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Antifungal Properties of Electrically Generated Metallic Ions

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A qualitative and quantitative investigation was undertaken to study the susceptibility of unicellular eucaryotic organisms (yeasts) to metallic cations generated by low levels of direct current. Results were characteristic of effects obtained previously using clinical and standard bacteria test organisms. The present study demonstrated that anodic silver (Ag^+) at low direct currents had inhibitory and fungicidal properties. Broth dilution susceptibility tests were made on several species of *Candida* and one species of *Torulopsis*. Growth in all isolates was inhibited by concentrations of electrically generated silver ions between 0.5 and 4.7 μg/ml, and silver exhibited fungicidal properties at concentrations as low as 1.9 μg/ml. The inhibitory and fungicidal concentrations of electrically generated silver ions are lower than those reported for other silver compounds.

We have previously examined the bactericidal activities of a variety of metallic ions generated from pure metallic anodes by direct currents. We reported (3, 4, 11) that the silver ion was an effective antibacterial agent when liberated by low levels of direct current. Quantitatively it was more effective against both gram-positive and gram-negative organisms than silver sulfadiazine. All other metals tested lacked bactericidal properties except when associated with high levels of direct current (400 μA) and the accompanying major pH changes.

Lukens (6) reviewed the work of S. E. A. McCallan and F. Wilcoxon, who examined the fungitoxicity of the elements in relation to their position in the periodic table. Generally, toxicity within a group increased with atomic weight. Silver and osmium were the most toxic elements. Data from J. G. Horsfall have been summarized (7) and show that metals are arranged in the following descending order of fungitoxicity: Ag > Hg > Cu > Cd > Cr > Ni > Pb > Co > Zn > Fe > Ca. Practically all work has involved the fungitoxicity of the metals as salts in combination with various anions. Since the electrically generated silver ion appeared to be superior to the silver compounds in antibacterial activity, we have attempted to determine the antifungal properties of several electrically generated metallic ions including silver. This article reports the results of this study.

MATERIALS AND METHODS

Qualitative studies. A 0.5-ml portion of nutrient broth (Gibco, T1859) or fluid Sabouraud medium (Gibco, 1401500) containing exponential-phase yeast (optical density at 500 nm, 0.1 to 0.3) was spread with a glass rod onto brain heart infusion agar (BHI; Gibco, T1904) or Sabouraud agar (Gibco, 1402240). Electrode culture plates (60 by 15 mm) were prepared as previously described (3). Silver, copper, zinc, and titanium wires that were at least 99.9% pure were used. Battery-operated constant-current generators (11) were applied to all electrodes (except controls) after yeast inoculation and throughout incubation in the dark (37°C, 24 h). The current levels employed were 0.4, 4.0, and 40.0 μA.

The following parameters were recorded for all control and experimental culture plates: (i) currents and applied potentials were taken at 0 and 24 h; (ii) subcultures, after 24 h, were taken aseptically at three points along each electrode and at 3-mm intervals out from each electrode (+ and −); (iii) pH measurements were made using pH test paper (Hydrion) at the same points as above; (iv) general observations such as zone size (radius), gas production, medium discoloration, and tarnishing of metal electrode were recorded, and photographs were taken.

The experimental organisms employed were *Candida albicans* I and *C. krusei*; both were derived from the VA hospital (Syracuse, N.Y.) and the Upstate Medical Center (Syracuse, N.Y.). *C. albicans* was maintained on BHI agar at 4°C, and *C. krusei* was maintained on Sabouraud dextrose agar (Gibco, 1402240) at 4°C, and both were transferred onto fresh media every 2 months.

Quantitative studies. The broth dilution susceptibility test (2) was employed to determine minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of electrically generated silver in nutrient broth. The silver ions used in the yeast susceptibility test were liberated in nutrient broth from pure silver wire anodes via battery-operated constant-current generators at 75 μA for 4 h. The current and voltage levels were monitored at

the beginning and end of silver generation. The chamber used to generate silver and the determination of the silver concentrations via emission spectroscopy have been previously described (3). The organisms used in the quantitative studies were *C. albicans* I, *C. albicans* II, *C. parapsilosis*, *C. pseudotropicalis*, *C. tropicalis*, and *Torulopsis glabrata*. These yeasts were obtained from the Upstate Medical Center and maintained on BHI as above. The final concentrations for all organisms used in the susceptibility test were between 10^4 and 10^6 cells/ml. All organisms were incubated in the dark at 37°C for 24 h. All inoculations and testing in the quantitative studies were performed in nutrient broth.

RESULTS

Qualitative studies at low current levels. Initially the four metals (silver, copper, zinc, and titanium) were tested against *C. albicans* using BHI plate cultures. When silver was shown to be the most reactive, experiments were then repeated using silver against *C. krusei*. The results presented in Table 1 were based on the sterility of stab tests from three points along each electrode. The extent of sterility depended on the number of points that demonstrated a lack of viable organisms. All data are the average of at least two separate experiments with the following two exceptions: the copper electrode experiment and the *C. krusei* inoculated in nutrient broth, which were performed only once. The yeasts were grown in an initial inoculating medium of nutrient broth with subsequent plating on BHI agar plates.

At 0.4 and 4.0 μA, copper and zinc showed no effect on *C. albicans* at either electrode, whereas 1/3 of the test points at the titanium cathode (negative) were sterile. However, all test points were sterile at the silver anode (positive) for both *C. albicans* and *C. krusei* in the same current ranges. There were also clear zones present, having a 6-mm radius from the wire (see Fig. 1a and b). Interestingly, in one of two experiments with *C. albicans*, the Ag cathode also showed clearing zones of 1.5-mm radius at 0.4 μA and 5.5-mm radius at 4 μA. These clearing zones had spots of yeast growth randomly throughout, and these zones proved to be nonsterile (Table 1). We had previously

TABLE 1. *Sterility near metal electrodes with weak direct currents*

Inoculating medium[a]	Current (A)	Electrode	Potential[b] (V)	No. of sterile subcultures near electrode[c]			
				C. albicans I		*C. krusei*	
				Pos.	Neg.	Pos.	Neg.
NB	0.4	Silver	0.47	+++	0		
		Silver	0.46			+++	0
		Copper	0.04	0	0		
		Zinc	0.03	0	0		
		Titanium	2.15	0	+		
NB	4.0	Silver	0.91	+++	0		
		Silver	0.58			+++	0
		Copper	0.54	0	0		
		Zinc	0.35	0	0		
		Titanium	3.12	0	+		
NB	40.0	Silver	1.65	+++	0		
		Silver	1.20			+++	0
		Copper	1.10	0	0		
		Zinc	0.50	0	0		
		Titanium	8.0+	+	+		
Sabouraud	0.4	Silver	0.53			0	0
Sabouraud	4.0	Silver	0.84			+++	0
Sabouraud	40.0	Silver	2.05			+++	++

[a] NB, Nutrient broth; Sabouraud, fluid Sabouraud medium. The yeasts were initially grown in either of these two media with subsequent plating on BHI agar or Sabouraud agar, respectively.

[b] Potential at 24 h.

[c] Sterility of the region near the electrode was measured by subculturing tests, which were taken at three points along each electrode (+ and −). The degree of sterility was categorized as follows: 0, lack of sterility at all three points along the electrode with no stab tests sterile; +, 1/3 of stab tests sterile; ++, 2/3 of stab tests sterile; +++, all stab tests sterile. Pos., positive; Neg., negative.

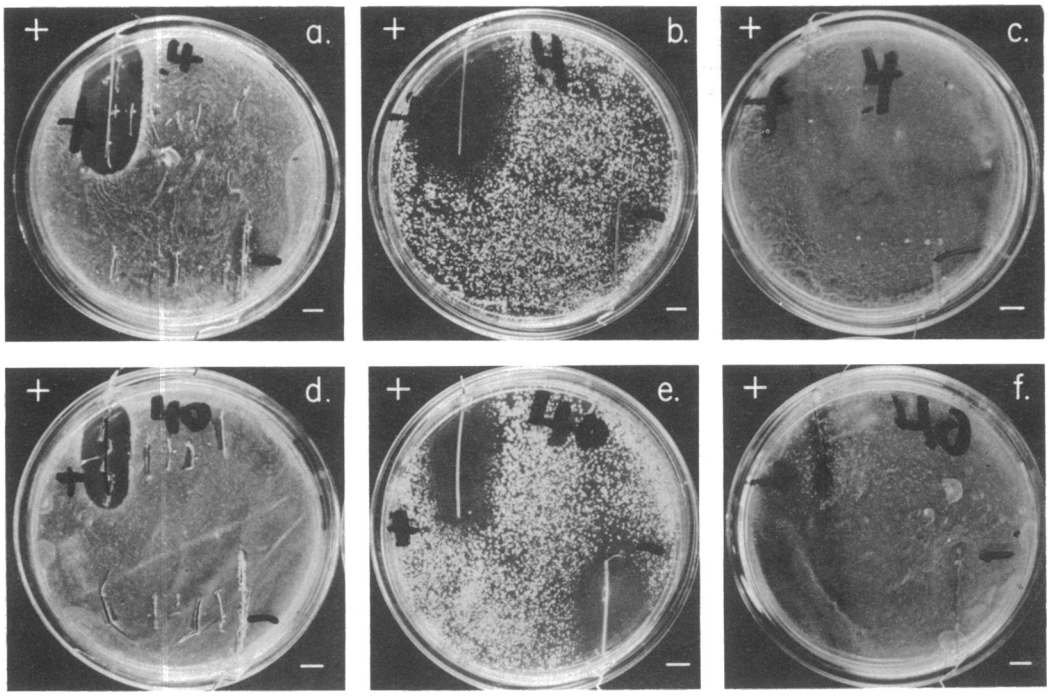

FIG. 1. *(a and d) Culture plates of C. albicans with Ag electrode at 0.4 and 40 μA after 24 h of incubation; (b and e) C. krusei with Ag at 0.4 and 40 μA; (c and f) C. albicans with titanium at 0.4 and 40 μA. Note the presence of clear sterile zones at all Ag anodes (+). C. krusei also has a nonsterile clear zone at the Ag cathode. Titanium has a small clear zone at both 40 μA positive and negative.*

seen similar clearing zones at the Ag cathode (only at the 4-μA level) with some bacteria (11). *C. krusei* at the Ag cathode exhibited no clear zones at 0.4 μA but also had a partially clear zone of 3-mm radius at 4 μA. This also proved to be nonsterile (Table 1). The control electrode (no current) generally had no clear zones, but occasionally a small clear zone of 1-mm radius or less was observed around the control Ag wire. These small zones proved to be nonsterile but do indicate some diffusion of Ag ions away from the Ag wire.

The silver, copper, and zinc electrode pairs at these current levels had potentials of less than 1 V. There were no pH shifts, gas formation, or agar discoloration seen for any of these metals at either current. However, at 4 μA a very slight tarnishing of the metal electrode was noticed for all three metals.

The titanium electrodes appeared slightly tarnished relative to the control and had an interelectrode potential greater than 2.0 V at 0.4 and 4 μA. Other than the appearance of a slight gas formation at the titanium anode in the 4.0-μA plate, there were no other changes observed (see Fig. 1c).

Qualitative studies at higher current. At 40 μA, all metals except zinc had a final interelectrode potential greater than 1 V. Silver again had a clear zone at the anode with a 4-mm radius for *C. albicans* and a 6-mm radius for *C. krusei*. The Ag wire appeared tarnished, and the zones proved to be sterile in both cases (Table 1). At the Ag cathode there was no clear zone produced with *C. albicans*, but with *C. krusei* there was a 6-mm-radius clear zone present that on subculture proved to be nonsterile. Both culture plates of the above had gas bubbles present at the cathode (see Fig. 1d and e). There were no substantial pH shifts except at the points directly adjacent to the cathode, where the pH shifted about +0.5 units as compared to the control.

The copper anode at 40 μA produced a 3-mm clear zone that proved to be nonsterile. A bluish discoloration appeared at the anode, along with gas formation at the cathode. A pH shift of +1.0 unit was observed at the cathode relative to the control.

Zinc at 40 μA had no zones, and no other changes were noted. Titanium at 40 μA exhibited 1/3 of the test points sterile at the anode and cathode with slight tarnishing, and no pH shift or discoloration of the medium was ob-

served. At both positive and negative electrodes gas formation was evident (see Fig. 1f).

Fluid Sabouraud medium was also used to grow the inoculum of *C. krusei*. Once turbid growth was achieved, the inoculum was plated onto Sabouraud agar and treated with silver electrodes. The potentials increased with increasing currents, as seen with *C. krusei* grown from an inoculum in nutrient broth. However, no test points were sterile until the 4.0-μA level, and at 40 μA 2/3 of the test points were sterile at the cathode. There was a brownish discoloration of the medium around the anode at all three current levels, with the greatest discoloration at 40 μA. At 40 μA there was a pH shift of +2.5 units at both positive and negative poles, with gas formation evident only at the negative. Thus *C. krusei* was inhibited at a higher current level at the Ag·anode when the inoculum was prepared in Sabouraud medium.

Quantitative studies. Since copper, zinc, and titanium showed no significant killing at the current levels used, attention was focused primarily on the inhibitory and fungicidal properties of the anodically released silver. The quantitative studies confirm the results obtained in the qualitative experiments using electrically generated silver ions.

The MIC and MFC of silver for six yeasts are summarized in Table 2. All data are averages of two or three experiments. All organisms were inhibited by a silver concentration of 4.7 μg/ml or less, and the fungicidal concentration of silver was as low as 1.9 μg/ml. These values were generally lower than the values reported for other silver compounds against *Candida* (1, 5, 12).

TABLE 2. *MICs and MFCs of electrically generated silver for some yeasts in nutrient broth*

Organism	Anodic Ag (μg/ml)	
	MIC[a]	MFC[b]
Candida parapsilosis	4.7	–[c]
C. pseudotropicalis	2.4	4.0
Torulopsis glabrata	1.6	2.6
C. tropicalis	1.0	–[c]
C. albicans I	0.5	1.9
C. albicans II	3.5	13.8

[a] A 0.5-ml portion (10^4 to 10^6 organisms) was added to the twofold serial dilution of silver-treated broth. The MIC was interpreted as the lowest concentration of Ag not associated with turbidity after the control tube appeared turbid at 37°C (between 4 and 50 h).

[b] A 0.5-ml portion was removed from the nonturbid tubes and mixed with a tube of agar. The MFC was defined as the minimum concentration of Ag at which all yeast cells were killed.

[c] Silver concentration greater than 15 μg/ml.

DISCUSSION

Golubovich completely interrupted the growth of *C. utilis* (10^6 cells/ml) with 4.7 μg of $AgNO_3$ per ml (5). Our findings show that electrically produced silver not only stops growth at concentrations below that used by Golubovich but also kills some species of *Candida* in the same range and without nitrate.

Avakyan (1) reported that silver (in the form of $AgNO_3$), in comparison to other heavy metals, was the most toxic to yeasts and bacteria. But overall the yeasts were less sensitive to the action of the heavy metals than the bacteria. Our results with yeasts show that their MIC and some MFC values are in the same range as those reported for 16 bacterial species (4). Thus, electrically released silver-ion therapy seems to be just as effective against some unicellular eucaryotic organisms as with procaryotic ones. Wlodkowski (12), using *C. albicans* isolates, reported an MIC of 100 μg/ml with Ag-sulfadiazine, and only 2% of the isolates had an MIC lower than 3.12 μg/ml. With the method reported here the MIC has been found to be 4.7 μg/ml or less for all six organisms tested so far in nutrient broth.

The data reported here illustrate that the use of silver wire in conjunction with low electrical current is more effective for inhibiting and killing yeasts than other contemporary silver compounds in use. The effectiveness of anodic silver against *Candida* even compares favorably with its inhibition of relatively resistant bacterial organisms. We have tested, for example, two strains of *Enterobacter cloacae* (7779 and 7780, gifts of H. S. Rosenkranz) that were isolated from a burn ward in which Ag-sulfadiazine was used. These two organisms had an MIC of 400 μg/ml with Ag-sulfadiazine (10). In tests with anodic Ag, the MICs obtained for the same two organisms were less than 3.0 μg of Ag per ml.

There is now strong evidence in the literature that the active component of any silver compound is the silver itself (5, 8, 9, 11). Our results confirm these findings, since they show the action of killing yeast residues specifically at the anode (+) where silver ions are produced. Whether we are dealing with just free silver ions or some electrochemical silver complex has yet to be determined. The data show that electrically generated silver cations are more effective than silver sulfadiazine or silver nitrate.

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