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Effects of static magnetic fields in biology: role of free radicals

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1. ABSTRACT

Biological systems can respond to a wide range of static magnetic fields (SMF). Some of these responses seem to be mediated partly through free radical reactions. For example, in magnetic sense and navigation using the geomagnetic field, one of the most promising mechanisms for explaining magnetic compass is "a radical pair mechanism". Biological free radicals are most commonly oxygen or nitrogen based with an unpaired electron, leading to the terms "reactive oxygen species (ROS)" or "reactive nitrogen species (RNS)". When applying SMF to medical treatment, coupling SMF exposure with possible chemotherapy of cancers is a novel fascinating area that SMF could enhance agent-induced ROS production against tumors. In addition, one of the potent mechanisms of SMF effects on hemodynamics and blood pressure has sometimes been linked to nitric oxide pathway. However, health and environmental concerns have been raised because the SMF effects on oxidative stress leading to genetic mutation and apoptosis/necrosis have been found. It seems to take place from free radical generation.

2. INTRODUCTION

All living organisms have been continuously exposed to natural magnetic fields. It seems likely that the exposure can cause biological effects, although the precise effects provoked are not well known. It has been argued that the controversial or inconsistent effects of SMF reported so far are primarily due to the diversified and versatile responses of living organisms, and various intensity ranges of SMF: weak-intensity SMF in the microtesla (microT) range, including the geomagnetic field (typically around 50 microT, range 20-90 microT), moderate-intensity SMF in the millitesla (mT) range, and strong-intensity SMF in the tesla (T) range, including ultrastrong-intensity SMF (more than 5 T).

As recently reviewed by Ueno and Shigemitsu (1), possible biophysical and biochemical effects can be expected when biological systems are simultaneously exposed to both SMF and other forms of energy such as light and radiation (2-3). Photochemical reactions produced by a radical pair intermediate can be expected to show SMF

effects that arise from an electron Zeeman interaction, electron-nuclear hyperfine interaction (Fermi-contact interaction), or a hyperfine interaction mechanism, including an electron-exchange interaction in a radical pair intermediate (4-10). Free radical reactions are ubiquitous in biology, and recent developments of the radical pair mechanism of the low-field effects (less than 1 mT), including the effects of SMF and electromagnetic fields (EMF) (11-17), and the consideration of detailed biochemical and biophysical systems (18-20) make this mechanism a prime candidate for the effects down to the geomagnetic field strength, as recently reviewed by Engström (21). According to theoretical estimates, the physical transduction step induced by low fields is not vulnerable to thermal perturbations (22-23). In the spin states of radicals, the theory predicts that an applied magnetic field perturbs the interconversion of the singlet and triplet states, resulting in an increase in the proportion of the triplet state, and thus the free-radical concentration (16).

Biological free radicals are most commonly oxygen or nitrogen based with an unpaired electron, leading to the terms "reactive oxygen species (ROS)", such as superoxide anion (O_2^-), hydroxyl radical (OH•) and singlet oxygen ($^1O_2$), or "reactive nitrogen species (RNS)", such as nitric oxide (NO) (21). The ROS and RNS play significant roles in immunological defense (24), intracellular signaling (25) and intercellular communication (26). It is assumed that SMF could change the lifetime of radical pairs, yields of cage products and escape products. If a SMF affects cells through the radical pair mechanism, a SMF influences the spin of electrons in free radicals, which may lead to changes in chemical reaction kinetics and possibly alter cellular function (13).

Concerning magnetic spin effects in radical enzymatic reactions involving at least one ROS, the Grissom research group (27) has shown experimentally that the activity of the B_{12}-dependent enzyme ethanolamine ammonia lyase changes with SMF of 100 mT. This finding is the first SMF effect on an enzyme-catalyzed reaction with known radical pair intermediates in a cell-free solution. It was a milestone in biomagnetism research on free radicals. Extensive experiments have also been carried out with the heme enzymes, horseradish peroxidase (HRP) and cytochrome C oxidase. The Grissom research group (28) reported that the rate of HRP increases by 20% at fields as low as 1 mT. However, a recent detailed reinvestigation by the Woodward research group (29) demonstrated that the reported effects of SMF on HRP were not observed up to 75 mT. Instead, the Woodward research group (30) studied the radical recombination reaction of radicals generated from the photolysis of 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (alpha-HP) on a microsecond time scale using time-resolved mid-infrared spectroscopy. The reaction was found to exhibit opposite biphasic magnetic field dependencies at 2 and 21 mT, and the effect at low fields was the first such observation for neutral free radicals in isotropic solution. The Hore research group (20) demonstrated that the yield of $^1O_2$ sensitized by chemically modified, carotenoid-less (quinine-depleted) bacterial photosynthetic reaction centers from the R-26 mutant of *Rhodobacter sphaeroides* and as a consequence the stability of the reaction center protein are strongly affected by SMF of a few mT: a 50% reduction for fields of 20–100 mT and a 10% reduction for 1 mT.

The biological systems are considered to be one of the nonlinear systems most sensitive to various external magnetic fields, including SMF and EMF (31), and it has been estimated that some of these responses seem to be mediated through free radical reactions (FRR). With particular reference to SMF in mT level fields, this is a field strength that would benefit from increased investigation because SMF therapy could be useful for vascular and circulatory diseases, including ischemic pain and hypertension, primarily due to the modulation of blood flow and/or blood pressure, partly through FRR such as NO pathway (32). However, recent studies have implicated ROS/RNS in the pathogenesis of vascular dysfunction, hypertension and activation of the sympathetic nervous system (SNS) (33-34), even if the lifetime of ROS and RNS in biological systems is extremely short, e.g., nano to microseconds for ROS (35) and a few seconds for NO (one of RNS) (36). Since NO exerts a tonic inhibition of central SNS activity (37), increased production of ROS enhanced oxidation/inactivation of NO, at least in part, through downregulation of neural NO synthase (nNOS), and consequently reduced availability of NO in the brain resulted in activation of the SNS (33). In addition, ROS/RNS are generated after ischemia/reperfusion from intracellular oxidases present in the myocardium and in infiltrating leukocytes (38). Although the relationship between the oxidative stress and activation of the SNS (in particular, hypertension) and/or ischemia-reperfusion injury has been implicated, the underlying mechanisms of SMF-induced ROS/RNS generation have not been clarified.

There is no established effect that would lead to a cumulative disorder with repetitive exposures to SMF (39). Nonetheless, there might be safety concerns of magnetic resonance imaging (MRI) for diagnostic imaging and magnetic levitation for transportation using strong intensity SMF in the T range. Based on advanced studies of the EMF effects on oxidative stress reactions, potential hazard SMF effects on living organisms are that SMF could increase the activity, concentration and lifetime of paramagnetic free radicals, which might cause an oxidative stress, genetic mutation and/or apoptosis (40-41). In particular, SMF exposure initiates an iron-mediated process that increases free radical formation in brain cells, leading to DNA strand breaks and cell death.

With reference to the iron ion compounds related to pathogenesis, although iron ions are important components of normally functioning organisms, in some cases, it can also be toxic (42). Because of its redox potential, Fe (II) generally has the potential to do more damage than oxidized Fe (III). For this reason, iron ions are primarily stored as Fe (III) within ferritin in organisms. The association of abnormal accumulation of metal ions with specific neurodegenerative disorders, such as Alzheimer's,

Parkinson's and Huntington's diseases, has been known for over 50 years (43). Various forms of metal ions may play a significant role in the biochemical processes that lead to the progression of neurodegenerative diseases (42). Although there is much speculation on that role, the primary mechanism is thought to be the result of oxidative stress: the free radical generation via the Fenton reaction, which is the oxidation of the iron-catalyzed hydrogen peroxide (H_2O_2) (44-46).

Therefore, knowledge of SMF effects on FRR is extremely important when considering human health, and the relation of immunological and neurodegenerative diseases, and stress response. Several attempts have been made to explore the parameters of FRR when living organisms, cells and biochemicals have been exposed to SMF. In this review, we introduce and focus on many recent studies, including our own, describing the effects of SMF on FRR. The influences of SMF on spin-dependent physiological processes are also discussed.

3. WEAK-INTENSITY SMF

3.1. Magnetic compass is based on "a radical pair mechanism"

Weak-intensity SMF effects (in the microT range), including the geomagnetic field, can affect the orientation and navigation behaviors in different kinds of living organisms partly through FRR: one of the most promising mechanisms for explaining magnetic compass is "a radical pair mechanism" via FRR (47-55). The radical pair mechanism for magnetic compass has been proposed by Ritz *et al*. (47-48) as a biophysical mechanism. This mechanism implies that *in vivo* magnetoreception involves radical pair processes, which are governed by anisotropic hyperfine coupling between (unpaired) electron and nuclear spins. One particularly interesting aspect of this mechanism is a link between photosensitivity and magnetoreception: "non-visual" magnetoreception is based on photoreceptor in the visual system and in the pineal gland (47-48). From behavioral, physiological and theoretical studies, the possible sources of free radicals have been recently identified in certain blue light receptors and circadian proteins, cryptochromes (18-19, 51-57), including flavin-tryptophan (18-19), flavin adenine dinucleotide (FAD)-tryptophan (57), and semiquinone/flavosemiquinone radicals (54) in their intermediate radical pair systems, and a photopigment, melanopsin (an opsin based photopigment) (58). However, there has been no report that a specific neurotransmitter in the nervous system is part of the radical-pair system involved in magnetoreception. Several experimental results obtained from various animal species have indicated that the magnetic compass mechanism such as cryptochrome-radical pair mechanism seems to be limited to avian magnetic compass of migratory birds. In contrast to the magnetic compass mechanism of birds, that of rodents does not involve FRR. Instead, it seems to be based on a fundamental different principle, which probably involves magnetite, other magnetically sensitive chemical reactions and electromagnetic induction, as reviewed by Thalau *et al.* (50) and Johnsen and Lohmann (51).

3.2. Impact of geomagnetic activity on melatonin

Studies on the other effects of geomagnetic activity have been reported on a potent endogenous free radical scavenger, melatonin. The geomagnetic field is often categorized as a SMF because it seems to be static on the physiological time scale. However, it is not temporally stable and has natural spatiotemporal variation. In this regard, the geomagnetic field is not a real SMF (it is a quasi SMF), but it is considered to be essential to living organisms as a natural and ubiquitous weak magnetic field. It has been reported that the natural variation in geomagnetic activity or geomagnetic storms might be related to the occurrence of sudden death in seizure and epilepsy patients (59), sudden infant death syndrome (60), depression (61) and suicide (62), whereas humans do not appear to have the ability to sense magnetic fields (51). The mechanisms by which changes in geomagnetic activity cause alterations in human physiology and behavior are still uncertain. The postulated mechanisms imply an interaction between geomagnetic disturbance and melatonin regulation in the pineal gland in the brain. The pineal gland represents the largest source of melatonin in the central nervous system (CNS) and SNS, and it may also function as a plausible receptor for detecting geomagnetism in humans. Melatonin is known as an effective free radical scavenger and was often examined for its antioxidant activities. In both humans and rats, there is evidence that geomagnetic disturbance is significantly associated with a decrease in melatonin release from the pineal gland (63-64). The possibility arises that the occurrence of a geomagnetic storm may result in decreased melatonin release and increased production of free radical species, thereby causing some mental disorders, neurological disorders, and abnormal behaviors. However, the exact mechanisms have not been elucidated.

3.3. Weak SMF effects on biochemical reactions

The studies of the influence of weak SMF (less than 1 mT) have been examined on several enzymatic reactions that are important in human biochemistry. The heme enzymes such as HRP, NOS and cytochrome C oxidase, and the B_{12}-dependent enzyme methionine synthase, are reasonable candidates for the site of interaction of less than 1 mT SMF with biological systems. It has been predicted that a SMF-induced change in the levels of NO (as produced by NOS) could alter intracellular Ca^{2+} levels and thereby change Ca^{2+} efflux rates. This would provide the first causal link between molecular effects and a cellular or physiological endpoint of SMF exposure. The Grissom research group (28) has shown that the redox cycle of a heme enzyme, HRP, is altered by a SMF as weak as 1 mT. The biphasic effects of SMF coincide exactly with predictions of the semi-classical model of SMF-dependent radical pair recombination. This work may be an overarching research challenge and an important contribution toward a complete understanding of the possible health effects of SMF exposure. In all cases, the postulated mechanism of interaction is through changes in radical pair recombination, in which one radical species is a transition metal ion (iron in heme or cobalt in B_{12}).

Prior to the Grissom research, Nossol *et al*. (65) investigated the effects of SMF ranging 50 microT-100 mT on cytochrome C oxidase activity *in vitro* by strictly controlled, simultaneous polarographic measurements of the enzyme's high- and low-affinity redox reaction. Significant changes as high as 90% of the overall cytochrome C oxidase activity resulted during exposure to a SMF at 300 microT and 10 mT in the high-affinity range. No changes were observed at other flux densities. After exposure to a change-inducing SMF, normal activity returned. These results were interpreted as effects of SMF on electron and proton translocation or on reactions with the cytochrome C oxidase and O_2 substrates. Later Mnaimneh *et al.* (66) assumed that these effects might be explained by using the radical pair mechanism if one of the elementary reactions involves a radical pair.

Noda *et al*. (67) examined the effects of SMF (250 microT and 2 mT) on the phorbol 12-myristate 13-acetate (PMA)-induced oxidative burst in rat peritoneal neutrophils. Both SMF showed enhancement of fluorescence production during the respiratory burst. Moreover, the combination of pre-treatment of lipoic acid (a free radical scavenger) and a 250 microT SMF exposure further enhanced fluorescence. They speculated that the enhancement of fluorescence production might be due to the increased lifetime of free radicals induced by SMF.

3.4. Summary of weak SMF effects on magnetic compass, melatonin release and biochemical reactions

In summary, the radical pair mechanism theory has been developed, which provides insight into the magnetic compass of living organisms using the geomagnetic field. A blue-light photoreceptor, cryptochrome, is the most promising candidate magnetoreceptor based on the radical pair mechanism. However, the magnetic compass based on the radical pair mechanism seems to be limited to avian magnetic compass of migratory birds. In contrast to the magnetic compass mechanism of birds via FRR, that of rodents does not involve FRR. Instead (except for migratory birds), magnetite and other magnetically sensitive chemical reactions might play a dominant role in animal orientation or navigation system in various animal species. The underlying mechanisms of the effects of geomagnetic disturbance on decreased melatonin release remain unknown. It is assumed that SMF could lengthen the time necessary for free radical recombination, and therefore prolong their effective lifetimes. In particular, there is increasing evidence that the redox cycles of metal ion-containing enzymes are modulated by weak SMF.

4. MODERATE-INTENSITY SMF

4.1. Control of biochemical reactions with moderate SMF

A change in radical pair recombination rates is one of the few mechanisms by which a SMF can interact with biological systems such as a cell-free system. As mentioned in introduction section, the Grissom research group (27) demonstrated that the kinetic parameter V_{max}/K_m (where K_m is the Michaelis constant) for the coenzyme B_{12}-dependent enzyme ethanolamine ammonia lyase was decreased 25% by a SMF near 100 mT with unlabeled ethanolamine and decreased 60% near 150 mT with perdeuterated ethanolamine. This effect is likely caused by a SMF-induced change in intersystem crossing rates between the singlet and triplet spin states in the (cob (II)alamin:5'-deoxyadenosyl radical) spin-correlated radical pair.

Mohtat *et al*. (40) examined the behavior of radical pairs derived by hydrogen abstraction of triplet benzophenone and some of its derivatives from bovine serum albumin, human serum albumin and calf thymus DNA. The SMF strength was as high as 150 mT with durations as long as 10 milliseconds. The results indicated that radical pair behavior is sensitive to SMF, and this effect can be interpreted by using the radical pair mechanism theory. The radical pair mechanism theory postulates that external SMF only influences radical pairs, generally by slowing down processes that require a change of spin. When a SMF is applied, less radicals can change their spin and more succeed in separating from their partner. The consequence of this is that, on average, radicals live longer and their overall concentrations increases when a SMF is applied (12). These effects are more pronounced when the radicals are "encouraged" to stay together by some chemical link or physical boundary. This boundary can be provided by a micelle, a bilayer membrane, or the organized structure of a cell.

Scaiano (68) examined a radical pair confined to a small space (provided by a micelle) generated in the triplet state using laser techniques. The two radicals in this case are derived from benzophenone and melatonin; the latter is a hormone produced by the pineal gland that controls the circadian rhythm (69). The trace without SMF contains a fast and slow decay. The fast decay corresponds to those radicals that decay by reaction with their partner in the pair, while the slow component shows that many radicals separate and then lack a reaction partner (i.e., they are no longer a radical pair). Those fast-decaying radicals have succeeded in changing their spin to achieve the magnetic balance.

Chignell and Sik (70) investigated the effect of SMF on the photohemolysis of human erythrocytes using ketoprofen. Ultraviolet (UV) irradiation (more than 300 nm) of the nonsteroidal anti-inflammatory agent ketoprofen (KP, 3-benzoyl-alpha-methylbenzoacetic acid) in aqueous solution (pH 7.4) resulted in heterolytic decarboxylation of the agent to give 3-ethylbenzophenone (EtBP). KP caused the photohemolysis of human erythrocytes probably from the lipid peroxidation, which is an indicator of ROS generation. Application of SMF (25-150 mT) during UV irradiation of KP and erythrocytes significantly decreased the time required for photohemolysis. This observation suggests that KP-induced photohemolysis involves the initial generation of a triplet radical pair derived from the reaction of triplet state KP or 3-EtBP with erythrocyte component (s) probably lipids. The SMF increases the concentration and/or lifetime of free radicals that escape from the radical pair so that the critical radical concentration, needed to initiate membrane damage and cause cell lysis, is reached sooner. Spin-trapping studies

with 2,6-dibromo-1-nitrosobenzene-4-sulfonate confirmed that the SMF increased the concentration of radicals released during the photolysis of either KP or 3-EtBP dissolved in organized media such as sodium dodecylsulfate micelles.

Some retinoids or porphyrins can form radical pairs, alter transfer of electrons, and increase synthesis of ROS in the mitochondrial respiratory chain. This leads to cell damage and apoptosis. Waliszewski *et al*. (71) reported that co-application of moderate-intensity SMF with some retinoids or porphyrins can enhance those effects by further alteration of electron flow in the mitochondrial respiratory chain, facilitation of forbidden transitions from the triplet to the singlet state of retinoid or porphyrin radicals, and modification of radical pair amount. Since electron flow in the mitochondrial respiratory chain seems to possess fractal dynamics, the SMF can initiate self-organization of the flow into more regular patterns, and create optimal conditions for damage of cancer cells without any detriment for the normal counterparts. The SMF should improve effectiveness and selectivity of retinoid chemoprevention or porphyrin photodynamic therapy.

Katz *et al*. (72-73) examined that SMF effects on cytochrome C-mediated bioelectrocatalytic transformations. This was exemplified by the bioelectrocatalyzed cytochrome C-mediated reduction of oxygen and oxidation of lactate in the presence of cytochrome C oxidase and lactate dehydrogenase (LDH), respectively. SMF induced a 3-fold increase in the rates of bioelectrochemical transformations at the functionalized interfaces in the strength up to 1 T.

Afanasyeva *et al*. (74) described that the effects of SMF (1-400 mT) on elementary kinetic steps of the enzymatic oxidation of nifedipine (NF) catalyzed by HRP. The measurement of kinetic traces was carried out at least 45 sec using the spectrophotometer. It was shown that the first step of the catalytic cycle is single electron transfer resulting in formation of $NF^{\bullet +}$ radical cation and ferroperoxidase (Per^{2+}). It could be concluded that both enzymatic oxidations of NADH (native HRP's substrate) and NF (its synthetic analogue), catalyzed by HRP, proceeded via formation of similar paramagnetic pairs. The mechanisms of NADH and NF oxidations catalyzed by HRP are considered to be identical.

4.2. Moderate SMF effects on ROS

Several experiments have been shown and discussed how SMF can influence the immune function or oxidative DNA damage via the ROS formation process. Danielyan and Ayrapetyan (75) found the changes of (^{3}H)ouabain (5×10^{-9} M) binding to its receptors after *in vivo* exposure of rats to a 200 mT SMF for 30 min. A decrease of ouabain binding was measured in brain, spleen and liver. In contrast, an increase of ouabain uptake by kidney was detected. The ouabain binding by heart was not significantly changed. It was reported that ouabain is a specific inhibitor of Na^+/K^+ ATPase and ROS generator (76). Therefore, it is speculated that the SMF could influence the ROS modulation of different organs, depending on their specificity and sensitivity to ouabain binding.

Gray *et al*. (77) evaluated the ability of SMF to enhance the *in vivo* action of a chemotherapeutic agent, adriamycin, against transplanted mammary tumors in mice. Treatment with 10 mg/kg adriamycin + repetitive four 4 h (16 h) exposures to a gradient SMF of 110 mT achieved greater tumor regression than the group treated with adriamycin alone lasting throughout a 20 d period. Since recent evidence supports the concept that adriamycin cytotoxicity may be caused by generating semiquinon free radicals and superoxide anion radicals (78), a possible explanation for the synergistic SMF effect with adriamycin is that SMF could enhance the ROS production bringing about changes in tumor cell membrane permeability, influencing positively the drug uptake (79). Alternatively, or in addition to this, it is assumed that SMF might increase locally the rate of conversion of adriamycin to ROS able to bind to DNA (79).

Chater *et al*. (80) investigated the effects of a uniform SMF (128 mT, 1 h/d, for 14 d) on some parameters of oxidative stress and on oxidative DNA damage in pregnant rats. Female rats were exposed to the SMF for 14 d (from d 6 to d 19 of pregnancy) and were allowed to deliver normally. The effects of SMF on oxidative states were assessed by the measurements of a ROS product, malondialdehyde (MDA), and ROS scavengers, such as superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPX) and catalase (CAT). The SMF exposure failed to alter plasma GPX, MDA, CAT and SOD, respectively in liver and kidney. By contrast, the SMF increased total GSH and reduced GSH in liver. The results showed that the SMF exposure did not induce oxidative DNA lesions in liver and kidney. The data did not provide evidence that the SMF exposure could cause DNA damage in liver and kidney in pregnant rats. The results suggest that hepatic GSH plays an important role in protection against SMF during pregnancy. These changes in antioxidant status (induced by GSH) lead to some adaptive responses due to activation of systems controlling the body oxidative mechanism balance.

Amara *et al*. (81) examined the effects of a uniform SMF (128 mT, 1 h/d, for 30 d) and/or zinc (Zn) treatment ($ZnCl_2$, 40 mg/L, *per os*, for 30 d) on hematological and biochemical some parameters in male rats. Plasma LDH, aspartate aminotransferase (AST), alanine transaminase (ALT), creatinine and urea concentrations were measured. The SMF exposure alone induced hematological changes and hepatic damage (increased LDH, AST and ALT levels) probably due to the ROS generation, whereas the SMF did not change the renal and hepatic Zn levels. The SMF exposure alone also induced a ROS scavenger, metallothionein (MT) synthesis in the liver and kidney. The Zn administration prevented the increases in the plasma AST and ALT activities, and the leukocyte and platelet counts induced by the SMF exposure. The Zn administration potentiated the increase in the MT concentrations induced by the SMF exposure in both tissues. It is suggested that the Zn supplementation could

prevent these adverse effects of SMF probably by its antioxidant properties.

Amara *et al*. (82) investigated the effect of co-exposure to a SMF (128 mT, 1 h/d, for 30 d) and cadmium (Cd) on the biochemical parameters, activities of antioxidant (ROS scavenging) enzymes and the extent of DNA damage in rat tissues. Animals were treated with Cd compounds ($CdCl_2$, 40 mg/L, *per os*) in drinking water during 4 wk. Cd treatment induced increases in plasma LDH, AST, ALT, creatinine and urea concentrations. Moreover, Cd treatment increased levels of oxidative DNA damage products, such as MDA and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) (a product of nuclear and mitochondrial DNA oxidation) in rat tissues. In contrast, the ROS scavengers, such as the GPX, CAT and SOD, were decreased in liver and kidney, while the hepatic and renal Cd contents were increased drastically. The combined effect of the SMF and Cd compounds resulted in decrease in the GPX and CAT activities in liver. However, the association between SMF and Cd compounds failed to alter AST, ALT, MDA and 8-oxo-dG concentrations. Cd treatment altered the activities of antioxidant enzymes and extent of DNA damage in liver and kidney of rats. Moreover, SMF combined with Cd disrupted this antioxidant response in liver compared to Cd-treated rats.

Amara *et al*. (83) investigated the effect of a SMF (128 mT, 1 h/d, for 30 d) on testicular function, antioxidant status and DNA oxidation in male rats. The 8-oxo-dG and MDA levels and the MT, GPX, CAT and SOD activities were used as markers of oxidative stress in testis. The SMF exposure had no effect on epididymal sperm count, spermatozoa motility and genital organ weight. In contrast, the SMF induced a decrease of testicular and plasmatic testosterone levels, respectively. Exposed rats displayed an increase of a ROS scavenger, MT concentrations in the testis, whereas the concentrations of ROS products, MDA and 8-oxo-dG, were simultaneously increased. In the gonad, the SMF decreased the CAT, GPX and mitochondrial manganese (Mn)-SOD activities. However, the cytosolic copper-zinc (CuZn)-SOD activity was unaffected. The SMF exposure failed to alter spermatogenesis in rat testis. In contrast, the same treatment decreased testosterone levels and induced DNA oxidation. From these results, they concluded that the sensitivity of testicular tissues to SMF was marked by the reduction of the antioxidant potential associated with an increase in the DNA oxidation and an impairment of testosterone production without affecting spermatogenesis and genital organ weight.

As for the SMF effects on the muscle heat shock protein (HSP) 72 and norepinephrine response, Abdelmelek *et al*. (84) found that a SMF of 128 mT, 1 h/d, for 5 d induced increases in norepinephrine and LDH contents in rat gastrocnemius muscles but a 67 mT SMF did not. The results suggested that the SMF could induce a stimulatory effect on the noradrenergic system in muscles. Although the SMF did not increase HSP 72 levels, they speculated that the cells might have developed antioxidant strategies, including HSP induction, to protect themselves against oxidative damage.

Flipo *et al*. (85) investigated that the effects of SMF (25-150 mT) on the cellular immune function of macrophages, spleen lymphocytes and thymic cells isolated from the C57BI/6 mice and cultured *ex vivo*. After the SMF exposure for 24 h, phagocytic uptake of fluorescent latex microspheres was decreased, intracellular Ca^{2+} influx $[Ca^{2+}]_i$ was increased in macrophages, and mitogenic responses were decreased in lymphocytes, while $[Ca^{2+}]_i$ and apoptosis were increased in a ROS generator, concanavalin A (Con A)-stimulated lymphocytes and thymocytes, respectively. The authors did not discuss the mechanisms, but concerning the synergistic SMF effects with Con A, SMF potentiated the ROS production and apoptosis induced by Con A.

Salerno *et al*. (86) examined that the effects of a 500 mT SMF on *in vitro* expression of activation markers and cytokine release in human peripheral blood mononuclear cells (PBMC). The SMF for 2 h reduced the expression of CD69 from PBMC that was enhanced after phytohaemagglutinin (PHA) stimulation. An increased release of IFN-gamma and IL-4 was also found, which was reduced after PHA stimulation. The release of TNF-alpha, IL-6 and IL-10 was not modified. The SMF modified activation marker expression and cytokine release from human PBMC. The effects might be related to the ROS formation process.

Fanelli *et al*. (87) examined the effects of SMF (66 mT or less) on the apoptosis induced by several agents in different human cell systems, U937 and CEM cells. The minimal intensity required to detect an antiapoptotic effect was 0.6 mT. The SMF intensities of 0.6 mT or more for 4 h decreased the cell apoptosis in an intensity-dependent fashion, reaching a plateau at 6 mT. The protective or antiapoptotic effect was mediated by the SMF to enhance $[Ca^{2+}]_i$ from the extracellular medium. In addition to the SMF-enhancing effect on $[Ca^{2+}]_i$, as a mechanism of the rescue of damaged cells, it was recently proposed that SMF-produced redox alterations may be part of the signaling pathway leading to apoptosis antagonism (88).

Buemi *et al*. (89) investigated the effect of a SMF (0.5 mT for up to 6 d) on the cell proliferation/cell death balance in monkey renal cells and rat cortical astrocytes. This SMF exposure has different effects on the two cell lines, and, in addition to having a tumorigenic effect, SMF may also play a nephropathogenic role at least partially due to ROS generation.

Danielyan *et al*. (90) examined the effect of a SMF (200 mT for 1 h) on (^{3}H)ouabain binding in human normal and cancer tissues. The SMF exposure led to a decrease of ouabain binding in both normal and cancer tissues when ouabain concentrations in the external medium were 10^{-9} M range, while in higher concentrations of ouabain (10^{-7}-10^{-6} M), an increase of ouabain binding was observed. Since ouabain is a ROS generator (74), it is inferred that the SMF

could influence the ROS modulation (generation/reduction) through ouabain binding, dependent on its concentrations.

Yokoi *et al*. (91) examined the effects of SMF (340 mT or less for 15 min) on monoamine oxidase (MAO) activity in rat brain homogenates. MAO catalyzes the oxidation of biogenic amines and amines with non-acidic hydrogens. Monoamine oxidation by MAO is thought to involve spin-correlated radical pair intermediates (92), and therefore there is a possibility that oxidation by MAO is a magnetically sensitive enzymatic reaction (91). However, the SMF exposure did not induce any change in the activities of two MAO isoenzymes (A and B).

The Jajte research group (93-94) reported that simultaneous exposure of rat lymphocytes to a 7 mT SMF for 3 h and $FeCl_2$ (10 microg/mL) caused an increase in the number of cells with DNA damage (apoptosis or necrosis). In contrast, the SMF exposure alone did not increase the number of cells with DNA damage. Incubation of lymphocytes with $FeCl_2$ did not produce a detectable damage of DNA either. To clarify the mechanism of DNA damage, the levels of lipid peroxidation were measured as the amount of MDA + 4-hydroxynonenal (4-HNE), as major lipid peroxidation end products. The amount of MDA + 4-HNE were increased following the simultaneous exposure to the SMF and $FeCl_2$, compared with the control and the SMF exposure alone. This suggests that the SMF exposure in the presence of Fe^{2+} could increase the ROS concentrations, and thus may lead to cell death.

Ishisaka *et al*. (95) investigated the effects of a SMF (25 mT for 1 h) on the apoptosis of human leukemic cell line (HL-60) induced by exogenous H_2O_2. The H_2O_2 induced a rapid DNA fragmentation and a slow decrease in viability of HL-60 cells. However, the SMF itself (6 mT for 18 h) did not exert any effect on the H_2O_2-induced DNA fragmentation or viability.

Teodori *et al*. (96) also reported a uniform SMF (6 mT for 5-18 h) itself did not affect viability of HL-60 cells. The SMF accelerated the rate of cell transition from apoptosis to secondary necrosis after induction of apoptosis by the DNA-damaging agent, camptothecin (a mammalian DNA topoisomerase I inhibitor). In addition, subsequent works (97-98) reported that a uniform SMF (6 mT for 18 h; 8 and 30 mT for 3h) did not affect viability of human glioblastoma cells, which possess an excitable membrane. However, a uniform SMF of 300 mT for 3 h increased apoptosis (98). They (97) evaluated the interference of the SMF (6 mT for 18 h) with physical (heat shock) or chemical (etoposide, VP16) induced apoptosis related to oxidative stress. The SMF exposure dramatically reduced the extent of heat shock- and VP16-induced apoptosis. It might be due to the ROS reduction.

Ishisaka *et al*. (95) investigated the effects of SMF (60-200 mT for 1 h) on human oral polymorphonuclear leukocytes (PMN). The luminol chemiluminescence of PMN responding to the ROS concentrations decreased during the incubation, concomitant with the superoxide generation. This luminol chemiluminescent intensity was further suppressed by the SMF exposure in a strength dependent manner. The degree of suppression by 200 mT was about 30% compared to that of non-exposed PMN. The suppressive effect of the SMF on endogenous superoxide generation measured by the reduction of cytochrome C oxidase was also observed. In addition, they examined the effects of a 200 mT SMF for 1 h on the Fe^{2+}-induced lipid peroxidation of mitochondrial membranes of isolated rat liver mitochondria. The lipid peroxidation was measured by oxygen consumption or by accumulation of thiobarbituric acid reactive substance (TBARS). However, the extent of lipid peroxidation was not significantly changed by the SMF exposure.

Kabuto *et al*. (99) showed that a SMF (1 and 5-300 mT for 40 min) had no effect on the accumulation of TBARS in mouse brain homogenates induced by $FeCl_3$. In contrast, when the homogenates were incubated with $FeCl_3$ in a SMF (2-4 mT), the accumulation of TBARS was decreased. The accumulation of TBARS in phosphatidylcholine solution incubated with $FeCl_3$ and H_2O_2 was also inhibited by the SMF exposure. These results suggest that the SMF could have an inhibitory effect on Fe^{2+}-induced lipid peroxidation and the effectiveness of this SMF suppression on Fe^{2+}-induced ROS generation is restricted to a "window" of field intensity of 2-4 mT.

Amara *et al*. (100) investigated the effect of a 250 mT SMF for up to 3 h on antioxidant enzyme activity, intracellular labile Zn (II) (a putative ROS scavenger) and DNA damage in human THP-1 cells (monocyte line). The SMF exposure did not alter the MDA concentrations and the GPX, CAT and SOD activities but induced a decrease of the Zn (II) in the cells stained with Zn-specific fluorescent probes zinpyr-1. The results showed that the SMF exposure did not cause oxidative stress and DNA damage in THP-1 cells presumably due to the depletion of the Zn (II). This mechanism might be associated with the antioxidant action of Zn against SMF-induced ROS generation.

Sahebjamei *et al*. (101) investigated the effects of a SMF (10 and 30 mT, 5 h/d, for 5 d) on the activities of antioxidant enzymes of suspension-cultured tobacco cells. The SMF exposure increased the SOD activity. In contrast, the SMF decreased the activity of other ROS scavengers, such as CAT and ascorbate peroxidase (APX). Levels of lipid peroxidation were also increased by the SMF exposure. These results suggested that the SMF could deteriorate antioxidant defense system of plant cells. This study points to a mechanism by which the increased SOD activity might enhance cytotoxicity by ROS generation, likely owing to the accelerated H_2O_2 generation and subsequent overproduction of OH• by the metal catalyzed Haber-Weiss reaction (102).

Yuge *et al*. (103) examined the effect of a magnetic pulling force on differentiation of cultured human osteoblasts. Magnetic microparticles (MMP) (0.05 microm in diameter and suspended in a medium at a concentration of 20,000 particles/mL) were introduced into the cytoplasm of a normal human osteoblast (NHOst) cell line, and the

cells were cultured in a SMF of 10, 30, and 50 mT for 21 d. Osteoblast differentiation was accelerated by a magnetic pulling force between MMP and a SMF of 30, and 50 mT. This acceleration was attributed mainly to the activation of p38 phosphorylation. It was reported that the p38 phosphorylation pathways can be rapidly activated by oxidative stress (104). Therefore, it is inferred that the SMF may have effects on the activation of p38 phosphorylation through ROS generation. However, the effects of SMF alone without MMP have not been tested.

The Dini research group (105-107) undertook a comparative study of the bio-effects induced by exposure to a uniform SMF (6 mT for up to 6 days) on several primary cultures and cell lines. Cell viability, proliferation, $[Ca^{2+}]_i$ concentration and morphology were examined. Primary cultures of human lymphocytes, mice thymocytes and cultures of mouse 3DO, rat FRTL-5, human U937, HeLa and Hep G2 cells were grown in the presence of 6 mT SMF and different apoptosis-inducing agents, cycloheximide (CHX), H_2O_2 and puromycin (PMC), or pro-apoptotic conditions induced by heat shock or glutamine deprivation. A common SMF effect was the promotion of apoptosis and mitosis, but not of necrosis or modifications of the cell shape. Increase of the $[Ca^{2+}]_i$ were also observed. When pro-apoptotic agents, heat shock or glutamine deprivation were combined with SMF, some cell types rescued from apoptosis. In contrast, apoptosis of 3DO cells was significantly increased upon simultaneous SMF exposure and incubation with CHX. It is conclude that the SMF exposure interfered with apoptosis in a cell type- and exposure time-dependent manner, while the effects of SMF on the apoptotic program were independent of the agents used or heat shock. Dini and Abbro (108) speculated in their review that the possible effects of SMF leading to perturbation of the apoptotic rate might be partially due to modulation of ROS generation. However, other research groups reported no significant SMF effects on cell proliferation (109-110).

As for the mutagenic effects of moderate SMF, Koana *et al*. (111) estimated the genetic effects of a homogeneous SMF (600 mT for 24 h) by a somatic cell test using a mutagen-sensitive mutant of *Drosophila melanogaster*. The SMF increased developmental lethality of mutant larvae. One of possible mechanism is the interaction of the SMF with mutagenic free radicals, which occasionally appear in larval somatic cells. It was reported that a 600 mT SMF increased the lifetime of free radicals in a micelle (7). Since a micelle can be considered a simple model of a cell, it is plausible that the lifetime of free radicals in living cells is also affected by the same intensity of SMF. If its lifetime is increased, the same amount of free radicals may have more mutagenic activity.

Kohno *et al*. (112) investigated SMF effects on three species of bacteria: *Streptococcus mutans*, *Streptococcus aureus*, and *Escherichia coli*. The results showed that a uniform SMF (30, 60, 80 and 100 mT for 24 h) caused strength-dependent decreases in the growth rate and growth number of bacteria for *S. mutans* and *S. aureus* when cultured under anaerobic conditions, but not under aerobic conditions. No growth effects were detected on *E. coli* cultures. Although participation of ROS was not demonstrated, it is at least suggested that there are significant differences in the SMF effects on bacteria depending on whether dissolved oxygen is present.

Potenza *et al*. (113) investigated the effects of a gradient SMF (200 and 250 mT for up to 5 h) on different DNA sources in *E. coli*. The bacterial strain XL1-Blue of *E. coli*, cultivated in traditional and modified Luria-Bertani medium, was exposed to the SMF for 5 h of growth. The *in vivo* assays did not reveal any DNA alterations following the SMF exposure, demonstrating the presence of cell dependent mechanisms, such as the repair system and the buffering action of the heat shock proteins DNA K/J (HSP 70/40). In contrast, the *in vitro* assays displayed interactions between the SMF and DNA, revealing that the SMF exposure induces DNA alterations in terms of point mutations. SMF of 200 and 250 mT for 90 min in the presence of H_2O_2 induced different DNA damage, probably related to the size and conformation of different DNA sources. They speculated that the SMF could perturb DNA stability interacting with DNA directly or increasing the ROS activity and this genotoxic effect of the SMF might be minimized in living organisms due to the presence of protective or defensive cellular responses.

Potenza *et al*. (114) studied that the effects of a gradient SMF (300 mT for up to 50 h) on cellular growth and gene expression in *E. coli* (XL1-Blue). The results showed alterations induced by the SMF in terms of increased cell proliferation and changes in gene expression. One clone, expressed only in the exposed cells, corresponds to a putative transposase. It suggests that the SMF exposure may stimulate transposition activity due to accumulated mutations most likely through ROS generation.

Morrow *et al.* (115) reported antioxidant protective effects of a uniform SMF (300 mT for 15 h) on *Streptococcus pyogenes* by measuring 8-hydroxyguanin, which is a sensitive marker of oxidative DNA damage. The cells exposed to the SMF had significantly less 8-hydroxyguanine compared with the unexposed control cells because of reduced ROS generation. The results suggest that a certain magnetic flux density could possibly protect normal cells against damage. They proposed that if SMF exposure is found to increase apoptosis via free radical mechanisms in transformed cells, with protecting or without damaging normal cells, SMF therapy could be applicable to the treatment of various cancers.

Wang *et al.* (116) reported that SMF of 10-35 mT for 12 h could promote the growth of *Chlorella vulgaris* and regulate its antioxidant defense system to protect cells efficiently. SMF of 45 and 50 mT for 12 h also could regulate antioxidant defense system but could not enhance the growth of *C. vulgaris*. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability was decreased, the TBARS levels was increased, and the activities of SOD, CAT, and peroxidase were increased. It was suggested that the scavenging and detoxification of H_2O_2 produced by SOD were achieved by either

nonenzymatic antioxidants or scavenging enzymes such as CAT and peroxidase, which therefore increased in SMF-exposed cells.

4.3. Moderate SMF effects on RNS

When NO is produced, there might be different mechanisms corresponding to the three major NOS isoforms: the neuronal (nNOS), endothelial (eNOS) and inducible (iNOS) isoforms. In the three NOS isoforms, electron transfer, resulting from heme iron reduction and from NADPH oxidation, has been reported, but differences in the final reaction yielding NO may still exist (117). Mnaimneh *et al*. (66) investigated the *in vitro* effects of SMF ranging 1-100 mT on NO production by murine BCG-activated macrophages. No significant differences were observed in NO levels after a 14-h exposure. In spite of their results, however, they made note that SMF could potentially modify an isoform of NOS-dependent response or "down-stream" from NO formation.

The mechanisms of *in vivo* SMF effects on blood flow and/or blood pressure could be mediated by suppressing or enhancing the action of biochemical effectors, thereby restoring homeostatic equilibrium and hemodynamic equilibrium (118). One of the potent mechanisms of SMF effects has sometimes been linked to NO pathway, as reviewed by McKay *et al*. (119).

An investigation by Okano *et al*. (120) indicates that the homeostatic effect of SMF might influence NO pathways. When genetically hypertensive rats were exposed to a gradient SMF of 1 and 5 mT for up to 12 wk, the blood pressure and/or the concentrations of NO metabolites, angiotensin II and/or aldosterone were reduced. Specifically, the SMF exposure reduced mean blood pressure during 3-6 wk. Young spontaneously hypertensive rats (SHR) are known to have increased levels of NO metabolites, presumably due to the upregulation of NOS. Exposure to a 5 mT SMF for 6 wk significantly reduced the concentrations of NO metabolites. A 1 mT SMF did not affect the NO metabolites. A 5 mT SMF reduced angiotensin II and aldosterone during 3-6 wk. Similar significant reductions in angiotensin II and aldosterone were seen with the 1 mT SMF. However, until the 9th wk of exposure, irrespective of the longer duration of exposure, all significant antihypertensive effects of SMF disappeared, due to the development of hypertension in young SHR.

The higher basal NO levels found in SHR relative to normotensive rats are most likely mediated by both iNOS (121-122) and nNOS (123-124) rather than by eNOS. Here, two of the isoforms (eNOS and nNOS) are constitutive, Ca^{2+}/calmodulin regulated, while the third isoform (iNOS) is inducible, Ca^{2+}/calmodulin independent. More importantly, these isoforms differ in the amounts of NO that they generate: picomoles for constitutive (eNOS and nNOS) and nanomoles for inducible (iNOS) (125). Therefore, NO reduction by SMF, in part, might be due to the downregulation of iNOS rather than nNOS.

Another mechanism for SMF influence on NO reduction could be explained by the antioxidant effect associated with decreased angiotensin II-induced superoxide reduction pathway. It is postulated that SMF might have antagonizing effects on RNS production in SHR because angiotensin II stimulates superoxide formation by activating NADPH and NADH oxidase *in vitro* (126) and *in vivo* (127), and increased oxidative stress induces elevated NO levels in non-exposed SHR (116). Therefore, the suppression and delayed onset in blood pressure elevation during exposure to a 5 mT SMF are presumed to be due to angiotensin II-mediated modulation of NO levels.

The above findings were partially elucidated when SHR were exposed to a 180 mT SMF (a magnet was implanted in the neck) for up to 14 wk (128). The SMF enhanced the hypotensive effect of nicardipine and caused a further increase in NO metabolites during the 6th wk of exposure compared with rats that also received nicardipine but were exposed to a sham SMF. Thus, the synergistic effect of the SMF appeared to be related to NO. The SMF alone (without nicardipine), however, did not induce any change in NO metabolite concentrations. In contrast to the previous study (120), in this study, it is speculated that NO increase induced by SMF, in part, might be due to the upregulation of iNOS rather than nNOS or eNOS.

Other research by these investigators, however, reported a lack of change in measured NO upon SMF exposure. Okano *et al*. (129) used a gradient SMF of 10 and 25 mT for up to 12 wk to counter reserpine-induced hypotension in rats. It was reported that the SMF did significantly counter the reserpine-induced effects. This effect was not mediated by NO production. They found no significant differences in the concentrations of NO metabolites between any tested groups. In another experiment using norepinephrine or a Ca^{2+} channel blocker, nicardipine, Okano and Ohkubo (130) reported similar findings. After exposing conscious rabbits to a gradient SMF of 5.5 mT for 30 min, bidirectional antagonizing effects were shown for the action of the pharmacologically modified vessel tone and blood pressure, but no changes in NO metabolites. It is suggested that the site of SMF interaction may be biochemical mechanisms involving baroreflex sensitivity and signal transduction pathways involving Ca^{2+}.

In contrast to the results of *in vivo* studies, however, no significant effects have been reported in *in vitro* studies (66, 131). In addition to Mnaimneh *et al.* (66) as described above, Noda *et al.* (131) also reported that SMF (3 and 30 mT for 1h) did not show any significant change in NOS activity in rat brain homogenates (supernatant solutions of whole brain and cerebellum homogenates).

4.4. Summary of moderate SMF effects on FRR

There are several reports that moderate SMF could influence the ROS modulation (generation/reduction) from enzymatic reactions in cell-free solutions. The SMF effects also play significant roles in the endogenous and exogenous ROS modulation in biological systems, *in vitro* and *in vivo* (Table 1). The SMF effects could affect the RNS modulation, in particular, endogenous NO modulation *in vivo* (Table 2).

Table 1. Summary of moderate-intensity SMF effects on ROS

Authors	Exposure Conditions	Results
Danielyan & Ayrapetyan (75)	200 mT + ouabain (5×10^{-9} M); 30 min; rats (kidney function)	I[5]
Gray *et al.* (77)	110 mT + adriamycin; 16 h; tumor-transplanted mice	I[5]
Chater *et al.* (80)	128 mT; 1 h/d, 14 d; pregnant rats (liver & kidney function)	I[5]
Amara *et al.* (81)	128 mT; 1 h/d, 30 d; rats (liver & kidney function)	I[5]
Amara *et al.* (82)	128 mT + Cd^{2+}; 1 h/d, 30 d; rats (liver function)	I[5]
Amara *et al.* (83)	128 mT; 1 h/d, 30 d; rats (testicular function)	I[5]
Abdelmelek *et al.* (84)	128 mT; 1 h/d, 5 d; rats (gastrocnemius muscle metabolism)	I[5]
Flipo *et al.* (85)	25-150 mT + concanavalin A; 24 h; mouse Ly[1] & Th[2]	I[5]
Salerno *et al.* (86)	500 mT; 2 h; human PBMC[3]	I[5]
Buemi *et al.* (89)	0.5 mT; 6 d; monkey renal cells & rat cortical astrocytes	I[5]
Danielyan *et al.* (90)	200 mT + ouabain (10^{-7}-10^{-6} M); 1 h; human normal & malignant tissues	I[5]
Zmyslony *et al.* (93)	7 mT + Fe^{2+}; 3 h; rat Ly[1]	I[5]
Jajte *et al*. (94)	7 mT + Fe^{2+}; 3 h; rat Ly[1]	I[5]
Teodori *et al*. (96)	6 mT + camptothecin; 5-18 h; human HL-60 cells	I[5]
Teodori *et al.* (98)	300 mT; 3 h; human glioblastoma cells	I[5]
Kabuto *et al*. (99)	2-4 mT + Fe^{2+}; 40 min; mouse brain homogenates	I[5]
Sahebjamei *et al.* (101)	10 & 30 mT; 5 h/d, 5 d; suspension-cultured tobacco cells	I[5]
Yuge *et al.* (103)	30 & 50 mT + magnetic microparticles; 21 d; normal human osteoblasts (NHOst)	I[5]
Chionna *et al.* (105)	6 mT; 24 h; human Hep G2 cells	I[5]
Tenuzzo *et al.* (106)	6 mT; 24-48 h; mouse 3DO, rat FRTL-5 & Hep G2 cells	I[5]
Tenuzzo *et al.* (106)	6 mT + cycloheximide; 24 h; 3DO cells	I[5]
Koana *et al.* (111)	600 mT; 24 h; *Drosophila melanogaster* (mutant strain)	I[5]
Potenza *et al.* (113)	200 & 250 mT; 5 h; *Escherichia coli* (mutant strain)	I[5]
Potenza *et al.* (113)	200 & 250 mT + H_2O_2; 90 min; *E. coli* (mutant strain)	I[5]
Potenza *et al.* (114)	300 mT; 15 h; *Streptocuccus pyogenes*	I[5]
Danielyan & Ayrapetyan (75)	200 mT + ouabain (5×10^{-9} M); 30 min; rats (brain, spleen & liver function)	D[6]
Danielyan *et al.* (90)	200 mT + ouabain (10^{-9} M); 1 h; human normal & malignant glandular tissues	D[6]
Fanelli *et al.* (87)	0.6-66 mT; 4 h; human U937 & CEM cells	D[6]
Ishisaka *et al.* (95)	60-200 mT; 1 h; human oral PMN[4]	D[6]
Teodori *et al.* (97)	6 mT + heat shock; 18 h; human glioblastoma cells	D[6]
Teodori *et al.* (97)	6 mT + etoposide; 18 h; human glioblastoma cells	D[6]
Tenuzzo *et al.* (106)	6 mT + H_2O_2; 24 h; mouse Th[2], human Ly[1], U937, Hep G2 & HeLa cells	D[6]
Tenuzzo *et al.* (106)	6 mT + cycloheximide; 24 h; mouse Th[2], FRTL-5, U937, Hep G2 & HeLa cells	D[6]
Tenuzzo *et al.* (106)	6 mT + puromycin; 24 h; mouse Th[2], FRTL-5, U937, Hep G2 & HeLa cells	D[6]
Tenuzzo *et al.* (106)	6 mT + heat shock; 24 h; mouse Th[2], human Ly[1] & U937 cells	D[6]
Tenuzzo *et al.* (107)	6 mT + glutamine deprivation; 5-6 d; U937 cells	D[6]
Morrow *et al.* (115)	300 mT; 15 h; *E. coli* (mutant strain)	D[6]
Wang *et al.* (116)	10-50 mT; 12 d; *Chlorella vulgaris*	D[6]
Danielyan & Ayrapetyan (75)	200 mT + ouabain (5×10^{-9} M); 30 min; rats (heart function)	NC[7]
Amara *et al*. (81)	128 mT+ Zn^{2+}; 1 h/d, 30 d; rats (liver & kidney function)	NC[7]
Abdelmelek *et al.* (84)	67 mT; 1 h/d, 5 d; rats (gastrocnemius muscle metabolism)	NC[7]
Fanelli *et al.* (87)	< 0.6 mT; 4 h; U937 & CEM cells	NC[7]
Yokoi *et al.* (91)	≤ 340 mT; 15 min; rat brain homogenates	NC[7]
Zmyslony *et al.* (93)	7 mT; 3 h; rat Ly[1]	NC[7]
Jajte *et al*. (94)	7 mT; 3 h; rat Ly[1]	NC[7]
Ishisaka *et al.* (95)	25 mT + H_2O_2; 1 h; HL-60 cells	NC[7]
Ishisaka *et al.* (95)	200 mT + Fe^{2+}; 1 h; human oral PMN[4]	NC[7]
Teodori *et al.* (96)	6 mT; 5-18 h; HL-60 cells	NC[7]
Teodori *et al.* (97)	6 mT; 18 h; human glioblastoma cells	NC[7]
Teodori *et al.* (98)	8-30 mT; 3 h; human glioblastoma cells	NC[7]
Kabuto *et al.* (99)	1 & 5-300 mT + Fe^{2+}; 40 min; mouse brain homogenates	NC[7]
Amara *et al.* (100)	250 mT; 3 h; human THP-1 cells	NC[7]
Yuge *et al.* (103)	10 mT + magnetic microparticles; 21 d; NHOst	NC[7]
Tenuzzo *et al.* (106)	6 mT; 24-48 h; mouse Th[2], human Ly[1], U937 & HeLa cells	NC[7]
Tenuzzo *et al.* (106)	6 mT + cycloheximide; 24 h; human Ly[1]	NC[7]
Tenuzzo *et al.* (106)	6 mT + puromycin; 24 h; 3 DO cells & human Ly[1]	NC[7]
Tenuzzo *et al.* (106)	6 mT + heat shock; 24 h; 3 DO cells	NC[7]
Bodega *et al.* (109)	1 mT; 1-4 h, 11 d; rat astroglial cells	NC[7]
Gamboa *et al.* (110)	5 mT; 24 h; rat Schwann cells	NC[7]

Abbreviations: [1]Ly, lymphocytes; [2]Th, thymocytes; [3]PBMC, peripheral blood mononuclear cells; [4]PMN, polymorphonuclear leukocytes; [5]I, increased; [6]D, decreased; [7]NC, not changed.

5. STRONG-INTENSITY SMF

5.1. Control of biochemical reactions with strong SMF

Using fireflies, *Hotaria parvula* and *Luciola cruciata*, as bioluminescence systems, Iwasaka and Ueno (132) studied the effects of a homogeneous ultrastrong SMF of 8 and 14 T on the light emission. They showed that the SMF exposure enhanced the inclination of the light emission intensities within 750 sec after addition of ATP, and the changes in the light emission intensities upon the SMF exposure were related to the change in certain biochemical systems of the firefly, such as the enzymatic process of luciferase and the excited singlet state responsible for subsequent light emission. They also demonstrated that SMF exposure up to 14 T could change the oxidation level of cytochrome aa3 periodically depending on the magnetic flux density (133).

Table 2. Summary of moderate-intensity SMF effects on RNS

Authors	Exposure Conditions	Results
Okano & Ohkubo (128)	180 mT + nicardipine; 6-8 wk; SHR[1]	I[2]
Okano *et al.* (120)	5 mT; 6 wk; SHR[1]	D[3]
Okano *et al.* (120)	1 mT; 6-12 wk; 5 mT; 12 wk; SHR[1]	NC[4]
Okano & Ohkubo (128)	180 mT; 6-8 wk; SHR[1]	NC[4]
Okano *et al.* (129)	10 & 25 mT; 12 wk; rats	NC[4]
Okano *et al.* (129)	10 & 25 mT + reserpine; 12 wk; rats	NC[4]
Okano & Ohkubo (130)	5.5 mT; 30 min; rabbits	NC[4]
Okano & Ohkubo (130)	5.5 mT + norepinephrine; 30 min; rabbits	NC[4]
Okano & Ohkubo (130)	5.5 mT + nicardipine; 30 min; rabbits	NC[4]
Mnaimneh *et al.* (66)	1-100 mT + BCG; 14 h; mouse macrophages	NC[4]
Noda *et al.* (131)	3 & 20 mT; 1 h; rat brain homogenates	NC[4]

Abbreviations: [1]SHR, spontaneously hypertensive rats; [2]I, increased; [3]D, decreased; [4]NC, not changed.

5.2. Strong SMF effects on ROS

The Nakagawa research group (134-135) measured and evaluated a ROS scavenger, MT, and a ROS product, lipid peroxidation, in the liver, kidneys, heart, lung and brain of 8-wk-old male BALB/c mice *in vivo*. The mice were exposed to a SMF of 3.0 and 4.7 T for 1-48 h. A 4.7 T SMF exposure for 6-48 h increased both MT and lipid peroxidation levels in the liver alone. A 3.0 T SMF exposure for 1-48 h did not induce any changes in both MT and lipid peroxidation levels in all the tissues. A single subcutaneous injection of CCl_4 (0.5 mL/kg) increased both MT and lipid peroxidation levels in the liver and the combination of CCl_4 administration and a 4.7 T SMF for 24 h potentiated both MT and lipid peroxidation levels. The increase in activities of both GOT and GPT caused by CCl_4 administration were also enhanced by the SMF exposure. It is concluded that exposure to a high SMF induces the increase of both MT and lipid peroxidation levels in the liver of mice and enhances the hepatotoxicity caused by CCl_4 injection.

The Shimizu research group (136) reported that a SMF exposure of mice to 3.0 T for 48-72 h and 4.7 T for 24-72 h increased the frequency of micronucleated polychromatic erythrocytes (a measure of chromosome breaks) in mouse bone marrow cells. The increase in micronucleus frequency was time and dose dependent. They consider that the increased numbers of micronuclei may be partially attributable to an oxidative stress caused by SMF.

The Nakagawa research group (137) evaluated the effects of a 4.7 T SMF on the frequency of micronuclei in Chinese hamster lung (CHL)/IU cells induced by an anti-neoplastic agent, mitomycin C (MMC) *in vitro*. The cells were simultaneously exposed to the SMF and MMC for 6 h, and then the cells were cultured for up to 66 h in normal condition for the micronucleus expression. The SMF exposure for 6 h decreased the frequency of MMC-induced micronucleated cell expression after culture periods of 18-66 h. Since MMC generates ROS (138) and subsequently the MMC-induced oxidative DNA damage results in increased micronucleus formation, in this study, it is speculated that the SMF exposure might interfere with the MMC-induced ROS generation.

Sabo *et al*. (139) examined the effects of a homogeneous SMF (1 T for 72 h) on HL-60 cells. The SMF exposure induced metabolic activity retardation in HL-60 cells. The decrease in metabolic activity reflects the concomitant decrease in cell number. Without SMF, the mixture of the anti-neoplastic agents, 5 fluorouracil (5-FU), cisplatin (CP), doxorubicin (DOX) and vincristine (VCR), induced the metabolic activity retardation, depending on the concentrations applied. The SMF exposure enhanced the cytotoxic effect of anti-neoplastic agents. All the anti-neoplastic agents tested, 5-FU (140), CP (141), DOX (142) and VCR (143), generate ROS, and subsequently the agents-induced oxidative DNA damage results in decreased metabolic activity. Therefore, in this study, it is postulated that the SMF exposure could potentiate the agents-induced ROS generation.

The Miyakoshi research group (144) reported that an ultrastrong SMF of 10 T for up to 4 d did not exert any effect on the cell growth rate or cell cycle distribution in Chinese hamster ovary (CHO)-K1 cells. The SMF exposure alone did not affect micronucleus formation. The micronucleus formation was enhanced by X-ray radiation in dose-dependent manner (up to 4 Gy). However, a 1 T SMF did not affect X-ray-induced micronucleus formation, but a 10 T SMF resulted in a significant increase in the micronucleus formation induced after a 4 Gy exposure (not 1 and 2 Gy exposure). One of the mechanisms of this effect is attributable to the 10 T SMF-induced oxidative DNA damage.

Aldinucci *et al*. (145) examined the effects of a 4.75 T SMF for 1 h on *in vitro* human peripheral blood mononuclear cells (PBMC) and Jurkat cells (human acute T-cell leukemia cells), which are a human leukemia cell line prone to go into apoptosis. The same study was also performed after activation of cells with 5 microg/mL PHA. The SMF did not induce any change in proliferation of intact and PHA-stimulated PBMC. In contrast, the SMF exposure of intact Jurkat cells caused a decrease in proliferation (5% of cell necrosis). In PHA-challenged Jurkat cells, however, the SMF exposure did not affect the proliferation.

Onodera *et al*. (146) also studied the effects of an ultrastrong SMF of 10 T for 3 h on PBMC alone. Consistent with the study of Aldinucci *et al*. (145), the SMF exposure alone did not influence immunological responses of PBMC. In contrast, when PBMC were treated with PHA (5 microg/mL), the SMF exposure resulted in a significant increase of the number of apoptotic cells. The SMF exposure reduced the number of both CD^{4+} and CD^{8+}

T-cells pretreated with PHA. The naive CD^{8+} T-cells were the most sensitive T cell subclass to the SMF exposure when stimulated with PHA. Since it was reported that the intracellular ROS production in lymphocytes, as determined by DCF peroxidation, was increased markedly by the PHA-stimulation (147), this study suggests that the SMF exposure may potentiate the PHA-stimulated ROS production.

With regard to the mutagenic effects of strong SMF, Zhang *et al*. (41) reported that a 5 T SMF for 24 h was sufficient to induce mutation in a mutant strain of *E. coli*, which lacked the superoxide stress-response gene. This study suggests that exposure to a 5 and 9 T SMF induces mutation through an elevated production of intracellular superoxide radicals.

Koana *et al*. (148) examined the genotoxic effects of a 5 T SMF for 24 h in a DNA-repair defective mutant of *D. melanogaster* using the somatic mutation and recombination test (SMART) (149), because this test was useful to detect the mutagenic activity of SMF and EMF. They reported that the SMF exposure increased the frequency of mutation in *mei-41* heterozygotes, and that the increase was suppressed to control levels by supplementation with vitamine E, which is a lipid-soluble antioxidant and a non-specific radical scavenger.

Ikehata *et al*. (150) reported that a SMF of 2 and 5 T for up to 48 h did not have mutagenic potential in a bacterial mutation test using various mutant strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *E. coli* (WP2 *uvrA*). They also reported that the SMF of 2 or 5 T for 48 h resulted in an increased mutation rate of the WP2 *uvrA* strain when induced by mutagenic agents, N-ethyl-N0-nitro-N-nitrosoguanidine (ENNG), N-methyl-N0-nitro-N-nitrosoguanidine (MNNG), ethylmethanesulfonate (EMS), 4-nitroquinoline-N-oxide (4NQO), 2-amino-3-methyl-3H-imidazo-(4,5-f)-quinoline (IQ), and 2-(2-furyl)-3-(5-nitro-2-fulyl) acrylamide (AF-2). The mutagenicities of 2-aminoanthracene (2-AA), 9-aminoacridine (9-AA), N-4-aminocytidine, and 2-acetoamidofluorene (2-AAF) were not affected by the SMF exposure. The SMF exposure *per se* did not change the bacterial growth. They suggested that the mechanism of the co-mutagenic effects of SMF combined with the agents might be related to *in vitro* interactions between the chemicals and DNA, and to repair systems with altered radical behavior in the test strains.

Schreiber *et al*. (151) reported no mutagenic and co-mutagenic effects of magnetic fields used for MRI. The effects of SMF (1.5 T for 1-24 h, and 7.2 T for 1 h), pulsed bipolar gradient, and high-frequency magnetic fields, and combinations of them, were examined using the Ames test. The Ames test was performed using a wild-type strain of *S. typhimurium* bacteria (RTA), as a preincubation assay, without metabolic activation. All combinations of magnetic fields were tested with and without co-exposure to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), benzo (a)pyrene-4,5-oxide (BPOX), ethylene oxide (EO), carboplatin (CAP), or cisplatin. As expected, chemical mutagens caused a clear-cut increase of the revertants in the Ames test. However, neither the SMF nor a combination of the SMF with the time-varying bipolar gradient field or a pulsed high-frequency magnetic field caused an alteration in the number of revertants in the Ames test. No co-mutagenic effect of any magnetic field combination was observed.

Takashima *et al*. (152) also examined the genotoxic effects of moderate to ultrastrong SMF (14 T or less for 24 h) on a DNA-repair defective mutant of *D. melanogaster* using SMART (148). A dose-response relationship was found between magnetic flux density ranging 0.5-2 T and somatic recombination rate but the linear increase was saturated above 2 T.

5.3. Strong SMF effects on RNS

For the clinical safety aspect of MRI, Sirmatel *et al*. (153) examined that plasma NO production immediately after 30 min-exposure to a 1.5 T SMF in an experimental group, comprising of healthy young male volunteers. The results indicated that endogenous NO concentrations in post-exposed samples were higher than those in pre-exposed samples. It is assumed that a possible mechanism of the SMF influence might be attributed to Ca^{2+} flux and subsequently to the activation of NOS/NO oscillation pathway related to calcium/calmodulin.

5.4. Summary of strong SMF effects on FRR

There are several reports that strong SMF effects play significant roles in the endogenous and exogenous ROS generation (Table 3). In contrast, only one report on the endogenous RNS (NO) generation in biological systems has been described (Table 4). However, there are safety concerns about the mechanisms suggesting that SMF might induce potentiation of endogenous ROS/RNS-induced apoptosis and/or necrosis. As for the physical effects of gradient SMF (magnetic forces), it has been reported that a gradient SMF with the maximum intensity of 8 T (the "force product" of the magnetic flux density and its gradient was 400 T^2/m) affected the dynamic movement of paramagnetic oxygen bubbles and restrained the evaporation of dissolved oxygen molecules (molar magnetic susceptibility = 3449×10^{-6} cm^3 mol^{-1}) from a reaction mixture such as a decomposition of H_2O_2 (154). Consequently, the dissolved oxygen levels in solution might be increased. Moreover, it has been recently reported that greater enhancement of chemical reaction rate occurs in solution resulting from the strong magnetic force attracting oxygen molecules in the air (the respective maximum values of magnetic flux density and the "force product" of the magnetic flux density and its gradient were 0.63 T and 44 T^2/m when the solution depth was less than 2.6 mm and the duration of exposure was more than 150 min) (155). This effect is considered to be further enhanced in aqueous solutions containing paramagnetic metallic complexes such as stable Cu (II) complexes and heme Fe (III) complexes (156). Therefore, in examining the effects of strong gradient SMF on FRR, the magnetic force (or force product) acting on the oxygen molecules should be considered.

Table 3. Summary of strong-intensity SMF effects on ROS

Authors	Exposure Conditions	Results
Satoh *et al.* (134)	4.7 T; 6-48 h; mice (liver function)	I[3]
Satoh *et al.* (134)	4.7 T + CCl_4; 24 h; mice (liver function)	I[3]
Watanabe *et al.* (135)	4.7 T; 3-48 h; mice (liver function)	I[3]
Watanabe *et al.* (135)	4.7 T + CCl_4; 24 h; mice (liver function)	I[3]
Suzuki *et al.* (136)	3 T; 48-72 h; mice (bone marrow cells)	I[3]
Suzuki *et al.* (136)	4.7 T; 24-72 h; mice (bone marrow cells)	I[3]
Okonogi *et al.* (137)	4.7 T + mitomycin C; 6 h; Chinese hamster lung (CHL)/IU cells	I[3]
Sabo *et al.* (139)	1 T; 72 h; human HL-60 cells	I[3]
Sabo *et al.* (139)	1 T + anti-neoplastic agents; 72 h; HL-60 cells	I[3]
Nakahara *et al.* (144)	10 T + X-ray (4 Gy); 4 d; Chinese hamster ovary (CHO)-K1 cells	I[3]
Aldinucci *et al.* (145)	4.75 T; 1 h; human Jurkat cells	I[3]
Onodera *et al.* (146)	10 T + PHA[1]; 3 h; human PBMC[2]	I[3]
Zhang *et al.* (41)	5 & 9 T; 24 h; *Escherichia coli* (mutant strain)	I[3]
Koana *et al.* (148)	5 T; 24 h; *Drosophila melanogaster* (mutant strain)	I[3]
Ikehata *et al.* (150)	2 & 5 T + mutagenic agents; 48 h; *E. coli* (mutant strain)	I[3]
Takashima *et al.* (152)	0.5-14 T; 24 h; *D. melanogaster* (mutant strain)	I[3]
Satoh *et al.* (134)	3 T; 1-48 h; mice (liver function)	NC[4]
Satoh *et al.* (134)	4.7 T; 1-3 h; mice (liver function)	NC[4]
Watanabe *et al.* (135)	3 T; 1-48 h; mice (liver function)	NC[4]
Watanabe *et al.* (135)	4.7 T; 1 h; mice (liver function)	NC[4]
Suzuki *et al.* (136)	2 T; 24-72 h; mice (bone marrow cells)	NC[4]
Suzuki *et al.* (136)	3 T; 24 h; mice (bone marrow cells)	NC[4]
Suzuki *et al.* (136)	4.7 T; 1-6 h; mice (bone marrow cells)	NC[4]
Nakahara *et al.* (144)	1 & 10 T; 4 d; CHO-K1 cells	NC[4]
Nakahara *et al.* (144)	1 T + X-ray (1-4 Gy); 4 d; CHO-K1 cells	NC[4]
Nakahara *et al.* (144)	10 T + X-ray (1-2 Gy); 4 d; CHO-K1 cells	NC[4]
Aldinucci *et al.* (145)	4.75 T; 1 h; human PBMC[2]	NC[4]
Aldinucci *et al.* (145)	4.75 T + PHA[1]; 1 h; human PBMC[2]	NC[4]
Aldinucci *et al.* (145)	4.75 T + PHA[1]; 1 h; human Jurkat cells	NC[4]
Onodera *et al.* (146)	10 T; 3 h; human PBMC[2]	NC[4]
Koana *et al.* (148)	5 T + vitamine E; 24 h; *D. melanogaster* (mutant strain)	NC[4]
Ikehata *et al.* (150)	2 & 5 T; 48 h; *E. coli* & *Salmonella typhimurium* (mutant strain)	NC[4]
Schreiber *et al.* (151)	1.5 T; 1-24 h; *S. typhimurium* (wild-type strain)	NC[4]
Schreiber *et al.* (151)	7.2 T; 1 h; *S. typhimurium* (wild-type strain)	NC[4]
Schreiber *et al.* (151)	1.5 & 7.2 T + mutagenic agents; 1 h; *S. typhimurium* (wild-type strain)	NC[4]

Abbreviations: [1]PHA, phytohemagglutinin; [2]PBMC, peripheral blood mononuclear cells; [3]I, increased; [4]NC, not changed.

Table 4. Summary of strong-intensity SMF effects on RNS

Authors	Exposure Conditions	Results
Sirmatel *et al.* (153)	1.5 T; 30 min; healthy humans	I[1]

Abbreviations: [1]I, increased.

6. PERSPECTIVES

Many important issues remain to be elucidated regarding the dosimetry that could exhibit significant effects on the FRR. It has been shown that it is more appropriate to consider biological responses to SMF through the hypothesis of intensity windows, instead of intensity-response dependence (157). Furthermore, it has been reported that the gradient component of SMF at the target site might be responsible for the physiological responses *in vivo* (120, 128-130, 158-159), since the *in vitro* effects of gradient fields on action potential generation (160-161), myosin phosphorylation (162) and arteriogenesis (163-165) have been found using a North-seeking magnet or a quadrupole magnet (an alternating combination of North and South pole) mostly in the absolute field gradient range of more than 1 mT/mm (1 T/m) in the target tissues or cells. However, this hypothesis has not been proven, and the effects and underlying mechanisms remain elusive. In particular, to reveal and clarify the effects and mechanisms of spatial magnetic flux gradient, it is necessary to carry out the experiments comparing the spatially homogeneous SMF (generating magnetic torques) and inhomogeneous SMF (generating magnetic forces). In addition, reversibility or irreversibility of induced effects should be clarified.

Concerning the biochemistry of ROS/RNS in ionizing radiation such as gamma-ray and X-ray, it has been proposed as a signal transduction pathway that radiation-induced ROS are the initiators and that NO or derivatives are the effectors activating the signal

transduction pathway (166). However, in non-ionizing radiation such as SMF and EMF, the radiation-induced signal transduction mechanisms between ROS and RNS have not been clarified.

There has been little research to date on the SMF effects on mitochondrial ATP synthesis and membrane potential through ROS. The SMF effects on the mechanisms of ATP synthesis by mitochondrial oxidative phosphorylation system would be a fascinating and promising area for future research because the source of energy of ATP synthesis is the transmembrane electrochemical potential of protons created by primarily at the expense of downhill electron transfer in mitochondrial respiratory chain.

Recently, state-of-the-art superconducting quantum interference device (SQUID) magnetometry for detection of endogenous magnetic fields, nuclear forward scattering (NFS), and synchrotron X-ray fluorescence imaging have been employed to evaluate which specific metal ion compounds are present in specific neurodegenerative diseases and to map them to structures in the tissue (167-170). The early results of these studies indicate that several metal iron compounds are associated with specific diseases.

In addition, electron paramagnetic resonance (EPR) or electron spin resonance (ESR) spectroscopy is an extremely powerful tool for direct detection and quantification of free radicals (171-172). An introduction to EPR/ESR spectroscopy has been well documented. It enables direct detection and quantification of free radicals in biological systems, *in vitro* and *in vivo*. It also offers the advantage to detect both the radicals and intermediates involved in the reaction. In addition to being a valuable tool for characterization of the radicals, the EPR/ESR technique is also useful for examining tissue metabolism, oxygenation, redox state, perfusion and so forth. This has led to increasingly widespread applications of the technique to experimental animal studies in physiology, pharmacology and pathophysiology. In addition, in the studies on chlorophyll from photosynthetic bacteria to plant cells, the recent advances in EPR/ESR spectroscopy on electron transfer processes in chlorophyll photosynthesis has been reviewed (173-174). It is expecting that the effects of SMF on electron transfer in photosynthetic reaction centers could be understood. Moreover, an EPR study on the radical pair mechanism on the possible basis of a magnetoreceptor has recently been undertaken (175). Although the EPR/ESR technology is by far limited to non-clinical applications, current developments in molecular probes and instrumentation may enable potential clinical applications of EPR/ESR. Among the types of measurements that are clinically important, measurement of tissue oxygenation (EPR/ESR oximetry) represents one of the most feasible applications. Depending on its species, ROS and RNS have a different reactivity and lifetime, and therefore they could play different roles in neurodegenerative and cardiovascular diseases. It is anticipated that clinical applications of EPR/ESR could identify its species in each disease.

7. CONCLUSIONS

The findings presented in this review suggest that FRR are modulated by the SMF of different intensity ranges and duration of exposures: many with excitatory effects, some with inhibitory action and others with none. The SMF could influence (increase/decrease/maintain) the endogenous and exogenous ROS/RNS in *in vivo*, *in vitro* and cell-free systems. However, reversibility or irreversibility of induced effects has little been investigated and reported. The recent data related to the mechanisms of action of SMF on FRR are diverse and multifold but elusive. The proposed mechanisms of the magnetic sense have a missing link in the pathway: FRR, the neural processing of the magnetic receptors, transducers and behavioral outputs. Given the importance of electron transfer processes in the energy metabolism of cells, it will be of great interest to examine the effects/mechanisms of the SMF on ROS in mitochondria and chlorophyll. Moreover, the SMF effects on FRR is extremely important when considering human health, and the relation of immunological and neurodegenerative diseases, and stress response. However, the SMF effects on the pathogenesis and clinical relevance via ROS/RNS have not been resolved. The underlying mechanisms of the modulation of ROS/RNS induced by the SMF have not been clarified, although the relationship between the oxidative stress and hypertension and/or ischemia-reperfusion injury has been implicated. Further studies are necessary to explore the mechanisms of the SMF action on FRR in more detail. From these findings, it seems plausible that our understanding of the SMF effects on FRR will continue to grow as a focus of increasing attention and importance.

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Abbreviations: SMF, static magnetic fields; EMF, electromagnetic fields; T, tesla; ROS, reactive oxygen species; RNS, reactive nitrogen species; FRR, free radical reactions; NO, nitric oxide; NOS, nitric oxide synthase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine transaminase; HRP, horseradish peroxidase; MDA, malondialdehyde; 8-oxo-dG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; SOD, superoxide dismutase; GSH, glutathione; GPX, glutathione peroxidase; CAT, catalase; TBARS, thiobarbituric acid reactive substance; MT, metallothionein; SHR, spontaneously hypertensive rats; HSP, heat shock protein; PHA, phytohaemagglutinin; PBMC, peripheral blood mononuclear cells; MRI, magnetic resonance imaging; EPR, electron paramagnetic resonance; ESR, electron spin resonance

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