Study the effect of extremely low frequency electromagnetic fields on some blood serum's lipoproteins, liver enzymes and P448/P450 cytochrome enzyme system in NMRI female mice

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ABSTRACT

Arterial diseases are the first causes of death in the developed countries. It seems necessary to investigate the role of enzymes, lipids, proteins and para-oxidation lipids in these diseases. The tribulation of fats is one of the most risk factors which results in atherosclerosis of coronary arteries. In this study, the short time effects of Extremely Low Frequency Electromagnetic Fields with the frequency of 50Hz, on the amount of some blood lipo-proteins, liver enzymes, P448 and P450 cytochrome enzyme system are investigated in the NMRI female mice. The results indicate that the blood serum's levels of lipoproteins decrease significantly, in comparison with control and sham groups ($P<0.05$). Experimental groups of 3 and 5 showed significant increase of HDL compare with control and sham groups ($P<0.001$). While levels of Aspartate aminotransferase (AST) increased in liver, Alanin aminotransferase (ALT) enzyme levels did not show any significant changes in experimental groups ($P<0.01$). In addition, Ames test shows similarity in control and experimental liver extract in the reveres colonies, so the electromagnetic field with intensity of 0.06 militesla ($P<0.01$), does not disturb the P448/P450 enzyme system.

Keywords: Extremely Low Frequency Electromagnetic Field (ELF-EMF); Serum Lipoprotein; Liver Enzymes; Ames Test; NMRI Mice

INTRODUCTION

The arterial diseases (with high rate of incidence) are the result of coronary arterial blockage problems. Arterial diseases increase rapidly in the world, due to some disadvantages of the industrial life [1]. Athero-sclerosis is the most prevalent type of heart diseases and is the first cause of death in the modern countries [1]. Regarding the high rate of arterial diseases incidence in the world and their deleterious backwashes on quality of human life, it is necessary to understand the parameters involved in formation and progression of the disease. Studies showed that alteration of the lipids and lipoproteins, such as triglyceride, cholesterol, LDL-c, HDL-c, and activity of AST and ALT enzymes, result in athero-sclerosis and arterial diseases [1]. On the other hand, application of electromagnetic fields has been dramatically increased in this century, by technological advances [2,3]. Production and usage of electricity, has played an important role in the evolution of the devices, patents and confirmed technologies which did not exist in past years [4]. Beside their positive effects on quality of human living, these technologies have resulted in an overwhelming exposure of the human being to the different electromagnetic frequencies generated by electrical devices, such as power lines, electricity coils, seashores, mixers, refrigerators, ovens and televisions, from one to thousands gigahertz frequencies [4,5].

The effects of these fields on human body depend on amperage, frequency and exposure duration [6]. For a better understanding of the effects of these foregoing technologies, many different researchers studied the effects of electromagnetic fields on human health [3,6]. In this article, the effects of 50 Hz extremely low frequency electromagnetic fields on levels of cholesterol, triglyceride, LDL, VLDL, HDL, the P448/P450 cytochrome enzyme, the lipids and trans-aminase liver enzymes in the blood serum of NMRI mice were investigated.

MATERIALS AND METHODS

A total of 30 white mice from a same colony were used (supplied by Tehran Pastur Institute). Animals were housed in groups of 6 per cage with free access to food and water and maintained on an artificial light dark cycle (14 hours light and 10 hours dark) and room temperature (25 ± 2). Animals were divided into 5 groups of 6 members: Group1 (control group): animals with normal feeding diet. Group2 (sham group): animals with high-cholesterol diet. (2.5% cholesterol in oleic acid solution) the animals were fed with mice pellets and cholesterol in experimental and sham group gavaged in 30 days. These animals were exposed to a power off electromagnetic field for 4 hours a day, in a period of 5 days. Groups 3-5(experimental groups): Animals of these groups, received high cholesterol diet, for 4 weeks (Figure on high level of cholesterol). Then the animals were exposed to 50HZ electromagnetic fields with frequency of 50 Hz and intensities of 0.04, 0.05 and 0.06 militesla, respectively 4 hours a day in 5 consecutive days. A pair of solenoids was made in Plasma Physics Complex. The bobbin was designed with 1000 rounds wire, 50 centimeters abscissa, and 11 centimeters in diameter. Two mice were housed in each cage with $7_{cm} \times 7_{cm} \times 17_{cm}$ dimensions for electromagnetic field exposure, while they could freely move. The foregoing intensities of electromagnetic fields, was adjusted by changing the voltage of the AC power source (ED-345BN) and gauged with a tesla-meter. After 5 days, animals were etherized and exsanguinated from their heart by 1cc insulin syringes. Blood was transferred from the rim of laboratory tube into the 1.5cc Ependorf tube. After complete coagulation (after 15 min at lab temperature), the Ependorfs were centrifuged at 3000 g for 15 minutes and blood serum was separated. The sera were kept at -20 $^{\circ}c$ for further experiments and the following parameters were evaluated. The amount of cholesterol in the serum or plasma was evaluated by the quantitative identification kit, with photometric method (Pars Azmoon co.). In this method the samples were centrifuged. The amount of all parameters in the serum or plasma was evaluated by the kit. The Ames test was applied for investigating the effects of a 50 Hertz electromagnetic field with 0.06 mili-tesla intensity, on P448/P450 cytochrome enzyme system. Mutant salmonella typhimurium TA100 bacterium which needs histidine for survival was used for accomplishment of Ames test. The optimum concentration of bacteria was adjusted at $1-2\times10^{9}$ cells per milliliter. Each tube of Ames test consisted of:

Nightly fresh cultivation of 0.5 ml bacteria, 0.5 ml biotin 0.5 mM and 0.5 mM histidine solution, 10 ml top agar (50 g/lit Agar + 50 g/lit NaCl), the carcinogen sodium azide (1.5 mg/m sodium azide).

Tubes were shacked for 3 seconds, and then extended on Agar with 40% glucose surface. The resulted samples were incubated at 37oC for 48 hours. After 24 hours of hunger, livers of 10 female mice were departed for investigation of enzyme actions. Hunger excites the secretion of liver enzymes and results in increasing of them. The livers were homogenized in 0.15M Potassium chloride solution and centrifuged at 9000 rpm, 4^{o}C for 10 minutes.

Statistical analysis:

Data were analyzed by SPSS software using one way variance analysis ANOVA; Tukey test was used for analysis. In all tests, a p-value of <0.05 was considered statistically significant.

RESULTS

The results of experiments are shown in Fig.1. The amount of cholesterol in experimental groups was observed with lower value in comparison with sham control group at P<0.01 and P<0.001. There is meaningful difference between experimental groups and sham control group in all cases. The obtained value of LDL from the above test is shown in Table1. The amount of LDL in experimental groups (3-5) had lower value in comparison with sham control group respectively at P<0.001, P<0.05 and P<0.001.

There was no significant difference between the amount of triglyceride in experimental group 0.06 mt and sham control group. The results are shown in Table 2. The amount of VLDL in experimental group 0.06 mt was considered different in comparison with sham control group at P<0.001. For other groups there were no significant differences.

The results of TG/HDL ratio, obtained from foregoing test, are shown inTable 4. The result of TG/HDL ratio in experimental group, shows that the 0.05 mili-tesla electromagnetic field, has less effect on decreasing that value(P<0.001).

The results of LDL/HDL ratio, obtained from foregoing test, are shown in Table 4 the LDL/HDL value in the mousses that had high-cholesterol diet, and exposed to a 0.06 mili-tesla electromagnetic field, shows significant decrease at P<0.05. But the LDL/HDL value, in experimental group 0.05 mt shows significant increase at P<0.001.

The results of comparison between AST and ALT liver enzymes, in experimental groups and sham control group are shown in Table 5. As shown in Fig. 5, the AST value in experimental groups has significant increase in comparison with sham control group. But there is no significant difference between the ALT value in experimental and sham control group.

Table 1. Comparison of the average cholesterol and LDL in groups.

Cholestrol	Mean	SD	LDL	Mean	SD
control	110.75	18.03	control	23.25	4.35
sham	153.00	5.29	sham	48.50	3.42
exp1	114.25	9.32	exp1	15.25	9.54
exp2	100.50	12.77	exp2	32.50	4.43
exp3	106.50	18.08	exp3	6.00	6.78

Table 2. Comparisons of average triglyceride and VLDL in groups.

VLDL	Mean	SD	TG	Mean	SD
control	23.50	5.26	control	115.25	24.97
sham	44.50	4.51	sham	230.75	21.16
exp1	32.75	4.57	exp1	164.25	23.47
exp2	44.50	5.92	exp2	222.00	26.98
exp3	28.25	4.57	exp3	141.00	22.64

Table 3. Comparison of HDL value in groups.

HDL	Mean	SD
control	66.50	9.95
sham	32.50	0.58
exp1	65.00	4.32
exp2	36.25	0.50
exp3	72.25	7.27

Table 4. Comparison of average TG/HDL and LDL/HDL in groups.

TG/HDL	Mean	SD	LDL/HDL	Mean	SD
control	1.65	0.21	control	0.35	0.06
sham	7.03	0.57	sham	1.45	0.06
exp1	2.50	0.46	exp1	0.23	0.15
exp2	6.10	0.79	exp2	0.88	0.15
exp3	1.90	0.22	exp3	0.10	0.00

Table 5. The average value of AST and ALT of serum in all groups.

ALT	Mean	SD	AST	Mean	SD
control	237.50	86.06	control	94.50	10.97
sham	473.50	207.87	sham	252.75	118.99
exp1	421.00	302.05	exp1	386.25	576.05
exp2	639.00	227.33	exp2	312.00	257.85
exp3	1057.50	140.33	exp3	229.00	100.82

The results of numeration of colonies in the Ames test (carcinogen: S9 in normal liver %97and Distilled water%2, carcinogen: S9 in fielded liver %94 and Distilled water %3) show that there is no actuarial difference in mutational growth of colonies, after addition to two types of liver, in both cases of sodium azide and distilled water ($P<0.01$).

DISCUSSIONS

The effects of electromagnetic fields on living microorganisms have been investigated for a long time [7]. The initial effect of electromagnetic field is to actuate the key biochemical processes in different metabolic pathways. The effect of electromagnetic field on the live microorganisms seems to be very complicated [7]. Due to daily exposure of electromagnetic fields produced by electrical devices, it's important to study various biological effects of different electromagnetic fields. These studies show that only some electromagnetic fields could interact with an electrical fields of human body and may cause some physiological changes [8,9]. It was shown that electromagnetic fields affect the function of human body organs such as heart, brain and nerves [9,10]. It is proposed that these fields are able to change the cell membrane potential and ions distribution. These changes may affect some biochemical processes and result in changing some serum biochemical parameters and enzymes [4,8]. Different mechanisms proposed for EMF action on biological systems include exchange of electrons, ions and dipoles [7,9] increasing tissue temperature more than $1\,^{\circ}c$ which can affect some immunological factors and hematopoietic system and subsequently, initiates the biochemical reactions, opening calcium canals and changing the electrochemical equivalency which results in initiation of signaling cascade inside the cells and affects the different metabolisms of cell such as lipid metabolism [11,12]. Furthermore, Pila et al. (1993) have reported that, weak electromagnetic fields could change the possibility of molecules collision, and results in starting the self-regulation changes in molecule. This phenomenon causes physiological answers at the cell metabolism level[4].

Verugo-Diaz (2007) reported that when calcium canals open, nitric oxide synthase converts Arginin to NO (nitric acid). The produced NO is non-polar and transports through a cell membrane to the other side easily. NO produces CGMP by activating the guanilil cyclase and transfers the message[13]. When level of NO increases, TBARS (Thio Barbituric Acid Reaction Substances) acts as a biomarker of produced NO, for lypolitic actions at fatty cells. These cells (fatty cells), inhibit high degrees of lypolysis, with controlling the NO synthase. Different signals that actuate the lypolysis are completed by NO [14]. Sakly (2006) has reported the effect of exposing to electromagnetic fields on production of hipoxy (reduced oxygen) in body and tissues[15]. Here, presence of lypolysis in tissues could be the results in hipoxy production. Exposition to electromagnetic fields causes changes in fields of the body and nerves. This problem results in changing the voltage of gates and ion channels and affects their operation [15,16]. Exposure to these fields causes sending signals into the body. During this process, signals for reducing cholesterol, triglyceride and VLDL in serum could be sent [15,16]. Controlling the HMGCOA (3-hydroxy 3-methyl glutaryl CoA) reductase enzyme and initial effects of electromagnetic field may result in decreasing the cholesterol and LDL of the blood serum of NMRI adult female mice, in comparison with the control sham group.

LDL is the basic carrier of cholesterol which provides necessary cholesterol for nervous tissues, synthesis-steroids and cell membrane. After removing of LDL and cholesterol, sending messages to cells, causes the plasmatic cholesterol to decrease. Accordingly, cholesterol is used for production of biliary acids, mitralocorticoids, androgens and cell membrane. After this stage, removal of cholesterol cloud is seen[2]. Decrease of Cholesterol and LDL in experimental groups at this work, is similar to the works of Ebadi (2006) on guinea pigs and B. Kulla (1999) on metal-workers blood serum [7,17].

Decreasing the amount of triglyceride and VLDL could be resulted from earlier effects of lypolysis in the fat cells and delivering the fatty acids after gluconeogenesis. Our results show that, electromagnetic fields cause decrease of the triglyceride and VLDL of serum, in the experimental groups No. 3 and 5. These results are similar to the results of ebadi (2006), Kulla (1999) and Leuo (2004) [7, 17,18].

Verugo-Diaz (2007) and. Riddle (2005) reported that electromagnetic fields have no effect on serum triglyceride levels. Their results do not agree with some other works [13, 19]. However, results of the present work confirm the results of sakly (2006) on the mice with blood vessels blockage. The optimum intensity of electromagnetic fields which decrease triglyceride and VLDL was observed in intensity of 0.04 to 0.05 mili-tesla [15]. The amount of HDL-c decreases with decreased level of triglyceride and the expresion of CETP gene (Cholesterol Ester Transfer Protein) increases with increased triglyceride. Thus increased levels of triglyceride increases the HDL-c and $apoA_1$ sequentially, triglyceride inside the liver lipase hydrolyses. Thus, HDL decreases and could be filtered in the kidney [8], then the amount of HDL-c in serum decreases. Therefore the main work of HDL-c is to clean the athrogenic plaques and inverses transportation of cholesterol [15]. In this work, HDL of serum in the experimental groups is higher than that in sham control group. These results is similar to works of Leuo (2004), on the hyperlipidemic rabbit's but do not agree with results of Invanova (2000, 2007) who studied the people reside on television stations [16,18]. The results of our experiments show that 0.04 and 0.06 mili-tesla electromagnetic fields cause increase in HDL ($P<0.001$). HDL didn't increase in the case of high cholesterol sham group. This study shows meaningful relation between the TG/HDL value in experimental groups and sham control group [20-22]. The importance of the TG/HDL ratio, on the amount of lipids of serum was reported in the literature and has been shown at the present work. This study showed that the electromagnetic fields with foregoing intensities cause the TG/HDL ratio to decrease. These results are similar to studies of Breslow and LeoEP [18,23]. The meaningful comparison of LDL/HDL amount of serum between experimental groups and sham control group at this work is consistent with the studies of Chiristophem, decreasing

the LDL/HDL ratio will prevent athero-sclerosis [5,24]. Exposing to electromagnetic fields in humans or animals result in increasing glucorticoids (cortisol), stress oxidative compounds and produced hipoxy. This is an important reason for increasing of amount of trans-aminases in experimental groups. Hipoxy production could increase the AST and ALT value in serum, up to thousands of units in liter [15,25]. Results of the present study show that, electromagnetic fields affect AST value. Electromagnetic fields cause increase of trans-amination process. This process results in gluconeogenesis for accessing to sugar in brain and hemoglobin. Production of stress oxidative compounds could be the reason of foregoing process which is in association with Dicarlo. A.L (2002) reports [26].

The investigations of Keshava (2004), on the electromagnetic fields with 50-60 Hz frequencies, show that increasing the norepinephrine value maybe causes to increase the value of AST and ALT [27]. The results of present work are similar to the results of Kulla (1985, 1988) while the studies of sakly (2006) show that electromagnetic fields have no effect on the AST enzyme production. These result doses not agree with this study [15,28,29].

The electromagnetic fields that are created during the application of electrical instruments could have some carcinogenic effects. These fields could also affect DNA involvements such as transcription, translation and other functions. If the cells do not enter to program of cell death, the changes could remain as mutations and result in cancer [30-32]. At the present work, the carcinogenic effects of a 0.06 mili-tesla electromagnetic field were studied using Ames test. The Ames test TA100 Salmonella Typhimurium bacteria in presence of S_9 liver microsome. The investigation on average percentage of growth of colonies shows that two types of livers have similar effects on the growth of colonies. These results are similar to the study of Guillosson (1993)[33]. The results of experiments show that the electromagnetic fields with foregoing intensities affect the lipoproteins of plasma, triglyceride and VLDL, and have more effects on LDL and cholesterol. But it doesn't have any effect on ALT and P448/P450 cytochrome system. These fields cause the HDL and AST to increase by different mechanisms such as molecular excitation, biochemical activation, production of free radicals, and debilitation of chemical bonds, hydration exchange and rotation of dipoles.

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