The efficacy of sodium hypochlorite antiseptic: a double-blind, randomised controlled pilot study

Objective: According to a recent clinical trial, 82% of hard-to-heal wounds harbour levels of bacteria that impede healing. A follow-up analysis of trial data revealed that the use of antiseptic cleansers did not correlate with bacterial burden. At a minimum, these findings suggest the need for clinical research into the efficacy of antiseptics in reducing bacterial burden. Evidence supporting the bacterial killing ability of antiseptics is largely derived from preclinical and laboratory studies. Few clinical trials have examined bacterial levels and healing rates in hard-to-heal wounds. Fortunately, the advent of fluorescence imaging to detect bacterial burden has simplified the conduct of clinical research examining the effectiveness of antiseptics in the clinic setting. The aim of this study was to evaluate the efficacy of a modified sodium hypochlorite (NaOCl) solution in reducing wound size and bacterial load in hard-to-heal wounds.

Method: In this randomised, double-blind pilot study, patients were randomised to one of two groups: daily wound cleansing with either normal saline solution (NSS) or NaOCl. Patients and investigators were blinded to the allocation. All wound types were included.

Results: A total of 16 patients consented to participate. At the initial visit, the target ulcer was measured and a fluorescence image to evaluate bacterial load obtained. The wound was then cleansed with either NSS or NaOCl and fluorescence imaging repeated. Patients cleansed the wound daily in accordance with the randomisation schedule. They returned to the clinic weekly for four weeks, and on each visit the wound was measured and a fluorescence image captured. Patients receiving NaOCl had a greater percent reduction in wound area versus NSS; although the first phase of the study was not powered for statistical significance, there was a strong trend favouring NaOCl. In addition, there was greater bacterial reduction in the NaOCl group.

Conclusion: Based on the results of this pilot study, enrolment has continued in order to increase the study's power. This pilot study suggests that sodium hypochlorite is efficacious in reducing bacterial burden and promoting healing.

Declaration of interest: This research was sponsored by Anacapa Technologies Inc., US. The authors have no conflicts of interest.

antiseptic • bacterial infection • chronic wound • diabetes • hard-to-heal wound • infection • sodium hypochlorite • ulcer • wound • wound care • wound healing

A recent clinical trial demonstrated that 82% of hard-to-heal wounds have bacterial levels that impede wound healing (>10^4 colony forming units (CFU)/g).[1] Subsequent analysis of the trial data showed that the use of antiseptics does not correlate with bacterial burden at any level. The authors concluded that antiseptic use is haphazard at best.[2] Clinicians across the US have embraced antiseptic cleansers in the treatment of hard-to-heal wounds. The evidence for cleansers is based on bacterial kill rates in laboratory studies and data from pre-clinical research. This new evidence highlights the need to study cleansers and topical antimicrobials in human clinical trials on hard-to-heal wounds.

In the past, conducting clinical trials on bacterial levels required biopsies for quantitative tissue culture. This invasive and expensive technique hindered research efforts. The advent of fluorescence imaging, that inexpensively detects bacterial burden in real-time, has simplified research on the efficacy of antiseptic agents. Fluorescence imaging (MolecuLight, US) is a validated technique that accurately identifies bacterial levels >10^4 CFU/g.[1,3] Incorporating fluorescence imaging obviates the need for biopsies and the cost associated with quantitative analysis in many clinical trials.

This investigation examined the efficacy of a modified sodium hypochlorite (NaOCl) solution (Anasept Antimicrobial Skin & Wound Cleanser, Anacapa Technologies Inc., US) in healing and reducing bacterial levels in hard-to-heal wounds. During World War I, Henry Dakin introduced the hypochlorite-based antiseptic to treat battlefield wounds.[4] In the hundred years since, NaOCl has emerged as a popular antiseptic. Although clinicians today occasionally prescribe the original 0.5% Dakin's solution, it suffers from several drawbacks. It is unstable, lasting only 30 days,[5] and there are also concerns with regard to cytotoxicity at high concentrations.[6] Modern formulations have addressed these shortcomings. The antiseptic wound cleanser (AWC) examined in this study has a shelf-life

Thomas E Serena,[1] MD, FACS, President and Trustee*; **Laura Serena**,[1] LPN, Vice-President of Research Operations; **Omar Al-Jalodi**,[1] MD, Natrox Wound Care Fellow; **Keyur Patel**,[2] DO, Medical Director; **Kristy Breisinger**,[1] Research Analyst
***Corresponding author email:** serena@serenagroups.com
1 SerenaGroup Research Foundation, Cambridge, MA, US. **2** SerenaGroup Advanced Wound and Hyperbaric Center, Monroeville, PA, US.

Table 1. Patient demographics and wound types

Patient	Age (years)	Sex	Comorbidities	Wound type	Wound age (weeks)
1	59	Female	Anxiety, depression, psoriasis, insomnia, hyperlipidaemia, hypomagnesaemia, DM II, gastroesophageal reflux disease, CVI, COPD, hypertension	VLU	6
2	62	Male	Lymphoedema, CVI, VLU	VLU	20
3	55	Male	Hypertension, CVI	VLU	8
4	52	Female	Abdominal wound, DM II, hypertension	Surgical wound	35
5	63	Male	Hyperlipidaemia, chronic pain—general body, hypertension, lymphoedema, peripheral neuropathy, gastroesophageal disease, DM II, CVI	VLU	20
6	72	Male	Coronary artery disease, hypertension, venous insufficiency, VLU, lymphoedema of leg, stasis dermatitis of leg, onycholysis	VLU	30
7	57	Male	CVI, VLU	VLU	140
8	57	Female	Hyperlipidaemia, chronic pain—general body, hypertension, lymphoedema, neuropathy, gastroesophageal disease, DM II, CVI	VLU	4
9	69	Female	Chronic pain—bilateral legs, CVI, PAD, COPD, morbid obesity	DFU	12
10	72	Male	Coronary artery disease, hypertension, venous insufficiency, VLU, lymphoedema of leg, stasis dermatitis of leg, onycholysis	VLU	12
11	59	Female	Anxiety, depression, psoriasis, insomnia, hyperlipidaemia, hypomagnesaemia, DM II, gastroesophageal reflux disease, CVI, COPD, hypertension	VLU	8
12	69	Female	Postoperative surgical wound	Surgical wound	56
13	57	Female	Hyperlipidaemia, chronic pain—general body, hypertension, lymphoedema, neuropathy, gastroesophageal disease, DM II, CVI	VLU	4
14	69	Female	Chronic pain—bilateral legs, CVI, PAD, COPD, morbid obesity	VLU	4
15	59	Male	DM II	DFU	4
16	41	Male	DM II	DFU	16

COPD—chronic obstructive pulmonary disease; CVI—chronic venous insufficiency; DFU—diabetic foot ulcer; DM II—type 2 diabetes mellitus; PAD—peripheral arterial disease; VLU—venous leg ulcer

of two years. In addition, the concentration of the cleanser is only 0.057%, which is far below cytotoxic concentrations.[7,8]

When the basic solution of NaOCl reacts with water the active ingredient, hypochlorous acid (HOCl), is formed:

$$NaOCl + H_2O = HOCl + NaOH$$

A portion of the HOCl dissociates into hypochlorite ions (OCl^-). Both HOCl and OCl^- are potent antioxidants that enter the microbe, disrupting several cellular functions and leading to death of the organism. The multiple modes of action may explain the lack of resistance to NaOCl.[9]

Methods

This prospective, randomised, double-blind pilot study, conducted at a single outpatient wound care centre located in Western Pennsylvania, US, evaluated the efficacy of a topical antiseptic (Anasept) versus normal saline solution (NSS) in the healing and reduction of bacterial burden in hard-to-heal wounds.

Adult patients drawn from a single wound clinic's patient population were prospectively enrolled after signing an institutional review board (IRB)-approved consent (Western Institutional Review Board Study Number: 1282225). This included permission to publish the photographs. Hard-to-heal wounds (venous leg ulcers (VLU), diabetic foot ulcers (DFU), as well as nonhealing surgical wounds) that had been present for a minimum of four weeks were included. Patients were excluded if, in the opinion of the investigator, they had conditions that might compromise safety or if the wounds had not been present for four weeks. Table 1 details patient demographics.

Patients were randomised to one of two groups: wound cleansing with NSS and standard of care (SoC), or wound cleansing with NaOCl and SoC. Patients, investigators and staff were blinded to the treatment. NaOCl has a slight odour of chlorine. The exterior of the bottles containing the solutions, NSS and NaOCl, were coated with bleach by an unblinded study coordinator. Therefore, all of the containers smelled of chlorine. The SoC varied by wound type, but consisted of removal of all nonviable tissue, maintenance of proper moisture balance, offloading for DFUs and compression stockings for VLUs.

Patients were seen weekly for four weeks, and on each visit the wound was cleansed with the assigned solution, debrided, measured using digital photographic planimetry (before and after debridement) and imaged for bacterial load, using the MolecuLight device. Using the solution provided by the research site, patients

cleansed their wounds daily and applied a primary dressing chosen by the investigator.

This pilot study was not powered to detect a difference in healing or bacterial reduction. Descriptive statistics were planned.

Results

A total of 16 patients with hard-to-heal wounds were enrolled over a three-month period. A total of 11 VLUs, three DFUs and two surgical wounds were randomised (nine in the NaOCl group and seven in the NSS group). Of the 16 patients, 15 completed the study: one of the NSS patients was lost to follow-up. There were no serious adverse events and the blind was not broken during the trial.

Percent area reduction (PAR) at four weeks is a well-established surrogate endpoint in wound healing clinical trials. For the 15 patients who completed the pilot, the PAR at four weeks was 71.8% for the NaOCl group compared with 21.3% for the NSS group. The PAR for the completed patients is shown in Fig 1. Interestingly, despite the low number of patients, this difference approached statistical significance (p=0.14, Mann–Whitney test) suggesting a trend toward healing for the NaOCl group. In the NaOCl group, three patients were healed at week four compared to two in the NSS group.

Weekly fluorescence imaging was used to follow bacterial load. None of the patients had complete resolution of fluorescence following cleansing and debridement on the first treatment visit; however, the NaOCl group had a decrease in fluorescence of 25–75%, while the saline arm ranged from no decrease to a 25% decrease. Complete closure at four weeks was achieved in six wounds. They were not included in the end-of-study bacterial load analysis. Fluorescence imaging of the remaining nine wounds revealed that all of the NSS group, four patients, had residual bacterial fluorescence at the end of the study. In the NaOCl group, two of the remaining five patients had some residual red fluorescence (40%) at four weeks. Fig 2 details a case treated with NaOCl with resolution of bacterial fluorescence.

Fig 1. Percent area reduction versus patient (x-axis)

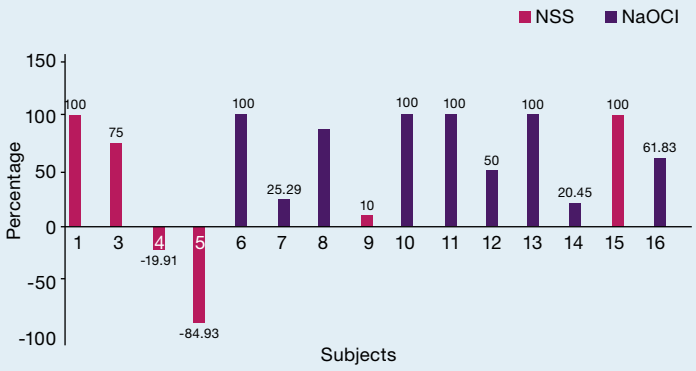
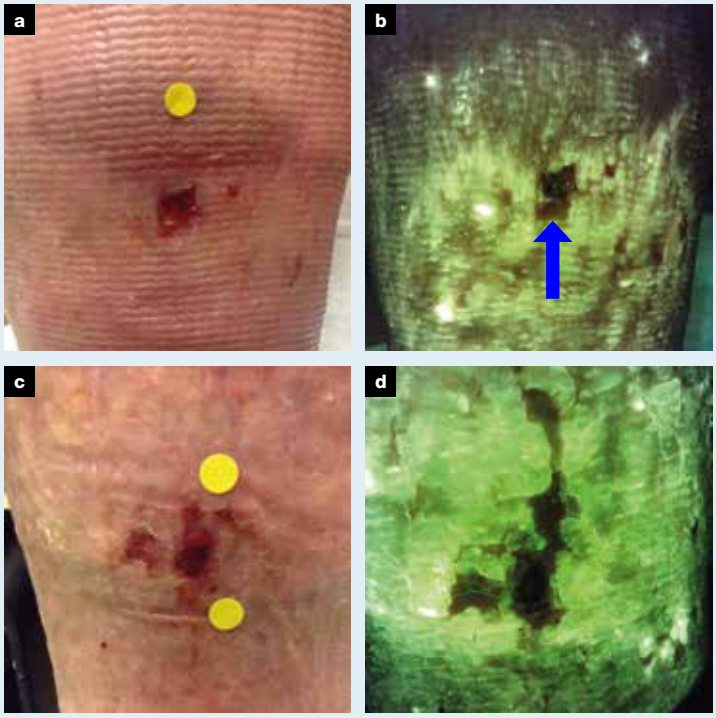


Fig 2. Venous leg ulcer. Treatment day 0 **(a)**; standard image after cleansing with sodium hypochlorite solution, fluorescence imaging with red fluorescence at arrow **(b)**; treatment day 28, standard image **(c)**; and fluorescence image with resolution of fluorescence **(d)**



Discussion

Controlling bacterial burden is essential in the treatment of hard-to-heal wounds; however, recent evidence on the indiscriminate use of antiseptics[3] points to the need to conduct clinical trials evaluating antiseptic cleansers and topical antimicrobials. This pilot study design allows investigators to conduct simple, cost-effective trials to evaluate antiseptics in patients with hard-to-heal wounds. Most clinical trials in hard-to-heal wounds are unblinded. The use of blinding in this and future trials will decrease bias and increase clinician confidence in the results.

This pilot evaluated the efficacy of a modified 0.057% NaOCl solution compared to a commonly used cleanser, NSS. This is not the first study to compare NaOCl to NSS. A 2004 trial also demonstrated that NaOCl decreased bacterial bioburden in most patients and, as in this pilot, NSS was ineffective as an antiseptic cleanser.[10] The 2004 trial had drawbacks. First, the author chose patients based on clinical signs and symptoms (CSS) of infection. A large clinical trial published in 2020, demonstrated that sensitivity of CSS is <20% for all wound types.[1] In addition, the investigator used semiquantitative swab cultures. A recent study demonstrated that swab cultures taken from hard-to-heal wounds are unreliable.[11] The improved trial design presented in this pilot

incorporated fluorescence imaging, enabling the investigators to detect clinically significant levels (>10^4 CFU/g)[1] of bacteria, decreasing reliance on CSS and obviating the need for culturing. A study evaluating hypochlorous acid (HOCl), the primary active ingredient in the NaOCl solution examined in this study, demonstrated that HOCl was superior to NSS in reducing bacterial burden when combined with ultrasonic debridement.[12]

The NaOCl-treated wounds in this study tended to have a more rapid wound healing and decreased bacterial levels by the end of the study, compared with the NSS-treated wounds. The results have encouraged the research team to modify and expand the trial: increase the number of subjects to reach statistical power, focus on a single wound type (VLU) and increase the number of research sites. In addition, the pilot suggests that the use of a topical antimicrobial between visits may improve efficacy. A trial examining the effectiveness of an antibiofilm agent in combination with negative pressure wound therapy (NPWT) revealed that application of the antimicrobial every three days eliminated bacterial fluorescence in a shorter time.[13]

In this study, persistence of bacteria following debridement and antiseptic use on the first visit is consistent with recent observations. It was also true in the antibiofilm NPWT trial mentioned above[13] and in a study on debridement using quantitative tissue cultures.[14]

Limitations

A major limitation of this trial, like the other antiseptic studies conducted to date, is that they are underpowered. If antiseptic cleansers are to remain a part of the wound clinician's armamentarium, fully powered clinical trials demonstrating benefit must be forthcoming. The goal of the pilot was to evaluate NaOCl in a variety of wounds; however, different wound types heal at different rates and the ease of eliminating bacterial burden may vary between wound types. In the first author's experience, reducing the bacterial burden in diabetic ulcers seems to be more difficult than in other wound types. Future investigators conducting similar trials may want to focus on a single wound type. Another limitation was the inclusion of smaller wounds. Of the wounds evaluated in this study, six healed during the four-week trial. The selection of larger wounds may have resulted in better data, particularly on the bacterial reduction end point.

A detail which was not measured in this study was the wound pH, both before and after cleansing, and it is intended to address this in future studies.

Conclusion

The trend toward improved wound healing, decreased bacterial burden and the absence of adverse events in the NaOCl-treated wounds suggests that NaOCl may be a suitable choice as an antiseptic cleanser. **JWC**

References

1 Le L, Baer M, Briggs P et al. Diagnostic accuracy of point-of-care fluorescence imaging for the detection of bacterial burden in wounds: results from the 350-patient fluorescence imaging assessment and guidance trial. Adv Wound Care (New Rochelle) 2021; 10(3):123–136. https://doi.org/10.1089/wound.2020.1272

2 Serena TE, Gould L, Ousey K, Kirsner RS. Reliance on clinical signs and symptoms assessment leads to misuse of antimicrobials: post hoc analysis of 350 chronic wounds. Adv Wound Care (New Rochelle) 2021 [ahead of print]; https://doi.org/10.1089/wound.2021.0146

3 Serena TE, Harrell K, Serena L, Yaakov RA. Real-time bacterial fluorescence imaging accurately identifies wounds with moderate-to-heavy bacterial burden. J Wound Care 2019; 28(6):346–357. https://doi.org/10.12968/jowc.2019.28.6.346

4 Hartley P. Dr. H. D. Dakin, F.R.S. Nature 1952; 169:481–482. https://doi.org/10.1038/169481a0

5 Rutala WA, Cole EC, Thomann CA, Weber DJ. Stability and bactericidal activity of chlorine solutions. Infection Control and Epidemiology 1998; 19(5):323–327. https://doi.org/10.2307/30141372

6 Barsoumian A, Sanchez CJ, Mende K et al. In vitro toxicity and activity of Dakin's solution, mafenide acetate, and amphotericin B on filamentous fungi and human cells. J Orthop Trauma 2013; 27(8):428–436. https://doi.org/10.1097/BOT.0b013e3182830bf9

7 Heggers JP, Sazy JA, Stenberg BD et al. Bacterial and wound healing properties of sodium hypochlorite solutions: the 1991 Lindenberg Award. J Burn Care Rehabil 1991;12(5):420–424. https://doi.org/10.1097/00004630-199109000-00005

8 Cotter JL, Fader RC, Lilley C, Herndon DN. Chemical parameters, antimicrobial activities, and tissue toxicity of 0.1 and 0.5% sodium hypochlorite solutions. Antimicrob Agents Chemother 1985; 28(1):118–122. https://doi.org/10.1128/AAC.28.1.118

9 Jones IA, Joshi LT. Biocide use in the antimicrobial era: a review. Molecules 2021; 26(8):2276. https://doi.org/10.3390/molecules26082276

10 Lindfors J. A comparison of an antimicrobial wound cleanser to normal saline in reduction of bioburden and its effect on wound healing. Ostomy Wound Manage 2004; 50(8):28–41

11 Serena TE, Bowler PG, Schultz GS et al. Are semi-quantitative clinical cultures inadequate? Comparison to quantitative analysis of 1053 bacterial isolates from 350 wounds. Diagnostics 2021; 11(7):1239. https://doi.org/10.3390/diagnostics11071239

12 Hiebert JM, Robson MC. The immediate and delayed post-debridement effects on tissue bacterial wound counts of hypochlorous acid versus saline irrigation in chronic wounds. Eplasty 2016;16:e32

13 Serena T, Al-Jalodi O, Serena L et al. Evaluation of the combination of a biofilm-disrupting agent and negative pressure wound therapy: a case series. J Wound Care 2021; 30(1):9–14. https://doi.org/10.12968/jowc.2021.30.1.9

14 Schwartz JA, Goss SG, Facchin F et al. Surgical debridement alone does not adequately reduce planktonic bioburden in chronic lower extremity wounds. J Wound Care 2014; 23(9):S4, S6, S8 passim. https://doi.org/10.12968/jowc.2014.23.Sup9.S4

Reflective questions

- Is it important to trial antiseptics in vivo as well as in vitro? And if so, why?
- How does blinding increase the reliabilty of the trial?
- Should sodium hypochlorite be considered as a cleanser for hard-to-heal wounds? And if so, why?