancer [3]. Many national and international agencies have established safety guidelines for exposure to RF fields [4, 5]; however, concerns remain about the potential adverse health outcomes because of increasing RF field exposures, so the significance of investigating non-thermal effects at the molecular level cannot be underestimated. Radiation can affect the body in many ways, and the health effects may not become apparent for many years. These effects range from mild symptoms, such as skin reddening, to serious effects such as cancer and death. *In vitro* research shows that membrane structure and functionality can be altered upon exposures to RF fields [8]. *In vitro* studies also revealed that RF/ MW at the particular frequency and power induce changes in the enzyme's kinetics [9–13]. In addition, there are also other studies which have reported that the modulating effects of MW can also cause destruction at DNA level which resulted in the inhibition of cell proliferation [14, 15].

2. MATERIAL AND METHODS

2.1. Experimental Set-up

The commercial Transverse Electro-Magnetic (TEM) cell (No. TC-5062A UHF-TEM Cell) was used in this study to irradiate the selected example systems. TEM cell is an enclosed box made of a conductor material, with its dimensions varied depending on the operating frequency used. One end of the box is connected to signal generator from which external signal was applied to generate

Findings of latest research studies on the effects of low power MW radiation			
Study	**Exposure**	**Frequency**	**Effect**
J. Tang, et al., 2015 [16]	0.016 W/Kg	900 MHz	Impaired spatial memory and damages BBB permeability in rats
A. H. Eris, et al., 2015 [17]	608 mW/m^2	900 MHz	Retarder learning and deficit in special memory in rats.
H. J. Li, et al., 2015 [18]	5, 10, 20 mW/cm^2	2.856 GHz	Long-term, chronic MW exposure could induce dose dependent deficit of spatial learning and memory in rats
F. Aydogan, Unlu, 2015 [19]	0.4 W/Kg	2.1 GHz	Exposure to 2100 MHz RF radiation causes salivary gland damage to some extent and especially with longer exposure duration.

a predictable field inside the TEM Cell. The absorption coefficients of the analysed samples were measured using an Ocean Optics USB2000 spectrometer.

2.2. Model Proteins

In order to study the influence of RF/MW radiation on proteins *in vitro*, LDH [EC1.1.1.27] and Catalase [EC 1.11.1.6] were selected as model systems. LDH enzyme plays a central role in metabolic pathways of almost every cell. LDH catalyses the reversible reduction of Pyruvate to L-lactate using NADH as a co-enzyme. LDH activity is calculated from the rate of change in NADH absorbance at 340 nm. Catalase is responsible for decomposition of H_2O_2 to water and O_2. Catalase activity is calculated by measuring the total amount of H_2O_2 decomposed to form O_2. The reaction catalysed by Catalase enzyme is comparatively different from other enzymes as the rate of decomposition of H_2O_2 is proportional to the amount of Catalase present, whereas unlike other enzymatic reaction Catalase undergoes spontaneous decomposition during the reaction process.

2.3. Measurement Procedure for LDH

Tris HCl, 0.2 M, pH 7.3, 2.8 ml + 6.6 mM NADH, 0.1 ml + 30 mM Sodium Pyruvate were mixed and a final aliquot was prepared for each of the three test and three control sample. Amount of 0.1 ml of LDH solution was added to the cuvette and the absorption of the solution at 340 nm was recorded, and immediately after that the cuvettes were transferred to TEM cell for irradiation. After irradiation the cuvettes were removed from TEM cell and the absorbance was measured again at 340 nm. This procedure was repeated for both irradiated and control samples after every 5 minutes. The spectrophotometer was set to record the absorbance at every 2 s. The temperature was maintained at 25°C (Temperature Controller, Quantum Northwest, Inc.).

2.4. Measurement Procedure for Catalase Reagents

The Phosphate Buffer (50 mM Potassium Phosphate Buffer, pH 7.0 at 25°C) was prepared in a ultrapure ionized water using potassium phosphate, dibasic, trihydrate (Catalog Number P5504).

Hydrogen Peroxide Solution [0.036% (w/w)] was prepared in Phosphate Buffer using hydrogen peroxide (30% (w/w), Catalog Number H1009).

An initial Catalase solution of 10 mg/ml was prepared and immediately before use it was diluted to100 units/ml in cold Phosphate Buffer. 1.45 ml of Hydrogen Peroxide Solution was pipetted into the test cuvettes, and 500 µl of previously prepared Catalase Solution were added in the cuvette. The activity of Catalase was measured with help of spectrophotometer and the absorbance values were recorded at 240 nm (A240). Immediately after recording of absorption the cuvettes were placed inside the TEM cell for irradiation. Same procedure was repeated after 2 min, 7 minutes and 9 minutes and readings were recorded. During the experiment the temperature was maintained at 25°C.

In order to relate the rate of reaction, dissociation constant of hydrogen peroxide K was calculated using the following formula:

$$K = \frac{1}{t} \log \left(\frac{A}{A - x} \right)$$

where t is the time interval of second reading, 'A' is the amount H_2O_2 absorbed at the end of reaction and 'x' is the H_2O_2 absorbed at the time of second reading.

Both LDH and Catalase enzyme solutions were exposed at the selected frequencies of 2.1 GHz, 2.3 GHz and 2.6 GHz at two different powers of 17 dBm and −10 dBm.

3. RESULTS AND DISCUSSION

The experimental evaluation of catalytic activities in selected enzymes (Catalase and LDH) exposed to MW of different power and frequency range was conducted and the changes in rate of absorption (rate of reaction) of irradiated samples were compared with non-irradiated samples in order to understand the modulating effects of MW at different combination of frequency and power. The test samples were exposed to MW at 2.1 GHz, 2.3 GHz and 2.6 GHz at powers of −10 dBm and 17 dBm. In the second experiment, the test samples of Catalyse were exposed at the frequency 2.6 GHz and different powers to understand the effect of changing power (power-dependence) at the particular frequency.

The results are presented in Figures 1, 2 and 3 respectively. Figure 1 clearly shows a consistent modulating effect of irradiation on dissociation constant K of H_2O_2: a 20% increase at 2.1 GHz exposure, and a 25% decrease at 2.6 GHz exposure versus non-irradiated sample at 17 dBm. At the power of −10 dBm relative change in K value is inconsistent. As can be seen from Figure 1, at 2.1 and 2.3 GHz it increases by 70% as compared to non-irradiated sample but at 2.6 GHz it is

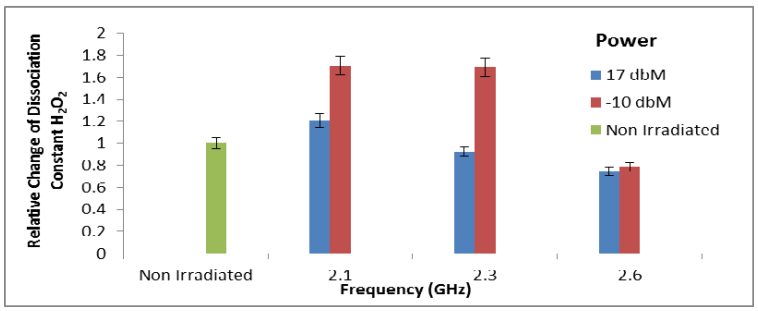

Figure 1: Relative change of Dissociation constant of H_2O_2 for irradiated vs. non-irradiated Catalase at the different frequencies.

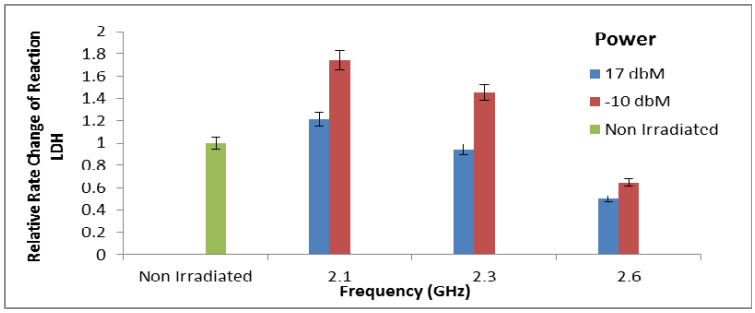

Figure 2: Relative change in rate of reaction of NADH for irradiated vs. non-irradiated LDH at the different frequencies.

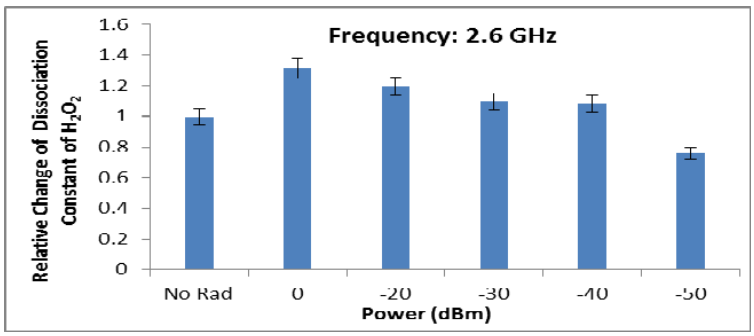

Figure 3: Relative change of Dissociation constant of H_2O_2 for irradiated vs. non-irradiated Catalase at 2.6 GHz and different powers.

reduced by 22%. However, 2.1 GHz exposures for both power levels induce maximum increase on dissociation constant K. At 2.6 GHz a decrease in activity for almost 25% is observed.

Figure 2 shows modulating effects of irradiation on LDH enzyme. Consistent decrease in rate of reaction at 17 dBm: a 21% increase at 2.1 GHz and almost 50% decrease at 2.6 GHz in comparison with non-irradiated sample were observed. Further at the power of −10 dBm under similar experimental conditions a steep change in rate of reaction was observed. At −10 dBm and 2.1 GHz reaction rate was increased by 75% and decreased by 35% at 2.6 GHz when compared to non-irradiated sample. Important to note, in both the enzymes the relative changes induces by exposures at −10 dBm and 2.3 GHz are not significant.

Finally, the effect of power on the activity of Catalase enzyme was investigated at the frequency of 2.6 GHz (Figure 3). The reason for studying the effects induced by exposures at 2.6 GHz is that this frequency is used as a carrier frequency by many telecommunication systems. To calculate the dissociation constant K, the same methodology was used as described above.

An interesting pattern is observed in relative change of dissociation constant, K, of H_2O_2 for irradiated versus non-irradiated samples. At 2.6 GHz and 0 dBm power, K value of the exposed sample was 32% higher than of non-irradiated sample. For −20 dBn the increase of almost 20% is recorded. For 30 dBm, an increase of 9% in K value is observed. Less significant increase is observed for −40 dBm. However, for −50 dBm, K was decreased by 25% compared to non-irradiated sample.

These findings confirmed our hypothesis that enzymatic activities can be changed by external exposures at low power MW irradiation, with the observed effects being power and frequency-dependant.

4. CONCLUSIONS

This study was aimed to test the hypothesis that the external low power MW radiation can affect catalytic activity of LDH and Catalase enzymes. Further to the analysis we also tested the relation and effect of frequency and power of applied irradiation on the enzyme reaction. The results obtained show that the MW radiation at selected frequencies and powers can produce modulating effects on the catalytic activity of selected enzymes by either increasing or decreasing the reaction rates.

Our second phase of experiment suggest that both frequency and power contribute separately for the modulating effects in the studied Catalase enzyme. The experimental findings highlight that even low power MW can induce modulating affects at the fixed frequency. However it requires further detailed investigation on a wide range of combinations of frequency and power to establish safe limits of MW exposures. Our findings reveal the significant relationship between the low power microwave exposures and catalytic activities in Catalase and LDH enzymes.

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