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

Suriani Abdul Rani, Russell Hoon, Ramin (Ron) Najafi, Lu Wang, Dmitri Debabov

NovaBay Pharmaceuticals, Inc., 5980 Horton Street, Suite 550, Emeryville, CA 94608

Abstract

Objective: To compare the antibacterial activities of commercially available skin, wound, and skin/wound cleansers at cell safe (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 *S.aureus* ATCC 33591. **Results**: The experimental design allowed calculation of relative cytotoxicity indexes ranging from 0 to 100,000. Two poloxomer 188 solutions and saline were found to be the least toxic (toxicity index 0); Chlorhexidine gluconate solution (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 reduction 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* toxicity indexes provide helpful guidelines for subsequent *in vivo* evaluations and clinical applications. The study findings suggest that pure hypochlorous acid (0.01%)[e] – in contrast to many commercially available wound cleansers – 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 cleansers 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 cleansers. Cleansers 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. Cleansers were serially diluted 1:10 with PBS.

Modified antimicrobial effectiveness testing. *S. aureus* ATCC 33591 was grown to log phase, centrifuged and re-suspended to 10^9 CFU/mL in PBS. 100 uL of this bacterial suspension was added to 1 mL of cleanser solutions diluted to non-cytotoxic concentrations in PBS. Cleansers were incubated with bacteria for 1, 5, 15, 30, 60 min, 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

Agents Tested	Use	Manufacturer	Nontoxic dilution	Toxicity Index	Time to 1 Log_{10} Kill	Time to 4 Log_{10} Kill
Restore Wound Cleanser	Wound	Hollister Woundcare, Libertyville, IL	10^0	1	24 hrs	>24 hrs
Saline (0.9% sodium chloride)	Wound		10^0	1	>24 hrs	>24 hrs
Shur-Clens®	Wound	ConvaTec, Skillman, NJ	10^0	1	>24 hrs	>24 hrs
Puracyn® OTC	Wound	Oculus Innovative Sciences for Innocyn, Inc., Rialto, CA	10^1	10	<1 min	30 min
WoundClenz OTC Wound Cleanser	Wound	Century Pharmaceuticals, Inc., Indianapolis, IN	10^1	10	24 hrs	>24 hrs
3M™ Wound Cleanser	Wound	3M Health Care, St. Paul, MN	10^1	10	24 hrs	>24 hrs
Dermagran® Wound Cleanser	Wound	Derma Sciences Inc., Princeton, NJ	10^1	10	24 hrs	>24 hrs
NeutroPhase®	Wound	NovaBay Pharmaceuticals, Inc., Emeryville, CA	10^1	10	<1 min	<1 min
Biolex™ Wound Cleanser	Wound	C.R. Bard, Inc., Covington, GA	10^1	10	>24 hrs	>24 hrs
CarraKlenz™ Dermal Wound Cleanser	Wound	Carrington Labs, Irving, TX	10^1	10	>24 hrs	>24 hrs
SAF-Clens™ AF	Wound	ConvaTec, Skillman, NJ	10^2	100	60 min	24 hrs
Prontosan™ Wound Irrigation Solution	Wound	B. Braun Medical Inc., Bethlehem, PA	10^2	100	15 min	>24 hrs
Allclenz® Wound Cleanser	Wound	Healthpoint, San Antonio, TX	10^2	100	60 min	24 hrs
Hydrogen peroxide (3%)	Wound	Hydrox Laboratories,Elgin, IL	10^3	1,000	>24 hrs	>24 hrs
Elta® Perineal Wash	Wound	Smith & Nephew, Largo, FL	10^3	1,000	>24 hrs	>24 hrs
Dermal Wound Cleanser	Wound/Skin	Mahtycke, Norcross, GA	10^3	1,000	>24 hrs	>24 hrs
Johnson & Johnson's® Baby Shampoo	Skin	Johnson & Johnson, Skillman, NJ	10^3	1,000	>24 hrs	>24 hrs
Hibiclens® (Chlorhexidine gluconate solution 4.0% w/v)	Skin	Hollister Woundcare, Libertyville, IL	10^4	10,000	>24 hrs	>24 hrs
Restore Skin Cleanser	Skin	Purdue Products L.P, Stamford, CT	10^4	10,000	>24 hrs	>24 hrs
Betadine® Surgical Scrub (Povidone-iodine, 7.5%)	Skin	Swiss-American Products, Inc., Dallas, TX	10^4	10,000	>24 hrs	>24 hrs

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

Each cleanser was serially diluted 1:10 with PBS (from 0- to 10,000-fold dilutions) and each dilution was tested for cytotoxicity until the results of the cells exposed to the diluted test solutions were similar to those cells exposed to PBS alone.

Shur-Clens®, Restore Wound Cleanser (poloxamer 188 solutions) and saline were found to be the least toxic to fibroblasts, requiring no dilution to maintain viable cells (a toxicity index of zero). Several agents (NeutroPhase®, Puracyn® (mixture of hypochlorous acid and sodium hypochlorite at pH 7.4), WoundClenz OTC, Biolex™, CaraKlenz™, 3M™ Wound Cleanser, and Dermagran®) required only one "10-fold" dilution (a toxicity index of 10). SAF-Clenz™ AF, Prontosan™ and Allclenz® each had a toxicity index of 100. Dermal Wound Cleanser, Johnson & Johnson's® Baby Shampoo, Elta® Perineal Wash and hydrogen peroxide had indices of 1000, while the toxicity index of Betadine® Surgical Scrub (povidone-iodine 7.5%), Hibiclens® (chlorhexidine gluconate solution 4.0 w/v), and Restore Skin Cleanser (polysorbate 20 solution) was 10,000.

The time to 4 log kill at the non-cytotoxic dilution of NeutroPhase® (10-fold dilution) was less than 1 min, followed by Puracyn® (10-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 isolate.

Antibacterial Activity	Low Cytotoxicity (toxicity index 1-100)	High Cytotoxicity (toxicity index 1,000-10,000)
Antibacterial activity at 'cell safe' concentration <1 minute	NeutroPhase®	none
Antibacterial activity at 'cell safe' concentration >1 minute	Puracyn® OTC SAF-Clens™ AF Allclenz® Wound Cleanser	none
Non-antibacterial in 24 hours at 'cell safe' concentration	Restore Wound Cleanser Saline (0.9% sodium chloride) Shur-Clens® WoundClenz OTC Wound Cleanser 3M™ Wound Cleanser Dermagran® Wound Cleanser Biolex™ Wound Cleanser CarraKlenz™ Dermal Wound Cleanser Prontosan™ Wound Irrigation Solution	Hydrogen peroxide (3%) Elta® Perineal Wash Dermal Wound Cleanser Johnson & Johnson's® Baby Shampoo Hibiclens® (Chlorhexidine gluconate solution 4.0% w/v) Restore Skin Cleanser Betadine® Surgical Scrub (Povidone-iodine, 7.5%)

Table 2. Cytotoxicity and antimicrobial effectiveness of skin and wound cleansers.

Potent wound cleansers with a high toxicity index (for example: Betadine, Hibiclens®, hydrogen peroxide) will likely have deleterious effects to living tissue. At the same time a non-cytotoxic wound cleanser (examples: saline, Shur-Clens®, and Restore Wound Cleanser) without antimicrobial effectiveness, will likely provide minimal reduction in bacterial burden.

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 cleansers at non-cytotoxic dilution 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) 0.023%, sodium hypochlorite (NaOCl) 0.004%, 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 cleansers 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

***Disclaimer: NeutroPhase® is a 510k registered product for wound cleansing only. While this study describes the antimicrobial effectiveness in diluted solutions, reduction in microbial growth in the NeutroPhase® solution has not been shown to correlate with a reduction in infections in patients. Clinical studies to evaluate reduction in infection have not been performed.**

References

1. Wilson JR, Mills JG, Prather ID, Dimitrijevich SD. A toxicity index of skin and wound cleansers used on in vitro fibroblasts and keratinocytes. *Advances In Skin & Wound Care* 2005; 18:373 – 378
2. European Pharmacopeia. EP <5.1.3>Efficacy of antimicrobial preservatives