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The effect of electromagnetic fields on oxidative DNA damage

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Abstract

Many recent studies have focused on the investigation of the biological effects of electromagnetic field. Although the several types of biological effects of electromagnetic fields have been shown, the molecular mechanisms of these effects have not been explained yet. Some epidemiological studies have suggested that exposure to ambient, low-level 50-60 Hz electromagnetic fields increase risk of disease including cancer such as leukemia among children who live close to power lines or among men whose jobs expose them to electromagnetic field, while others have suggested that electromagnetic fields exposure could increase both the concentration of free radicals and oscillating free radicals. Electromagnetic fields are known to affect radical pair recombination and they may increase the concentration of oxygen free radicals in living cells. In this study, oxidative stress was formed by the oxidation of ascorbic acid and the effect of 50 Hz, 0.3 mT electromagnetic fields on the oxidative DNA damage has been investigated. The results of the study showed that extremely low-frequency electromagnetic fields enhanced the effect of oxidative stress on DNA damage and supported the idea obtained from the previous studies on an increasing effect of electromagnetic fields on the concentration and the life-time of free radicals.

Key words: Electromagnetic fields, DNA damage, ascorbic acid, vitamin C, oxidative stress

Elektromanyetik alanın oksidatif DNA hasarı üzerindeki etkisi

Özet

Günümüzdeki birçok çalışma, elektromanyetik alanın biyolojik etkilerinin araştırılması üzerinde odaklanmıştır. Elektromanyetik alanın biyolojik etkilerinin bazı türlerinin gösterilmiş olmasına rağmen, bu etkilerin moleküler mekanizmaları henüz açıklanamamıştır. Bazı epidemiyolojik çalışmalar, 50-60 Hz dolayındaki düşük düzeyli elektromanyetik alana maruz kalmanın yüksek gerilim hatlarına yakın yaşamakta olan çocuklarda veya elektromanyetik alana maruz kalarak çalışanlarda görülen lösemi gibi kanser vakalarını kapsayan hastalıklara ilişkin riski artırdığını öne sürerken, bazı çalışmalar ise elektromanyetik alan maruziyetinin serbest radikal konsantrasyonunu ve serbest radikallerin izlenebilirliğini artırabileceğini ileri sürmüştür. Elektromanyetik alanın radikal çifti rekombinasyonunu etkilediği bilinmektedir ve bu da, hücrelerdeki oksijene dayalı serbest radikal konsantrasyonunu artırabilir. Bu çalışmada, askorbik asit oksidasyonu ile oksidatif stres oluşturulmuş ve 50 Hz, 0.3 mT düzeyindeki elektromanyetik alanın, oksidatif DNA hasarı üzerindeki etkisi araştırılmıştır. Bu çalışmanın sonuçları, oldukça düşük frekanslı elektromanyetik alanın, oksidatif stresin DNA hasarı üzerindeki etkisini artırdığını göstermiş ve önceki araştırmalardan elde edilen, elektromanyetik alanın serbest radikal konsantrasyonu ve yarı ömrü üzerindeki artırıcı etkisine dair düşünceleri desteklemiştir.

Anahtar sözcükler: Elektromanyetik alan, DNA hasarı, askorbik asit, C vitamini, oksidatif stres

Introduction

There are many reports on the biological effects of electromagnetic fields (EMF) and there have been many attempts to develop a theoretical explanation of this phenomenon. Some epidemiological studies have suggested that exposure to ambient, low-level 50/60 Hz EMF increases risk of disease including cancer such as leukemia among children who live close to power lines or among men whose jobs expose them to EMF (Wertheimer and Leeper, 1979; Tomenius, 1986; Savitz et al., 1988; London et al., 1991). EMF firstly affects the cell membrane. Some ion channels such as Na-K ATPase have been affected according the level of EMF. The alteration in the activity of these proteins causes an increasing or decreasing intracellular concentration of many ions such as Na^+, K^+, Mg^{2+} and Ca^{2+} which plays very important roles in cell signaling. Therefore, the biological effects of EMF expand among the cellular systems (Goodman et al., 1995). Although the several types of biological effects of EMF have been shown, the molecular mechanisms of these effects have not been explained yet. Some studies have suggested that EMF exposure could be due to both the increase in the concentration (Jajte, 2000) and oscillating of free radicals (Scaiano et al., 1995). EMF is known to affect radical pair recombination and they may increase the concentration of oxygen free radicals in living cells (Jajte, 2000). Increasing the concentration of free radicals creates oxidative stress and some biological reactions such as DNA damage occur under this condition. Metabolic energy production or effects of chemicals and radiation can form oxidative stress.

In this study, oxidative stress was formed by the oxidation of ascorbic acid with Cu^{2+} ions and the effect of 50 Hz, 0.3 mT EMF on the oxidative DNA damage was investigated.

Materials and methods

DNA isolation

High molecular weight (app. 10 kb) human genomic DNA was isolated from the white blood cells with the modified method of Poncz et al. (1982) by using MBI Fermentas genomic DNA isolation system. Molecular weight and purity of DNA samples were controlled by agarose gel electrophoresis. The concentration of DNA samples was spectrophotometrically determined. All DNA samples were free from proteins, RNAs and solvents used for extraction.

Oxidative DNA cleavage reactions

Cleavage reactions were carried out in a medium containing 0.5 µg DNA, 20 mM Tris-HCl (Sigma) pH 7.8, 0.25 mM ultra-pure ascorbic acid (Merck) and $CuCl_2$ (Sigma) in the final concentrations of 2.5, 5, 7.5 and 10 µM, in a final volume 10 µl. Other antioxidants (glutathion, cystein and dithiothreitol, Sigma) and metal chelator (EDTA, Merck) were added to the reaction mixtures at a final concentration of 0.5 mM. The mixtures were incubated at room temperature for 10, 20 and 30 minutes. Adding EDTA at a final concentration of 25 mM stopped reactions. DNA cleavages in the reaction mixtures were analyzed on the 1% agarose gel (Promega) electrophoresis.

EMF exposure system

Electromagnetic fields were applied by using the Helmholtz coil. The coil system was constructed by using the polyester sphere that was surrounded by copper wire with 0.75 cm diameter (Galt et al., 1995). The diameter and height of the sphere were 16 and 26 cm, respectively. 50 Hz, 4.5 V electricity was applied to coil system. As a result, 0.3 mT EMF was generated at the center of the coil system which includes handles for the sample tubes.

Results and discussion

The oxidative DNA damage was induced by the concentration of cupric ions (Figure 1). In the constant ascorbate concentration (0.25 mM), oxidative DNA breakage was started in the presence of 2.5 µM copper(II) ions and EMF also induced the DNA breakage at this condition (Figure 1, lanes 4 and 10). Therefore, main DNA band in the lane 10 of Figure 1 is thinner than the lane 4. In the presence of high cupric ions concentration, excess scission of DNA molecules occurred at the EMF when compared to normal conditions (Figure 1, lanes 6 and 12). Electromagnetic fields did not have an effect on the oxidation of ascorbic acid in the absence of cupric ions (specific data was not shown, but the sample in Figure 3, lane 12 had reflected this result, because EDTA was

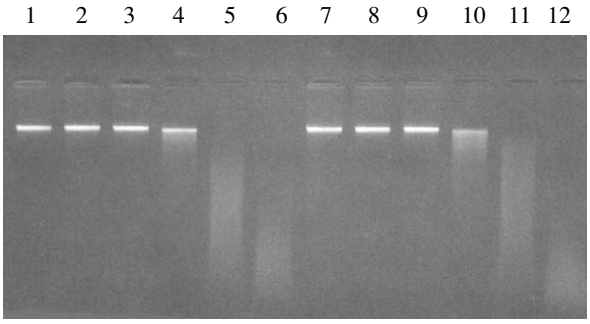

Figure 1: The effect of EMF and Cu(II) concentrations on oxidative DNA damage. All lanes include 0.5 µg DNA. The DNA samples in the lanes from 1 to 6 were incubated under normal condition, 7 to 12 were incubated in EMF at room temperature in the presence of 0.25 mM ascorbic acid except the 1 and 7 which were control lanes. Cu(II) concentrations were 1.25 µM in 2 and 8, 2.5 µM in 3 and 9, 5 µM in 4 and 10, 7.5 µM in 5 and 11, 10 µM in 6 and 12 lanes. Incubation time was 30 min for all samples.

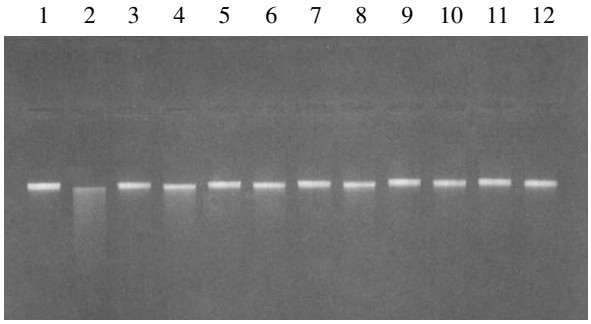

Figure 2: The effect of incubation time on oxidative DNA damage depends in electromagnetic fields. All lanes include 0.5 µg DNA. Incubation times were 30 min from 1 to 4, 20 min from 5 to 8 and 10 min from 9 to 12. Ascorbic acid concentration were 0.25 mM in all lanes. Cu(II) concentrations were 2.5 µM in 1, 3, 5, 7, 9 and 11 while 5 µM in 2, 4, 6, 8, 10 and 12. The samples in 1, 2, 5, 6, 9 and 10 were incubated in EMF. Other samples were incubated at normal conditions.

chelating cupric ions and eliminated their oxidative effects).

The results of this study showed that the oxidative DNA damage depends on the incubation time (Figure 2). DNA breakages could be observed at the 20th minute of incubation time (Figure 2, lanes 6 and 8). EMF exposure enhances the oxidative DNA damage after the 20th minute (Figure 2, lanes 2 and 4).

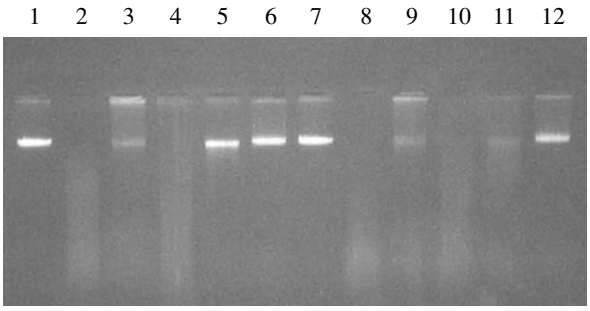

Figure 3: The effect of some antioxidants (glutathione, cystein, dithiothreitol) and metal chelator (EDTA) on excessive oxidative DNA damage in EMF. All lanes include 0.5 µg DNA. Ascorbic acid and Cu(II) concentrations were 0.25 mM and 7.5 µM in all lanes except the control DNA lanes 1 and 7, respectively. Lanes 2 and 8 were scission controls. Glutathione (3 and 9), cystein (4 and 10), dithiothreitol (5 and 11) and EDTA (6 and 12) concentrations were 0.5 mM. The samples in 1 to 6 were incubated at normal condition. The others were incubated in EMF. Incubation time was 30 min for all samples.

In the presence of EDTA as a cationic metal chelator, oxidative DNA damage was not observed. This result showed that ascorbate oxidation and oxidative DNA damage depend on cupric ions as an oxidizing agent (Figure 3, lanes 6 and 12). As an antioxidant, cystein did not block the oxidative DNA damage (Figure 3, lanes 4 and 10). Glutathione reduced the oxidative stress. Therefore, the DNA damage was formed as aggregation rather than fragmentation in the presence of glutathione (Figure 3, lanes 3 and 9). Dithiotreitol (DTT) was the most effective antioxidant of all investigated but EMF exposure inhibited the effectiveness of DTT (Figure 3, lanes 5 and 11).

The oxidative species produced by ascorbate oxidation in the presence of copper(II) ions damage the DNA molecules (Figure 1). Previously DNA damage depending on ascorbate oxidation had been studied (Erdem et al., 1994; Zareie et al., 1996). Oxidative DNA damage was observed as fragmentation or aggregation. The degree of oxidative DNA damage varies in the levels and reactivity of free radicals produced in the reaction medium. In the presence of oxygen, the hydroxyl and peroxyl radicals such as superoxide anion and hydroperoxyl radical are produced by the reaction between radical form of ascorbic acid (ascorbyl radical) and molecular oxygen (Fuchs et al., 1990).

These radicals attack to electrophilic nuclei on the targets and create secondary carbon radicals. At the

high level or high reactivity of these radicals, excess formation of secondary carbon radicals on the same DNA molecule causes a reaction between each other and then the DNA damage occurs as fragmentation. Therefore, DNA size became smaller and gave the smeared patterns on gel electrophoresis (lanes 5 and 6 in Figure 1) However, at the low level or low reactivity of oxygen species, the oxidative DNA damage results in aggregation of the DNA molecules with the intermolecular reaction of the secondary carbon radicals. Thus, the DNA samples became heavier and were retarded on the gel electrophoresis (lanes 3 and 9 in Figure 3).

Our results showed that extremely low-frequency EMF enhanced the effect of oxidative stress on DNA damage and supported the idea obtained from previous studies on an increasing effect of EMF on the concentration and the life-time of free radicals (Jajte, 2000; Scaiano et al., 1995; Jajte and ZmySlony, 2000). Especially the comparisons of lane 2 to lane 4 in Figure 2 and lane 5 to lane 11 in Figure 3, indicate that the degree of the oxidative stress under the EMF is greater than the normal condition.

In the brain cells of rats, an increase in DNA single- and double-strand breaks had been found after acute exposure to a sinusoidal 60 Hz magnetic field. When the experiment was carried out in the presence of melatonin or a radical scavenger compound N-*tert*-butyl-alpha-phenylnitrone (PBN), the effect of magnetic fields on brain cell DNA was not observed (Lai and Singh, 1997). Melatonin is a neurohormone and it is also an antioxidant and a free radical scavenger. Therefore, this hormone could protect biological systems against oxidative damage. The increasing effect of EMF on the concentration of free radicals has been suggested that melatonin suppression in humans may increase the probability of mutagenic and carcinogenic risk (Jajte and ZmySlony, 2000).

EMF (≥1 mT) increases the concentration of free radicals that escape from the alkyl sulphate and sulphonate micelles. The effect of extremely low-frequency EMF on the radicals formed from singlet precursors is larger than triplet precursors. Some radicals such as hydroxyl and peroxyl radicals generated in the biological reactions are formed from singlet precursors (Eveson et al., 2000).

In conclusion, the results obtained from our study suggest that the effects of extra low frequency EMF on the concentration of free radicals and the recombination of radical pairs might trigger the carcinogenesis in the populations living close to the overhead electric power distribution lines.

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