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The effect of magnetotherapy and magnetostimulation on cytokine release by human peripheral blood lymphocytes *in vitro*

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Abstract

Introduction:.Owing to its anti-inflammatory, anti-oedematous, anaelgesic, and angiogenetic properties, the electromagnetic field (EMF) has multiple medical applications, mainly in the physiotherapy of various conditions. In many cases, physiotherapy with EMF can exert a direct or indirect effect on the immune system. The aim of the project was to assess the impact that magnetotherapy and magnetostimulation may have on the activity of peripheral blood lymphocytes from healthy individuals. M***aterial and Methods***: Human peripheral blood lymphocytes obtained from 28 healthy male subjects were exposed to EMF via magnetostimulation (2 programmes, Viofor JPS device) or magnetotherapy (2 programmes, Magnetronik MF-10). Cytokine release (TNF-α, IL-10, IL-1β, IL-12, IL-8) by resting and phytohemagglutinin-activated lymphocytes was determined. ***Results:*** Magnetostimulation programme M2P3 was found to induce increased TNF-α and IL-8 levels. Mitogen stimulation had no effect on the secretory activity of peripheral blood lymphocytes. Magnetotherapy contributed to increased concentration of IL-10 released by resting and PHA-activated peripheral blood lymphocytes in both the programmes under study. ***Conclusions***: Cytokine release by peripheral blood lymphocytes exposed in vitro to EMF depends on the physical parameters of the EMF applied. When appropriate therapeutic programmes are used, both magnetotherapy and magnetostimulation have influence on the activity of pro- or anti-inflammatory cytokines. ***(Clin Exp Med Lett 2007; 48(2): 111-115)***

Keywords: electromagnetic fields, magnetostimulation, magnetotherapy, immunity, lymphocytes, cytokines

Introduction

The ubiquity of electromagnetic field (EMF) in the communal environment and the growing range of its technical and medical applications make it necessary to elucidate the mechanisms of activity and the possible effects on human health. Of particular importance seem to be the potential effects on the immune system which not only has a protective role against pathogenic microorganisms, but also plays an essential part, along with the endocrine and nervous systems, in maintaining internal homeostasis [1].

It has been observed that EMF may enhance the autoregulatory functions of the system when appropriate physical parameters: induction, frequency, and application time, are provided. It also exhibits anti-inflammatory, anti-oedematous, analgesic and angiogenetic potential. This finding made it possible to introduce EMF therapy in medicine [2]. Over the last several years, EMF has been commonly applied to the treatment of musculoskeletal diseases (post-traumatic conditions, delayed (retarded) synostosis**,** pseudarthrosis, osteoporosis, osteoarthrosis), neurologic conditions (stroke, neuralgias, migraine, multiple sclerosis, Parkinson's disease, Alzheimer's disease) and skin diseases (crural ulceration) [3]. Apart from magnetotherapy employing 1-40 Hz EMF with 1-25 mT induction level, magnetostimulation in which magnetic field intensity is 10 to 1000 times as low, has been more and more common [3]. Although the latter method has been used for a rather short time, recent studies point to its positive biological effects. Particularly interesting are the reports on the impact of pulsed low-frequency magnetic fields on internal homeostasis [2,3,4]. The scarce literature reports, sometimes providing inconsistent findings, make one presume that low frequency EMF may stimulate immunologic reactivity as well as cellular and humoral immunity of the system [4].

Many of the experimental animal studies are performed on models employing EMF with physical parameters much different from those encountered in the communal environment or used in physiotherapy devices [1].

The present project focused on the assessment of the impact of EMF used in routine magnetotherapy and magnetostimulation on TNF-α, IL-10, IL-1β, IL-12, IL-8 release by peripheral blood lymphocytes from healthy individuals.

Material and Methods

The study was conducted on 28 healthy males classified into two groups: Group I – 13 subjects aged 29-44

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(mean 36.68 ± 3.58) and Group II – 15 subjects aged 31-42 (mean 37.14 ± 2.87). Blood samples were collected with Vacutainer on heparin or EDTA and processed no later than 4 h since the collection time.

Human peripheral blood lymphocytes were isolated by centrifugation in Gradient G (Polfa, Poland), specific gravity 1.114 g/cm^3, according to commonly adopted analytic protocol [5]. After collecting the isolated lymphocytes and washing them twice in PBS, a cellular suspension 1x10^6/ml in density was prepared. Cell homogeneity amounted to 96-98% and their vitality assessed with tripane blue was 95-98%. The obtained immune cells were exposed to EMF of the same physical parameters as those routinely used in physiotherapy devices.

The tubes containing lymphocytes were placed in a coil applicator, 200 mm in diameter. The cells deriving from Group I were subject to magnetostimulation using Viofor JPS (Med&Life, Poland). Two therapeutic programmes: M2P3 and M3P3 were applied. EMF impulses, V-shaped (as the saw-like), were emitted as a series of several impulse packets. The following physical parameters of EMF were used: magnetic induction 45 μT, basic impulse frequency 180-195 Hz, impulse packet frequency 12.5-29 Hz, series frequency 0,08-0,3 Hz, and application time 12 min. in both the programmes..

The cells coming from Group II were exposed to EMF via magnetotherapy with Magnetronik MF-10 (ZEE Otwock, Poland). Two therapeutic programmes were used: programme I "analgesic" (impulse shape: quadrangle, magnetic induction 20 mT, frequency 40 Hz) and programme II "vascular" (impulse shape: sinusoidal, magnetic induction 20 mT, and frequency 10 Hz). In both the programmes, the application time was 12 min.

To assess proliferation, a part of the lymphocytes were stimulated with phytohemagglutinin (PHA) at 5μg/ml. Thus activated lymphocytes were exposed to EMF either via magnetotherapy (Magnetronik) or magnetostimulation (Viofor).

In both the study groups, lymphocyte activation and cytokine release (TNF-α, IL-10, IL-1β, IL-12, IL-8) was assessed. Following 24-h incubation, the plates with lymphocytes were centrifuged at 2000 rpm for 10 min. and the supernatant was collected, divided into portions and stored at -80°C till further analysis. Cytokine concentration was determined with ELISA immunoassay using commercial kits: Bender Medsystems (IL-12), BD OptEIA Set Human Becton Dickinson (IL-8), Becton Dickinson (TNF-α, IL-10, IL-1β) according to manufacturer's procedure.

Lymphocytes not exposed to EMF or stimulated with PHA were used as the controls.

The study was approved by the local Ethics Committee. A descriptive statistical analysis was performed using Student's t-test, Cochran Cox test, Shapiro-Wilk test and Mann-Whitney test in a univariate analysis. Two-sided P value <0.05 was considered statistically significant.

Results

Assessment of cytokine release by resting lymphocytes exposed to EMF

The results regarding resting lymphocytes exposed to EMF via magnetostimulation are displayed in Table 1. The findings revealed significantly decreased concentrations of TNF-α and IL-8 when the M2P3 programme was applied. TNF-α concentration decreased by 20.88% (p<0.02) and IL-8 by 28.27% (p<0.007). For the other cytokines (IL-10, IL-1β, IL-12), the changes in concentration levels were statistically non-significant. As regards the M3P3 programme, no statistically significant differences in cytokine concentration could be found for any of the cytokines studied.

The results of magnetotherapy performed on resting lymphocytes are presented in Table 2. IL-10 concentration was found to increase significantly in both the programmes: by 20.64 % (p<0.04) in programme I and by 49.64% in programme II.

Table 1. Cytokine release by resting peripheral blood lymphocytes exposed to EMF through magnetostimulation (Viofor JPS)

	TNF-α (pg/ml)	IL-10 (pg/ml)	IL-1β (pg/ml)	IL-12 (pg/ml)	IL-8 (pg/ml)
Control	661.20 ± 486.77	1453.54 ± 1263.52	1231.30 ± 665.38	145.09 ± 56.99	60582.29 ± 16516.62
M2P3 programme (Viofor)	523.12 * ± 486.08	1471.73 ± 686.23	1106.17 ± 657.98	115.41 ± 63.11	43450.14 ** ± 22939.95
M3P3 programme (Viofor)	578.00 ± 682.66	1169.58 ± 659.03	1123.35 ± 661.89	111.47 ± 79.43	51579.29 ± 31624.25

* p<0.02; ** p<0.007

Table 2. Cytokine release by resting peripheral blood lymphocytes exposed to EMF through magnetotherapy (Magnetronik MF-10)

	TNF-α (pg/ml)	IL-10 (pg/ml)	IL-1β (pg/ml)	IL-12 (pg/ml)	IL-8 (pg/ml)
Control	1059.66 ± 359.74	860.61 ± 484.14	1574.13 ± 668.51	430.21 ± 162.00	30653.38 ± 26569.88
Programme I (Magnetronik)	944.31 ± 350.64	1038.26 * ± 667.95	1472.97 ± 838.62	417.36 ± 112.33	15807.38 ± 11829.74
Programme II (Magnetronik)	1053.99 ± 412.52	1287.87 ** ± 1008.22	1670.65 ± 914.92	421.18 ± 162.02	32210.75 ± 19188.65

* $p<0.04$; ** $p<0.03$

Assessment of secretory activity of PHA-stimulated lymphocytes exposed to EMF

Table 3. isplays the results regarding lymphocytes stimulated with PHA and exposed to EMF via magnetostimulation. No statistically significant changes in cytokine concentration was found for any of the cytokines in either the M2P2 or M3P3 programme.

The results regarding PHA-stimulated lymphocytes subject to EMF exposure via magnetotherapy are shown in Table 4. statistically significant increase in IL-10 concentration was noted in both the programmes: by 26% ($p<0.002$) in programme I and by 42.44% ($p<0.03$) in programme II. The concentrations of the other cytokines did not change significantly in either of the programmes.

Table 3. Cytokine release by PHA-stimulated peripheral blood lymphocytes exposed to EMF through magnetostimulation (Viofor JPS)

	TNF-α (pg/ml)	IL-10 (pg/ml)	IL-1β (pg/ml)	IL-12 (pg/ml)	IL-8 (pg/ml)
Control	1335.41 ± 1037.27	1465.42 ± 1119.72	1901.55 ± 1130.80	183.50 ± 58.08	82919.71 ± 26616.11
M2P3 programme (Viofor)	1124.60 ± 939.58	1506.71 ± 883.75	1558.04 ± 935.70	154.90 ± 74.92	56211.86 ± 32811.61
M3P3 programme (Viofor)	1094.59 ± 970.45	1315.18 ± 782.39	1779.29 ± 1325.78	154.87 ± 105.73	68559.29 ± 40865.41

Table 4. Cytokine release by PHA-stimulated peripheral blood lymphocytes exposed to EMF via magnetotherapy (Magnetronik MF-10)

	TNF-α (pg/ml)	IL-10 (pg/ml)	IL-1β (pg/ml)	IL-12 (pg/ml)	IL-8 (pg/ml)
Control	1872.22 ± 683.16	822.19 ± 423.30	1694.69 ± 720.82	538.41 ± 191.68	36567.25 ± 29844.79
Programme I (Magnetronik)	1657.32 ± 518.62	1036.96 * ± 393.80	1675.52 ± 997.20	481.29 ± 123.99	23654.13 ± 15545.52
Programme II (Magnetronik)	1905.73 ± 720.45	1171.13 ** ± 599.95	1958.57 ± 1168.86	542.15 ± 140.18	42231.00 ± 21239.43

* $p<0.002$; ** $p<0.03$

Discussion

The findings of the present study revealed that peripheral blood lymphocytes subject to a short-term in vitro stimulation with EMF of low magnetic induction (Viofor) showed a decreased secretory activity. Decreased TNF-α and IL-8 levels were observed when resting lymphocytes were stimulated with EMF emitted during the M2P3 while this stimulation had no effect on the concentration of all the other cytokines. The use of the M3P3 programme had no influence on the secretory activity of the resting lymphocytes.

Stimulating the resting lymphocytes with phytohemagglutinin resulted in an increased cytokine release. Thus activated lymphocytes exposed to EMF with higher induction level (Magnetronik) produced increased concentration of IL-10 in both the programmes, whereas the concentration levels of the other cytokines remained unchanged.

The findings regarding the impact of EMF exposure on the secretory activity of the immune cells differ significantly depending on the type of cells and the physical characteristics of EMF under study. The studies performed on 18 physiotherapy workers occupationally exposed to high frequency EMF: shortwave diathermy (27.12 MHz), decimeter waves (433.92 MHz) and microwaves (2.45 GHz) did not reveal any significant changes regarding the immune system either after a several hours' exposure or two weeks out of work [6]. In another study [7], peripheral blood lymphocytes and monocytes were exposed to very high frequency EMF (microwaves 1300 MHz) pulsed at 330 pps, 5 μs impulse of 1mW/cm^2, and then cultured for 72 h. An increase in the secretory activity of both lymphocytes and monocytes could be noted: increased IL-10 and IL-1β concentrations, respectively.

The studies performed among medical staff operating EMF devices with very high levels of magnetic induction (MRI, induction 0.5 T) did not reveal any significant differences in TNF-α concentration for the lymphocyte subpopulations studied [8]. Similar results were obtained in a study on the impact of EMF with very high induction levels on the activity of peripheral blood lymphocytes [9]. A decreased expression of CD 69 antigen was noted as well as no differences in IL-10 and TNF-α concentration. Also a 60-min. in vitro exposure of resting and PHA-stimulated (5 mg/ml) lymphocytes to high intensity EMF (MRI, 4.75 T) did not induce any changes in IL-1β or TNF-α concentrations [10].

The findings of the studies on peripheral blood mononuclear cells (PBMC) exposed to low frequency (50/60 Hz) EMF did not reveal its influence on cytotoxicity or cytokine concentration (TNF-α, IL-10). These studies were performed using a variety of impulse shapes: rectangular, sinusoidal, elliptic, and EMF dose with magnetic induction level ranging from 2 to 500 μT [11]. In another study on PBMC exposed to constant and pulsed EMF at 15-min. cycles (with a 105-min. interval and 6 h exposure time), no impact of such field on TNF-α concentration could be found. This referred both to the resting and PHA-activated (1 μg/ml) lymphocytes [12]. An increased cytokine release by peripheral blood lymphocytes was also detected in a study on exposure to low frequency (50/60 Hz) EMF [9]. The findings referred both to the resting and PHA-stimulated lymphocytes. In the study on PBMC exposed to 50 Hz EMF with modulated induction ranging from 50-200 μT, a decreased TNF-α synthesis was noted [14]. An inhibition of TNF-α release but with no impact on IL-1β secretion by resting and PHA-activated peripheral blood lymphocytes (exposure to 50Hz and 3 μT EMF) was also reported [11]. A 72-h stimulation of PBMC with 50 Hz EMF of 1-30 μT produced significant inhibition of TNF-α synthesis and increased IL-1β concentration. This study did not reveal any effect of EMF exposure on IL-10 or IL-6 concentrations [16].

In experimental models used for assessment of the impact of EMF on the secretory activity of the cells, the tumour cells can sometimes be used. A study performed on J774.2 tumour cells revealed that a 24-h exposure to 25Hz and 80 μT EMF produced an increase in TNF-α synthesis [17].

Clinical studies conducted among children with recurrent upper airway infections demonstrated abnormal lymphocyte and monocyte activity (low IL-10 concentrations and high IL-1β levels). Forty of the children were subject to 10 sessions of magnetostimulation (Viofor) as an auxiliary treatment to pharmacotherapy. The M1P2 programme was applied where the basic impulse frequency was 180-190 Hz and magnetic induction ranged from 3-40 μT. The EMF applicator was a ring, 500 mm in diameter, which was placed in such a way that it would cover the child's chest during EMF emission. Ten sessions of 10 min. each were conducted. After the series of sessions, the children showed normal immune parameters under study [18,19].

A number of reports point to the fact that EMF exposure may impair the lymphocyte capacity to respond to different stimuli as well as affect other immune functions [20-24]. The findings of our study indicate that when appropriate therapeutic programmes are used, both magnetotherapy and magnetostimulation have influence on the activity of pro- or anti-inflammatory cytokines, thus inhibiting the inflammatory response of the system. Moreover, they can motivate health professionals to a wider use of these physical methods in immunotherapy.

Conclusions

1. EMF exposure via M2P3 programme of magnetostimulation (Vioform JPS) induces decreased concentration of TNF-α and IL-8 released by resting peripheral blood lymphocytes
2. Stimulating the resting peripheral blood lymphocytes with phytohemagglutinin, and their exposure to EMF via magnetostimulation (Viofor JPS) do not affect cytokine release of these cells.

3. EMF exposure via magnetotherapy (Magnetronik MF-10), in both the programmes: 'analgesic' and 'vascular', results in concentration increase of IL-10 released by resting and PHA-activated peripheral blood lymphocytes.
4. The secretory activity of peripheral blood lymphocytes exposed in vitro to EMF is dependent on the physical parameters of EMF.

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