Bactericidal Effect of Hydrogen Peroxide on Urinary Tract Pathogens

ANTHONY J. SCHAEFFER,* JOANNE M. JONES, AND SUSAN K. AMUNDSEN

Department of Urology, Northwestern University Medical School, Chicago, Illinois 60611

Bacterial contamination of urinary drainage bags is a frequent source of bladder bacteriuria in patients with indwelling catheters. Previous work demonstrated that the addition of 30 ml of 3% H2O2 prevented bacterial contamination of urinary drainage bags for up to 8 h in patients with urinary infections (>10^5 colony-forming units per ml). Survival curves of a variety of organisms in filter-sterilized urine with various concentrations of H2O2 (0.6 to 0.01%) were constructed. Organisms with high cellular catalase activity (Staphylococcus aureus, Serratia marcescens, and Proteus mirabilis) required 30 to 60 min of exposure to 0.6% H2O2 for a reduction of 10^6 to <1 colony-forming unit per ml, whereas Escherichia coli, Streptococcus sp., and Pseudomonas sp. required only 15 min of exposure. The efficacy of H2O2 in urine was maintained despite exposure to room temperature for 5 days and reinoculation with bacterial suspensions. H2O2 is inexpensive and relatively nontoxic, and these data suggest that periodic instillation of H2O2 into urinary drainage bags may eliminate a source of bladder bacteriuria and environmental contamination.

Hydrogen peroxide has been used in a variety of situations as a bactericidal agent (11). Catheter-associated urinary tract infections account for approximately 30% of all nosocomial infections (13), and contaminated urinary drainage bags are a frequent reservoir for bacteria that may colonize the urinary tract (8a) and the hospital environment. In previous work, we determined that the periodic instillation of 30 ml of 3% H2O2 into urinary drainage bags prevented bacterial contamination before bladder bacteriuria and also significantly reduced drainage bag bacteriuria in patients with urinary infections (8a). The purpose of this study was to determine survival curves for common urinary tract pathogens exposed to various H2O2 concentrations. The results of this study demonstrated that bacteria were effectively reduced from 10^6 to <1 colony-forming unit (CFU) per ml at low H2O2 concentrations and that the efficacy of H2O2 was maintained even with prolonged exposure to room temperature and bacterial reinoculation.

MATERIALS AND METHODS

Bacterial strains. Escherichia coli, Serratia marcescens, Proteus mirabilis, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus faecalis, and Pseudomonas sp. were isolated from patients with urinary tract infections.

Survival experiments. The bacterial isolates were grown on blood agar plates for 18 h. Each isolate was suspended in saline to an optical density of 0.9 (540 nm) with a Coleman spectrophotometer (model 6/20), so that when 1 ml was inoculated into 25 ml of sterile urine, there would be approximately 10^6 CFU/ml. This standard inoculum was used in all experiments. Each morning urine specimens were collected and had a mean pH of 6.0. This pH value is similar to previously reported mean urinary pH values of 5.85 (6), 5.98 (7), 6.02 (9), and 6.03 (8). A growth curve for each isolate in urine served as the control for the survival studies with hydrogen peroxide (Fisher Scientific Co.) concentrations of 0.6, 0.3, 0.1, 0.02, and 0.01%. All flasks were incubated in a water bath shaker at 37°C, and 1-ml samples were aseptically removed at time intervals of 1, 5, 15, 30, 60, 120, 240, and 360 min and 24 h. All flasks were reinoculated at 24 h to determine whether H2O2 activity was still present. H2O2 in sterile urine (filter sterilized with a 0.4-μm-pore-size polycarbonate Nuclepore filter) was also stored at room temperature for 5 days to determine whether the H2O2 retained its activity (H2O2 activity was tested by sampling for viable bacteria).

Viability counts. Several dilutions of each sample were plated on Difco brain heart infusion agar (E. coli, S. marcescens, and S. faecalis), Difco tryptic soy agar (S. epidermidis, S. aureus, and Pseudomonas sp.), or Difco MacConkey agar (P. mirabilis). Plates were incubated at 37°C for 24 h, and colonies were counted to determine cell survival after H2O2 exposure. Before diluting and plating for cell survival, samples were treated with 10,000 U of bovine liver catalase per ml (Sigma Chemical Co.) to inactivate all H2O2 present.

RESULTS

The exposure times for reduction of 10^6 to <1 CFU/ml for seven common urinary tract pathogens with various H2O2 concentrations are shown in Table 1. Concentrations of 0.6 and 0.3% H2O2 resulted in an eight-decimal reduction of
the inoculum within 30 min for all isolates, except *S. marcescens*, which required 60 min of exposure to 0.6% H$_2$O$_2$ and 2 h of exposure to 0.3% H$_2$O$_2$. Lower concentrations of H$_2$O$_2$ (0.1 to 0.01%) required exposures of up to 6 h for an eight-decimal reduction of *P. mirabilis*, *Pseudomonas* sp., and *E. coli*. The remaining organisms were reduced to <1 CFU/ml after exposures of up to 24 h.

Representative survival curves showing the growth characteristics of *E. coli* and *S. aureus* in urine with and without H$_2$O$_2$ are shown in Fig. 1 and 2, respectively. *S. aureus*, *S. epidermidis*, and *S. faecalis* had similar growth curves with little or no increase in numbers of viable bacteria for the first 2 h; *S. marcescens*, *Pseudomonas* sp., *E. coli*, and *P. mirabilis* had a one-decimal decrease in viable bacteria during h 1 of incubation. During the entire 24-h incubation period, maximum increases of 1- to 1.5-decimal increases above the original inoculum were observed for all organisms. The generation times ranged from 45 to 90 min.

### Table 1. Time required to reduce $10^8$ to <1 CFU/ml with various concentrations of H$_2$O$_2$ in urine

<table>
<thead>
<tr>
<th>Organism</th>
<th>Time required at following H$_2$O$_2$ concn (%)</th>
<th>0.6</th>
<th>0.3</th>
<th>0.1</th>
<th>0.02</th>
<th>0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. marcescens</em></td>
<td>1 h</td>
<td>2 h</td>
<td>6 h</td>
<td>24 h</td>
<td>24 h</td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>30 min</td>
<td>30 min</td>
<td>1 h</td>
<td>6 h</td>
<td>6 h</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>30 min</td>
<td>30 min</td>
<td>2 h</td>
<td>6 h</td>
<td>24 h</td>
<td></td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>15 min</td>
<td>15 min</td>
<td>1 h</td>
<td>24 h</td>
<td>24 h</td>
<td></td>
</tr>
<tr>
<td><em>S. faecalis</em></td>
<td>15 min</td>
<td>15 min</td>
<td>2 h</td>
<td>6 h</td>
<td>24 h</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>15 min</td>
<td>15 min</td>
<td>30 min</td>
<td>1 h</td>
<td>2 h</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>15 min</td>
<td>15 min</td>
<td>15 min</td>
<td>2 h</td>
<td>4 h</td>
<td></td>
</tr>
</tbody>
</table>
The stability of \( \text{H}_2\text{O}_2 \) in urine was tested by reinoculating the flasks used for survival experiments with \( 10^6 \) CFU/ml. The number of viable bacteria after 5- to 6-h exposures was determined. The data show that flasks originally containing 0.6 to 0.1% \( \text{H}_2\text{O}_2 \) yielded no surviving \( \text{E. coli, S. epidermidis, Pseudomonas} \) sp., or \( \text{S. faecalis} \), whereas \( 10^5 \) CFU of \( \text{S. marcescens} \) per ml survived exposure to 0.6% \( \text{H}_2\text{O}_2 \). Concentrations of 0.02 and 0.1% effectively reduced the initial inoculum to <1 CFU/ml for \( \text{E. coli} \) and \( \text{S. faecalis} \) and reduced the number of viable \( \text{Pseudomonas} \) sp. to \( 10^2 \) CFU/ml, but had little effect on \( \text{S. epidermidis} \) and \( \text{S. marcescens} \).

The stability of \( \text{H}_2\text{O}_2 \) was further tested by storing urine containing 0.6% \( \text{H}_2\text{O}_2 \) at room temperature for 5 days. Suspensions of \( \text{E. coli} \) or \( \text{S. aureus} \) were added, and samples were plated after various periods of exposure to determine viability. \( \text{E. coli} \) and \( \text{S. aureus} \) were reduced to <1 CFU/ml after exposures of 30 min and 3 h, respectively, even after \( \text{H}_2\text{O}_2 \) had been stored in urine for 5 days.

**DISCUSSION**

The results of this study indicate the bactericidal effectiveness and stability of \( \text{H}_2\text{O}_2 \) in urine against a variety of nosocomial pathogens. Although the precise mechanism of cell destruction is not known, \( \text{H}_2\text{O}_2 \) has been shown to cause membrane fragility (10), sensitivity to induced breaks in deoxyribonucleic acid strands (14), mutation (5), and inhibition of deoxyribonucleic acid repair in \( \text{E. coli} \) (15). The sensitivity of various strains of bacteria to \( \text{H}_2\text{O}_2 \) varies (3) and may be affected by levels of cellular catalase (an enzyme responsible for the degradation of \( \text{H}_2\text{O}_2 \) to water and oxygen), the growth phase of the organism, the culture medium, and temperature (1, 2, 16). Catalase is found in most aerobic and facultative anaerobic bacteria that possess cytochrome systems (4). This enzyme is probably involved in the breakdown of the \( \text{H}_2\text{O}_2 \) produced as a product during the aerobic degradation of sugars. Taylor and Achazar (12) demonstrated differences in the catalase activity of a variety of gram-negative organisms. Catalase production is high in \( \text{Serratia} \) spp. and \( \text{Proteus} \) spp., whereas \( \text{Escherichia} \) spp. and \( \text{Klebsiella} \) spp. have little activity. Gram-positive \( \text{Staphylococcus} \) spp. also produce catalase, whereas \( \text{Streptococcus} \) spp. do not.

These observations seem to correlate with the general pattern of \( \text{H}_2\text{O}_2 \) susceptibility of the strains tested in this study. \( \text{S. aureus, S. marcescens, and P. mirabilis} \) required exposure times of 30 to 60 min to 0.6% \( \text{H}_2\text{O}_2 \) for an eight-decimal reduction, whereas \( \text{E. coli, S. faecalis, S. epidermidis, and Pseudomonas} \) sp. required a 15-min exposure. Lower concentrations of \( \text{H}_2\text{O}_2 \) were most effective against \( \text{E. coli} \) and \( \text{Pseudomonas} \) sp., whereas longer exposure times to low \( \text{H}_2\text{O}_2 \) concentrations were required to obtain <1 CFU/ml for \( \text{P. mirabilis} \) and the gram-positive organisms. \( \text{S. marcescens} \) was not inactivated after 24-h exposures to 0.02 or 0.01% \( \text{H}_2\text{O}_2 \). The age of cultures, growth medium, and temperature may also affect the levels of catalase production and sensitivity to \( \text{H}_2\text{O}_2 \). \( \text{S. epidermidis} \) was more sensitive than \( \text{S. aureus} \) to 0.6% \( \text{H}_2\text{O}_2 \), whereas with 0.02% \( \text{H}_2\text{O}_2 \) the sensitivity was reversed. \( \text{S. epidermidis} \) may produce more catalase than \( \text{S. aureus} \) during the longer period of growth permitted at lower \( \text{H}_2\text{O}_2 \) concentrations. The breakdown of \( \text{H}_2\text{O}_2 \) by catalase would effectively decrease the \( \text{H}_2\text{O}_2 \) concentration, thereby enhancing bacterial resistance.

If 100 ml of 3% \( \text{H}_2\text{O}_2 \) is added to a urinary drainage bag and urine is produced at a rate of 1 ml/min, even after 8 h, 0.6% \( \text{H}_2\text{O}_2 \) should be present in the drainage bag. This level should be sufficient to prevent bacterial contamination of the drainage bag even with increased urine output. \( \text{H}_2\text{O}_2 \) in urine retained its bactericidal effectiveness despite exposure to sunlight and room temperature for up to 5 days. The stability in urine, low cost, and minimal toxicity suggest an important role of \( \text{H}_2\text{O}_2 \) in the management of patients with indwelling urethral catheters. Periodic instillations of 3% \( \text{H}_2\text{O}_2 \) into urinary drainage bags should effectively prevent bacterial growth and contamination of the urinary tracts of catheterized patients and of the hospital environment.

**LITERATURE CITED**


