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Bio-effects of non-ionizing electromagnetic fields in context of cancer therapy

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1. ABSTRACT

Bio-effects mediated by non-ionizing electromagnetic fields (EMF) have become a hot topic of research in the last decades. This interest has been triggered by a growing public concern about the rapid expansion of telecommunication devices and possible consequences of their use on human health. Despite a feasibility study of potential negative impacts, the therapeutic advantages of EMF could be effectively harnessed for the treatment of cancer and other diseases. This review aims to examine recent findings relating to the mechanisms of action underlying the bio-effects induced by non-ionizing EMF. The potential of non-thermal and thermal effects is discussed in the context of possible applications for the induction of apoptosis, formation of reactive oxygen species, and increase of membrane permeability in malignant cells. A special emphasis has been put on the combination of EMF with magnetic nano-particles and ultrasound for cancer treatment. The review encompasses both human and animal studies.

2. INTRODUCTION

Over the last few decades, researchers started to pay more attention to the bio-effects induced by EMF. The growth of interest in this area has been influenced by public concern about the possible negative impact of technologies employed for telecommunication and mobile telephones (1-7). The term non-ionizing 'electromagnetic fields' encompasses a broad spectrum of frequencies of electromagnetic waves between 3 kHz and 300 GHz. In this review the abbreviation 'EMF' strictly refers to non-ionizing electric and/or magnetic fields.

The last two centuries are characterized by an extensive research and rapid expansion of EMF applications for various commercial and technological needs. Some of them have been already successfully implemented in health care as a basis for diagnostic and therapeutic modalities. However some frequencies classically reserved for technological applications are still waiting for exploration and further clinical use. Research has already begun in this area. The feasibility of use of EMF for treating of various pathologies has been discovered in many *in vivo* and *in vitro* studies. Some studies exploited mainly the electric component of EMF (electroporation (8); electro-chemotherapy (9, 10); pulsed electric fields (11)), while others have focused on the magnetic features of EMF (static and pulsed magnetic fields of different frequencies and powers) (12, 13). The magnetic aspect has been routinely utilized to induce thermal effect with assistance of metal-containing nanoparticles (14-16).

3. NON-THERMAL BIO-EFFECTS INDUCED BY ELECTROMAGNETIC FIELDS

3.1. Possible mechanisms of action

In spite of numerous studies up to date, the precise mechanism underlying the non-thermal bio-effects mediated by EMF remains vague. First of all, it concerns the impact of EMF at genetic level and its consequences for all cellular components. Unlike other physical modalities, such as ultrasound, EMF is unable to provide sufficient thermal or mechanical energy to directly trigger the breakage of the DNA molecular structure (17). Because of this, possible DNA damage has been linked to the induction of free radicals and oxidative stress. This assumption has been supported by various experimental studies (18-22).

Lai and Singh hypothesized that the increase of production of free radicals caused by EMF might be associated with the Fenton reaction (5).This phenomenon was discovered by Henry John Horstman Fenton (1894), and it is directly related to unbound iron inside cells. According to the classic theory, the iron ion can catalyze hydrogen peroxide decomposition with the generation of hydroxyl radicals (23) (Eq.1):

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

The reaction is highly pH-dependent and under normal physiological conditions the reduction potential of Fe^{3+} to Fe^{2+} is 772 mV (23, 24). It should be noted that iron maintains a high catalytic activity despite its predisposition to actively form chelate complexes in organic environment (23). As a result of the Fenton reaction, hydrogen peroxide is reduced to hydroxyl-free radicals by glutathione peroxidase and catalase, where iron plays a pivotal role. The formed free radicals can cause the DNA double-strand breaks, which can lead to accumulation of mutations due to inaccurate repair of damaged sites (25), and also to apoptosis (26-29).

There are reports indicating an abundance of iron in cancer cells and its cardinal role in formation of hydroxyl radicals and promotion of tumor growth (30-35). In order to harness this phenomenon for clinical purposes, researchers have been attempting to develop new strategies (36-39). As of today, most of the proposed iron-based therapeutic concepts are oriented on chemical or genetic approaches. However, in this context, the potential of bio-physical modalities such as EMF has not yet been fully explored. First of all, it relates to the ability of EMF to directly influence intracellular iron, thus providing an opportunity for precise targeting and selective destruction of tumors at different locations.

Another possible therapeutic strategy is targeting iron-containing cellular structural elements such as holotransferrin. In some studies, it has been experimentally demonstrated that EMF (60 Hz) affects an expression of human transferrin receptors, which play a crucial role in iron homeostasis in an organism (40-42). Apart from transferrin, other metal-proteins (cytochromes) and metal-enzymes (catalases) might be a subject of interest for future electromagnetic applications (43, 44).

All aforementioned discoveries provide a foundation for possible clinical implementation of EMF-based therapies. On the other hand, there are reports about the negative effects of EMF on DNA and cellular structures (5, 45-47). It was revealed that EMF can directly affect hydrogen bonds of DNA thus disturbing the DNA structure (48). As mentioned above, the production of free radicals and stimulation of oxidative stress is feasible under the application of extremely-low EMF (ELF EMF) (49-53). Thus the genotoxic potential of EMF must be considered in the context of possible therapeutic applications.

3.2 Potential for permeabilization of cellular membrane and drug delivery

Electroporation is an established technique for the permeabilization of cells (54-57). Current review is more focused on recent insights on application of ELF magnetic and radio-wave components of EMF rather than on electrical conductivity. In this context, the work of Stratton and colleagues deserves special attention (58). The researchers used acute monocytic leukemic THP-1 cells (AML-M5) as cell model in order to investigate the response of cellular membrane to applied alternating current, pulsed ELF EMF (0.3 µT at 10 Hz, 6 V AC). To quantify the cellular damage, researchers utilized versatile bio-physical and bio-chemical analytic techniques such as flow cytometry, calcium chelation, detection of intracellular calcium and transmission electron microscopy. The results revealed that ELF EMF has a capacity to induce disruption of plasma membrane. Stratton *et al* assumed that the underlying mechanism could be linked to the formation of areas of low lipid density at the membrane due to re-alignment of charged phospholipid groups (58). In turn, it leads to the creation of pores in the plasma membrane and consequently to the influx of molecules from the extracellular space.

The deliberate poration of plasma membrane has been a basis for introduction of therapeutic and genetic compounds into the cytoplasm and nucleus. The mechanism involved in EMF induced membrane permeabilization completely differs from other non-viral methods of membrane poration such as microinjection (59-61), biolistics (62, 63), photoporation (64, 65), and sonoporation. (66-69). Most of those approaches involve the mechanical breakage of cellular membrane due to applied external forces. The disruption of the membrane can result in either resealing the damaged site and survival of the cell or it dies due to the launching of an apoptotic reaction. In this sense, the membrane poration by means of EMF offers a method of drug delivery that does not cause a trauma to the cell.

The fundamental mechanism of EMF mediated molecular uptake has some similarities with the well-known technique of 'electroporation' (70, 71). The term 'electroporation' refers to re-arrangement of membrane lipids and formation of pores under applied electrical pulses. In the case of EMF, the main driving force of re-arrangement of phospholipid groups is the magnetic field, and direct application of electric pulses is not required. Thus the permeabilization of the plasma membrane by means of EMF is, by its nature, completely non-invasive. This feature significantly extends its clinical application for gene and drug delivery. However further extensive research in this area is ultimately needed.

3.3. Feasibility of modulation of apoptosis

The non-invasive modulation of apoptotic reactions is an area of great potential in cancer management. Apoptosis ('programmed cell death') is a natural process occurring in cells and it plays a fundamental role in maintaining and regulating vital functions of an organism (72-75). According to current knowledge and understanding of this phenomenon, apoptosis has two signaling cascades: intrinsic (mitochondrial) and extrinsic pathways (76, 77). The intrinsic pathway is linked to permeabilization of mitochondrial membrane resulting in escape of cytochrome C from mitochondria. It triggers a cascade of reactions, including the binding of cytochrome C to apoptotic protease activating factor 1 (Apaf-1), activation of the caspase chain and consequently leading to the breakdown of the nucleus (77-79). The extrinsic pathway relies on the ligation of Tumor Necrosis Factor (TNF) death receptors on the surface of cellular membrane with Fas ligand and the formation of a signaling complex containing Fas-Associated Death Domain protein (FADD). It triggers an activation of the caspase family (8, 3, 6 and 7), and finally nuclear cleavage (77, 80). Both pathways could be triggered by chemical, biological or physical stimuli (81-83).

In malignant cells, the apoptosis process is suppressed or deregulated, which promotes uncontrolled tumor growth and resistance to anti-cancer therapy (83). Restoring apoptotic reactions may amend the effect of traditional chemotherapy, making tumor cells more susceptible to pharmacological agents.

In this context, EMF provides an exciting opportunity for the targeting of the apoptotic pathways in a controllable, non-invasive and reliable manner. This might be achieved by the exploiting the magnetic component of EMF and the new generation of iron-based nano-carriers. Recently, Cho and colleagues conducted a study, in which they used magnetic nanoparticles (zinc-doped iron oxide) conjugated with antibody for death receptor 4 (DR4), which acted as a 'magnetic switch' for the induction of apoptosis in colon cancer cells (84). The magnetic nanoparticles were bound to death-inducing signaling complex (DISC) containing the FADD, which launched the cascade of apoptotic reactions involving the activation of caspase 8 and caspase 3 therefore following the extrinsic apoptosis pathways. Apart from *in vitro* studies, the authors conducted experiments on animal models (zebra fish) in order to validate the effectiveness of induction of apoptosis by magnetic nanoparticles. The choice of zebra fish was dictated by the genetic similarity of zebra fish ovarian TNF receptor to human DR4 receptor (84). It was found that embryo development of fish subjected to magnetic field (0.5 Tesla) was significantly disturbed. Those findings demonstrated a potential of magnetic fields to be employed for apoptosis induction and may have an application for the treatment of various pathologies, including cancer.

The last decade has been characterized by extensive research addressing the induction of apoptosis by means of pharmacological compounds. Therapeutic agents were found to be able to induce or suppress apoptotic events in the cells by targeting apoptosis pathways (80, 83). Some agents, such as monoclonal antibodies agonist to Dr4 and Dr5 and all trans retinoic acid (ATRA), are active in the induction of an extrinsic pathway such as tumor necrosis factor-related apoptosis-inducing ligand receptor (TRAIL), while others such as arsenic trioxide, lonidamine and antisense Bcl-XL, Bak, Bax have been targeting the intrinsic (mitochondrial) pathway (80). There is also a range of agents that target the modulators of apoptosis pathways, e.g., proteasome inhibitors (bortezomib), NFnB inhibitor, and mTOR inhibitors. Most of these agents are currently in clinical trials or under development.

Taking into account the high therapeutic potential of the above-mentioned agents in cancer management, it would be worthwhile to investigate the effect of their combined use with bio-physical modalities such as EMF. Some attempts have been already made for other non-invasive and non-ionizing physical methods such as ultrasound (85), but to date only a few studies have been carried out on EMF.

4. THERMAL BIO-EFFECTS CAUSED BY ELECTROMAGNETIC FIELDS: APPLICATIONS FOR CANCER THERAPY

The thermal bio-effects induced by EMF have been mainly associated with microwave radiation, where the energy has been absorbed and distributed by tissues resulting in heat production. The elevation of temperature has a huge potential for clinical implementation, in particular for tumor elimination. However, it must be noted that the thermal effect depends on many factors such as the parameters of applied EMF, the duration of exposure, and the compensatory mechanisms of the tissue. In order to increase the efficiency and safety of thermal therapy, the researchers started paying close attention to exploiting magnetic nanoparticles. It has been demonstrated that the utilizing of nanoparticles in combination with EMF allows an effective destruction of malignant cells and provides a platform for drug and gene delivery (15, 86-91). Various types of nano-carriers such as magnetic microsphere-methotrexate conjugates (92), micro-particles containing metallic iron and activated carbon (93), and magnetoliposomes have been proposed (94). Although all of them vary in size, structure, magnetic and thermal properties the application of magnetic nanoparticles mostly relies on generation of heat upon exposure to EMF, thus providing a basis for developing thermal bio-effects and release of loaded substances to the tissues. Apart from the potential for drug delivery, magnetic particles can facilitate target-navigation by means of Magnetic Resonance Imaging (MRI).

The thermal effects of EMF enhanced by iron-containing carriers have attracted interest among oncologists and biophysicists in the last decades. Giustini *et al* have exploited iron oxide magnetic nanoparticles (MNP) in combination with alternating magnetic field (169 kHz) to validate their radio-sensitization potential for cancer treatment (95). It was revealed that the coupling of magnetic field with MNP provided the optimal level of tumor regrowth compared with their solitary applications and micro-wave induced hyperthermia.

The organ targeting of nanoparticles could be significantly improved by combining with targeting ligands. Derfus and co-workers proposed the use of superparamagnetic nanoparticles conjugated to 30 bp DNA with oligonucleotides assembled on the surface for remote activation (96). In their study, the molecular release was triggered by dissociation of DNA oligonucleotides under EMF (400 kHz) induced heating.

Another promising area, which deserves attention and extensive research, is the incorporation of targeting ligands to the magnetic nano-carriers for binding to various factors on the tumor surface such as Fibroblast Growth Factor (FGF) or the family of TNF's. This method would provide a high concentration of therapeutic agents in the desired location. It has some similarities with the utilization of the new generation of ultrasound micro-bubbles for drug/gene delivery (97-100). However it should be mentioned that the ultrasound drug delivery systems suffer from the same issues as magnetic nano-carriers such as problems of controlling of size at fabrication stage, *in vivo* stability, biocompatibility, and bio-distribution.

Despite the challenges, which are mainly related to the development phase, magnetic nano-carriers provide promising material for the development of a new multi-functional platform for diagnostics, drug delivery, modulation of apoptosis, image-guided targeting, and tumor ablation.

Historically hyperthermia has been considered as a standard method for apoptosis induction (101). In this perspective, the hyperthermic potential of EMF could open a new avenue in the targeted activation of apoptosis in tumor tissues. Apart from this, EMF provides the means for non-thermal modulation of apoptosis, particularly in conjunction with the use of pharmacological apoptotic inducers. Potentially these modulators could be incorporated into the magnetic nano-carriers. Once injected the carriers are activated by applying extracorporeal EMF, which would result in local release of apoptotic inducers. Such an approach can guarantee a synergistic effect and a lowering of the administrated dose.

At the same time, the potential of non-thermal induction of apoptosis by EMF should not be ignored. It has been shown that ELF EMF can non-invasively trigger apoptotic cascades without involvement of magnetic nanoparticles and high EM frequencies. Berg *et al* demonstrated the *in vitro* efficacy of ELF EMF and pulsed EMF (PEMF) in the induction of apoptosis, inhibition of angiogenesis, suppression of proliferation and direct death of cancer cells (102).

One of the latest technologies for the induction of apoptosis in cancer cells is Tumor Treating Fields (TTF) therapy, which has been proposed by Prof. Yoram Palti. This method exploits alternating electric fields with the frequency specific for the targeted cell type. At present, TTF is undergoing extensive studies, and the first results are promising.

5. FUTURE DIRECTIONS

The unique bio-physical features of EMF provide a mechanism for its combined use with chemical, biological and physical methods for treating cancer and other diseases. Both thermal and non-thermal bio-effects elicited by EMF could be effectively coupled with other non-invasive therapeutic modalities such as ionizing radiation, ultrasound, UV, photo-dynamic and laser therapies (7).

In this context, the combination of EMF and high intensity focussed ultrasound (HIFU) is an exciting direction of research, where synergistic properties of both modalities could be effectively utilized for tumor reduction, induction of apoptosis and remotely-activated drug delivery. HIFU has already proven its capability for non-invasive and precise eradication of tumors without damaging unaffected tissues (104-106). Apart from the induction of hyperthermic reaction, HIFU is able to cause the cavitation effect, which has been considered a driving force for ultrasound-assisted drug and gene delivery (67, 106, 107). For the latter, the exploitation of ultrasound contrast agents with paramagnetic properties might provide a new platform for cancer management. Stride and co-workers have already demonstrated the usefulness of such a strategy for gene delivery (108), where phospholipid micro-bubbles containing magnetic nanoparticles were synthesised. These micro-bubbles were found to be more effective compared with phospholipid micro-bubbles and micelles containing magnetic nanoparticles.

Double stranded DNA chain, where one chain is bonded to magnetic particle, and another is linked to therapeutic molecule, could be incorporated into the phospholipid micro-bubbles. This would protect the drug from immune arresting and prolong its lifetime in the circulation system. In addition, it provides visualization either by using MRI or ultrasound. After detection on the target zone, the micro-bubbles could be subjected to an external magnetic or acoustic field in order to elevate local temperature. It would result in disruption of hydrogen bonding between two DNA strands and the release of therapeutic molecule. In order to enhance site specific gene delivery, the phospholipid micro-bubbles could be equipped with targeting ligands on their surface. This approach significantly broadens the EMF-associated applications for various clinical situations, where gene therapy is needed.

It must be noted that the EMF-based anticancer therapy can be utilized in other areas, where other physical modalities cannot provide an appropriate level of safety and effectiveness. For example, the treatment of brain tumors by means of ultrasound and laser therapy requires direct access to the affected brain tissues, because the skull acts as an impenetrable barrier for any type of waves except electromagnetic ones. Although the researchers have been trying to overcome this obstacle by developing multi-array ultrasonic transducers, there are still problems with the heating effect and necessity for total depilation. In this regard, EMF offers a unique opportunity to non-invasively suppress tumor growth by induction of apoptosis, site-specific delivery of chemotherapeutic agents and the direct ablation of malignant cells.

Apart from these features, EMF has potential to inhibit the growth of cancer cells in a highly selective manner without damaging normal tissues. Recent studies conducted by Zimmerman *et al* have provided experimental proof that a very low level of amplitude-modulated EMF is able to suppress the growth of hepatocellular carcinoma and breast cancer cells (109). This conforms to the results of another study conducted on patients (single-group, open-label, phase I/II) by Costa *et al* (110). The researchers noted the anti-tumor efficacy of amplitude-modulated EMF in treatment of hepatocellular carcinoma.

The sensitivity of different types of tumor to specific EM frequencies is a crucial factor for effective anti-cancer therapy. In this context, the work of Barbault *et al* deserves special attention (111). In the study, 1524 types of EM frequencies (from 0.1 Hz up to 114 kHz) were scrutinized in order to determine tumor specific frequencies on patients diagnosed with various types of cancer, including colorectal cancer, hepatocellular carcinoma, breast cancer and others. It was revealed that 77.6 % of used frequencies were tumor-specific. The application of EMF was not accompanied by reports of adverse effects.

To summarize, EMF-based technology possesses all the features to become a new therapeutic modality. It particularly concerns the use of a new generation of magnetic nano-carriers for both diagnostic and therapeutic purposes. In the initial phase of disease, the application of these agents equipped with specific targeting ligands would significantly facilitate the imaging of affected tissues by means of MRI. At the same time, the nano-carriers might be loaded with anti-cancer drugs or apoptotic modulators, which could be released under externally applied EMF or ultrasound. Beside the drug release, EMF provides a means for local hyperthermia, the stimulation of production of free radicals, induction of apoptosis, and direct damage DNA in cancerous cells. Moreover, EMF allows immediate post-treatment evaluation. Such an approach will allow the maximising of the therapeutic efficacy of anti-cancer drugs along with the decreasing probability of side effects.

Notably EMF provides a wide range of options for its use either alone or in combination with different pharmacological, genetic, and bio-physical modalities for the treatment of cancer and various disorders. However, further research for the optimization of treatment protocols is required.

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