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Exposure to Static and Extremely-Low Frequency Electromagnetic Fields and Cellular Free Radicals

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ABSTRACT

This paper summarizes studies on changes in cellular free radical activities from exposure to static and extremely-low frequency (ELF) electromagnetic fields (EMF), particularly magnetic fields. Changes in free radical activities, including levels of cellular reactive oxygen (ROS)/nitrogen (RNS) species and endogenous antioxidant enzymes and compounds that maintain physiological free radical concentrations in cells, is one of the most consistent effects of EMF exposure. These changes have been reported to affect many physiological functions such as DNA damage; immune response; inflammatory response; cell proliferation and differentiation; wound healing; neural electrical activities; and behavior. An important consideration is the effects of EMF-induced changes in free radicals on cell proliferation and differentiation. These cellular processes could affect cancer development and proper growth and development in organisms. On the other hand, they could cause selective killing of cancer cells, for instance, via the generation of the highly cytotoxic hydroxyl free radical by the Fenton Reaction. This provides a possibility of using these electromagnetic fields as a non-invasive and low side-effect cancer therapy. Static- and ELF-EMF probably play important roles in the evolution of living organisms. They are cues used in many critical survival functions, such as foraging, migration, and reproduction. Living organisms can detect and respond immediately to low environmental levels of these fields. Free radical processes are involved in some of these mechanisms. At this time, there is no credible hypothesis or mechanism that can adequately explain all the observed effects of static- and ELF-EMF on free radical processes. We are actually at the impasse that there are more questions than answers.

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Static and extremely-low frequency electromagnetic fields; free radicals; Fenton reaction; cell proliferation; evolution

Introduction

This is a review of the research on the effects on cellular free radicals after exposure to static- and extremely-low frequency (ELF, 0–300 Hz) non-ionizing electromagnetic field (EMF). In 1997, we first reported that melatonin, a potent antioxidant, and the spin-trap compound N-tert-butyl-alpha-phenylnitrone (that neutralizes free radicals) blocked a 60-Hz magnetic field-induced DNA strand break in cells of the rat brain (Lai and Singh 1997a, 1997b). Further experiment (Lai and Singh 2004) demonstrated similar inhibitory effects of Trolox (a vitamin E-analog anti-oxidant) and 7-nitroindazole (a nitric oxide synthase inhibitor). In addition, the effect could also be blocked by the iron chelator deferiprone suggesting the involvement of the iron-catalyzed Fenton Reaction that produces the potent cytotoxic hydroxyl free radical. These data indicated that the ELF magnetic field affected free radicals in cells leading to cellular molecular damages. There are now more than 200 papers published showing that static and ELF-EMF affect cellular free radical processes. A list of the papers is in the "supplementary material" included in the online version of this paper. There are rather strong indications that exposure to static- and ELF-EMF affects oxidative status in cells and animals. Many of the cellular oxidative and anti-oxidative components have been shown to be affected by the fields.

Effect on cellular free radical processes is probably the most consistent biological effect of non-ionizing electromagnetic fields (EMF). It has been reported in many different animal and plant species after exposure to EMF from static to radiofrequency (see Yakymenko et al. (2016) and a 2017-update in the "oxidative effects of ELF-EMF and radiofrequency radiation (RFR) section" in the Bioinitiative Report (2012)).

Free radicals

Reactive free radicals (mainly, reactive oxygen species (ROS) and reactive nitrogen species (RNS)) are produced as a result of cellular metabolism, particularly in the mitochondria. Reactive oxygen species (ROS) include mainly singlet oxygen, superoxide, peroxides,

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and hydroxy radical and reactive nitrogen species (RNS) including mainly peroxtnitrite, nitrogen dioxide, which are products of the reaction between nitric oxide and superoxide. Nitric oxide is generated in cell by nitric oxide synthases. Presence of free radicals in cells can lead to macromolecular damages (in DNA, proteins, and lipids), disturbance in cell functions, and cell death. Damage in DNA is a cause of cancer. Under normal conditions, free radical levels are kept in check by various inducible antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). In instances, when there is an excessive increase in free radical production or a deficit in anti-oxidant capacity, oxidative/nitrosative stress results leading to cellular damage and functional deficits. However, free radicals also serve important cellular functions and are involved in cellular signaling cascades that govern normal cell functions and in immune defense against bacteria. They are also involved in cellular chemistry that triggers apoptosis. Thus, it is essential to keep free radicals at a critical physiological homeostatic level. Any disturbance could lead to detrimental biological consequences (cf. Pizzino et al. 2017; Valko et al. 2007).

Cellular free radical processes is a complex physiological mechanism. It involves feedbacks and compensatory responses of different cellular components to maintain homeostasis. EMF could disturb different components of the process leading to a cascade of changes. Since exposure to EMF leads to disturbance in free radical production and excessive presence of free radicals in cells can be considered as a stress, living organisms under chronic EMF exposure probably go through the three phases of the "general-adaptation-syndrome" of stress, i.e., alarm, resistance, and exhaustion phases (Selye 1951). Thus, the characteristic of the free-radical responses depends on how long exposure has been occurring. In addition, effects observed could depend on the cell type and organ studied; time when the changes were studied, and exposure conditions (such as intensity, cumulative duration of exposure, and characteristics of the field). Thus, it is not surprising that the changes described in the "supplementary material" show a complex pattern, i.e., changes are not always in the same direction. Research on the effects of static- and ELF-EMF is mainly on ROS. There are only few studies on RNS (~5%).

Effects of static and ELF EMF-induced changes in cellular free radical processes

Several papers reported changes in biochemistry, physiology, and general functions as a consequence of changes in cellular oxidative status resulting from exposure to static- and ELF-EMF. These include: DNA damage (Giorgi et al. 2017; Jajte et al. 2001; Koyama et al. 2008; Lai and Singh 1997a, 1997b; Lai and Singh 2004; Yokus et al. 2005, 2008); immune response (Akan et al., 2010; Kim et al. 2017); inflammatory response (Kim et al. 2017; Zhang et al. 2017); apoptosis (De Nicola et al. 2006; Ding et al. 2004; Garip and Akan 2010; Ghodbane et al. 2015; Koh et al. 2008; Solek et al. 2017; Wartenberg et al. 2008; Yang and Ye 2015); protein misfolding and generation of prions (Lian et al. 2018); cell proliferation and differentiation (Ehnert et al. 2017; Hajipour Verdam et al., 2018; Lee et al. 2010; Patruno et al. 2010; Song et al. 2018; Van Huizen et al. 2019; Wolf et al. 2005); rhythmic slow activity in hippocampal slices of the brain (Bawin et al. 1996); visual evoked potentials (Akpinar et al. 2012); auditory event-related potentials (Akpinar et al., 2016); visual and somatosensory evoked potentials (Akpınar et al. 2016); heart rate (Ciejka and Goraca 2009); wound healing (Glinka et al. 2013; Patruno et al. 2010, 2011); bone formation (Zhang et al. 2018); post-stroke recovery (Cichoń et al. 2017a, 2017b, 2018); hyperalgesia (Jeong et al. 2006); opioid-induced antinociception (Kavaliers et al. 1998); spatial memory and learning (Cui et al. 2012; Deng et al. 2013; Karimi et al. 2019); cognitive impairment (Duan et al. 2013); mismatch-negativity response (Kantar-Gok et al., 2014); depressive disorder (Ansari et al. 2016); anxiety-like behavior (Djordjevic et al. 2017); and obsessive compulsive disorder-like behavior (Salunke et al. 2014). However, in most of these studies, the cause–effect relationship was not well established. Do EMF-induced changes in oxidative status cause these effects? Or, are they effects of EMF caused by mechanisms unrelated to oxidative changes? One powerful proof of a free-radical effect is to establish whether an effect, e.g., DNA damage, could be blocked by antioxidants or pro-oxidants. An effect caused by a change in free radicals should be able to be blocked by antioxidants or pro-oxidants. There are several studies that employed this strategy (see "Supplementary material").

In most of the ELF-oxidative effects studies, the intensities used were relatively high (i.e., more than 0.1 mT) compared to ambient levels of static- and ELF-EMF (in μT levels) in the human environment. However, effects at high intensities could possibly occur in occupational exposure situations where the levels are relatively high. In addition, the exposure durations in most of these studies are short-term (from hours to several days), whereas environmental exposure is generally chronic. Can most of the research results applicable to real-life exposure situation? Do oxidative changes occur after exposure to ambient levels of static- and ELF-EMF?

Table 1. Free radical effects observed at low intensities of static and ELF-EMF.

	Effect observed	Exposure conditions
Bediz et al. (2006)	Oxidative changes in rat blood and brain	50 Hz; 5 μT
Belova et al. (2010)	Changes in ROS and activation of mouse peritoneal neutrophils	Continuous-wave (31 Hz) and pulsed (15 Hz); 74.7 μT
Budziosz et al. (2018)	Change in SOD activity in the rat brain	50 Hz; 4.4 pT
Calcabrini et al. (2017)	Increased ROS in human keratinocytes	50 Hz; 50 μT
Ehnert et al. (2017)	Changes in oxidative status and differentiation in human osteoblasts	16 Hz pulses; 6 – 282 μT
Fernie and Bird (2001)	Increase in oxidative stress in male American kestrels	50 Hz; 30 μT
Hajnorouzi et al. (2011)	Decreased SOD and Growth promotion of maize seedlings	Static and 10 KHz; 22 μT
Karimi et al. (2019)	Increases in total oxidant status and antioxidant activity and change in memory retention in rats	50 Hz; 1–2000 μT
Kesari et al. (2016)	Increase in superoxide in SH-SY5Y human neuroblastoma cells pretreated with menadione	50 Hz; 10 μT
Mannerling et al. (2010)	Increase in superoxide in k562 human leukemia cells	50 Hz; at or below 25 μT
Manikonda et al. (2014)	Oxidative stress in rat brain	50 Hz; 50 μT
Martino and Castello (2011)	Changes in proliferation and SOD in human umbilical vein endothelial cells	Static; 30 μT
Naarala et al. (2017)	Increase in superoxide in rat glioma C6 cells	Static and 50 Hz; 30 μT
Poniedzialek et al. (2013)	Change in ROS in human neutrophils	EMF tuned to calcium ion cyclotron frequency; 10 μT
Regoli et al. (2005)	Decrease in CAT in snail digestive gland	50 Hz; 2.88 μT
Sharifian et al. (2009)	Decreases in SOD and GPx in human serum and red blood cells	50 Hz; 8.8–84 μT
Van Huizen et al. (2019)	Changes in ROS and regeneration in planarian	Static; 100 μT
Zhang et al. (2018)	Changes in nitric oxide activity in bone monocytes	50 Hz; 0.5 μT
Zmyslony et al. (2004)	Decrease in ROS in rat lymphocytes stimulated by $FeCl_2$	50 Hz; 40 μT

There are studies that showed effects on free radical processes at low static- or ELF-EMF intensities (at low μT levels). They are listed in Table 1. Furthermore, related to this is a paper by Kapri-Pardes et al. (2017) showing effects on cellular signal cascades in eight cell lines exposed to ELF-EMF at 0.15 μT at a similar level that has been suspected to cause childhood leukemia. Though the authors did not investigate the oxidative status of their cells, they concluded that the effects were mediated by NADP oxidase, an enzyme that can generate superoxide free radicals. In addition, similar to the finding of Kesari et al. (2016), Maes et al. (2016) also reported an increase in micronucleus formation in SH-SY5Y human neuroblastoma cells after exposure to a 50-Hz magnetic field at 10 μT, but without pretreatment with menadione as in the Kesari et al. (2016) study. Thus, disturbance of oxidative processes can possibly occur at ambient levels of static- and ELF-EMF (e.g., see ambient levels reported by Abuasbi et al. 2018a, 2018b; Bürgi A et al., 2017; Gourzoulidis et al. 2018; Ilonen et al. 2008; Lindgren et al. 2001; Yitzhak et al. 2012).

Effects of electric fields

A few words have to be said on exposure to electric fields. The exposure set-ups for the electric field are quite different from those of magnetic fields. There are at least 16 electric-field exposure studies on free radical processes: Akpinar et al. (2012, increase ROS in rat brain and retina; 2016, increased rat brain lipid peroxidation); Calota et al. (2006, decreased human serum ROS); Fitzsimmons et al. (2008, increased nitric oxide in human chondrocytes); Gok et al. (2016, increased lipid peroxidation in mouse brain and retina); Güler et al. (2008, increased lipid peroxidation in guinea pig liver); Güler et al. (2009a, no significant effect on guinea pig plasma protein carboxylation; 2009b, increased protein carboxylation in guinea pig lung); Harakawa et al. (2005, no significant effect on rat plasma); Kantar-Gok et al. (2014, increased rat brain protein carboxylation and lipid peroxidation); Luo et al. (2019, decreased insect antioxidant enzyme); Miliša et al. (2017, increased ROS in paramecium); Pakhomova et al. (2012, increased ROS in Jurkat cells); Türközer et al. (2008, no significant effect on guinea pig brain lipid peoxidation); Wartenberg et al. (2008, increased oral mucosa cancer cell SOD); and Wu et al. (2016, increased mouse liver SOD). In most studies, 50-Hz electric field at kV/m intensity (2–21.8 kV/m) and exposure time from hours to days were investigated. Most studies reported effects indicative of an increase in free radicals, e.g., increases in lipid peroxidation. Effects could occur at a very low level of electric field exposure. A study by Fitzsimmons et al. (2008) using a pulsed electric field at 0.00002 kV/m, reported an increase in nitric oxide after 30 min of exposure. Wartenberg et al. (2008) used a 0.004 kV/m DC-electric field and reported changes in activities in the antioxidant enzyme SOD. This is actually quite interesting. Since electric fields do not penetrate into cells, do electric and magnetic fields act on different mechanisms leading to changes in cellular free radical processes?

Static- and ELF-EMF, free radicals, and cell proliferation, viability, and differentiation

Free radicals can cause damages to cellular macromolecules (DNA, protein, and lipid). These damages can affect

cell functions. Mutation in DNA can lead to cancer development. However, too much damage to a cell can cause cell death. And death to precancerous and cancer cells decreases the incidence of cancer. This may be a possible non-invasive method for cancer prevention and treatment. It is, of course, not known how much EMF exposure is needed to push cancerous cell over the edge to death. It may depend on the type of cancer cell.

On the other hand, the death of cells that cannot reproduce and be replaced leads to dysfunction in organs. This is particularly true for nerve cells. The connection between EMF exposure and neurodegenerative diseases are still not yet well established. There are several recent studies indicating a possible correlation with Alzheimer's disease, amyotrophic lateral sclerosis, dementia, and motor dysfunctions (Gunnarsson and Bodin 2018; Huss et al. 2018; Jalilian et al. 2018; Koeman et al. 2017; Pedersen et al. 2017). There are, however, two interesting points that need to be pointed out. First, static- and ELF-EMF have been shown to reverse and improve cognitive performance in animal models of neurodegenerative disorders (Akbarnejad et al. 2018; Bobkova et al. 2018; Hu et al. 2016; Li et al. 2019; Liu et al. 2015; Sakhaie et al. 2017; Tasset et al. 2012). Can these be related to the effects of static- and ELF-EMF on protein folding and prion production (Lian et al., 2018) and induction of heat-shock proteins (Laramee et al. 2014; Zeni et al. 2017)? Second, there is an inverse correlation between cancer risk and Alzheimer's and Parkinson diseases (Poprac et al. 2017). An increase in cellular free radicals is a common factor of these diseases. This supports the notion that static- and ELF-EMF exposure can kill cancer cells and cause neurodegenerative diseases.

Harnessing cellular oxidative status using static and ELF-EMF could also be beneficial in the treatment of certain diseases. Several papers have suggested such possibilities including: improvement of immune responses (Akan et al. 2010; Belova et al. 2010; Frahm et al. 2006; Kim et al. 2017); treatment of osteoarthritis (De Mattei et al. 2003); attenuation of ischemic brain injury (Duong et al., 2016; Rauš Balind et al. 2014); increasing antioxidant properties in cells and tissues (Falone et al. 2016); treatment of myopathies (Vignola et al. 2012); wound healing and tissue regeneration (Glinka et al. 2013; Patruno et al. 2010, 2011; Van Huizen et al. 2019); cytoprotection (Osera et al. 2015, 2011); inducing differentiation of stem cells (Haghighat et al. 2017b, 2017a; Marycz et al. 2018; Park et al. 2013; Van Huizen et al. 2019); and protective effect on Huntington's disease (Tasset et al. 2012; Túnez et al. 2006). One interesting prospect is the use of static and ELF-EMF in the treatment of cancer. EMF can selectively kill cancer cells (Lai and Singh 2010). Many years ago, we (Lai and Singh 2004) speculated that cancer cells are more vulnerable to EMF than normal cells and that EMF kills cancer cells by free radical formation. Since it is much easier to produce ELF-EMF than RFR, and ELF-EMF gives a more uniform distribution and better tissue penetration that RFR, it is more advantageous to use ELF-EMF for cancer treatment. Let us look at the studies on static- and ELF-EMF exposure on free radicals and cell proliferation, differentiation, cell cycle, and cell death in cancer and normal cells, summarized in Table 2. These are important cellular processes that determine cancer development and treatment, growth, development, wound healing and regeneration in living organisms.

Several studies on cancer cells listed in Table 2 suggested a possible beneficial effect on cancer treatment under static- or ELF-EMF exposure by increasing apoptosis and decreasing proliferation and viability (Benassi et al. 2016; Ding et al. 2004; Errico Provenzano et al. 2018; Hajipour Verdom et al. 2018; Koh et al. 2008; Lai et al. 2016; Mannerling et al. 2010; Osara et al., 2011; Wartenberg et al. 2008; Yang and Ye 2015). However, others suggested a protective effect by decreasing apoptosis and increasing proliferation and viability that would allow cancer to grow faster (De Nicola et al. 2006; Falone et al. 2007, 2017; Garip and Akan 2010; Martinez et al., 2016; Osera et al. 2015; Song et al. 2018; Wolf et al. 2005), whereas no significant effect on cell viability and proliferation was reported by some studies (Consales et al. 2019; Morabito et al. 2010; Naarala et al. 2017; Pakhomova et al. 2012; Sadeghipour et al. 2012). Interestingly, A study (Ayşe et al. 2010) showed opposite effects depending on the duration of exposure. This reflects the discussion above on the dynamic of cellular free radical processes and their ability to compensate. Cell type probably plays a significant role. Cell-type-specific responses to ELF-EMF have been reported by Sullivan et al. (2011), Kesari et al. (2016), Koziorowska et al. (2018), Makinistian et al. (2019), and Wang et al. (2018). The conditions of exposure probably cause the diversity of responses, but the conditions of exposure described in the table do not reveal a clear pattern on how different exposure parameters affect cellular free radical processes and changes in cell proliferation, differentiation, and apoptosis. There is a slight tendency of an inverse relationship between free radical activity and cellular proliferation, i.e., an increase in free radicals causes a decrease in cell proliferation and vice versa. Also, increase in free radical activity tends to enhance apoptosis. This uncertainty is actually not surprising because, in each study, we are looking at only some components of the free radical processes and not the whole pattern of changes. Feedback and compensatory

Table 2. Static- and ELF-EMF-induced free radical changes and cell proliferation, differentiation, viability, and cell cycle in (a) cancer and (b) normal cells.

(a)	Cancer cell type	Exposure conditions	Oxidative effects	Effects observed
Ayşe et al. (2010)	K562 human leukemia cells	50 Hz MF, 5 mT, 1 h or 1 h/day for 4 days	Increased superoxide, effect disappeared at 2 h after exposure	Single exposure decreased differentiation, repeated exposure increased differentiation
Benassi et al. (2016)	SH-SY5Y human neuroblastoma cells	50-Hz MF, 1 mT, 6–72 h	Increased protein carboxylation	Enhanced neurotoxin-induced apoptosis
Consoles et al. (2019)	SH-SY5Y human neuroblastoma cells	50-Hz MF, 1 mT, 24–72 h	No change in superoxide and hydrogen peroxide	Decreased iron content and iron-gene expression in a mutant cell type; no effect on cell viability and proliferation
De Nicola et al. (2006)	U937 human lymphoma cells	Static MF, 0.6 mT, 2 h; 50-Hz MF 0.07–0.1 mT, 2 h	Increased ROS, decreased GSH	Reduced apoptosis
Ding et al. (2004)	HL-60 human leukemia cells	60-Hz MF, 5 mT, 24 h		Enhanced apoptotic effect of H_2O_2
Errico Provenzano et al. (2018)	Human acute promyelocytic leukemia NB4 cells	50-Hz MF, 2 mT, 8, 16, 24 h	Increased ROS	Decreased proliferation and enhanced differentiation of all-trans retinoic acid (ATRA)-treated NB4 cells
Falone et al. (2007)	SH-SY5Y human neuroblastoma cells	50-Hz MF, 1 mT, 96–192 h	No change in ROS, SOD and CAT; increased GST and GPx	Increased viability, no change in cell cycle and apoptosis; enhanced oxidative effects of H_2O_2
Falone et al. (2017)	SH-SY5Y human neuroblastoma cells	50-Hz MF, 0.1 or 1 mT, 5 and 10 days	Decreased protein carboxylation and DNA oxidation; Increased antioxidation defense and GPx activity	Increased proliferation and survival advantage of cells
Garip and Akan (2010)	K562 human leukemia cells	50-Hz MF, 1 mT, 3 h	Increased ROS	Decreased and increased apoptosis in untreated and H_2O_2-treated cells, respectively
Hajipour Verdom et al. (2018)	Human MCF-7 breast adenocarcinoma cells	Static magnetic field, 10 mT, 24 and 48 h	Increased ROS	Decreased viability and differentiation; synergistic with doxorubicin
Koh et al. (2008)	Human prostate cancer cells (DU145, PC3, and LNCaP)	60-Hz MF, 1 mT, 6, 24, 48, or 72 h	Increased hydrogen peroxide	Apoptosis and cell cycle arrest
Lai et al. (2016)	Human Molt-4 leukemia cells	0.2 Hz pulses, carrier frequency modulated 134 KHz field from radiofrequency ID chip, 1 h		Cell death blocked by the spin-trap compound N-tert-butyl-alpha-phenylnitrone and the iron chelator deferoxamine
Mannerling et al. (2010)	K562 human leukemia cells	50-Hz MF 0.025–0.1 mT, 1 h		Accumulation of cells in the G2 phase
Martínez et al. (2016)	Human NB69 neuroblastoma cells	50-Hz MF, 0.1 mT, 3-h on/3-h off for 24, 42, or 63 h, or continuously for 15–120 min		MF activated MAPK-p38 and ERK ½, increase in cell proliferation
Morabito et al. (2010)	Rat PC-12 pheochromocytoma cells	50-Hz MF, 0.1 or 1 mT, 30 min or 7 days	Increased ROS in 30 min 1 mT exposure; decreased CAT in 0.1 and 1 mT 30 min and increased catalase in 1 mT 7 day exposure	All effects were observed in undifferentiated and not in differentiated cells; no significant effect on cell proliferation
Naarala et al. (2017)	Rat C6 glioma cells	Nearly vertical 33 μT static MF plus a horizontal or vertical 50-Hz 30 μT MF, 2 h	Increased cytosolic superoxde in vertical static field horizontal 50-Hz MF (but not vertical 50-Hz MF)	Cell viability not affected.
Osera et al. (2011)	SH-SY5Y human neuroblastoma cells	72-Hz pulsed MF, 2 mT, 24 h	Increased SOD-1	Decreased cell proliferation with higher quiescence
Osera et al. (2015)	SH-SY5Y human neuroblastoma cells	72-Hz pulsed MF, 2 mT, 10 min for 4 times over 7 days or 72 h	Increased Mn-SOD	Increased protection against oxidative stress; pulsed MF prevented H_2O_2-induced decrease in cell number and HSP-70 expression
Pakhomova et al. (2012)	Jurkat cells derived from human T-cell leukemia	Nanosecond pulsed electric field (300 ns, 1–12 kV/cm)	Increased ROS proportional to pulsed number	No effect on U-937 cells (also derived from human lymphoma)
Sadeghipour et al. (2012)	Human T47D breast carcinoma cells	100 and 217 Hz pulsed EMF, 0.1 mT, 24–72 h	Increased ROS in 217-Hz 72 h exposure	No significant change in apoptosis
Song et al. (2018)	Human cervical cancer cells (HeLa)	60-Hz EMF, 6 mT, 72 h	Decreased ROS	Increased cell proliferation

(*Continued*)

Table 2. (Continued).

(a)	Cancer cell type	Exposure conditions	Oxidative effects	Effects observed
Wartenberg et al. (2008)	Human UM-SCC-14-C oral mucosa cancer cells	DC EF, 4 V/m, 24 h	Increased Cu/Zn SOD, decreased GSH, no change in CAT	Increased apoptosis and decreased cell proliferation
Wolf et al. (2005)	Human HL-60 leukemia cells	50-Hz MF, 0.5–1 mT, 24–72 h	Increased DNA oxidative damage, increased ROS in Rat-1 fibroblasts	Dose-dependent increase in cell proliferation
Yang and Ye (2015)	Human osteosarcoma MG-63 cells	50-Hz EMF, 1 mT, 1, 2 or 3 h	Increased ROS	Decreased viability and cell growth, increased apoptosis
(b)	Type of normal cells	Exposure conditions	Oxidative effects	Effects observed
Di Loreto et al. (2009)	Rat cortical neurons	50-Hz MF, 0.1 or 1 mT, 7 days	No effect on lipid peroxidation, total ROS, and GSH	Increased cell viability, decreased apoptosis
Ehnert et al. (2017)	Human osteoblasts	Pulsed EMF, 16-Hz, 6–282 μT; 7 min or 7min/day (>3 days)	Increased ROS after single exposure, decreased after repeated exposure; increased GPX3, SOD2, CAT, GSR	EMF promoted osteoblast differentiation
Emre et al. (2011)	Wistar rat in vivo, liver	Pulsed EMF (0.5 ms rise time, 9.5 ms fall time) EF 0.6 V/m, MF 1.5 mT, each frequency train of 1 Hz, 10 Hz, 20 Hz and 40 Hz was given for 4-min and with 1-min interval between each frequency (together 20 min.); on each day, three exposure cycles performed (1 h), 1 h per day for 30 days	Increased lipid peroxidation, increased SOD	No effect on apotosis, decreased necrosis
Feng et al. (2016)	Human amniotic epithelial cells	50-Hz MF, 0.2–2 mT, 30, 60, 120 min	Increased mitochondrial ROS	activation of Akt and anti-apoptotic effect
Ghodbane et al. (2015)	Wistar male rat in vivo, brain and liver	Static MF, 128 mT, 1 h/day, 5 days	No effect on lipid peroxidation, increased CAT in liver	Increased apoptosis in liver through a mitochondrial caspase-independent pathway
Hajipour Verdom et al. (2018)	Human HFF normal fibroblasts	Static MF, 10 mT, 24 and 48 h	Increased ROS and GSH	Decreased viability and differentiation
Hu et al. (2016)	Hippocampus of exposed 3xTg AD mice	50-Hz MF, 0.5 mT, 20 h/day for 3 months	Decreased ROS and molecules involved in oxidative stress	Decreased apoptosis
Mohammadi et al. (2018)	Tobacco cells	Static Magnetic field, 0.2 mT, 24 h	↑ H_2O_2, ↑ NO	Delayed G1-S transition, increased cyclic nucleotides
Patruno et al. (2010)	Human epidermal keratinocyte cell HaCaT	50-Hz MF, 1 mT, 3, 18, 48 h	Decreased superoxide, increased nitric oxide and nitric oxide synthase, decreased CAT	Increased cell proliferation
Romeo et al. (2016)	Human fetal lung fibroblast	Static magnetic field, 370 mT, 1 h/day for 4 days	No change in ROS	No effect on viability, DNA damage and apoptosis
Solek et al. (2017)	Mouse spermatogenic cell lines	2, 50, 120 Hz pulsed (1 sec on/1 sec off) and continuous-wave EMF, 2.5–8 mT, 2 h	Increased superoxide and nitric oxide	Cell cycle arrest and apoptosis
Song et al. (2018)	Human fetal lung fibroblasts (IMR-90)	60-Hz EMF, 6 mT, 72 h	Decreased ROS	Increased cell proliferation
Van Huizen et al. (2019)	Schmidtea mediterranea (planarian), regeneration after amputation	Static magnetic field;100–400 μT and 500 μT; 12, 24 or 48 h	↓ ROS after 100–400 μT and ↑ ROS after 500 μT exposure	Decreased regeneration at 100–400 μT, increased at 500 μT; increased ROS promotes stem cell proliferation and differentiation
Zhang et al. (2018)	RAW264.7 bone monocytes	Static MF, 500 nT, 0.2 T, 16 T; 12 h to 4 days	Increased NO (16 T); decreased NO (500 nT and 0.2 T); increased NOS (16 T) decreased NOS (500 nT and 0.2 T)	Nitric oxide mediates SMF effects on osteoclast formation; effect depends on intensity of MF- decreased at 16T and increased at 500 nT and 0.2 T

mechanisms further complicate the picture. It is like the predicament of the "blind men and the elephant": "each was partly in the right, and all were in the wrong!" Thus, it is imperative to understand the conditions under which static- and ELF-EMF could cause a consistent increase/decrease in free radical activity in cells.

However, one must also keep in mind that free radical is by no means the only mechanism by which static- and ELF-EMF affect cell proliferation and viability. Other mechanisms could be involved, e.g., activation of the ERK1/2 signaling pathway (Qiu et al. 2019); and heat shock proteins (Zeni et al. 2017).

Magnetic field and the Fenton reaction

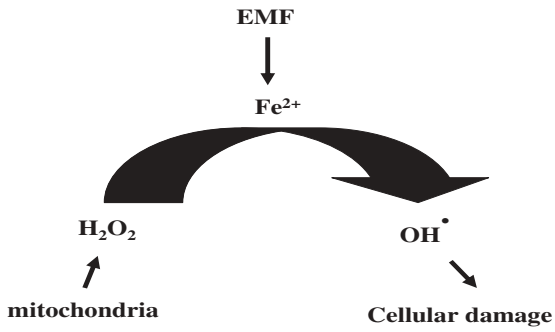
The iron-involved Fenton reaction may play a role in the generation of free radicals after EMF exposure. In previous research, we (Lai and Singh 1997a) found that an acute (2 hr) exposure to a 60-Hz magnetic field caused DNA single and double strand breaks in brain cells of rats. The effects were mediated by free radicals (Lai and Singh 1997b). Data also showed that the effects involved iron, since they can be blocked by pre-treating rats before magnetic-field exposure with the iron chelator deferriprone. More recently, we found that pulsed ELF-EMF field could also kill human Molt-4 leukemia cells via an iron-dependent free radical process (Lai et al. 2016).

Iron plays a vital role in cell growth, e.g., in energy metabolism and DNA synthesis. For DNA synthesis, riboses are converted into deoxyriboses, a component of the DNA molecule, by the enzyme ribonucleotide reductase, of which iron is a cofactor. Immediately before cell division, iron is taken up into cells. In vertebrates, a cellular iron transport system involves a specific interaction between the iron-binding protein transferrin in the extracellular fluid and cell surface transferrin receptors that results in a facilitated transport of iron across the cell membrane via endocytosis (Trowbridge et al. 1984). Due to their rapid rate of division, most cancer cells have high rates of iron intake (Karin and Mintz 1981) and express a much higher cell surface concentration of transferrin receptors (May and Cuatrecasas 1985) than normal cells. In general, the aggressiveness of a tumor is positively correlated with cell surface transferrin receptor concentration of its cells. For example, breast cancer cells have 5–15 times more transferrin receptors on their cell surface than normal breast cells (Reizenstein 1991), and they take up more iron than normal breast cells (Shterman et al. 1991). This is basically true in many different types of cancer cells.

Since cellular responses to magnetic fields involve an iron-dependent process, we hypothesize that cancer cells are more responsible to magnetic fields than normal cells. The more potent hydroxyl free radicals are formed by the Fenton Reaction from hydrogen peroxide produced mainly in the mitochondria and in the cytoplasm by superoxide dismutase (SOD) (Figure 1).

Indeed, there are studies indicating that cancer cells are more responsive to EMF than normal cells (Crocetti et al. 2013; Curley et al. 2014; Kamalipooya et al. 2017; Lai and Singh 2010; Morotomi-Yano et al. 2014; Tofani et al. 2001; Zimmerman et al. 2012). Various studies demonstrated that iron chelators blocked the effect of ELF-EMF on oxidative processes (Calcabrini et al. 2017; Lai et al. 2016; Lai and Singh 2004). In addition, static- and ELF-EMF have been shown to affect iron metabolism and iron-related gene expressions in cells (Consales et al. 2019; Dey et al. 2017; Fitak et al. 2017; Hajnorouzi et al. 2011; Lee et al. 2015; Shokrollahi et al. 2018). One can speculate that magnetic field causes a type of cell death know as ferroptosis, which can be blocked by iron chelators and lipid-soluble antioxidants and is being explored as a mean for cancer therapy (Shen et al. 2018; Wang et al. 2018b).

This mechanism provides a non-invasive mean of using ELF-EMF to selectively kill cancer cells (thus less side effects) by taking advantage of a fundamental property of cancer cells, i.e., high uptake of iron and increased the production of cytotoxic free radicals. This also led to the development, by us, a group of anticancer compounds that produce carbon-based free



The Fenton Reaction

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\bullet} + H^{+}$$

Figure 1. The Fenton Reaction and effect of EMF exposure that enhances the conversion of hydrogen peroxide into hydroxyl radical catalyzed by a transition metal such as iron. Ferrous iron (Fe^{2+}) is converted into ferric iron (Fe^{3+}) in the formation of hydroxyl radical (OH) from hydrogen peroxide (H_2O_2). Ferric iron is converted back to the ferrous form by reacting with hydrogen peroxide.

radicals in the presence of iron (Lai et al. 2013). These compounds have been shown to be very selective and effective in killing many types of cancer cells. In addition, the concept of the Fenton Reaction also led to the idea of using ELF-MF for treatment of malaria (Feagin et al. 1999; Lai and Singh 2010).

The evolutionary aspects of static- and ELF-EMF in nature and free radicals

For millions of years, living organisms evolved in the presence of environmental static geomagnetic field and natural extremely low frequency electromagnetic fields, such as the lightning-generated Schumann Resonance. It is not surprising that these fields can play important roles in the survival of living organisms, e.g., in food foraging, directional cues, and reproduction. Living organisms are very sensitive and responsive to low levels of these EMFs. This has been shown in many animal species and affect various biological functions, e.g., exploratory behavior in rodents (Malewski et al. 2018); body alignment of dogs (Hart et al. 2013a); magnetic alignment in carps (Hart et al. 2012); landing direction of water birds (Hart et al. 2013b); orientation of grazing and resting cattle and deer (Begall et al. 2008); and cardiovascular and brain activities in humans (Pishchalnikov et al. 2019; Wang et al. 2019). Some animals can differentiate north and south poles of a magnetic field (known as polarity compass) (Begall et al. 2008; Hart et al. 2012, 2013b, 2013a; Malkemper et al. 2015). Another function-related reproduction is the "natal homing behavior", i.e., an animal returns to its birthplace to reproduce, in some animal species. It is observed in sea turtle (Brothers and Lohmann 2015); eel (Naisbett-Jones et al. 2017); and salmon (Putman et al. 2014b). Apparently, newborns of these animals are imprinted with the memory of the intensity and inclination angle of the local geomagnetic field. This information will later be used to locate their place of birth. All these traits confer some survival competitiveness to the organisms.

In order for an environmental entity to affect the functions of an organism, the following criteria have to be met. First, the organism should be able to detect the entity. Second, the level of the entity should be similar to those in the normal ambient environment. Third, the organism must have response mechanisms tuned to certain parameters of the entity to allow immediate detection of the presence and changes of the entity. Immediate detection and response to the entity are essential for the survival of the organism.

For the detection of changes in static- and ELF-EMF in the environment, several mechanisms have been evolved. Some organisms evolved special organs (receptors) to detect environmental EMF. For example, the platypus has thousands of electric sensors on its bill skin. Using these electroreceptors, in interaction with another type of sensor the mechanoreceptor, the monotreme platypus can detect an electric field of 20 μV/cm (Manger and Pettigrew 1996), which is similar to that produced by the muscles of a shrimp. The information is processed by the somatosensory cortex of the platypus to fix the location of the prey. This type of electroreception is common in all three species of monotremes and short bill echidna. Electric fish (Elasmobranch) emits EMF that covers a distance of several centimeters (Montgomery and Bodznick 1999; von der Emde 1999). Again, this allows the location of a potential prey by comparing its electrical properties with the vicinity. Their electroreceptors have been shown to detect a field of 5 nV/cm. These EMF sensing mechanisms are highly sensitive and efficient.

Two other mechanisms have been proposed to account for electroreception: magnetite involved in iron-oxidation and radical pair production in certain cellular molecules. In both cases, the generation of reactive oxidative species is involved. The radical-pair reaction hypothesis and conversion of the form of radicals (singlet-triplet interconversion) in a group of flavoproteins known as cryptochromes (Hore and Mouritsen 2016) in animal species have been intensively studied. There are reports of the presence of cryptochromes in plants, which may be responsible for the effect of EMF on plant growth (Ahmad et al. 2007; Mohammadi et al. 2018). A comprehensible description of this topic is beyond the scope of this paper and the expertise of this author. Readers are referred to several papers on the topic: Barnes and Greenebaum (2015); Binhi and Prato (2017); Galler et al. (2005); Dodson et al. (2013); Hore (2019); Hore and Mouritsen (2016); Kirschvink et al. (2001); Landler and Keays (2018); Sheppard et al. (2017); and Sherrard et al. (2018).

Thus, the mechanisms described above, electro-receptors, magnetites, and radical-pair, enable living organisms to immediately detect the presence and changes in environmental electromagnetic fields of very low intensity. An effect that could have dire consequences on species survival is that man-made EMFs, with ubiquitous presence in the recent environment, could disrupt the natural responses to nature static- and ELF-EMF. Disruption of directional senses in insects has been reported (Shepherd et al. 2018). Polarity compass also can be disturbed by man-made EMF (Burda etal. 2009; Malkemper et al. 2015; Putman et al. 2014a). A study by Engels et al. (2014) showed that magnetic noise (at 2 KHz – 9 MHz, i. e., within the range of AM radio transmission) could disrupt magnetic compass orientation in migratory European robins. The disruption can occur at a very low noise level of 0.01 V/m (0.0000265 μW/cm^2). Similar effects of RFR interference on magnetoreception have

also been reported in a night migratory songbird (Aher et al. 2016) and European robin (Wiltschko et al. 2015).

Electro-hypersensitivity in humans (Baliatsas et al. 2012) also showed an instantaneous response to EMF and at low intensities. One wonders whether the underlying mechanisms of electro-hypersensitivity are related to the processes described above. It may be the remnant of a primordial evolutional response of humans to static- and ELF-EMF in the environment. Free radicals may play a role. There is a report of increased oxidative stress in electro-hypersensitivity self-reporting patients (Irigaray et al. 2018).

From the discussion above, it is apparent that static- and ELF-EMF that can affect free radical processes at very low intensities that in turn affect evolution and species survival. In the electromagnetic spectrum, the only other frequency range that biological responses can occur at very low intensity is the light spectrum. It has been shown that the human visual system is sensitive to one photon (Hecht et al. 1942; Pugh 2018), i.e., the reaction of one photon with one rhodopsin molecule in the retina. Apparently, beneficial selection outcomes in evolution have made the living organism extremely sensitive to these fields.

Here, let us digress to an unrelated but equally important topic: i.e., on the biological effects of radiofrequency radiation (RFR), another segment of the EMF-spectrum that is being intensively studied. Since the presence of RFR in the ambient environment is new in the evolutionary time scale, how are living organisms responsive to RFR? No specific cellular detection and response-mechanisms, other than heating, have been discovered. Biological responses to RFR at very low intensities have been reported (e.g., see Table 1 in Levitt and Lai (2010)). But, most of those studies were on modulated fields. Is it possible that the observed biological responses to RFR are actually caused by its ELF-modulations, since almost all environmental RFR sources are modulated (see discussion in section 9 "Effects below 4W/kg: thermal versus nonthermal" in Levitt and Lai (2010))? In the literature, biological effects of ELF-EMF and RFR are found to be very similar (e.g., compare the neurological effects of RFR described in Lai (2018) to those of ELF-EMF (see section on "neurological effects of ELF-EMF" Bioinitiative Report (2012))). In that sense, one can deduce that there is no other significant RFR effect other than thermal effect. Effects of low-level non-modulated continuous-wave (CW) RFR may be a counterargument. However, it is difficult to produce non-modulated RFR in the laboratory, or micro-thermal effect can occur under CW-RFR exposure. There are only several studies showing effects of very low-level CW-RFR (e.g., with specific absorption rate (SAR) ~10 mW/kg). Positive results were reported by de Pomerai et al. (2003) (aggregation of bovine serum albumin and changes in protein conformation, 1 GHz CW, 15–20 mW/kg); Marinelli et al. (2004) (cell self-defense responses, 900 MHz CW, 3.5 mW/kg); D'Inzeo et al. (1988) (acetylcholine-related ion channel, 10.75 GHz CW, 8 mW/kg); Persson et al. (1997) (blood-brain barrier permeability, 915 MHz CW, 0.4 mW/kg); and Tattersall et al. 2001) (hippocampal functions, 700 MHz CW, 1.6 mW/kg). Somosy et al. (1991) (molecular and structural changes in cells of mouse embryos, 2450 MHz) reported that modulated radiation (effect observed at 2.4 mW/kg) is more potent that CW radiation (effect observed at 240 mW/kg). Navakitkia and Tomashevskaya (1994) (behavioral and endocrine changes, 2450 MHz, 2.7 mW/kg with modulation), Schwartz et al. (1990) (calcium movement in heart, 2450 MHz, 0.15 mW/kg with modulation) and Wolke et al. (1996) (calcium concentration in heart muscle cells, 900 MHz, 1 mW/kg with modulation) reported effects with modulated radiation and not with CW radiation. The SARs given were averaged SARs. There is a study that showed DNA damage in human glial cells after exposure to a 50 Hz-modulated 900-MHz RFR but not to CW field. In that study, only an exposure power density of 26 $\mu W/cm^2$ was provided. Other than the Persson et al. (1997) study, all the other studies were carried out in vitro. Interestingly, the Persson et al. (1997) paper reported that CW- is more potent than modulated-915 MHz radiation on increasing blood-brain barrier permeability. Certainly, the RF-carrier could affect the distribution of energy in the exposed subject. And, the pattern of energy distribution can affect biological responses to EMF (Lai et al. 1984). The concept of interaction of modulation with the RF-carrier is not new. It was shown in an earlier study (Bawin et al. 1978) that 6- and 16-Hz amplitude-modulated 147-MHz RFR (0.8 mW/cm^2) increased calcium efflux from chick cerebral tissues, whereas 6- and 16-Hz fields alone caused a decrease in efflux. Lastly, I like to point out that there are not enough research data to support the popular belief that "modulated is more biologically potent that non-modulated RFR." More experiments using the same exposure set-up with continuous-wave and modulated RFR of the same frequency and with intensities that produce the same averaged SAR are needed to reach such a conclusion.

Concluding remarks

(1) Change in cellular free radical activity is one of the most consistent effects of static- and ELF-EMF on living organisms.
(2) The mechanisms by which static- and ELF-EMF affect cellular free radical processes is not well understood. The "radical pair" hypothesis is a likely candidate, particularly the

involvement of cryptochromes. It allows immediate detection and response to changes in static- and ELF-EMF in the environment, which likely play important roles in the evolution of living organisms.

(3) Oxidative responses to static- and ELF-EMF are probably dependent on the characteristic of the field and exposure (such as frequency, modulation, and duration) and the exposed object (such as cell type, and states of biological activities),

(4) However, chronic exposure that leads to the excessive and persistent presence of free radicals can cause oxidative stress and should be avoided.

(5) Effects of static- and ELF-EMF on free radicals probably have beneficial health effects particularly relating to cell proliferation, differentiation, cell death, and cell cycle. These are effects that could influence cancer development and treatment, growth and development, regeneration, and healing.

(6) In future research, it is imperative to identify the field parameters that can selectively cause beneficial or detrimental health effects.

Conflict of interest statement

The author declares no conflict of interest.

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