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Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields

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Abstract

Extremely low frequency (ELF) electromagnetic field (EMF) is thought to prolong the life of free radicals and can act as a promoter or co-promoter of cancer. 8-hydroxy-2′-deoxyguanosine (8OHdG) is one of the predominant forms of radical-induced lesions to DNA and is a potential tool to asses the cancer risk. We examined the effects of extremely low frequency electro magnetic field (ELF-EMF) (50 Hz, 0.97 mT) on 8OHdG levels in DNA and thiobarbituric acid reactive substances (TBARS) in plasma. To examine the possible time-dependent changes resulting from magnetic field, 8OHdG and TBARS were quantitated at 50 and 100 days. Our results showed that the exposure to ELF-EMF induced oxidative DNA damage and lipid peroxidation (LPO). The 8OHdG levels of exposed group (4.39 ± 0.88 and 5.29 ± 1.16 8OHdG/dG.10^5, respectively) were significantly higher than sham group at 50 and 100 days (3.02 ± 0.63 and 3.46 ± 0.38 8OHdG/dG.10^5) ($p < 0.001$, $p < 0.001$). The higher TBARS levels were also detected in the exposure group both on 50 and 100 days ($p < 0.001$, $p < 0.001$). In addition, the extent of DNA damage and LPO would depend on the exposure time ($p < 0.05$ and $p < 0.05$). Our data may have important implications for the long-term exposure to ELF-EMF which may cause oxidative DNA damage.

Keywords: *DNA damage, magnetic field, 8-hydroxy-2′-deoxyguanosine, 8-oxoguanine, thiobarbituric acid, TBARS*

Abbreviations: *ELF, Extremely low frequency; EMF, Electromagnetic field; 8OHdG, 8-Hydroxy-2′-deoxyguanosine; dG, 2′-deoxyguanosine; TBARS, Thiobarbituric acid reactive substances; LPO, Lipid peroxidation; UV, Ultraviolet; HPLC, High performance liquid chromatography; ECD, Electrochemical detection; ROS, Reactive oxygen species*

Introduction

In modern society, the use of electricity is so widespread that it is impossible to avoid exposure to magnetic fields (MF) including extremely low frequency electro magnetic field (ELF-EMF) produced by power lines and many kinds of electrical appliances. During the last 25 years, there has been growing public and scientific interest in possible health risks associated with exposure to ELF-EMF (less than 200–300 Hz) which may affect biological systems [1–4].

Not only epidemiological but also experimental studies suggest that there is an association between exposure to ELF-EMF and increased risk of cancer [5,6]. In order to explain the epidemiological observations associated with ELF-EMF exposure, experiments have been conducted in multiple laboratories to examine alterations of biological functions by EMF at the cellular and molecular levels. Cellular

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studies have described a variety of EMF effects on biological and biochemical responses such as cell proliferation [6,7], cell surface properties [8], gene expression [9], apoptosis induction [10], calcium transport [6,11], signal transduction, enzyme regulation and DNA damage [12].

Among the putative mechanisms, by which ELF-EMF may affect biological systems is by increasing free radical life span and the concentration of free radicals (or other reactive oxygen species, ROS) in cells [1,2,13–15]. It is well known that ROS, leads to oxidative damage in major cell macromolecules such as lipids and nucleic acids. Oxidative DNA damage has been implicated in aging, carcinogenesis and other degenerative diseases. Although more than 20 different oxidative modifications of DNA bases have been identified, main attention has been focused on 8-hydroxy-2′-deoxyguanosine (8OHdG) [16]. 8OHdG is one of the predominant forms of radical-induced lesions to DNA due to the $HO^{\cdot}$ attack at the C8 of guanine and it is frequently analyzed as a marker of cellular oxidative stress relevant to carcinogenesis, because 8OHdG has been shown to cause G-to-T transversions [16]. Therefore, the importance of 8OHdG is that it reflects mutagenity under *in vivo* conditions. Investigators suggested the analysis of 8OHdG in leukocyte DNA as a potential tool to asses the cancer risk. However, it increases not only in cancer cases but also in other ROS mediated diseases [5,16–18].

The present study employs 8OHdG assays, based on HPLC-EC, to address the question whether *in vivo* exposure of rat leukocyte DNA to ELF-EMF leads to genetic effects associated with increased risk of cancer. To examine possible time-dependent changes in oxidative DNA damage resulting from MF, the effects of 50 and 100 days exposure to MF and sham conditions were quantitated for rats.

The magnetic field strength used in present study is within the limits contained in occupational and public environment MF exposure guideline standards and it exists in both the public and the occupational environments [19]. MF intensity in this study is in the range of MF emitted from some electrical appliances [20,21]. In order to support our observation, we also evaluated the possible influence of ELF-EMF on the lipid peroxidation (LPO) in their plasma samples.

Material and methods

Chemicals

Chemicals were HPLC grade where available and otherwise were reagent grade (Sigma, St. Louis, MO). 8OHdG and deoxyguanosine (dG) standards were a generous gift from Dr Miral Dizdaroglu (National Institute of Standards and Technology, Gaithersburg, MD, USA).

Animals

The experiments were performed on 48 female Wistar rats obtained from Medical Science Application and Research Center of Dicle University (DÜSAM), aged 2 months at the beginning of the study, weighing 183–203 g, and fed with standard pelleted food (TAVAS Inc. Adana, Turkey). The rats were divided four groups of twelve: Two controls (sham) and two experimental. The experimental groups have been exposed to 0.97 mT ELF in methacrylate boxes (17 × 17 × 25 cm). The first group ($n = 12$) were exposed to EMF throughout 50 days, the second group ($n = 12$) throughout 100 days, 3 h a day. Third ($n = 12$) and fourth ($n = 12$) groups were sham groups that were treated like experimental group except ELF-EMF exposure (corresponding to first and second groups, respectively). The animals were kept in 14/10 h light/dark environment at constant temparature of 22 ± 3°C, 45 ± 10% humidity. This protocol was approved by the local ethics committee.

Magnetic field generation and exposure of rat to magnetic field

The MF was generated in a device designed by us that had two pairs of Helmholtz coils of 25 cm in diameter. This magnet was constructed by winding 225 turns of insulated soft copper wire with a diameter of 1.0 mm. Coils were placed vertically and horizontally as facing one another. The distance between coils was 25 cm. An AC current produced by an AC power supply (DAYM, Turkey) was passed through the device. The current in the wires of the energized exposure solenoid was 0.65 A, which resulted 50 Hz MF. The MF intensity was measured as mean 0.97 ± 0.136 mT in different 15 point of methacrylate cage by using digital teslameter (Phywe, 209101074, Germany) by a person who is not involved in the animal experiment. No temperature differences were observed between exposure and sham coils during the exposure. Ambient fields were varied from 0.1 to 0.2 μT (1–2 mG). We measured ambient fields with a cell sensor (Cell Sensor EMF detection meter, Tec Health Corp, USA, sensitivity 0.1–5 μT [1–50 mG]).

The animals were sacrificed after the last exposure by anaesthesia with ketalar (50 mg/kg, im) and the blood of the animals was withdrawn into ethylenediaminetetraacetic acid (EDTA)-containing syringes by cardiac puncture. Approximately 10 ml whole blood centrifuged at 1000*g* at 15°C for 10 min to separate the plasma from the infranatant. Plasma was stored at −80°C until TBARS measurement. The buffy coat fraction was collected from the interface between plasma and red blood cells (RBC) and transferred to a 20 ml centrifuge tube on ice. The remaining RBCs were removed by hypotonic lysis

with 20 ml of 20 mM tris (hydroxymethyl)-aminomethane (Tris)-HCl buffer (pH 8.0) containing 5 mM of EDTA.

DNA isolation methods

Isolation of leukocytes from DNA was performed using the method of Lodovici et al. [22] with some modifications. Briefly leukocytes were resuspended in 4 ml of 10 mM Tris-HCl buffer (pH 8.0) containing 10 mM EDTA, 10 mM NaCl, and 0.5% sodium dodecyl sulphate and then were incubated at 37°C for 1 h with RNAse (20 μg/ml). Leukocytes were then incubated at 37°C for 3 h with proteinase K (200 μg/ml). After incubation, the mixture was extracted with chloroform/isoamyl alcohol (10:2, v/v) in the presence of a 0.2 volume of 10 mM ammonium acetate, and DNA was precipitated from the aqueous phase as reported [22]. DNA concentrations were estimated spectrophotometrically using a specific absorption of 20 A_{260} U/mg. Portions of DNA samples (3–4 A_{260} units) were dissolved in 200 μl of 20 mM sodium acetate buffer (pH 4.8).

Enzymatic hydrolysis of DNA and analysis of steady-state levels of 8OHdG

8OHdG measurement was performed by a modification of the method of Kasai [16]. Briefly, DNA samples (150–200 μg) were denatured at 90°C for 5 min, and immediately placed in an ice water bath. The samples were incubated with 20 μg of nuclease P1 (E.C. 3.1.30.1) for 1 h at 65°C. Finally, the mixture was digested for 2 h at 37°C with 10 μl of calf intestine alkaline phosphatase (E.C.3.1.3.1) (2 IU) in the presence of 10 μl of 1 M Tris-HCl buffer (pH 8.5). In all these procedures the samples were protected from light with aluminium foil. The hydrolyzed mixture was filtered with vacuum prior to HPLC analysis to remove enzymes and other macromolecules (Millipore, Bedford, MA, USA), and 100 μl was injected into the HPLC apparatus. The nucleosides were separated by C18 reverse-phase column (Supelco, 5 μm, ID 0.46 × 25 cm) equipped with a guard column under isocratic conditions. The eluting solution was 90% 50 mM KH_2PO_4 (pH 5.5) containing 10% methanol at a flow rate of 1 ml/min. The solution was filtered by vacuum through a 0.22 μM cellulose acetate filter. For analysis a Shimadzu (Japan) Series LC-10AD pump system equipped with pulse damper and autosampler (SIL 10A, Shimadzu, Japan) was used, connected to DECADE (Antec Leyden, Leiden, Netherlands) digital electrochemical amperometric detector and Shimadzu UV detector (SPD 10 V, Japan) serially. The 8OHdG and dG were detected using electrochemical (600 mV) and UV (254 nm) detector, respectively. Deoxyguanosine (0.5 mg/ml) and 8OHdG (5 ng/ml) solutions were used as standard samples. Identification and quantification of 8OHdG and dG were performed by comparison with retention time and by the method of peak-area measurement using a linear regression curve for standard solutions. Data acquisition was performed by Shimadzu Class-LC10 software (Japan). The retention time for dG was about 5.5 min and that for 8OHdG was 7.5 min. A representative chromatogram is presented in Figure 1. The 8OHdG levels were expressed as the ratio of 8OHdG per 10^5 dG.

TBARS assay

Plasma TBARS levels were determined by spectrophotometric method and expressed as nmol/ml using a molar extinction coefficient of $1.56 \times 10^5\,M^{-1}\,cm^{-1}$ at 535 nm [23].

Statistical analysis

Statistical analysis was performed with SPSS 8.0 package (SPSS Inc., Illinois, USA). The analysis of differences between exposed groups and sham groups for each variable on given days was performed using independent sample t test. In order to determine the significance of interactions between variables, linear regression analysis was made for each group.

Results

Data concerning steady state levels of 8OHdG and TBARS levels in exposed and sham groups on 50 and 100 days are shown in Table I. Determined statistical differences was shown in the same table.

When data processed at 50 and 100 days, the steady-state levels of 8OHdG in MF-exposed rats were found to be significantly different from sham group ($p < 0.001$ and $p < 0.001$, respectively). The higher TBARS levels were also detected in the exposure group both on 50 and 100 days ($p < 0.001$ and $p < 0.001$, respectively).

In addition, prolonging the duration of exposure time from 50 to 100 days significantly increased both steady state levels of 8OHdG and TBARS levels ($p < 0.05$ and $p < 0.05$, respectively). When sham groups compared at 50 and 100 days, nor 8OHdG neither TBARS levels were changed statistically ($p > 0.05$).

By linear regression analysis, significant correlations were observed in the exposed group (50 days) between 8OHdG/dG ratio and TBARS concentrations ($r = 0.672$, $p < 0.05$). But a correlation between 8OHdG/dG ratio and TBARS levels was not found in 100 days ($r = 0.023$, $p > 0.05$).

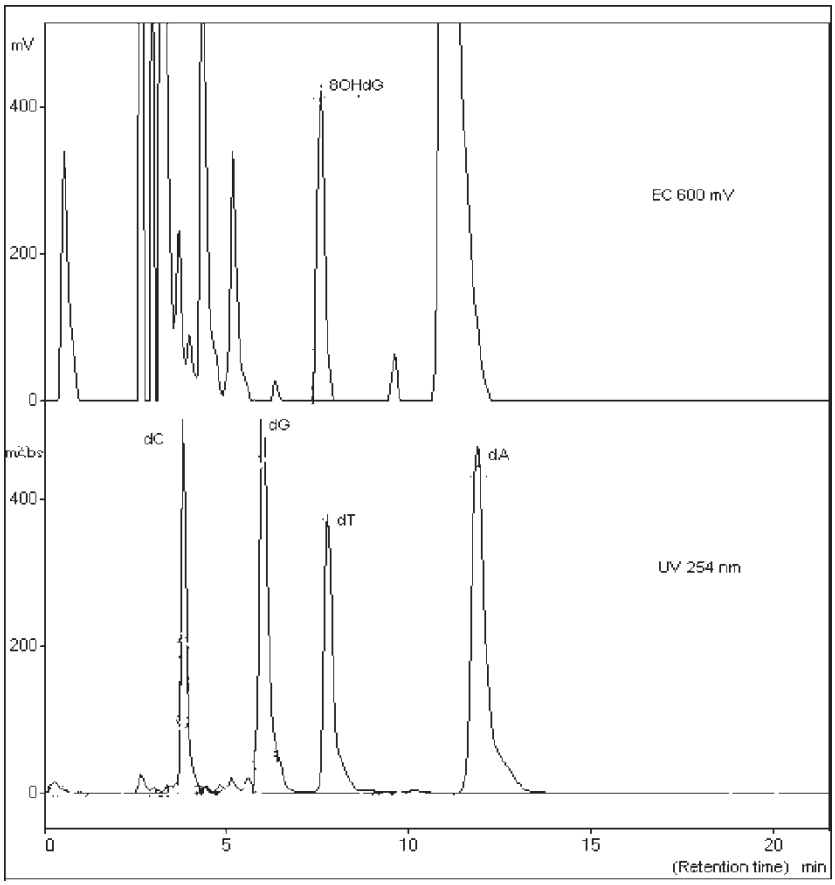

Figure 1. Typical HPLC profiles obtained during simultaneous 254 nm optical density and electrochemical analysis (600 mV) from enzymatic hydrolysate of DNA (100 μl injections) from leukocytes of female Wistar rat exposed ELF-EMF.

Discussion

Knowledge concerning any genotoxic potential of EMF is an important basis for the assessment of EMF-induced cancer risk. In this study, our main objective was to investigate possible effects of ELF-EMF on the oxidative DNA damage. As far as we know, few studies have been investigated related with the effect of ELF-EMF on the DNA damage.

A series of studies by Lai and Singh [1,2,24] reported that whole-body exposure to 0.1–0.5 mT power frequency MF (60 Hz) can result in DNA strand breaks and DNA-protein crosslinks in the brain cells of rodents. Similarly, Svedenstal et al. observed an increase in DNA double strand breaks in brain cells of rat after 32 days of exposure to MF of 7.5 μT [25] and after 14 days at 0.5 mT [26]. In Ahuja et al. [27] and Phillips et al. [3] studies, an increase in DNA single strand breaks was observed after exposure to MF in human lymphocytes (50-Hz, 0.2–2 mT, 1 h) and human Molt-4 cells (60-Hz, 0.1 mT, 24 h). Similar findings have been reported by other researchers. The results of Zmyslony et al. [28] and Jajte et al. [29] indicated that 3 h exposure to 7 mT MF, static or 50 Hz, can induce DNA damage in rat lymphocytes if the cells were simultaneously treated with iron ions. More recently, Ivancsits et al. [5,30] suggested intermittent (5 min on/10 min off) exposure

Table I. The 8OHdG/dG.10^5 ratio and TBARS concentration of the exposed and sham exposed groups in 50 and 100 days.

	Exposed groups		Sham (control) groups	
	50 Days (n=12)	100 Days (n=12)	50 Days (n=12)	100 Days (n=12)
8OHdG/dG.10^5	$4.39 \pm 0.88^{a,b,d,\star}$	$5.29 \pm 1.16^{a,c,e}$	$3.02 \pm 0.63^{b,c}$	$3.46 \pm 0.38^{d,e}$
TBARS (nmol/ml)	$26.37 \pm 4.44^{a,c,e,\star}$	$33{,}03 \pm 8.42^{b,d,e}$	$19.59 \pm 2.27^{a,b}$	$19.96 \pm 1.64^{c,d}$

All values are given as mean ± SD. The measured variables were compared with both the others groups in same row (Independent-samples t test) and with the other variable in the same group (Linear-regression analysis).

[a,b,c,d,e] The differences between mean values having same superscripts in each row is important (at least $p < 0.05$).

[⋆] There was a significant correlation between 8OHdG/dG ratio and TBARS level ($p < 0.05$).

to MF (50 Hz, 20–1000 μT-24 h) causes a reproducible increase in DNA strand breaks in human fibroblasts, whereas continuous exposure had no significant effect.

In those studies, it is mainly supported that ELF-EMF can cause DNA damage; however, some authors suggested controversial results. Miyakoshi et al. [31] indicated that ELF (5–400 mT, 30 min) alone did not induce DNA strand breaks in human glioma cells. Reese et al. [32] and Fairbairn et.al. [33] reported that EMF did not cause significant effect on DNA strand break in rat ovary cell (60 Hz, 0.1–2 mT, 1 h) and human cell, respectively. Fiorani et al. [34] and Stronati et al. [35] did not observed significant difference between ELF-EMF exposed and unexposed samples (50 Hz, 0.2–200 μT and 1 mT, 2 h, respectively). Furthermore, McNamee et.al. [36] determined that acute MF exposure (60 Hz, 1 mT, 2–24 h) did not cause DNA damage in the cerebellums of immature mice.

The reason for the conflicting results probably occur because of the difference of exposure setups, experimental conditions such as an alternative or static MF, the frequency, intensity and duration time of MF, the time of recovery, investigation targets and assay methods [5,30]. Although similar exposure time and intensity of MF have been used to determine the DNA damage in those studies, contradictory results attracted attention. If some researchers give different answers to the question "whether the MF can influence the DNA damage" another possible reason for this is that used methods for analysis of DNA damage.

The most of the authors mentioned above used the comet assay (Single-cell gel electrophoresis, SCGE) to determine strand breaks or other DNA damages. It is important to recognize that ELF-EMF is nonionizing because its intrinsic (quantum) energy is too low to dislodge an electron from a struck molecule [37]. Because of this reason, ELF-EMF can not directly affect macromolecules such as DNA and lipids. Most of the authors suggested that MF could increase the concentration of the free radicals (ROS) in cells and affect the biological systems by prolonging the life of free radicals [1,2,13–15]. If it is thought that MF causes oxidative DNA damage by ROS, it will be more appropriate to assay 8OHdG and the other products of oxidative DNA damage which is formed specifically for ROS attack. Whereas, Comet technique not only indicate direct ROS damage, but also indicates indirect damage such as alkali labile sites, single and double-stranded DNA breaks, incomplete excision repair sites and inter strand cross-links. The most of the indirect damages are formed not only by ROS, but also by several other factors such as "ionizing radiations" i.e. ultraviolet light, X-rays, and nuclear emissions that strike a molecule have far greater intrinsic energy [37].

Some authors suggested that ELF-induced damage could be removed by DNA repair mechanisms at extended off-times (>15 min) in intermittent exposure [30], and after termination of exposure the induced comet tail factors returned to normal level within 9 h by repair mechanism [5]. Therefore, obtained results can vary depending on when evaluated due to the recovery time. Whereas, 8OHdG assay has advantage that reflects *in vivo* balance between DNA damage and cellular repair.

Although we have not been able to see a published value about the possible influence of ELF-EMF on the 8OHdG levels, our results bear some resemblances with some of the other studies on the effects of ELF-EMF on DNA damage [1–3,24–30]. In addition, our study is approved by previous investigator that they found that the extent of DNA damage would depend on the duration of exposure time [5,30]. These observations suggest that MF lead to accumulative effects. The reason of the increased 8OHdG levels may be due to increased ROS concentration and/or decreased DNA repair enzyme activites.

Several studies have suggested a relationship between increased levels of 8OHdG and aging [38–40]. Shigenaga and Ames [38] determined that the 8OHdG level was increased approximately two-fold in some tissues of 2 month and 24-month-old Wistar rats because of the aging. According to our results, sham group of 100 days had higher median 8OHdG level than sham group of 50 days, although this difference was not significant. The absence of an age-related increase in 8OHdG levels might be dependent on the short interval of the ages among our sham groups.

On the other hand, our experimental results showed there was a positive effect of EMF on LPO and a correlation with exposure time. Similar findings have been reported by other researchers [41–43].

If our results are totally evaluated, it may provide more evidence for the hypothesis that ELF-EMF can damage macromolecules increasing perhaps ROS induction. However, some authors suggested that ELF-EMF can cause DNA damage by affecting DNA repair process and antioxidant enzyme status [24,44].

However, 8OHdG is not the only product resulting from oxidative DNA damage, there are other techniques such as GC-MS capable of measuring other DNA damage products as mutagenic as 8OHdG [45] that we only measure in our studies and those techniques can be applied. Also, further examination of the interaction of MF with DNA, and DNA repair enzymes (i.e. ogg, fpg, endonucleases and their analogues), and antioxidant enzyme status is required.

Because of the differences in body size, geometry and physiological responses of rats used in this study, it is difficult to express that results obtained in this study will create same effects on humans and any such

comparison between the rats and human should be made carefully.

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