A Relative Toxicity Index for Wound Cleansers

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ABSTRACT: Sixteen commercial wound and/or skin cleansers were evaluated for their toxicity towards polymorphonuclear leukocytes (PMN). PMN were attached to glass cover slips and exposed to the test solutions or physiologic solution for thirty minutes. Following exposure, the PMN were evaluated for viability with the trypan exclusion test and functionality by quantitating their phagocytic efficacy. PMN were exposed to serial, ten-fold dilutions of each cleanser until the resulting cellular viability and functionality were similar to cells run simultaneously but exposed to physiologic solution. A relative index of toxicity was derived from the dilution required to eliminate toxicity. The toxicity index for the sixteen cleansers ranged from 10 to 100,000, indicating one ten-fold dilution to five, ten-fold dilutions. This index may provide useful information for making a decision of which cleanser to utilize in wound care.

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Effective wound management involves the removal of foreign material, bacteria, and necrotic debris from the wound. Isotonic saline is effective in most cases, but clinicians often employ more effective wound cleansing solutions in wounds that are heavily contaminated or are the result of traumatic injury. These cleansing solutions often contain surfactants and/or antiseptic agents which theoretically improve their efficacy. Many surfactants, however, have been shown to be toxic to cells, delay wound healing, and inhibit the wound's defenses against infection.[1,2,3,4] All antiseptics have been show to be toxic to cells.[5,6]

Because the FDA does not regulate wound cleansers, it is the responsibility of each practitioner to select a wound cleansing solution that has been documented to be safe for use in open wounds. Documentation of safety is difficult because standardized tests for wound cleansers have not been established. However, estimates of safety can be made by comparing the results of various cleansers in a standardized reproducible model of cellular toxicity.

The purpose of this study was to develop an easy and reliable screening test which would identify those cleansers which are potentially deleterious to wound cells. By comparing the results, it was anticipated that a useful toxicity index would be developed which would allow easy comparison of the potential toxicity of these wound cleansers.

Methods

Skin and wound cleansers. Sixteen commercial wound and/or skin cleansers were evaluated. All agents were obtained from the manufacturer or their distributor. All agents were used as received except liquid Ivory® soap, which was diluted to 0.5% as the initial use dilution. In order to eliminate any bias in testing, all agents were transferred to separate sterile containers that were only identified by a code number. The identities of the test solutions were revealed only after the results had been finalized. (Exhibit A)

Experimental design. Polymorphonuclear leukocytes (PMNs) were isolated from fresh rabbit blood onto glass coverslips. These PMNs were exposed to the various cleansing solutions for 30 minutes and then assayed for viability and functionality. Viability was accessed by the trypan blue dye exclusion test, and functionality was assessed by the ability of the exposed cells to phagocytize yeast cells. Serial 1:10 dilutions of the cleansers were tested until the results for the cells exposed to the diluted cleansing solution were similar to those of cells exposed to Hank's balanced salt solution (HBSS).

Testing of each cleanser involved blood from 3 different rabbits. Each test agent and each of its 1:10 dilutions being tested were evaluated on 6 coverslips from blood from each reabbit for a total of 18 coverslips per concentration; 9 coverslips for testing of viability and 9 coverslips for testing of functionality. During each experiment, 18 coverslips (6 coverslips from each rabbit's blood sample) were used as control samples and were exposed only to HBSS.

Cell Viability and Functionality Following Exposure to Cleansers

Preparation of cells. This procedure was modified from that of Patselas et al.[7] Unheparinized blood was obtained from anesthetized (Halothane), adult, female New Zealand White rabbits by intracardiac puncture. Aliquots of blood (0.8 ml) were immediately placed onto clean, #2 coverslips and incubated for 30 minutes @ 37°C in humidified chambers. During this incubation, the red blood cells formed a clot that was separated from the glass coverslip by a layer of serum. PMNs and monocytes adhered to the coverslip and remained attached while the blood clot and serum were removed by carefully rinsing the coverslips in HBSS. PMNs comprised 90% of the adherent cells and were the only cells quantitated during this evaluation. Each coverslip with its adherent PMNs was placed cell side up into its own container of HBSS with 10% added autologous serum. Coverslips were stored in this solution at room temperature until utilized (not to exceed 4 hours).

Exposure to test agent. Coverslips were carefully removed from their container, and excess HBSS drained away by placing guaze to the lower edge of the vertically-held slip. Then each coverslip was placed cell side up in a humidified chamber and received 0.3 mls of a test agent. The test agent was either a cleanser, one of the cleanser's 1:10 dilutions, or HBSS as a control. Coverslips were incubated 30 minutes at 37°C with the PMNs in contract with the test agent. After the incubation, the test agent was drained from each coverslip, and the coverslips were gently swirled in a HBSS bath to remove excess test agent before testing for viability and functionality.

Viability assay – Trypan blue dye exclusion. Following exposure to a test agent for 30 minutes, each coverslip was then placed cell side down onto a slide containing a drop of 0.125% Trypan blue dye (wt/vol in sterile isotonic saline). Using gentle pressure with gauze at the coverslip edges only, the excess dye solution was removed, and the coverslip edges were sealed with melted paraffin. The slides were viewed within 10 minutes with a light microscope (40X) and 50-100 cells counted. Viable cells remained unstained, while the nucleus of non-viable cells was stained blue. The ratio of viable cells to the total number of cells counted was recorded as percent viability for each coverslip. The mean percent viability the 9 coverslips exposed to each concentration of cleanser was determined. The concentration of cleanser that resulted in mean viability of ≥ 85% was considered not significantly different than samples exposed to HBSS, and thus was considered non-toxic.

Functionality assay – phagocytic efficiency. Following exposure to a test agent for 30 minutes, the test agent was gently rinsed away and the cells on each coverslip were exposed to 0.5 ml of

saline containing 10^5 yeast cells (see Yeast Preparation). The PMNs were incubated in the presence of the yeast cells for 30 minutes at 37°C in humidified chambers before the yeast solution was rinsed away. The coverslips were then fixed for one minute in absolute methanol, stained for 15 minutes in Giemsa (0.325 mg/ml in methanol), rinsed in distilled water, and air dried. Coverslips were then placed cell side down onto microscope slides and the edges sealed with melted paraffin.

The slides were examined, using 100X oil immersion microscopy. The number of yeast injested per PMN was recorded for 50-100 cells. Then the mean number of yeast injested per PMN was calculated for each coverslip. Each coverslip's result was compared to the results of HBSS-treated controls for that rabbit, and the coverslip result expressed as a percentage of control cell functionality. For each concentration of cleanser tested, the percent functionality for all 9 separate coverslips was used to calculate mean phagocytic efficiency for that concentration of cleanser. When the phagocytic efficiency of a concentration of test cleanser was ≥ 85% of that of cells exposed only to HBSS, that concentration of cleanser was considered not to be significantly different than the controls, and thus was considered non-toxic.

Yeast preparation. One colony-forming unit of a clinical isolate of *Candida albicans* (B311-University of Virginia) was incubated for 24 hours in 30 mls of trypticase soy broth. The yeast were then collected by centrifugation and the broth decanted. The pellet was washed twice with sterile saline and then resuspended in 2 mls of saline. The yeast was diluted in sterile saline, and the final solution was made in HBSS with 10% autologous serum. The final concentration was 10^5 yeast/0.5mls.

Toxicity Index

A toxicity index was assigned to each test agent based upon the results from the viability and phagocytic efficiency assays. The dilution of each test agent was identified in which both the viability and phagocytic efficiency of treated cells was similar to HBSS-treated (control cells). The toxicity index was the denominator of that dilution. Thus, if the non-toxic dilution was 1/1000, the toxicity index would be 1000.

Results

There was a wide range in the number of dilutions required to eliminate toxicity from the sixteen cleansers tested (See Table 1). Shur Clens® was least deleterious to PMNs, requiring no dilution to maintain viable cells and only a 10^{-1} dilution to allow full phagocytic function. Biolex™, Saf Clens™, and Cara Klenz™ were slightly more deleterious, requiring a 10^{-2} dilution to be similar to controls. The majority of the cleansers required either a 10^{-3} or 10^{-4} dilution before losing their deleterious effects. The Bard™ and Hollister™ skin cleansers required a 10^{-5} dilution before PMNs were fully viable and functional.

The toxicity index was defined as the dilution required for both viability and phagocytic efficiency to be similar to cells exposed to HBSS. The toxicity indexes for the 16 cleansers ranged from 10 to 100,000. (See Table 2).

Discussion

Many wound and skin cleansers are now available for use in patient care. Unfortunately, the Food and Drug Administration (FDA) does not require manufacturers to provide safety and efficacy results for review prior to distribution. Each manufacturer is responsible for the safety

and efficacy of their product, but there are no standardized criteria for what is safe and what is efficacious.

This study was designed to suggest one preliminary screen for determining relative safety of wound cleansers. The PMN was selected as the test cell because its function of phagocytizing microbes can be readily quantitated. PMNs provide the first line of defense for the open wound against microbial contamination. Thus, inhibition of PMN function predisposes the wound to the development of infection.

In this study, the PMNs were exposed to the wound cleanser solution for 30 minutes. This exposure time was an arbitrary decision based on a compromise of several clinical practices. Some clinicians irrigate wounds for a brief period and then rinse the wound surface. Others apply the cleanser to the wound with saline to remove the cleanser from the wound surface. Others apply the cleanser to the wound and allow it to soak and soften the wound debris for a while before they irrigate or cleanse the wound. Although most of the chemical trauma is probably induced upon acute contact, 30 minutes of exposure was selected to insure that cellular toxicity from extended contact was not missed. A wound cleanser should not be toxic to the wound cells not matter how long it is left in the wound, since most practitioners do not rinse the wound cleanser from the wound following irrigation or cleansing.

The results of this study indicated that the relative toxicities of wound cleansers ranged from 10 to 10,000. Shur Clens® was the least toxic wound cleanser with a score of 10. Shur Clens® did not alter cell viability in its use concentration, but because of its surface active properties, it did reduce phagocytic efficacy compared to cells suspended HBSS. Thus, the score of 10 does not represent toxicity but inhibition of phagocytosis. Wound Cleansers with scores greater than 10 were associated with inhibition of cell viability and function. A score of 10,000 suggests that the wound cleanser is 1000 times more toxic to PMNs than Shur Clens® under these *in vitro* test conditions. The *in vivo* consequences of these toxicity values need to be determined.

Skin cleansers are formulate to be stronger cleansers than wound cleansers. Thus, it is not unexpected that the skin cleansers were more toxic to PMNs than wound cleansers. The toxicity indexes for the skin cleansers ranged from 1,000 to 100,000. It might be suspected that the harsher the skin cleanser, the greater the chance for skin damage and irritation. Thus, the selection of a skin cleanser needs to be a balance between cleansing efficacy and skin irritation.

Skin cleansers should never be allowed to contact the wound surface. Skin cleansers are too harsh to contact the sensitive tissue of the open wound. However, wound cleansers may be used as gentle skin cleansers when the cleansing action required is not too demanding. Products that are indicated for use as both a wound and skin cleanser should be evaluated with caution. Such cleansing products cannot be formulated to be effective skin cleansers yet be mild enough not to harm the cells of the open wound.

The primary rule of patient care is “first, do no harm.” With respect to cleansing wounds, this means choosing a wound cleanser that is compatible with the healing wound cells. Since the FDA does not regulate wound cleansers, it is the responsibility of the practitioners to know which wound cleansers are the most biocompatible. The test results reported in this study are just the beginning of the types of studies required to evaluate the relative toxicities of the available wound cleansers.

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Exhibit A

The sixteen cleansers were:

Agent	Intended Primary Use	Manufacturers
Shur Clens®	Wound	Calgon Vestal Labs St. Louis, MO
SAF Clens™	Wound	Calgon Vestal Labs St. Louis, MO
Biolex™	Wound	Catalina Biomedical Corp. Duarte, CA
Cara-Klenz™	Wound	Carrington Labs, Inc. Dallas, TX
Ultra Klenz™	Wound	Carrington Labs, Inc. Dallas, TX
Constant-Clens™	Wound	Sherwood Medical St. Louis, MO
Clinical Care™	Wound	Care-Tech® Laboratories St. Louis, MO
Dermal Wound Cleanser	Wound	Smith & Nephew United, Inc. Largo, FL
Puri-Clens™	Wound	Sween Corporation Mankato, MN
Uni Wash®	Skin	Smith & Nephew United, Inc. Largo, FL
Betadine® Surgical Scrub	Skin	Purdue Frederick, CO. Norwalk, CT
Hibiclens®	Skin	Stuart Pharmaceuticals Wilmington, DE
Hollister™ Skin Cleanser	Skin	Hollister TM Inc. Libertyville, IL
Bard™ Skin Care Deodorizing Cleanser	Skin	Bard® Home Health Div. Murray Hill, NJ
Techni-Care™ Surgical Scrub	Skin	Care Tech® Laboratories St. Louis, MO
Liquid Ivory® Soap (0.5%)	Skin	Proctor & Gamble Cincinnati, OH

Table 1.

Dilution of wound or skin cleanser required to result in cellular performance similar to that of control cells following 30 minutes of exposure.

Cleanser	Intended Primary Use	Non-Toxic Dilution	
		Viability	Phagocytosis
Shur-Clens®	Wound	None	10^{-1}
Biolex™	Wound	10^{-1}	10^{-2}
Saf Clens™	Wound	10^{-2}	10^{-2}
Cara Klenz™	Wound	10^{-2}	10^{-2}
Ultra Klenz™	Wound	10^{-2}	10^{-3}
Clinical Care™	Wound	10^{-3}	10^{-3}
Uni Wash®	Skin	10^{-3}	10^{-2}
Ivory Soap®	Skin	10^{-3}	10^{-2}
Constant-Clens™	Wound	10^{-3}	10^{-3}
Dermal Wound Cleanser	Wound	10^{-3}	10^{-4}
Puri-Clens™	Wound	10^{-3}	10^{-4}
Hibiclens®	Skin	10^{-4}	10^{-4}
Betadine® Surg Scrub	Skin	10^{-4}	10^{-4}
Techni-Care™ Scrub	Skin	10^{-4}	10^{-5}
Bard™ Skin Care Deodorizing Cleanser	Skin	10^{-5}	10^{-5}
Hollister™ Skin Cleanser	Skin	10^{-5}	10^{-5}

Table 2.

Toxicity Indexes for Sixteen Wound or Skin Cleansers

Cleanser	Intended Primary Use	Toxicity Index
Shur Clens®	Wound	10
Biolex™	Wound	
Saf Clens™	Wound	100
Cara Klenz™	Wound	
Ultra Klenz™	Wound	
Clinical Care™	Wound	
Uni Wash®	Skin	1,000
Ivory Soap® (0.5%)	Skin	
Constant Clens™	Wound	
Dermal Wound Cleanser	Wound	
Puri-Clens™	Wound	10,000
Hibiclens®	Skin	
Betadine® Surg Scrub	Skin	
Techni-Care™ Scrub	Skin	
Bard™ Skin Cleanser	Skin	100,000
Hollister™ Skin Cleanser	Skin	