Silver Anode- Induced Phenotypical Changes in Bacteria

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Background: It has been definitely proven that the silver lons emitted from a silver electrode are highiy bactericidal. When silver electrodes are embedded in a culture medium, generally, the area immediately around the anode is found to be sterile but a certain bacterial population remains outside of this inhibition zone.

Methods: In the following study, four randomly chosen bacterial samples were incubated into Endo agar by the presence of a silver anode. Inhibition zones occurred and bacterial specimens were taken from area which is outside of the boundary of inhibition zone and analyzed using standard physiological and biochemical tests.

Results: It was found that the bacteria remaining in this area were changed phenotypically. They lost their genus-specific characteristics and were identified as different strains. These phenotypical deviations were interpreted from a taxometric perspective by a computer program. The changes were between 10-32 Operational Taxonomic Units.

Conclusion: These specimens were then recultured on Endo agar. They continued to change their biochemical identity with the exception of two samples and all of them became quinolone-sensitive even if they had been previousiy resistant. Electricaliy released silver ion therapy seens to be just as effective method at least for bacterial infections.

Keywords: Silver anode, numerical taxonomy, quinolones

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Silver compounds are used to treat eye infections and burn wounds topically as bacteriostatic agents. When a pure silver metal is polarized using electricity, silver ions are emitted from the electrode surface. İt is found that positively polarized silver ions are approximately 100 times more lethal for bacteria than nonpolar silver.[1] The same bacterial inhibition cannot be obtained by using an equal amount of silver without an electric current. The term, silver anode (SA), means a positively driven pure silver electrode. Its antibacterial effect has a large spectrum which includes many facultative bacteria and anaerobes.[1-7] Of the metal electrodes (Ag,Pt,Au,Cu and steel) tested, only silver anodes were inhibitory at low currents.[7,8]

Moreover, the anodic silver ions are also found to be antifungal and antiviral both in-vitro and in-vivo.[1,2,9] However, the mechanism causing this effect is not yet fully understood. Further, the antibacterial effect of emitted silver continues even when the electric current is stopped. If low electric current is applied to silver electrodes after incubation of the seeded agar plate, a zone of inactive bacteria is created, which upon subculture are not viable.[2] This demonstrates that, the silver anode is bactericidal as well as inhibitory. In contrast to the anode, the silver cathode contributes to osteogenesis, but not microbicidal.[8,10]

SA has been used in the treatment of deep bone infections in orthopaedics.[11,12,13] There has been no indication of deleterious or irreversible effects on mammalian cells unless the current exceeds 2 coulombs per day. It has been found to be non-carcinogenic, non- antigenic and minimally toxic when implanted in living tissue at a current of 5-20 μA.[3,8,11,13,14]

It is well known that, when SA is applied to living bacteria in a culture medium, a large inhibition zone appears around the anode after an incubation period. Generally, the inner part of the inhibition zone is found to be sterile. Whether or not the phenotyoic profiles of the bacteria remaining outside the zone were changed has not been investigated taxonomically.

In this study, the phenotypic profiles and antibiotic susceptibilities of four bacterial samples in the presence of a silver anode were compared before and after silver anode applied into the culture medium. The observed changes were interpreter program.

Materials and Methods

Citrobacter freundii (B1), Enterobacter aerogenes (B2), Pseudomonas aeruginosa (B3) and Proteus vulgaris (B4) were isolated from specimens taken from patients and purified. Each of the samples was prepared using3 ml of broth medium (GIBCO 1-t-1904) containing exponential-phase bacteria (optical density in the range of 0.11 to 0.20 at 460 nm, 4 x 10^4 - 10^5 CFU) One ml of the bacterial suspensions was of Endo nutrient agar at approvimately 45 ^oC. Each solution was blended and equally divided into two standard petri dishes, ane of which contained no electrodes and served as the control. All the dishes were incubated for 48 hours. The surface area of each electrode was 0.4 cm^2, the direct constant current was 15μA, the thickness of the agar was 3 mm, the total charge was 1.29 coulomb per day and the charge density was 3.24 coulombs per cm^2 for each electrode. The electrode posilion and experimental setup has been described previously.[15]

Bacterial samples were taken from the control plates (first example) and from the SA treated plates near the boundaries of the inhibition zones (second example). Then, each of the second examples of four bacterial samples were recultured without electricity for 48 hours. These growths represented the third examples of the bacterial samples.

Standard physiological and biochemical tests and antibiotic susceptibility tests were performed on each of the three examples of the four bacteriall samples. The test patterns are shown in Table 1 and Table 2.

Table 1. Standard physiological and biochemical tests which were performed on the three examples of the four bacterial samples, *C. frenduii* (B1), *E. aerogenes* (B2), *P. aeruginosa* (B3), *P. vulgaris* (B4). Two of four bacterilal sample phenotypes (B1 and B2) highly deviated after silver anode (SA) treatment. 1st, 2nd, and 3rd examples represnt the initial, after SA treatment and subsequent generation respectively. (+, positive; -, negative;?, difficult to decide test results)

	Bacterial Samples			
Test Pattern	B1	B2	B3	B4
Gram	- - -	- - -	- - -	- - -
catalase	+ - +	+ +	+ + +	
oxidase	- + -	- -	+ + +	- - -
capsule	+ - +		- - -	- -
motility	+ - -	+ ? -	+ - +	+ - +
indole production	+ - -	- -	- - -	+ - +
Methyl red	+ + +	- + -	- - -	+ + +
Voges-Proskauer	- - -	+ + -		
Citrate utili.	+ + +	+ ?	+ - +	+ - +
H_2S production	- + -	- - -	+ - -	+ - -
urease	+ - ?	- -	+ + -	+ + +
nitrate reduction	+ + +	+ +	+ + +	+ + +
KCN, growth in	- - +	+ ?	+ + +	+ + +
bile tolerance	- -			
aesculin hidr.	- - +	+ + +	-	+ - +
D-mannitol	+ + +	+ + -	+ - +	- - -
dulcitol	? - -	- + -	-	- - -
adonitol	+ - -	+ - -	- - -	- - -
sorbitol	+ + +	+ + -	- - -	- - -
salicin	- - +	+ + +	+ +	+ - +
eriythritol	- - -	- -	- - -	- - -
inositol	- - -	+ - -	- - -	- - -
glycerol	+ - -	+ + +	+ - +	+ - +
L-rhamnose	+ + -	+ + -	- - -	- - -
trehalose	+ + +	+ + +	- - -	- + -
arabinose	+ + +	+ + +	- - -	- - -
raffinose	- - +	+ + +	- -	- - -
cellobiose	+ - +	+ + +	- - -	- - -
melibiose	- + -	+ +	- -	- - -
D-xylose	+ + +	+ + +	- - -	+ + +
D-glucose	+ + +	+ + +	+ - -	+ + +
sucrose	- - +	+ ? +	- - -	+ + +
lactose	- + +	+ + +	- - -	- - -
D-mannose	+ + +	+ + +	- - -	- - -
maltose	+ ? + +	+ + +	- - -	+ + +
maltose	+ ? + +	+ + +	- - -	+ + +
Examples	1. 2. 3.	1. 2. 3.	1. 2. 3.	1. 2. 3.

Table 2. The antibiotic susceptibility test results of four bacterial samples before (1st example), after (2nd example) silver anode application and their recultured forms (3rd example). They gave almost completely to be sensitive to quinolone group antibiotics after silver anode treatment.
C. frenduii (B1), *E. aerogenes* (B2), *P. aeruginosa* (B3), *P. vulgaris* (B4).

Antibiotics	**Inhibition zone radius (mm)**			
(alphabetically order)	(1st, 2 nnd and 3 rd examples respectively)			
Amikasin	10, 9, 13	12, 10, 12	13, 12, 8	8, 10, 0
Amoxicillin + Clav.	10, 8, 8	11, 11, 8	9, 8, 0	15, 16, 0
Ampicillin	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Ampicillin+Sulbactam	8, 9, 8	9, 11, 10	8, 8, 0	19, 8, 0
Ceftazidime	9, 10, 8	12, 11, 10	12, 10, 10	18, 14, 14
Ciprofloxacin	13, 12, 30	16, 16, 24	17, 10, 21	8, 8, 24
Clindamycin	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Gentamicin	8, 9, 8	11, 12, 8	9, 8, 0	9, 10, 0
Netilmicin	8, 8, 8	10, 10, 8	13, 8, 8	9, 10, 16
Penicillin	0, 0 0	0, 0, 0	0, 0, 0	..0, 0, 0
Tetracycline	11, 10, 10	11, 10, 14	0, 0, 8	10, 8, 10
Tobramycine	10, 9, 14	12, 12, 14	9, 8, 0	11, 10, 0
Bacteria	**B1**	**B2**	**B3**	**B4**

B3 and B4 almost completely returned to their initial phenotypes in their third examples. The antibiotic susceptibility tests indicated that quinolone (ciprofloxacin) sensitivity was rather enhanced in the third examples of all samples despite the fact that the samples became resistant in their first examples to quinolones (Table.2.) Third examples of B3 and B4 acquired a pronounced resistance to all antibiotics except to quinolone. Although, tetracycline, penicillin, netilmicin, ceftazidime and ampicillin sensitivities did not show significant alterations for all bacterial specimens.

Assessment of the results was made by a computer program which was prepared using the Quick Basic 4.5 and assembler software language.[16] This computer program includes the phenotypic profiles of 431 clinically important bacteria (207 Gram negative) based on sixty- two standard physiological and biochemical test responses of bacteria. It is capable of placing an unknown bacteria in the bacterial phenogram when the user inputs its biochemical test responses. Also, it is capable of calculating the taxonomic distance of two bacteria by means of their phenotypic characteristics by the following formula:$d^2 = 1 - (OTU \times 10^{-2})$ where d = taxonomic distance; OTU = Operational Taxonomic Unit (percentage similarities).

Results

After incubation, inhibition zones appeared around the anodes in all four dishes. The radius of the inhibition zones varied from 20 to 24 mm around all anodes (Fig 1). No corrosion or colorization was observed on the surfaces of either the anodes or the cathodes. End of experiment, 15 μA of current was still delivering despite medium impedances were increased 23 +- 5 Ω because of ion saturation on the electrode surfaces.

The phenotypic characteristics of the bacterial samples changed, but their Gram reactions and colonial morphologies did not. The alteration of phenotypic identities of the bacterial samples can be seen in Table 1. If the phenotypic profiles of samples are considered by themselves, it suggests the following results;

1. Citrobacter freundii was identified as Salmonella arizona subgroup 3 B in its second example with 18 OTU between them, and as Enterobacter asburiae in its third example with a difference of 32 OTU.
2. Enterobacter aerogenes was identified as Enterobacter intermedium in its second example with a distance of 14 OTU and as Actinobacillus suis in its third example with a distance of 30 OTU.
3. B3 and B4 were both briefly depressed in their second examples. Pseudomonas aeruginosa was identified as Alcalifaciens denitrificum and Proteus vulgaris as Proteus penneri in their second examples with 12 and 10 OTU between their first examples
4. respectively.

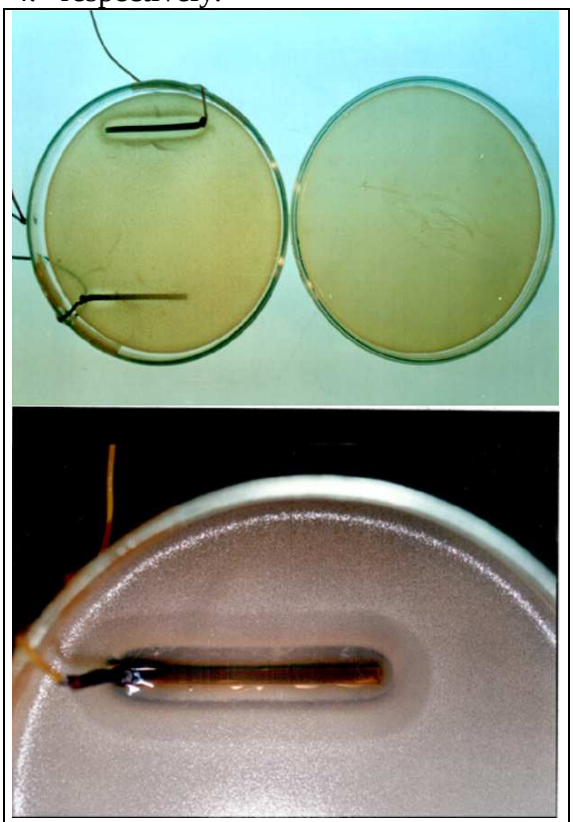

Figure 1. The inhibition zones around the silver anode. A certain partial inhibition zone can be observed around the clear inhibition zone. The material which is taken inside the inhibition zone is found our to be sterile.

Discussion

Inside of the inhibition zone, the anodic silver concentrations alter from 0.9 to 280.3 μg/ml and are sufficient for antibacterial action.[15] Many factors operate in this area. In bacterial cells, the oxidation of glucose, glycerol, fumarate, succinate, D- and L-lactate are inhibited by silver ions.[17] Enzymes are inactivated and free sulphydryl groups and NAD are oxidized, ATP is destroyed[17], but DNA shows no molecular distortion.[18] Consequently, both the respiration and energy metabolism of the bacteria collapses.[17,19,20] Also, certain pleomorphism, pili defects, and vacuolizations[15] and mesosomal dysfunctions appear in the bacterial cell after SA treatment.[1]

However,nearby or outside the nhibition zone, the silver concentration is not completely lrthal for bacteria. Nevertheless, the electric current spreads over the entire agar medium. For this reason, it can be concluded that the phenotypic changes in the bacteria are produced mainly by electricity.

An electrical potential already exists on the surface of bacterial cells (zeta potential). These charges on the membrane reflet the interent genetic expression (DNA content) and are specific to bacterial species. It is most likely that the bacterial DNA is affected by the alteration of surface charge during external electrical interventions.[21,22] Some species can undergo genetic changes to a nearby species under different environmental conditions.[23] Also, both ATP and cytoplasmic content can leak out when bacterial cells are exposed to electricity. These leaks lead to an electrotransformation process which is correlated with electropermeabilization.[21,22] In this study, al of the phenotypic changes of the treated samples may not be real genetic transformations. Nevertheless,if these changes had occurred bacause of phenotypic adaptation only, then all silver anode treated strains would have returned to their initial phenotypes after they were incubated without electricity. However, the phenotypes of both *B1* and *B2* were apparently changed by SA application even though they were hypothethic members of their own genus. The phenotypes of *B3* and *B4* were briefly depressed only in their second examples and then they almost completely returned to their initial phenotypes. Aydın et al. Demonstrated that *P*. aeruginosa showed vauolization and pili defects but did not changed phenotypically under same condition.[15] Already, both B3 (P. aeruginosa) and B4 are centrotype microorganisms and they were less affected than B1 and B2. The question of whether these changes are a genotypic or a phenotypic adaptation will remain until DNA sequence analysis is performed on such bacterial species. Meanwhile, the term ‘ Identity Crisis’ will be used to describe these genus-specific deviations.

It has been frequently demonstrated that direct or alternating electric currents increase the permeability of cellulas membranes.[20,21] Low electrical applications readily amplify the biocidal effect of antibiotics on bacteria in similar ways. The quinolone molecule is attracted by silver. The Sulfo-Quinolone Fluorescence Technique is a well known practical method used in the micro-analysis of silver.[24] On the other hand, the quinolone molecule’s proximity to DNA is necessary for the biocidal effect as it migrates through the medium and cellular barriers by an active transport mechanism.[25] It has been proven that the major proportion of the sılver present was bound to the bacterial DNA (40 μmol Ag per 100 mg DNA).[18] It is possible that the bound silver on bacterial DNA plays an important role in the attraction and orientation of quinolone molecules toward bacterial DNA.

Our results showed that the self-atavistic structures of the bacteria were obviously disturbed and that most bacterial strains became sevsitive to quinolone by silver anode application even if they had been previously resistant. Thus, electrically released silver ion therapy seems to be just as effective method at least for bacterial infections.

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References

1. Berger TJ,Spadaro JA, Chapin SE, Becker RO. Electrically generated silver ions: quantitative effects on bacterial and mammalian cells. Antimic Agents Chemotherapy, 1976;9:357-8.
2. Spadaro JA, Berger TJ, Barranco SD, Chapin SE, Becker RO. Antibacterial effects of silver electrodes with weak direct current. Antimic Agents and Chemotherapy, 1974;6:637-642.
3. Spadaro JA, Webster DA, Chase SE. Direct current activation of bacteriostatic silver electrodes. 3rd Annual meeting BRAG Soc., California, October 1983.
4. Spadaro JA. Silver anode inhibition of bacteria. Proceedings of Int. Congree on Gold and Silver in Medicine. Syracuse,1987.
5. Uezono H. Effect of weak direct current with the silver electrodes on bacterial growth. J Jpn Orthop Assoc, 1990;64:860-867.
6. Falcone EA, Spadaro JA. Inhibitory effects of electrically activated silver material on cutaneous wound bacteria. Plastic and Reconstructive Surg, 1986;77:455-458.
7. Barranco SD, Spadaro JA, Berger TJ, Becker RO. In vitro effect of weak direct current on Staphylococcus aureus. Clin Orthop Rel Res, 1974;100:250-255.
8. Spadaro JA. Bone formation and bacterial inhibition with silver and other electrodes. Reconstr Surg Traumat, 1985;19:40-50.
9. Berger TJ, Spadaro JA, Bierman R, Chapin SE, Becker RO. Antifungal properties of electrically generated metallic ions. Antimic Agents and Chemotherapy, 1976;10:856-860.
10. Dueland R, Spadaro JA, Rahn BA. Silver antibacterial bone cement. Clin Orthop, 1982;169:264-268.
11. Tamura K. Some effects of weak direct current and silver ions on experimental osteomyelitis and their clinical application. J Jpn Orthop,1983;57:187-197.
12. Webster DA, Spadaro JA, Becker RO, Kramer S. Silver anode treatment of chronıc osteomyelitis. Clin Orthop Rel Res, 1981;161:105-114.
13. Becker RO, Spadaro JA. Treatment of orthopaedic infections with electrically generated silver ions. J Bone and Joint Surg, 1978;60-A:871-881.
14. Spadaro JA, Chase SE, Webster DA. Bacterial inhibition by electrical activation of percutaneous silver implants. Biomed Mat Res, 1986;20:565-577.
15. Aydın M, Köksal F, Günay İ, Serin MS, Polat S. The effect of antibacterial silver electrodes and the nature of ion emission in the outer side of inhibition zone. Ann Med Sci, 1996;5:52-57.
16. Aydın M, Günay İ, Köksal F, Serin MS. Taksometri ve

bakteriyel identifikasyonda bilgisayar kullanımı. Mikrobiyol BÜLT, 1996;30:281-287.
17. Bragg PD, Rainnie DJ. The effect of silver ions on the respiratory chain of Escherichia coli. Can Microbiol, 1974;20:883-889.
18. Modak SM, Fox CL. Binding of silver sulfadiazine to the cellular components of Pseudomonas aeruginosa. Biochem Pharm, 1973;22:2391-2404.
19. Cowlishaw J, Spadaro JA, Becker RO. Inhibition of enzyme induction in E. Coli by anodic silver. J Bioelec, 1982;3:295-304.
20. Rowley BA, McKenna J, Chase G. The influence of electrical current on an infecting microorganism in wounds. Ann NY Acad Sci, 1974;238:543-551.
21. Sixou S, Eynard N, Escoubas JM, Werner E, Teissie J. Optimized conditions for electrotransformation of bacteria are related to the extent of electropermeabilization. Biochimica et Biophysica Acta, 1991;1088:135-138.
22. Eynard N, Sixou S, Duran N, Teissie J. Fast kinetics studies of E. Coli electrotransformation. Eur J Biochem, 1992;209:431-436.
23. Noel RK, John GH. Bergey's Manual of Systematic Bacteriology. Barbara Tansill (Ed.), Williams&Wilkins, 1986, Vol.1,pp.45-300.
24. Berman E: Silver. Heyden International Topics in SCIENCE. Edited by Thomas LC, 1980, Chapter 25,pp.121-145.
25. 25. Bergan T. Quinolones. Antimicrobial Agents. Edited by Peterson PK and Verhoef J, Elsevier Science Publishers BV,1988, Chapter 16,pp177-202.
26. Aydin M. , Harvey-Woodworth CN. Halitosis: a new definition and classification. British Dental Journal, 2014; 217: E1 doi 10.1038/sj.bdj.2014.552