Effects of Microamperage, Medium, and Bacterial Concentration on Iontophoretic Killing of Bacteria in Fluid

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Prevention of nosocomial urinary tract infections by iontophoresis is addressed. An iontophoretic generator was used to provide microamperage (10 to 400 µA) to vials containing either synthetic urine or supplemented synthetic urine. Bacteria were added to vials, and parameters of growth, bacterial killing, and microamperage were examined. Escherichia coli and Proteus species were both inhibited and killed at various microamperages and with several electrode types, the most efficient being gold-gold as the anode-cathode combination. Klebsiella pneumoniae in supplemented synthetic urine was least inhibited in growth, and higher microamperage (200 to 400 µA) was most effective in killing the bacteria. Bacterial growth reduction and killing were directly related to increasing microamperage and were inversely related to bacterial concentration.

Prevention of nosocomial urinary tract infections (UTI) has long been a goal of clinicians. Urinary catheters are the leading cause of nosocomial UTI, and UTI is the most common predisposing factor for cases of fatal gram-negative sepsis that originate in hospitals (5, 7). Consequently, prevention of UTI related to urinary catheters could lead to a substantial reduction in morbidity and mortality from nosocomial gram-negative sepsis. Such prevention could also decrease morbidity and mortality in geriatric patients, because those who require catheterization can have a high incidence of bacteriuria (9, 15).

Advancements have been made in catheter design to include a completely closed drainage system. Some have suggested that silver oxide, coated onto catheters, may protect patients from catheter-associated bacteriuria (14). In a previous study, we examined iontophoresis as a method to generate ions that would kill bacteria both in static fluid and in a model catheter system (4). In the study reported here, we further define parameters that influence bacterial killing and survival during iontophoresis in an artificial urine.

**MATERIALS AND METHODS**

**Constant current-constant voltage generator.** A microamperage generator was built in the Medical Electronics Division at the University of Texas Medical Branch by D. Arnett (4). The instrument provided constant current (1 to 450 µA) or constant voltage (0.01 to 12.50 V) to 10 independently controlled channels. (A wiring diagram is available upon request.) Vented, stoppered vials were attached by metal connectors to the microamperage generator. A diagram of our system has been published elsewhere (4). Wires (gold, silver, copper, nickel, platinum, or gold-palladium [50:40 alloy]), all approximately 0.2 mm in diameter, were attached to the base of the metal connectors so that 2 cm of each wire or rod was covered by 10 ml of synthetic urine. Variations in microamperage were small (average variation, ±2 µA) in each channel when the generator was set to maintain constant microamperage (10, 50, 200, or 400 µA per channel).

**Bacteria and media.** The following bacteria were used: Escherichia coli (clinical isolate; identified by the Clinical Laboratories, University of Texas Medical Branch), Proteus mirabilis, and Klebsiella pneumoniae (the latter two organisms were donated by Johnny Peterson). These bacteria were chosen because gram-negative bacteria are responsible for 80 to 90% of all UTI, with E. coli being responsible for more infections than all other genera combined (7). Frozen (−70°C) stocks were inoculated into brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and incubated at 37°C for 24 h before being used. Bacteria were washed three times by centrifugation in cold filter-sterilized synthetic urine (6). Bacterial concentrations were determined with a Petroff-Hauser counting chamber and diluted with sterile synthetic urine to obtain the desired concentration of bacteria (2 × 105 or 2 × 104/10 ml, depending on the experiment). Viable counts were made by diluting 0.1-ml samples in sterile synthetic urine and plating onto brain heart infusion agar plates. Because K. pneumoniae grew poorly in synthetic urine, Trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, Md.) was added to synthetic urine as previously described (6). Standard growth curves for the three genera of bacteria (without applying current) were determined. Preliminary results showed no difference in growth, either with or without metal wires in the vials.

**Model catheter system for static fluid.** Bacterial inocula were added to glass vials containing 10 ml of synthetic urine (5% TSB was added to synthetic urine for K. pneumoniae only) to give the desired concentration (2 × 106 or 2 × 104 cells per 10 ml). The vented rubber stopper containing the desired electrode combination was then placed into the bacterial suspension. Constant current was applied and monitored for any changes. Samples (0.1 ml) were withdrawn from each vial at designated intervals (4 and 8 h and 1, 2, 3, 4, 5, 6, and 7 days). These samples were serially diluted in sterile synthetic urine, plated onto brain heart infusion agar (E. coli and K. pneumoniae) or MacConkey agar (P. mirabilis), and incubated at 37°C for 24 h to determine the CFU. In the gold-gold electrode reinoculation assay, one channel was maintained at 50 µA and one was maintained at 400 µA, and the vials were reinoculated every other day with the desired concentration of bacteria. Controls consisted of bacterial suspensions in contact with the

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desired electrode combination without application of constant current.

RESULTS

Growth of bacteria in artificial urine. All three organisms used in this study grew in artificial urine (Fig. 1). However, *K. pneumoniae* grew at a very low rate and reached a low total-population level. Consequently, TSB-enriched artificial urine was used, and *K. pneumoniae* then grew very well (Fig. 1). In the remainder of the experiments, described below, the basic artificial urine was used as a growth medium for *E. coli* and *P. mirabilis*, but for *K. pneumoniae* only enriched artificial urine was used.

Prevention of bacterial growth with iontophoresis. Gold iontophoresis was effective at preventing growth of *E. coli*, *P. mirabilis*, and *K. pneumoniae* at the inoculum size of 2 x 10^2 cells per 10 ml. In general, iontophoresis at 10, 50, 200, and 400 µA killed the bacterial inocula within 2 days. Usually, the organisms were undetectable within 4 h (Fig. 2a and b), except for *K. pneumoniae*, for which survivors were detected after 1 to 2 days (Fig. 2c). Investigations of bacteria inoculated at 2 x 10^2/10 ml did not continue after 2 days, as pilot studies showed that no survivors were cultured at periods greater than 2 days.

Bacteria in the denser inoculum (2 x 10^4/10 ml) survived better than bacteria in the less-dense inoculum. However, if the microamperage was kept at 200 to 400 µA, bacterial growth was reduced, slowed, or eliminated. Although growth of *E. coli* and *P. mirabilis* was frequently eliminated by 200 to 400 µA, only 400 µA killed *K. pneumoniae* (Fig. 2).

Reinoculation of bacteria into vials undergoing gold iontophoresis showed again that 400 µA was somewhat more effective than 50 µA at reducing or eliminating the organisms at either inoculum size (Fig. 3). *K. pneumoniae* was eliminated more rapidly in this series of experiments than in the previous series.

Prevention of bacterial growth with multiple-electrode iontophoresis. Other electrodes were combined with gold to determine whether they also were capable of reducing or eliminating bacterial growth. Several electrode combinations with gold as a component of the anode or cathode were effective in eliminating growth of *E. coli* and *P. mirabilis* (Fig. 4a and b). The most effective electrodes were carbon-gold, platinum-gold, and a gold-palladium alloy. Although these electrodes were effective initially in slowing *K. pneumoniae* growth, the effect was not sustained (Fig. 4c). By day 4, either the electrodes had broken (by day 2) or they were no longer able to suppress or eliminate *K. pneumoniae* growth (Fig. 4c). Of the electrode combinations silver-gold, nickel-gold, and copper-gold, only the anodes (silver, nickel, and copper) broke.

DISCUSSION

The study confirms and extends our previous work and the work of others showing that bacterial populations of different genera can be reduced or eliminated by iontophoresis (1, 3, 4, 16, 17). Our earlier study (4) utilized a worst-case situation which allowed optimal growth conditions for the bacteria. However, in this study, synthetic urine provided less than optimal conditions for the growth of bacteria. Synthetic urine was chosen because it more closely mimics the growth conditions found in human urine than do microbiologic growth media. Synthetic urine can be consistently synthesized with the same compounds and avoids the variation in constituents typical of human urine (6). However, *K. pneumoniae* grew very poorly under these conditions, and, as has been done by others (6), TSB was added to the synthetic urine (Fig. 1). Growth of *K. pneumoniae* and the other genera should be related to the results given in Fig. 1 because even those electrodes that we describe as being less effective than gold-gold electrodes in suppressing or eliminating bacterial growth were still able to significantly suppress growth. For example, Fig. 4c shows that *K. pneumoniae* growth is only approximately 10^3 to 10^5 CFU/ml by day 3, while Fig. 1 shows that normal growth would be approximately 10^7 CFU/ml, a highly significant difference.
FIG. 2. Effect of gold electrodes on *E. coli* (A), *P. mirabilis* (B), and *K. pneumoniae* (C). Inocula were placed into 10-ml vials at 0 h, and measurements of CFU were made at the indicated intervals. For *K. pneumoniae* (C), the CFU per milliliter represent logarithmic growth.
FIG. 3. Growth of bacteria reinoculated into vials undergoing gold iontophoresis at 50 µA (A) and 400 µA (B). Inocula were placed into 10-ml vials at 0 h, and measurements of CFU were made at the indicated intervals. Asterisks indicate days on which vials were reinoculated with organisms, and the CFU for those days represent inocula placed into 10-ml vials.

This work suggests that even very low microamperage can be effective in reducing or eliminating bacterial growth. This effect is strongly dependent on at least two parameters. If the inoculum size is above approximately 2 × 10⁴/10 ml, 10 to 50 µA generally will not control the population regardless of electrode composition; consequently, one parameter is inoculum size. This is in agreement with the results of our previous work (4). Although we do not claim to know exactly how many microamperes will kill a given bacterial population, we do know that the trend of increasing microamperage can reduce or eliminate denser bacterial inocula. Iontophoresis of metals or their salts may be related to interference with DNA replication or alteration of proteins associated with cell membranes, as suggested by previous investigators (12, 13), but this remains to be determined. The second parameter that affects bacterial killing is the rate of bacterial growth. This is shown by the relative increase in the rate of growth of _K. pneumoniae_ in TSB-enriched artificial urine. We speculate that other parameters (e.g., growth medium composition or innate genetic resistance to ions) play a role in bacterial killing. Other investigators have provided some evidence that electrical treatment may inhibit or terminate bacterial growth by generating metallic salts or chloride-containing salts (2, 10, 11). In our system and in others, multiple types of bacterial inhibition may occur simultaneously. Lower microamperage (<400 µA) may not generate enough ions or salts to overcome bacterial growth.

Included in the experimental design was an attempt to determine whether other electrodes could function as well as or better than gold. In general, gold wire made the best electrode pairs, as it had excellent killing characteristics and gave a relatively long electrode life. This study included trials of other electrodes that were apparently not well suited for iontophoresis because they broke early in the experimental procedures (copper and nickel). Another electrode, silver, did fairly well when it was utilized as the anode. Early breakage of electrodes in the solutions is probably related to rapid oxidation, as the usual place for electrode breakage was at the junction of electrolyte solution (synthetic urine) and metal with the atmosphere. We are investigating electrode life span in relation to variable voltage and microamperage (G. M. Rao, S. Weinberg, C. P. Davis, M. D. Anderson, and M. M. Warren, unpublished results); preliminary results show that decreasing microamperage increases
FIG. 4. Effect of mixed electrodes on *E. coli* (A), *P. mirabilis* (B), and *K. pneumoniae* (C). Inocula were placed into 10-ml vials at 0 h, and measurements of CFU were made at the indicated intervals. For *K. pneumoniae* (C) the CFU per milliliter represent logarithmic growth. The electrode combinations Ag-Au, Ni-Au, and Cu-Au broke in solutions after days 1 to 2; consequently, these electrodes were not evaluated after day 1.