eISSN: 09748369, www.biolmedonline.com

Bioeffects of electromagnetic base station on glutathione reductase, lipid peroxidation and total cholesterol in different tissues of Wistar rats

***AC Achudume, B Onibere, F Aina**
Institute of Ecology and Environmental Studies,
Obafemi Awolowo University, Ile-Ife, Nigeria.

*Corresponding author: aachudum@yahoo.com

Abstract
Assessment of potential health risk of electromagnetic field (EMF) includes numerous uncertainties. The radio waves emitted by a GSM base stations, can have a peak power of 2 watts, and there are relatively few known reports linking biological effects to enzymatic and macromolecules of basal biochemical activity. The aim of the present study is to determine the bioeffects of base station on glutathione reductase (GR), lipid peroxidation (LP) and total cholesterol levels in different tissues of rats exposed to base station radiation. Animals (20 male Wistar rats) were randomly located in the vicinity of base station <10m on ground zero. Exposure was in three forms: continuous waves, or modulated at 900MHz or modulated GSM-nonDTX. The radio frequency radiation (RFR) was 1800 MHz, specific absorption radiation (SAR) (0.95-2W/kg) for 40 and/or 60days continuously. Control animals were located > 300m from base station, while sham control animals were located in a similar environmental conditions, but in the vicinity of a non-functional base station. Results of bioeffects of base station on enzymatic activity and macromolecules showed insignificant effects on the rat kidney, liver and brain at 40days. However, at 60days, decreased activity of GR, decreased levels of lipid peroxidation as measured by malondialdehyde and total cholesterol were observed. The sham controls have relative values as the controls, same as far field exposure. The difference was not statistically significant except in the brain ($P<0.05$), when compared to sham and far field. The decrease in LP in the tissues could be attributed to increased oxidative stress leading to depletion of tissues contents with diminution of antioxidative defense system. These subtle bioeffects at 60 days could mean greater potential health risk at much longer period of exposure.

Keywords: Electromagnetic; antioxidant; glutathione reductase; cholesterol; lipid peroxidation.

Introduction

Several studies on the relationship between biological health effects and electromagnetic fields (EMF) exposure has been widely recognized from epidemiological and experimental studies (Oberfeld et al, 2003, Lönn et al, 2005 and Abdel-Rassoul et al, 2007). Assessments of potential risks of electromagnetic frequency include numerous uncertainties referred to as 'idiopathic environmental intolerance' in order to avoid the implication of causation (WHO 2000). There have been a number of contradictory reports on overall effect of base station on public health (Shoemaker et al, 2005). Combined with growing body of evidence the information available today on the potential health effects of base stations gives strong support to the notion that base station may affect multiple facets of brain function (Salford et al, 2003), behavior (Abdel-Rassoul 2007), and health in general (Lönn et al, 2004, Blank 2007 and Mishra et al, 2008)

The risk level of exposure to radiation depends on the type, frequency of exposure, amount of energy absorbed and duration (Balmori 2005). Although, some exposure may affect people differently depending on age and pre-existing health conditions (Santini et al, 2003). A fundamental intensity threshold signal that is emitted by mobile base station may be appropriate to its physiological temperature and biological reactions within the cells. During lingering exposure, the effects can change from stimulant to inhibition, depending on the pulse shape (Farrel et al, 1997), during development, differentiation and physiological condition or health of the receiving organism (Kusunoki and Hayashi 2008).

A study on electromagnetic radiation and oxidative stress in rats showed widespread biological effects at a power density of 3.67 W/m^2 with specific absorption rate (SAR) of 1-3mW/kg in reduced glutathione concentration (Yurekli et al,2006). In addition, studies have shown some of the biological effects include

increase in levels of enzyme ornithine carboxylase which has been implicated in tumor promotion (Hyland 2003) and alterations in thyroid hormone levels and behavior (Sinha 2008). Other studies have indicated that irradiation induces reactive oxygen specie (ROS), which play an important role in radiation damage of the cell (Cemek et al, 2006). The same study showed reduced glutathione (GSH) level as having antiperoxidative effect on different tissues and a scavenger effect on ROS. In the case of GSM base stations, there are few reports of evidence of such health problems as a result of enzymatic and macromolecules basal biochemical parameters. It is the aim of this present study to determine the bioeffects of base station on glutathione reductase, lipid peroxidation and cholesterol level.

Materials and Methods

Male Wister rats (75-100g wt) were caged and grouped randomly in clean plastic containers having saw dust chips for bedding. Animal treatments and protocol employed in this study was according to the institutional Ethical Committee and in the principles of laboratory Animals Care (NIH publication No 85-23). All animals were fed standard pellet (Ladokun feed, Nigeria Ltd) and de-ionized distilled water ad libitum.

Animals (n=20) were separately located in the vicinity of the base station 700ft or less, estimated distance was less than 10m on ground level zero. The exposure to radiofrequency radiation (RFR) was in three forms-continuous waves, or modulated at 217 Hz or modulated GSM-nonDTX the RFR was 1800 MHz. The second group was located further than 300m of the cellites, while the third was placed in a similar environment and conditions as the experimental but in non-operating base station thus served as sham control. The specific absorption rate (SAR) in the animals range from 0.98-3.9 W/cm^2. The electric field intensity was measured (radiofrequencies and microwaves) in V/m, using a Model Rados RDS-120 Universal Survey Meter, range 0.05-10 µV/m (Rados Tech, Finland) with automatic selection of measuring range was used to measure radiofrequency and microwaves The specific absorption rate (SAR) in the animals range from 0.98-3.9µV/m. Comfort 30s Reliable Digital Thermometer (REF 0T11-121c, 070502) was used to measure the temperature around the base station with 10% sensitivity from a unidirectional antenna (range: 1MHz-3GHz). The average exposure duration for EMF was continuous for forty and /or sixty days. At the end of the first 40 days half of the animals were sacrificed by cardiac puncture. Livers, kidneys and brain were dissected out, rinsed and homogenized for biochemical analysis. The homogenate served as the enzyme source. At the end of sixty days the rest animals were treated as previously described.

Biochemical analysis

Glutathione reductase (GR) activity was measured in three different tissues following the methods of Goldberg and Spooner (1983). The 3ml of reaction mixture contained 2.6 ml phosphate buffer solution (PBS) 0.12M, pH 7.2; 0.1 ethylenediaminetetraacetate solution (EDTA) 15mM; 0.1 ml oxidized glutathione (GSSG) 65.3mM. To this, 10 µl of homogenate was added and the volume was made up to 2.95ml with distilled water. After incubation at room temperature for 15min, 0.05 ml of nicotinamide adenine dinucleotide phosphate (NADPH) 9.6mM was added. Decrease in absorbance/min was recorded immediately at 340nm for 3min. Control was run without GSSG. The activity of GR has been expressed as unit/g tissue.

Lipid peroxidation (LPO) was assessed by measuring malondialdehyde (MDA) levels based on the reaction of MDA with thiobarbituraric acid (Shafiq-Ur-Rehman 1984). In brief, 1ml of each of the tissue homogenate was incubated at 37±0.5^0 C for 2h. The sample was mixed with 1ml of 10% w/v trichloroacetic acid (TCA) to precipitate protein. The mixture was centrifuged at 2000 rpm for 10min, and aliquot of 1ml supernatant was reacted with 1ml of 0.67% thiobarbituric acid in boiling water bath for 10min. Results have been expressed as nmolMDA/g tissue.

Total cholesterol was estimated following the methods of Abell et al, 1962. Sample of each tissue homogenate 200 µl was added to 2.0ml of alcoholic potassium hydroxide (KOH). Another 5ml of petroleum ether was added to the mixture after cooling to room temperature followed by distilled water (2.0ml). The mixture was centrifuged at low speed. Aliquots (4.0ml) of the petroleum ether layers were vaporized by heating to 60^0 C and thermostated to 25^0 C allowing 10min for the temperature to stabilize. At time interval of 30s, 6.0 ml of Lieberman-Burchard reagent was added. The mixture was allowed to stabilize to room temperature, the absorbance (A) was read at 620nm in timed intervals of 30s.

Statistical analysis
Data were subjected to Barlett's test for homogeneity, followed by analysis of variance (ANOVA). For post hoc comparison Student Newman Keul's test was employed.

Results and Discussion
The results of the bioeffects of EMF base station on biochemical activity of glutathione reductase, lipid peroxidation and total cholesterol level in different tissues of rats is presented in Tables 1-3.The study demonstrates slight decrease in the activity of glutathione reductase, lipid peroxidation as measured by malondialdehyde and total cholesterol in all three tissues investigated when rats were exposed to radiation emitted from base station in 40 days which further decreased when the period of exposure was extended to 60 days. The sham had relative values when compared to control and those of far field. The numerical values for GR are greater than those of LP and Total cholesterol. At 40 days, the LP in the brain increased while at 60 days, the level decreased substantially (Table 2). The decrease observed in total cholesterol at 60 days was significant at $P< 0.05$ (Table 3).The bioeffect of EMF base station on rats have been well documented for more than a decade (Yurekli et al, 2006, Cemek et al, 2007, Sinha 2008). These effects are thermal and can penetrate the cell membrane to inhibit the activity of cell membrane bound enzymes and thereby affect the cell function, metabolism and signal transduction (Ke et al, 2008). The nephrotoxic and hepatotoxic actions of EMF radiation in the present study may have contributed to the decrease in activity of glutathione reductase (enzymatic antioxidant marker), as a result of enhanced production of reactive oxygen species (ROS) (Table 1). With the disruption of the internal cellular structure and decreased permeability or disintegration of the cell wall, many of the cellular enzymes may leak in to the interstitial fluid and find their way into the blood (Gregus and Klaassen 2001). Decrease activity of GR could only be associated with EMF radiation and could therefore be due to oxidative reactions in biological macromolecules. Again, the possible mechanisms by which radiation decreased the level of GR might be due to conjugation with pro-oxidants resulting in inhibition of GR synthesis Gregus and Klaassen (2001).

The bioeffect of radiation with the lipids of biological membranes may have significant consequences for the structural and functional properties of cells (Yurekli et al, 2006). Cell membrane lipids may be damaged due to peroxidation of unsaturated fatty acids (Eritsland 2000). Several studies have shown that radiations are known to cause lipid peroxidation, increased reactive oxygen species and perturbation of antioxidant systems in rats (Yurekli et al 2006), such alterations in the structure and functions of the cell membrane include decreases in membrane-bound enzymes, and loss of essential fatty acids (Van Ginkel and Sevanian 1994). The decrease in LPO in different tissues in the present study could be attributed to increase oxidative stress caused by induced generation of ROS and depletion of tissue contents coupled with diminution in enzymatic antioxidative defense mechanism (Table2).

There are evidences that exposure to radiation might have subtle effects on biological functions, including the brain (Salford et al, 2003, Mishra et al, 2008). This does not mean that health is affected, but it is equally possible that exposure to radiation even at levels below WHO guidelines, may not be totally without potential adverse health effects (REFLEX 2004). Reduction in enzyme activity and total cholesterol levels in different tissues and in particular the brain may be related to enhanced stress. In the present study, GR activity were decreased by 6.9% in the kidney, 6% in liver and 17% in the brain, while total cholesterol level decreased 65% in kidney, 33% in liver and 29% in brain and showed such subtle bioeffects on these tissues respectively. Thus, the reductions of biomolecules are indicative of multifactor mechanisms being involved in radiation related oxidative stress (Hutter et al, 2006). Though, it is difficult to understand the implicit mechanism for radiation related oxidative stress, but going by the decreased activity of GR, decreased levels of LP as measured by malondialdehyde and decrease in total cholesterol indicate potential abnormalities in response to EMF radiation and could be linked to electromagnetic hypersensitivity syndrome being experienced by humans living in the vicinity of EMF base stations. Finally, these observations are consistent with the idea that EM fields affect many biological systems by interacting with electrons moving during redox reactions and also within DNA (Blank, 2008).

In conclusion, the present study on potential hazards associated with exposure to EMF base stations demonstrate reduction in enzymatic GR, lipid peroxidation and total

cholesterol in different tissues of rats exposed to EMF base station radiation for 60 days continuously. Short time duration exposure (40) days did not potentates bioeffect of consequences. The results further indicate that longer exposure duration may lead to depletion of these parameters and could be considered important factors in electromagnetic hypersensitivity syndrome.

Table 1: Bioeffect of electromagnetic base station radiation on glutathione reductase, lipid peroxidation and total cholesterol in rats

Treatment unit/g tissue	Kidney	Liver	Brain
Control	4.32±0.07	4.21±0.01	4.10±0.08
40 days	4.26±0.02	4.19±0.05	4.85±0.03
60days	4.02±0.24	3.96±0.08	3.09±0.01
Sham	4.33±0.01	4.20±0.00	4.11±0.00

Table 2: Bioeffects of electromagnetic base station radiation on lipid peroxidation in rats

Treatment	Kidney	Liver	Brain
		nmol MDA/ml tissue	
Control	0.28±0.04	0.30±0.03	0.29±0.04
40 days	0.28±0.02	0.33±0.00	0.36±0.01
60 days	0.26±0.04	0.26±0.03	0.24±0.08
Sham	0.37±0.08	0.30±0.01	0.30±0.06

Table 3: Bioeffects of electromagnetic base station radiation on total cholesterol in rats

Treatment	Kidney	Liver	Brain
Control	0.17±0.09	0.18±0.04	0.21±0.39
40 days	0.17±0.04	0.19±0.01	0.18±0.02
60 days	0.06±0.00	0.12±0.06	0.15±0.08*
Sham	0.17±0.05	0.18±0.00	0.22±0.02

* Significantly different from control P<0.05

References

Abdel-Rassoul G.,.Abou El-Fateh O., Abou Salem M., Michael A Farahat F., El-Batanouny M., Salem E, 2007. Neurobehavioral effects among inhabitants around mobile phone base stations. Neurology and Toxicology 28(2): 434-440

Abell LL., Levy BB., Ken A, 1962. A simplified method for the estimation of cholesterol in serum and

demonstration of specificity. Journal of Biological Chemistry 196,360

Balmori A, 2005. Possible effects of electromagnetic fields from phone masts on a population of white stork (*Ciconia ciconia*). Electromagnetic Biology and Medicine 24: 109-119

Blank M, 2007. Evidence for stress response (stress protein). In Bioinitiative Report: A Scientific Perspective on Health Risk of Electromagnetic fields. On-line 31st August 2007 pp. 1-40

Blank M, 2008. Protein and DNA reaction stimulated by electromagnetic fields. Electromagnetic Biology and Medicine
27:1-21

Cemeek M, Enginar H., Karaca T, Unak P, 2006. In vivo radio protective effects of Nigella sativa L oil and reduced glutathione against irradiation induced oxidative injury and number of peripheral blood lymphocytes in rats. Photochemistry and Photobiology 82(6):1691-1696

Eritsland J, 2000. Safety considerations of polyunsaturated fatty acids. American Journal of Clinical Nutrition 71:1975-2015

Farrel ,JM, Litovitz TL, Penafiel M, 1997. The effect of pulsed and sinusoidal magnetic fields on the morphology of developing chick embryos. Bioelectromagnetism 18:431-438

Goldberg DM, Spooner RJ, 1983. Glutathione reductase.. In: Bergmeyer HU, Bergmeyer J GraBl M. (ed). Methods of enzymatic analysis, 3rd ed., vol. 111. Verlag Chemie, Weinheim, pp 258-265

Gregus Z, Klaassen CD, 2001: In Casarett and Doull's toxicology: the basic science of poisons. 6th ed. McGraw-Hill, New York, pp 35-81

Hutter, H.P., Moshammer, H., Wallner, P., Kundi, M. 2006. Subjective symptoms, sleeping problems and cognitive performance in subjects living near mobile phone base stations. Occupational and Environmental Medicine 63(5):298-799

Hyland, G. 2000. Physics and biology of mobile telephony. Lancet 356:1833-1836

Ke, X.Q., Sun, W.J.., Lu, D.Q., Fu, Y.T, Chiang, H. 2008. 50-Hz magnetic field induces EGF-receptor clustering and activates RAS. International Journal of Radiation Biology 84(5):413-420

Kusunoki, Y. and Hayashi, T. 2008 Long-lasting alterations of the immune system by ionizing radiation exposure: Implications for disease development among atomic bomb survivors. International Journal of Radiation Biology 84(1): 1-14

Lönn, S., Ahlbom, A., Hall, P. Feychting, M. 2005. Long-term mobile phone use and brain tumor risk. American Journal of Epidemiology 161 (6): 526-535

Lönn, S., Ahlbom, A., Hall, P., Feychting, M. 2004. Mobile phone use and the risk of acoustic neuroma. Epidemiology 15(6): 653-659.

Meral, I.. Mert, H., Mert, N., Deger, Y., Yoruk, I., Yetkin, A., Keskin, S.2007. Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. Brain Research 1169: 120-124.

Mishra, K.P., Ahmed,M., Hill, R.P. 2008. Low radiation effects on human health with implications to radioprotection and cancer radiotherapy. International Journal of Radiation Biology 84(5):441-444

Oberfeld, G., Navarro, A.E., Portoles, M., Maestu, C., Gomez-Perretta, C. 2003. The microwave syndrome. Further aspects of a Spanish study. Electromagnetic Biology and Medicine 22(2): 161-169

Salford, L.O., Brun, A.E., Eberhardt, J.L., Malmgren, L., Person, B.R.R. 2003. Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environmental Health Perspectives 111(7): 881-883

Salminen, T., Thomsen, J., Tynes, T. 2005. Mobile phone use and risk of acoustic neuroma: results of the interphone case-control study in five North European countries. British Journal of Cancer. 3; 93(7):842-848

Santini, R., Santini, P., Danze, J.M., LeRuz, P., Seigne, M. 2003. Symptoms experienced by people in vicinity of base stations: 11/ incidences of age, duration of exposure, location of subjects in relation to the antennas and other electromagnetic factors. Pathology and Biology 51:412-415

Shafiq-Ur-Rehman 1984. Lead-induced regional lipid peroxidation in brain. Toxicology Letters 21: 333-337

Shoemaker, M.J., Swerdlow, A.J., Ahlbom, A., Auvunen, A., Blaasaas, K.G., Cardis, E., Christensen, H.C., Feychting, M., Hepworth, S.J., Johansen, C., Klaeboe, L., Lonn, S., McKinney, P.A., Muir, K., Raitanen, J. Sinha, R.K. 2008. Chronic non-thermal exposure of modulated 2450MHz microwave radiation alters thyroid hormones and behavior of male rats. International Journal of Radiation Biology 84(6): 505-513

Van Ginkel, G., Sevanian, A. 1994. Lipid peroxidation-induced membrane structural alterations. Methods in Enzymology 233:273-288

WHO, 2000. Electromagnetic fields and Public health. Fact sheet No193

Yurekli, Al. Ozkan, M., Kalkan, T., Saybasili, H 2006. GSM base station electromagnetic radiation and oxidative stress in rats. Electromagnetic Biology and Medicine 25:177-188.