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Nondrug Antimicrobial Techniques: Electromagnetic Fields and Photodynamic Therapy

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ABSTRACT

Developing nondrug antimicrobial and antibacterial treatment techniques are necessary because of the emergence of antibiotic resistance worldwide. Photodynamic therapy (PDT) and electromagnetic therapy (EMFT) are two examples of these approaches. Antimicrobial photodynamic therapy is a novel and promising technique that involves the simultaneous presence of visible light, oxygen and a photosensitizer (PS). It can be applied for eradicating pathogenic microorganisms such as Gram-positive and Gram-negative bacteria, yeasts and fungi. Moreover, electric fields, magnetic fields and pulsed EMFs (PEMFs) are common approaches showing promising antimicrobial effects. These treatments can be used as alternative or adjunctive treatment for some infections. This paper reviews the recent developments and basic principles of nondrug antimicrobial techniques focusing on EMFs and PDT techniques. The future perspectives of these techniques as well as clinical considerations are discussed.

Key words: Photodynamic Therapy, Electromagnetic Fields, Antimicrobial Effect.

INTRODUCTION

One of the most worrying threats to public health is spread of multi-resistant infections[1]. Several antimicrobial treatments are starting to be considered as a promising alternative technique to resistant infections[2, 3]. Photodynamic therapy and electromagnetic therapy are two examples of these approaches. Antimicrobial PDT is particularly useful for dental[4] and dermatological[5] applications. Three main components are involved in this technique include photosensitizer (PS), light with appropriate wavelength and oxygen. This PS should be able to produce reactive oxygen species (ROS) in the presence of light and oxygen[6]. The formation of ROS as a consequence of PDT follows two main pathways characterized by different photochemical mechanisms called “type I” and “type II”. Type I mechanism will let to superoxide anion that can go on to create more reactive ROS such as hydroxyl radicals whereas, Type II will generate singlet oxygen. Microbial cell death is related to disruption of proteins. Gram-negative bacteria, superficial fungal and Helicobacter infection can be removed by PDT[7, 8].

Electromagnetic fields (EMFs) have therapeutic effects for a wide variety of diseases including tumors[9], musculoskeletal diseases[10] neurological disorders[11] and wounds[12]. Today there are several fields for biological interactions of EMFs. Electromagnetic (EM) waves are time varying electric and MFs that have different frequencies and the biological effects vary with frequency. The most energetic ‘ionizing radiation’, such as cosmic and X-rays (1018-1022 Hz) damage cells and even much lower frequencies of ultraviolet (1016 Hz) waves can damage skin. Lower frequency waves are ‘non-ionizing’, but microwaves (109 -1011 Hz) that cook foods obviously are harmful to the living organisms. Several factors such as the features of

bacterial metabolism, intensity of irradiation and combination of growth and irradiation media have strong impact on these effects[13-15]. This paper is divided in two parts. In the first part, the applications of PDT are described. The characterization and applications of EMFFs are reported in second part.

Photodynamic therapy

Background of anti-microbial photodynamic therapy

Light has been employed to treat various diseases such as psoriasis, vitiligo and cancer for more than three thousand years[16-18] the concept of 'phototherapy' has been developed at the end of the nineteenth century by Niels Finsen. He discovered that red-light exposure is useful to treat smallpox pustules disease. in 1903 he won the Nobel Prize for his finding[19].

More than 100 years ago, the researchers have demonstrated cell death can be induced when the light is combined with certain chemical compounds. In the 20th century, Oscar Raab reported the certain wavelengths of light in the presence of acridine have toxic effect against Paramecia caudatum[17].

In 1903, Tappeiner reported that the heat, is not responsible for inducing this toxic effect[20]. He introduced the term "photodynamic reaction" in 1904 [21]. The effect of oxygen on cell killing has been demonstrated by additional experiments. In these experiment in the absence of oxygen could not be observed[22].

Inactivation of microorganisms by Photodynamic is based on production of free radicals or singlet oxygen. In this phenomenon chemical agent known as a photosensitizer (PS) is excited by absorbing low doses of visible light at certain wavelength. In this process the toxic singlet oxygen are produced[23].

Since the middle of the last century anti-microbial photodynamic therapy has not attracted much attention due to the discovery of antibiotics. However, during recent decades, the level of antibiotic resistance has been increasingly raising worldwide which has highlighted the role of new nondrug antimicrobial methods[24-26].

Mechanism of Action of PDT

Photodynamic therapy has three main components including light, photosensitizer and oxygen. When a photosensitizer absorbs light of certain energy, it may undergo a transition from ground state to a higher energy triplet state. It may then lose its energy by two types of reactions. In type I reaction, photosensitizer can be transferred from the triplet state PS to a substrate. In this reaction free radicals and/or radical ions are generated[6]. These elements can react with other biomolecules and oxygen to yield Singlet-state oxygen. In type II reaction photosensitizer may back to the ground state molecular oxygen to generate excited singlet-state oxygen. Singlet-state oxygen is very reactive and in can induce cell damage and death in biomolecules such as proteins, nucleic acid and lipids. Both mechanisms can occur in the cell simultaneously, but the main pathway is type II. Microbial cell death is not related to DNA damage because the DNA repair systems protect the cell. The main cause of microbial cell death that induce by PDT is disruption of proteins[27].

Light Sources for Anti-microbial Photodynamic Therapy

The light source with sufficient intensity is essential for treatment of bacterial infectious diseases. During passage through the various skin layers the intensity of light is decreased because of attenuation[28, 29].

Several light sources such as coherent and incoherent light can be used for PDT. The absorption peak of PpIX as photosensitizer is 405 nm. Therefore it can be excited by Blue light with 405 nm. The wavelength of this light is relatively short; therefore the penetration of this light is confine. For deeper and thicker lesions the red light (635 nm) is applied. The last Q band is targeted by this light, because it does not excite PpIX as efficiently as blue light. The higher dose of red light is needed to induce same effect[30].

The fluence and irradiance are two important factors for efficient PDT. Dose of light source at 503 nm is 10 J/cm^2 and 100 for 635 nm. Moreover, to avoid reducing the efficiency the appropriate rate of fluence is essential duo to quickly consuming of oxygen. For this reason, about 30

minutes is needed for treatment with red light and treatment with blue light takes about 15 minutes[31].

Anti-microbial Effects of Photodynamic Therapy
Gram-negative Bacteria

Gram-negative bacteria are the cause of many infections especially in elderly people. The cell wall of these bacteria shows a low permeability because of their double lipid bilayer[32, 33]. Whereas, Gram-positive bacteria have a single lipid bilayer[34, 35]. This additional layer is responsible for their resistance to antibiotics. Therefore the prevalence of gram-negative bacteria is higher than Gram-positive bacteria in the modern hospital environment[36, 37]. The simple diffusion of PS into the cytosol of Gram-negative bacteria is confine. Therefore, the PDT of Gram-negative bacteria is more difficult than Gram-positive bacteria. The uptake of anionic and neutral PS is prevented due to their membrane structure[38, 39]. The permeability of cell membrane can be increased by PMBN or Tris/EDTA for performing PDT with non-cationic PS[40].

Superficial Fungal Infections

Infections by Candida albicans and other similar fungi are highly resistant to traditional antifungal agents such as fluconazole especially in immunocompromised patients[41].

According to in vitro studies, Candida species are sensitive to PDT with photofrin or the porphyrin precursor 5-aminolaevulinic as a photosensitizer. Therefore, this technique can be used for killing these cells[42, 43].

The first in vivo study has been performed by Teichert *et al*.where they have used diode laser to activate methylene blue to treat oral candidiasis[44]. n PDT process macrophages and neutrophil granulocytes are activated to kill cells[45].

Helicobacter Infection

The International Agency for Research on Cancer (IARC) and WHO reported that Infections by Helicobacter pylori were a causal link with gastric ulcer, chronic gastritis and gastric cancer. For gastric cancer the failure of treatment is increasing because of the drug resistance, side effects, and compliance and expense of therapy. Therefore, the eradication of Helicobacter pylori is very important issue[46].

Ganz and coworkers have delivered blue light to induce lethal damage in H. Pylori in regions of the gastric antrum. This study, conducted in ten patients, showed 91% of H. Pylori can be killed by blue light[47].

Electromagnetic Therapy
Electromagnetic Fields

Due to discovering great antimicrobial effect of EMF, in this section we aim to overview the effects of EMFs on bacteria. EMF can be divided into seven categories including: (1) Extremely low frequency (ELF)(0-300 Hz), utilized for biological applications; (2) very low frequency (300-30 KHz); (3) low middle frequency (30 KHz-30 MHz); (4) ultra high (30-300 MHz), used in radio and TV; (5) very high frequency (300 MHz-30 GHz), used in satellite communication; (6) extremely high frequency (30-300 GHz); (7) infrared (300 GHz-300THz); and visible light (429-750THz), used in light spectrum.

Along with the development in the application of EMF, the antibacterial influences of EMF in low intensity have been the focus of a host of studies which can be expressed into 2 categories including high frequency and low frequency.

High Frequency Low Intensity EMFs

Living cells and bacteria utilized a complex network of sensing and responding to physical and chemical factors for both communicate with each other and survive under various environmental conditions[48]. It was realized that applying electromagnetic irradiation (EMI) at extremely high frequency (30-300 GHz) with low intensity at specific frequencies.

(70-73GHz) can affect bacteria in the manner of energy transformation into informative signals. Some researchers reported the great potential of low intensity EMI of resonant frequencies leading to depressing effects on E. coli which is one of the great characterized bacteria[49-52].

These effects can be affected by some factors such as the features of bacterial metabolism , intensity of irradiation, the combination of growth

and irradiation media, and other factors[53-55]. Furthermore, these mentioned effects can adjust the interaction of living organs versus physical and chemical factors[14, 54]. Also, metabolic process or mechanical resonance caused to mutation in the growth cycle of bacteria[56, 57].

It was reported that applying EMF at specific frequencies (45-53 GHz and of 70-75 GHz) with low intensity can reduce the growth of *E. coli*[14, 52]. One of important mutual reaction of EMI with organisms is Genome targeting. However, at these frequencies, the induced energy cannot break a chemical chain in DNA. Scientists have found that EMF stimulating at these frequencies have great potential to produce oxygen radicals, or disorder process of DNA-repair processes[58]. The cell membrane has elastic forces in its wall. These forces will participate in coherent self-sustained oscillation which causes conformational transmission of macromolecules that are fed with metabolic energy. This process weakens oscillatory forces[48]. These forces have biological origin and they required ATP. Therefore, the proton F_0F_1-ATPase as the principal enzymatic complex of the bacteria's membrane has a significant effect in membranous mechanisms of applied EMI. Trchunian *et al*. (2000) found that the change in the oxidation-reduction potential (Eh) of the surface of bacteria had a considerable effect where bacteria can survive, particularly in the F0F1-ATPase adjustment[57]. In addition , water molecules at their resonant frequencies including 41.5, 51.8 and 53 Hz can mediate the effects of applied EMF on bacteria[59]. Similarly, some studies have reported inhibiting effect of EMF at resonant frequencies of water molecules (41.5, 51.8 and 53 Hz) on E. coli growth[14, 53, 54, 57]. The fluctuation of water molecules in this level can changes the protein composition and the degree of hydration and other properties of proteins[49, 51, 53, 55]. In this regard , Tadevosian *et al*. (2006) demonstrated that applying EMF at 70.6 and 76 GHz frequencies can affect the E. coli growth and properties of water molecules[53].

Extremely Low Frequency

Several studies have investigated the effects of ELF-EMFs on biological systems[60, 61]. The outcomes of ELF-EMF research are antithesis. However, there are little knowledge about the mechanisms of mutual reaction between ELF-EMF and organisms. In this context, some procedures, such as growth and protein synthesis, were employed to expressed the effect of ELF-EMFs on living organisms[62].ELF-EMF has two remarkable effects on bacteria: (1) effect associated with the applied fields; these fields should be spatially and temporally coherent as well as undisturbed by incoherent electric or magnetic noise[63]; ELF-EMFs are different in frequency, wave form and strength; a sharp "window" (i.e. a discrete combination of frequency and strength) can be used to produce a visible effect; (2) prokaryotes, which are intact organisms, completely functional, also may be more resistant as compared with cell cultures and as well as can compensate the EMF reduction

Protein Synthesis

Bacteria can produce stress proteins, for example induced by heat. It was reported that applying EMFs lead to considerable change in the protein pattern of *Proteus vulgaries* at 41°C whereas at 37 °C, applying EMF had no effect. EMF combined with the heat (41 °C) can produce distinct changes at pH 6. However, there was no effect by using E. coli and SDS-PAGE either at 37 °C or 43 °C. With respect to protein synthesis, this can maintain the temperature higher without producing change in protein pattern[64].

Scientists investigated the effects of ELF-EMF on the protein synthesis of eukaryotic cells[65, 66]. In this regard, Goodman *et al*(1993) revealed that applying sinusoidal Magnetic fields (MFs) at 72Hz can affect the E. coli protein synthesis[67]. Similarly, it was reported that PEMFs in vivo using highly sensitive electrophoresis affect the E. coli protein synthesis[68]. In another research, Kropinski *et al* (1994) showed that using 60 Hz sinusoidal MFs produce no effect on protein synthesis[69]. It can be said that only if heat is applied in addition to the MF, changes in bacterial protein pattern is appeared. It means that applying heat play a significant role in the combination act with ELF-EMFs. It seems that the physiological reaction of eukaryotic cells with applied heat are similar to those induced by ELF-EMF[67, 70].

Enzymatic Activity

Applying EMFs can affect the activity of membrane enzyme but their effects on Triton

solubilized disk membranes or on soluble isoforms of adenylate kinase is insignificant. Also, it was demonstrated that ELF-EMFs caused to little effects on the activities of soluble enzymes[71, 72];It means that the membrane have a significant role in mediating the effect of the ELF-EMF on the enzymatic activity. In this relation, several studies explained extraordinary results about the effects of ELF-EMF on biological membranes[73-76].

Morelli *et al* (2005) reported that applying ELF-EMFs at 75 Hz with the amplitudes above a threshold decreased the enzymatic activities of three membrane-bound enzymes including phosphoglycerate kinase, alkaline phosphates and acetyl cholinesterase from blood cell or from synaptosomes[77]. Falone *et al* (2007) demonstrated that applying ELF considerably increased the activities of glutathione S-transferase and glutathione peroxidase whereas treatment did not affect superoxide dismutase, catalase and glutathione reductase activities[78]**.**

Antioxidant Effects

Falone *et al* (2007) compared the grade of cellular vulnerability by using ELF-EMF with a well-characterized pro-oxidant treatment. Their results indicated that induced mortality of hydrogen peroxide in cells exposed and in control group are same. Nevertheless, long-term ELF-EMF exposure to the neuroblastoma cells significantly increased in the creation of ROS after H_2O_2 incubation. It was reported that co-treatment with the well known antioxidant N-acetylcysteine could revert this rise[78]. Thus, applying ELF-EMF may affect the free radicals production or increased the activity of the hydroxyl radicals produced by H_2O_2.

Growth Curve Assessments

Falone *et al* (2007) reported that ELF-EMF exposure could not affect the SH-SY5Y growth curve, while it increased the viability of SH-SY5Y[78].

Majority of the studies demonstrated no considerable effect of ELF-EMFs exposure on the growth of E. coli K12, the protein synthesis rate of E. coli B leu-3 and the luminescence of Photo bacterium phosphorus and photobacterium fischeri. It is important to say that EMFs per se cannot affect intact bacterial cultures. Indeed, if any effects were observed, they were so negligible. Some studies explained that using ELF-EMF caused to decrease of the growth of E. coli by a maximum of 3.8% [79, 80]. In this context, Mittenzwey *et al* (1996) concluded that bacteria are resistant to applying ELF-EMF. It may because of compensation and self-regulation[62]

CONCLUSION

This study has reviewed the most current techniques of EMFs and PDT in antimicrobial studies and mechanisms of actions of these methods. These approaches show the promising antibacterial effects. These techniques in appropriate parameters can be used for some bacterial and microbial pathogens as alternative and adjunctive treatment options. For establishing new EMFs and PDT based techniques for antimicrobial and antibacterial purposes further control studies should be performed.

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