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Effect of long-term pulsed electromagnetic field exposure on hepatic and immunologic functions of rats

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Summary
Background In this report, the effects of long-term pulsed electromagnetic field (PEMF) exposure on hepatic and immunologic functions were examined.

Methods Male rats were randomly divided into four groups: a control group and three experimental groups exposed to a 50-Hz PEMF at 5, 10, or 20 mT for 10 weeks.

Results Compared with the control group, activities of serum alanine aminotransferase and aspartate aminotransferase and concentrations of serum, liver, and spleen Metabolism of lipid peroxidation (MDA) in the 10- and 20-mT PEMF groups were significantly increased. The activities of Glutathione peroxidase (GSH-Px) and Superoxide Dismutase (SOD) in the serum, liver, and spleen and concentrations of serum immunoglobulins were significantly decreased.

Conclusion These results demonstrate that long-term exposure to PEMF can lead to oxidative damage of the liver and spleen.

Keywords Pulsed electromagnetic fields · Liver · Spleen · Rat · Immune system

Bao-lin Li and Wei Li contributed equally to this work.

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Introduction

Pulsed electromagnetic field (PEMF) is abundant in nature and therefore commonly encountered in daily life. Such an exposure can lead to injury of organisms. In particular, the liver, being the largest organ of the body and mainly responsible for the metabolism of xenobiotics and biotransformation, is prone to injury [1]. The effects of PEMF can accumulate in the liver and inhibit hepatic function. In rats, long-term exposure to PEMF can enhance lipid peroxidation, lessen antioxidation function, initiate oxidative stress in hepatic tissue, and contribute to injury of the liver and damage to hepatic function. PEMF can also restrict growth of the spleen and inhibit the immune regulation of cellular factors in the spleen, thus suppressing its immunologic function. As shown in rats, long-term exposure to PEMF can decrease CD3+, CD4+, CD4+/CD8+ T lymphocytes and levels of interleukin-2, tumor necrosis factor-α, and Alpha naphthyl acetate esterase (ANAE+), while CD8+ T lymphocytes increase, ultimately suppressing T lymphocyte immune function [2–5]. In this experiment, the effects of long-term exposure to PEMF on hepatic function and the immune system of rats were examined. To accomplish this goal, the activity of hepatic enzymes, GSH-Px, and SOD and concentrations of immunoglobulins in the serum and the content of MDA in the serum, liver, and spleen were assessed following long-term exposure to PEMF.

Materials and methods

Preparation of experimental animals

In total, 32 male Wistar rats at 6 weeks of age (purchased from the Heilongjiang University of Chinese Medicine) were used in this study. All experimental procedures were in compliance with the "Laboratory Animal

Administration Rules of Harbin, June, 2009," and were approved by the committee of animal administration of Harbin Medical University. All rats were allowed free access to water and food during the 10-day pre-experiment period. They were then divided into four groups by randomized block design according to weight: one control (sham) and three experimental groups. The three experimental groups (PEMFI, PEMFII, PEMFIII) were exposed to 50-Hz PEMF at 5, 10, or 20 mT throughout a 10-week period. The control (sham) group was treated like the experimental groups but was not subjected to PEMF exposure. All groups were treated at the same time and maintained under the same conditions of light (12-h light/dark cycle) and food (standard laboratory chow) throughout the experimental period. The temperature (22 °C) and humidity were monitored continuously throughout the experimental period. During the experimental period, all animal groups were maintained in clean cages in a separate laboratory that was under daily veterinary inspection to prevent exposure to or transmission of any pathogens [6].

Application of PEMF

PEMF exposure consisted of a pair of Helmholtz coils (Shenzhen Shenxian Technology, Shenzhen, China), with a mean radius of 15.0 ± 0.3 cm. The coil was positioned vertical to the horizontal plane. The mean horizontal distance between the coils was 15.0 ± 0.5 cm. The generator (Model 734A, Shenzhen Shenxian Technology) was able to generate an effective magnetic field frequency of 50 Hz, with a sinusoidal wave at 5, 10, or 20 mT. The magnetic flux density at the center of coils was measured with a magnetoresistive sensor (Model CT3-1, Shanghai Fourth Ammeter Factory, Shanghai, China) and was adjusted by varying the coil current. The wave shape was visualized using an oscilloscope (RIGOL, Beijing, China). In this study, a 50-Hz low-frequency PEMF was used at 5 (PEMFI), 10 (PEMFII), or 20 (PEMFIII) mT to induce a continuous, low-frequency PEMF exposure for a 10-week period. Sham-exposed controls were maintained under similar conditions without any exposure to the low-frequency PEMF [7].

ALT and AST activity in serum

At the conclusion of the experiment, blood samples were taken from the eyes and the serum separated. Activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum was detected by a fully automatic biochemical analyzer. In addition, GSH-Px and SOD activity and the content of MDA (metabolism of lipid peroxidation) in the serum, liver, and spleen were determined. Aseptically separated liver and spleen were homogenized quickly on ice. Homogenates and the serum were used to measure the activity of GSH-Px and SOD and the content of MDA according to the directions of the reagent kit. The dithio-bis-nitrobenzoic acid method was used to determine the activity of GSH-Px, and the xanthine oxidase method was used to detect the activity of SOD. The thiobarbituric acid method was used to determine the content of MDA.

Table 1 Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity in serum

Group	ALT (U/L)	AST (U/L)
Control (sham)	63.45 ± 6.28	78.35 ± 14.17
PEMFI	78.25 ± 2.46*	136.52 ± 5.28*
PEMFII	105.24 ± 3.28*†	192.667 ± 15.42*†
PEMFIII	114.27 ± 4.28*†	246.32 ± 14.28*†

*Compared with the control group, $P < 0.05$
†Compared with the control group, $P < 0.01$

Table 2 GSH-Px activity in the serum, liver, and spleen

Group	GSH-Px (U/mg)		
	Serum	Liver	Spleen
Control (sham)	243.63 ± 5.17	43.478 ± 6.37	2.48 ± 1.45
PEMFI	235.73 ± 7.18	33.25 ± 3.42*	2.15 ± 1.84
PEMFII	184.63 ± 8.72*	29.772 ± 4.74*†	1.77 ± 0.85*
PEMFIII	163.53 ± 6.63*†	18.43 ± 5.27*†	0.74 ± 0.46*†

*Compared with the control group, $P < 0.05$
†Compared with the control group, $P < 0.01$

Measurement of immunoglobulins IgG, IgA, and IgM in serum

Immunoturbidimetry was combined with the American Beckman Coulter (CX4-PRO) fully automatic biochemical analyzer to measure IgG, IgA, and IgM in the serum.

Methods of data processing and statistics

Values were expressed as mean and standard deviation. Statistical analyses were performed using the SPSS program version 13.0 (SPSS, Chicago, IL). Group differences were determined using a one-way analysis of variance. A *P*-value less than 0.05 was required for the results to be considered statistically significant.

Results

ALT and AST activity in rat serum

The activity of ALT and AST in the serum increased gradually as a function of increasing intensity of PEMF exposure, with all levels of PEMF exposure being significantly greater than controls as shown in Table 1.

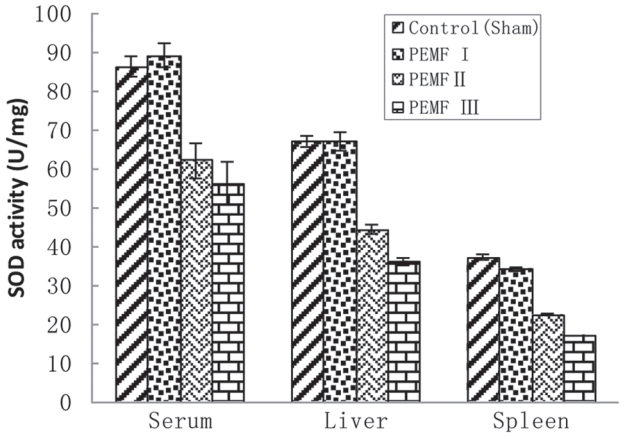

Fig. 1 With increasing intensity of PEMF, the activity of SOD in the serum, liver, and spleen decreased gradually; the activity in PEMFII and PEMFIII groups were lower than that in the control group ($P<0.05$)

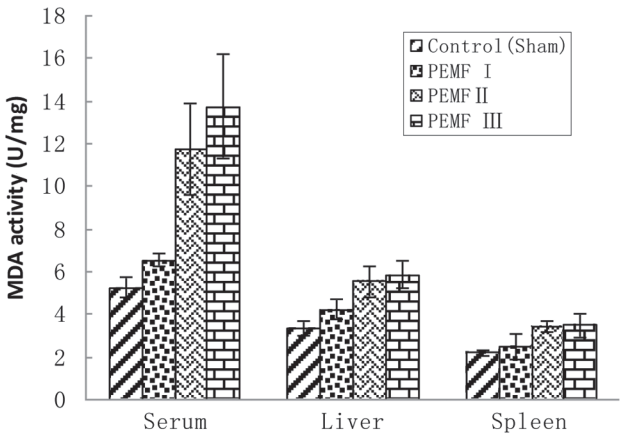

Fig. 2 With increasing intensity of PEMF, the content of MDA in the serum, liver, and spleen showed an upward trend. Compared with the control group, the content of MDA in the serum was apparently higher in PEMFII and PEMFIII groups ($P<0.01$)

GSH-Px and SOD activity and the content of MDA in serum, liver, and spleen

With an increasing intensity of PEMF, GSH-Px activity was correspondingly decreased and significantly lower than controls at all intensities in the liver and for the two higher intensities in the serum and spleen (Table 2). Similarly, as the intensity of PEMF increased, SOD activity in the serum, liver, and spleen decreased gradually, with significantly decreased levels in the PEMFII and PEMFIII groups as compared with the control group (Fig. 1). With an increasing intensity of PEMF, the content of MDA in the serum, liver, and spleen was correspondingly increased. As compared with the control group, the content of MDA in the serum was significantly increased in response to PEMFII and PEMFIII, while MDA content in the liver and spleen was significantly increased in response to each of the three intensities of PEMF (Fig. 2).

Table 3 Immunoglobulin content in the serum (mg/L)

Group	IgG	IgA	IgM
Control (sham)	25.33 ± 1.33	44.17 ± 0.46	46.41 ± 2.16
PEMFI	16.14 ± 1.26*	35.33 ± 0.37*	37.64 ± 1.72*
PEMFII	14.27 ± 2.17*†	32.16 ± 0.58*	28.36 ± 2.14*†
PEMFIII	12.68 ± 2.34*†	26.35 ± 0.35*†	24.23 ± 2.35*†

*Compared with the control group, $P<0.05$
†Compared with the control group, $P<0.01$

Concentrations of immunoglobulin in serum

Concentrations of immunoglobulins were significantly decreased in response to all PEMF intensities as compared with that of the control group (Table 3).

Discussion

ALT and AST are two significant enzymes synthesized in the liver that can directly reflect changes in hepatic function. ALT mainly exists in endochylema of liver cells, while AST exists in endochylema and chondriosome. Normally, ALT and AST are impermeable to liver cell membranes; consequently, their content in peripheral blood is low and constant. However, in response to cell membrane injury or chondriosome lyses, ALT and AST are released into hepatic sinusoids and enter the peripheral blood to increase serum content of ALT and AST. Therefore, the presence of serum ALT and AST can serve as an index of liver cell injury [8–10]. Our current results demonstrating increases in serum ALT and AST are in accordance with those of related studies. The findings that activity of ALT and AST in rat serum increases as a function of PEMF intensity indicates that long-term exposure to PEMF can produce injury of the liver and affect hepatic function.

SOD and GSH-Px represent important antioxidases that can accelerate and catalyze the process of oxygen free radical scavenging as well as inhibit the effect of lipid peroxidation. As a result, they play a key role in antioxidation, antitumor, and antiaging processes. MDA is a lipid peroxide formed during lipid peroxidation triggered by free radicals attacking polyunsaturated fatty acids on biomembranes [11–14]. Its content can reflect the degree of lipid peroxidation and indirectly mirror the degree of cellular injury. Results obtained from a number of studies have demonstrated that PEMF can cause lipid peroxidation in rat livers, significantly increasing MDA content in the liver and substantially reducing the activity of GSH-Px and SOD [15–17]. Our findings reveal that increasing intensities of PEMF result in corresponding decreases of GSH-Px and SOD activity in the serum, liver, and spleen and corresponding increases of MDA content. Such a relationship suggests that exposure to PEMF can initiate lipid peroxidation reactions in hepatic and splenic tissues. The decreases in this antioxidant capacity results in oxidative damage to the liver and spleen and contributes to the inhibition of hepatic function and immune function of the spleen.

Immunoglobulins secreted by B lymphocytes are important indicators of humoral immunity. Immunoglobulins, mainly IgG, IgA, and IgM, represent a pattern of manifestation and the physical basis of antibodies [18–20]. Findings from a number of studies have shown that PEMF can inhibit the formation of serum hemolysin and antibodies and results in abnormalities of immune function, thereby increasing the risk of disease [21–24]. The results of our experiment reveal that concentrations of IgG, IgA, and IgM in all PEMF-exposed groups were significantly decreased as compared with the control group. In this way, long-term exposure to PEMF can inhibit the secretion of immunoglobulin, weaken the humoral immune function, and subsequently reduce the organism's capacity to resist infectious diseases and responses to environmental stress.

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Conflict of interest

The authors declare that there are no actual or potential conflicts of interest in relation to this article.

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