ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1991, p. 2131–2134
0066-4804/91/102131-04$02.00/0

Vol. 35, No. 10

Bacterial and Fungal Killing by Iontophoresis with Long-Lived Electrodes

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Received 29 April 1991/Accepted 23 July 1991

Iontophoresis with gold, carbon, and platinum electrodes was shown to effectively reduce or eliminate gram-positive, gram-negative, and *Candida albicans* inocula in synthetic urine. Platinum and gold electrodes were more effective than carbon electrodes, but platinum showed the best longevity and may reduce or eliminate microbial colonization of catheters.

For decades, catherters have been associated with nosocomial infections and are thought to be the most common cause of sepsis in hospitals (6, 8). We and other investigators have evaluated the effect of iontophoresis on organisms, and we have suggested that iontophoretic technology may be used in urinary and, potentially, other types of catheters (1–5).

Long electrode life span and effectiveness in killing microbes are two major criteria for incorporation of electrodes into catheters, although other considerations are important (e.g., cost and effects on tissue or urine composition). The best electrodes developed to date for use in fluids have been gold electrodes (4, 5). The present study was done to evaluate gold, carbon, and platinum electrodes in terms of effectiveness of killing microbes and, for gold and platinum, electrode longevity.

A 10-channel microampere generator (synthesized at University of Texas Medical Branch; wiring diagram available on request) was used to provide constant amperage with variable current (4). Vented stoppered vials were connected to the generator by metal connectors. A diagram of our system has been published elsewhere (4). Wires (gold or platinum, 0.2-mm diameter) or carbon rods (approximately 3-mm diameter) were attached to the bases of metal connectors so that approximately 2 cm of each wire or rod was covered by synthetic urine (4, 5). Synthetic urine was used to avoid variations in constituents normally found in human urine (7).

The following microorganisms were used: *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Klebsiella pneumoniae*. The bacteria were chosen because gram-negative bacteria are responsible for more than 80% of all urinary tract infections, with *E. coli* being responsible for more infections than all other genera combined (6, 8). *Staphylococcus saprophyticus* and *C. albicans* were also utilized as representative members of gram-negative and fungal genera. Standard growth curves for the genera of microorganisms (without applying current) were determined. Preliminary results showed no difference in growth, either with or without metal wires (with no current applied) in the vials. Growth curves of several of the organisms cultivated under these conditions have been published previously (5).

Bacterial or fungal inocula were added to glass vials containing 10 ml of synthetic urine to give the desired concentration of CFU per milliliter. The vented rubber stopper containing the desired electrode combination was then placed into the bacterial suspension. Constant current was applied, and the set-up was monitored for any changes, such as electrode breakage. Samples (0.1 ml) were withdrawn from each vial at designated intervals. These samples were serially diluted and cultured. Controls consisted of bacterial suspensions in contact with the desired electrode combination without application of constant current. Comparison of numbers of CFU survivors was done with Student's *t* test (two-tailed).

Gold and platinum were examined for their longevity after multiple challenges with bacteria to stimulate the proposed frequent seeding of bacteria by the ascending route in clinical systitis (8). Vials were inoculated with *E. coli* CFU (10^8) every other day. Immediately before inoculation, the same inoculum volume (0.1 ml) was removed and the number of CFU per unit volume were determined. After 90 days, this procedure was changed to inoculation with 10^8 *E. coli* CFU once per week. In addition to 0.2-mm gold wire electrodes, braided gold wire (three strands of 0.2-mm wire) and 0.6-mm solid gold wire were also used.

In general, gold and platinum electrodes were better than carbon electrodes in reducing or eliminating an inoculum of microbes (Fig. 1 through 3). Gold electrodes were particularly effective at killing gram-negative organisms (Fig. 1 through 3). Platinum electrodes, while comparably effective against gram-negative organisms, were significantly ($P < 0.05$) more effective than carbon or gold electrodes in reducing or eliminating *S. saprophyticus* (Fig. 2), but only at the lower microamperage used. Carbon, gold, and platinum electrodes were about equally effective in reducing or eliminating a *C. albicans* inoculum (Fig. 3). Results with *P. mirabilis*, *K. pneumoniae*, and *P. aeruginosa* (data not shown) were similar to those with *E. coli* and *S. saprophyticus* (Fig. 1 and 2).

Carbon electrodes were the least efficient in killing microorganisms in the experiments. Even at the highest microamperage used (200 to 400 μA), these electrodes were not always able to kill the entire inoculum, even over a 24-h period (for example, see Fig. 2 and 3). Nonetheless, carbon electrodes were efficient in reducing the number of detectable CFU by several orders of magnitude (10 to 10^8 CFU) even after only 4 h of iontophoresis with all bacteria tested and *C. albicans* (Fig. 1 through 3).

Intermittent seeding of urine during infection could poten-

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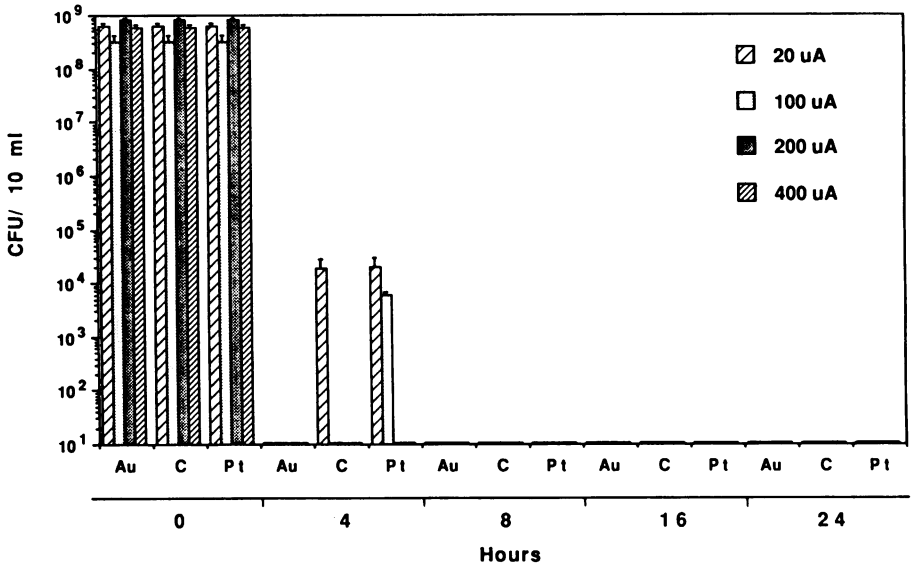

FIG. 1. Iontophoresis of *E. coli*. Abbreviations: CFU/10 ml, number of CFU of organisms per 10 ml of synthetic urine subjected to iontophoresis; μA, constant microamperage applied to the electrodes; Au, gold electrodes; C, carbon electrodes; Pt, platinum electrodes; Hours, length of time of iontophoresis.

tially decrease electrode life by increasing resistance in the iontophoresed artificial urine. To examine electrode longevity, gold and platinum electrodes were assayed to determine how long they would remain intact while being subjected to repeated inoculations of *E. coli* yet kept at a constant current of either 200 or 400 μA. Gold wire electrodes showed the shortest effective life span of about 9 to 10 days (Table 1). Platinum electrodes showed remarkable longevity; these electrodes did not break over 180 days. In addition, the longevity could not be related to either the diameter of the electrode or the microamperage (Table 1). Both gold and platinum electrodes showed no surviving *E. coli* CFU 24 h after the vials were inoculated during the length of the experiment.

This study supports and expands our work and the work of others (1–5, 10–12). In most studies, the inoculum levels have been modest (10^2 or 10^4) to mimic the initial infecting dose of organisms that presumably travel retrograde from the patient's urethral flora to the bladder in catheterized patients (5, 8). In this study, larger inocula were used

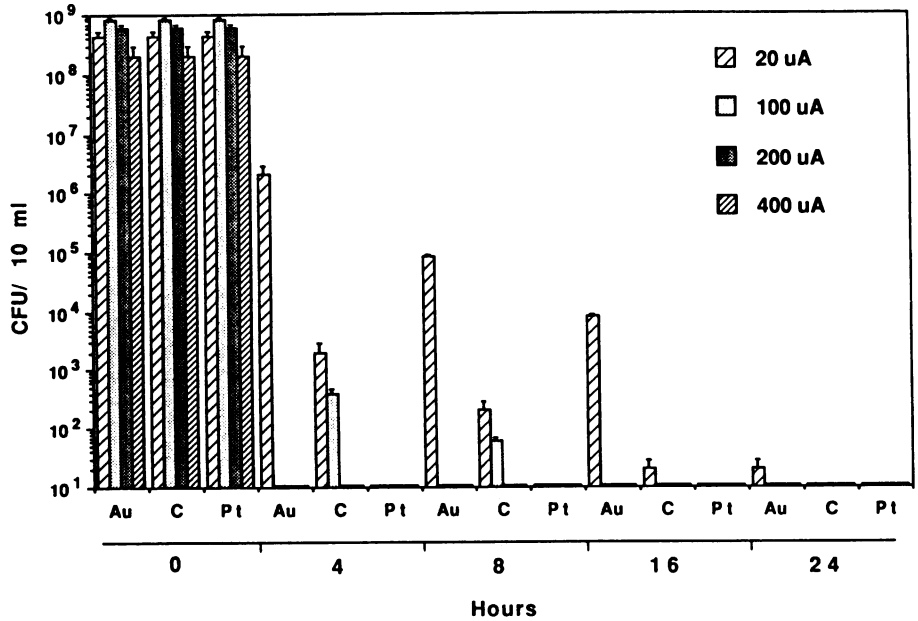

FIG. 2. Iontophoresis of *S. saprophyticus*. Abbreviations: CFU/10 ml, number of CFU of organisms per 10 ml of synthetic urine subjected to iontophoresis; μA, constant microamperage applied to the electrodes; Au, gold electrodes; C, carbon electrodes; Pt, platinum electrodes; Hours, length of time of iontophoresis.

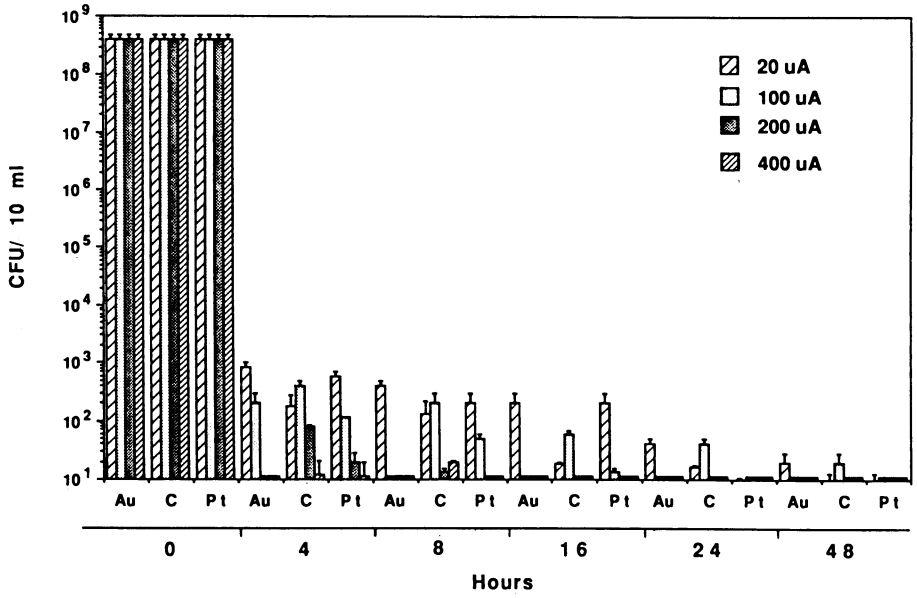

FIG. 3. Iontophoresis of *C. albicans*. Abbreviations: CFU/10 ml, number of CFU of organisms per 10 ml of synthetic urine subjected to iontophoresis; μA, constant microamperage applied to the electrodes; Au, gold electrodes; C, carbon electrodes; Pt, platinum electrodes; Hours, length of time of iontophoresis.

(approximately 10^8) to see whether iontophoresis could reduce or eliminate a large number of microbes. *C. albicans* was also killed. These findings indicate that iontophoresis may be useful, not only as a preventative tool but possibly as a therapeutic tool. Data presented here are similar to results of our previous studies with much smaller inocula (10^2 to 10^4 CFU) (5).

Longevity of gold electrodes was shown, in our test system, to be related directly to the diameter of the electrodes and inversely to the microamperage applied to the electrodes. Gold electrodes showed good longevity and might have lasted longer had they been completely covered by fluid. Inclusion of electrodes in catheters likely would keep them constantly immersed in urine and thus reduce or eliminate the exposure to air-mediated oxidation which, we believe, is the reason for egression of gold into the fluid phase.

Platinum electrodes provided the best longevity of the three electrodes tested. In contrast to the data for gold electrodes, our data did not relate longevity of platinum electrodes to the diameter of the electrodes or inversely to the microamperage applied. However, the experiment was terminated after 180 days without electrode breakage, so we cannot be certain that if longer experiments were done, similar relationships to gold electrode longevity would be found. This technology, with its low voltage requirements (2 to 3.5 V supplied by a 9-V battery), could easily be adapted and made portable for patient use. This microamperage and voltage level reportedly caused little or no tissue damage (2). Although it has been proposed that iontophoresis-generated metal ions are responsible for bacterial killing (1–3), the relative effectiveness of carbon electrodes suggests that other mechanisms are likely playing an important role. Bacteria did not apparently develop resistance to iontophoresis, but further experiments need to be done to determine whether any do develop resistance. Nonetheless, the lack of organisms surviving iontophoresis over months bodes well for potential use in catheters. Kunin has recently suggested that catheterization has not substantially advanced since the development of the closed drainage system (9). Development of iontophoretic urinary catheters may provide further advancements in catheterization techniques and thus benefit a large number of patients who need long-term catheterization.

TABLE 1. Longevity of gold and platinum electrodes after multiple inocula of *E. coli* in synthetic urine[a]

Electrode type (wire diameter [mm])	Current (μA)[b]	Survival time (days)[c]
Gold (0.2)	200	13–14
	400	9–10
Gold (0.6)	200	20–21
	400	10–11
Gold (0.2) (three wires braided together)	200	33–35
	400	29–31
Platinum (0.2)	200	180
	400	180

[a] Vials were inoculated with 10^8 CFU every other day, and equal volume samples were removed for determination of surviving CFU after 24 h. After 90 days, vials were inoculated with 10^8 CFU once per week.

[b] Constant current level, with voltage varying between approximately 2 and 3.5 V.

[c] Indicates how long electrodes usually lasted before anode broke off in three or more experiments. The extent of the experiment was 180 days. Platinum electrodes were effective in killing CFU and did not break after 180 days.

This study was supported, in part, by the Advanced Technology Program, 004952-010, State of Texas.

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