

The Effect of Antiseptic Solutions on Microorganisms in Venous Leg Ulcers

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The effect on the microbial ulcer flora of wet gauze dressings soaked in antiseptic solutions used for desloughing leg ulcers is not known. Quantitative cultures were therefore performed in 45 venous leg ulcers, before application and after 15 minutes' treatment with gauze dressings with four different antiseptic solutions: aluminium acetotartrate (Alosol) 1%, potassium permanganate 0.015%, acetic acid 0.25% and chloramine 0.25%. The percentage of ulcers with each type of microorganism did not differ before and after application of the antiseptic solutions. *Staphylococcus aureus* was found in 79% of the ulcers, gram-negative rods in 39%, *S. epidermidis* in 21%, *Proteus spp* in 21%, *Pseudomonas spp* in 14% and fungi in none.

Potassium permanganate reduced the mean number of bacteria per ulcer from 4.4×10^6 to 0.9×10^6 (ns), chloramine from 2.7×10^6 to 2.2×10^6 (ns), Alosol from 1.2×10^7 to 3.5×10^6 (ns) and acetic acid from 6.3×10^6 to 2.6×10^5 ($p = 0.007$).

S. aureus was reduced by acetic acid ($p = 0.002$), gram-negative rods by both chloramine ($p = 0.03$) and acetic acid ($p = 0.03$). The number of *Pseudomonas*, *Proteus*, *S. epidermidis* and *Streptococcus haemolyticus group G* was not reduced significantly ($p > 0.05$) by any of the solutions. **Key words:** varicose ulcers; leg ulcer therapy; local anti-infective agents; bacteria.

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Treatment with graduated elastic support is essential for the healing of venous ulcers (1), but local ulcer treatment is also important (2). In clean ulcers without necrosis and debris and with granulation tissue, healing conditions seem to be optimal. Wet dressings with saline or antiseptic solutions are commonly used in patients with sloughy leg ulcers (3). The effects on microorganisms of wet dressings with antiseptic solutions in venous leg ulcers have not been studied in detail.

In this investigation, the aim was to study the effect of four antiseptic solutions on the number of microorganisms in sloughy exudating venous leg ulcers.

MATERIAL AND METHODS

Patients, antiseptic solutions and dressings

Twenty-nine ambulatory patients (18 women and 11 men) with a mean age of 75 years were included in the study. They came for ambulatory treatment to the Department of Dermatology, Sahlgrens' Hospital, Göteborg, Sweden. The patients had at least one exudating sloughy venous leg ulcer larger than 1 cm², and altogether 45 ulcers were included in the study. All patients gave their informed consent to inclusion in the study and the Ethical Committee of Sahlgrens' Hospital, University of Göteborg, approved the study.

The following sterile solutions were used: aluminium acetotartrate (Alosol) 1%, acetic acid 0.25%, potassium permanganate 0.015% and

chloramine (N-chloro-benzene-sulphonamide-sodium) 0.25%. At least 10 ulcers (one ulcer per patient) were allocated to each solution in consecutive patients. All types of dressings were allowed to be included in the study, since the results of cultures were compared before and after the use of the solution in the same ulcer and not between ulcers.

Various dressings were used the week before the study. All patients had compression bandages: Wero normal non-adhesive elastic bandage (Wernlii, Switzerland) or Coban self-adherent wrap (Medical Products Division, 3M, USA).

Experimental procedure

In vitro. Solutions were tested in vitro for their inhibitory effect on *Streptococcus haemolyticus group A*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Candida albicans*. The organisms were grown on blood agar at 37°C. Suspensions containing 10⁴ cells/ml from 24 h-cultures of the microorganisms were made in phosphate-buffered saline (PBS), pH 7.4, and 0.3 ml of the suspensions containing the test organisms was spread on blood agar with a bent glass rod. Wells (5 mm) were cut in the agar and 0.1 ml of the test chemical solution added. Plates were incubated at 37°C and read after 24 h.

In vivo. Culture samples were obtained from the central part of the ulcer directly after the dressing was removed, after cleansing, after the antiseptic solution had been applied for 15 min and covered with Saran wrap and then removed, and in 12 ulcers also after 30 min. The ulcers were cleansed by wiping off the ulcer surface with a swab. Topical anaesthesia was not given. Debridement with a curette or scissors was not performed. Sterile cotton gauze was moistened with the antiseptic solution and applied to the ulcer as a wet dressing.

Culture method (4, 5)

A sterile cotton tip was moistened with Triton X (Triton X 0.1% in PBS 0.075 mol/l; pH 7.9) and, after application to the centre of the ulcer, rotated 20 full turns and then placed in 1 ml of the Triton X solution. The sample was then mechanically shaken in a Vortex Jr mixer for 20 s. Aliquots (0.1 ml) were removed and diluted in 10-fold steps in PBS and transferred to blood agar plates. Plates were incubated at 37°C for 24 h. After incubation, colonies of different morphological types were counted and selected for identification of the bacterial type. Gram staining was done and appropriate biochemical tests were performed for identification. Anaerobic cultures were not performed. Sabouraud plates were incubated and cultured for fungi.

Statistical methods

Descriptive statistics were used, and for comparisons between bacterial numbers before and after the solutions a non-parametric test (Wilcoxon's signed rank test) was used with two-tailed *p*-values.

RESULTS

The percentage prevalences of microorganisms in the leg ulcers after cleansing were as follows: *S. aureus* in 79%, *S. epidermidis* in 21%, *St. hemolyticus group B* in 2%, *St. hemolyticus group G* in 5%, *Non-hemolyticus st.* in 2%, *Enterococcus spp* in 2%, *Proteus spp* in 21%, *Pseudomonas spp* in 14%, *Klebsiella spp* in 4%, gram-negative rods (*Acinetobacter*, *Citrobacter diversus*, *Morganella morganii*, *Serratia marescens*, *Enterobacter spp*) in 39% and difteroids in 7%, and these numbers did not differ

Table I. The number of bacteria (mean values) after cleansing (A) compared with after the application of solutions for 15 min (B)

Number of ulcers	Solution	Mean number of bacteria/ulcer	SD	95% Confidence interval	p
10	Potassium perm. sol. 0.015%	A 4.4.10 ⁶ B 0.9.10 ⁶	1.2.10 ⁶ 2.1.10 ⁶	-3.8.10 ⁶ to 1.3.10 ⁷ -0.5.10 ⁶ to 2.5.10 ⁶	ns
11	Alsol sol. 1%	A 1.2.10 ⁷ B 3.5.10 ⁶	2.8.10 ⁷ 7.7.10 ⁶	-7.6.10 ⁶ to 3.1.10 ⁷ -1.7.10 ⁶ to 8.6.10 ⁶	ns
12	Chloramine sol. 0.25%	A 2.7.10 ⁶ B 2.2.10 ⁶	4.2.10 ⁶ 4.5.10 ⁶	3.6.10 ⁴ to 5.3.10 ⁵ -6.2.10 ⁵ to 5.1.10 ⁶	ns
12	Acetic acid sol. 0.25%	A 6.3.10 ⁶ B 2.6.10 ⁵	1.5.10 ⁷ 2.7.10 ⁵	-2.9.10 ⁶ to 1.6.10 ⁷ 8.3.10 ⁴ to 4.4.10 ⁵	p=0.0068

compared to either before cleansing or after antiseptic solutions (ns). There was no growth of fungi.

The in vitro studies with sodium chloride 0.9%, water, acetic acid 0.25% and potassium permanganate 0.015% did not show any inhibition zones. Alsol solution 1% showed an inhibitory zone for *St. hemolyticus* group A (10 mm). Chloramine 0.25% showed inhibitory zones for *S. aureus* (11 mm), *S. epidermidis* (11 mm) and *E. coli* (10 mm).

The effect of 15 minutes' treatment with the antiseptic solutions on the bacteria in the ulcers is shown in Table I. All the solutions had reducing effects, but only acetic acid significantly ($p=0.007$). In Table II the effect of each solution on *S. aureus* is shown; acetic acid was effective here also ($p=0.002$). The number of gram-negative rods, found in 20 ulcers, was reduced significantly by chloramine ($p=0.03$) and acetic acid ($p=0.03$). *Pseudomonas*, found in 8 ulcers, was reduced by acetic acid (4 ulcers) from 6.3.10⁵ to 1.9.10⁵, but not significantly (ns). *Proteus*, *S. epidermidis*, *St. haemolyticus* group B, G or *Non-hemolyticus* st. were not significantly reduced ($p>0.05$) by any of the solutions. There was no further reducing effect after 30 min.

DISCUSSION

Clinical infection (cellulitis or erysipelas) in ulcers is known to retard wound repair (6, 7), and should be treated with systemic antibiotics. Most leg ulcers seem to be colonised with bacteria and sometimes fungi (8-11). Some types of microorganisms might cause wound healing retardation by producing toxins or other products. The presence of large numbers of microorganisms without clinical signs of infection might also influence

ulcer healing, but this issue is still under debate. Reducing bacterial counts below 10⁵ per gram tissue or cm² surface area has been reported to affect ulcer healing favourably (12). However, the use of topical and systemic antibiotics should be minimised to avoid the development of resistant strains (13).

Wet saline dressings are used to cleanse and to dry ulcers from exudation. Antiseptics in the wet dressing could theoretically be of value to reduce the bacterial load and prevent infection.

The in vitro and the in vivo results were not in complete concordance in this study. Chloramine showed most inhibitory effects in the in vitro studies and acetic acid in the in vivo studies.

Acetic acid 0.25% reduced the total number of bacteria, *S. aureus* and gram-negative rods when applied for 15 min to venous leg ulcers. Acetic acid has previously been reported to eradicate *P. aeruginosa*, but no other bacteria, when applied repeatedly as a 5% solution to burns and superficial wounds (14). In the present study, no statistically significant reductive effects on the number of *Pseudomonas* were found. This lack of statistically significant effect could be explained by the low number of ulcers or a real lack of effect caused by too low a concentration of the acetic acid and/or indicate that it should be used repeatedly to be effective against *Pseudomonas*. Acetic acid 0.25% in this study showed surprisingly good effects on all other types of bacteria, especially *S. aureus* and the gram-negative rods. No microbial inhibition was seen with potassium permanganate 0.015% in vivo or in vitro, and the only effect of Alsol solution 1% was found in vitro on *St. hemolyticus* group A.

Table II. The number of *S. aureus* (mean values) after cleansing (A) compared with after the application of solutions for 15 min (B)

Number of ulcers	Solution	Mean number of <i>S. aureus</i> /ulcer	SD	95% Confidence interval	p
10	Potassium perm. sol. 0.015%	A 4.9.10 ⁵ B 9.1.10 ⁵	7.4.10 ⁵ 2.1.10 ⁶	-4.6.10 ⁴ to 1.0.10 ⁶ -5.4.10 ⁵ to 2.5.10 ⁶	ns
8	Alsol sol. 1%	A 1.1.10 ⁶ B 3.3.10 ⁵	2.4.10 ⁶ 5.2.10 ⁵	-9.5.10 ⁵ to 3.1.10 ⁶ -1.0.10 ⁵ to 7.6.10 ⁵	ns
10	Chloramine sol. 0.25%	A 8.1.10 ⁵ B 2.2.10 ⁶	1.3.10 ⁶ 4.5.10 ⁶	-8.9.10 ⁴ to 1.7.10 ⁶ -1.4.10 ⁶ to 5.0.10 ⁶	ns
10	Acetic acid sol. 0.25%	A 3.8.10 ⁶ B 1.4.10 ⁵	7.3.10 ⁶ 2.5.10 ⁵	-1.4.10 ⁶ to 9.1.10 ⁶ -3.6.10 ⁴ to 3.2.10 ⁵	p=0.002

The antiseptics were designed to reduce bacterial contamination on intact skin (15). Some have the opinion that solutions that cannot be tolerated in the conjunctival sac should not be used in an open ulcer (16). Antiseptic solutions have been reported to give irritative, toxic and allergic reactions (17–19). High concentrations (16) give more irritative and toxic reactions than low (17). Wound healing has been reported to be both promoted (20) and delayed (18). No clinically noticeable adverse reaction was observed in this study, but the effects on tissues or cells have not been investigated further.

The effects of low concentrations of chloramine, acetic acid, aluminium acetotartrate and potassium permanganate on the number of microorganisms in venous leg ulcers has not previously been studied. The circumstances differ in fibroblast cultures compared with venous leg ulcers. Antiseptic solutions are probably gradually inactivated by proteins in the ulcer exudate. This study does not answer the question of how long the reducing effects on the bacterial colonisation remain, since we only studied the effect after 15 and 30 min.

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