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Electrically Generated Silver Ions: Quantitative Effects on Bacterial and Mammalian Cells

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The inhibitory and bactericidal concentrations of electrically generated silver ions were 10 to 100 times lower than for silver sulfadiazine. Effects on normal mammalian cells were minimal.

Silver generated at the positive (anode) electrode with weak direct current has been demonstrated to be an extremely effective agent for inhibiting bacterial growth in vitro (2, 7). This has been due primarily to a specific effect of silver at low current densities with no other alteration of the medium. To date the antibacterial activities of electrically generated silver have not been compared with the activities of other antimicrobial agents, in particular silver sulfadiazine. Therefore, we determined the bacteriostatic and bactericidal concentrations of electrically generated silver against 16 clinical isolates and standard test organisms. We also report the effects of anodically generated silver on mouse bone marrow cell populations after 18 h in culture.

The broth dilution susceptibility test (1) was used to determine minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of electrically generated silver in nutrient broth (GIBCO). The silver used in the bacterial susceptibility test and tissue culture of bone marrow was liberated from pure silver wire anodes via battery-operated constant-current generators at 75 μA for 4 h. The apparatus used for silver generation and the emission spectroscopic method used for determination of its concentration were described previously (7).

The MICs and MBCs for 16 bacterial species using electrically generated silver are summarized in Table 1. The data indicate that all organisms were inhibited at 1.25 μg of silver per ml or less and killed at a level of 10.05 μg/ml or less. In comparison with the study of Carr et al. (3) with silver sulfadiazine (Table 1, last column), electrically generated silver had a significantly lower MIC for seven similar strains of bacteria tested. In four of these, the bactericidal concentration achieved with electrically generated silver was also lower than the inhibitory concentration determined for silver sulfadiazine. Recently, it was shown by Marengo et al. (4) that *Providencia stuartii* was resistant to all currently used antibiotics except amikacin. The data in this paper demonstrate that *P. stuartii* (burn wound isolate) is also susceptible to electrically generated silver with an MBC of 0.73 μg of silver per ml. The MIC for electrically generated silver against *P. stuartii* was about 100 times lower than the MIC for silver sulfadiazine (Table 1).

Of the total silver concentration measured, significant portions were probably bound or inactivated by components of the medium. If so, the effective MICs or MBCs for silver would be much lower than the concentrations recorded in Table 1. Furthermore, silver derived from electrodes with current applied for only 15 to 30 min gave rise to an MBC (*Staphylococcus aureus*) of 0.11 μg/ml as compared with 0.26 μg/ml for 4 h of current application. Thus, as has been observed by others (F. Ellis, personal communication), the initially evolved silver is probably the most effective.

A preliminary observation by electron microscopy of *S. aureus* (unreported data) indicated that electrically released silver ions act by altering the mesosomal function of the cell. If this is verified, then the action of Ag^+ is similar to that of Ag-sulfadiazine, whose bacteriostatic action is also apparently membrane related (6).

We chose a susceptible normal mammalian cell system, mouse bone marrow cultured in vitro, to determine the silver anode's effect on morphology and cell distribution. Marrow cells were aspirated and resuspended in McCoy 5A media (Flow Laboratories) with 20% fetal calf serum. Ten aliquots of the above, mixed with silver-treated nutrient broth (to a final silver concentration of 4 μg/ml), were cultured for 18 h and compared with 10 similarly prepared controls without silver. Three hundred cells were differentially counted per culture after fixation and staining.

Overall, the cells of the silver-treated series

TABLE 1. *Inhibitory and bactericidal concentrations of electrically generated silver for some microbial species*

Organism	Strain identification no.	Anodic Ag (μg/ml)		MIC (μg/ml) of Ag-sulfadiazine[c]
		MIC[a]	MBC[b]	
Escherichia coli	ATCC 25922	0.50	2.02	
E. coli	Dental	1.03	8.25	3.13
Providencia stuartii	A 21471	0.13	0.73	12.50
Proteus mirabilis	Clinical	0.08	2.51	1.56
Pseudomonas aeruginosa	ATCC 27853	0.31	2.51	1.56
Serratia	386A	0.08	0.51	3.13
Staphylococcus albus	Dental	0.12	8.25	
S. aureus	ATCC 25923	0.03	0.26	
S. aureus	Dental	0.25	8.25	25
Streptococcus group D	296	0.63	10.05	50
S. mitis	Dental	0.31	10.05	
S. monila	Dental	1.25	10.05	
S. mutans	GS-5	0.63	10.05	
S. mutans	GS-7	0.63	10.05	
S. pyogenes	ATCC 19615	0.24	0.48	
S. salivarius	Dental	1.03	8.25	

[a] A 0.5-ml aliquot (10^4 to 10^5 organisms) was added to the twofold serial dilution of silver-treated broth. The MIC was interpreted as the lowest concentration of silver not associated with turbidity after 24 h at 37 C.

[b] A 0.5-ml aliquot was removed from the nonturbid tubes and mixed with a tube of agar (GIBCO). The MBC was defined as the lowest concentration of silver giving a count of less than 10 colonies/plate after 48 h at 37 C.

[c] The MICs for Ag-sulfadiazine were reported by Carr et al. (3) for different strains of the organism listed above.

showed no obvious detrimental effects such as cell aggregation, distortion, lysis, or pH changes as compared with the controls. The medium containing anodic silver caused a decrease in the promyelocyte population, with a simultaneous increase in neutrophils and a decrease in the percentage of normoblasts of the erythrocyte series (Fig. 1). Thus silver may enhance the maturation sequence of one cell type each of the leukocyte (decreased promyelocytes) and erythrocyte (decreased normoblasts) series as compared with the controls. This same effect has been observed with bone marrow cells cultured on a metallic silver substrate (5).

In conclusion, Ag^+ generated at the anode seems to be a very effective bactericidal agent at low concentrations without any detrimental effects upon normal mammalian cells.

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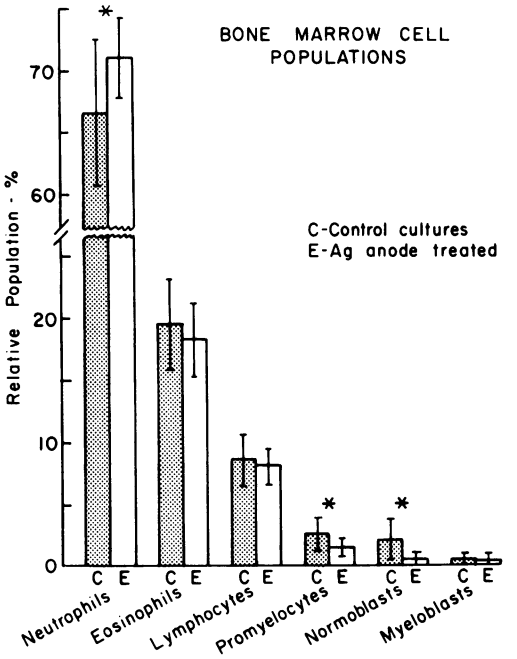

FIG. 1. *Relative percentage of marrow after culturing with silver ions (4 μg/ml) for 18 h. *, Means are significantly different from another ($P < 0.05$).*

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