What is the Antimicrobial Activity of Wound and Skin Cleansers at Non-Toxic Concentrations?

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Abstract

Objective: To compare the antibacterial activities of commercially available skin, wound, and skin/wound cleansers at non-toxic concentrations. Methods: Saline and 19 commercial cleansers were evaluated for cytotoxic effects on L929 mouse dermal fibroblasts. Cells were exposed to serial 10-fold dilutions of each cleanser until treatment-induced cytotoxicity was comparable to the baseline cytotoxicity of unexposed control fibroblasts. Time-kill kinetics of these test concentrations of cleansers was tested against methicillin-resistant Staphylococcus aureus ATCC 35919. Results: The experimental design allowed calculation of relative cytotoxicity indexes ranging from 0 to 100,000. Two placebo 188 solutions and saline were found to be the least toxic (cytotoxicity index 0). Chlorhexidine gluconate solution (4.0% w/v), polyborate 20 solution and povidone-iodine (7.5%) most toxic (cytotoxicity index 10,000). At non-cytotoxic concentrations, pure hypochlorous acid (0.01%) was the most rapidly bactericidal, achieving a 4 log kill in CFU in less than 60 seconds. A mixture of hypochlorous acid / sodium hypochlorite (in molar ratio 50:50) at pH 7.4 was next at 30 minutes, while most of the agents tested required > 24 hours. Conclusions: Wound healing depends on maintaining bacterial balance while not damaging the viability of the healing tissues. In vitro toxcity indexes provide helpful guidelines for subsequent in vivo evaluations and clinical applications. The study findings suggest that pure hypochlorous acid (0.01%–) in contrast to many commercially available wound cleaners – has rapid bactericidal activity at concentrations that are safe for human cells.

Introduction

Chronic non-healing wounds, such as venous ulcers and pressure ulcers cause tremendous patient suffering. Treatment of such wounds presents a serious unmet medical need. Strategies that optimize the wound healing have evolved with advances in understanding of the tissue repair process. An ideal wound cleanser provides periodic reduction of bacterial contamination and removal of debris without adversely impacting cellular activities crucial to the wound healing process. Therefore it is important to evaluate wound care products and their potential cytotoxicity.

In this study we determined the non-cytotoxic concentration of saline and 19 widely used skin/wound cleansers and compared the antimicrobial effectiveness of these cleaners at their non-cytotoxic concentrations using methicillin-resistant S. aureus (MRSA) isolate. MRSA infections are a growing concern in wound care.

Materials & Methods

Test Agents: Twenty commercial skin, wound and skin/wound cleansers were evaluated. Cleansers were obtained from manufacturers or distributors (see Table 1).

Cells and Testing: L929 mouse fibroblasts were obtained from ATCC. Cytotoxicity was evaluated by modified methods as described by Wilson et al. (1). Cells were cultured in 96-well plates to approximately 70% confluency prior to exposing them to various cleaners. Cleaners were aspirated off the wells after exposure for 30 min at 37°C. Cells were incubated in fresh media overnight prior to determining cell viability using CellTiter 96® Aqueous One Solution Cell cell proliferation assay. Cleaners were serially diluted 1:10 with PBS.

Modified antimicrobial effectiveness testing: S. aureus ATCC 35919 was grown to log phases, centrifuged and re-suspended to 10^7 CFU/mL, in PBS. 100 μL of the bacterial suspension was added to 1 mL of cleanser solutions diluted to non-cytotoxic concentrations in PBS. Cleaners were incubated with bacteria for 1, 5, 15, 30, 60 mins, 4 hr, and 24 hr at room temperature. Viable cell counts were determined by plating 10-fold serial dilutions of aliquots removed at the indicated times onto TSA. Plates were incubated overnight at 37°C and CFUs were counted.

Results

The time to 4 log kill at the non-cytotoxic dilution of NeutroPhase® (10-fold dilution) was less than 1 min, followed by Puracyn® (16-fold dilution) at 30 min. The time to 4 log kill at non-cytotoxic dilutions of all other tested products were greater than or equal to 24 hours. Nine of the cleansers evaluated met the preservative effectiveness criteria of 1 log reduction in 24 hours according to EP <5.1.3> (2) tested using an MRSA (4.0% w/v), polysorbate 20 solution and povidone-iodine (7.5%) most toxic (toxicity index 10,000). At non-cytotoxic concentrations, pure hypochlorous acid (0.01%) was the most rapidly bactericidal, achieving a 4 log kill in CFU in less than 60 seconds. A mixture of hypochlorous acid / sodium hypochlorite (in molar ratio 50:50) at pH 7.4 was next at 30 minutes, while most of the agents tested required > 24 hours. Conclusions: Wound healing depends on maintaining bacterial balance while not damaging the viability of the healing tissues. In vitro toxcity indexes provide helpful guidelines for subsequent in vivo evaluations and clinical applications. The study findings suggest that pure hypochlorous acid (0.01%–) in contrast to many commercially available wound cleaners – has rapid bactericidal activity at concentrations that are safe for human cells.

Table 1. Toxicity index and modified antimicrobial effectiveness testing of cleaners.

<table>
<thead>
<tr>
<th>Cleanser</th>
<th>Toxicity Index</th>
<th>Modified Antimicrobial Effectiveness</th>
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<tbody>
<tr>
<td>Placebo 188</td>
<td>0</td>
<td>No 4 log kill in 24 hours</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>No 4 log kill in 24 hours</td>
</tr>
<tr>
<td>Chlorhexidine gluconate</td>
<td>10,000</td>
<td>No 4 log kill in 24 hours</td>
</tr>
<tr>
<td>Polyborate 20</td>
<td>10,000</td>
<td>No 4 log kill in 24 hours</td>
</tr>
<tr>
<td>NeutroPhase®</td>
<td>75</td>
<td>&gt; 24 hours</td>
</tr>
<tr>
<td>Puracyn®</td>
<td>100</td>
<td>&gt; 24 hours</td>
</tr>
</tbody>
</table>

Discussion

In this study in vitro methods were used to evaluate the potential deleterious effects of cleansers on wound healing and the antimicrobial effectiveness of cleaners at non-cytotoxic dilutions in PBS, pH 7.9. Products maintained antimicrobial effectiveness. Measuring time to 4 log kill at additional time points allowed further differentiation of products. The time to 4 log kill at the non-cytotoxic dilution of NeutroPhase® was less than 1 min, followed by Puracyn® at 30 min. Both cleansers contain hypochlorous acid, a rapidly acting antimicrobial produced endogenously as part of the body’s innate immune system. NeutroPhase® is a pure hypochlorous acid (HOCl, 0.01%) solution in 0.9% saline at pH 4, while Puracyn® contains electrolyzed water (99.97%), sodium chloride (NaCl) 0.03%, sodium hypochlorite (NaOCl) 0.04%, and hypochlorous acid (HOCl) 0.003%. At a non-cytotoxic dilution of 1:10 in PBS pH 7 (both cleansers consist of an approximately 1:1 mixture of HOCl: NaOCl). Faster activity of NeutroPhase® is likely explained by higher total chlorine content (0.01%) compared to that of Puracyn® (0.007%).

This in vitro study demonstrates that many wound and skin cleansers may be toxic to fibroblasts, one of the key cells in wound repair, and suggests that these cleaners might also be toxic to other cells. Several of the cleansers studied are non-cytotoxic to cells even undiluted, while a single 10-fold dilution is sufficient to render another group non-cytotoxic.

Conclusions

• This study demonstrates that many wound and skin cleansers may be toxic to fibroblasts
• When diluted to concentrations non-cytotoxic to fibroblasts, 9 cleansers maintained antimicrobial effectiveness against MRSA
• NeutroPhase® and Puracyn® had fastest time to 4 log kill of MRSA at the concentrations non-cytotoxic to fibroblasts

References
2. European Pharmacopeia. EP <5.1.3>Efficacy of antimicrobial preservatives