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ORIGINAL ARTICLE

Experimental model for ELF-EMF exposure: Concern for human health

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Dedicated to the memory of our dear friend and colleague, Giovina Vianale.

KEYWORDS

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THP-1

Abstract Low frequency (LF) electromagnetic fields (EMFs) are abundantly present in modern society and in the last 20 years the interest about the possible effect of extremely low frequency (ELF) EMFs on human health has increased progressively. Epidemiological studies, designed to verify whether EMF exposure may be a potential risk factor for health, have led to controversial results. The possible association between EMFs and an increased incidence of childhood leukemia, brain tumors or neurodegenerative diseases was not fully elucidated. On the other hand, EMFs are widely used, in neurology, psychiatry, rheumatology, orthopedics and dermatology, both in diagnosis and in therapy.

In vitro studies may help to evaluate the mechanism by which LF-EMFs affect biological systems.

In vitro model of wound healing used keratinocytes (HaCaT), neuroblastoma cell line (SH-SY5Y) as a model for analysis of differentiation, metabolism and functions related to neurodegenerative processes, and monocytic cell line (THP-1) was used as a model for inflammation and cytokines production, while leukemic cell line (K562) was used as a model for hematopoietic differentiation.

MCP-1, a chemokine that regulates the migration and infiltration of memory T cells, natural killer (NK), monocytes and epithelial cells, has been demonstrated to be induced and involved in various diseases.

Abbreviations: AD, Alzheimer's disease; ELF, extremely low frequency; EMFs, electromagnetic fields; HD, Huntington disease; LF, low frequency; MCP-1, monocyte chemoattractant protein-1; PMA, phorbol-12-myristate-13-acetate; PEMF, pulsed EMF

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Since, varying the parameters of EMFs different effects may be observed, we have studied MCP-1 expression in HaCaT, SH-SY5Y, THP-1 and K562 exposed to a sinusoidal EMF at 50 Hz frequency with a flux density of 1 mT (rms).

Our preliminary results showed that EMF-exposure differently modifies the expression of MCP-1 in different cell types. Thus, the MCP-1 expression needs to be better determined, with additional studies, with different parameters and times of exposure to ELF-EMF.

1. Introduction

Exposure to electromagnetic fields (EMF) is a phenomenon that has always existed, nevertheless, during the 20th century, this is steadily increasing due to environmental exposure to man-made electromagnetic fields. Growing electricity demand, ever-advancing technologies and changes in social behaviors have created more and more artificial sources. Thus, both at home and at work everyone are exposed to a complex mix of weak electric and magnetic fields, arising from the generation and transmission of electricity by domestic appliances and industrial equipment, by telecommunications and broadcasting.

Generally the extremely low frequency (ELF) region of the electromagnetic spectrum is defined by frequencies from 3 to 3000 Hz (Poole and Ozonoff, 1996). These fields are produced by electrical devices, high tension electrical distribution networks, from residential and occupational sources and by power lines. 60 Hz (in the USA) and 50 Hz sine wave signals resemble the household alternating current electrical power supply in Europe and a large part of the world. Low-frequency electric fields influence all systems characterized by charged particles as the human body. In fact tiny electrical currents exist in the human body due to the chemical reactions that occur as part of the normal bodily functions, even in the absence of external electric fields. For example, nerves transmit signals through electrical impulses. Most biochemical reactions, from digestion to brain activities, are complying with the rearrangement of charged particles.

For several years, it has been considered that both residential and occupational exposures to ELF magnetic fields (MF) could be a possible carcinogen, based on several epidemiological studies reporting childhood leukemia and brain tumors in adult and leukemia following chronic exposure to MF (IARC 2002). Epidemiologic studies on EMF effect, reported evidence of association among childhood leukemia and postnatal exposures above 0.4μT. Previous studies concluded that residential exposures to EMFs carry an increased risk of leukemia, although other studies showed that there is no significant risk (Leitgeb, 2011). In contrast with earlier studies (Wertheimer and Leeper, 1979; Savitz et al., 1988; London et al., 1991), but in accord with others (Jirik et al., 2012; Auvinen et al., 2000) that have shown no significant increase in risk of the Acute Lymphoblastic Leukemia (ALL) for children exposed to residential levels of magnetic fields, Linet et al. show a lack of association between electromagnetic field exposure and ALL (Linet et al., 1997.

Harmful effect of EMF exposure on living tissue depends primarily on the frequency (wavelength) and density of the field and on the exposure time. Further important risk factors are the functional state and the sensibility of the exposed organism. The vascularization of the irradiated parts and the distance from the radiation source must be considered, too. EMFs of the magnitude to which we are now regularly exposed, have been implicated as a contributory factor to the childhood cancer incidence, particularly leukemia and brain cancer.

There are numerous publications describing various *in vitro* effects of EMF exposure, although the significance of these observations for clinical interpretation is unsubstantiated. A fundamental interaction mechanism between weak ELF magnetic fields and cells is also lacking, although several candidate mechanisms have been proposed. Numerous hypotheses have been suggested (IARC 2002; Davanipour et al., 2007; Draper et al., 2005; Gottwald et al., 2007), although none is convincingly supported by experimental data. A large number of cellular components, systems and processes such as proliferation, (Tsai et al., 2007) morphology, (Noriega-Luna et al., 2011) apoptosis, (Grassi et al., 2004) gene expression (Mayer-Wagner et al., 2011) and differentiation (Piacentini et al., 2008), can conceivably be affected by EMF exposure (Simkò and Mattsson, 2004). Although the role of increased intracellular Ca^{2+} was already well documented more than 20 years ago (Walleczek, 1992), recent studies have confirmed the role of increased intracellular Ca^{2+} following EMF exposure. Recently, it was suggested that a possible early biological response to EMF exposure, is the formation and prolonged survival of reactive oxygen species and other free radicals (Mannerling et al., 2010).

Different types of magnetic and electromagnetic fields are now used effectively in medicine (Markov 2007), such as in diagnostic (e.g. magnetic resonance imaging-MRI, scanner and microwave imaging) or therapy (Consales et al., 2012). Electromagnetic therapy carries the promise to be used in different diseases, in fact magnetotherapy provides an easy and non invasive method to treat the site of injury (Markov 2007). Pulsed electromagnetic fields in low frequency and intensity range (Gauss or micro-Tesla) increase oxygenation to the blood, improve circulation and cell metabolism, improve function, pain and fatigue from fibromyalgia (Sutbeyaz et al., 2009), help patients with treatment-resistant depression (Martiny et al., 2010), and may reduce symptoms from multiple sclerosis (Lappin et al., 2003). EMFs have been commonly used for the treatment of some pathological conditions to stimulate tissue regeneration and repair (Bertolino et al., 2006). Application in the area of orthopedics for the treatment of non-union fractures and failed fusions, takes advantage of the evidence that pulsed EMF (PEMF) accelerates the re-establishment of normal potentials in damaged cells (Fiorani et al., 1997), promotes the proliferation and differentiation of osteoblasts (Wei et al., 2008) and improves the osteogenic

phase of the healing process (Canè et al., 1993). Long-lasting relief of pelvic pain of gynecological origin has been obtained consistently by short exposures of affected areas with the application of a magnetic induction device, producing short, sharp, magnetic-field pulses of minimal amplitude (Jorgensen et al., 1994). EMFs improve cell survival and reduce ischemic damage (Grant et al., 1994).

2. EMFs and skin injury

Being the skin the largest organ that covers the surface of our body, it is frequently subject to the action of non ionizing MF. Keratinocytes, those are able to release immunomodulators and to play a key role in immune system function may be used as an *in vitro* model to evaluate the biological effects of non ionizing electromagnetic field on the skin.

Wound healing is a highly coordinated and complex process involving the proliferation and migration of various cell types (epidermal, dermal as well as inflammatory cell), chemical mediators and the surrounding extracellular matrix, resulting in a tightly orchestrated re-establishment of tissue integrity by specific cytokines. Wounds can be categorized as acute or chronic according to their healing time-frame. Acute wounds repair themselves and heal normally following the correct pathway. The chronic condition derives from non-healing wounds in a timely and orderly manner, that determines ulcers (Lazarus et al., 1994). Ischemia, diabetes mellitus, venous stasis and pressure can be at the root of the majority of non-healing wounds that are prone to complications including functional limitations, infections and malignant transformation (Eltorai et al., 2002; Chraibi et al., 2004).

Although there are many experimental and clinical evidences supporting the use of magnetic fields to help bone healing, its application for soft tissue healing, including skin and tendons is still ambiguous. Several authors, however, showed the ability of PEMFs in reducing the wound healing durations (Cheing et al., 2014; Athanasiou et al., 2007; Strauch et al., 2007) and improving tensile strength of scars (Goudarzi et al., 2010). Roland et al. used pulsed magnetic energy to stimulate neovascularization in a rat model (Roland et al., 2000). Weber et al. showed that rat groin composite flap survival increases when supported by an arterial loop, thus confirming that PEMFs promote neovascularization (Weber et al., 2004). The most rapid wound healing exposed to EMF may be dependent on the anti-inflammatory effects caused by the change in the coagulation system, in the improvement of microcirculation and in immunological reactiveness (Matic et al., 2009). Conversely, Milgram found that PEMF did not produce any beneficial effects on wound healing. So the effects of PEMF on wound closure varied among the studies, possibly due to different treatment protocols that were applied (Milgram et al., 2004).

Callaghan et al. (2008) confirmed the results of Tepper et al. (2004), that demonstrated the increase in proliferation and tubulization of endothelial cell cultures and the increase in the expression of fibroblast growth factor 2 (FGF-2), a potent stimulator of angiogenesis, after exposure to electromagnetic field.

Vianale et al. showed that ELF-EMF modulates production of RANTES, MCP-1, MIP-1α and IL-8, and keratinocyte growth through the inhibition of the NF-kB signaling pathway and they hypothesized that ELF-EMF may inhibit inflammatory processes (Vianale et al., 2008).

Recently, several reports have supported the anti-inflammatory effects of EMFs on tissue repair. Pesce et al. reviewed the effect of EMFs on cytokines that drive the transition from a chronic pro-inflammatory to an anti-inflammatory state of the healing process (Pesce et al., 2013). Patruno's results showed the ability of ELF-EMF to induce keratinocyte proliferation and to up modulate NOS activities and to down-regulate COX-2 expression and PGE-2 production, involved in the modulation of inflammatory reaction (Patruno et al., 2010). *In vitro* study of Huo et al. showed that the noninvasive EMFs have a strong effect on normal human keratinocytes and fibroblast migration while only weakly promote keratinocyte proliferation (Huo et al., 2009). The observations of Manni et al. confirm the hypothesis that ELF-EMF (50 Hz) may modify cell membrane morphology and interfere with initiation of the signal cascade pathway and cellular adhesion (Manni et al., 2002). ELF-EMF application modifies the biochemical properties of human keratinocytes (HaCaT) associated with different actin distributions as demonstrated by Lisi et al. (2006).

3. EMFs and neurodegenerative diseases

The term neurodegeneration indicates the progressive loss of neuronal function and structure until the neuron death. Many neurodegenerative diseases such as Parkinson disease (PD), Alzheimer's disease (AD), Huntington disease (HD), and Amyotrophic Lateral Sclerosis (ALS) result from neurodegenerative processes and many of these are classified as pathologies due to the aggregation of misfolded proteins. PD is a disorder of the central nervous system resulting from the death of dopaminergic cells in the substantia nigra. The basis of this mechanism may consist of an abnormal accumulation of the protein alpha-synuclein that forms insoluble fibrils, in the damaged cells. The beta-amyloid peptide (Aβ) is a small peptide that comes from the cleavage of a larger transmembrane protein called amyloid precursor protein (APP). Aβ is the major component of plaques in the cerebral cortex of AD and is critical to neuron growth, survival and is involved in the loss of synapses and the neuron death, as well as hyperphosphorylated tau protein, the main component of neurofibrillary tangles in AD brain. Sobel and Davanipour hypothesized the ability of the ELF-EMFs to increase the intracellular calcium concentration levels that are positively correlated with the cleavage of the APP to give the soluble Aβ (Sobel and Davanipour, 1996). Several studies seem to suggest a potential association between occupational exposure to ELF-EMFs (typical of electric power installers and repairers, power plant operators, electricians, telephone line technicians, welders, carpenters, and machinists) and AD onset (Garcìa et al., 2008; Röösli, 2008), although their biological nexuses remain unknown.

HD is a progressive neurodegenerative disorder whose underlying genetic defect lies in expanded trinucleotide (CAG)n of the Huntington ubiquitous protein. In HD, an autosomal dominant disease, the mutated gene leads neuronal dysfunction and degeneration, even though the mechanisms by which it acts are not fully understood. The potential correlation between EMF exposure and HD pathogenesis is not sustained by epidemiological evidence, while there is evidence that the improvement in behavior and the neuroprotective effect of ELF-EMF exposure may be due to enhanced neurotrophic

factor levels, and reduced both oxidative damage. The Amyotrophic Lateral Sclerosis is a fatal neurodegenerative disorder characterized by progressive degeneration of motor neurons in the spinal cord, motor cortex, and brainstem. Approximately 20% of patients were found to show mutation of the gene encoding the antioxidant Cu^{2+}/Zn^{2+} SOD (SOD1) (Julien and Kriz 2006), confirming the central role of oxidative stress in neurodegenerative diseases (Chang et al., 2008). On the basis of epidemiologic findings, evidence shows an association between amyotrophic lateral sclerosis and occupational EMF exposure although there is confounding (Davanipour et al., 1997; Savitz et al., 1998). The investigations of EMF effects on neurodegenerative diseases are now very interesting, although not well developed, in fact the experimental findings supporting this link are still controversial due to the field frequency applied and the disease investigated (Consales et al., 2012). Crasson et al. indicated that 50 Hz EMF may have slight influence on event-related potential and reaction time under specific circumstances of sustained attention in healthy male volunteers. (Crasson et al., 1999). Trimmel's study shows a reduction of cognitive performances in attention, perception and memory performances by a 50 Hz EMF of 1 mT (Trimmel and Schweiger, 1998).

Sulpizio et al. have demonstrated that ELF-MF exposure triggers significant changes in the protein global profile of SH-SY5Y cell line, experimental model for neurodegenerative disorders. In particular, the expression levels of common protein spots involved in cellular defense mechanisms, organization, and biogenesis increased as a consequence of ELF-EMF treatment. In ELF-EMF treated samples was observed the over-expression of proteins related to a high malignant potential, drug resistance, cytoskeleton re-arrangement, and enhanced defense against oxidative stress, in association with higher proliferative activity (Sulpizio et al., 2011; Xie et al., 2010). *In vivo* study showed that exposure to environmental ELF-EMF did not change the expression of α3, α5 and α7 nicotinic cholinergic receptors impaired in AD (Antonini et al., 2006). Falone et al. have demonstrated that a 50 Hz magnetic field induced a significant enhancement of the antioxidant defenses together with a major shift of redox homeostasis and they previously established that ELF-MF exposure improves cellular viability and induces significant adaptations in the redox-related biochemical machinery of the human neuroderived SH-SY5Y cell line (Falone et al., 2007).

4. EMFs and immune cells

Cells of the immune system regulate health on a systemic level, thus are plausible study targets. .In response to a pathogen challenge they must respond in a very sensitive, swift and effective way. Immune cells produce cytokines, important signaling molecules, which are key regulators of cell activation and inhibition.

Monocytes and macrophages have an important function as the first line of defense against pathogens and can act as antigen-presenting cells to trigger a specific response from lymphocytes, and are capable of producing several cytokines including interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin-10 (IL-10). Their recruitment to inflammatory sites and neoplastic tissues and their activation induce a wide range of intracellular signaling pathways and are crucial to the success of an immune reaction. Cytokine production and secretion patterns are modified upon differentiation of monocytes into macrophages (Bouwens et al., 2012). Several papers have demonstrated that the *in vitro* exposure of immune cells to nonthermal ELF-EMF can elicit molecular and cellular changes that might be relevant to the activity of the immune system *in vivo*. Years ago, it was demonstrated that only mutagen-activated lymphocytes are responsive to EMF exposure, in fact EMF do not interfere with activation and committeemen of cells (Cadossi et al., 1992). Nindl et al. have showed that 60 Hz sinusoidal EMFs induce an increase in anti-CD3 binding to T cell receptors (TcRs) of Jurkat cells, a T lymphocyte cell line, and that can regulate lymphocyte proliferation *in vitro* and *in vivo* (Nindl et al., 2000). Reale et al. have demonstrated that upon ELF-EMF activation, monocytes/macrophages increase the production of chemokines, peroxidases, cytolytic proteases, and nitric oxide (NO) enhancing their microbicidal/tumoricidal capacity (Reale et al., 2006). Akan et al. have demonstrated that field application increased NO, cGMP, and HSP levels, and caused a slight decrease in apoptosis (Akan et al., 2010; Frahm et al., 2010). Many *in vitro* and *in vivo* studies have evaluated the expression of free radicals in human monocytes and mouse macrophages after exposure to 50 Hz, 1 mT ELF-EMF (Simkò et al., 2001; Lupke et al., 2004; Rollwitz et al., 2004). NO, a free radical, is an important intracellular and intercellular signaling molecule, and an important host defense effector for the phagocytic cells of the immune system (Förstermann and Kleinert, 1995). Several studies have been conducted to evaluate the effect of ELF-EMF exposure on cytokine profiles, consistent and independently replicated laboratory evidence to support modulation of cytokines expression and production has not been obtained (Ikeda et al., 2003; Luceri et al., 2005; Miller et al., 1999; Natarajan et al., 2006; Reale et al., 2006; Lupke et al., 2006; Murabayashi et al., 2004). Ayşe et al. demonstrated that *in vitro* effect of ELF-EMF on the differentiation of K562 cells is time dependent. In fact single exposure to ELF-EMF resulted in a decrease in differentiation; ELF-EMF applied everyday for 1 h caused an increase in differentiation. These results imply that the time-course of application is an important parameter determining the physiological response of cells to ELF-EMF (Ayşe et al., 2010) and other authors have supported the hypothesis that the effect of ELF-EMF on biological systems depends on the conditions of the cell (Garip and Akan, 2010).

Numerous studies are still underway to try to understand the mechanism behind these alterations investigated as the gaming is very complex. Conflicting conclusion were showed in many EMF *in vivo* studies, due to small numbers of subject, distance from EMF source, time exposure or concomitant environmental risks. The *in vivo* study of Boscolo did find differences in cytokine levels in serum of subject exposed to ELF-EMF (Boscolo et al., 2001). In a set of experiments evaluating time courses for immediate early genes, stress response, cell proliferation and apoptotic genes, Kirschenlohr et al. showed no consistent response profiles after repeated ELF-EMF exposures (Kirschenlohr et al. 2012).

5. Cell line *in vitro* models

Cell lines have some advantages over human primary cells such as (a) homogeneous genetic background that minimizes

the degree of variability in the cell phenotype, a trait particularly important when studying the biological function with high variability; (b) ability to be stored indefinitely in liquid nitrogen to guarantee sufficient cells for DNA, RNA and protein; (c) reduced variability compared to primary cells; and (d) reproducibility of the results obtained.

SH-SY5Y cells were derived from immature neoplastic neural crest cells that exhibit properties of stem cells. The SH-SY5Y cell line is a thrice-cloned subline of SK-N-SH cells that were originally established from a bone marrow biopsy of a neuroblastoma patient and were widely used as model of neurons since the early 1980's (Biedler et al., 1973). These cells possess the capability of proliferating in culture for long periods without contamination, a prerequisite for the development of an *in vitro* cell model, posses many biochemical and functional properties of neurons, exhibits neuronal marker enzyme activity, express neurofilament proteins and also express opioid, muscarinic, and nerve growth factor receptors (Ciccarone et al., 1989). Consequently, the SH-SY5Y cell line has been widely used in experimental neurological studies, including analysis of processes related to neurodegeneration, neuroprotection and neurotoxicity. The processes of keratinocyte proliferation and differentiation represent the central and final event in tissue regeneration leading to the formation of a massive bulk of cells, necessary to cover the wounded area. It is widely accepted that *in vitro* keratinocyte model systems, such as HaCaT cell line, at low and high density can be compared with early and late phases of the re-epithelialization process. HaCaT cells are *in vitro* spontaneously transformed keratinocytes from histologically normal skin. Thus keratinocytes are the most likely cells to be impacted by electromagnetic radiation.

THP-1 is single, round suspension cells that after exposure to phorbol-12-myristate-13-acetate (PMA) or 1α,25-dihydroxyvitamin D3 (1α,25(OH)2D3) may start to adhere to culture plates accompanied by phenotype change into a macrophage. Based on phenotypic and functional features with human microglial cells, human monocyte-derived macrophages were called brain macrophages (Ulvestad et al., 1994). THP-1 cells, due to their functional and morphological similarities, have been widely used as a model of human monocytes/macrophages or microglia (Tsuchiya et al., 1982; Tsuchiya et al., 1980; McDonald et al., 1998) or as a valid model to mimic proliferation, adhesion and migration of monocytes and macrophages in the vasculature.

The human K562 cell line has been isolated and characterized by Lozzio (Lozzio and Lozzio, 1975) from a patient with chronic myelogenous leukemia (CML) in blast crisis. K562 has been used as a model of common progenitor of erythroblasts and megakaryocytes and can be differentiated into erythroid and megakaryocytic lineages thus has been used extensively as a model for the study of leukemia differentiation, molecular mechanism(s) regulating the expression of genes (Iyamu et al., 2000), as well as to determine the therapeutic potential of new differentiation-inducing compounds (Bianchi et al., 2001).

6. Effects of ELF-EMF exposure on MCP-1

Chemokines are low molecular weight chemotactic cytokines that have been shown to play a relevant role in inflammatory events, such as transendothelial migration and accumulation of leucocytes at the site of damage. In addition, they modulate a number of biological responses, including enzyme secretion, cellular adhesion, cytotoxicity and T-cell activation and tissue regeneration (Vianale et al. 2008).

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C–C chemokine family and is a potent chemotactic factor for monocytes. Located on chromosome 17 (chr.17, *q11.2*), human MCP-1 is composed of 76 amino acids and is 13 kDa in size (Van Coillie et al., 1999). A variety of cell types including endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytic, monocytic, and microglial cells (Cushing et al., 1990; Standiford et al., 1991; Brown et al., 1992; Barna et al., 1994), are able to produce MCP-1, either constitutively or after induction by oxidative stress, cytokines, or growth factors. Rolling of monocytes on endothelial cells is dependent on the binding of E-selectin and sialyl Lewis X, and adhesion to the endothelium is dependent on the interaction of integrin on monocytes and adhesion molecules on the endothelial cells. Although leukocytes have been considered the main targets for chemokines, recent evidence indicates that the actions of these proteins are not restricted to these cell types. The main function of MCP-1 consists of the establishment of chemotaxis driving the recruitment of cells at sites of inflammation, by integrin activation. Specifically MCP-1 attracts monocytes, natural killer cell and memory T cells, and influences expression of cytokines related to T helper responses. Its expression occurs in a variety of diseases characterized by mononuclear cell infiltration, and there is substantial biological and genetic evidence suggesting that it may contribute to the inflammatory component of diseases such as atherosclerosis, multiple sclerosis, Alzheimer's disease, or rheumatoid arthritis. In the central nervous system (CNS), MCP-1 is involved in the recruitment of the main resident immune cell types of the brain (astrocytes and microglia) and of infiltrating monocytes from the systemic bloodstream. There is strong evidence that MCP-1 plays a major role in myocarditis, ischemia/reperfusion injury in the heart, in transplant rejection, and in cardiac repair. After 24 h of chronic exposure to 50 Hz, 1 mT EMF, MCP-1 levels were reduced significantly in PHA-stimulated cells, while in non-stimulated cells no significant differences in MCP-1 levels were observed. The authors speculate the anti-inflammatory potency of electromagnetic fields and suggest that the inhibitory effect on MCP-1 release, evaluated by the ELISA assay, could be one of the mechanisms by which ELF-EMF is therapeutic in inflammatory diseases (Di Luzio et al., 2001).

Previous studies have suggested that magnetic field is involved in NO production. Thus, Reale et al. exposed LPS-stimulated peripheral blood adherent mononuclear cells to 50 Hz EMF. Results of RT-PCR showed that, while both mRNA and protein levels of MCP-1 were up-regulated, iNOS was down-regulated. The increase in MCP-1 is related to NF-kB protein expression and in agreement with previous results showing that the inhibition of nitric oxide production in endothelial cells increased the expression of MCP-1. The changes in MCP-1 and iNOS expression, evaluated through RT-PCR, after ELF-EMF are very interesting for their roles in the development of inflammatory responses. The authors suggest a non pharmacological role of EMF in maintaining the balance between MCP-1 and NO in inflammatory reaction (Reale et al., 2006).

Since the EMF effect is cell type-dependent and MCP-1 is produced which acts on different cell types, and very little is known about the influence of ELF-EMF on MCP-1 expression in different cell types, we studied the effect of ELF-EMF on MCP-1 expression and production in HaCaT, SH-5YSY, THP-1 and K562 cells.

In our ELF-EMF the flux density of 1 mT (rms) was produced by an electromagnetic generator (Agilent Technologies, Santa Clara, CA, mod. 33220A) with stability higher than 1% both in frequency and in amplitude. The generator was connected to a power amplifier (Nad Electronics Ltd, London, U.K., mod. 216). An oscilloscope (ISO-TECH mod. ISR658, Vicenza, Italy) was dedicated to the monitoring of output signals from the Gaussmeter (MG-3D, Walker Scientific Inc., Worcester, MA). A current flux passed through a 160 turns solenoid (22 cm length, 6 cm radius, copper wire diameter of 1.25×10^{-5} cm) generating a horizontal magnetic field. The achieved MF intensity (1 mT/rms) was measured continuously during exposure using a Hall-effect probe connected to the Gaussmeter. The solenoid was then placed inside the incubator. The environmental magnetic noise inside the incubator was related to the geomagnetic field (~40 mT), and to the 50 Hz disturbance associated with the working incubator [~7 mT (rms)]. The built-in digital thermometer of the incubator monitored the internal temperature, which resulted constant at 37 ± 0.38 °C. In addition, another digital thermometer (HD 2107.2; Delta OHM, Padova, Italy) was placed inside the solenoid and near the cell cultures to record local temperature variations. No significant temperature change related to applied ELF fields was observed (DT0.18C). However, no thermal effect on cells can be hypothesized for temperatures around 37.8 °C, because EMF interactions with biological molecules are known to be non thermal in nature. Low-level Joule heating was dissipated inside the incubator by a fan system. In all experiments cells were placed in the central part of the solenoid, which presented the highest degree of the field homogeneity (98%).

All experiments are performed at the same conditions of EMF intensity, frequency, chronical exposure, and temperature. In Table 1, we report the effects of the ELF-EMF exposure on MCP-1 expression in different cell lines.

In HaCaT cells, using RT-PCR we have evidenced a decrease in MCP-1 expression from 4 to 72 h in EMF-exposed cells with respect to non-exposed cells. This decrease was confirmed by additional Real Time PCR (basal exposed 0.9 ± 0.02 vs. basal non-exposed 1.6 ± 0.05). Also the ELISA immunoassay, performed to evaluate the release of MCP-1, confirmed the expression results. Since it is well accepted that an excessive or prolonged inflammatory response may interfere with wound healing and cause reduction of the inflammatory chemokines by ELF-EMF exposure, represents an interesting and new therapeutic approach in delayed healing.

Table 1 Effects of the ELF-EMF exposure on MCP-1 in different cell types.

Cell lines	Effect on MCP-1 expression
HaCaT	
Basal	Decreased
SH-SY5Y	
Basal	Decreased
THP-1	
Basal	No effect
LPS-stimulated	No effect
K562	
Basal	No effect
PMA-stimulated	Increased

In SH-SY5Y cell cultures exposed to ELF-EMF, genes involved in the stress response, cell growth and differentiation or protein metabolism have been reported to be generally down-regulated. Genes involved in Ca^{2+} metabolism, the PI3-kinase pathway are up-regulated. Likewise, key mediators of the inflammatory response appear susceptible to swift modulation, in SH-SY5Y.

MCP-1 is involved in the neuroinflammatory processes associated with diseases characterized by neuronal degeneration. To characterize the impact of ELF-EMF on early ongoing cellular processes, MCP-1 gene expression in SH-SY5Y, was evaluated in the presence and absence of ELF-EMF exposure by RT-PCR. After 24 h of ELF-EMF exposure MCP-1 expression was not significantly affected. Albeit our results on MCP-1 expression, despite differences in experimental conditions, are in line with several other ELF-EMF exposure results, while they are not in accord with a study reporting that ELF-EMF promotes cellular neurodifferentiation, as exemplified by neurite extension and number (Falone et al., 2007). In conclusion, our results showed that ELF-EMF exposure is well tolerated and has no relevant impact on MCP-1 gene expression.

The role of MCP-1 in human disease has been demonstrated by immunohistochemical studies in fact the adhesion of cells to the endothelium was induced by expression of adhesion molecules and chemotactic proteins, such as MCP-1. We have analyzed the effects of EMF on the expression of MCP-1 also in THP-1 cells. Since in THP-1 exposed to ELF-EMF no increase in basal levels of MCP-1 was observed, cells were treated or not with LPS and exposed to 50 Hz, 1 mT EMF for 24hr. Our data indicate that the presence of 10 μg/ml of LPS leads to an increase in expression of MCP-1 in both THP-1 cells non exposed or exposed to EMF. Thus, we hypothesized that MCP-1 mediated THP-1 migration is not affected by EMF exposure, and consequently the exposure to the fields is not a risk factor in diseases in which microglial migration plays a crucial role, such as atherosclerosis, multiple sclerosis and other neuroinflammatory diseases.

Although it is known that intracellular redox status modulates MCP-1 expression and that ELF-EMF exposure can act on redox state of K562 cells. No studies have evaluated the influence of EMF exposure on MCP-1 expression in K562 cell line. In K562 exposed to the ELF-EMF spontaneous expression of MCP-1, detected by RT-PCR, was not modulated in comparison to cells not exposed. PMA induces monocytic or megakaryocytic differentiation of K562 cells through the activation of MAP kinases. When PMA-stimulated cells were exposed to the field, we noticed a slight increase in the expression of the chemokines and particularly the increase in MCP-1. Next step of this study will be to evaluate if ELF-EMF exposure is able to modulate the activation of MAP kinases in comparison with PMA.

7. Conclusions

The results of *in vivo* and *in vitro* studies suggest that EMF may modulate the expression of some inflammatory molecules. The understanding of the influences of EMF on transcriptional events will lead to a better understanding of their mechanisms and to therapeutic interventions for diseases in which these inflammatory molecules play a key role. In spite of the fact that the mechanisms of action of EMF are still under investigation, some authors have supposed that exposure to ELF-EMF affects cell function through mechanical action on both intracellular and membrane proteins, which includes ion channels, membrane receptors and enzymes. All studies agree that the effect of the sinusoidal ELF-EMF varies in relation to cell type and other parameters, such as frequency, flux density and time exposure.

Our data confirm the cell-type dependent effects; in fact we observed increase, decrease or no effect on the MCP-1 expression in different cell lines grown under the same conditions (sinusoidal 50 Hz, 1 mT, 37 °C, 5% CO_2).

In order to assess if ELF-EMFs, associated with both industrial and domestic use, may play a role as adjuvant or causative factor in disease development or may play a role as therapeutic and diagnostic tool, further studies to evaluate a more complete list of genes that may be up- or down-regulated by ELF-EMF exposure must be preformed. New studies designed to evaluate the actions of ELF-EMF under multiple conditions, including chronic or sporadic exposure, in combination with common stressors pertinent to real life, appear warranted and may aid our understanding of their true biological impact.

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