renovar™

Advanced Skin and Cleanser
Advanced Wound Irrigation Solution
Clinical Summary

Topical Stabilized Hypochlorous Acid (HOCl) Overview
Breaks Down Biofilm

Wound and Biofilm:

Ortega-Peña S, Hidalgo-González C, Robson MC, Krötzsch E: In-vitro microbicidal, anti-biofilm and cytotoxic effects of different commercial antiseptics. Int Wound J. 2017 June; 14(3):470-479

- Topical antiseptics are widely used for wound treatment, with the goal of disrupting biofilm capacity.
- We analyzed the effectiveness of a variety of antiseptics to inhibit various stages of biofilm formation and remove biofilms in-vitro as well as the agents' cytotoxic effects on fibroblasts.
- We found that the chlorine-releasing agents exhibited immediate anti-biofilm effects only in the short term with some resistance.
- HOCl was effective in preventing biofilm formation within a short time period and showed virtually no toxicity.

Wound and Biofilm:

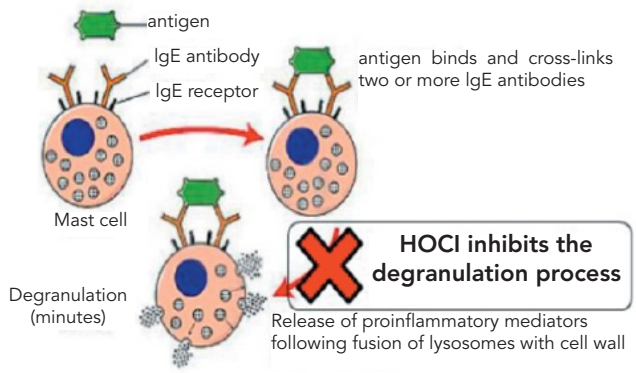
Sakarya S, Gunay N, Karakulak M, Ozturk B, Ertugrul B: Hypochlorous Acid: an ideal wound care agent with powerful microbicidal, antibiofilm, and would healing potency. Wounds. 2014 Dec; 26(12):342-350

- The aim of this study was to investigate the effect of stabilized HOCl on killing rate, biofilm formation, antimicrobial activity within biofilm against frequently isolated microorganisms, and migration rate of wounded fibroblasts and keratinocytes.
- All microorganisms were killed within 0 minutes, and the accurate killing time was 12 seconds. The effective dose for biofilm impairment for standard microorganisms and clinical isolates ranged from 1/32 to 1/16.
- The stabilized HOCl solution had dose-dependent favorable effects on fibroblast and keratinocyte migration compared to povidone-iodine and media alone.
- These features lead to a stabilized HOCl solution as an ideal wound care agent.

Anti-inflammatory:

HOCl has multiple anti-inflammatory effects:

- Decreases the activity of histamines.
- Directly decreases the activity of leukotrienes released by neutrophils.
- Directly increases the activity of TGF-beta, which is anti-inflammatory.
- Increased synthesis of growth factors.
- Decreases the activities of MMP-7, collagenases and gelatinases.
- Oxidizes alpha2-macroglobulin, which neutralizes pro-inflammatory cytokines, including TNF-alpha, IL-2, and IL-6.
- Decreasing mast cell activation:
    - HOCl prevents allergen- and calcium-induced mast cell degranulation for up to 8 hours after a single exposure.



- Phospholipase A2 Inhibition of Late-Phase Mast Cell Response even at 3-5 ppm, HOCl inhibited PLA-2 activity and blocked the Arachidonic cascade.

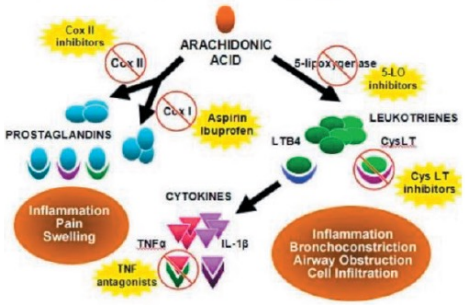


Figure 2. Arachidonic acid is metabolized to produce inflammatory mediators. Many current anti-inflammatory and pain medicines inhibit some portion of the arachidonic acid pathways.

Reduced Itch and Pain:

Pruritus

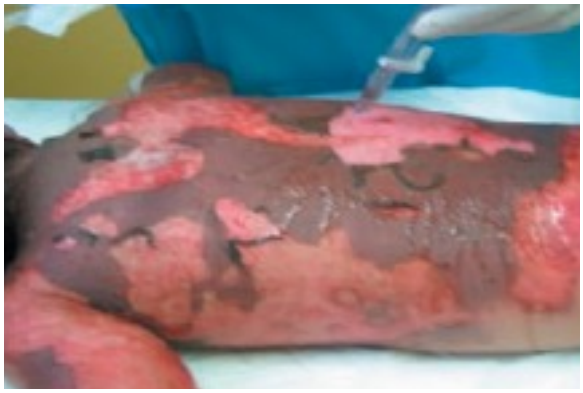
Pelgrift RY, Friedman AJ: Topical Hypochlorous Acid (HOCl) as a Potential Treatment of Pruritus Current Dermatology Reports 2 (3) 181-190, 2013

Topical HOCl has recently been proposed as a treatment of pruritus.
The article proposes two mechanisms by which HOCl may reduce pruritus:

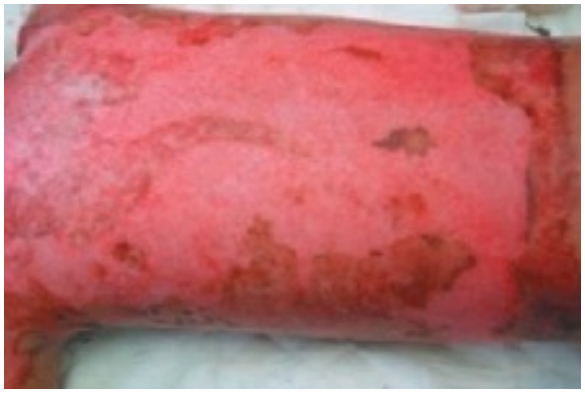
1. HOCl is microbicidal to cutaneous pathogens, especially Staphylococcus aureus in atopic dermatitis.
2. HOCl is anti-inflammatory and reduces the activities of histamine leukotriene B4 and interleukin-2, all of which are implicated in the pathophysiology of itch.

Increases Perfusion ($TCPO_2$)

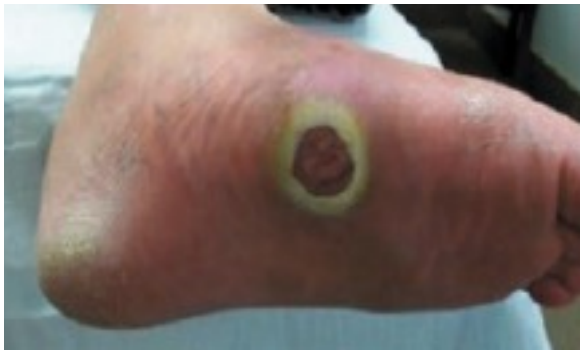
Persistent Improvement of Diabetic Foot Ulcer Perfusion Results from Application of Superoxidized Water, Cheryl M. Mongiovianni, PHd, RVT, CWS Lake District Hospital, Lakeview, OR



Two-year old girl with partial-thickness burns



Day three of treatment



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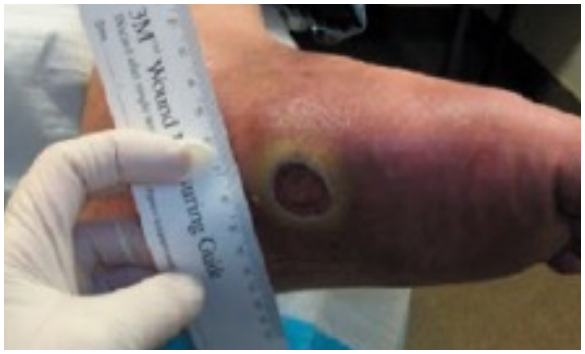

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Investigator Blinded Randomized Study, Evaluating HOCl in the Treatment of Atopic Dermatitis-Associated Pruritus

Authors: Brian Berman, MD, PhD, Mark Nestor, MD, PhD Center for Clinical and Cosmetic Research and University of Miami Miller School of Medicine

Results

Irritation:

- Investigator-assessed clinical improvement at day 1 – 28% reduction in irritation
- Improvement continued at day 3 to 46% reduction in irritation

Itch Relief:

- Day 1 – 50% of subjects reported improvement in itching
- Day 3 – 85% of subjects reported improvement in itching

Discussion

Subjects were enrolled into the study if they had AD as defined by the Hanifin criteria and had a score of >2 on an itch severity scale (0-4). 30 subjects were enrolled over the course of the study, with 20 randomized to the treatment group (HOCl), and 10 randomized to the untreated control group. Subjects who were randomized to the treatment group were instructed to use HOCl BID or PRN for 72 hours.

Three primary measures used in this study were the Participant Global Assessment (PGA), Investigator Global Assessment (IGA), and VAS itch score.

Table 1. The mean VAS itch score between the two groups were similar at the Baseline investigator-elicited itch score (0-4).

Study Group	n	Mean (μ) IGA Itch Score at Baseline	Standard of Deviation (σ) of mean
TREATED	20	No signs of toxicity	0.826
UNTREATED	10	Does not generate cytotoxic effects	0.483

Statistical Test	p-value (a+0.05; ß=0.20)	95% Confidence Interval
Independent Samples t-test	0.601	-0.732 – 0.432

29 subjects were included in the final analysis.

Mean change in PGA and IGA between baseline and 72 hours were both shown to be significantly different, with a decrease (improvement) in favor of the treatment group (PGA: p - value = 0.128; IGA: p – value = 0.012 (Figure 1).

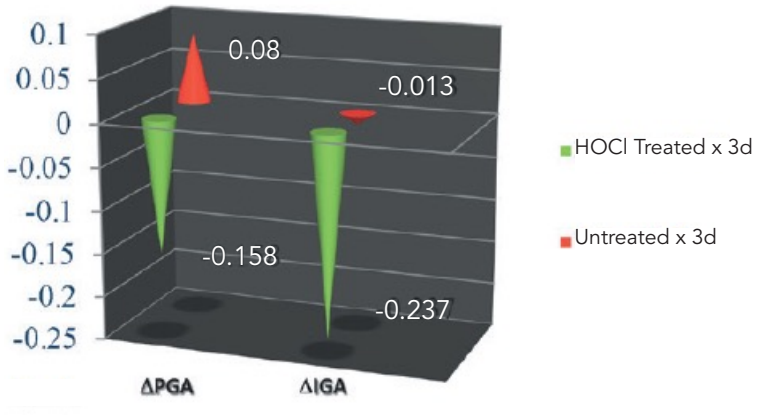

Figure 1.

The mean itch VAS scores were significantly lower in the treated group (Figure 2).

Figure 2. Mean % Change in itch VAS With and Without Treatment with HOCl Gel x 3 days.

The analysis showed 73.7% of the subjects in the treated and 30.0% of the subjects in the untreated group experienced a reduction in itching between baseline and 72 hours post-application. There were no treatment-related discontinuations or SAEs.

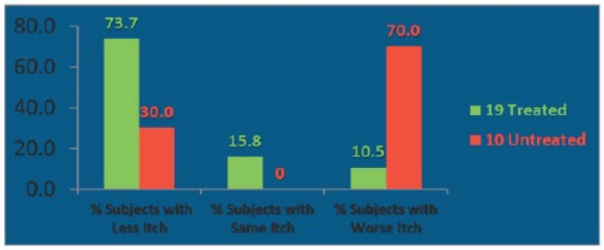

Figure 3. Effect of with HOCl Gel Treatment on Itch in Atopic Dematitis - day 3.

The study demonstrated that HOCl leads to a significant reduction in itching associated with AD in as little as 72 hours. The twice-daily regimen was manageable and easy to follow. HOCl is effective over a short period of time with few doses needed. This is a cost-efficient and effective method for improving the symptom of pruritus in patients with AD.

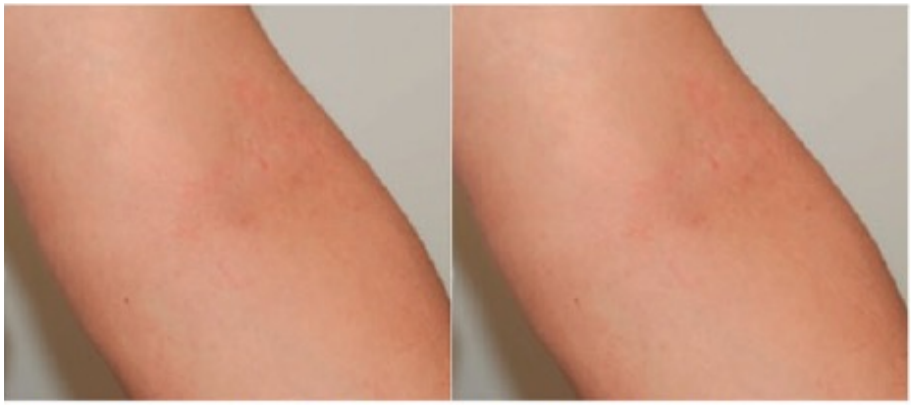

Figure 4. Subject treated with HOCl before (left) and 72 hours after (right) treatment.

Use in Clinical Practice

Clinical Dermatology

Based on the premise that the majority of clinical skin conditions are caused by infection and/or inflammation, Topical Stabilized HOCl is non-toxic, extremely safe, and has clinical benefits for a variety of skin conditions:

- *Acne Vulgaris*
- Atopic Dermatitis
- Seborrheic Dermatitis
- MRSA
- Dermatophytosis
- Folliculitis
- Psoriasis

Topical stabilized HOCl can treat both inflammation and infection. It does not have detrimental effects on the skin and is an optimal treatment option of ulcerations and open wounds.

Surgical and Aesthetic

Topical Stabilized HOCl optimizes wound healing and the creation of a scar from surgical prep to daily treatment. For surgical and aesthetic wounds (including laser), Topical Stabilized HOCl prevents infection (preparation) for cosmetically superior aesthetic outcomes and surgical scars and is not toxic to normal tissue.

Wound Healing – Surgical Preparation

Totoraitis K, Cohen JL, Friedman A.: Topical Approaches to Improve Surgical Outcomes and Wound Healing: A Review of Efficacy and Safety J Drugs Dermatol. 2017 Mar 1;16(3):209-212

In an attempt to mitigate risk and improve surgical outcomes, multiple topical products continue to be used both pre- and post-procedure. Traditionally, this includes both topical antibiotics and antiseptics. Topical antiseptics, including chlorhexidine and povidone-iodine, can have a cytotoxic effect on keratinocytes and may impede wound healing as a result. In addition, chlorhexidine, in particular, can produce both otologic and ocular toxic effects when used on the face. Emerging products, such as HOCl, are an ideal alternative to the more commonly used agents, as it has effective antimicrobial actions and minimal adverse effects.

The Role of Super Oxidized Solution in the Management of Diabetic Foot Ulcers: Our Experience

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ABSTRACT
Dressing or Preparation of the diabetic foot ulcer is very essential not only to reduce or prevent infection but also for the preparation for definite surgery, if necessary. Present article is about our experience in the evaluation of effectiveness of Super Oxidized Solution in local treatment of diabetic foot ulcers. Out of 20 cases Super Oxidized Solution helped in total healing in 8 cases, and prepared wounds for definite cover by reducing infection and promoting granulations which are prerequisites for definitive surgery in remaining 12 cases.
Keywords: Super oxidized solution, diabetic foot ulcer, healing.

INTRODUCTION
Diabetic foot ulcers have been managed by local dressings with various agents like povidone-iodine, EUSOL, acetic acid, hydrogen peroxide, silver sulfadiazine, local antibiotic ointments, etc. These dressings are meant to prevent infection or reduce bacterial load and promote granulations thus helping in wound healing.[1] Use of Super Oxidized Solution is a new concept in wound management. Super Oxidized Solution is an electrochemically processed aqueous solution manufactured from pure water and sodium chloride. During this electrolysis process, reactive species of oxygen and chlorine are formed. These released reactive species create an unbalanced osmolarity, so that it damages the integrity of the cell membrane, then reacts and denatures the lipids & proteins of single-cell organisms. This is a direct result of the osmolarity difference between the ion concentrations of the solution and single-cell organism. Multicellular organisms are not prone to such osmolarity changes.[2]

MATERIAL AND METHODS
In the present study, a total number of 20 patients with diabetic foot ulcers were included and managed during the period of January 2006 to November 2006. This study was done in the Dept. of Plastic Surgery, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, India.

All patients were carefully examined clinically. Basic investigations like Haemoglobin, Total Count, Differential Count, Bleeding Time, Clotting Time, Erythrocyte Sedimentation Rate, Random and/or Fasting Blood Sugar, S. Creatinine, Blood, Urea, HIV, HBsAg, Complete Urine Examination, etc. were done for fitness, for anaesthesia, and to rule out underlying systemic illness. For all cases, a wound swab was sent for culture and sensitivity to know the type of organism & its sensitivity to antibiotics.

All diabetic ulcers were irrigated daily with a Super Oxidized Solution and covered with gauze soaked in a Super Oxidized Solution. Blood sugar was controlled by insulin and/or oral hypoglycemic drugs. An Endocrinologist's opinion was taken for all cases.

Many wounds healed by the use of the Super Oxidized Solution only. For non-healing wounds definite wound cover (skin graft/flap) was provided once the wound became sterile & healthy granulation appeared after local treatment with Super Oxidized Solution. Those patients who were unfit for surgery/anaesthesia or not willing to have surgery were treated with local dressings using Super Oxidized Solution only.

Status of the bacterial growth, time taken for the wound to become sterile, time taken for the appearance of the granulation, time taken for healing, and complications were noted.
Once wounds healed completely, patients were followed bi-weekly for three months.

RESULTS
Complications - In our study no observable complications were noted.

DISCUSSION
The incidence of Diabetes Mellitus is increasing in the present scenario because of a sedentary lifestyle, changes in dietary habits, an increase in stress, and an increase in the lifespan of human beings. Management of Diabetes and its complications is a complex procedure involving all specialties as it involves all organs and systems of the body. If glycemic control is not adequate, patients present with various complications like Diabetic ulcers which are caused by trivial or noticeable trauma. Diabetic foot ulcers are a challenging problem for every clinician in day-to-day practice. These wounds have been managed by local dressings with various agents like povidone-iodine, EUSOL, acetic acid, hydrogen peroxide, silver sulfadiazine, local antibiotic ointments, or powders, etc. for a long time.[3]

Super Oxidized Solution is a newer concept in wound management. Researchers from many parts of the world have investigated Super Oxidized Solutions as a disinfectant for instruments. The literature also describes the use of this solution on humans for various indications like ulcers, mediastinal irrigation, peritoneal lavage, hand washing etc.[4,5]

European CE KEMA- Medical Device Class IIb (in 2004), and FDA (in 2005) approval was obtained for this Super Oxidized Solution for its use in acute and chronic wounds.[6] This solution has been used by many clinicians with positive results in the management of wounds of various etiology. No reaction or complication has been reported in literature.[7-9] In our study, we selected infected diabetic ulcers and used a Super Oxidized Solution as a local agent for cleansing and sterilizing. In our study, all age groups were included and males were more than females in number (Table 1) all sizes of wounds were included in this study (Table 2).

All 20 cases were infected (wound culture and sensitivity positive) at the time of presentation to our department. After 5 days of using the Super Oxidized Solution, 19 wounds became sterile and only 1 case came out positive for growth (Table 4). Of the 20 cases, 8 wounds were healed with simple local dressings with Super Oxidized Solution without any complications (Fig. 2, 4). Skin grafting was done for 11 cases and local flap was done for 1 case (Table 5). All the above definite procedures were done once wounds became sterile with healthy granulations after the use of Super Oxidized Solution.

Many authors have used a Super Oxidized Solution with no complications.[10,11] In our study none of the patients suffered from noticeable complications.

In the management of diabetic foot ulcers, a Super Oxidized Solution debrides necrotic tissue, reduces microbial load, promotes granulation, and decreases the healing time, without damaging the normal tissue or complications. Those patients, who have small superficial ulcers or not fit for definite surgery, can be managed conservatively with Super Oxidized Solution only. The moistening effect and minimum toxicity found with the use of this Super Oxidized Solution makes it a good choice for diabetic foot ulcer management. However, new controlled trials must be conducted to fully establish the antimicrobial, anti-inflammatory, and positive effects in wound healing.

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Table-1: Distribution of cases with respect to age and sex

Age	Male	Female	Total	Percentage
10-20 yrs	0	0	0	0%
20-30 yrs	1	0	1	5%
30-40 yrs	3	1	4	20%
40-50 yrs	4	2	6	30%
>50 yrs	5	4	9	45%
Total	13	7	20	100%

Table-2: Distribution of cases with respect to type of Diabetes

Type of Diabetes	No. of cases	Percentage
Type 1	1	5%
Type 2	19	95%
Total	20	100%

Table-3: Distribution of cases with respect to size of wound

Size of the wound	No. of cases	Percentage
<5 cm	10	50%
5-10 cm	6	30%
>10 cm	4	20%
Total	20	100%

Table-4: Distribution of cases with respect to infection

Growth in Culture	No. of cases	No. of cases positive for infection after use of Super Oxidized Solution		
		After 1 day	After 3 days	After 5 days
Staph. areus	6	4	1	
E. coli	4	1	0	
Enterococci	3	2	1	1
Pseudomonas	3	2	1	
Proteus. mirabilis	2	1	1	
Streptococci	2	2	0	
Total	20			

Table-5: Distribution of cases with respect to procedure

Procedure	No. of cases	Percentage
No. procedure	8	40%
Skin graft	11	55%
Flap	1	5%
Total	20	100%

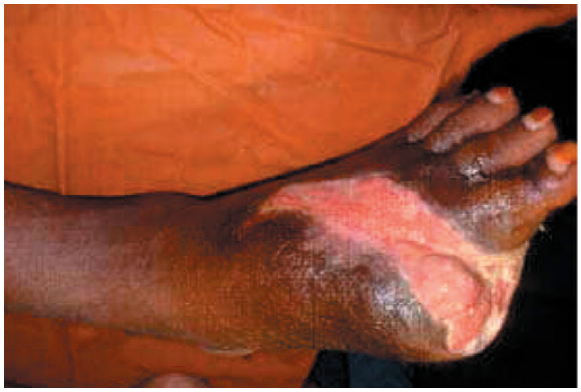

Figure 1. (a) A case of diabetic ulcer left foot

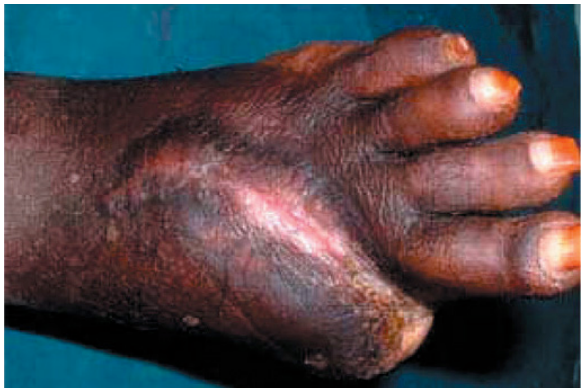

Figure 1. (b) Healed completely in 2 weeks with Super Oxidized Solution without any definite procedure

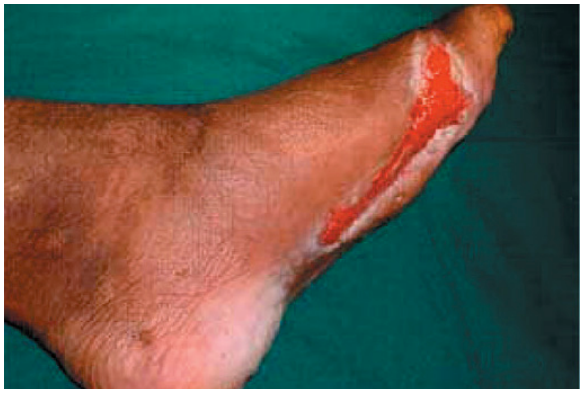

Figure 2. (a) A case of diabetic ulcer left foot

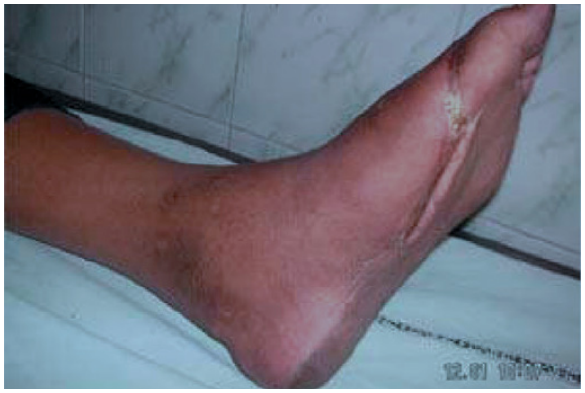

Figure 2. (b) Healed completely in 2 weeks with Super Oxidized Solution without any definite procedure

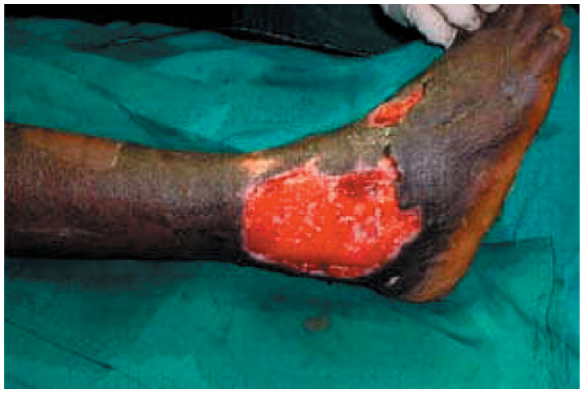

Figure 3. (a) A case of diabetic ulcer right foot

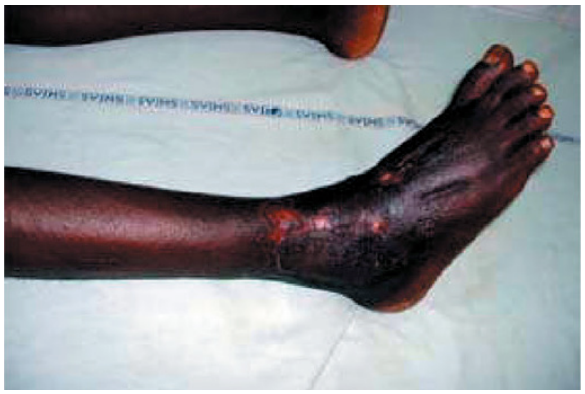

Figure 3. (b) A case of diabetic ulcer right foot prepared by Super Oxidized Solution and later covered

Efficacy and safety of neutral pH superoxidised solution in severe diabetic foot infections

Fermín R Martinez-De Jesús, Antonio Ramos-De la Medina, José Mariá Remes-Troche, David G Armstrong, Stephanie C Wu, Jose Luis Lázaro Martínez, Juan V Beneit-Montesinos

Martinez-De Jesus FR, Ramos-De la Medina A, Remes-Troche JM, Armstrong DG, Wu SC, Lazaro Martinez JL, Beneit-Montesinos JV. Efficacy and safety of neutral pH superoxidised solution in sever diabetic foot infections. Int Wound J 2007;doi:10.1111/j. 1742-481 X. 2007.00363.x.

ABSTRACT

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The use of antiseptics in wound care is often controversial and there is definitely a need for a non-toxic, highly disinfecting agent. This study assessed the efficacy of a neutral pH superoxidised aqueous solution (NpHSS) for infection control, odour reduction and surrounding skin and tissue damage on infected diabetic foot ulcerations. From November 2003 to March 2004, 45 patients with type 2 diabetes were randomized into a single-blind clinical trial comparing NpHSS (intervention group; n = 21) versus conventional disinfectant (control group; n = 16). All patients received comprehensive care including surgical debridement as appropriate, moist wound care, intensive glucose control, and broad-spectrum antibiotics. Treatment groups were matched in terms of sex, age (61.9 ± 11.9 versus 67.8 ± 11.6), years of diabetes duration (16.4 ± 8.1 versus 17 ± 10.2), obesity, HgAlc (7.1 ± 2 versus 6.7 ± 1.8), initial fasting glycaemia (163 ± 59 versus 152 ± 65.8 mg/dl), ulcer duration/week (13.7 ± 24 versus 15.1 ± 16.3), B/A Index (0.9 ± 0.5 versus 1.14 ± 0.7), depth and extent of infection/peri-wound cellulitis (groups B and C of the Tampico Hospital Classification) as well as aetiology ($P = 0.647$). Odour reduction was achieved in all NpHSS patients (100% versus 25%; $P < 0.01$) and surrounding cellulitis diminished ($P < 0.001$) in 17 patients (80.9% versus 43.7%). Nineteen patients in the NpHSS group showed advancement to granulating tissue stage (90.4% versus 62.5%; $P = 0.05$) with significantly less tissue toxicity (94% versus 31.2%; $P < 0.01$). A non-toxic, NpHSS, as part of a comprehensive care regimen, may be more efficacious in infection control, odour and erythema reduction than conventional disinfectants in treatment of diabetic foot infections.
Keywords: Antiseptics • Cellulitis • Diabetic foot • Infections • Wound healing

Key Points

- Most of the chemical disinfectants currently used in wound care can cause damage to the skin or granulating tissue.
- An effective, non-toxic antiseptic agent that cleanses the wound and removes bacterial contaminants without damaging healthy tissue is therefore needed to help diminish the bacterial burden and prevent the substantial long-term morbidity associated with amputations.
- In this randomized, controlled, single-blind study, our aim was to assess the efficacy of a non-toxic, neutral pH super oxidized aqueous solution (NpHSS) in terms of infection control, odour reduction, and peri-wound skin affection in infected diabetic foot ulcerations.
- Between November 2003 and March 2004, 45 consecutive patients with type 2 diabetes and infected, deep diabetic foot ulcers presented for care at the San Elian Diabetic Foot Salvage and Prevention Center (SEDFSPC) in Veracruz, Mexico; they were initially selected for the study by use of a randomly alternate assignment to either NpHSS or standard management (control group).
- Patients were randomized using randomly alternate assignment to either NpHSS or standard management (control group).
- Both groups received comprehensive care and the control group included the use of chemical antiseptics such as soap or povidone-iodine.
- All patients were treated using an outpatient ambulatory model.
- Forty-five patients were evaluated at baseline.
- Eight patients had severe arterial disease and did not meet the inclusion criteria.
- From the remaining 37 patients, 21 received the NpHSS and 16 received control treatment.
- Patients with diabetes have a higher risk of developing deleterious infections.
- Defects in host immune responses are at least partially responsible for their susceptibility and may include diminished polymorphonuclear leucocyte functions.
- Poor granulation tissue formation, prolonged persistence of abscesses, and impaired wound healing may also help predisposed diabetic persons to infectious complications.

- Many of these immunodeficiencies are related to the metabolic perturbations caused by poorly controlled diabetes.
- One must determine whether the failure to heal is secondary to persistent infection, antiseptic chemical damage, or because of the persistence of abnormal inflammatory response.
- Prompt empirical treatment with antibiotics can prevent the infection from spreading rapidly and reaching the blood and organs.
- Adequate debridement and the use of local antiseptics for wound cleansing are mandatory.
- NpHSS contains ROS and free radicals similar to those produced and released during the respiratory burst inside the mitochondria to produce energy (ATP) CO_2 and water.
- Further studies are needed to assess if NpHSS has additional properties on wound healing plus its disinfectant action.

INTRODUCTION

Infected foot ulcerations are a frequent cause of morbidity, hospitalization and amputations among persons with diabetes (1). The attributable cost of caring for a primary healing foot ulcer for 3 years after the initial diagnosis can amount to over $26,000 (2). The survival rate of patients who develop diabetic foot ulcers is reduced by about 15%, and at 5 years, these patients have approximately a 50% survival rate usually after arterial interventions and amputations (3,4).

Diabetic patients are prone to developing foot ulcerations because of the lack of neurological sensation and cycles of repetitive stress from ambulation. Rates of ulceration, infection, and amputation have declined dramatically in some centres that have developed programs and teams for foot care. Infection still remains a common problem in diabetic patients (5-8).

Ulcerations are pivotal events leading to diabetic foot infection, which has been shown to be the immediate cause of amputations in 25.50% of the cases (2). This is especially true of deep space infection, which is associated with an amputation rate of 52% (2). The resultant amputations are minor in 24-60% of the cases and major in 10-40% (2).

The spectrum of foot infections in diabetic patients ranges from simple superficial cellulitis to chronic osteomyelitis. Infections in patients with diabetes are often difficult to treat because of impaired microvascular circulation, which limits the access of phagocytic cells to the infected area and results in a poor concentration of antibiotics in the infected tissues (9). Wound healing is delayed when acute infection persists (10-12). Infection control along with appropriate debridement and good wound care are all essential factors in the treatment of diabetic foot ulcerations. Most of the chemical disinfectants currently used in wound care can cause damage to the skin or granulating tissue. Topical antiseptics such as hypochlorite (Dakin's solution), povidone-iodine (betadine), and others can cause significant cell damage. This may not only interfere with the wound healing process (13), but also could be cytotoxic or deleterious for the underlying tissues or proximal skin (14). Furthermore, normal sterile saline or water, although non-cytotoxic, does not adequately remove surface contaminants and does not have antiseptic properties. Some products as wound cleansers help break the bond between contaminants, necrotic tissue, and the surface of the wound (15,16).

An effective non-toxic antiseptic agent that cleanses the wound and removes bacterial contaminants without damaging healthy tissue is therefore needed to help diminish the bacterial burden and prevent the substantial long-term morbidity associated with amputations.

Super oxidized aqueous solutions are non-toxic, neutral pH water that contains reactive oxygen species (ROS) generated by the electrolysis of sodium chloride and water. Components of this solution include super oxidized neutral pH water, chlorine <85 parts per million (ppm), ROS/ free radicals, oxidized water H_2O; 99.99%, sodium hypochlorite (NaOCl) <50 ppm, HOCl <60 ppm, hydrogen peroxide (H_2O_2) <4 ppm, ozone (O_3) <0.2 ppm, chlorine dioxide (C_1O_2) <1.5 ppm, sodium hydroxide (NaOH) <8 ppm, sodium carbonate (Na_2CO_3) <21 ppm, sodium chloride (NaCl) <110 ppm. The bactericidal advantages and wound-healing properties of ROS have been documented (13). Super oxidized water has been shown to be a powerful non-toxic bactericidal against a variety of aerobics gram-negative and gram-positive rods (17) and is highly effective in treating infectious skin conditions and refractory ulcers associated with diabetes mellitus or peripheral circulatory insufficiency (18,19).

In this randomized, controlled, single-blind study, our aim was to assess the efficacy of a non-toxic, neutral pH, super oxidized aqueous solution (NpHSS) in terms of infection control, odour reduction, and peri-wound skin affection in infected diabetic foot ulcerations.

MATERIALS AND METHODS

Patients and baseline evaluations

Between November 2003 and March 2004, 45 consecutive patients with type 2 diabetes and infected, deep diabetic foot ulcers who presented for care at the San Elian Diabetic Foot Salvage and Prevention Center (SEDFSPC) in Veracruz, Mexico were initially selected for the study by use of a randomly alternate assignment to either NpHSS or standard management (control group). All patients gave written informed consent, and the study was reviewed and approved by the human subjects SEDFSPC Ethics Committee.

The inclusion criteria involved patients with type 2 diabetes older than 18 years of age with infected, deep wounds at or distal to the malleoli, presence of mal odour, active peri-wound cellulitis, loss of protective sensation, and at least one dopplerable pedal pulse. Wound size was evaluated by measuring the maximum length by the maximum width. Exclusion criteria included severe arterial disease (diagnosed by the criteria listed above based on the absence of both foot pulses on the affected extremity), brachial/ankle index below 0-5, the diagnosis of osteomyelitis total gangrene of the study foot or forefoot, severe cardiovascular and renal failure and severe neurological problems that would make the patient a poor candidate for the study (e.g. confined to the bed). Patients with no family assistance were also excluded from the study.

Baseline demographic measurements were performed at the first visit. The diagnosis of diabetes was made prior to enrollment and confirmed by either communication with primary care providers or a review of medical records. Doppler studies were performed, and branch/ankle index calculated. Neuropathy was evaluated by the Michigan (20), and the Rydel-Seiffer scores. The Michigan test included vibratory and 10 g Semmes-Weinstein sensitivity protective response (21). Neuropathic, vascular assessments and wound characteristics were included in the Tampico Hospital Diabetic Foot Classification system, which grades wounds according to depth, the extent of peri-wound cellulitis, and the aetiology of injury.

Stage A ulcer corresponds to skin surface disruption, without cellulitis or bone affection, stage B is a deep wound with probable bone involvement and 2 cm of peri-wound cellulitis extension and a stage C ulcer encompasses all foot depths with abscess, necrosis, bone infection, and cellulitis greater than 2 cm in width. This is in correlation to the Definitions and Criteria of the International Working Group on the Diabetic Foot International Consensus (2). All patients in the study had either a stage B or stage C ulcers. The second part of the grading system defines the aetiology, be it vascular, neuropathic, or both. X-ray radiography of the affected foot was also performed in order to help with the assessment of osteomyelitis.

Standard of care treatment and therapeutic intervention

Patients were randomized using randomly alternate assignment to either NpHSS or standard management (control group). Both groups received comprehensive care and the control group included the use of chemical antiseptics such as soap or povidone-iodine. Patients were blinded about the differences in treatment.

All patients were treated using an outpatient ambulatory model, which included appropriate surgical debridement, aggressive parenteral/intramuscular broad-spectrum antimicrobial administration, appropriate offloading and strict glycaemic control.

Broad-spectrum antimicrobial use was started empirically and immediately at the first visit to cover polymicrobial infection. Appropriate changes or adjustments were made after microbiological assessments of deep wound cultures were determined (2,22,23). Antibiotics were used for a minimum of 10 days in all patients in both groups. Antibiotics were used for more than 10 days if clinical signs of infection continued to be present. All patients received pentoxifylline at a dose of 1200 mg/day as a haemorheologic as part of San Elian's outpatient ambulatory model irrespective of their degree of vascular compromise.

Patients in both treatment groups were initially followed on a daily basis and depending on the condition of the wound, were seen every third day or once a week.

Patients randomized to the intervention group received an initial 15 to 20-minute immersion of the affected foot in NpHSS. Following appropriate debridement, the affected foot soak was repeated either weekly or biweekly depending on the severity of the peri-wound cellulitis followed by subsequent wound cleansings with NpHSS spray between immersions. NpHSS was also used instead of saline solution to remove gauze. Foot immersions were discontinued on clinical improvement of the wound and surrounding skin or when the first sign of maceration was observed. NpHSS spray applications were continued until either the complete resolution of wound infection or the end of the 20-week study.

The control group received the same treatment with the exception that saline was used in place of NpHSS. Povidone-iodine was initially used after debridement to cleanse the wound. When the infection resolved and the formation of granulation tissue was observed, the patient was switched to a surgical soap (Dermo Clean) with a saline rinse to minimize the cytotoxic effects of povidone-iodine. If clinical signs of infection returned, the use of povidone-iodine was resumed.

Both groups received conventional standard method of wound care consisting of gauze saturated with Triticum vulgare (Italdermol, Italmex, S.A., Mexico) to moisten the wound followed by adhesive covering. Heavily exudating wounds were dressed with calcium alginate (e.g. Kaltostat, Bristol-Mayer Squib, Mexico).

All patients in both groups were instructed to reduce weight bearing on the affected foot by using a wheelchair or crutches and resting as much as possible. We are aware that most patients have difficulty complying with these modalities. Compliance was assessed by directly questioning the patient and his/her caretaker and by inspection of the dressings by the healthcare team.

Primary objective and measurements

The primary outcomes were odour reduction, infection control as indicators of antiseptic efficacy, and wound safety in the treatment of diabetic foot infections. Infection control was assessed in terms of peri-wound cellulitis reduction and the percentage of patients advancing from infected, necrotic tissue to wound granulation. Cellulitis reduction was considered effective when the affected area of erythema decreased by more than 50% during the infection phase. Two members of the staff assessed odour reduction independently at the wound level. The Kappa agreement index was performed via 2 x 2 tables to detect differences between the two observers for odour perception. A kappa value (K = Po-Pe/1-Pe; Po= observed agreement, Pe = expected agreement by random) between 0-61 and 1-0 was representative of substantial to excellent agreement.

Granulating tissue is the pink/red, moist tissue comprised of new blood vessels, connective tissue, fibroblast, and inflammatory cells, which fill an open wound when it starts to heal.

Erythema of surrounding skin and granulating tissue was assessed by direct clinical observation. Abnormal peri-ulcer skin conditions such as dryness, erythema, induration, rash, epidermolysis, or blister formation may be caused by the use of chemical disinfectants. The absence of this damage along with the presence of healthy tissue surrounding the ulcers was considered as clinical signs of no or minimal tissue toxicity.

Daily pictures and weekly measurements of the wound, peri-wound cellulitis, local skin affection, and granulation tissue were recorded in a database as part of systematic data collection previously designed independently of this study.

For statistical analysis, significance was considered at $P < 0.05$. Values of chi-squared with Yale's correction or Fisher exact for 2 x 2 tables and of variance ratios for natural and treatment analysis of variance were calculated.

RESULTS

Forty-five patients were evaluated at baseline. Eight patients had severe arterial disease and did not meet the inclusion criteria. They were sent to a vascular surgeon for either vascular intervention or amputation, and their data were not included in the report. Of the remaining 37 patients, 21 received the NpHSS, and 16 received control treatment. All subjects completed the full length of the 20-week study. Subjects in both treatment groups were matched in terms of age, sex, years of diabetes duration, obesity, mean HbAlc, ulcer duration/week, and Yao B/A index (Table 1). No differences were noted in the staging of ulcers (Table 2) of the Diabetic Foot Tampico Hospital Classification and the wounds were similar in aetiology (P = 0.873). The mean average of oral antibiotics treatment was 26 ± 3.1 days for intervention versus 30 ± 5.2 for the control group (NS).

Table 1 Baseline demographic and clinical characteristics of NIDDM patients with severe infected foot ulcers

Characteristic	NpHSS (n = 21)	Control (n = 16)	P value
Age (years)*	61.9 ± 11.9	67.8 ± 11.6	NS
Sex†			
Male	9 (45)	8 (50)	NS
Female	12 (55)	8 (50)	
Years of diabetes duration*	16.4 ± 8.1	17 ± 10.2	NS
Mean of HbA1c*	7.1 ± 2	6.7 ± 1.8	NS
Mean of fasting glycaemia* (mg/dl)	163 ± 59	152 ± 65.8	NS
Obesity†	6 (30)	4 (25)	NS
Ulcer duration/week*	13.7 ± 24	15.1 ± 16.3	NS
B/A index (Yao)*	0.9 ± 0.5	1.14 ± 0.7	NS

Obesity is defined as body mass index > 27 kg/m^2.
HbA1c, haemoglobin A1c; NS, non-significant; NIDDM, non-insulin dependent diabetes mellitus; NpHSS, neutral pH super oxidized solution.
*Values are given as mean ± SD or actual (percent).
†Chi-squared and Yates' correction.

Table 2 Stage severity of diabetic foot Tampico Hospital grading

Stage	NpHSS (n = 21)	Control (n = 16)	P value
B	12	9	0.873
Vascular	4	1	NS
Neuropathic	7	3	
Both	1	5	
C	9	7	
Vascular	2	1	
Neuropathic	2	2	
Both	5	4	

All patients (100%) in the NpHSS group versus only 25% (n = 4) of the control group showed odour reduction (Table 3). The NpHSS group also showed a statistically significant reduction in cellulitis, 81% compared with 44% for the control group. The peri-wound cellulitis remained unchanged or worsened for four patients in the NpHSS group and for nine patients in the control group. Nineteen of the 21 patients (90.4%) in the NpHSS group showed good outcomes advancing from infected, necrotic tissue to granulating tissue; this was seen in only 62.5% of the control group. Tissue damage was observed to be less severe in the intervention versus control group.

Ninety percent (n = 19) of patients in the NpHSS group showed improvements in peri-wound conditions compared with 31% (n = 5) of controls (Table 3). The majority of the patients in the control group showed granulation as well as skin damage. Two of six patients in the intervention and control group, respectively, showed no change in the percentage of granulation tissue at the start and at the end of the 20-week study. Seventy-five percent of control subjects had a malodorous wound at the end of the study compared with zero patients in the NpHSS group. The absolute risk reduction (ARR) is 75% [95% confidence interval (95% CI): 53-7&-96-22] with a number needed to treat (NNT) of 2. In 56% of the control subjects, peri-wound cellulitis either worsened or remained unchanged; this was seen in only 19% of NpHSS subjects. The ARR is 37% (95% CI:7.66-66.75). The NNT was 3, meaning that one in every three patients with peri-wound cellulitis will benefit from this treatment. Thirty-seven percent of control subjects showed either no improvement of infected tissue to granulating tissue or presented with worsening of the wound (Table 3), while only 9.5% of the NpHSS group had similar results. The ARR was 27.9% with a 95% CI for this difference ranges from 1.14% to 54.82% and the NNT of 4. Sixty-nine percent of control subjects had tissue toxicity, while only 9.5% of NpHSS subjects had skin and tissue damage around the ulcer. The difference or the ARR was 59.2 (95% CI: 33.28 - 85.18) with a NNT of 2.

Table 3 Total of patients with good outcomes with NpHSS versus control group and clinical efficacy assessment by NNT

Outcome	NpHSS (n = 21)	Control (n = 16)	P value*	NNT	95% CI (min-max)
Fetid odour reduction	21 (100)	4 (25)	0.001	2	1 - 1.9
Infection control					
Cellulitis reduction	17 (80.9)	7 (43.7)	0.01	3	1.5 - 13.1
Advances from infection to granulating tissue	19 (90.4)	10 (62.5)	0.05	4	1.8 - 87.9
Improvement of skin around the ulcer	19 (90.4)	5 (31.2)	0.001	2	1.2 - 3

Percentage values are given in parenthesis.
NNT, number needed to treat. NNT significant clinical efficacy range = 2-4.
*Yates correction for chi-squared; significant results for $P < 0.05$.

DISCUSSION

The use of antiseptics in wound care is often controversial, and there is definitely a need for a non-toxic, highly disinfecting agent. In this randomized, controlled, single-blind study, we found that the use of a non-toxic, NpHSS, is more effective in infection control, odour reduction and causes less skin affection than conventional disinfectants in the treatment of diabetic foot infections.

Patients with diabetes have a higher risk of developing deleterious infections (24,25). Defects in host immune responses are at least partially responsible for their susceptibility and may include diminished polymorphonuclear leucocyte functions such as abnormalities of migration, phagocytosis (26), intracellular killing, and chemotaxis (27). Some evidence suggests that cellular immune responses may be reduced (28). Poor granulation tissue formation, prolonged persistence of abscesses, and impaired wound healing may also help predispose diabetic persons to infectious complications. Many of these immunodeficiencies are related to the metabolic perturbations caused by poorly controlled diabetes (28-30).

Deleterious wound healing complicated by infections are often a challenge to clinicians even with the use of antibiotics. One must determine whether the failure to heal is secondary to persistent infection, antiseptic chemical damage, or because of the persistence of abnormal inflammatory response. Antibiotics are usually continued for 10 days or longer even though clinical symptoms of infection may disappear earlier. Prompt empirical treatment with antibiotics can prevent the infection from spreading rapidly and reaching the blood and organs (2,21,23,31). Symptoms of cellulitis usually disappear after a few days of antibiotic therapy in non-diabetic patients, but in diabetic foot infections, not only are broad-spectrum antimicrobials necessary, adequate debridement and the use of local antiseptics for wound cleansing are mandatory.

Prior to the introduction of superoxidised solutions to our facilities, the use of antiseptic agents had been a topic of controversy and debate. There are pros and cons to the use of cytotoxic but potent antiseptic solutions like povidone-iodine or less cytotoxic but weak antiseptics like soap. The resultant dry, dark, indurated skin from povidone-iodine applications made it difficult to distinguish whether the delayed wound healing was secondary to infection or chemical damage. Consequently, povidone-iodine is often employed at the initial surgical debridement for severe deep infections with the primary focus on infection treatment, while the possible associated cytotoxicity and tissue damage become a secondary issue until this phase ends. This study showed that the use of NpHSS as compared with conventional chemical antiseptic agents provides higher infection control while minimizing skin and tissue damage. Once the severity of the infection has been reduced, more patients advanced to the granulating tissue phase with NpHSS treatment as compared with those who received conventional chemical antiseptics.

The subjects in the two groups were matched in terms of their metabolic status, severity of wound depth, cellulitis extension, and vascular/neuropathic aetiology. This prevented bias and showed that NpHSS, compared with conventional disinfectants, was an effective antiseptic agent with greater odour reduction and less tissue toxicity. The absence of mal odour following cellulitis reduction was seen in 100% of the patients who were treated with NpHSS. The reduction of odour can be explained by the action of free radicals in the NpHSS that react highly with putrefaction substances from necrotic tissues and anaerobes. Hydrogen peroxide, chlorine, hypochlorite, and hypochlorous acid all have known deodorant properties. NpHSS' bactericidal actions and minimal cytotoxicity over granulating tissue increased the number of patients advancing to the next wound healing stage, while patients in the control group had clinical signs of wound infection for a longer period of time. The transition stage between inflammatory and granulating phases where ulcers, although apparently uninfected, are not granulating and are often highly time and resource-consuming. Unlike an invasive infection, which produces the classic signs of erythema, oedema, and induration, indicators for surface-level infections are much more subtle: persistent high-volume exudate, sudden deterioration in the quality or quantity of granulation tissue, continual formation of a thin layer of vascular tissue and increased pain (32). The reinforcement of patient care with NpHSS along this transition stage showed better control of this subtle infection, increasing the percentage of patients that advanced to the granulating phase.

The preliminary NpHSS success in this trial could be explained by its ROS properties that simultaneously act to kill bacteria and react with odour putrefaction substances while causing less cytotoxicity and tissue damage to granulating tissue and surrounding healthy tissue.

NpHSS contains ROS and free radicals similar to those produced and released during the respiratory burst inside the mitochondria to produce energy (adenosine triphosphate, ATP), CO_2, and water. Microbial killing requires the ability of leucocytes to generate ROS, as well as the action of various microbicidal enzymes and peptides contained in leucocyte secretory granules. Superoxide anion and granule microbicidal enzymes are the mechanisms phagocytic leucocytes use to kill their targets. Super oxidized solutions act as degradative oxygen free radicals killing directly by their oxidizing capacity, they are highly reactive with bacterial cell wall and membrane components, and 'signaling' protease activation via the highly pH-dependent nicotinamide adenine dinucleotide phosphate (NADPH) oxidase electrogenic process (32).

Dismutation of the superoxide generates OH-, the accumulating negative charge must be compensated by an influx of H+ and/or K+. The hypertonicity resulting from K+ transport promotes the release of inactive cationic granule proteases bound to an anionic sulfated proteoglycan matrix. The highly K+ hypertonic environment solubilizes bacterial proteases and allows them to participate in the killing response.

Superoxide anion dismutes to form H_2O_2 and several other ROS and reactive intermediates (Figure 1). The reactive intermediates are products of nitrogen or oxygen reactions. The reactive nitrogen intermediates are derived from endothelial cell nitric oxide (NO) metabolism.

The properties of NO on wound healing and immune response become deleterious when it reacts with superoxide to produce peroxynitrite, a compound involved in DNA damage to cell and bacteria walls. Fortunately, NO must compete to react with superoxide molecules that are continuously producing reactive oxygen intermediates (ROD, thereby reducing the production of peroxynitrite. Instead of reacting with NO to form peroxynitrite, superoxide produces H_2O_2, other ROI, and ROS that are scavenged or reversed by primary and secondary antioxidant defenses. Catalyzed by the granule enzyme myeloperoxidase (MPO), H_2O_2 combines with chloride to form the highly microbicidal chemical, hypochlorous acid. Organisms such as Staphylococcus aureus and Candida albicans require NADPH oxidase activity to be effectively killed and are particularly susceptible to the action of ROS and MPO in both in vitro and in vivo experiments (33). Both oxidase activity and protease action are necessary to destroy these organisms.

The electrical energy of the superoxide solutions destroys microorganisms including fungi, viruses, mycobacteria, spirochetes, and bacteria (32). Former acid solutions lose both its electrical potential and its germicidal action when it comes into contact with multicellular organic matter, and it reverts to ordinary water. Conversely, NpHSS appears to be more effective in retaining its germicidal action in this condition because of the trigger of ROS in a potassium- and pH-dependent mechanism. Further studies are needed to assess if NpHSS has additional properties on wound healing plus its disinfectant action. We hypothesized that the increased granulating tissue patients proportion on the intervention group may be explained as an additional property of the ROS that might trigger early wound healing through fibroblast migration and proliferation (34). One basic study shows that the H_2O_2 neutrophil and macrophage production acts on human keratinocytes causing Rac1 gene overexpression to improve histological architecture, enhance deposition of connective tissue, higher cell density, and a hyperproliferative epithelial region (13).

The success of treatment modalities must be determined in order to choose a new proposal of treatment, significant statistical results are not always enough to make clinical decisions. For this reason, we calculated the NNT to assess the clinical impact of our statistical outcomes. The relative benefit of an active treatment over a control is usually expressed as the relative risk, the relative risk reduction, or the odds ratio. For clinical decision-making, however, it is more meaningful to use the NNT (35). Of clinical importance in decision-making is the ability of NpHSS to reduce odour in one patient of every three treated; its significant cellulitis reduction in one of every three patients, and advancement from infection to the granulating stage with less tissue toxicity in one of every two patients.

Diabetic foot infections are a common and complex problem with serious economic and medical consequences. Fortunately, much progress has been made in the past two decades to treat and prevent diabetic foot infections. The results of this study have shown that patients with mild to severe, non-limb-threatening infections can be treated as outpatients with antibiotics and NpHSS local antiseptic therapy. Accumulating evidence suggests that infection may be controlled by proper wound care; optimal metabolic control; and early, aggressive, surgical, and antibiotic therapy (36,37).

All these factors or components work synergistically to help with limb preservation in the large majority of patients (Figures 2, 3). The continued development of new treatments like NpHSS may take us one step closer to a more efficacious and cost-effective algorithm to treat diabetic foot ulcerations.

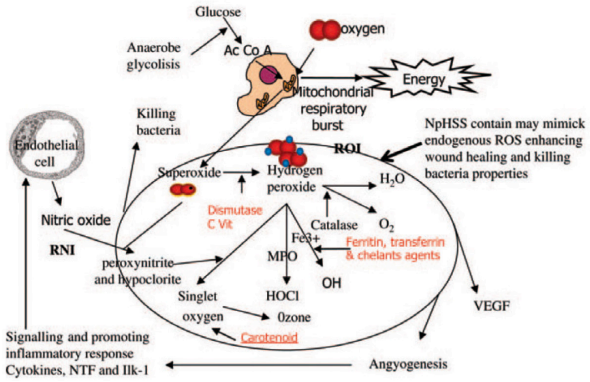

Figure 1. Oxidative stress and body primary and secondary defences HOCI, hypochlorous ac d; ILK-1, interleukin-1; MPO, myeloperoxidase; NpHSS, neutral pH superoxidised aqueous solution; NTF, necrosis tumor factor; ROI, reactive oxygen intermediates; ROS, reactive oxygen species; RNI, reactive nitrogen intermediates; VEGF, vascular endothelial growth factor.

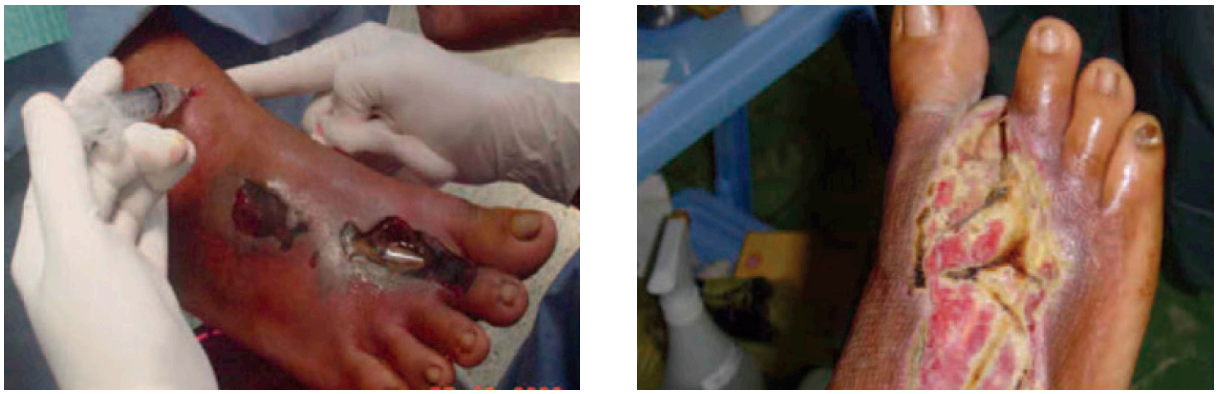

Figure 2. A 48-year-old woman with severe diabetic foot infection condemned previously to our assessment for below-the-knee amputation. Left picture shows the patient foot at initial surgical debridement. Fetid odour, necrosis, cellulitis area of more than 10 cm deep abscess, necrotic injuries and 'browned' colored purulent discharge were present. Right picture shows 2 -3 weeks later that granulating tissue increased and improving of skin condition around ulcer after neutral pH super oxidized aqueous solution treatment.

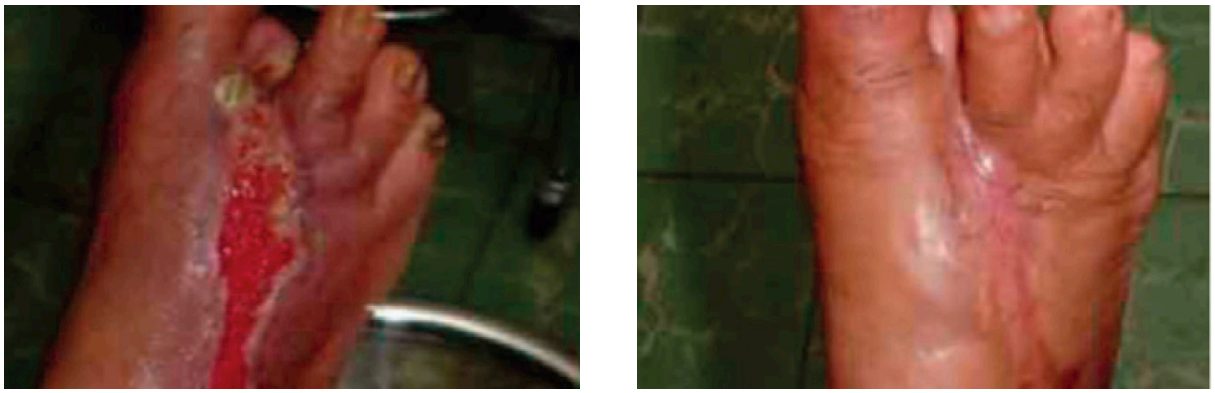

Figure 3. Nine weeks later, after use of neutral pH superoxidised aqueous solution. Granulating tissue, wound contraction and healthy skin around the ulcer. Right picture shows total wound healing in a patient visit 4 months later.

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Renovar™ Advanced Skin and Cleanser and Advanced Wound Irrigation Solution In-Vitro Study Summary

Acute Dermal Toxicity Testing:

The test substance, Renovar, was evaluated for its dermal toxicity potential and relative skin irritancy when a single undiluted dose of 5050 mg/kg was applied to the intact skin of albino rabbits. No mortality occurred during the study. There were no clinical signs of toxicity or signs of dermal irritation at any time throughout the study. There was no effect on body weight gain in animals surviving to termination, with the exception of three animals that lost or failed to gain weight during the second week. The gross necropsy conducted at the termination of the study revealed no observable abnormalities. The estimated LD_{50} as indicated by the data, was determined to be greater than 5050 mg/kg.

Acute Oral Toxicity Testing:

The test substance, Renovar, was evaluated for its acute oral toxicity potential in albino rats when administered as a gavage dose at a level of 5000 mg/kg. The study was terminated following the stopping rules of this procedure. No mortality occurred during the study. There were no clinical signs of toxicity during the study. There was no effect on body weight. The gross necropsy conducted at the termination of the study revealed no observable abnormalities. The acute oral LD_{50} was estimated to be greater than 5000 mg/kg.

Acute Inhalation Toxicity Testing:

The test substance, Renovar, was evaluated for its acute inhalation toxicity potential in albino rats. Five males and five females were exposed for four hours to an aerosol generated from the undiluted liquid test substance at a level of 2.16 mg/l. There was no mortality during the study. Clinical signs included activity decrease and piloerection, which were no longer evident by Day 1. Body weights were unaffected by exposure, except in one animal during the first week. The gross necropsy revealed no observable abnormalities. As indicated by the data, the acute inhalation LC50 is greater than 2.16 mg/l.

Ocular Irritation Testing:

Three New Zealand White rabbits were used to determine the eye irritation potential of a test article. In this test, a 100 mg or 0.1 ml portion of the test article or an extract of a test article is instilled into one eye of each animal. In the case of an aerosolized test article, a single 1-second burst is sprayed from a 10 cm distance at the open eye. The other eye is left untreated to serve as a control. The animals' eyes are gently held closed for one second after dosing to prevent loss of the test article. Both of the animals' eyes are observed and scored at 1, 24, 48, and 72 hours after dosing.

Acute Dermal Irritation Testing:

A primary dermal irritation study was conducted on three albino rabbits. There was one intact test site per animal. Each test site was treated with 0.5 ml of the undiluted test substance and covered with a semi-permeable dressing. The test substance was maintained in contact with the skin for 4 hours. Observations for dermal irritation and defects were made at 1, 24, 48, and 72 hours after removal of the dressings. Irritation scores were derived from the respective erythema and edema scores through the 72-hour observations for each animal.

Skin Sensitization Testing:

A skin sensitization study was conducted on 15 male and 15 female short-haired albino guinea pigs to determine if the test substance produced a sensitizing reaction. Males and females were assigned to each of two groups, designated Groups I (5/sex) and II (10/sex). Group I animals remained untreated during the induction phase of the study and served as a naive control group. Group II animals, the test group, were treated with 0.4 ml of undiluted test substance (selected from previous screening). The animals were treated once weekly for three weeks, for a total of three treatments. After a two-week rest period, all animals (Groups I and II) were challenged at a virgin test site with an application of 0.4 ml of undiluted test substance. The test substance produced no irritation in the naive control animals (Group I) after the single treatment at challenge. The test substance likewise produced no irritation in the test animals (Group II) after the challenge and therefore did not elicit a sensitizing reaction in guinea pigs.

Cytotoxicity Testing:

An in-vitro biocompatibility study based on the United States Pharmacopeia (USP) test was conducted on the test article to determine the potential for cytotoxicity. A filter disc with a 0.1 ml aliquot of the test article, a filter disc control with 0.1 ml of 0.9% Sodium Chloride Irrigation USP, a negative control, and a positive control were each placed on an agarose surface directly overlaying a confluent monolayer of L-929 mouse fibroblast cells. The samples were prepared in duplicate. After incubating at 37° C in 5% CO_2 for 24 hours, the cell culture was examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any). The culture was then examined microscopically (100X) to verify any decolorized zones and to determine cell morphology in proximity to the articles. Under the conditions of this study, the test article showed no evidence of causing cell lysis or toxicity. The test article met the requirements of the USP since the grade was less than a grade 2 (mild reactivity). The filter disc control, the negative control, and the positive control performed as anticipated.

1.0 Safety | Biocompatibility Testing

Animal Model	Results
Genotoxicity	No signs of toxicity
Cytotoxicity	Does not generate cytotoxic effects
Dermal Sensitization (Animal)	No skin sensitization or irritations
Skin Irritation (Rabbit)	No skin irritation detected throughout the study
Ocular Irritation (Rabbit)	No ocular irritation detected
Acute Oral Toxicity (Rat)	No mortality or clinical/behavioral signs of toxicity were detected. Does not cause oral toxicity.
Acute Dermal Toxicity (Rabbit)	No signs of dermal irritation at any time. Does not generate dermal toxicity.
Acute Inhalation Toxicity (Rat)	No signs of any toxic effects when inhaled

2.0 Microorganism Kill Chart

Name of Organism	Log Reduction (30 sec.)	Time to Kill	Percent Reduction
Acinetobacter baumannii	6.3692	30 seconds	99.9999%
Bacteroides fragilis	7.6435	30 seconds	99.9999%
Candida albicans	6.3345	30 seconds	99.9999%
Enterobacter aerogenes	6.0881	30 seconds	99.9999%
Enterococcus faecalis-VRE	6.3646	30 seconds	99.9999%
Enterococcus faecium-VRE MDR	6.5119	30 seconds	99.9999%
Escherichia coli	5.699	30 seconds	99.9998%
Haemophilus influenzae	5.1775	30 seconds	99.9993%
Klebsiella oxytoca-MDR	6.0492	30 seconds	99.9999%
Klebsiella pneumoniae	6.143	30 seconds	99.9999%
Micrococcus luteus	5.842	30 seconds	99.9999%
Proteus mirabilis	6.2028	30 seconds	99.9999%
Pseudomonas aeruginosa	5.8096	30 seconds	99.9998%
Serratia marcescens	5.9978	30 seconds	99.9999%
Staphylococcus aureus-MRSA	6.3454	30 seconds	99.9999%
Staphylococcus aureus	6.2266	30 seconds	99.9999%
Staphylococcus epidermidis	6.0233	30 seconds	99.9999%
Staphylococcus haemolyticus	5.9112	30 seconds	99.9999%
Staphylococcus homins	5.4456	30 seconds	99.9996%
Staphylococcus saprophyticus	5.959	30 seconds	99.9999%
Streptococcus pyogenes	6.716	30 seconds	99.9999%

Renovar demonstrates in-vitro activity against a broad spectrum of gram-negative and gram-positive bacteria while breaking down biofilm in just 30 seconds, allowing healthy tissue to form and reducing the risk of infection.

3.0 Performance | Safety | Compatibility Study

Testing	Results
Bactericidal: (carrier test)	Pass - 1 in 60 samples or less contained surviving bacteria
Bactericidal: (suspension test)	Pass - all three tested organisms a reduction of the bacterial load of more than 105 was achieved within 15 minutes
Bactericidal: (MRSA)	Pass - all 20 samples showed no growth
Bactericidal: Resistant strains (VRE)	Pass - all 20 samples showed no growth
Tuberculocidal (Mycobacterium)	Pass - during the 20-day incubation time period, no growth was observed
Virucidal	Pass - Renovar demonstrated complete inactivation of HIV-1 following a 10-minute exposure time
Fungicidal	Pass - Renovar was fungicidal against Trichophyton mentagrophytes following a ten-minute exposure at 20° C
Sporicidal	Pass - Renovar was sporicidal against Bacillus atrophies spores. The reduction in spores was log 6.5 on average.
AOAC Available Chlorine in Disinfectants	Pass - Renovar with available chlorine levels of approximately 60 ppm demonstrated germicidal equivalence to a control sample with 200 ppm available chlorine

The hypochlorous acid (HOCl) in Renovar contains broad spectrum, antimicrobial activity that is directly toxic to many bacteria and fungi and might also impart antiviral properties.

4.0 Preservative Testing – Successfully meets USP Category 1 Criteria

Organisms	Result Day 7	Result Day 14	Result Day 27
P. aeruginosa ATCC 9027	> 1 log reduction from the initial calculated count	> 3.0 log reduction from initial calculated count	No increase from Day 14
E. coli ATCC 8739			
S. aureus ATCC 6538			
C. albicans ATC 10231	No increase from the initial calculated count	No increase from the initial calculated count	No increase from the initial calculated count
A. niger Atcc 16404			

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