THE EFFECT OF STATIC ELECTROMAGNETIC FIELD ON CEPHALOTHIN-RESISTANT *PSEUDOMONAS AEROGINOSA*

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Abstract

Pseudomonas aeroginosa is among the most common significant etiological agents of hospital-acquired diseases. Due to their multiple resistance against a variety of antibiotics, these bacteria pose serious threats especially in burned patients. Sometimes burned patients lose their lives due to multiple-resistant pseudomonal infections. In this project, efforts were made to resensitize cephalothin resistant *Pseudomonas aeroginosa* to this antibiotic by static electromagnetic field (SEMF). Sixty samples of cephalothin-resistant *Pseudomonas aeroginosa,* collected from the burn unit of Ahwaz Taleghani hospital, were subjected to 0.35 Tesla of SEMF with and without cephalothin. All the *Pseudomonas aeroginosa* strains became resensitized to 16µg/ml cephalothin. SEMF by itself did not exert any effect on this bacterium. If *in-vivo* experiments confirm this *in-vitro* results, SEMF could be used as a valuable tool for treatment of serious cutaneous pseudomonal infections in burned patients.

Keywords:

Static Electromagnetic Field (SEMF) Cephalothin-resistant *Pseudomonas aeroginosa*

Introduction

Multiple resistant strains of bacteria are rampant. Immediately after introduction of a new antibiotic, resistant strains of bacteria emerge, so that a stronger antibiotic is necessary for treatment of the communicable diseases(1).

Mycobacterium tuberculosis was once considered to be under control is now emerging as a major health threat again because of its resistance to administered antibiotics (2). In addition multiple resistant enterobacteriacea to antibiotics are frequently being reported (3).

Introduction of novel antibiotics has ended up with emergence of resistant strains. Among the resistant bacteria, *P. aeroginosa* is notorious for its capability to resist against many antibiotics (4). Especially in burn units this bacterium is a significant infectious agent.

The tag of war between human and infectious agents seems endless. To get out of this vicious cycle, novel approaches need to be devised. Static electromagnetic field (SEMF) seems a promising one for this purpose.

Materials & Methods

The SEMF apparatus and reagents:

The device for creating static electromagnetic field applied in our project was a home made apparatus, planned, developed and set up by my colleague in department of Biophysics,

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faculty of medicine, Ahwaz Jundishapur University of Medical Science. This apparatus is made up of a bobbin with a 20000 rounds and 0-24 Amp with dynamic poles of soft iron. The efficiency and precision of the apparatus was checked by a gauss-meter (Gaussmeter 410, Brokhaus Messtechnik, Germany). During application, the system can be adjusted for creation of different intensities of magnetic field by adjusting its ampre button.

The culture media used in this project were purchased from Oxoid Company, (UK) and all chemicals were purchased from Merck, (Germany). An eppendorf tube containing 1ml of bacterial suspension was placed in the middle of the magnetic field so that the field could cover all part of the tube unanimously.

Sample preparation

Sixty five samples of cephalothin-resistant *Pseudomona aeroginosa* strains were collected from the burn unit of Ahwaz Taleghani hospital. To check the species of each sample, O/F and carbohydrate test were performed for them as described (5). *Pseudomonas aeroginosa* are oxidase positive, produce pigments on Muller Hinton agar, grow well at 42ºC and reduce nitrate. Also they oxidise glucose, lactose and mannitol. Based on these criteria sixty samples out of 65 collected specimens were confirmed as *Pseudomonas aeroginosa* and were included in this study.

Cephalothin resistency of each bacterial strain was determined by Bauer-Kirby disk diffusion test. For this purpose a colony of bacteria was dissolved into 2-3ml of TBS containing 16µg/ml of cephalothin (Sigma lot no.20k0842). The concentration of the antibiotic was determined according to minimal inhibitory concentration of the cephalothin (6). The tubes were incubated at 37ºC till the turbidity reached to tube number 0.5 McFarland. A lown of bacteria from each tubes were spread on Muller-Hinton agar separately and a disk of cephalothin was placed on each inoculum and incubated at 37ºC overnight. If the diameter of zone of inhibition around the disk was less than 18mm, the strain was considered as a resistant strain (6).

To determine the effect of SEMF on the collected samples, a colony of each bacterial specimen was dissolved in 4-5 mls trypticase soy broth and incubated at 37˚C till the turbidity of the liquid media reached to the tube 0.5 McFarland set. An eppendorf tube of such suspension was subjected to SEMF.

SEMF Application

Practically in 4 groups of sterile eppendorf tubes, 1ml of the bacterial suspension (0.5 McFarland) was emptied. In tubes group 1, 16 µg/ml cephalothin was added and subjected to 0.35 Tesla of electromagnetic field for 15 minutes. This time and intensity of SEMF was the most effective condition and has been achived by a serial pilot test.

In pilot tests the standard strain of *Pseudomonas aeroginosa* (PTCC1074) provided from Iran Industrial and Scientific Research Organization, Tehran, was subjected to variable intensities of SEMF (3, 6, 12, 18, 24 and 30 Amp) and different duration (5, 10, 15, 20, 25 minutes).

Eppendorf tubes group 2 were treated as group 1 except that group 2 were not subjected to magnetic field but ambient environment. The third group contained only 1ml bacterial suspension without antibiotic but subjected to magnetic field (0.35 Tesla). Group 4 contained 1ml bacterial suspension without antibiotic and no subjection to magnetic field. Colony count was performed for 4 preparations of each sample. In each set of experiment the standard strain of *Pseudomonas aeroginosa* (PTCC 1074) resistant to cephalothin was treated as the same as collected hospital strains.

Statistical Analysis

The results of colony count were analyzed by ANOVA test for analysis of obtained colony counts, Tuky and HSD for determination of significant difference values between test and control groups.

Results

Static magnetic field by itself (in the absence of antibiotic) had no effect on *Pseudomonas aeroginosa* strains, but cephalothin-resistant bacterial suspension supplied with 16 μg/ml cephalothin and subjection to electromagnetic field duration reduced the biomas of bacteria to less than 1/6 of its original population (about 83.4% reduction).

Discussion

In recent years efforts have been made to apply static magnetic field for treatment of infectious diseases. Liang and his colleagues reported that the efficacy of Daunorubicin (an anticancer drug) enhances under SEMF (7). Therefore resistant bacteria could be susceptible to antibiotic by exposure of the patients to the SEMF.

Magnetic field affects on the ions of the environment and alters their directions. Cell milieu is full of variety of ions. In bacterial metabolic pathways, many ions emerge and consumed permanently. The emergence and consumption of ions are especially prominent during glycolysis, Krebs and cytochrome oxidase cycles.

It was assumed that because SEMF could change the direction of bacterial ions especially cytochrome oxidase ions, in turn this alteration of ion direction should lead to the death of the subjected bacterial suspension. But the results showed that the effect of static magnetic field (SEMF) on bacterial growth in the absence of antibiotics in their milieu is not similar for various bacterial species and strains of bacteria on one hand and the condition of magnetic field on the other hand. Kahno and his colleagues reported that static electromagnetic field does not impose any impact on the growth of *Staphylococcus aureus*, *Streptococcus mutans* and *E. coli* cultured under aerobic condition. However if *Staphylococcus aureus* and *Streptococcus mutans* were cultured anaerobically, SEMF would inhibit their growth considerably (8, 9). Lack of effect of SEMF on the growth of those bacteria in the absence of antibiotic was in full concordance with our results with *Pseudomonas aeroginosa*. But the strains of bacteria play a role in this effect. For instance, the growth of mutant *E. coli* K-12 (lon mutant) was reduced at a rate of 20% under SEMF (10). SEMF could inhibit the growth of *Mycobacterium tuberculosis* compeletely (11). In this bacterium SEMF blocks the enzymatic reaction of iron reduction (11). In another report, Zhang demonstrated that by regulation of the intensities of the SEMF versus the time of the exposure of bacterial samples, the growth of bacteria could be inhibited (12).

In our project all the 60 cephalothin resistant strains of *Pseudomonas aeroginosa* (of 65 samples) collected from burn unit were used for the experiment. SEMF by itself (0.5 Tesla for 15 minutes without antibiotic in bacterial suspension) did not affect the growth of the bacteria at all. But if the bacterial suspension was supplied with 16μg/ml cephalothin and subjected to the same condition of SEMF, the population of the bacteria was reduced to 1/6 of their original biomass (about 86% reduction). That is SEMF sensitized the bacteria to cephalothin. Our result was similar to Benson and colleague (13). They have performed their experiment with *Pseudomonas aeroginosa* and demonstrated that 0.0005-0.01Tesla of magnetic field enhances the activity of gentamycin against *P. aeroginosa.*

On possible mechanism of action of SEMF on the cells, is that SEMF alone does not

damage DNA (14, 15, 16), but simultaneous application of 2-5 Tesla of SEMF increases the mutagenicity of some substances such as N-methyl-N'-Nitro-N-nitrosoguanidine(ENNG), N-ethyl-N'-Nitro-N-nitrosoguanidine (ENNG),ethylmethanesulfonate (EMS), 4-nitroquinoline-N-oxide (4-NQO), 2-amino-3-methl-3H-imidazo[4,5-f]quinoline (IQ) or 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2). On the other hand, simultaneous exposure of other mutagenic substances like 2-aminoanthracene (2-AA), 9-aminoacridine (9-AA), N4-aminocytidine and 2-acetoamidofluorene (2-AAF) with SEMF did not elevate their mutagenic effects (17). Therefore SEMF could be considered as a probable co-mutagenic factor at least with some of the mutagenic substances.

In *Pseudomonas aeroginosa*, efflux pumps play a prominent role in multi-drug resistancy of this bacterium. In the efflux-mechanism of resistancy, a group of proteins named efflux proteins covalently link to accumulated antibiotics inside the cell milieu and propel the attached antibiotics outside the bacteria (18).

It is assumed that SEMF alters the directions of both the efflux carrier proteins and antibiotics so that the chemical attachment of these molecules does not occur, transfer of antibiotics to outside of the cell diminishes and the bacteria die off because of accumulation of antibiotic inside its milieu.

Further work is warranted to elucidate the efficacy of SEMF under clinical conditions on burn patients.

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