CLINICAL STUDY REPORT

A Prospective, Randomized, Single-Blind, Clinical Investigation of a Neutral pH Superoxidized Water in Patients with Infected Diabetic Foot Ulcers

FINAL: 25 June 2004

Study Conducted by: **Dr. Fermin Rafael Martinez-De Jesus**

Study Performed at: **La Clinica del Pie Diabetico**
Orizaba 198, Colonia Zaragoza
Veracruz, Veracruz, Mexico C.P. 91910

Study Enrollment Period: **November 2003 to March 2004**

CONFIDENTIAL

SIGNATURE PAGE

The undersigned declare that this clinical report accurately reflects the conduct of the study and the analyses of the data collected during the study.

June 25 2007.

Fermin Rafael Martinez-De Jesus, M.D. Date

Table of Contents

1. LIST OF TABLES

2. EXECUTIVE SUMMARY

2.1. Overview: Effectiveness and Safety

The purpose of the study "**A Prospective, Randomized, Single-Blind, Clinical Investigation of a Neutral pH Superoxidized Water in Patients with Infected Diabetic Foot Ulcers"** was to evaluate the effectiveness of Microcyn, a topical antimicrobial agent, for the treatment of infected diabetic foot ulcers as compared with conventional wound therapy. Patients treated with Microcyn showed an important, clinical benefit with respect to the reduction of fetid odor, cellulitis, and tissue toxicity (cytotoxicity) when compared to patients treated with conventional therapy alone.

Forty-five patients were enrolled into the 20 week study. Patients were eligible to be screened if they presented with an infected diabetic foot ulcer. The patients signed an informed consent prior to receiving any study related treatment. Patients in each treatment group received similar treatment of the wound.

There were no statistically significant differences with respect to any demographic characteristics between the Microcyn and Control groups. Patients underwent sharp debridement of the study ulcer to remove necrotic or hyperkeratinized tissue during the study. Patients in the two study arms received similar treatment regimens with the exception that soap and Microcyn were used in place of the povidone iodine and saline rinses. All study wounds received identical dressings, consisting of an application of Italdermol (gel used in providing a moist wound environment), gauze, and adhesive covering. In addition to instructions to avoid weight bearing as much as possible, patients were provided with off weight bearing custom molded inserts to relieve pressure at the ulcer site, if the ulcer was on a weight bearing area. All patients in both treatment groups were seen daily initially, then depending on the condition of the wound, were required to be seen every third day or once a week.

The Endpoints for the study were as follows:

Primary: - Reduction in fetid odor, cellulitis, cytotoxicity

Safety: - Serious Adverse Events

Analysis of the data did reveal a relationship between treatment and odor reduction, cellulitis, and cytotoxicity. All patients (100%) in the Microcyn intervention group showed a reduction in fetid odor, compared to only a quarter (25%) of the patients in the Control group. The percentage of patients in the Microcyn intervention group that showed a reduction in cellulitis was approximately 81% compared to about 44% in the Control group. Cytotoxicity, defined as 1) advancement from infection to the formation of granulation tissue in the wound and 2) development of healthy tissue peri-wound, was observed for the Microcyn intervention group to be about 90% and 94%, respectively. For the Control group, the values were found to be 63% and 31%, respectively.

Overall, the patients treated with Microcyn showed a more positive outcome compared to the Control group in regards to the reduction in fetid odor, cellulitis, and cytotoxicity.

There were no serious adverse events that were directly related with the use of Microcyn in the intervention group during the 20 week study. The number of patients who experienced no change in cellulitis or worsening of the wound was less in the Microcyn intervention group (19%) compared to that in the Control group (56%).

2.2. Design

This study was a prospective, single blind, randomized, controlled investigation that compared a Microcyn regimen to a Control regimen in the treatment of infected diabetic foot ulcers.

Patients were randomized when they met the criteria for the study and when they presented to the diabetic foot clinic. Randomization was by alternate assignment to either Microcyn or Control. Patients were not informed as to whether they were receiving the Microcyn treatment or the Control treatment. However, if a patient happened to become aware of which treatment they were receiving, they were not disqualified from the study.

The group randomized to Microcyn treatment received their first application after qualifying for the study and after debridement. Additional applications of Microcyn were performed at the prescribed visits. Evaluations for both the Microcyn and Control groups were made weekly for the 20 week study.

2.3. Patient Disposition

Within the Study Population (Table 1), eight patients (18%) out of the 45 randomized were excluded from the study immediately after the initial assessments due to severe arterial obstruction in the study leg. The patients were transferred to a vascular surgeon for either limb salvage or major amputation. No other patients dropped out during the study.

2.4. Conclusions

Summary statement: Microcyn is a safe and effective treatment for the reduction of fetid odor, cellulitis, and cytotoxicity of infected diabetic foot ulcers.

Effectiveness: Analyses of the study demonstrated the following:

- Patients treated with Microcyn showed an important clinical benefit with respect to the reduction of fetid odor. All of the patients (100%) showed a reduction in fetid odor compared to only 25% of the Control patients.

- Microcyn patients achieved a significant 81% reduction of cellulitis compared to only 44% of the Control patients.

- Patients in the Microcyn intervention group showed 90% had an improved outcome of the wound (infection to granulation tissue development) compared to 62% for the Control patients.

- Microcyn patients had improved tissue and skin around the wound (94%) compared to Control patients (31%).

Safety: Analyses of the study demonstrated the following:

- Application of Microcyn to the wound was shown to be safe.

- No serious adverse events occurred that were deemed to be causally related to the use of Microcyn.

3. ETHICS

3.1. Ethical Conduct of the Study

The study was performed in accordance with the Helsinki statement.

3.2. Patient Informed Consent

The investigator and/or designated staff were responsible for obtaining the written patient informed consent from each eligible patient prior to enrollment in the study. The patient was provided with both a verbal and written description of the study that covered the objectives, procedures, and possible risks involved. Each patient was informed that he or she was free to withdraw from the study at any time without jeopardizing the future direction of his or her treatment.

3.3. Investigator and Study Administrative Structure

This study was conducted at one center in Mexico (La Clinica del Pie Diabetico, Orizaba 198, Colonia Zaragoza, Veracruz, Veracruz, Mexico C.P. 91910).

The product for the study was prepared by Oculus Technologies of Mexico S.A. de C.V. Salvador Pineda No. 214, Colonia Dr. Miguel Silva, Morelia, Michoacan, Mexico C.P. 58200.

The collection, input, and output of the data for the study was performed by Nicolas Martinez Montanez.

4. BACKGROUND

Oculus Innovative Sciences, Inc. has developed Microcyn, a topical antimicrobial agent designed to help reduce bacteria that potentially can cause skin and wound infections. Microcyn is an electrochemically processed aqueous solution manufactured from pure water and salt (NaCl). Electrical energy is used to produce a change in the aqueous

solution resulting in a highly oxidized, pH neutral water with a controlled amount of free radical ions.

Microcyn is designed to reduce the bacterial counts in the infected diabetic foot ulcers, thereby improving the wound conditions, which may then stimulate the wound healing process and closure of the wound. Wounds that remain infected cannot proceed to the granulation tissue phase of wound healing. Yahagi, et al. hypothesized that reactive oxygen species, shown to be the electron spin resonance spectra present in the anode chamber of the water, might trigger early wound healing through fibroblast migration and proliferation(1).

The safety of Microcyn has been assessed in numerous animal studies and it appears to be safe for human contact and its intended use as an antiseptic. Microcyn has shown significant *in-vitro* rapid-onset antimicrobial activity against a variety of bacterial pathogens, including those most commonly responsible for infections.

Microcyn has been tested against a variety of organisms, including the five most common bacteria found in hospitals: *Staphylococcus aureus,* Coagulase-negative *Staphylococcus* spp. (such as *Staphylococcus epidermidis*), *Enterococcus* spp. (such as *Enterococcus hirae*), *Escherichia coli*, and *Pseudomonas aeruginosa*. Microcyn demonstrated a 10^6 reduction after 15 seconds of exposure against all five organisms.

Additionally, Oculus has conducted several bactericidal tests using Association of Analytical Communities (AOAC) test methods. Using these methods Microcyn was shown to kill *S. aureus, P. aeruginosa, S. choleraesuis,* in one to 10 minutes. Lastly, Microcyn has been shown to be effective against mycobacterium *M. bovis*, HIV-I and Canine Parvovirus.

The Microcyn formulation used in the toxicology studies was the same that was used in the clinical study on human subjects.

Since Microcyn is produced at only a single strength, the dose of Microcyn can only be varied by changes in the volume applied per unit of skin surface area. The doses of Microcyn topically applied to intact skin in the toxicology studies ranged from 0.05 to 0.07 mL/cm^2 in the acute dermal toxicity study to 8.0 mL/cm^2 in the skin irritation study. In full thickness dermal wounds in rats Microcyn was applied at a dose of .09mL/cm^2.

The toxicology studies of Microcyn topically applied to intact skin were conducted using a single application with exposure for 4 to 24 hours. Multiple applications of Microcyn, once or twice daily, over a period of 7 days were assessed in full-thickness dermal wound in rats.

Two studies were conducted to assess the effect of Microcyn on intact skin; acute skin irritation in rabbits and acute dermal toxicity in rabbits. No clinical signs, dermal irritation or abnormalities on gross necropsy were noted in any animals exposed to Microcyn on intact skin.

Characterization of local and systemic toxicities from topically applied Microcyn to a full thickness dermal wound was assessed in rats. No abnormal clinical observations, significant differences in serum chemistry or hematology parameters, or abnormalities on gross necropsy, were noted in any of the animals. Skin irritation scores and histopathology of the wounds and surrounding tissues did not reveal any differences between the Microcyn treated wounds and the saline control treated wounds.

Systemic toxicity of Microcyn was also assessed by intraperitoneal injection in mice. No mortality or evidence of any systemic toxicity was observed in any of the animals receiving a single intrperitoneal dose of Microcyn.

Microcyn was administered by the oral route to rats to permit absorption and characterize any inherent toxicities of the product. No mortality, clinical signs, or abnormalities on gross necropsy were noted in any animals exposed to Microcyn in a single oral dose.

The potential for ocular irritation from topically applied Microcyn was assessed in rabbits. No ocular irritation or other clinical signs were noted in any animals exposed to Microcyn via topical ocular administration.

Microcyn was administered by inhalation to rats to determine the acute inhalation toxicity potential. All animals showed very slight or slight decreased activity and very slight piloerection after exposure but all were asymptomatic by the following day. No mortality or abnormalities on gross necropsy were noted in any animals exposed to Microcyn by inhalation.

The potential for skin sensitization from Microcyn was assessed in guinea pigs using a modified closed patch (Buehler) method. No irritation was seen in the naive control animals after a single treatment challenge or in the test (induction treated) animals after the challenge treatment. Therefore, Microcyn did not elicit a sensitizing reaction.

Genotoxicity, carcinogenicity and reproductive toxicity studies with Microcyn have not been performed.

When applied to animals topically to intact dermis, open full thickness dermal wounds, the conjunctival sac, orally, by inhalation, or by intraperitoneal injection, Microcyn has shown no product related adverse effects. This data supported the belief that Microcyn, applied topically to human skin and wounds, should be well tolerated with regard to both systemic toxicity and local tolerance.

5. OBJECTIVES

The purpose of this study was to assess the safety and effectiveness of Microcyn for the treatment of infected diabetic foot ulcers as compared with conventional wound therapy.

Effectiveness Endpoint was as follows:

Primary: - Reduction in fetid odor, cellulitis, cytotoxicity

Safety Endpoint was as follows:

- Serious Adverse Events

6. METHODOLOGY

6.1. Patient Population

The study was designed to enroll Type II diabetic patients with infected foot ulcers.

To be eligible for enrollment and randomization, patients had to meet the study's inclusion/exclusion criteria.

6.2. Inclusion / Exclusion Criteria

Inclusion Criteria

- Patients age older than 40 years
- Patients with severely infected type II diabetic foot ulcers
- Patients with fetid odor presence
- Patients with ulcers confined to the foot or upon the ankle secondary to the initial foot ulcer
- Patients with various degrees of tissue involvement (e.g., surface to bone)
- Patient has neuropathy
- Patient is willing to participate in the clinical study and can comply with the follow-up regimen.
- Patient has read and signed the clinic's Informed Consent form before treatment.

Exclusion Criteria

- Patients with severe arterial obstructions
- Patients with brachial/ankle index below 0.5
- Patients with total gangrene of the study foot
- Patients with total gangrene of the study forefoot
- Patients with severe cardiovascular and renal failure
- Patients with no detectable pulses in the study leg
- Patients with severe neurological conditions that would make the patient a poor candidate for the study (e.g., confined to the bed)
- Patients with no additional family assistance

- Patients with known allergies to antibiotics

6.3. Treatment

Upon presentation at the clinic the study patients underwent screenings by an ophthalmologist, othorrinolaringologyst, gastroenterologist, and endocrinologist. The patients also underwent orthopedic, vascular, and neurologic assessments. Doppler studies were performed and branch/ankle index was calculated. Neuropathy was assessed using the Michigan test and the Rydel-Seiffer scoring system. The wounds were assessed using the Diabetic Foot Tampico Hospital Classification. This system graded the wounds into three different groups (Groups A, B, and C). Group A are those wounds with a skin disruption without cellulitis and bone infection. Group B are deep wounds with probable bone involvement with 2 cm of cellulitis extension. Group C are all foot depth wounds with cellulitis more than 2cm and the presence of abscess, necrosis, and bone infection. This grading system also assisted in determining the nature of the ulcer (e.g., vascular, neuropathic, or both).

Cellulitis was defined in this study as spreading bacterial infection of the skin and the tissues immediately beneath the skin, which may be caused by many different types of bacteria. Osteomyelitis was identified by radiographic film, bone probe test, or visual diagnosis of bone condition.

All study ulcers were treated according to an outpatient ambulatory model. The study ulcers received sharp debridement of necrotic tissue and drainage of abscess (if required). Patients also received at the first visit a parenteral intramuscular broad spectrum antibiotic (pentoxiphilline) to prevent the systemic spread of bacteria. In addition, the patients were required to be totally non weight bearing until the cellulitis resolved and the formation of granulation tissue was observed. Once granulation tissue was observed in the ulcer, the patient could resume weight bearing activities by utilizing the pressure reducing orthotic provided by the physician. The physician also made modifications to correct for hyperglycemia.

The ulcers in the Microcyn intervention group received a standardized method for treating the wound. After sharp debridement of the ulcer, the wound was washed with surgical soap (Dermo Clean [benzalconium chloride]), rinsed with Microcyn and the ulcer was immersed in Microcyn for 15 – 20 minutes. The surgical soap was used only after the initial debridement of the infected ulcer and while the severe infection was present. If the infected ulcer was located around the ankle region, then a plastic boot filled with Microcyn was utilized. If the infected ulcer was located below the ankle then the ulcer was immersed in a bucket filled with Microcyn. If the wound showed signs of a severe infection (i.e., abscess, tendon spread, or tissue necrosis) then the ulcer was immersed in Microcyn weekly (2- 3 times a week) and between the immersions Microcyn was sprayed on the ulcer until the infection resolved. Once the infection resolved or edema in and around the ulcer was observed, the immersions ceased and only spray applications were continued.

The Control group also received a standard method for wound care. From the onset of the study povidone iodine was used after debridement to clean the wound. When the infection resolved and formation of granulation tissue was observed, the clinic switched to a surgical soap (Dermo Clean) with saline rinse to prevent the cytotoxic effects caused by the use of povidone iodine. If severe deep infection returned, then the use of povidone iodine was resumed.

All of the study wounds (Microcyn intervention group and Control group) were dressed with gauze saturated with trichum vulgare (Italdermol) to moisten the wound followed by adhesive covering. Heavy exudating wounds were dressed with a calcium alginate (e.g., Kaltostat).

6.4. Assessments

Evaluations of the ulcers in both the Microcyn and Control groups were made weekly until the Week 20 visit. Wounds were evaluated for fetid odor, cellulitis, and cytotoxicity. Photographs were taken at every visit to provide a pictorial record.

6.5. Statistical Tests Utilized

For the statistical analysis, the significance level was set at the traditional 5% level. Values of chi squared with Yates correction or Fisher exact for 2x2 tables and of variance ratios for natural and treatment analysis of variance were calculated.

Fetid odor reduction was calculated by the the Kappa agreement index to detect differences between two observers was performed in a 2 x 2 table for foul odor perception. The “K” was calculated by K = Po-Pe/1-Pe, where Po = observed agreement (Pe = expected agreement by random). The calculation result for each observation was confirmed again online at the Decisional Analysis section in the Medical Algorithm Project sponsored by the Institute for Algorithmic Medicine.

To detect if the p value with significant differences (alpha level) had an impact in the clinical decision making the number needed to treat (NNT) was calculated. (2) In a 2x2 table good and bad outcomes in control and experimental groups were used to calculate the absolute risk reduction with a 95% confidence interval. This calculation provide how many patients are necessary to treat to obtain for one of them the benefits of Microcyn for wound cleansing. We calculated, % standard treatment - % new treatment = absolute risk reduction. Then, 100/absolute risk reduction=NNT. Our own calculation of number needed to treat was tested again online at Graphpath.com free calculator for scientist. A large treatment effect, in the absolute scale, leads to a small number needed to treat. NNTs for effective treatments is in the range of 2 – 4.

6.6. Effectiveness Endpoint

6.6.1. Effectiveness Endpoints and Analysis

The endpoints for the study were reduction of fetid odor, cellulitis, and cytotoxicity.

6.7. Safety Analyses

Only serious adverse events occurring after initial treatment with Microcyn or the Control treatment are included in the safety analyses.

7. EFFECTIVENESS RESULTS

7.1. Patient Enrollment and Disposition

Table 1 presents a summary of patient status through Week 20 by treatment group. Forty-five (45) patients were enrolled into the study (24 in the Microcyn intervention group and 21 in the Control group). Eight patients (3 in the Microcyn intervention group and 5 in the Control group) were excluded from the study immediately after the initial assessments due to severe arterial obstruction in the study leg. The patients were transferred to a vascular surgeon for either limb salvage or major amputation. No other patients dropped out during the study.

Table 1
Summary of Patient Status Through Week 20
by Treatment Group

	Microcyn n (%)	**Control n (%)**	**Total n (%)**
Number of Patients	24	21	45
Number and Percent of Discontinued Patients	3 (12.5)	5 (23.8)	8 (17.8)
Reason for Discontinuation			
Adverse Event	0 (0.0)	0 (0.0)	0 (0.0)
Fatal	0 (0.0)	0 (0.0)	0 (0.0)
Non-Fatal	0 (0.0)	0 (0.0)	0 (0.0)
Serious Adverse Event	0 (0.0)	0 (0.0)	0 (0.0)
Patient Requested Discontinuation	0 (0.0)	0 (0.0)	0 (0.0)
Patient Lost to Follow-up	0 (0.0)	0 (0.0)	0 (0.0)
Other	3 (12.5)	5 (23.8)	8 (17.8)
Number and Percent of Patients Completed	21 (87.5)	16 (76.2)	37 (82.2)

7.2. Patient Demographics and Wound Characteristics

As noted in the demographic tables below, there were no statistically significant differences with respect to any demographic characteristics (i.e., age, years of diabetes, glycemia, ulcer duration, branch/ankle index, gender, and body mass index) between the Microcyn intervention group and Control groups.

Table 2 presents a summary of demographic and other patient information at Screening by parameter and by treatment group.

Table 2a
Summary of Continuous Demographic and Other Patient Information at Screening by Treatment Group

Parameter	Treatment Group	N	Mean	S.D.	P-Value
Age (years)	Microcyn Control	21 16	61.9 67.8	11.9 11.6	NS
Diabetes Duration (years)	Microcyn Control	21 16	16.4 17.0	8.1 10.2	NS
Mean Fasting Glycemia	Microcyn Control	21 16	163.0 152.0	59.0 65.8	NS
Ulcer Duration (weeks)	Microcyn Control	21 16	8.58 8.67	8.50 8.50	NS
Branch/Ankle Index	Microcyn Control	21 16	0.9 1.14	0.5 0.7	NS

Table 2b
Summary of Discrete Demographic and Other Patient Information at Screening by Treatment Group

Parameter	Category	Microcyn n (%)	Control n (%)	P-Value
Gender	M F	9 (45.0) 12 (55.0)	8 (50.0) 8 (50.0)	NS
Obesity	≤ 27 kg/m > 27 kg/m	15 (71.4) 6 (28.6)	12 (75.0) 4 (16.0)	NS

7.3. Severity Stage of Diabetic Foot Ulcers

The breakdown of the severity of the study ulcers by treatment group and by classification according to the Diabetic Foot Tampico Hospital Classification is shown in Table 3. Group A wounds were those with a skin disruption without cellulitis and bone infection. Group B wounds were deep wounds with probable bone involvement with 2 cm of cellulitis extension. Group C wounds were all foot depth wounds with cellulitis more than 2cm and the presence of abscess, necrosis, and/or bone infection. The analysis of staging the study ulcer using the Tampico classification and by treatment group was observed to be similar regarding the type of ulcer (vascular, neuropathic, or both) (p=0.873).

Table 3
Stage Severity of Diabetic Foot
Tampico Hospital Grading

Stage	Microcyn Group	Control Group	p-value
Group A	8	5	
Vascular	3	0	
Neuropathic	4	1	
Both	1	4	
Group B	4	4	
Vascular	1	1	0.873
Neuropathic	3	2	NS
Both	0	1	
Group C	9	7	
Vascular	2	1	
Neuropathic	2	2	
Both	5	4	

7.4. Effectiveness Evaluations

The Effectiveness Endpoints for this study were:

- Reduction in fetid odor, cellulitis, and cytotoxicity

Table 4 shows the outcomes of the study ulcers by treatment group for reduction of fetid odor, cellulitis, and cytotoxicity.

Fetid odor reduction was assessed on every wound by two physicians of the staff. Each physician reviewed the wound separately and made the determination if fetid odor was present or not. Then, the physicians confirmed their results. A Kappa value in range of 0.61 – 1.0 was considered between substantial to excellent agreement. Analysis of the data showed that all patients (100%, n=21) treated with Microcyn had a reduction in wound odor versus only 25% (4/16) in the Control group. Controlling fetid odor highlights Microcyn's antimicrobial action in killing bacteria that produce odor in the wound bed. The reduction in fetid odor is almost immediate upon application of Microcyn over necrotic tissue in the wound. Further studies are needed to determine the mode of action of on how it works on the offensive odor produced by necrotic tissue.

Cellulitis reduction was visually assessed by a physician and documented via photographs at each visit. Cellulitis reduction was observed in approximately 81% (17/21) of the Microcyn treated patients compared to about 44% (7/16) in the Control group.

Cytotoxicity parameters were visually assessed by a physician and documented via photographs at each visit. Analyzing for cytotoxicity, the Microcyn intervention group had over 90% (19/21) advanced from the infection stage to having the formation of healthy granulation tissue in the wound compared to about 63% in the Control group. In addition, over 90% (19/21) of the Microcyn treated ulcers demonstrated a clear improvement of tissue and skin around the study ulcers as compared to only 31% (5/16) in the Control group.

Table 4
Effectiveness Analysis for Fetid Odor Reduction, Cellulitis, and Cytotoxicity
Comparison of Treatment Groups

Outcome	Microcyn N (%)	Control N (%)	P-value[1]	NNT[2]
Fetid Odor Reduction	21 (100.0)	4 (25.0)	0.001	2
Cellulitis Reduction	17 (80.9)	7 (43.7)	0.01	3
Cytotoxicity				
Advances from infection to granulating tissue	19 (90.4)	10 (62.5)	0.05	4
Improvement of tissue and skin around the ulcer	19 (90.4)	5 (31.2)	0.001	2

[1] P-values based on Yates correction for chi-squared.

[2] NNT = Number needed to treat. NNT significant clinical efficacy range = 2-4

8. SAFETY EVALUATIONS

Data from all randomized patients are included in the safety analyses.

8.1. Serious Adverse Events

Table 5 presents a summary of serious adverse events that were reported during the 20 week study. No serious adverse events directly related with the use of Microcyn in the intervention group during the 20 week study.

Table 5
Number and Percent of Patients Who Had a
Serious Adverse Event

Treatment Group	N	n (%)
Microcyn	21	0 (0.0%)
Control	16	0 (0.0%)

9. DISCUSSION

9.1. Introduction

The purpose of this pilot study was to evaluate the safety and effectiveness of Microcyn, a topical antimicrobial agent, for the treatment of infected diabetic foot ulcers as compared with conventional wound therapy.

This prospective, randomized, controlled study provided patients in the two study arms the following treatment regimen: Patients underwent sharp debridement of the study ulcer to remove necrotic or hyperkeratinized tissue during the study. Patients in the two study arms received similar treatment regimens with the exception that soap and Microcyn were used in place of the povidone iodine and saline rinses. All study wounds received identical dressings, consisting of an application of Italdermol (gel used in providing a moist wound environment), gauze, and adhesive covering. In addition to instructions to avoid weight bearing as much as possible, patients were provided with off weight bearing custom molded inserts to relieve pressure at the ulcer site, if the ulcer was on a weight bearing area. All patients in both treatment groups were seen daily initially, then depending on the condition of the wound, were required to be seen every third day or once a week. All patients were instructed to be totally off weight bearing until granulation tissue was observed in the ulcer. Then, the patients could be ambulatory with a custom pressure reducing insert.

The primary endpoints for this study were reduction in fetid odor, cellulitis, and cytotoxicity by Week 20.

9.2. Background and Rationale for Use

Patients with diabetes mellitus are prone to the development of foot ulcers due to the pathophysiology of their disease. The development of peripheral neuropathy and the resulting loss of sensation render the foot extremely vulnerable to even minor trauma. Repetitive stress to the insensate foot, an increased susceptibility to infection, and an increased likelihood of having peripheral vascular disease, all serve to impair the healing process in the diabetic patient once an injury has occurred. The combination of peripheral sensory neuropathy, infection, and compromised vascular supply often result in the development of gangrene and the need for amputation.

Studies have shown that patients with diabetes have a higher risk of acquiring or having more severe presentations of various infectious diseases. Defects in host immune defenses are at least partially responsible for this susceptibility. Immune problems include diminished polymorphonuclear leukocyte functions such as abnormalities of migration, phagocytosis, intracellular killing, and chemotaxis. Some evidence suggests cellular immune responses are reduced as well. Poor granuloma formation, prolonged persistence of abscesses, and impaired wound healing may also predispose to infectious complications. Many of these immunodeficiencies are directly related to the metabolic perturbation caused by poorly controlled diabetes.

In the diabetic foot, frequently symptoms often get worse before they get better, probably because with the death of the bacteria, substances that cause tissue damage are released. When this occurs, the body continues to react even though the bacteria are dead. Antibiotics are continued for 10 days or longer even though the symptoms may disappear earlier. Prompt empirical treatment with antibiotics can prevent the infection from spreading rapidly and reaching the blood and organs. (3)

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 parenteral antimicrobials necessary, in addition, local antiseptics for wound cleansing are mandatory.

Previously to the introduction of treatment with superoxidized solution severe problems existed in making the right treatment decisions between a more toxic, but potent antiseptic like povidone-iodine and a safe but weak antiseptic like soap. A dry, dark, epidermal lysis and hardened skin resulted when povidone-iodine was used delaying wound healing and causing confusion about the wound condition (if the injury or local worsening was caused by infection or chemical damage). In this study, we used povidone iodine only at initial surgical debridement or when the wound became a severe deep infection, when damage to tissues is not yet important.

Once the severity of the infection was reduced, more patients advanced to granulating tissue stage with Microcyn treatment. A necrotic and infected wound shows progress toward healing when the volume of necrotic tissue is reduced and signs of infection are eradicated, even if the dimensions of the wound have increased. In contrast, a granulating wound should exhibit a steady reduction in wound depth, wound dimensions and volume of exudates. (4,5)

The similar characteristics between groups mainly in metabolic control and severity of wound in depth and cellulitis extension and vascular or neuropathic etiology avoided bias and determined that antisepsis was more effective and showed less tissue toxicity and better control of odor with Microcyn than the conventional group treated with povidone-iodine, saline and soap. Fetid odor absence, followed cellulitis was the main source of good outcomes showen in this study. Less toxicity over granulating tissue increased the rate of patients advancing to the next wound healing stage, meanwhile patients in Control group remained infected to chronicity or even worsening. A common reason for failure to progress is excessive bioburden, i.e., bacterial counts on the wound surface sufficient to retard the repair process. One difficulty in managing these wounds is the fact that clinical indicators for surface infections are different than the signs associated with invasive infection, many times overcoming when povidone-iodine was used. Whereas invasive infection produces the classic signs of erythema, edema 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, repeated formation of a thin layer of avascular tissue, and increased pain. (6,7) The success of modalities of treatment must be determined in order to choose a new proposal of treatment, significant statistic results are not always enough to take clinical decision.

For this reason we calculated the Number Needed to Treat (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. These measures are used extensively in both clinical and epidemiological investigations. For clinical decision making, however, it is more meaningful to use the measure "Number Needed to Treat." This measure is calculated on the inverse of the absolute risk reduction. It has the advantage that it conveys both statistical and clinical significance to the doctor. Furthermore, it can be used to extrapolate published findings to a patient at an arbitrary specified baseline risk when the relative risk reduction associated with treatment is constant for all levels of risk. More emphasis is now being put on effective use of biomedical literature to guide clinical treatment. As a result accessing, critically appraising, and incorporating the results of clinical investigations into clinical practice are becoming higher priorities for doctors and medical students. In the ideal treatment situation every patient would receive 100% of drugs, surgery or any other interventional benefits. Unfortunately, the real condition in clinical practice is that this benefit could be obtained in less proportion. Good or bad outcomes for interventions must be compared with a control group and determine the difference of absolute risk reduction and then the NNT. In this study NNT provided us with information about significant difference in absolute risk reduction on the intervention group. Of clinical importance in decision making is Microcyn's reduction of foul odor in one patient of every three treated, its significant cellulitis reduction in one of three patients in intervention group or advances from infection to granulating stage and less tissue toxicity in one of two patients, respectively.

Diabetic foot infections are a frequent and complex problem with serious financial and medical consequences. Fortunately, much progress has been made in the past two decades. Careful studies in our own experience (8,9) have shown that patients with mild to severe, non-limb-threatening infections can be treated as outpatients with intramuscular or oral antibiotic and 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. In the large majority of patients, a functional limb may be preserved. The continued development of new treatments like Microcyn as well as the synthesis of existing ones in a cost-effective, evidence-based, multidisciplinary manner is the challenge currently facing all healthcare professionals who care for diabetic foot infections.

The purpose of the study upon which this report is based was to generate data on the safety and effectiveness of Microcyn in the setting of a prospective, randomized, controlled study.

9.3. Effectiveness of Microcyn

The effectiveness of Microcyn could be obtained by three simultaneous actions: 1) its steriliant properties, 2) enhancing wound healing, and 3) less cytoxicity to new granulating tissue and surrounding healthy tissue. The solution contains a high concentration of hypochlorous acid, a well-known and potent germicidal agent but ineffective to accelerate the healing of full-tickness cutaneous wounds in rats. (1) The

enhancement of wound healing by a mechanism unrelated to the antibacterial action of HOCI, may be explained by the presence of reactive oxygen species that might trigger early wound healing through fibroblast migration and proliferation. The hydroxy radicals also have a germicidal effect. The electrical energy of the solution itself destroys microorganisms including fungi, viruses, mycobacteria, spirochetes, and bacteria. Acid solution loses both its electrolitical potential and its germicidal action when it comes into contact with multicellular organic matter, and it reverts to ordinary water. Conversely, Microcyn appears to be more effective to retain its germicidal action in this condition.

10. SAFETY PROFILE OF MICROCYN

The data from this study demonstrated that Microcyn was safe to use and was not associated with the development of any specific type of serious adverse events.

Case studies have been conducted to evaluate the use of Microcyn in a number of different indications (venous ulcers, diabetic foot ulcers, oral infections, surgical wounds, and burns). In these studies, there have been no reported incidences of serious adverse events reported by investigators to date.

The data from this study showed Microcyn to have a safety profile comparable to that of the Control treatment. Specifically, treatment with Microcyn was shown to be safe and its use did not lead to the occurrence of any serious adverse events.

11. CONCLUSIONS

Microcyn is a safe and effective treatment for the resolving infected diabetic foot ulcers.

- Patients treated with Microcyn showed an important clinical benefit with respect to the reduction of fetid odor. All of the patients (100%) showed a reduction in fetid odor compared to only 25% of the Control patients.

- Microcyn patients achieved a significant 81% reduction of cellulitis compared to only 44% of the Control patients.

- Patients in the Microcyn intervention group showed 90% had an improved outcome of the wound (infection to granulation tissue development) compared to 62% for the Control patients.

- Microcyn patients had improved tissue and skin around the wound (94%) compared to Control patients (31%).

Safety: Analyses of the study demonstrated the following:

- Application of Microcyn to the wound was shown to be safe.

- No serious adverse events occurred that were deemed to be causally related to the use of Microcyn.

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