Effects of Radiation Emitted from Base Stations on Bilirubin, Transaminases and Lipid Peroxidation in Exposed Rats

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Abstract

Purpose: Exposure to non-ionizing radiation emitted from base station have been reported to have some evidence of alterations in the activity of certain cells leading to unspecific health symptoms referred to as idiopathic environmental intolerance. The objective of the present study was to evaluate bilirubin, amino transferases, lipid peroxidation, total cholesterol and High density lipoprotein (HDL) Cholesterol in whole blood as indices of stress-related idiopathic environmental intolerance.

Materials and Methods: Male rats were randomized and exposed to non-ionizing radiation emitted by base station between three mobile telephone masts for up to 60 days.

Results: Results showed that at 40 days of exposure, there were no observable differences in the levels of alanine and aspartate transaminases. However, at 60 days of exposure, there were significant decreases in amino transaminases and did not cause any significant stress in bilirubin. The intracellular level of lipid peroxidation as measured by malondiadehyde in the liver and kidney decreased by 15% and 43%, respectively. There was no difference in level of cholesterol at 40 days of exposure while the increased levels at 60 days were not significant.

Conclusion: The parameters evaluated indicate stress-related unspecific symptoms which may be associated with non-ionizing radiation emitted from base stations.

Keywords: base station, bilirubin, amino transaminases, lipid peroxidation, non-ionizing radiation

1. Introduction

Exposure to high level non-ionizing radiation emitted by base stations has been recognized to cause a variety of diagnostic entities which manifest as diffuse hypersensitivity syndrome not easily recognizable (H. P. Hutter, H. Moshammer, P. Wallner, & M. Kundi, 2006). Several studies of effects of magnetic fields on cell growth and proliferation have been carried out using mixture of in vitro models showed that acute exposure at 200mT decreased incorporation of [3H] thymidine into the DNA of cancer cells (J. Wiskirchen, et al., 2000; C. Y. Li, et al., 2003). Sabo et al. (2002) reported that the metabolic activity of HL-60 (Hela) cells was reduced by exposure to static magnetic field. In other studies, exposure to transient electromagnetic pollution in its various forms may account for higher plasma glucose levels and may contribute to the misdiagnosis of diabetes (M. Havas, 2008). Other studies have reported overall effects of base stations on public health (M. J. Shoemaker, et al., 2008; O. I. Alatise, et al., 2009). These effects are thermal and can penetrate the cell membrane to inhibit the activities of cell membrane bound enzymes and thereby affect the cell functions, resulting in massive cell death, organ damage and possibly death to the individual (X. Q. Ke, et al., 2008).

General adverse health effect associated with human exposure to non-ionizing radiation emitted by base stations includes developmental abnormalities (S. Lönn, et al., 2004), neurologic and neurobehavioral disorders (G. Abdel-Rassoul, et al., 2007). Sex-related differences (R. Santini, et al., 2002), fatigue, headache, sleep disruption and loss of memory were among the effects found (R. Santini, et al., 2003).

Lipid peroxidation has largely been considered as a molecular mechanism involved in deleterious effect of a variety of xenobiotics (M. D. Stringer, et al., 1986). Because of the body membrane chemical composition, lipoproteins especially high density lipoprotein (HDL), are highly susceptible to oxidations (H. A. Schwertner, et al., 1994; A. C. Achudume, et al., 2010). It was further suggested that bilirubin, a naturally occurring antioxidant,
could have a role in protecting lipids and lipoproteins against oxidation and hence reduce accumulation of cholesterol plaque (T. W. Wu, 1994; H. Esterbauer, et al., 1992). Given that oxidized lipids and lipoproteins are known to be atherogenic (H. A. Schwertner, et al., 1994; D. Steinberg, et al., 1989), low bilirubin concentrations could be associated with increases in oxidized lipids and lipoproteins (H. A. Schwertner, et al., 1994).

There is little experimental information describing possible effects of chronic exposure to non-ionizing electromagnetic waves. So far, long term adverse effects have become apparent in increased telephone masts in the environment. In the present study we examined bilirubin, amino transferases, lipid peroxidation, total cholesterol, and high-density lipoprotein cholesterol in whole blood with inverse association to base station-emitted non-ionizing radiation exposure to power frequency magnetic fields and discuss idiopathic environmental intolerance of the non-ionizing radiation.

2. Materials and Methods

2.1 Chemicals

Alanine aminotransferase (ALT) and aspartate amino transferase (AST) 10x50ml were procured from Boebringer Mannheim GmbH Mannheim, Deutschland, Germany. Direct bilirubin kit 475 ml; HDL cholesterol 5x5 ml; Dextran sulfate, phosphotungstic acid and other reagents were supplied by Sigma Chemical Co. (St Louis, MO, USA). Stock standards were prepared gravimetrically and checked spectrophotometrically (Base, Switzerland) after dissolution in absolute ethanol. Relevant wavelengths and absorptivities were adapted from the literature (Ng. Kwan-Hoong, 2003). We prepared working standard solutions by dilution with absolute ethanol and stored all standards at -20°C. Under these conditions they were stable for at least three months.

2.2 Animals

Male Wister rats (150 ± 20g) were procured from the animal holding facility of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria. They were housed in polypropylene cages under standard laboratory conditions, (room temperature 25 ± 5°C, relative humidity 50 ± 10%). Rats were offered food pellets (Ladokun Feeds, Ibadan, Nigeria Ltd) and water was provided ad libitum. Animal treatments and protocol employed in this study were according to the institutional Ethical Committee and the principles and guidelines as contained in the “Principles of Laboratory Animal Care” (NIH publication No 85-23) were followed.

2.3 Study Groups

Rat of group A were exposed for 40 days and rat of group B were exposed for 60 days. Rats in group C were exposed for sixty days in a similar condition as the experimental in a non operating tower base station thus serving as sham and D were placed in a free zone 2km from the nearest telephone mast towers and served as controls for 40 and 60 days, respectively. Forty healthy male Wistar rats were randomized for the study. After five days of acclimatization period, rats were divided into four groups, each containing ten rats.

2.4 Exposure System and Design

The experimental rats were exposed to non-ionizing radiation emitted by base station in a small house between three mobile telephone mast towers with a distance not greater than 20 meters apart and three shelters which contain electric power generators (electrical field intensity of 2.3±0.82 μV/m ) and radio- frequencies of 900 or 1800 MHz. A model Rados RDS-120 Universal Survey Meter, range 0.05±0.10 μV/m (Rados Tech, Jane, Finland) with automatic selection of measuring range was used to measure radiofrequency and microwaves. The specific absorption rate (SAR) in the animals was 0.6 W/kg. Comfort 30s Reliable Digital Thermometer (Mainland, China) (REF 0T11-121c, 070502) was used to measure the temperature around the base stations. The dose-response relationships (non-thermal) are nonlinear (A. A. Marino, E. Nilsen, & C. Frilot, 2003).

2.5 Biochemical Analysis

At the end of each experimental period rats were starved overnight and sacrificed by cardiac puncture, blood was collected in heparinized tube and processed for the estimation of serum bilirubin, and amino transferases. Small pieces of liver and kidney were carefully removed from each rat and processed for the estimation of microsomal lipid peroxidation, total cholesterol and high-density cholesterol.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were determined following the method of Acliya et al., (G. S. Achliya, S. G. Wadodkar, & A. K. Dorle, 2003) using a kit procured from Boebringer Mannheim GmbH Mannheim, Germany.

Total bilirubin was determined with diazotized sulfanilic acid reagent with blank correction following the methods of Bartis (C. A. Burtis & R. A. Edward, 1996) and Ashwood.
Peroxidized membrane lipids were estimated by a method described by Radi et al. (R. Radi, et al., 1991). Microsomal pellets were precipitated by calcium according to the method suggested by Schenkman and Cinti (J. B. Schenkman & D. L. Cinti, 1978). Malondialdehyde and other reactive oxidize products of membrane lipids, under acid conditions, react with thiobarbituric acid and form a pink colored chromogen which is strongly absorbed at 532nm.

Cholesterol was estimated in the liver and kidney homogenates separately following the methods of Liebermann and Burchard as described by (J. P. Peters & D. D. Vanslyke, 1946). Phosphotungstate-magnesium reagents, prepared in the laboratory were used for high density cholesterol (HDL) – cholesterol analyses. HDL cholesterol was measured after samples of low density (LDL) and very low density (VLDL) lipoproteins were selectively precipitated and removed by centrifugation. The supernatant contained the cholesterol associated with the soluble HDL fraction was assayed for cholesterol by an enzymatic method. The amount of color produced was directly proportional to the concentration of HDL cholesterol in the sample. Protein content was determined by the standard method of Lowry et al. (1951). The copper ions in alkaline reagent react with peptide bonds of samples to form a purple color with an absorbance maximum at 540 nm. The intensity of the color is proportional to the total protein concentration.

2.6 Statistical Analysis

The data for the experimental groups and their respective controls were analyzed to evaluate the effect of non-ionizing radiation using the two-way analysis of variance (ANOVA). Mean values were compared using Dunnett’s test at 5% level of significance to analyze significant differences between each treatment and the control (C. W. Dunnett, 1955).

3. Results

Effects of short and long term exposure to non-ionizing radiation emitting base station on various stress related parameters in blood, bilirubin and amino transferases are presented in Table 1. These results demonstrate relative alterations after 40 and 60 days of exposure to non-ionizing radiation emitted bases station.

Table 1. ALT, AST (UI/L) and bilirubin (mg/dL) of rats exposed to radiation

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>ALT</th>
<th>AST</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.81±0.27</td>
<td>1.35±0.06</td>
<td>0.290±0.21</td>
</tr>
<tr>
<td>40</td>
<td>1.79±0.15*</td>
<td>1.15±0.27</td>
<td>0.179±0.20*</td>
</tr>
<tr>
<td>60</td>
<td>0.83±0.59*</td>
<td>0.076±0.05*</td>
<td></td>
</tr>
</tbody>
</table>

* p< 0.05 compared to control

At 40 days of exposure, there were no significant decreases in the levels of alanine and aspartic transferase activities after 60 days exposure, the activities of both enzymes were significantly decreased by 36% and 39%, respectively, in comparison to control group (P<0.05). In a similar manner after 60 days, bilirubin significantly decreased by 59% of the control (p<0.05).

Table 2. Microsomal Malondialdehyde (nmoles/mg) in liver and kidney of rats exposed to radiation-emitting base station

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.26±0.03</td>
<td>0.42±0.04</td>
</tr>
<tr>
<td>40</td>
<td>0.23±0.02</td>
<td>0.27±0.00*</td>
</tr>
<tr>
<td>60</td>
<td>0.22±0.05*</td>
<td>0.24±0.02*</td>
</tr>
</tbody>
</table>

* p<0.05 compared to control

The effects of exposure to non-ionizing radiation emitted by telephone base station on intracellular level of lipid peroxidation (LPD) as measured by malondialdehyde in the liver and kidney were presented in Table 2. At 40
days of exposure there were marked decreases of lipid peroxidation in liver and kidney. However, the levels of malondialdehyde after 60 days were significantly (P<0.05) decreased by 15% in liver and 43% in kidney. Long term exposure of rats to radiation-emitting base station produced time-depended decreases in LPO compared to the control group (p < 0.05)

Table 3. Total cholesterol and HDL—cholesterol (mmole/mg) in liver and kidney of rats exposed to radiation-emitting base station

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>Liver Cholesterol</th>
<th>Liver HDL</th>
<th>Kidney Cholesterol</th>
<th>Kidney HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.18 ± 0.04</td>
<td>0.03 ± 0.00</td>
<td>0.17 ± 0.02</td>
<td>0.09 ± 0.0</td>
</tr>
<tr>
<td>40</td>
<td>0.18 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.17 ± 0.05</td>
<td>0.08 ± 0.0</td>
</tr>
<tr>
<td>60</td>
<td>0.19 ± 0.01</td>
<td>0.02 ± 0.00</td>
<td>0.18 ± 0.00</td>
<td>0.09 ± 0.0</td>
</tr>
</tbody>
</table>

Table 3 shows the total cholesterol and HDL in both liver and kidney of rats exposed to non-ionizing radiation emitting base station. There was no significant difference in the level of cholesterol at 40 and 60 days exposure. The same was true for HDL in both liver and kidney.

4. Discussion

The non-ionizing radiation emitted from base stations, while generally of low level, is continuous. It is suggested that chronic low level non-ionizing radiation emitted overtime, may be as harmful as higher-level, acute non-ionizing radiation exposure (A. Bortkiewicz, et al., 2004).

The present study shows subchronic exposure to non-ionizing radiation emitted from base station for 40 days did not cause significant stress in rat ALT (2.51±0.5) and AST (1.15±0.27) compared to control (2.81±0.27) and (1.35±0.06) respectively (Table 1). However, after 60 days of exposure, there were significant decreases in bilirubin (0.076±0.05), ALT (1.79±0.5) and AST (0.83±0.59) compared to control (2.81±0.27), (1.35±0.06) and (0.290±0.21) respectively indicating gradual subtle effects. It is worth noting that prolonged exposure to radiation emitted from base stations has been reported to induce symptoms of causal environmental syndrome (R. Santini, et al., 2003). Decreases in rat bilirubin concentrations were found in individuals with cardiac artery disease (CAD) (H. A. Schwertner, et al., 2004). Concerned that low level bilirubin might indicate a liver disease, which somehow protected against CAD, we compared a related subset, amino transferases in exposed rats. ALT and AST are reliable determinants of liver parenchymal injury (D. W. Moss, A. R. Handerson, & J. F. Kachmar, 1987). Activities of both transferases in this study significantly decreased as a result of radiation emitted by base station, indicating liver dysfunction.

This study showed low levels of microsomal malondialdehyde in liver and kidney of rats exposed for 60 days (Table 2). The role of lipid peroxidation and antioxidants in oxidative modification show lipid peroxidation has been considered as a molecular mechanism involved in deleterious effects of a variety of xenobiotics (M. D. Stringer, et al., 1986; H. Esterbauer, et al., 1992). Since biomembranes and subcellular organelles are the major site of lipid peroxidation (B. Halliwell, and B. Gutteridge, 1989), present observations on the decreases of lipid peroxidation in both liver and kidney suggest that non-ionizing radiation emitted from base station may have caused some damages to these lipid membranes. This is in agreement with earlier results (A. C. Achudume, B. Onibere, & F. Aina, 2009; A. C. Achudume, et al., 2010).

Bilirubin is a naturally occurring antioxidant of physiological importance (R. Stocker, et al., 1987) and as such, could have a role in protecting lipid and lipoproteins against oxidation. The decreased bilirubin concentrations in this study in addition to being a reflection of hepatic dysfunction could be associated with decreased levels in lipid peroxidation (Table 2). Ockner (R. K. Ockner, 1982) reported that a low level of bilirubin prevented solubilization of cholesterol and its clearance through the bile, thereby giving rise to elevated blood cholesterol concentration. The results in this study do not support this report since no significant changes were found in both total cholesterol and HDL (Table 3).

A large study on the connection between non-ionizing radiation emitted by base station and a variety of subjective symptoms reported by people living in the vicinities of cellular phone base stations (R. Santini, et al., 2002; G. Abdel-Rassoul, et al., 2007; O. I. Alatise, et al., 2009) and transient electromagnetic fields (M. Havas, 2008) implied a new diagnostic entity. The World Health Organization (WHO, 2000), however, prefers to name
it “idiopathic environmental intolerance”, in order to avoid the implication of causation (H. P. Hutter, et al., 2006). We do not know of any association of non-ionizing radiation emitted from base station with biochemical alterations in humans. A prospective study is needed to determine whether bilirubin and amino transferases can help predict idiopathic environmental intolerance.

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

**References**


