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EFFECTS OF 300 mT STATIC MAGNETIC FIELD ON IL-8 SECRETION IN NORMAL HUMAN COLON MYOFIBROBLASTS*

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Abstract: Intestinal subepithelial myofibroblasts play a crucial role in the growth and development of the intestine. Colitis, small bowel injury, gastric ulcer disease and inflammatory bowel disease (IBD) accompany the increase in the count of activated myofibroblasts. In the last few years, the increasing production of electromagnetic (EMF) and static magnetic (SMF) fields due to the expanding use of electronic devices in everyday life, has led to a number of studies on the effects of these fields on living organisms. Because of its anti-inflammatory properties, EMF therapy may be of medical use as an IBD treatment. This mechanism has not been elucidated yet. In the present work normal human colon myofibroblasts were exposed to SMF with a flux density of 300 mT for 96 h and then the cells were cultured for 24 and 48 h with 25 mM sodium butyrate (NaB) and 10 mM 5-aminosalicylic acid (5-ASA) in either the presence or absence of SMF. Tumor necrosis factor α (TNF-α) – dependent IL-8 secretion was determined with ELISA kit. Cell viability was determined with XTT assay. It was shown that SMF has no effect on TNF-α – dependent IL-8 secretion in control cells and in cells cultured in the presence of 5-ASA and NaB.

Keywords: static magnetic field, normal human colon myofibroblasts, interleukin 8, 5-aminosalicylic acid, sodium butyrate, cell viability

In the last few years, the increasing production of electromagnetic (EMF) and static magnetic (SMF) fields due to the expanding use of electronic devices in everyday life and also in medicine, has led to a number of studies on the effects of these fields on living organisms. There are fewer studies conducted on SMF than on EMF. SMFs are usually classified as weak (< 1 mT), moderate (1 mT to 1 T), strong (1–5 T), and ultrastrong (> 5 T). SMFs have a variety of effects on living organisms ranging from an enhanced rate of enzymatic reaction to increased transcription levels and alteration in cellular growth (1). They can change biophysical properties of membranes that include hyperpolarization, redox potential, and fluidity, thereby altering flux through sodium (Na^+) and calcium (Ca^{2+}) channels (2-6). The biological effects of magnetic field depend on its properties and on different cell types (transformed or primary cells), which are exposed as single cell suspensions, monolayers or spheroids (7). The best documented in medicine are the anti-inflammatory properties of magnetic field (8).

Inflammation in inflammatory bowel diseases (IBD) is histologically characterized by a predominantly polymorphonuclear infiltrate. The recruited and activated neutrophils and macrophages are thought to be responsible for the mucosal damage (9). Intestinal subepithelial myofibroblasts play a crucial role in the growth and development of the intestine, its protection from noxious agents and repair after damage, they also contribute to wound healing and fibrosis. They are located in the lamina propria under the epithelial cells layer (10). Much attention in IBD has been focused on epithelial cells damage and TNF-α and IL-1β production by T cells and macrophages (9). These cytokines can affect the other cells of the intestinal mucosa, e.g., myofibroblasts. Many factors are secreted by the activated

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myofibroblasts in the intestine in various disease states: experimental colitis, small bowel injury, gastric ulcer models or disease and also in naturally occurring IBD. Examples include prostaglandins, TNF-α, epidermal growth factor, transforming growth factor β, basic fibroblast growth factor, interleukins (IL-1β, IL-6, IL-10) and chemokines (IL-8) (11, 12). IL-8 play a complementary and sequential role in neutrophil recruitment in the inflamed tissue in ulcerative colitis patients (13). IL-8 secretion depends on nuclear factor κB (NF-κB) activity which is increased in the intestinal lamina propria of patients with IBD. According to Lushnikov et al. (7), a therapy involving magnetic waves may be used in medicine in IBD treatment because of its anti-inflammatory properties,. Its mechanism has not been elucidated yet. Our earlier studies showed that the anti-inflammatory properties of SMF may depend on the influence on the IL-6 secretion (14). The aim of these investigations was to evaluate the SMF influence on viability, and IL-8 production in normal human colon myofibroblasts.

EXPERIMENTAL

The source of static magnetic field

The source of SMF was 48 small arranged sequentially neodymium cylinder magnets S-N-S (7 mm diameter and 3 mm thickness). The individual magnets were placed next to each other with alternating polarity. Each 96-well plate was placed over the magnets. Both, the exposed and the control samples were kept in the same incubator at 37°C. The magnetic flux density measured near the surface inside the wells using a teslameter, model LZ-641H (ENES Magnesy, Paweł Zientek Sp. k., Poland) was equal to 300 ± 30 mT.

Cell cultures

Normal human colon myofibroblasts CCD-18Co were obtained from American Type Culture Collection. The cells were cultured in a minimum essential medium (MEM, Sigma) supplemented with 10% fetal bovine serum (FBS, Sigma), 100 IU/mL penicillin G, 100 mg/mL streptomycin and 10 mM HEPES buffer (Gibco). The cell cultures were maintained at 37°C in 5% CO_2 atmosphere.

Cytotoxicity assay

The XTT (*In Vitro* Toxicology Assay Kit XTT Based, TOX-2, Sigma) assay was used to assess cell viability. The method is based on the ability of mitochondrial dehydrogenases of live cells to cleave the tetrazolium ring of XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt), yielding orange formazan crystals, which are soluble in aqueous solutions. After removing the supernatant, the cells were washed

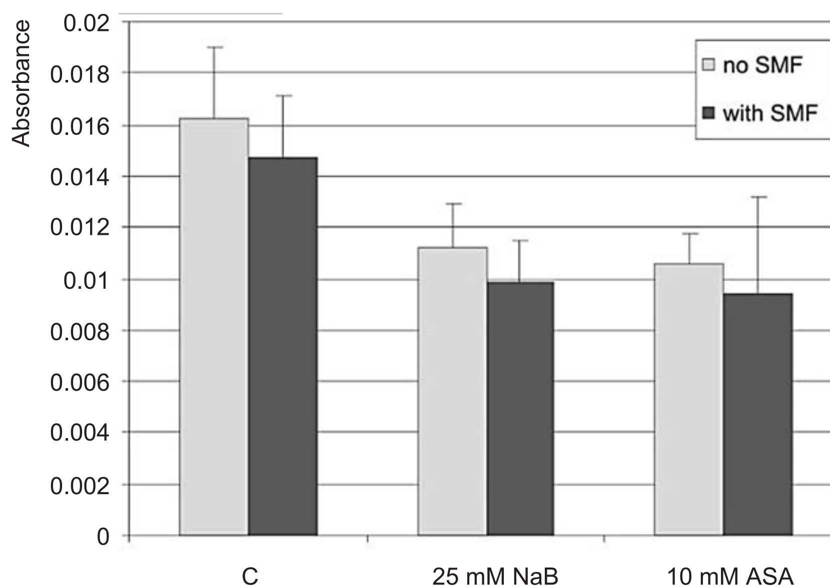

Figure 1. The effects of the SMF with a flux density of 300 mT on the myofibroblasts viability. Cell viability was expressed as a value of absorbance. Myofibroblasts were cultured for 48 h with 50 ng/mL TNF-α in the presence of 25 mM NaB and 10 mM 5-ASA. The results represent the mean ± SD (n = 6); * $p < 0.05$ compared with the control (C)

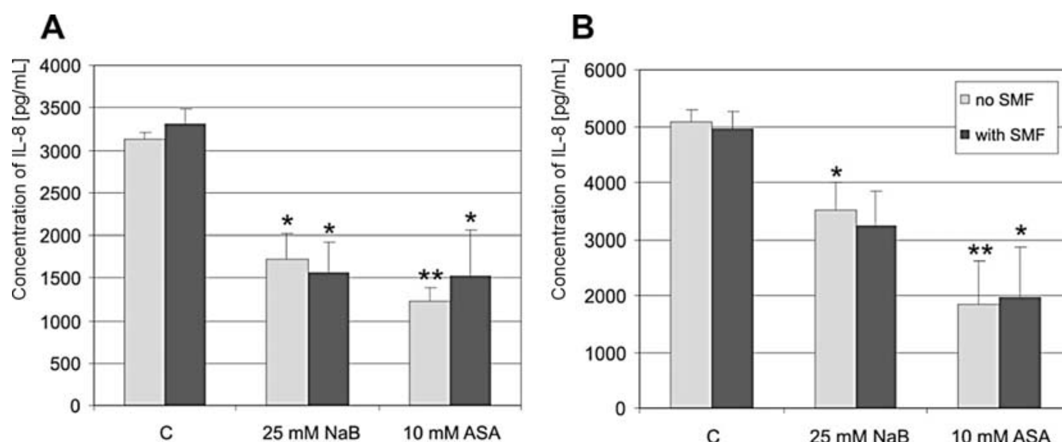

Figure 2. Influence of the SMF with a flux density of 300 mT on TNF-α-dependent IL-8 secretion in myofibroblasts. The cells were cultured for 24 h (A) and 48 h (B) in the medium containing 50 ng/mL TNF-α, 25 mM NaB and 10 mM 5-ASA in the presence and absence of SMF. The results represent the mean ± SD (n = 6); * $p < 0.05$ compared with the control (C)

three times with RPMI (without phenol red dye) and then 150 μL XTT solution was added into each well for 4 h. The absorbance of samples was measured at 450 nm (reference 690 nm) using a plate reader (MRX Revelation, Dynex). The absorbance was directly proportional to amount of the live cells.

IL-8 assay

Myofibroblasts were dispensed with a density of 20000 cells / 0.2 mL into 96-well plates. The cells were grown for 4 days with MEM supplemented with 10% FBS in either the presence or absence of 300 mT SMF. Twenty four hours before initiation of the proper experiment, the medium was replaced with a medium containing 1% FBS and then the cells were cultured with 50 ng/mL TNF-α, 25 mM sodium butyrate (NaB) and 10 mM 5-aminosalicylic acid (5-ASA) for 24 and 48 h in the presence and abstence of SMF. After this time, the supernatants of the cells were frozen at -70°C. The concentrations of IL-8 in the supernatants (diluted 1 : 10) were determined by measuring an ELISA MAX™ according to the instructions of the manufacturer (Biolegent). The absorbance at 450 nm (570 nm reference) was measured after stop reaction with the plate reader (MRX Revelation, Dynex). IL-8 concentration was determined on the basis of a standard curve and the resulting values were converted to the amount of living cells.

Statistics

The difference between independent sample means were tested using the analysis of variance (ANOVA, *post hoc* Tukey's test) or by a nonparametric Kruskal-Wallis test. A value of $p < 0.05$ was considered statistically significant. The results are expressed as the mean values ± standard deviation from number of experiments. Statistical analysis was performed using data analysis software system STATISTICA 10 (StatSoft, Inc. 2011).

RESULTS

The cells were incubated in the presence and absence of magnetic field for 6 days, while for the last two they were additionally exposed to 50 ng/mL TNF-α, 25 mM NaB and 10 mM 5-ASA. All of the medicinal products have shown a proven favorable action in the treatment of IBD. In response to TNF-α, the cells secreted IL-8. No significant impact of magnetic field was seen on the robustness of both control myofibroblast and those exposed to NaB and 5-ASA (Fig. 1). The detected action of 25 mM NaB on the secretion of IL-8 was differentiated and depended on incubation duration. Upon 24 h, NaB inhibited the secretion of IL-8, however to a minor degree (Fig. 2A). Following 48 h, it proved to have no significant effect (Fig. 3). In the latter case, the amount of IL-8 protein was assessed per live cell count. The apparent decrease in the amount of IL-8 (Fig. 2B) may be due to the simultaneous impact of NaB on cell viability. Ten mM 5-ASA probably had a slight inhibitory effect on the seretion of IL-8 after both 24 and 48 h. However, under no circumstances was magnetic field found to affect the aforementioned processes.

DISCUSSION AND CONCLUSIONS

Active ulcerative colitis (UC) and Crohn's disease (CD) belong to inflammatory bowel diseases which are characterized by increased monocyte secretion of pro-inflammatory cytokines. Experimental models of IBD and remission of CD induced by chimeric anti-TNF-α antibodies have demonstrated that TNF-α has a major role in mucosal inflammation. In IBD, TNF-α action extends beyond pro-inflammatory properties. TNF-α can activate endothelial cells, induce chemokines, recruit neutrophils to the inflamed gut mucosa, induce edema, activate coagulation and participate in granuloma formation (15). Intestinal subepithelial myofibroblasts, because of their location, can play a crucial role in IBD (10). In response to the TNF-α, activated myofibroblasts secrete numerous pro-inflammatory cytokines such as IL-8. In general, the influence of EMFs on wound healing and inflammatory processes in tissue are the best documented. Magnetic field exposure enhances wound healing by reducing inflammatory cell infiltration (16). According to Kaszuba-Zwoińska et al. (17), the direct effect of EMF on inflammatory cells viability may offer additional therapeutic opportunities in the management of CD.

Our previous studies on the same cells have shown anti-inflammatory properties of SMF associated with reduced TNF-α-induced IL-6. TNF-α (50 ng/mL)-stimulated myofibroblasts exposed to 300 mT SMF showed decreased IL-6 secretion. The same effect was observed in present mixture of Fe^{2+}/ascorbate. The exposure to SMF for 72 h caused also an increase of myofibroblast proliferation (14). The aim of these investigations was to evaluate the influence of SMF on IL-8 secretion in myofibroblasts cultured additionally in the presence or absence of 5-ASA and NaB. Both of the agents show anti-inflammatory properties on the intestinal mucosa and are used in IBD therapy. 5-ASA is a first-line drug in the treatment of IBD (18) and NaB is a product of fiber fermentation in colon (19). Both of them had influence on myofibroblasts viability after 48 h. Ten mM 5-ASA and 25 mM NaB weakly inhibited TNF-α-dependent IL-8 secretion. In the concentration used, i.e., 25 mM, NaB inhibited the secretion of IL-8 only upon 24 h, whereas, within 48 h of incubation, the effect was not seen probably due to its impact on cell viability. In any case, there was no effect of the static magnetic field on cell viability and TNF-α-induced IL-8 secretion.

Not many publications are available on the effect of SMF on the intestinal mucosa cells. Vianale et al. (20), showed that the exposure of human keratinocytes (HaCat cell line) to 50 Hz EMFs induced an early reduction of NF-κB levels, down-regulating mRNA expression and release of IL-8, MCP-1, MIP-1 and RANTES. Also, they reported an increase in keratinocyte growth. According to the authors, a selective

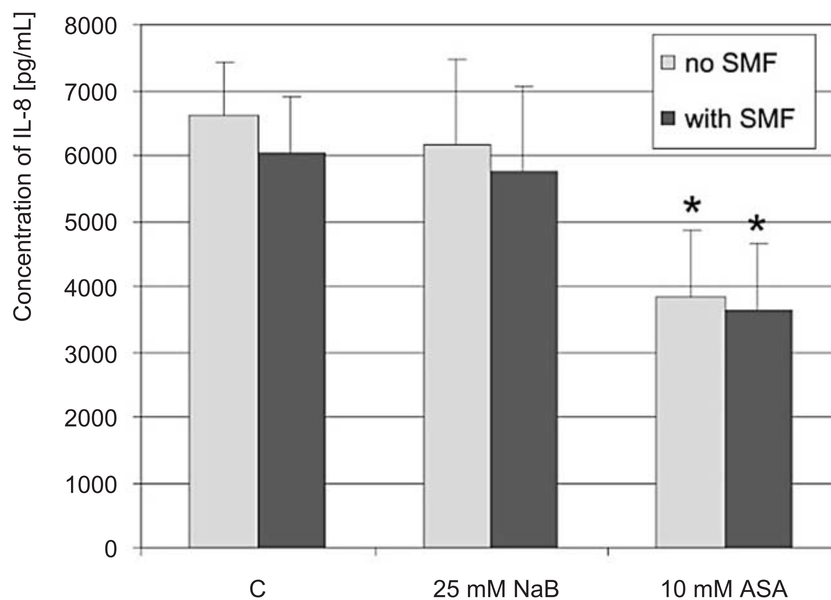

Figure 3. Influence of SMF with a flux density of 300 mT on TNF-α-dependent IL-8 secretion in myofibroblasts. The cells were cultured for 48 h in the medium containing 50 ng/mL TNF-α, 25 mM NaB and 10 mM 5-ASA in the presence and absence of SMF. The amount of IL-8 was converted into the number of live cells by means of a dedicated XTT test. The results represent the mean ± SD (n = 6); * $p < 0.05$ compared with the control (C)

inhibition of the NF-κB signalling pathway by extremely low frequencies EMF may be involved in the decrease of chemokine production (20). Several studies show that field exposure also has anti-inflammatory effects on fibroblast-like cell populations. Ongaro et al. (21) demonstrated that EMF at 75 Hz (1.5 mT) decreased PGE_2 and the production of pro-inflammatory cytokines IL-6, respectively, by 42 and 29% in human synovial fibroblasts activated with IL-1β. EMF had no effect on IL-8 secretion (21). The pulsing electromagnetic field decreased viability of human peripheral blood mononuclear cells (PBMC) isolated from CD patients and healthy donors by about 10 and 5%, respectively. PBMC from CD patients were characterized by lower production of pro-inflammatory cytokine such as interferon γ and higher concentration of anti-inflammatory cytokine such as IL-10 (17). Some studies report that repetitive SMF exposure had no effect on cell growth rate, whereas others show that SMF alone altered cell proliferation or cell death balance. Wiskirchen et al. (5), for example, found that repetitive exposure to SMF of 1.5 T exerted no effects on proliferation of human fetal lung fibroblast cells, while Raylman et al. (6) reported that prolonged exposure to 7 T SMF produced a reduction in live cell number in melanoma, ovarian carcinoma, and lymphoma cell lines. SMF exposure significantly reduced lipopolysaccharide (LPS)-induced cytotoxicity. No statistically significant differences in cell count were demonstrated upon cell exposure to SMF without LPS treatment (22). It seems that the effect of magnetic field on live cells depends upon cell type, cell line, strength and wave form of the magnetic field, time of exposure and range of radiofrequency in case of EMF. Our studies imply that SMF has no effect on IL-8 secretion in human colon myofibroblasts. Nor does it affect the action of either 5-ASA or NaB on IL-8 secretion.

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