Effects of static and electromagnetic fields on human serum paraoxonase-1 activity in vitro

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Abstract

Introduction: In recent years the relationship between electromagnetic fields and coronary artery disease is attracted a considerable attention. Low density lipoprotein (LDL) oxidation is the initial step in the development of atherosclerosis. Paraoxonase1 (PON1) protects LDL and High density lipoprotein (HDL) against oxidative processes, thus preventing the formation of atherogenic (oxidized-LDL) ox-LDL molecules. In this study we investigated the effects of static magnetic fields (SMFs) and extremely low frequency electromagnetic fields (ELF-EMFs) on PON1 activity as one of the independent risk factors for cardiovascular disease.
Materials and methods: Pooled serum sample of 20 healthy men were exposed to SMFs and EMFs flux densities of 0.125, 0.25, 0.5, 1, 2, 3 and 4 mT for 60, 120 and 180 minutes at 25°C and then PON1 activity was measured spectrophotometrically using paraoxon as substrate.
Results: EMFs of 0.125-1 mT had no effect on PON1 activity. Exposure to magnetic flux density of 2 mT leads to a significant increase in PON1 activity in 1 hour ($P < 0.05$). Magnetic flux density of 3 and 4 mT, after 1hour of exposure, lead to an increase of PON1 activity to 1.2% and 2.8%, respectively ($P < 0.01$).
Conclusion: PON1 activity is influenced by a variety of agents like environmental, pharmacological, and lifestyle factors as well as age and sex. According to the finding of this study ELF-EMFs can alternate the serum activity of PON1 in vitro. If this effect of EMFs on PON1 activity has proven in vivo, it can be considered as an effective factor in coronary artery disease.

Keywords: Static magnetic field, Extremely low frequency electromagnetic field, Paroxonase-1

Introduction

In today's world, with the progression of technology and continuous exposure of human to a diverse range of electromagnetic fields (EMFs), investigation on the biological effects and health implications of EMFs matters a lot. Meanwhile evaluation of the effects of EMFs on cardiovascular disease (CVD) has attracted considerable attention (1). Coronary artery disease (CAD) is the most common type of CVD, the greatest current public health problem, in the 21st century (2). CAD is a state of degenerative and dynamic alteration in the arterial walls of heart which results from the accumulation of lipids, especially cholesterol and formation of atherosclerosis plaques (3). Among the lipoproteins, high density lipoprotein (HDL) and low density lipoprotein (LDL) play a significant role in atherosclerosis process (4). LDL modification, especially formation of

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oxidized LDL (ox-LDL), is an early step in promotion of atherosclerosis which leads to the macrophages accumulation in the arterial wall and transformation in to the foam cells (5). On the other hand, HDL has antioxidant activities (6) and inhibit the oxidation of LDL (7). This antioxidant property of HDL, in part, resulted from the capacity of paraoxonase-1 (PON1), an enzyme associated with HDL structures, to hydrolyze the oxidized phospholipids and hydroxides of cholesteryl linoleate contained in ox-LDL molecules (8). PON1 also limits the process of foam cell formation and reduces the formation of atherosclerotic plaques through suppressing the differentiation of monocytes into macrophages (9).

Decreased PON1 activity has been seen in several diseases such as CAD (10). It is evident that PON1 activity is influenced by a variety of agents like pharmacological, environmental, and lifestyle factors (11-13). It is evident that EMFs can influence the activity of enzymes involved various metabolic pathways in plants (14-16), cultured cells (17), and animals (18).

Investigation on rats shown that exposure to extremely low frequency magnetic fields (ELF-MF) could impair oxidant-antioxidant function and might increase oxidative stress and lipid peroxidation in a time dependent manner (18). The aim of this study is to investigate the effects of static magnetic fields (SMFs) and ELF-MFs on human serum PON1 activity in vitro in a common intensities we expose to daily.

Materials and methods

Serum preparation and enzyme activity assay: A pooled serum was prepared from 12 h fasting blood samples of 20 donors. Blood samples (2 ml) were collected by venepuncturing after 12-14 h fasting. Serum was separated by centrifugation at 3000 rpm for 15 min. A 100 µl aliquot of serum was stored at -80°C until the measurement of serum PON1 activity. PON1 activity was determined using 2 mM paraoxon (diethyl p-nitro-phenyl phosphate, Sigma chemical Co., USA) as the substrate in 100 mM tris buffer, pH 8.0, containing 2 mM of CaCl2. A 40 µl aliquot of pooled serum was added to 500 µl of the substrate medium. The generation of p-nitrophenol (initial rate of hydrolysis) was determined at 412 nm using UV-VIS-3100 spectrophotometer (Shimadzu, Kyoto, Japan) over a period of 2 min (19). The molar extinction coefficient of p-nitrophenol was considered 17,000 M/cm (20). All assays were performed in duplicate at 25°C. The between assay and within assay coefficient of variation (CV) for the method was 3.6% and 5.1%, respectively.

SMF and EMF Exposure System: A solenoid cylinder with a diameter of 12 cm, height of 30 cm, and 1200 turns was used to generate SMF and EMF. For producing suitable static magnetic flux densities, a regulated direct current (DC) power supply (Model 7321, Sanjesh, Tehran, Iran) and for producing suitable electromagnetic flux densities, a voltage regulator AC power supply (model: TDGC2, 220v, 50-60 Hz, Delta International Electric Co, Shanghai, China) have been applied. The solenoid was located inside a 25 ºC ventilated incubator (Parsazma, Tehran, Iran), so the temperature inside the solenoid was controlled exactly during the exposure. Before enzyme activity measurement, serum samples (inside a quartz cuvette) were put in the centre of the solenoid for certain times. The produced SMF and EMF at the exact site of cuvette location was measured using a digital teslameter with a 3-D sensor (Holaday, Eden Prairie, MN) (21, 22).

Statistics analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL). The results are presented as mean values ± standard deviation. A probability of 0.05 was considered statistically significant. Non-parametrical Mann–Whitney–Wilcoxon test was used to compare the data

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between control and exposed samples. Comparison of data between different magnetic flux densities and different exposure times was examined with the non-parametric Kruskal–Wallis H-test for several independent samples.

Results

The impact of SMFs of 0.125, 0.5, 1, 2, 3 and 4 mT on serum PON1 activity is shown in Table1. Static magnetic flux density of 0.125 mT had no effect on PON1 activity. Static magnetic flux densities of 0.25 and 0.5 mT lead to an increase (8.1% and 1.7%, respectively) in PON1 activity at the first hour of exposure. Exposure of serum to 1 mT SMF for 3 hours decreased the PON1 activity to 1.7% ($P < 0.05$). As indicated in Table 1, PON1 activity significantly increased only at the first hour of exposure to 2mT SMF ($P < 0.05$). SMFs of 3 and 4 mT lead to the increase in PON1 activity in all applied times of exposure.

Table1. Effect of SMFs on serum PON1 activity after 3 hours of exposure.

	Time of exposure (min)			
SMF (mT)	0	60	120	180
0.125	77.93 ± 0. 68	77.82 ± 0.5	78.56 ± 0.23	78.26 ± 0.4
0.25	77.40 ± 0.4	83.66 ± 0.87**	82.01 ± 0.35**	83.83 ± 0.51**
0.5	77.85 ± 0.4	79.21 ± 0.58*	80.32 ± 0.61**	79.82 ± 0.61**
1	78.09 ± 0.41	78.42 ± 0.42	77.94 ± 0.18	76.77 ± 0.68*
2	78.27 ± 0.42	79.26 ± 0.33*	78.07 ± 0.45	77.48 ± 0.28
3	78.15 ± 0.38	82.55 ± 0.33**	82.49 ± 0.30**	80.25 ± 0.24**
4	78.45 ± 1.34	87.41 ± 0.35**	83.35 ± 0.38**	82.51 ± 0.40**

Serum PON1 activity was determined using paraoxon as substrate at 25 ˚C within two minutes. The data shows the level of PON1 activity in U/l and the values expressed as mean ± standard derived from the five separate tests. *P <0.05, **P <0.01

The impact of extremely low frequency EMFs of 0.125, 0.5, 1, 2, 3 and 4 mT on serum PON1 activity is indicated in Table 2. Electromagnetic flux densities of 0.125 to 1 mT had no effect on PON1 activity even after 3 hours of exposure. Magnetic flux density of 2 mT increases the activity of PON1 just at the first hour of exposure ($P < 0.05$). The magnetic fields of 3 and 4 mT result in a significant increase in enzyme activity, reaching a maximum of 1.2% and 8.2%.

Table2. Effect of EMFs on serum PON1 activity after 3 hours of exposure.

	Time of exposure (min)			
EMF (mT)	0	60	120	180
0.125	84.63 ± 0. 63	85.0 ± 0.36	84.96 ± 0.35	84.83 ± 0.4
0.25	84.96 ± 0.57	84.64 ± 0.61	84.98 ± 0.51	84.56 ± 0.44
0.5	85.26 ± 0.53	85.12 ± 0.29	84.76 ± 0.45	84.69± 0.35
1	85.15 ± 0.49	85.76 ± 0.51	85.73 ± 0.40	85.80± 0.34
2	84.75 ± 0.40	85.93 ± 0.45*	85.81 ± 0.50	85.89 ± 0.29
3	85.31 ± 0.49	87.09 ± 0.39**	87.01 ± 0.48**	86.88 ± 0.43**
4	85.11 ± 0.49	87.16 ± 0.5**	87.48 ± 0.45**	87.51± 0.31**

Serum PON1 activity was determined using paraoxon as substrate at 25 ˚C within two minutes. The data shows the level of PON1 activity in U/l and the values expressed as mean ± standard derived from the five separate tests. *P <0.05, **P <0.01

Discussion

This study was undertaken to investigate the effects of SMFs and extremely low frequency EMFs on human serum PON1 activity in vitro. According to the result of our study, SMFs and ELF-EMFs can impact the activity of PON1, although

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changing the activity of PON1 under the SMF doesn't follow a specific pattern and is not dependent on the time of exposure. Exposure to magnetic flux density of 2 mT leads to a significant increase in PON1 activity in 2 hours. Magnetic flux density of 3 and 4 mT lead to an increase in PON1 activity to 1.2% and 2.8% after 1 h of exposure respectively.

The earth's magnetic field is about 0.03 to 0.07 mT, which is a natural component of the environment for living organisms. Magnetic fields much weaker than the earth's field can affect living organisms through the chemical processes and effect on the radical pair recombination (23). A review by Belyavskaya suggested that prolonged exposure of plants to a weak magnetic field might cause different biological effects at the cellular, tissue and organ levels (24).

Induction of electrical charges and currents is a primary action of MF in biological systems (25). Influence on nuclear spins of paramagnetic molecules is one of the major molecular effects of MFs which plays an important role in chemical reaction when two molecules with unpaired electrons are formed following the disruption of chemical bound (26).

Study on the impact of electromagnetic fields on the enzymes activity is a new concept in science and there are only a few publications on this subject in accessible literature. Enzymes contain protein structures that organize the dipoles and charges into an especial pattern that can orient specifically the electric field they exert onto particular regions of their bound substrates (27-30). This effect is called electrostatic preorganization. A reorganized enzyme active site can create an electric field with a particular orientation which is controlled by the protein's folded structure and optimizes a particular electrostatic interaction (28).

The impact of a frequent magnetic field on the activity of peroxidase, catalase and superoxide dismutase (respiratory enzymes) was investigated before (31, 32). According to the report of Seifirad et al, acute ELF-MF exposure increased lipid peroxidation and antioxidant serum activity (PON1 activity) in rat. These changes are irreversible in chronic exposure. They believe that the effects of ELF-MF evoke antioxidant system to recompense toxic effects of produced reactive oxygen species (18).

Shaoyi et al, investigate the effects of magnetic field on the activity of α-amylase purified from Bacillus subtilis. They report that magnetic field had a considerable effect on activity, Km, Vm and secondary conformation of α-amylase in a dose and time dependent manner. The values of Km and Vm reduced with increase in the intensity of magnetic field (33).

Conclusion

ELF- MF exposure could impair oxidant antioxidant balance and might increase oxidative stress and lipid peroxidation. Antioxidant defect could be repaired after exposure; however it might depends on the duration and continuity of ELF-MF exposure. This investigation indicates that PON1 activity can affected by static and electromagnetic fields in vitro, whereas increases of magnetic field intensity and exposure time do not cause linear increases for PON1 activity. The possible reason for change in PON1 activity and kinetic parameters may be the secondary conformation changed in enzyme structure following the exposure to static and electromagnetic fields.

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Conflict of interest

Authors declare that there is no conflict of interest.

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