Use of wet-to-moist cleansing with different irrigation solutions to reduce bacterial bioburden in chronic wounds

Objective: The influence of different irrigation solutions, in conjunction with wet-to-moist cleansing, on the reduction of sessile, non-planktonic bacteria which colonise wounds, has not been investigated. In this study, the antibacterial effect of different irrigation solutions, during a 20-minute wet-to-moist cleansing, has been evaluated in chronic wounds.

Methods: This study was designed as a prospective cohort study with 12 study arms and was conducted between June 2011 and April 2016. Patients with chronic wounds present for more than three months, irrespective of previous treatments, were recruited into this study. Quantitative wound swabs were obtained before and after a 20-minute, wet-to-moist cleansing, using different wound irrigation solutions. Sterile 0.9% saline served as a control.

Results: We recruited 308 patients, of which 260 patients with 299 chronic wounds were eligible for analysis. *Staphylococcus aureus* was the most common recovered (25.5%) microorganism, of which 8% were meticillin-resistant *Staphylococcus aureus* (MRSA) strains. Although 0.9% saline supported cleansing of the wound bed, it did not significantly reduce the bacterial burden. The highest reduction of bacterial burden was achieved with an aqueous solution containing betaine, zinc and polyhexamethylene biguanide (polihexanide; ln RF=3.72), followed by a 3% saline solution containing 0.2% sodium hypochlorite (ln RF=3.40). The most statistically significant reduction of bacterial burden, although not the highest, was achieved with povidone-iodine (ln RF=2.98; p=0.001) and an irrigation solution containing sea salt 1.2% and NaOCl 0.04% (ln RF=2.51; p=0.002).

Conclusion: If a reduction of bacterial burden is warranted, wound irrigation solutions containing a combination of hypochlorite/hypochlorous acid, or antiseptics such as polihexanide, octenidine or povidone-iodine, ought to be considered.

Declaration of interest: Professor Assadian was member of the Hutchinson Santé's medical advisory board and Mölnlycke Medical Advisory Board. He declares having received consulting and lecture fees travel compensation, and speakers' honoraria from Altrazeal Europe Ltd., Antiseptica chem. GmbH, B. Braun Melsungen AG, Ethicon Ltd., Lohmann & Rauscher GmbH & Co. KG, Mundipharma GmbH, Nawa Heilmittel GmbH, Quantum Management & Service GmbH, Schülke & Mayr GmbH and 3M Deutschland GmbH in the past. Professor Leaper has received travel and accommodation expenses, together with honoraria for teaching and participation in advisory/consultation groups from Ethicon Ltd., Pfizer Ltd., Carefusion, and Smith & Nephew. Dr. Eberlein is a member of Zuellig Pharma's medical advisory board. He declares having received consulting and lecture fees travel compensation, and speakers honoraria from Altrazeal Europe Ltd., B. Braun Melsungen AG, Lohmann & Rauscher GmbH & Co. KG, Nawa Heilmittel GmbH and 3M Deutschland GmbH in the past. None of the companies had any influence on the design of the study or interpretation of results. All other authors have no potential conflict of interest relevant to this article to report.

bacteria • chronic wound • wet-to-moist cleaning phase • wound irrigation

Wound bed preparation (WBP) is the first step in the care of any chronic skin defect after identification and management of underlying, responsible, pathological factors, such as ischaemia, pressure or diabetes.[1,2] The aim of WBP is to eliminate necrotic tissue, debris and microbial bioburden from the wound bed through techniques of maintenance debridement. Furthermore, it is to safeguard the wound edges, protect the periwound skin, and to support and facilitate optimal healing.[3]

The most effectively used step of WBP is sharp mechanical debridement, followed by other methods including autolytic, enzymatic, biologic (larval debridement), or wet-to-dry debridement.[3,4] Other than debridement, a wet-to-dry method of wound cleansing (or more correctly, a 'wet-to-moist technique') has been also described,[5] which should not be confused with the wet-to-dry method of debridement.[3]

The aim of a wet-to-moist cleansing of a wound is to facilitate subsequent removal of dry necrotic tissue, fibrinous material, non-viable external contamination, and to some extent decontamination/suppression of

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microbial colonisation. This technique involves the use of a gauze pad, which is wetted thoroughly with a sterile solution poured from a container, spray bottle or a syringe. After a minimum of 15 to 20 minutes, optimally up to 60 minutes, the gauze pad should still be moist and can be removed, causing minimal trauma to the underlying wound bed and, in sensitive patients, with less pain.[5]

An additional aspect to be considered is reduction of the bacterial bioburden in the wound bed, although this is not the primary aim of the wet-to-moist cleansing technique. Efforts aimed at reducing bacterial load have a number of clinical goals, including infection prevention, reduction of malodour and augmentation of healing. Consequently, the reduction of bioburden, as well as the elimination of virulence factors produced by certain bacterial strains, has attracted the attention of wound care professionals. However, questions have been raised regarding the choice of the most appropriate irrigation solution, which should be chosen for its antimicrobial effect, as well as its effectiveness during the wet-to-moist wound cleansing phase.[6,7]

This cleansing technique was initially described using lukewarm, sterile 0.9% saline or Ringer's solution,[5] but now a number of different wound irrigation solutions, including several which contain antimicrobial preservatives, are available. However, the influence of these different irrigation solutions, used in conjunction with a wet-to-moist cleansing of wound care, on the reduction of sessile, non-planktonic bacteria which colonise wounds, has not been investigated. In this study the antibacterial effect of different irrigation solutions during a 20-minute wet-to-moist treatment of chronic wounds has been studied.

Methods

All data analysed were collected as part of routine diagnosis and treatment, and patients were diagnosed and treated according to national guidelines and standards.[8–10] All included patients gave written informed consent to treatment, data collection and participation. Following informed consent, eligible patients were recruited prospectively from the Wound Competence Centre Linz (WCC, part of the Academy for Certified Wound Management (ACWM) Zurich, Switzerland) between June 2011 and April 2016.

Individuals were included if one or more arterial ulcer, pressure ulcer (PU), venous leg ulcer (VLU), mixed arterial-venous leg ulcer or diabetic foot ulcer (DFU) had been present for more than three months, irrespective of previous treatments. Subjects were not eligible if they were pregnant, had received systemic antibiotics within 14 days before dressing change, had a known allergy to the applied wound irrigation solutions, or were unable to give their consent.

Outcome measure

The primary outcome measure of the study was the difference in the quantitative number of microorganisms per 1cm^2 of wound surface harvested before and after a 20-minute wet-to-moist[5] cleansing with different irrigation solutions.

Randomisation

Patients were randomised to one of the 12 wound irrigation solutions investigated, using a computerised, randomisation programme, dynamic allocation and stratification by wound size. In the event of bilateral wounds, both wounds were wetted with the identical test compound. Both the patient and the wound care-givers were aware which irrigation solution was being used. However, neither the microbiologist processing samples, nor the individual conducting the statistical analysis, had any knowledge on the assigned treatment arm.

Intervention and investigated irrigation solutions

After aseptically removing the dressing in place at dressing changes, wounds were gently cleansed with physiological saline (0.9% NaCl) and gauze, and a first quantitative wound swab (COPAN eSwab 480CE, COPAN Diagnostics Inc.,US) was obtained. A sterile gauze (Lohmann & Rauscher, Germany) was then wetted with either 0.9% saline (B. Braun AG, Melsungen, Germany) or one of the following wound irrigation solutions containing antimicrobial preservatives:

- Sea salt 3% and 0.2% sodium hypochlorite (NaOCl) Actimaris forte, Actimaris AG, Switzerland)
- Sea salt 1.2% and NaOCl 0.04% (Actimaris solution, Actimaris AG, Switzerland)
- Hypochlorite (ClO^-) and hypochlorous acid (HCIO) 57mg/l (Anosteralyt 30%; Aquis GmbH, Germany)
- NaCl 0.023% and NaOCL 0.004% + hypochlorous acid 0.003% (Microdacyn60, Oculus, US)
- A 10% povidone-iodine (PVP-I) solution with 10% free iodine (1% povidone-iodine; Betaisodona; Mundipharma, Austria)
- H_2O, lithium-magnesium-sodium-silicate, Sal Maris, nascending O_2 (Biosept; patch 2012; or patch 2013, GlucoMetrix, Germany)
- H_2O, cocamidopropyl betaine, zinc, iron, polyhexamethylene biguanide (polihexanide; PHMB; Nawalution; NAWA Heilmittel GmbH, Germany)
- 0.1% octenidin dihydrochlorid (OCT), ethylhexylglycerin, H_2O (Octenillin; Schulke & Mayr, Germany)
- An aqueous solution of 0.1% PHMB and undecylenamidopropyl-betaine (Prontosan; B.Braun AG, Germany).

The wetted gauze was placed on a wound without mechanical stress on the wound bed. After 20 minutes' application time, the moist gauze was gently removed and a second quantitative wound swab was obtained from the identical location.

Microbiological processing

Samples were obtained following the Levine wound swab technique.[11] All obtained samples were immediately transported to the microbiological laboratory of the Keppler University Hospital, Linz,

Table 1. Baseline characteristics of patients and wounds

	Male	Female	All	p-value
No. of patients (%)	104 (40.0)	156 (60.0)	260 (100.0)	<0.01*
Age mean±SD years	60±8	76±11	72±12	0.76
Duration of wound mean±SD years	1.2±3.8	2.7±4.4	72±12	0.18
Wound size (mean cm^2±SD)	11±9	9±5	10±8	0.63
No. of wounds (%)	118 (39.5)	181 (60.5)	299 (100.0)	<0.01*
Venous leg ulcer (%)	35 (36.1)	62 (63.9)	97 (32.4)	<0.01*
Mixed leg ulcer (%)	17 (19.3)	71 (80.7)	88 (29.4)	<0.01*
Arterial leg ulcer (%)	23 (58.9)	16 (41.1)	39 (13.0)	0.28
Pressure injury (%)	23 (63.9)	13 (36.1)	36 (12.0)	<0.01*
Diabetic foot ulcer (%)	18 (56.3)	14 (43.7)	32 (10.7)	0.49
Other wounds (%)	2 (28.6)	5 (71.4)	7 (2.5)	0.34

SD—standard deviation; *significant difference p≤0.05

Table 2. Yielded bacterial spectrum of 299 wounds in 260 patients

Organism	Number (n) of positive wounds	Proportion (%) of all wounds
Staphylococcus aureus	75	25.5
meticillin-resistant Staphylococcus aureus (MRSA) subset	6	(8%)
Proteus mirabilis	52	17.7
Enterococcus spp.	48	16.3
Pseudomonas aeruginosa	42	14.3
Escherichia coli	28	9.5
Klebsiella sp.	13	4.4
Acinetobacter sp.	7	2.4
Candida sp.	4	1.4
Gram-negative, other	30	10.2
Gram-positive, other	22	7.5

Austria. Swabs were streaked on Columbia agar with 5% sheep blood (Becton Dickinson GmbH, Germany) and McConkey agar (BioMérieux SA, France). Agar plates were incubated aerobically at 35°C in 5% CO_2 for 48 hours. Growth was identified at the genus and species level (Vitek 2, BioMérieux SA) and reported quantitatively as colony forming units (CFUs). Count of CFUs was logarithmically transformed and results were compared as natural logarithm (ln) CFU.

Sample size

A priori power analysis based on previous measurements (data not shown) demonstrated that 11 participants of each intervention and a minimal bacterial bioburden of 10^3 CFU per wound would be sufficient to test the null-hypothesis of equality (α=0.05, power 95%) of planktonic bacterial bioburden before and after the wet-to-moist cleansing phase.

Statistical analysis

All CFU counts were logarithmically transformed into the natural logarithm (ln) and further processed. Two-sample comparisons at per wound level (CFU count before and after wet-to-moist wound cleansing) were performed using two-sample t-tests (two-tailed homoscedastic paired t-test) or Wilcoxon rank-sum test for continuous and ordinal variables, where appropriate. Statistical significance was considered at p≤0.05. Analysis was performed using SAS (Version 9.2, SAS Institute Inc., US).

Results

We enrolled and randomised 308 patients. Of these, 48 patients withdrew or were excluded from further analysis because a first or second wound swab was missing; 260 (84%) patients with 299 wounds had complete data on the primary endpoint. Enrolled patients had 97 VLUs (32.4%; n=72 were bilateral VLUs), 88 mixed venous-arterial ulcers (29.4%; n=59 were bilateral ulcers), 39 arterial leg ulcers (13%; n=12 were bilateral ulcers), and 32 (10.7%) DFUs (n=3 were bilateral ulcers).

In addition, 36 wounds (12%) were sacral PUs and seven chronic wounds had other underlying causes (three lymphatic wounds and four unknown). Compared with male patients, female patients presented significantly more frequently with venous (p<0.01) or mixed arterial-venous (p<0.01) leg ulcers, while male patients had significantly more PUs (p<0.01). Characteristics of the study cohort are summarised in Table 1.

Microbial colonisation spectrum

A wide variety of different bacteria were identified in wounds, with 8% of cultures demonstrating multiple organisms. *Staphylococcus aureus* was the most common recovered (25.5%) microorganism, of which 8% were meticillin-resistant *Staphylococcus aureus* (MRSA) strains; The MRSA proportion reflects the current MRSA frequency in Austria.[12] *Enterococcus* spp. were the second most frequent Gram-positive bacteria (16.3%) colonising wounds. *Proteus mirabilis* (17.7%), *Pseudomonas aeruginosa* (14.3%) and *Escherichia coli* (9.5%) were the most frequently isolated Gram-negative rods. The bacterial spectrum is summarised in Table 2.

Reduction of bacterial bioburden

Using 0.9% saline during wet-to-moist cleansing did not significantly reduce the planktonic bacterial burden on wounds. The highest reduction of bacteria was achieved with an aqueous solution containing cocamidopropyl betaine, zinc, iron, and the antiseptic PHMB (ln RF=3.72) (ln RF denotes for the bacterial reduction factor, expressed as natural logarithm), followed by a 3% sea salt solution with 0.2% NaOCl (ln RF=3.40). However, while not the highest, the most significant reduction in bacterial burden was achieved with povidone-iodine (ln RF=2.98; p=0.001) and an irrigation solution based on NaCl 0.023% and NaOCl 0.004% in combination with hypochlorous acid 0.003% (ln RF=2.51; p=0.002; Table 3). Interestingly, the results also demonstrate that combining two different

irrigation solutions may not necessarily result in higher reduction factors than using single irrigating solutions alone. This was shown, trend-wise, for the combination of 0.1% OCT and an aqueous solution containing 0.1% PHMB and undecylenamidopropyl-betaine. While the OCT-based solution achieved an ln RF of 2.90 within 20 minutes of application, and the undecylenamidopropyl-Betaine cleansing solution an ln RF of 1.54, the combination decreased the efficacy of the OCT-based solution to ln RF 2.88. This difference, however, was not statistically significant (p=0.856). Furthermore, in the case of two different batches of aqueous lithium-magnesium-sodium-silicate oxygen, manufactured one year apart, the fresh batch achieved a reduction of ln RF 1.01, and the one-year older batch only ln RF 0.74. This difference reached borderline significance (p=0.049).

In summary, except for 0.9% NaCl solution, a 0.1% PHMB and undecylenamidopropyl-betaine solution, and aqueous lithium-magnesium-sodium-silicate oxygen, all investigated solutions achieved measurable and significant bacterial reduction when used as irrigation solutions during a wet-to-moist cleansing over 20 minutes' application time.

Table 3. Results of bacterial reduction after 20 minutes' application of test solutions or control

Test compound	No. of patients	No. of wounds	V (ln)	N (ln)	ln RF	p-value
Nawalution	11	13	13.11	9.39	3.72	0.005*
ActiMaris forte 3%	20	23	11.21	7.81	3.40	0.005*
Povidone-Iodine 1%	22	27	10.57	7.59	2.98	0.001*
Anosteralyt	14	14	11.18	8.22	2.96	0.014*
Octenilin	22	23	9.41	6.51	2.90	0.015*
Prontosan + Octenilin	16	16	10.79	7.92	2.88	0.047*
ActiMaris sensitive 1.2%	31	33	9.91	7.40	2.51	0.002*
Microdacyn 60	17	31	13.44	11.59	1.86	0.031*
Prontosan	33	36	11.90	10.36	1.54	0.051
Biosept (2013)	37	41	10.95	9.94	1.01	0.251
Biosept (2012)	25	28	11.02	10.28	0.74	0.512
NaCl 0.9%	12	14	11.51	11.02	0.49	0.761

V—before wet-to-moist cleansing; N—after wet-to-moist cleansing; ln—natural logarithm; ln RF—natural log reduction factor; p-value based on two-tailed homoscedastic t-test; *significant difference p≤0.05

Discussion

Although previously it has been reported that wet-to-moist cleansing with 0.9% NaCl might reduce the number of planktonic bacteria on a wound surface,[5] this study showed that physiological saline alone is not able to significantly reduce the microbial bioburden. If an additional antimicrobial effect is desired, irrigation with wound antiseptic solutions should be used for the wet-to-moist cleansing. Antiseptics based on PHMB, OCT, PVP-I or some hypochlorite/hypochlorous acid compositions may be applied for this purpose. However, our results also demonstrate that microbial reduction on wound surfaces is significantly lower than that observed against planktonic bacteria during *in vitro* experiments.

Limitations

In view of the results it also has to be pointed out that the irrigation solution with the highest $\log_{10}$ reduction factor is not necessarily the most suitable solution to be used in conjunction with wet-to-moist cleansing. The study design does not allow conclusions on wound healing or prevention of development of wound infection to be drawn. Furthermore, the role and significance of bacteria during the wound healing process is controversial. While some authors consider the bacterial density to be critical in wound healing and development of infection,[13,14] others consider the type and virulence of bacteria to be of greater relevance.[15,16] These and other factors such as microbial synergism, the host immune response and the quality of tissue must be considered holistically in assessing the probability of wound infection.[17]

Another limitation of this study is the bacterial sampling technique. While wound swabs taken follow the Levine technique,[11] it is suggested that tissue biopsy is the gold standard for determining wound bacterial bioburden.[17,18] However, only a few studies have compared wound swabs with biopsies for the diagnosis of chronic infected wounds or the ability to identify bacterial bioburden.[19,20] Obtaining swabs and curetted tissue from chronic wounds has been shown to yield similar recovery rates for common wound bacteria.[19] This is in line with the results of a review in which the Levine technique showed a high positive (77%) and negative (91%) predictive value close to that found with wound biopsy.[20] However, we feel that the best sampling technique for detecting bacteria in a wound has not yet been identified and validated. This may include questioning if culturing samples under aerobic conditions is sufficient or if culture conditions for detection of anaerobe bacteria should also be conducted. Indeed, our processing methods may further limit our results, since the presence and faith of anaerobe bacteria were not investigated. It may be possible that only polymerase chain reaction (PCR) techniques are able to provide an accurate picture of the microbiome of a wound, yet they do not allow distinction of viable from non-viable organisms.

Finally, in a subset of patients, our study also demonstrated that combining different irrigation solutions does not increase the antibacterial efficacy. Indeed, in 16 patients, a combination of a 0.1% OCT and an aqueous solution containing 0.1% PHMB and undecylenamidopropyl-betaine had been used. While the logarithmic reduction factor of the OCT-based solution was significantly higher than the bacterial reduction of a cleansing solution containing PHMB and betaine, the combination of both reduced the antimicrobial efficacy of the OCT-based solution.

However, there is little-to-no justification in combining different irrigation solutions and no manufacturer of irrigation solutions recommends such an approach. This study provides objective results that combining different irrigation solutions containing antimicrobial compounds does not increase the antimicrobial efficacy of certain mixtures, and that the opposite may occur.

Conclusion

In conclusion, this study demonstrates that using 0.9% NaCl during wet-to-moist cleaning shows no effect on reducing bacteria on a wound. If bacterial reduction is warranted, wound irrigation solutions based on a hypochlorite/hypochlorous acid combination, polihexanide (a preservative in the US), octenidine or povidone-iodine should be considered. **JWC**

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