

Impact of Low-Frequency Pulsed Magnetic Fields on Defensin and CRP Concentrations in Patients with Painful Diabetic Polyneuropathy and in Healthy Subjects

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Aim. The aim was to assess whether magnetic field influences defensin and CRP concentrations in patients with diabetic polyneuropathy and in healthy subjects.

Methods. 61 diabetic patients were randomly divided into 2 groups: study group—32 patients exposed to low-frequency magnetic field; and control group—29 patients with sham exposure. Additionally, 20 healthy subjects exposed to low-frequency magnetic field. Exposures were performed during 3 weeks, 5 days in a week. Defensin and CRP concentrations were measured at baseline, after 3 weeks and at the end of the study.

Results. There were no significant changes in defensin concentration in patients with diabetes in both the real and sham exposure group. We observed increased concentration of defensin in healthy subjects in week 5 vs. baseline value ($P < 0.02$).

Conclusions. Magnetic field has no impact on defensin concentration in diabetic patients but has positive influence on this parameter in healthy subjects.

Keywords Defensin; CRP; Magnetic field; Diabetes.

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Introduction

The effect of low-frequency magnetic fields on human tissues has been reported in many studies. Pulsed magnetic fields (PMFs) have, among others, analgesic, vasoactive, neurostimulatory, and trophic effects in humans (Musaeov, 2003). By inducing low-frequency currents, PMF can depolarize, repolarize, and hyperpolarize neurons and modulate neuropathic pain in this way. For many years, diabetes has been a contraindication to magnetic therapy mainly due to lack of research on its influence on glucose metabolism. The results of recently conducted trials showed a positive effect of such therapy on glucose utilization (Banaszkiewicz, 1992). Several experimental studies on extremely low-frequency pulsed magnetic fields showed they can enhance cellular immune functions (Zhang, 1997; Scarfi, 1991). Exposure to PMF of human peripheral blood mononuclear cells was contributed with diminished synthesis of TNF- α and TNF- β with no effect on IFN- γ (Bersani, 1999).

Due to huge resistance of many bacterial tribes to classical antibiotics, in the 1980s and 1990s researchers started intensive examination of oxygen-independent mechanisms of neutrophil bacteria killing (Spitznagel, 1985). Highly developed system of antibacterial substances was found in primary and secondary granules (azurophil) of neutrophils. The former are rich in: cathelicidins, defensins, bactericidal permeability increasing protein (BPI), serprocidins, calprotectins, etc. Human neutrophil alpha-defensins (Human Neutrophil Peptides, HNP) belong to the family of cationic trisulfide-containing microbicidal peptides. Three highly homologous HNP, HNP1–3, stored in azurophilic granules of polymorphonuclear leukocytes comprise about 99% of total defensin content of the neutrophils with traces of HNP-4. HNP 1–3 are able to kill and/or inactivate a broad spectrum of bacteria, fungi, some enveloped viruses, parasites, or host cells (Ganz, 1997). They are able to modulate immune response and inflammation by inducing the chemokine interleukin-8 (IL-8) in epithelial cells and modulating cytokine expression in several cell types and causing chemotaxis to monocytes, T cells and immature dendritic cells. There is data that defensins play regulatory role in classical pathway of complement. They have the capacity to modulate the inflammatory responses through regulation of cytokine production and adhesion molecule expression (Chaly, 2000). Neutrophils, while activated, release HNP rapidly, which can be measured in plasma and other fluids during infection or inflammation. Very low levels of HNP are also observed in normal plasma. Recently, researchers found that serum concentration of α -defensin 1–3 is increased in Type 1 diabetic patients with nephropathy (Saraheimo, 2008). There is a link between diabetic nephropathy and low-grade inflammation and activation of the complement system (Saraheimo, 2003). There is also a suggestion that α -defensins play role in pathogenesis of atherosclerosis (Higazi, 2000). On the other hand, α -defensins inhibit pathologic retinal neovascularization in patients with diabetes (Economopoulou, 2005). Other researchers claim that α 1-defensin can inhibit hepatic glucose production through a pathway different from that mediated by insulin (Liu, 2008).

The aim of this randomized, placebo-controlled, double-blind study was to assess whether magnetic field influences defensin and C-reactive protein (CRP) concentrations in patients with diabetic polyneuropathy and in healthy subjects.

Material and Methods

Study Design and Patients

The study had a randomized, double-blind, placebo-controlled design.

Table 1
General characteristics of the subjects

	Study group <i>n</i> = 32	Control group <i>n</i> = 29	Healthy <i>n</i> = 20
Age (yrs)	53.6 ± 13.6	55.5 ± 10.4	40.2 ± 10.3
Duration of diabetes (yrs)	17.1 ± 9.1	12.9 ± 8.5	
Type of diabetes I/2 (number of patients)	12/20	9/20	
Gender M/F	12/20	13/16	6/14
BMI (kg/m ²)	29.1 ± 6.0	29.3 ± 6.1	23.6 ± 3.7
WHR	0.910 ± 0.093	0.908 ± 0.092	0.82 ± 0.8
Baseline HbA1c (%)	8.7 (7.0–9.7)	8.1 (7.2–9.4)	

Values are means ± standard deviation or medians (interquartile range).

No significant differences between study and control group in subjects' characteristics at entry into the study.

From February 2004 through October 2005, 61 subjects with symptomatic diabetic polyneuropathy were recruited from the Silesia region in Poland. Enrollment criteria required that all patients have diabetes (of any type) diagnosed and were suffering from painful diabetic polyneuropathy with pain disturbing their sleep at night. Diagnosis of diabetic polyneuropathy was also confirmed by electroneurography (NC). All the subjects had to mark at least 40 mm on 100 mm Visual Analog Scale (VAS) of pain intensity, where zero (0) means no pain and 100 mm the worst possible pain (Melzack, 1987). Subjects were excluded if there were other causes of neuropathic pain (e.g., alcohol, drugs, etc.). As a safety precaution, pregnant women, subjects with diagnosed neoplasm, or subjects having cardiac pacemakers were also excluded. Analgesic or other drugs administered for the treatment of chronic neuropathic pain were continued during the study period and no new drugs were allowed during the study. Patients were randomly divided into two groups. Study group consisted of 32 people with painful diabetic polyneuropathy, exposed to low-frequency magnetic field, and average pain duration in this group was 23 months. Placebo group consisted of 29 subjects with sham exposure, with average pain duration of 28 months. There were also 20 healthy subjects (no history of diabetes mellitus) exposed to low-frequency magnetic field using the same magnetostimulation scheme.

Data regarding the influence of PMF on pain, quality of life and sleep and HbA1c, and lipids profile was already published by Wróbel (2008).

Table 1 shows the general characteristics of subjects. Study protocol was approved by the ethics committee of Medical University of Silesia in Katowice, and written informed consent was obtained from all patients before enrollment.

Experimental Treatment

Magnetic exposure was performed with use of VIOFOR JPS (Med&Life, Poland), a commercially available Polish device (with a bed shape), generating a low-frequency magnetic field of magnetic induction values up to 100 µT (www.vioforjps.pl). Any

interaction at this level is defined as magnetostimulation in contrast to magneto-therapy, where induction values are above 100 μT . Electromagnetic wave generated by VIOFOR JPS is a complex sequence of pulses of a frequency about 180–195 Hz. Electric field intensity is about 130 V/m and is similar to the intensity of Earth's electric field. Construction of VIOFOR JPS allows performing the study with use of the double blind method. Depending on the type of code pre-entered, the device works in real or sham exposure mode. During its action both the observer and the subject do not know which mode is active. The device was coded by Med&Life Company prior to the beginning of the study. Active and placebo codes were randomly divided into two equal parts (randomization in blocks for ten people). Codes were disclosed after completing the study by all the subjects.

Magnetostimulation Scheme. Each patient was exposed to magnetic field or had sham exposure in a period of 15 days (3 weeks without Saturdays and Sundays). A single session lasted 20 min and consisted of two 10 min-long exposures with the following application parameter components:

- trunk—M1, P2, intensity 4
- lower limbs—M1, P2, intensity 6.

where M1 is an application with constant intensity of selected field throughout the whole time of exposure and P2 is a JPS system, utilizing ionic cyclotron resonance.

Outcome Measure

The primary outcome measure was the change in defensin and CRP concentrations. The study period consisted of three weeks of real (low-frequency magnetic field) or sham exposure and two weeks of follow-up observation. Defensin was measured by Human HNP 1-3 Neutrophil Defensins Elisa test kit (*Hycult, Biotechnology, The Netherlands*) at baseline, after three weeks of exposure and at the end of the study (week 5). The serum CRP was determined by using immunoturbidimetric assay—C-Reactive Protein (Latex) test (*Cobas Integra 400/400 Plus, Roche Diagnostics, Germany*) at the same points of the study. In each study point, 2 ml serum samples were collected from all the participants.

Statistical Analysis

Data is presented as means \pm standard deviation for parametric data and as medians (interquartile range) for nonparametric data. Shapiro-Wilk normality test was used to evaluate the distribution of data. Differences between the groups were tested with Mann-Whitney *U*-test. To assess the differences between baseline and each week of the study, the Wilcoxon test was used.

Results

Efficacy

The baseline characteristics of all the participants are presented in Table 1. Baseline characteristics for 61 subjects with diabetes were similar in both groups. Baseline values of defensin and CRP in diabetic patients were higher compared to healthy subjects.

Table 2

Defensin [ng/ml] and CRP [mg/L] concentrations at baseline, after 3 and 5 weeks

	Group	Baseline	Week 3	Week 5
Defensin [ng/ml]	Study $n = 32$	114.8(42-372.7)	138.5(64.2-245)	124.2(0.04-173.6)
	Control $n = 29$	108(0.04-215.3)	149.6(77.5-265)	114.8(65.7-245)
	Healthy $n = 20$	56.8(44.8-85.1)	67.8(51.5-93.1)	109.7(61.3-190.7) ^{&}
CRP [mg/L]	Study $n = 32$	3.0(1.3-5.9)	2.4(0.7-3.8) [*]	2.9(1.4-5.2)
	Control $n = 29$	2.8(1.25-5.8)	2.6(1.50-5.1)	3.8(2.1-6.3)
	Healthy $n = 20$	1.0(0.6-2.2)	1.2(0.7-2.3)	1.2(0.6-1.6)

Values are medians (interquartile range) ^{*} $P < 0.01$; [&] $P < 0.05$ vs. baseline.

We did not observe significant changes in defensin concentration in patients with diabetes in both real and sham exposure group (Table 2). In healthy subjects exposed to magnetic field, we observed increased concentration of defensin only in week 5 in comparison to baseline value ($P < 0.02$). There were no significant changes in CRP value while comparing real and sham exposure group of diabetic patients (Mann-Whitney U test). However, we observed CRP reduction after 3 weeks in real exposure group ($P < 0.005$) but not in week 5 (Wilcoxon test). We did not observe any significant changes in CRP concentration in healthy subjects at any point of the study.

Safety

No side effects were recorded during the study.

Discussion

Our results show that low-frequency magnetic field has no impact on defensin concentration in diabetic patients. On the contrary, in healthy subjects, elevation of serum defensin was observed two weeks after exposure period (week 5). The literature search revealed no study in which the effect of low-frequency magnetic fields on defensin and CRP levels in diabetic patients and healthy subjects was assessed in a randomized, double-blind, placebo-controlled trial.

Baseline elevation of CRP values in diabetic patients in our study is in line with other trials. C-reactive protein (CRP) levels rise with inflammation of blood vessels. Both atherosclerotic process and Type 2 diabetes are linked to higher CRP levels (Greenfield, 2004). Chronic inflammatory process caused by cytokines action on the hepatocytes, monocytes/macrophages, adipocytes, and endothelial cells leads to development of atherotrombosis and metabolic syndrome. Low-grade tissue inflammation may be a cause of insulin resistance and a common basis for Type 2 diabetes, atherosclerosis, and cardiovascular disease (Yudkin, 1999). In Type 1 diabetes, elevated CRP levels are associated, among others, with poor glycaemic control, larger body habitus, gender, use of contraceptive pills, and factors comprising insulin-resistance syndrome (Jenkins, 2008).

Defensins belonging to antimicrobial peptides play a complex role in human immune system. They have, among others, antibacterial, antifungal, and antiviral

properties. Defensins can either enhance or inhibit an inflammation in many ways. Their role in diabetes has not been clearly defined yet. In our study, baseline elevation of defensin level in diabetic patients correlate with increased CRP value. Both might be explained by low-grade inflammation accompanying diabetes.

There is data from experimental studies that pulsed magnetic fields (PMF) increases thymus weight in mice. PMF modifies electrophoretic mobility of lymphocytes which is linked to proliferation and maturation of these cells (Bellosi, 1991). In other words, in vitro study on human lymphocytes PMF inhibited their proliferative response to mitogenic stimulation depending on exposure time and intensity of PMF (Roman, 2005). In view of positive effect of pulsed magnetic fields in rheumatoid arthritis, the researchers analyzed its effect on activity of matrix metalloproteinases in synovial fluid cells in vitro. The study showed no uniform effect of low-frequency pulsed magnetic fields on different proteinases (Mohamed, 1994). One of the hypotheses explaining the effect of magnetic field on human tissues is that it affects plasma-membrane transport of calcium ions—a resonant interaction at cyclotron frequency for calcium ions at geomagnetic densities of magnetic field takes place (Blackman, 1982; Halle, 1988).

Our study showed that defensin concentration in a healthy subject exposed to PMF increases not directly during exposure period, but after two weeks of follow up, which suggests a distant effect of PMF on this parameter. In conclusion, PMF of exposure pattern used in our study has enhancing impact on immune system in healthy subjects. Lack of effect of PMF on defensin concentration in diabetic patients might be linked to higher baseline value of this parameter in comparison to subjects without diabetes. It must be emphasized that in humans biological response is dependent on exposure at definite levels of magnetic field strength. A “window” is defined as a biological response that occurs only in a specific amplitude/frequency range, being moderate or absent beyond this range. It is not excluded that the use of various parameters of magnetic field exposure (specific “windows”) could influence defensin level in people with diabetes. In view of the complex role of defensin in the immune system of diabetic patients, the important question is: What effect would be favorable? This requires further elucidation.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

References

- Banaszkiewicz, W., Straburzynski, G. (1992). Influence of pulsed magnetic fields on selected parameters of metabolic control and acid - alkaline balance. *Baln. Pol.* 34:109–119 (in Polish).
- Bellosi, A. (1991). Effect of pulsed magnetic fields on healthy mice, a study through cellular electrophoresis of thymic cells. *Acta Med. Hung.* 48(1–2):95–102.
- Bersani, F., Ed. (1999). *Electricity and Magnetism in Biology and Medicine*. New York-Boston-Dordrecht-London-Moscow: Kluwer Academic/Plenum Publishers.
- Blackman, C., Benane, S., Kinney, L., et al. (1982). Effects of ELF fields on calcium-ion efflux from brain tissue in vitro. *Radiat. Res.* 92:510–520.
- Chaly, Y. V., Paleolog, E. M., Kolesnikova, T. S., et al. (2000). Neutrophil alpha-defensin human neutrophil peptide modulates cytokine production in human monocytes and adhesion molecule expression in endothelial cells. *Eur. Cytokine Netw.* 11(2):257–66.
- Economopoulou, M., Bdeir, K., Cines, D. B., et al. (2005). Inhibition of pathologic retinal neovascularisation by alpha-defensins. *Blood* 1;106(12):3831–3838.

- Ganz, T., Lehrer, R. I. (1997). Antimicrobial peptides of leukocytes. *Curr. Opin. Hematol.* 4:53-58.
- Greenfield, J. R., Samaras, K., Jenkins, A. B., et al. (2004). Obesity is an important determinant of baseline serum c-reactive protein concentration in monozygotic twins, independent of genetic influences. *Circulation* 109:3022-3028.
- Halle, B. (1988). On the cyclotron resonance mechanism for magnetic field effects on transmembrane ion conductivity. *Bioelectromagnetics* 9:381-385.
- Higazi, A. A-R., Nasar, T., Ganz, T., et al. (2000). The α -defensins can stimulate proteoglycan-dependent catabolism of low-density lipoprotein by vascular cells: a new class of inflammatory apolipoprotein and possible contributor to atherogenesis. *Blood* 96:1393-1398. http://www.vioforjps.pl/_index.html
- Jenkins, A. J., Rothen, M., Klein, R. L., et al. (2008). Cross-sectional associations of C-reactive protein with vascular risk factors and vascular complications in the DCCT/EDIC cohort DCCT/EDIC Research Group. *J. Diabetes Complic.* 22(3):153-163.
- Liu, H. Y., Collins, Q. F., Moukdar, F., et al. (2008). W. Suppression of hepatic glucose production by human neutrophil α -defensins through a signaling pathway distinct from insulin. *J. Biol. Chem.* 283:12056-12063.
- Melzack, R. (1987). The short-form McGill pain questionnaire. *Pain* 30:191-197.
- Mohamed Ali, H., Kolkenbrock, H., Ulbrich, N. (1994). Influence of electromagnetic fields on enzyme activity of rheumatoid synovial fluid cells in vitro. *Eur. J. Clin. Chem. Clin. Biochem.* 32(4):319-326.
- Musaev, A. V., Guseinova, S. G., Imamverdieva, S. S. (2003). The use of pulsed electromagnetic fields with complex modulation in the treatment of patients with diabetic polyneuropathy. *Neurosci. Behav. Physiol.* 33(8):745-752.
- Roman, A., Zyss, T., Nalepa, I. (2005). Magnetic field inhibits isolated lymphocytes' proliferative response to mitogen stimulation. *Bioelectromagnetics* 26(3):201-206.
- Saraheimo, M., Forsblom, C., Pettersson-Fernholm, K., FinnDiane Study Group (2008). Increased levels of alpha-defensin (-1, -2 and -3) in type 1 diabetic patients with nephropathy. *Nephrol. Dial. Transplant* 23(3):914-918.
- Saraheimo, M., Teppo, A. M., Forsblom, C., et al. (2003). Diabetic nephropathy is associated with low-grade inflammation in type 1 diabetic patients. *Diabetologia* 46:1402-1407.
- Scarfi, M. R., Bersani, F., Cossarizza, A., et al. (1991). Spontaneous and mitomycin-C-induced micronuclei in human lymphocytes exposed to extremely low frequency pulsed magnetic fields *Biochem. Biophys. Res. Commun.* 15:176(1):194-200.
- Spitznagel, J. K., Shafer, W. M. (1985). Neutrophil killing of bacteria by oxygen-independent mechanisms: a historical summary. *Rev. Infect. Dis.* 7(3):398-403.
- Wróbel, M. P., Szymborska-Kajane, A., Wystrychowski, G., et al. (2008). Impact of low frequency pulsed magnetic fields on pain intensity, quality of life and sleep disturbances in patients with painful diabetic polyneuropathy. *Diabet. Metabol.* 34:349-354.
- Yudkin, J. S. (1999). Abnormalities of coagulation and fibrinolysis in insulin resistance: evidence for a common antecedent? *Diabet. Care* 22:25-30.
- Zhang, H., Ye, H., Zhang, C., et al. (1997). Experimental studies on extremely low frequency pulsed magnetic field inhibiting sarcoma and enhancing cellular immune functions. *Sci. China C Life Sci.* 40(4):392-397.