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Short-term Exposure to 50-Hz Electromagnetic Field and Alterations in *NQO1* and *NQO2* Expression in MCF-7 Cells

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Abbreviations: AMI – acute myocardial infarction, MI – myocardial infarction, BP – blood pressure, HF – heart failure, EF – ejection fraction, STEMI – ST-Segment Elevation Myocardial Infarction, AF – atrial fibrillation.

Abstract

AIM: Extremely low-frequency electromagnetic fields (ELF-EMFs) have some genotoxic effects and it may alter the mRNA levels of antioxidant genes. The NAD(P)H: quinone oxidoreductase-1 (*NQO1*) and *NQO2* are ubiquitously expressed. Considering that there is no published data on the effect(s) of ELF-EMF (50-Hz) exposure and expression levels of *NQO1* and *NQO2* in the human MCF-7 cells, the present study was carried out.

METHODS: The ELF-EMF (0.25 and 0.50 mT) exposure patterns were: 5 min field-on/5 min filed-off, 15 min field-on/15 min field-off, and 30 min field-on continuously. In all exposure conditions, total exposure time were 30 minutes. The RNA extraction was done at two times; immediately post exposure and two hours post exposure. The effect of ELF-EMF on gene expression was assessed by real-time PCR.

RESULTS: The *NQO1* mRNA level (at 0h) decreased in the cells exposed to 5 min field-on/5 min filed-off condition at 0.25 mT EMF when compared with the unexposed cells. The *NQO2* mRNA level (at 0h and 2h) increased in the cells exposed to 5 min field-on/5 min filed-off condition at 0.50 mT EMF when compared with the unexposed cells.

CONCLUSIONS: Alterations in the *NQO1* and *NQO2* mRNA levels seem at the "5 min field-on/5 min field-off" condition.

Introduction

Today electromagnetic fields (EMFs) are commonly present in our daily life. There are many reports in relation to effects of the extremely low-frequency electromagnetic fields (ELF-EMFs) on biological systems such as genotoxic effects [1-3] and alterations in expression levels of many genes [4, 5]. A relationship between elevation of reactive oxygen species (ROS) production and/or increasing the lifetime of ROS and the EMFs effects on biological systems has been reported [3]. Therefore, studies with respect to the alterations in mRNA levels of antioxidant genes are highly important to human public health. Very recently it has been reported that when the human breast cancer MCF-7 cells were exposed to the ELF-EMF, the mRNA levels of several antioxidant genes showed significant alterations [5].

NAD(P)H:quinone oxidoreductase-1 (NQO1; EC 1.6.5.2; OMIM: 125860) is a flavoprotein that catalyses the 2-electron reduction of various quinones and redox dyes, such as phylloquinone and the vitamin K menadione [6]. It has been reported that *Nqo1*-null mice exhibited increased toxicity when administered menadione compared with the wild-type mice, indicating a role for NQO1 in protection against quinone toxicity [7]. NAD(P)H: quinone oxidoreductase-2 (NQO2; EC 1.10.99.2; OMIM: 160998) is another member of NQOs and at the protein level, it shows 49% similarity with the liver cytosolic NQO1 protein. It has been stated that NQO1 and NQO2 are ubiquitously expressed [8, 9]. It has been reported that genetic polymorphisms in the NQO1 and NQO2 altered the risks of several complex multifactorial diseases which are associated with oxidative stress [10-14]. On the other hand, alterations of the mRNA levels of the NQO1 and NQO2 were observed in the SH-SY5H cells after exposure to

morphine and methadone [15, 16].

Considering that there is no published data on the effect(s) of ELF-EMF exposure and expression levels of *NQO1* and *NQO2* in the MCF-7 cells, the present study was carried out.

Materials and Methods

Cell culture

Human breast adenocarcinoma cell line MCF-7 was obtained from National Cell Bank of Iran (NCBI) (Pasteur Institute, Iran). Cells were seeded approximately 24h before EMF exposure at the density of 3×10^5 cells/ml in 100 mm surface treated Petri dishes (Jetbiofil).

Electromagnetic field exposure system and exposure conditions

Apparatus generated the 50-Hz EMF and exposure conditions were reported previously [5]. Three conditions of exposure were designed; two intermittent and one continuous. In all three exposure conditions, we tried to have a total exposure time of 30 minutes. The EMF exposure conditions were: 1) 5 min field-on/5 min filed-off, 2) 15 min field-on/15 min field-off, 3) 30 min field-on continuously. Control cultures were located in the exposure apparatus when the power was off. Control Petri dishes for each of three conditions were kept in disconnected solenoid for an equal time to EMF exposure. The RNA extractions were done at two times; immediately post exposure (0 h) and two hours post exposure (2 h).

RNA extraction, cDNA synthesis and Real-time RT-PCR

RNA extraction, cDNA synthesis and real-time PCR were performed as reported previously [5]. All samples had a high quality of RNA ($OD_{260/280}$ = 1.8-2.1). Primers specific for the studied genes and TATA box-binding protein (*TBP*, OMIM: 600075; used as a reference gene) were designed using Allele ID software (v.7.5, Premier Biosoft International, Palo Alto, CA, USA). The primer sequences are shown in Table 1. The primers were specific for mRNAs and could not amplify genomic DNA. Relative gene expression was calculated according to the $2^{-\Delta\Delta Ct}$ method based on the threshold cycle (Ct) values.

Table 1: The sequence of primers used in real-time PCR

Genes	Forward 5'---3'	Reverse 5'---3'	Product size (bp)
NQO1	ACTATGCCATGAAGGAGGCTG	CCTTCAGTTTACCTGTGATGTCC	129
NQO2	TTACGCTATGGCAGGTAAGAAAGT	CCTCGGCTCAAGGTTCATGG	157
TBP	CCCGAAACGCCGAATATAATC	TCTGGACTGTTCTTCACTCTTG	134

Statistical analysis

Data are shown as the mean ± SD of three independent experiments. Statistically, significant differences were assessed using one sample student *t*-test by SPSS software (SPSS Inc.). $P < 0.05$ was considered statistically significant compared to the controls.

Results

Table 2 showed the alterations of the *NQO1* and *NQO2* mRNA levels after the cultured MCF-7 cells were exposed to 0.25 and 0.50 mT 50-Hz EMF. The *NQO1* mRNA level (at 0 h) significantly decreased in the cells exposed to 5 min field-on/5 min filed-off condition at 0.25 mT EMF when compared with the unexposed cells. The *NQO2* mRNA level (at 0 h and 2 h) significantly increased in the cells exposed to 5 min field-on/5 min filed-off condition at 0.50 mT EMF when compared with the unexposed cells.

Table 2: mRNA levels (mean ± SD) of *NQO1* and *NQO2* genes in MCF-7 cells after exposure to 50-Hz electromagnetic field

Genes	Field intensity (mT)	Times after exposure	Exposure conditions		
			30 min cont.	15 On/15 Off	5 On/5 Off
NQO1	0.25	0h	0.78 ± 0.15	0.99 ± 0.05	0.91 ± 0.02*
	0.25	2h	0.98 ± 0.12	1.02 ± 0.07	0.90 ± 0.15
NQO1	0.50	0h	1.17 ± 0.11	1.06 ± 0.14	1.21 ± 0.21
	0.50	2h	1.16 ± 0.18	1.06 ± 0.09	1.01 ± 0.19
NQO2	0.25	0h	0.68 ± 0.14	0.91 ± 0.15	0.95 ± 0.07
	0.25	2h	1.06 ± 0.15	0.96 ± 0.08	0.91 ± 0.11
NQO2	0.50	0h	1.08 ± 0.07	1.09 ± 0.26	1.18 ± 0.06*
	0.50	2h	1.10 ± 0.06	1.07 ± 0.19	1.24 ± 0.08*

*P < 0.05 all values compared with untreated controls (=1) using one sample *t*-test.

Discussion

Previously we showed that the EMFs had some significant effects on the alterations of antioxidant genes and the alterations maximally seem at "the 15 min field-on/15 min field-off condition" [5]. The present data indicated that alterations in the *NQO1* and *NQO2* mRNA levels seem at "the 5 min field-on/5 min field-off" condition.

Some prodrugs used as antitumor agents could be activated by endogenous tumor-selective human enzymes such as NQO1 and NQO2 [8]. The elevated level of NQOs in some primary human tumours in comparison with normal surrounding cells and the fact that the NQO1 and NQO2 are ubiquitously expressed [8, 9] make them good target enzymes for bioreductive activation of prodrugs [8, 10]. Many efforts have been done on up-regulating these genes especially *NQO1* to increase the

effectiveness of cancer chemotherapy with prodrugs and also in order to decrease the side effects of chemotherapy.

β-lapachone is a σ-naphthoquinone originally extracted from the Brazilain lapacho tree. Its mechanism of action involved the reduction of the 1,2-carbonyl sites by NQO1. It has been shown that β-lapachone has great potential for the treatment of cancers with high levels of NQO1 [10].

To our knowledge, this study is the first to show the alteration of mRNA levels of the *NQOs* in human cultured cells due to ELF-EMFs. Further experiments should be done on other intensities of ELF-EMFs and conditions of exposure to find the most effective and long-lasting exposure on these genes and other oxidoreductases. Besides, the ELF-EMFs in combination with quinone-based antitumor prodrugs (such as β-lapachone) should be used to investigate the effect of these two treatments.

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