Effects of low Amperage Current on the Protein Pattern of Potential Pathogen

Tuhiram Dixit[1,*], Dr. Mukta Sharma[2] and Mayur Sharma[3]
[1,*]Technologist, Asian Institute of Medical Sciences, Faridabad, Haryana, India.
[2]Department of Microbiology, S.B.B. Dental College and Research Centre, Ghaziabad, U.P., India.
[3]Department of Microbiology, S.B.S.P.G.I., Dehradun, India.

Electrical current is known for bactericidal activity by influencing the inhibition of biological processes of organism at the cellular level. Electrical current has been reported to alleviate pain, probably by altering endorphin concentration, and speed up wound healing by increasing cell proliferation. Infection is a major clinical complication associated with the use of central venous catheters (CVCs). Treatment of these infections by antibiotics is relatively expensive. Not always successful and frequency necessitates catheter removal. Bacteria attached to devices such as biofilms are quite typical, resistant both to antimicrobial agent and to host defense. Despite the fact that endogenous bacteria are beneficial to the host in their natural habitat (e.g. prevent the overgrowth of opportunistic pathogens and colonization by antibiotic resistant bacteria), many of these micro-organism are potentially pathogenic.

Keywords: Inhibition, Endorphin concentration, Cell proliferation, Central Venous Catheters (CVCs), Antimicrobial agent, Endogenous bacteria.

1. INTRODUCTION

An infection is the detrimental colonization of a host by a foreign organism. In an infection, the infecting organism seeks to utilize the host´s resources to multiply usually at the expense of the host. A wound infection happens when microorganism enter to tissues through a break in the skin. Wound can be introduces through punctures, lacerations, incisions or burns. Bacterial infection caused by *Staphylococcus* species, *Corynebacterium* spp., *Brevibacterium* spp. and Acinetobacter live on normal skin as a commensel. Each class of antibiotic-β- lactams, cephalosporin's, tetracyclines, amino glycosides, quinolones, macrolides –targets a specific bacterial process. There are some of the side effects of the use of antibiotic such as antibiotic are rarely specific to one type of bacteria. Also we all have "friendly" bacteria in the gut and other part of the body which do useful jobs for us such as helping digest our food and preventing certain infection such as 'thrush". These are often killed by antibiotics, allergic reactions can also develop. As an alternative of antibiotics, low amperage electric current can be used by various workers for the treatment of wound infection [1]. A historical review of literature on the use of low intensity direct current (LIDC) revealed that LIDC enhance wound healing. Two reasons cited for this beneficial effect are the bactericidal effect of electrical current and the stimulation of granulation tissue growth by the use. Inactivation of microorganism is important in wound healing. Many methods, such as heat sterilization, U-V, irradiation and the addition of chemicals (antibiotics, metabolic inhibitors, biocides and preservative), are currently used to inactivate microorganism. However, heat

treatment causes a loss of organoleptic properties in food and hence, the development of efficient alternative food preservation system is required, such as pulsed electric field, have been widely studied [2]**.**The application of high electric field serial pulse to liquid foods has been shown to be an efficient method of sterilization which is much simpler and cheaper to implement. Use of electric current to sterilize food products by jules effect is well known. Some investigators [3].have assimilated the lerhal effect of electricity to a simple thermal effect called ohmic heating.The effect of electrical stimuli on tissue has been the subject of considerable experimental attention. Cells have complex electrical systems which are sensitive to electric field change. Varied effect, both specific and general, behavioral, physiologic, and immunologic changes, in a wide variety of system including bacteria, insect, plant and mammalian cells in culture have been observed after exposure to electric field. Direct current has also been shown to affect nerve tissue, tumor growth, and skin wound healing [4].Proliferation after a certain threshold. High electrical current will result in overt tissue damage and provoke cell necrosis, but lower current may stimulate cells to go in to apoptosis. The bactericidal effect of low intensity electric field on microorganisms may be speculated due to loss or modification of some vital proteins of the microbial cells which can be observed by protein profiling. By protein profiling we can see the change in protein content before and after passing the electric current on the bacterial and fungal strains isolated from wound infections. The system most people use for separating proteins by gel electrophoresis was formulated [5].

Therefore the present study is designed with the following aims and objective:

- Determine the effect of low amperage direct current on microbial protein content.
- Extraction of protein from strain.
- Analyze SDS-PAGE profile of whole cell protein from the isolated strains.

2. MATERIALS AND METHODS

2.1. Strains Used

Bacterial and fungal cultures which were used in the project were obtained from the departmental stock culture of Microbiology of the culture strains include *Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. Fungal culture such as *Candida albicans* was grown in 10 ml glucose-peptone broth, in screw capped poly propylene tubes, for 24-36 hr at 37°C to yield a growth of late log to stationary phase. The tubes were aerated by shaking intermittently [6]. We used Bacterial protein extraction. Fungal protein extraction and Protein analysis were measured by Lowry's method [7].

2.2. Chemicals Used

Table 1: All the chemicals used were of analytical and molecular grade. Source of the chemicals used are given below in the table1:

S. No.	Chemicals	Source
1.	Peptone	Hi-Media Pvt. Ltd., Mumbai.
2.	Beef extract	Hi-Media Pvt. Ltd., Mumbai.
3.	NaCl	Merck Specialities Pvt. Ltd., Mumbai.
4.	Dextrose	Hi-Media Pvt. Ltd., Mumbai.
5.	SDS	Hi-Media Pvt. Ltd., Mumbai.
6.	APS	RANBAXY Laboratory Reagent S.A.S Nagar.
7.	TEMED	Hi-Media Pvt. Ltd., Mumbai.
8.	Tris	SISCO Research laboratory Pvt. Ltd., Mumbai.
9.	Coomassie Brilliant Blue (R-250)	Hi-Media Pvt. Ltd., Mumbai.
10.	Glysine	Hi-Media Pvt. Ltd., Mumbai.
11.	EDTA	Hi-Media Pvt. Ltd., Mumbai.
12.	TrisHCl	Hi-Media Pvt. Ltd., Mumbai.
13.	Glycerol	Renkem Pvt. Ltd., New Delhi
14.	Bromophenol blue	Qualigens Fine Chemicals, Mumbai.
15.	Sodium carbonate	MerkSpecialities Pvt. Ltd., Mumbai.
16.	NaOH	MerkSpecialities Pvt. Ltd., Mumbai.
17.	Copper sulphate	Renkem Pvt. Ltd., New Delhi.
18.	Sod Pottasium tartrate	RANBAXY Laboratory Reagent S.A.S Nagar.
19.	Folin	MerkSpecialities Pvt. Ltd., Mumbai.
20.	Acrylamide	Hi-Media Pvt. Ltd., Mumbai.
21.	Bisacrylamide	Hi-Media Pvt. Ltd., Mumbai.
22.	Methanol	Renkem Pvt. Ltd., New Delhi.
23.	Glacial acetic acid	Renkem Pvt. Ltd., New Delhi.
24.	Formaldehyde	Samir Tech. Chem., Vadodara.
25.	Sodium thiosulphate	RANBAXY Laboratory Reagent S.A.S Nagar.
26.	Isopropanol	Renkem Pvt. Ltd. New Delhi.
27.	Acetic acid	Renkem Pvt. Ltd., New Delhi
28.	$AgNO_3$	RANABXY Laboratory Reagent S.A.S Nagar.

The electrophoresis kit:-GeNei, Banglore

2.3. SDS-PAGE Profile

PAGE was performed by Tris-glycine system [5] using slab apparatus manufacture by GeNei, Banglore, India. Routinely a 7.5% stacking gel and 13.5% separating gel were used. Concentrated solution of 30% ammonium per sulphate and TEMED were mixed in appropriate amount to give the required final concentration. Final volume was made by distilled water. The proteomic analysis of bacterial strain was done on 13.2% separating gel and for fungal strain 10% separating gel was used.

2.4. PAGE Chemicals

a. Stacking buffer (pH 6.8)
b. Separating buffer (pH 8.8)
c. Acrybis solution
d. Electrode/Runing buffer
e. Staining solution
f. Destaining Solution
g. APS (10%) solution

2.5. Loading of Sample

40µl of all sample were loaded in the walls with the help of micropippete. Molecular weight protein marker treated similarly as sample and 20µl of it was loaded in one of the well and we used electrophoretic Condition. The samples were run on gel till they reached line above the base. The voltage applied was 100V.

2.6. Staining of the Gel

We used Coomassie Brilliant Blue Staining. Staining was made by adding Coomassie Brilliant Blue R-250 (2.4 gm), methanol (100 ml), water (100 ml), glacial acetic acid (40 ml). After electrophoresis the gel was taken in a petridish and stained for 1hr with the staining solution. Destaining solution was made by adding 15 gm NaCl in 500 ml water. Destaining was done overnight and Silver staining procedure done.

3. RESULTS AND DISCUSSION

3.1. Analysis of Whole Cell Proteins

Total amount of whole cell protein were estimated following the Lowry's method [7]. It was clearly depicted from the Table 2, that amount of proteinin *Staphylococcus aureus* was in the range of 86.4µg/0.1ml to 82.9µg/0.1ml and in *Escherichia coli*, it varied from

87.05µg/0.1ml to 82.6µg/0.1ml but in *Canada albicans* the amount of protein was very low it was from 70.8µg/0.1ml to 70.6µg/0.1ml.

Table 2: Total whole cell protein content in 0.1ml of Bacteria and fungal strains after the exposure to low amperage direct current.

S. No.	Duration of exposure to current	Amount of protein in *Staphylococcus aureus* (µg/0.1ml)	Amount of protein in *Escherichia coli* (µg/0.1ml)	Amount of protein in *Candida albicans* (µg/0.1ml)
1.	Control	86.4	85.6	70.8
2.	One day exposure	84.7	85	77.6
3.	Two days exposure	82.9	87.5	74.1
4.	Three days exposure	85.2	82.6	❖

Note: no protein estimation was done in 3rd day sample of *Canada albicans* (C3).

3.2. SDS-PAGE Profile

Whole cell protein extract from the bacterial and fungal strains after treatment with low amperage direct current were resolve through 13.2% SDS-PAGE in bacterial strain and 10% SDS-PAGE in fungal strain and electrophoretogram are presented in Table 3,4,5 and 6.

Table 3: SDS-PAGE profile of whole cell protein of *Staphylococcus aureus* after electric current exposure.

Name of sample	Molecular weight of whole cell proteins (kDa)
Standard protein marker	97.4, 66, 43, 20.1, 14.3, 3.0
S0	97.4, 74, 55.6, 50, 33.5, 19.8, 14.3, 7, 3.0
S1	97.4, 74, 55.6, 50, 33.5, 19.8, 14.3, 7, 3.0
S2	97.4, 74, 55.6, 50, 33.5, 14.3, 7, 3.0
S3	97.4, 74, 55.6, 50, 33.5, 19.8, 14.3, 7, 3.0

Where, S0 –*Staphylococcus aureus* with no exposure to current (control).

S1 –*Staphylococcus aureus* after one day exposure to current for two hours.

S2 –*Staphylococcus aureus* after two days exposure to current for two hours.

S3 –*Staphylococcus aureus* after three days exposure to current for two hours each day.

Table 4: SDS-PAGE profile of whole cell protein of *Escherichia coli* after electric current exposure.

Name of sample	Molecular weight of while cell proteins (kDa)
Standard protein marker	97.4, 66, 43, 20.1, 14.3, 3.0
E0	51.5, 36, 16, 11
E1	51.5, 36, 16, 11
E2	51.5, 36, 16, 11
E3	51.5, 36, 16, 11

Where, E0- *Escherichia coli* strain with no exposure to direct current given (control)

E1- *Escherichia coli* strain after one day exposure to direct current for two hours.

E2- *Escherichia coli* strain after two day exposure to direct current for two hours each day.

E3- *Escherichia coli* strain after three days exposure to direct current for two hours each day.

Table 5: SDS-PAGE profile of whole cell protein of *Candida albicans* after electric current exposure.

Name of sample	Molecular weight of whole cell proteins(kDa)
Standard protein marker	97.4, 66, 43, 20.1, 14.3, 3.0
C0	10, 7.5, 5.2
C1	10, 7.5, 5.2
C2	10, 7.5, 5.2

Where, C0- *Candida albicans* without any exposure to direct current (control)

C1- *Candidaalbicans* after exposure to current for two hour in day 1.

C2- *Candida albicans* after exposure to current for two hours in day 2.

Table 6: Mean and SD values of *Staphylococcus aureus*, *E. coli* and *Candida albicans* before and after exposure to current.

S. No.	Duration of current exposure	*Staphylococcus aureus*		*Escherichia coli*		*Candida albicans*	
		Mean	SD	Mean	SD	Mean	SD
1.	Control	7.39	0.19	7.25	0.00	7.50	0.25
2.	One day exposure	6.88	0.70	6.78	0.51	7.17	1.03
3.	Two days exposure	5.37	0.95	5.01	0.71	5.10	1.45
4.	Three days exposure	4.48	0.40	5.28	0.16	3.69	1.93
5.	MD(0-3 days)	2.90	0.50	1.97	0.16	3.80	1.71

The mean value for control group of *Staphylococcus aureus* was 7.39±0.19 and the protein content found in this sample was 86.4µg/0.1ml. In Sl sample it was 5.37±0.95 and the protein content found was 84.7µg/0.1ml. In S2 sample mean value, 6.88±0.70 and protein content was 82.9µg/0.1ml similarly in S3 mean value was 4.88±0.40 and its protein concentration was 85.2µg/0.1ml. In E. coli case the mean value for E0 sample was 7.25±0.0, in E1 sample was 6.78±0.51, in E2 sample it was 5.01±0.71 and in E3 sample it was 5.28±0.16. Protein found in all samples were 85.6, 85, 87.05 and 82.6µg/0.1 ml respectively. Similarly in case of *Candida albicans* the mean value for C0 was 7.50±0.23 which was control, in C1 sample it was 7.17±1.03, in C2 sample it was 5.10±1.45 and in C3 it was 3.69±1.93. The protein contents in all these samples were 70.8, 77.6 and 74.1 respectively. In C3 sample no protein estimation was done. The result incorporated in this paper provided complementary information in terms of protein changes in the samples of *Staphylococcus aureus, E. coli,* and *Candida albicans* when subjected to low amperage direct current treatment. Staphylococcus aureus is a major human pathogen causing significant morbidity and mortality in both community and hospital as well [8]. The Fungi especially yeast belonging to the genus *Candida* are potential opportunistic pathogen. Yeast is the most common fungi isolated from human patients*. Candida* an opportunistic pathogenic fungus in humans usually causes either septicaemic or mucosal infections [9].Human being carry the yeast *Candida albicans* and other *Candida* species as part of their commensal microflora. However, the host predisposed to *Candida* infections is usually immune compromised such as AIDS, diabetes, organ transplant, tumors and others [10]. Commensal *Candida* species inhibiting the oral cavity, vaginal canal, and gastrointestinal tract of host and may begin the infectious process under certain condition such as immune compromised state of the patients [11]. Their incidence has greatly increased over the past several decades with the introduction of broad-spectrum antibiotics, immune suppressive corticosteroid, and antitumor agents as well as an increasing number of AIDS patients [12, 13]. For instance, *C. albicans* is the second cause of nosocomial urinary tract infections in the intensive care unit according to the National Nsocomial Infection Surveillance System reports [14]. Another commensal bacterium *Escherichia coli*, inhabiting in the gastro intestinal tract of the human body and may cause disease during unfavorable condition of the host. SDS-PAGE of polypeptide or protein has been using increasingly concerning bacterial systematics both at genus and the species level and more recently used for type determination [15,16]. The initial selection of these proteins was based on the requirement that the radioactive counts must differ by a factor of three. Preliminary examinations of these data indicate that the newly expressed protein was not related to the heat-shock proteins of *E. coli* [17].

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