J. Egypt. Acad. Soc. Environ. Develop., (C-Molecular Biology)
Vol. 6. No. (1): 41 – 60 (2005) ISSN 1110 - 8762

THE ELECTROMAGNETIC SPECTRUM INDUCES MUTATION IN MITOCHONDRIAL NADH UBIQUINONE OXIDOREDUCTASE SUBUNIT GENE

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Key words: electromagnetic spectrum, mitochondrial NADH ubiquinones oxidoreductase subunit gene, PCR/RFLP.

ABSTRACT

The Polymerase Chain Reaction/Restriction Fragment Length Polymorphisms (PCR/RFLPs) technique was selected as a biomarker to evaluate the effect of exposure to electromagnetic speculum radiation in three groups of albino rats (*Rattus rattus*) exposed to electromagnetic spectrum radiation (10,16 and 20 pulses/day; three days a week for three weeks). A fourth group of rats was kept as untreated control. The primary DNA damage was evaluated by monitoring the fragmentation of mitochondrial NADH ubiquinone oxidoreductase subunit gene in the hepatocytes. The inter-individual differences in gene damage between exposed subjects were compared with the mitochondrial NADH ubiquinone oxidoreductase subunit gene of the unexposed rat. It was found that rats which were exposed to different doses of electromagnetic spectrum radiation showed highly significant increases in levels of gene damage compared with controls. However, influences of the different doses absorbed on the levels of' DNA damage, assessed by use of the PCP/RFIPs. might be excluded in the majority of subjects. A new diagnostic hypothesis for detecting the effect of electromagnetic spectrum on the liver of albino rats is proposed based on the analysis of mitochondrial NADH ubiquinone oxidoreductase subunit gene/RFLP by using AflII, ApaI, PstI, HaeII, DsaI, SspBI and DraIII restriction endonucleases. The results obtained have confirmed the usefulness of the PCR/RFLPs as an additional complement to the standard biodosimetric methods.

ACKNOWOLEDGEMENT*: The authors indebted to* ***Prof. Dr. Nahed Mohamed El-Abiad,*** *Biological Application Dept. For her sincere guidance and valuable thanks to* ***Dr. Gamal Gaber Mostafa El-Argi,*** *Plasma Dept., who did his best during the exposure time.*

INTRODUCTION

The electromagnetic spectrum (ionizing and non-ionizing radiations) is a ubiquitous environmental physical agent whose DNA-damaging effects are fairly well established. Its physico-chemical interaction with cellular DNA produces a variety of primary lesions, such as single-strand breaks (SSBs). alkali-labile sites, double-strand breaks (DSBs), DNA - DNA and DNA - protein crosslinks and damage to purine and pyrimidine bases (Natarajan, 1993; Kruszewzki *et al*., 1998; Chaubey *et al*., 2001).

In mammalian mitochondria, complex I catalyses the proton-motive oxidation of NADH by ubiquinone. It consists of 43 Subunits (Walker, 1992 and Skehel et al., 1998). Seven subunits (ND1-ND6 and ND4L) are encoded by the mitochondrial genome (Chomyn *et al*., 1985 and 1986) the other subunits are encoded by nuclear genes.

A wide range of methods are presently used for the detection of early biological effects of DNA-damaging agents in environmental and occupational settings. Currently, unstable chromosomal aberrations in peripheral blood lymphocytes, in particular dicentrics, are the most fully developed biological indicators of ionizing radiation exposure (IAEA, 1986; Carrano and Natarajan, 1988; Bauchinger, 1995; Ramalho *et al*., 1998).

In the last few years, the single cell gel electrophoresis (SCGE) or Comet assay has been widely used for genotoxicity testing (Singh *et al*., 1988; Tice et al., 1990; Fairbairn et al., 1995 and Olive, 1999). In the molecular studies, DNA damage evaluated by the Comet assay and RFLPs technique have been. used as a biomarker of radiation exposure (Betti *et al*., 1994; Collins *et al*., 1997; Garaj-Vrhovac and Kopjar, 1998; Sram *et al*., 1998; Piperakis *et al*., 1999; Kopjar and Garaj-Vrhovac, 2001; Maluf *et al*., 2001; Garaj-Vrhovac *et al*., 2002).

There is widespread scientific and public interest in possible health hazards of exposure to electromagnetic, fields (EMFs) associated with radiofrequency (RF) and microwave (MW) radiation. This interest has resulted in numerous studies designed to assess both the occupational and residential health risk of' EMFs (Cleveland and Ulcek, 1999 and WHO, 2003).

Most *in vivo* evidence suggests that RF and MW radiation are not mutagenic and are therefore unlikely to initiate cancer. The majority of studies reported lack of a clastogenic effect (Verschaeve and Maes, 1998), but there were some studies reported an increase in the number of single-

strand and double-strand DNA breaks in the brain cells of rats exposed to pulsed or continuous wave 2.45 GHz radiation at specific absorption rates (SARs) of 0.6 and 1.2 W/kg (Sarkar *et al*., 1994 and Lai and Singh, 1996). These studies have been criticized on the basis of deficiencies in the procedures used to process the DNA and the gel electrophoresis methods used to determine the presence of strand breaks (Williams, l 996).

Oxidation of fuel molecules produces NADH and FADH2, and electrons generated from NADH/FADH2 are transferred between complexes I - IV to molecular oxygen, the final electron acceptor. The transfer of electrons releases energy that is stored in the form of a proton gradient across the inner mitochondrial membrane, and this energy is used to produce ATP. Individual cells contain a large number of mitochondria (e.g., ~1500 in a liver cell), and each mitochondrion has hundreds to thousands of DNA molecules. mtDNA is a circular, intronless molecule ~ 6.6 kb in size that encodes 13 polypeptide components of the ETC, 22 tRNAs and 2 rRNAs (Wallace, 1999). Mitochondrial DNA has a higher mutation rate than nuclear DNA (nDNA) as the mtDNA molecule is not protected by histones, is exposed to reactive oxygen species generated during oxidative phosphorylation. and is replicated by DNA pol-γ that copies with low fidelity due to the absence of a proof-reading function (Kirches et al.. 2001).

To further understand the effect of electromagnetic spectrum on the environmental organisms, we planned to determine the spectrum effect on liver mitochondrial NADH ubiquinone oxidoreductase subunit gene of albino rats digesting them by certain restriction endonuclease.

MATERIAL AND METHODS

Biological materials: In this study mature male albino rats (*Rattus rattus*) aged approximately two-three months and weighing 100-120 g were used. Rats were bred in the animal house of The Nuclear Research: enter. They were kept in plastic cages, each contain ten rats. They were given food and water a*d-libitum* and were kept under constant laboratory conditions. The animals were divided into four groups each of ten rats: the first group represent unexposed control, and the other 3 groups were exposed to different number of pulses of electromagnetic radiations (10, 15 and 20 pulses/day, respectively) from plasma focus device (PF-01 device). Rats were exposed three days/week for three weeks. 30 seconds was the time between each pulse. Electromagnetic

spectrum includes radiowaves, infrared, optical (visible light), ultraviolet and X-ray. The exposure system that was used to generate the magnetic radiations under static pressure of lmbar, frequency was from 8 to 12 GHz, and voltage was 15 kV, exposure was in the Plasma and Nuclear Fusion Department. The animals were scarified at the end of exposure time.

DNA Extraction: Total DNA was extracted from the liver of the rats as described by Awwad (2003). One pl of the resuspended pellet was checked by gel electrophoresis for the presence of DNA (Fig. I).

Polymerase Chain Reaction Amplification: A fragment of NADH ubiquinone oxidoreductase subunit gene of approximately 3090 base pairs length was amplified using the primers: (A) 5'- AAG CTT CAC TTA ACA GGC AAG ACA G -3' and (B): 3'- CCT ATA GCT GGC CAG AAG AGA GG -5'. Primers were designed from an alignment of the NADH ubiquinone oxidoreductase subunit gene sequences of rat, of which the whole mitochondrial genomes are known. The standard PCR reaction mixture was used (Kessing *et al*., 1989). The standard polymerase chain reaction program for ampli".ication of mitochondrial NADH ubiquinone oxidoreductase subunit gene was: 30 - 35 cycles; one minute, 94^{o}C; 2-3 minutes, 45^{o}C., and 3 minutes, 72^{o}C. Glassmilk DNA purification was used to purify the gene from the agarose gel. One µl of the resuspended pellet was checked by agarose gel electrophoresis for the presence of NADH ubiquinone oxidoreductase subunit gene (Fig. 2).

Restriction fragment length polymorphisms (RFLPs): Several restriction enzymes were used in this study; these are *Af1*II, *Apa*I, *Psa*I and *Hae*II (New England BioLabs; England) and *Dsa*I, *Ssp*BI and *Dra*III (Boehringer-Mannheim; Germany).

Restriction endonucleases were used to digest the NADH uhiquinone oxidoreductase subunit gene of the rats Digestion and RFLP analysis were performed as described by Vidigal *et al*., (1998).

RESULT

Mitochondrial NADH ubiquinone oxidoreductase subunit gene was obtained from the control animal group and the three treated rat groups (10, 15 and 20 pulses/day) obtained from the PCR. The sizes of NADH ubiquinone oxidoreductase subunit gene were approximately 3090 bp (Fig. 2).

*Af1*II, *Apa*I and *Pst*I restriction enzvmes did not differentiate the normal and the other three treated groups. *Af1*II restriction endonuclease

digested the NADH ubiquinone oxidoreduciase subunit gene of the normal and the three treated nit groups into four restriction fragments (~90, ~230, ~50 and ~2420 bp; Fig. 3, and Table 1). *Apa*I restriction enzyme cut the gene of the normal and the three groups into three restriction bands (~90, ~1130 and ~1870 bp: Fig. 4 and Table 2). Also, *Pst*I restriction endonuclease did not differentiate between the normal and the three treated groups where the enzyme cut the gene of the four groups into three bands (~450, ~880 and ~760 bp; Fig. 5 and Table 3).

*Hae*II restriction endonuclease clarified that the first group of the treated rats which exposed to 10 pulses/day of electromagnetic radiations when its NADH ubiquinone oxidoreductase subunit gene was digested into four restriction bands (~180, ~560, ~700 and ~1650 bp; Fig. 6, and Table 4), whenever the same restriction enzyme fragmented the gene of the normal and the other two groups into three fragments (~180, ~1260 and ~1650 bp; Fig. 6, and Table 4).

*Dsa*I and *Ssp*BI restriction enzymes elucidated the third treated group which was exposed to 20 pulses/day of electromagnetic radiations. *Dsa*I restriction endonuclease cut the gene of third treated group into four cuts (~500, ~650, ~830 and ~1010 bp; Fig. 7 and Table 5) and the normal and the other two treated groups into three bands (~650, ~830 and ~1610 bp; Fig. 7 and Table 5). Also, *Ssp*BI restriction endonuclease digested the gene of third treated group into four fragments (~470, ~500, ~590 and ~1630 bp; Fig. 8 and Table 6) and the normal and the other two treated groups into three bands (~370, ~1090 and ~1630 bp; Fig. 8 and Table 6).

*Dra*III enzyme clustered the normal and the three treated groups into two clusters when digested their NADH ubiquinone oxidoreductase subunit gene. *Dra*III restriction endonuclease cut the normal group into three restriction fragments (~450, ~1150 and ~1490 bp; Fig. 9 and Table 7) and fragmented the three treated groups into four restriction fragments (~100, ~450, ~1150 and ~1390 bp Fig. 9 and Table 7).

DISCUSSION

The PCR and RFLP analysis of the mitochondrial NADH ubiquinone oxidoreductase subunit gene, used here, has proven to be helpful in diagnostic studies of the effect of the electromagnetic spectrum on the liver of rats and to estimate genetic mutations in their DNA. The molecular data have been confirmed as an effective tool for studying DNA damages (Sachs *et al*., 2004).

A common theme of the animal studies on the effect of the electromagnetic spectrum is a serious DNA damage. In particular, electromagnetic spectrum inhibits cytochrome oxidase activity in the mitochondria of the brain and the liver (Dumanskij and Shandala, 1973; Webber *et al*., 1980; Schon, 2000; Mawrin and Dietzmann, 2001 and Lai and Singh, 2004). The liver became depleted of glycogen, the blood sugar curve was found to be affected, and the fasting blood glucose raised.

Mitochondrial activity of cytochrome oxidase was decreased under the effect of the electromagnetic spectrum exposure (Dumanskij and Shandala, 1973 and Belokrinitskiy, 1982). The later authors also found increased RNA and DNA in the liver and spleen, and structural changes in the liver, spleen, testes, and brain of white rats and rabbits exposed to electromagnetic spectrum.

In large clinical studies, Orlova (1960) noted decreased appetite, indigestion, epigastric pain, and enlargement of the liver in irradiated workers, while Gel´fon and Sadchikova (1960) also noted liver enlargement and tenderness in certain patients, with a decreased antitoxic function of the liver in a few workers. Trinos (1982) noted decreased appetite and indigestion, as well as chronic gastritis, cholecystitis, and decreased gastric acidity, especially in workers exposed to microwaves for more than ten years. Bachurin (1979) also note'd chrotuc gastritis and cholecystitis in workers occupationally exposed to electromagnetic spectrum.

The present study studied the effect of the electromagnetic spectrum on the mitochondrial NADH ubiquinone oxidoreductase subunit gene in liver cells of rats.

PCR/RFLP profile produced high variations between the normal mitochondrial NADH ubiquinone oxidoreductase subunit gene of control rat liver and three treated rat groups (10, 15 and 20 pulses/day) according to the differences of profiles obtained with the restriction endonucleases *Hae*II, *Dsa*I, *Ssp*BI and *Dra*III. On the other hand, the molecular results obtained with PCR/RFLP of mitochondrial NADH ubiquinones oxidoreductase subunit gene suggested that *Af1*II, *Apa*I and *Pst*I restriction enzymes did not detect the mutations of the gene which were exposed to the electromagnetic spectrum based on the similarity of profiles obtained with these restriction endonucleases.

The present study shows that PCR/RFLP is a simple and rapid technique representing an important progress in studies on the effect of electromagnetic spectnun on the organisms. The study demonstrated that

mitochondrial NADH ubiouinone oxidoreductase subunit gene contains useful genetic markers for the diagnosis and detection of the electromagnetic spectrum hazards.

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Table 1: Length of NADH ubiquinone oxidoreductase subunit gene fragments, resulting from digestion with *Af1*II enzyme of the normal and the three treated groups. (see Fig. 3)

Groups	Band #1	Band #2	Band #3	Band #4
Normal group	~90	~230	~350	~2420
10 pulses/day	~90	~230	~350	~2420
15 pulses/day	~90	~230	~350	~2420
20 pulses/day	~90	~230	~350	~2420

Table 2: Length of NADH ubiquinone oxidoreductase subunit gene fragments, resulting from digestion with *Apa*I enzyme of the normal and the three treated groups. (see Fig. 4)

Groups	Band #1	Band #2	Band #3	Band #4
Normal group	~90	~1130	~1870	--------
10 pulses/day	~90	~1130	~1870	--------
15 pulses/day	~90	~1130	~1870	--------
20 pulses/day	~90	~1130	~1870	--------

Table 3: Length of NADH ubiquinone oxidoreductase subunit gene fragments, resulting from digestion with *Pst*I enzyme of the normal and the three treated groups. (see Fig. 5)

Groups	Band #1	Band #2	Band #3	Band #4
Normal group	~450	~880	~1760	--------
10 pulses/day	~450	~880	~1760	--------
15 pulses/day	~450	~880	~1760	--------
20 pulses/day	~450	~880	~1760	--------

Table 4: Length of NADH ubiquinone oxidoreductase subunit gene fragments, resulting from digestion with *Hae*II enzyme of the normal and the three treated groups. (see Fig. 6)

Groups	Band #1	Band #2	Band #3	Band #4
Normal group	~180	~1260	~1650	--------
10 pulses/day	~180	~560	~700	~1650
15 pulses/day	~180	~1260	~1650	--------
20 pulses/day	~180	~1260	~1650	--------

Table 5: Length of NADH ubiquinone oxidoreductase subunit gene fragments, resulting from digestion with *Dsa*I enzyme of the normal and the three treated groups. (see Fig. 7)

Groups	Band #1	Band #2	Band #3	Band #4
Normal group	~650	~830	~1610	--------
10 pulses/day	~650	~830	~1610	--------
15 pulses/day	~650	~830	~1610	--------
20 pulses/day	~500	~650	~830	~1010

Table 6: Length of NADH ubiquinone oxidoreductase subunit gene fragments, resulting from digestion with *SspB*I enzyme of the normal and the three treated groups. (see Fig. 8)

Groups	Band #1	Band #2	Band #3	Band #4
Normal group	~370	~1090	~1630	--------
10 pulses/day	~370	~1090	~1630	--------
15 pulses/day	~370	~1090	~1630	--------
20 pulses/day	~470	~500	~590	~1630

Table 7: Length of NADH ubiquinone oxidoreductase subunit gene fragments, resulting from digestion with *Dra*III enzyme of the normal and the three treated groups. (see Fig. 9)

Groups	Band #1	Band #2	Band #3	Band #4
Normal group	~450	~1150	~1490	--------
10 pulses/day	~100	~450	~1150	~1390
15 pulses/day	~100	~450	~1150	~1390
20 pulses/day	~100	~450	~1150	~1390

THE ELECTROMAGNETIC SPECTRUM INDUCES MUTATIOM IN MITOCHONDRIAL NADH UBIQUINONE OXIDOREDUCTASE SUBUNIT GENE

طيف الموجات الكهروماغناطيسية يحدث طفرات في جين وحيدة الناده أبيكوينون (عامل الأكسدة الاختزالي الموجود في الميتوكوندريا)

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** قسم التطبيقات ألبيلوجية – مركز البحوث النوويه – هيئة الطاقة الذريه

تستخدم الموجات الكهروماغناطيسية في مجالات علمية تطبيقية وطبية عديدة. ومن هذا كان من المهم دراسة تأثير طيف الموجات الكهروماغناطيسية على حيوانات التجارب كنموذج يوضح تأثير هذه الاطياف على الإنسان وخاصة على العاملين في مثل هذه الحقول العلمية والطبية المعرضين بكثرة لتأثيراتها لعمل الاحتياطات اللازمة.

وقد أجريت هذه الدراسة باستخدام بعض التقنيات الجزيئية الحديثه. في هذا البحث تم استخدام التغاير في طول القطع المحددة لجين وحيدة الناده ابيكوينون (عامل الأكسدة الاختزالي) الموجود في الميتوكوندريا باستخدام تقنية تفاعل البلمرة المتسلسل / تعدد أطوال قطع القصر في الجرذان الألبينو المعرضة لعدد مختلف من نبضات الموجات الكهروماغناطيسية كأساس لمعرفة تأثيرها على الكبد، حيث تم فصل جين الوحيدة الصغيرة للناده ابيكوينون (عامل الأكسدة الاختزالي) الموجودة إ الميتوكوندريا الموجودة في خلايا الكبد وأكثاره باستخدم تفاعل البلمرة المتسلسل وبعد ذلك تم تعريض هذا الجين لتسعة طرز مختلفة من أنزيمات القصر هي: *Af1*II, *Apa*I, *Pst*I, *Hae*II, *Dsa*I, *Ssp*BI, *Dra*III .

وقد تم تقسيم الجرذان إلى أربع مجموعة: المجموعة الأولى ضابطه والمجموعات الثلاث الأخرى تم تعريضها لعدد مختلف من نبضات الموجات الكهروماغناطيسية (١٠، ١٥ و ٢٠ نبضة/يوم) للمجموعة الأولى والثانية والثالثة على التوالي وبمعدل ثلاث أيام في الأسبوع ولمدة ثلاثة أسابيع وكان تردد التيار الكهربي من ٨ – ١٢ جيجاهيرتز من جهاز البلازما فوكس (PF-01 device) وتم تحديد الطفرات التي حدثت بالجين في المجموعات المعرضة وكانت أبرز النتائج كالتالي:

١ – لم تظهر فروق واضحة تبين أن هناك أي تأثير للموجات الكهروماغناطيسية على جين وحيدة الناده ابيكوينون (عامل الأكسدة الاختزالي) الموجود في الميتوكوندريا لخلايا الكبد عند إستخدام إنزيمات القصر (*Af1*II, *Apa*I, *Pst*I).

٢ – وجود درجات متفاوتة من الإختلاف بين المجموعات المدروسة عند إستخدام إنزيمات القصر (DsaI, *Ssp*BI, *Dra*II, *Hae*II).

وعلى ذلك توضح الدراسة أن إستخدام طرق البيولوجيا الجزيئية يعطي صورة دقيقة عن تأثير هذه الاطياف على المورثات وبالتالي وضع تصور عن تشوه البروتين الناتج من هذه المورثات. وبالرغم من أن الدراسة على جين وحيدة الناده ابيكوينون (عامل الأكسدة الاختزالي) الموجود في الميتوكوندريا ليست كافية إلا أنه يمكن إستخدام بعض الإنزيمات المستخدمة في هذه الدراسة خاصة تلك التي أظهرت فروقا بين المجموعات كمجسات لمعرفة تأثير هذه الاطياف.