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The use of detergent irrigation for musculoskeletal wounds

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Abstract The primary purpose of irrigation is to remove bacterial contaminants from the wound. Surfactants do that by disrupting the bonds of the organism to the surface. The use of this wound care strategy was studied in a series of investigations spanning several years. In vitro experiments revealed that surfactant irrigation was superior to saline or antibiotic solutions for removal of adherent bacteria from metallic surfaces, from bone, and from bovine muscle. An in vivo model of the complex orthopedic wound was developed. The superiority of surfactant irrigation over saline or antibiotic solution was demonstrated in animal wounds containing metal, bone injury, and soft tissue damage. Specificity of different surfactant irrigations for various bacterial species was demonstrated. A sequential surfactant irrigation protocol was developed and shown effective in the polymicrobial wound with established infection.

Résumé Le but fondamental de l'irrigation est d'enlever les contaminants bactériens de la plaie. Les surfactants font cela en interrompant les liaisons de l'organisme avec la surface. L'usage de cette stratégie du soin de la blessure a été étudié dans une série d'enquêtes qui couvrent plusieurs années. Les expérimentations in vitro ont montrées que l'irrigation par surfactant était supérieure aux solutions salines ou antibiotiques pour éliminer les bactéries adhérentes aux surfaces métalliques, à l'os, et au muscle bovin. Un modèle in vivo de la blessu-

re orthopédique complexe a été développé. La supériorité d'irrigation par surfactant sur les solutions salines ou antibiotiques a été démontrée chez l'animal dans des blessures comprenant des éléments métalliques, des lésions osseuses et des parties molles. La spécificité de différents surfactants vis à vis de différentes espèces bactériennes a été démontrée. Un protocole séquentiel de l'irrigation au surfactant a été développé et s'est montré aussi efficace dans les blessures polymicrobiennes avec infection établie.

Introduction

In modern times, wounds are commonly irrigated with sterile normal saline (NS), with or without additives. The most commonly studied additives have been antiseptics or antibiotics. Over the past 8 years, we have been studying a third category of irrigation additive – surface-active agents or detergents (also known as 'surfactants'). This research program began with the observation that, while irrigating an orthopedic wound with a solution containing an antibiotic, the solution foamed profusely. The foaming suggested that the disinfectant efficacy of the antibiotic irrigant could be due to the 'surface-active' soap-like properties of the antibiotic rather than to the antimicrobial properties. To test this proposition, we began an interdisciplinary investigation to improve our understanding of wound disinfection, particularly in regards to the contaminated and infected orthopedic wound.

Materials and methods

Based on hydrophobic interactions, we hypothesized that soap irrigation might be superior to other solutions at disrupting the initial surface attachments of bacteria. The soap chosen for study was liquid Castile soap, which is mostly potassium oleate – the alkali salt of oleic acid.

In experiment number 1, we selected an inanimate surface represented by 3.5 mm stainless steel screws, and a strain of *Sta-*

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phyllococcus epidermidis that produces a glycocalyx coating ("slime"). The screws were incubated overnight in bacterial broth to produce a uniform coating of adherent bacteria, and then irrigated with 1 l of solution by either jet lavage (Pulsavac wound debridement system, Zimmer, Inc., Warsaw, Indiana, USA) or by bulb syringe. One set of screws served as an unirrigated control. The solutions tested were sterile NS, bacitracin (50,000 u/l), neomycin sulfate 0.5% in saline, Polymyxin/neomycin (100,000 u Polymyxin B and 1 gm neomycin sulfate in 1 l), and liquid Castile soap (18 ml in 1 l normal saline [NS]). After irrigation, the screws were sonicated to remove all residual bacteria, and the number of bacteria remaining on each after irrigation was quantitated by culture of serial dilutions of the sonicate fluid.

In experiment number 2, we used the same methods with different bacterial species and different surfaces [3]. Stainless steel screws, titanium screws, and human cortical bone fragments of uniform size and shape were coated with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or *S. epidermidis* by incubation overnight in seeded broth. Irrigation was performed using a jet lavage system and a liter of one of four solutions: saline, bacitracin, neomycin, or Castile soap. A fifth group of specimens served as non-irrigated controls. After irrigation, each specimen was sonicated and quantitatively cultured. Colony counts were made on duplicate cultures of three dilutions and then averaged. The data was analyzed with a one-way ANOVA, with pair-wise multiple comparisons made using Tukey's method.

In experiment number 3, we surveyed the various types of surfactants for disinfectant efficacy. The goal was to test a variety of detergent types to see if there were differences between them. We tested nine different surfactant agents in five different *in vitro* assays [18]. The agents tested were Castile soap, oleic acid, sodium dodecyl sulfate (SDS), benzalkonium chloride (BK), cholamidopropyl-dimethyl ammonio-propanesulfonate (triton), Shur-Clens (a commercially available wound detergent), Pluronic F-68 (primary ingredient of Shur-Clens), dimethyl sulfoxide (DMSO), and glycerin. The assays used included a microtiter plate assay for bacterial attachment, bulb syringe irrigation of biofilm-coated screws, tube dilution techniques for bactericidal activity, activity against preformed biofilm, and effectiveness in jet lavage for colonized screws.

Experiment number 4, the first study of BK as a wound irrigant, compared a 1:2000 concentration of BK to NS in an *in vitro* model that consisted of muscle contaminated with three different species of bacteria. Strips of aseptically harvested bovine muscle were incubated for 15 min with 1×10^7 colony-forming units (CFU) of *S. aureus*, *S. epidermidis*, or *P. aeruginosa*. The muscle strips were then irrigated with 100, 1,000, or 10,000 ml of solution using a Water Pik. The samples were then emulsified in 10 ml of saline using sonication, and quantitatively cultured.

In experiment number 5, Sprague-Dawley rats were used to develop an animal model of the contaminated orthopedic wound that would include injured muscle and bone and a metallic implant [19]. Under sterile conditions, we created a 4-cm dorsal lumbar incision and pushed a 20-gauge needle through a hole in a lumbar spinous process. A 30-gauge stainless steel wire was passed through the hole, tied over the bone, and the ends cut. The wound was inoculated with 0.1 ml of bacterial suspension (usually 10^6 or 10^7 organisms) and the inoculum was allowed to sit for 15 min. We then irrigated the wound with the test solution using a Pulsavac power irrigator with the power setting at four, and closed the wound with nylon sutures. The animals were examined each day for signs of wound infection. Fourteen days after the procedure, we sacrificed the animals in a carbon dioxide chamber, reopened the surgical wound, and cultured the superficial wound (subcutaneous) tissue, the deep wound (submuscular) tissue, and the wire. We compared 1 l of 0.1% BK to the same volume of sterile NS in wounds contaminated with either 10^6 or 10^7 *S. aureus*.

In experiment number 6, this *in vivo* model of a complex, contaminated wound was used to compare BK to different irrigation solutions against different bacterial species. We contaminated the wound with 1×10^6 CFU of either *S. aureus* or *P. aeruginosa*, and irrigated the wound with 3 l of one of the following: saline, 0.05%

Castile soap solution, 0.03% BK solution, and 33,000 u/l of bacitracin solution.

In experiment number 7, we tried to restore the safety and efficacy of BK by first irrigating the wound with BK and then with Castile soap. A group of animals was inoculated with *P. aeruginosa* and the wounds were irrigated with either 3 l of NS or 1 l each of BK, Castile soap, and saline, in that order.

In experiment number 8, to test the efficacy of sequential irrigation for a wound with polymicrobial infection, we inoculated the animal model with both 10^3 *S. aureus* and 10^6 *P. aeruginosa*. This inoculum was based on the demonstrated ID_{50} of each bacterial species. After 15 min, we irrigated with 3 l of saline or 1 l each of Castile soap, NS, and BK, and a half liter NS rinse, in that order. The rationale for this order of surfactants was to first remove *P. aeruginosa* from the wound with Castile soap, the saline would remove residual cellular products, and the anionic soap (which may deactivate the BK), and the BK would address the *Staphylococcus* [6].

In experiment number 9, to examine the role of hardware in established wound infection, we modified our animal model to allow testing of the sequential irrigation protocol. After inoculating the wound with 10^4 *S. aureus*, we closed the wound for 24 h prior to irrigation with either saline or sequential surfactants (Castile soap, saline, BK). This allowed development of an established wound infection. The wire was then left in place, removed, or replaced with a new wire.

Experiment number 10 was designed to test surfactant irrigation and, in particular, the sequential surfactant irrigation (SSI) protocol against wild bacteria with demonstrated pathogenicity. Samples of bacterial species were collected from patients with osteomyelitis or orthopedic implant infection. We tested three strains of *S. aureus* and two of *S. epidermidis* in the animal model, as described previously, using a bacterial suspension of 1×10^8 organisms in 100 μ l to contaminate the wound. After allowing the inoculum to sit for 15 min, we washed the wound with 3 l of either saline or Castile soap, saline, BK (SSI). For the animals that received *S. epidermidis* inoculum, a group of animals was inoculated but not irrigated to serve as control. The animals were sacrificed and a culture of three sites was done at 2 weeks, as described above.

Results

The statistically significant results of experiment number 1 showed that: (1) Any irrigation was better than no irrigation; (2) Jet lavage was better than bulb syringe at removing this species of bacteria from this surface with any fluid; (3) Antibiotic was no more effective at removing bacteria than saline; (4) The use of Castile soap solution removed significantly more bacteria than saline or any antibiotic solution [2] (Fig. 1).

The results of experiment number 2 varied somewhat based on bacterial species and on surface type [3]. All irrigation solutions were better than nonirrigated controls on all surfaces for all bacteria. For the stainless steel surface, the soap solution was the best at removing all three bacteria and was always better than saline but not significantly different than antibiotic for *S. aureus* or *P. aeruginosa*. For titanium, the soap again was the best overall, and always better than saline. It was better than bacitracin for *S. aureus* but equivalent to the antibiotics for *P. aeruginosa*. The results for the bone surface showed less clear differences. There was no difference between solutions for *S. epidermidis*, soap was better than bacitracin but equivalent to neomycin and saline for *S. aureus*,

Fig. 1 Removal of *Staphylococcus epidermidis* from steel surfaces. Logarithmic plot of the number of residual bacteria after screw irrigation with different solutions and by different methods. All irrigation groups were significantly different than control, and all power irrigation groups were significantly less than bulb groups. Within the power and bulb groups, there was no significant difference between solutions, except the soap group, which was significantly lower than all others

Table 1 Removal of different bacteria from various surfaces. Irrigation solutions are displayed, arranged from left to right in order of decreasing efficacy in bacterial removal. Treatments that share a red underline are not significantly different; treatments in the

same row that do not share a red underline are significantly different ($P < 0.05$). *Neo* neomycin solution, *Baci* bacitracin solution, *NS* normal saline

Bacteria	Surface	Irrigation solution best → worst			
<i>Staphylococcus epidermidis</i>	Steel	Soap	<u>Neo</u>	<u>NS</u>	<u>Baci</u>
	Titanium	Soap	<u>Neo</u>	<u>Baci</u>	<u>NS</u>
	Bone	<u>Neo</u>	<u>Soap</u>	<u>Baci</u>	<u>NS</u>
<i>Staphylococcus aureus</i>	Steel	<u>Soap</u>	<u>Neo</u>	<u>Baci</u>	<u>NS</u>
	Titanium	<u>Soap</u>	<u>Neo</u>	<u>Baci</u>	<u>NS</u>
	Bone	<u>Soap</u>	<u>Neo</u>	<u>NS</u>	<u>Baci</u>
<i>Pseudomonas aeruginosa</i>	Steel	<u>Neo</u>	<u>Soap</u>	<u>Baci</u>	<u>NS</u>
	Titanium	<u>Neo</u>	<u>Soap</u>	<u>Baci</u>	<u>NS</u>
	bone	<u>Neo</u>	<u>Soap</u>	<u>NS</u>	<u>Baci</u>

and neomycin was better than the other three solutions for *P. aeruginosa*. Thus, the liquid soap solution removed at least as many or more bacteria for all three species on all three surfaces, except one situation – *P. aeruginosa* on bone (Table 1).

In experiment number 3, we found a significantly greater efficacy for surfactant-enriched solutions when applied by jet lavage than for NS alone. Three agents showed “first round” activity against *S. epidermidis*, *S. aureus*, and *P. aeruginosa*: oleic acid, SDS, and BK. Of those, only BK showed activity against preformed biofilm and against all three bacterial species. With jet lavage, BK increased bacterial removal by 100–100,000 times over bulb syringe irrigation.

The results of experiment number 4 showed that contaminated muscle samples irrigated with BK had significantly less bacteria recoverable for all three species at all volumes of irrigation ($P < .001$). Further, it appeared that increasing the volume of irrigation resulted in significant increases in bacterial removal with each increment for BK, while the bacterial removal with saline reached a plateau at 1 l. Increasing the irrigation volume to 10 l of saline resulted in no further decrease in recoverable bacteria. With BK, however, there were no recoverable bacteria after 10 l of irrigation for all three species [11] (Fig. 2).

In experiment number 5, there was no difference in the rate of culture-positive superficial wounds but, for all

Fig. 2 Effect of increasing volume of irrigation. Recoverable bacteria after irrigation of muscle contaminated with *Pseudomonas aeruginosa*. Similar curves were obtained for *Staphylococcus aureus* and *Staphylococcus epidermidis*

other comparisons (deep wound, wire, total positive cultures, and number of culture-positive animals), BK was significantly superior to saline irrigation ($P<.001$). The total number of positive cultures was 75% less in the BK irrigation group. In addition, histologic sections were made of wounds in animals irrigated but not inoculated with bacteria for evaluation by a veterinary pathologist who was blinded to the treatment group. Using a scoring system of seven histologic criteria to look for evidence of tissue toxicity, the pathologist found no significant difference between the tissue of the saline-irrigated rats and those of the BK-irrigated rats [19].

In experiment number 6, irrigation efficacy varied with bacterial species. For the *S. aureus*, only BK irrigation significantly lowered the culture-positive rate compared to saline. For *P. aeruginosa* contamination, Castile soap irrigation reduced the culture-positive rate com-

pared to saline. Furthermore, in the *P. aeruginosa*-contaminated animals, BK irrigation actually significantly increased the number of positive wound cultures. In fact, BK caused wound dehiscence and breakdown in 75% of the animals, which was severe enough that several had to be euthanized prematurely [6]. This finding of variable efficacy and complication rate introduced the concept of specificity in irrigation additives.

In experiment number 7, the combination irrigation regimen proved effective against *P. aeruginosa*, significantly lowering the positive culture rate compared to saline while at the same time preventing wound breakdown [6] (Table 2). Nevertheless, the infection rate with the combination regimen was greater than with Castile soap alone [6].

Experiment number 8 was successful, with sequential irrigation significantly reducing the number of culture-positive animals and culture sites. Quantitation of the CFU recovered with the culture swabs showed significantly lower numbers for sequential irrigation compared to saline for all three culture sites – superficial, deep, and wire [4] (Fig. 3).

In experiment number 9, with the implanted wire left in place, the SSI significantly – but modestly – reduced the percentage of positive cultures from all sites after 2 weeks compared to saline irrigation – 40% versus 58% ($P<.001$). Removal of the wire from the spinous process at the time of irrigation significantly ($P<.001$) reduced the number of positive cultures in both irrigation groups to the same level – approximately 20%. At least one site was positive in 35% of rats in the saline group and 41% of rats in the SSI group. When the wire was

Table 2 Specificity in irrigant/contaminant interactions. Number of positive cultures over total number of cultures. NS normal saline, BK benzalkonium chloride, CS Castile soap, Baci bacitracin

solution, SSI sequential surfactant irrigation (BK/CS/NS). Green highlight is significantly better than NS controls, yellow highlight is significantly worse than NS controls ($P<0.05$)

Irrigation Solution	Culture Site	Trial 1: <i>P. aeruginosa</i>	Trial 2: <i>S. aureus</i>	Trial 3: <i>P. aeruginosa</i>
NS	Superficial	9/20	18/20	4/9
	Deep	11/20	19/20	5/9
	Wire	7/20	19/20	5/9
BK	Superficial	16/20	2/20	5/10
	Deep	18/20	3/20	7/10
	Wire	18/20	4/20	6/10
CS	Superficial	2/20	15/20	0/10
	Deep	5/20	17/20	0/10
	Wire	3/20	18/20	1/10
Baci	Superficial	5/20	16/20	N/A
	Deep	8/20	19/20	N/A
	Wire	7/20	17/20	N/A
SSI	Superficial	N/A	N/A	0/10
	Deep	N/A	N/A	3/10
	Wire	N/A	N/A	3/10

Fig. 3 Cleansing the polymicrobial wound. Comparison of the number of positive cultures at each site in the polymicrobial (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) wound inoculation. Statistical significance between levels of positive culture: superficial $P=0.009$, deep $P=0.046$, wire $P=0.046$. NS normal saline, SSI sequential surfactant. Irrigation (Castile soap, NS, benzalkonium chloride)

Fig. 4 Comparison of normal saline (NS) and sequential irrigation (SSI) on wounds with established infection involving hardware. First pair, hardware left in place; second pair, all hardware removed; third pair, hardware exchanged (removed before irrigation, replaced with fresh after irrigation)

removed prior to irrigation and exchanged for a fresh, sterile wire placed after irrigation, the culture-positive rate went up again for the NS group but the SSI group demonstrated significantly – and again dramatically – better results. The percentage of all positive culture sites remained about 20% for the SSI group but the saline group culture-positive rate went up to 40% ($P<0.05$) (Fig. 4) [14].

In experiment number 10, for animals inoculated with *S. epidermidis*, saline irrigation alone decreased the percentage of animals with at least one positive culture site from 50% (ten of 20) to 15% (three of 20), a significant difference ($P<0.05$). For animals inoculated with *S. aureus*, saline irrigation alone was ineffective – 100% (12 of 12) of animals had at least one positive culture site. Sequential irrigation reduced that percentage to 50% (six of 12) [17]. The difference between the two bacterial species may reflect the enhanced and specific initial surface binding of *S. aureus* compared to the more nonspecific surface attachment mechanisms of *S. epidermidis* [5, 7].

Discussion

The use of soaps to cleanse traumatic wounds was once commonly advocated and practiced by surgeons [10, 16]. With the advent of the antibiotic age, the use of soaps in traumatic wounds has fallen by the wayside and is not a part of standard treatment, while antibiotic irrigation has been accepted in some areas with little experimental support.

Bacterial infection of a wound begins with adhesion of the bacteria to a surface, which occurs in four distinct stages: nonspecific attachment, specific attachment, in situ multiplication, and release/dissemination [12]. The forces involved in the early nonspecific attachment are gravitational, electrostatic, and hydrophobic in nature, and it is these forces that surfactant irrigation seeks to disrupt. Surfactants lower surface tensions and disrupt the hydrophobic or electrostatic forces that form the initial bond between bacteria and the surface. Surfactants accomplish this by virtue of fact that they consist of two molecular domains – a hydrophobic end and a hydrophilic end. A subcategory of surfactants, the detergents, have the additional capacity to form micelles which can surround organisms and prevent them from clumping together or attaching to the surface, effectively keeping them suspended where they can be rinsed from the wound. Surfactants can be categorized based on the nature of their hydrophilic moiety. If it is charged, it can be cationic (positive charge), also known as “invert soaps,” or anionic (negative charge), as in most soaps. Amphoteric surfactants have both charges present, and nonionic surfactants have uncharged hydrophilic portions.

While there are some potential detrimental effects of surfactants, particularly endothelial damage [21], and while there have been some in vitro cellular toxicity and occasional skin irritation observed [9], soaps are generally well tolerated by body tissues when used in mild concentrations. We saw no evidence of histologic changes in wounds contaminated with *Staphylococci* and irrigated with BK. There were wound-healing problems seen with BK irrigation when the wound had been contaminated with *P. aeruginosa*. That interaction was the only adverse effect of surfactant irrigation we ever observed in a long series of studies, and it was eliminated with the institution of the sequential irrigation protocol.

During experiments with the polymicrobial contamination of the orthopedic wound, we noticed that the ID_{50} was reduced when both organisms were present. The ID_{50} of *S. aureus* was reduced from 1×10^6 to 1×10^3 – a 1,000-fold decrease – when *P. aeruginosa* was present in the wound. Similarly, the ID_{50} of *P. aeruginosa* was reduced by ten times by the presence of *Staphylococcus*. This enhancement of pathogenicity by multiple organisms, known as bacterial synergy, had been observed before [8, 15], but this was the first demonstration of such interactions between these common orthopedic pathogens in a complex orthopedic wound model. Further exploration of this interaction in our model using varying amounts of inoculum of the two organisms led to intrigu-

Fig. 5 Bacterial synergy. Percentage of animals with culture-positive results after inoculation with 10^3 organisms of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or both organisms together. Infection rate in animals inoculated with both is significantly higher than either alone ($P=0.004$)

ing observations, suggesting a complex interaction between the two pathogens and the host. At low levels of inoculum (10^2 – 10^4) with both organisms, a significantly higher infection rate was observed than the additive rate with either organism alone, demonstrating bacterial synergy in the traditional sense (Fig. 5). At higher concentrations of inoculating organisms (10^5 – 10^7), the infection rate actually decreased, and declined to a level significantly below what would be expected for either organism alone. In all animals inoculated with both organisms, no matter how much *P. aeruginosa* was inoculated into the wound – even up to 10^7 CFUs (an inoculum that causes 100% infection rate by itself) – no *P. aeruginosa* was ever recovered from the wound by culture – only *S. aureus* [13]. These fascinating and perplexing observations have not yet been explained or further investigated.

Our studies have shown that surfactant irrigation is superior to antibiotic or saline irrigation at removing adherent bacteria from orthopedic wound materials such as soft tissue, metal, and cortical bone. This result is further strengthened by the addition of mechanical energy through high-pressure lavage, the “car-wash” effect. Surfactant irrigation is superior to saline at reducing the number of positive cultures in our animal model of the complex contaminated orthopedic wound, and there seems to be some specificity to the interaction between bacterial contaminant species and the type of surfactant used to irrigate the wound. Because of this specificity, we developed a sequential SSI protocol that incorporates an anionic fatty acid salt (Castile soap) with a quaternary ammonium cationic surfactant (BK) to optimize efficacy against Gram-negative and Gram-positive organisms, both individually and in the polymicrobial wound. We have demonstrated a complex and biphasic interaction between two bacterial species and a host wound, which may add another level of complexity to the specific in-

teraction between contaminant and irrigant. The SSI protocol has been shown to be effective not only in wounds contaminated with tame lab bacteria, which are less virulent [1, 20], but also in wounds contaminated with wild pathogens and in infected wounds with hardware. Future directions of study involve clinical utilization of a surfactant irrigation protocol. A human study presently is in progress comparing Castile soap to bacitracin irrigation for open fractures of the lower extremity. With more than 350 patients in the study so far, there is no significant difference in infection rate, nonunion rate, or wound-healing difficulties. Understanding the proper role of surfactant irrigation will await completion of this and other studies.

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