

An Open-label, Three-arm Pilot Study of the Safety and Efficacy of Topical Microcyn Rx Wound Care versus Oral Levofloxacin versus Combined Therapy for Mild Diabetic Foot Infections

Adam Landsman, DPM, PhD*
Peter A. Blume, DPM†
Douglas A. Jordan, Jr., DPM‡
Dean Vayser, DPM§
Andres Gutierrez, MD, PhD||

Background: This randomized, prospective, multicenter, open-label study was designed to test whether a topical, electrolyzed, superoxidized solution (Microcyn Rx) is a safe and effective treatment for mildly infected diabetic foot ulcers.

Methods: Sixty-seven patients with ulcers were randomized into three groups. Patients with wounds irrigated with Microcyn Rx alone were compared with patients treated with oral levofloxacin plus normal saline wound irrigation and with patients treated with oral levofloxacin plus Microcyn Rx wound irrigation. Patients were evaluated on day 3, at the end of treatment on day 10 (visit 3), and 14 days after completion of therapy for test of cure (visit 4).

Results: In the intention-to-treat sample at visit 3, the clinical success rate was higher in the Microcyn Rx alone group (75.0%) than in the saline plus levofloxacin group (57.1%) or in the Microcyn Rx plus levofloxacin group (64.0%). Results at visit 4 were similar. In the clinically evaluable population, the clinical success rate at visit 3 (end of treatment) for patients treated with Microcyn Rx alone was 77.8% versus 61.1% for the levofloxacin group. The clinical success rate at visit 4 (test of cure) for patients treated with Microcyn Rx alone was 93.3% versus 56.3% for levofloxacin plus saline-treated patients. This study was not statistically powered, but the high clinical success rate (93.3%) and the *P* value (*P* = .033) suggest that the difference is meaningfully positive for Microcyn Rx-treated patients.

Conclusions: Microcyn Rx is safe and at least as effective as oral levofloxacin for mild diabetic foot infections. (J Am Podiatr Med Assoc 101(6): 484-496, 2011)

Concern continues to grow over the development of drug-resistant bacteria, partly due to the lack of judicious use of oral antibiotics. It is widely suggested that topical antibiotics may be sufficient for the treatment of mildly infected wounds and

may offer additional benefits when the patient has systemic complications such as renal or hepatic disease. Mild diabetic foot infections are characterized by their involvement of only skin and soft tissues, without evidence of systemic factors, such as ascending cellulitis. Successful treatment of mild diabetic foot infections requires meticulous attention to local and systemic conditions.¹

Optimal wound care starts with cleansing, debridement, and off-loading of the lesion.² Historically, patients are then treated with oral antibiotics (Infectious Diseases Society of America guidelines/level B-1 evidence).^{1,3} Although the Infectious Diseases Society of America guidelines do not recommend a particular antibiotic regimen, fluoro-

*Podiatric Surgery, Harvard Medical School/Cambridge Health Alliance, Cambridge, MA.

†Surgery, Orthopedics, and Rehabilitation, Yale School of Medicine, New Haven, CT.

‡Clinical Research of Tampa Bay, Port Richey, FL.

§Scripps, San Diego, CA.

||Oculus Innovative Sciences, Petaluma, CA.

Corresponding author: Adam Landsman, DPM, PhD, Podiatric Surgery, Harvard Medical School/Cambridge Health Alliance, 1493 Cambridge St, Cambridge, MA 02139. (E-mail: adamlandsman@aol.com)

quinolones are commonly used as first-line therapy because these are drugs approved by the Food and Drug Administration for complicated skin and skin structure infections. In terms of efficacy, 78.0% to 83.4% of all diabetic foot infections respond satisfactorily to levofloxacin, the fluoroquinolone most commonly prescribed in these cases.³ However, the use of this and other systemic antibiotics is associated with adverse effects and increased costs of care. Moreover, researchers have recently found an increasing number of methicillin- and fluoroquinolone-resistant bacteria from diabetic foot infections against which this and other common antibiotics have no effect.^{4, 5} It has, thus, been suggested that topical biocides could overcome these problems, but the lack of randomized controlled trials has made it difficult or impossible to establish their role in the treatment of diabetic foot infections (Infectious Diseases Society of America guidelines, Nelson). Initial results from uncontrolled studies⁶ in other countries, however, suggest that electrolyzed topical solutions (Microcyn technology) may be effective in the treatment of mild diabetic foot infections.

Microcyn technology-based solutions are electrochemically processed aqueous solutions manufactured from pure water and US Pharmacopeia-grade sodium chloride. During this electrolysis process, water molecules are pulled apart, and hypochlorite or hypochlorous species are formed. The resultant electrochemically modified solution is superoxidized and has been shown to possess broad-spectrum activity against bacteria, mycobacteria, fungi, protozoa, and viruses *in vitro*,⁷⁻⁹ in animal models,^{10, 11} in intact skin,⁶ and in human wounds, particularly in diabetic foot infections.¹²⁻¹⁷

Although the effect of electrolyzed solutions on bacteria and other pathogens has been demonstrated previously, the use of these solutions has been significantly limited owing to difficulties in storing and transporting it in a manner that will preserve its efficacy. More recently, advances in the manufacturing process and in the development of new storage bottles has led to much greater availability and greatly extended shelf life. One such new product, Microcyn Rx (Oculus Innovative Sciences, Petaluma, California), is a topical wound antimicrobial with a shelf life of more than 12 months. This product, which is the focus of the present study, was recently thrust into the forefront of the media when it was discovered that Microcyn Rx was highly effective against the H1N1 virus associated with swine flu. Mexican health officials committed to buying vast quantities to treat swine flu and have

demonstrated that it is safe and effective for considerably reducing the potential for spreading this serious disease.

The open-label, multicenter, randomized controlled trial reported herein compared the use of topical Microcyn Rx alone versus topical saline solution plus levofloxacin versus Microcyn Rx plus levofloxacin for the treatment of mild diabetic foot ulcers.

For the purpose of this study, it is hypothesized that mildly infected diabetic foot ulcers treated with Microcyn Rx demonstrate clinical success, as defined by clinical cure or improvement, that is similar to that of wounds treated with either oral levofloxacin and saline irrigation or oral levofloxacin and Microcyn Rx. This would suggest that topical treatment with Microcyn Rx would be equivalent to oral antibiotics for the treatment of this type of wound.

Secondary study aims evaluated microbiologic cure between patients treated with Microcyn Rx alone versus Microcyn Rx with oral levofloxacin as well as microbiologic cure of Microcyn Rx versus oral levofloxacin plus saline irrigation, and these data are presented herein as well. These data demonstrate that the use of this electrolyzed topical solution is highly effective for treating mild diabetic foot infections. We believe that this could become the new standard of care for the treatment of this type of diabetic foot infection.

Materials and Methods

This article describes a randomized open-label, three-arm, prospective pilot study of the safety and clinical efficacy of topical Microcyn Rx wound care solution versus oral levofloxacin versus combined therapy for the treatment of mild diabetic foot infections. Patients were enrolled in the study at 16 study centers in the United States between May 1, 2007, and December 31, 2007, in accordance with the ethical principles of the current version of the Declaration of Helsinki. The study protocol and informed consent document were approved by the institutional review board of each participating institution or independent ethics committees. Written informed consent was obtained from each participant.

Patients

Individuals who met all of the inclusion criteria and none of the exclusion criteria and who were willing

to have microbiologic samples procured and otherwise comply with the schedule of assessments were eligible for the study. All of the patients were at least 18 years of age with diabetes mellitus (type 1 or 2) and had a mild diabetic foot infection. Eligible foot ulcers involved skin and deeper soft tissue and were classified by Infectious Diseases Society of America guidelines as mildly infected and by the University of Texas Classification as 1B. All infections were of presumed bacterial etiology. Ulcers could be located on the foot and malleolar areas, measured 1 to 9 cm², and were accessible for culture. Adequate circulation to the foot was required, as evidenced by an ankle-brachial index greater than 0.8 measured by Doppler scanning or transcutaneous oxygen pressure of at least 30 mm Hg and a palpable pulse on the study foot (either dorsalis pedis or posterior tibial artery).

Patients were excluded if they received antibiotic treatment for more than 24 hours within 72 hours of study entry. Patients with necrotizing fasciitis, deep abscesses in the soft tissue, sinus tracts, gas gangrene, or infected burns were excluded, as were those with superinfected eczema or other chronic medical conditions, those with ulcers located on the stump of an amputated extremity, and those with ulcers having a nondiabetic etiology. Infections complicated by the presence of prosthetic materials and osteomyelitis were also excluded. Women of childbearing potential who were unable to take adequate contraceptive precautions, had a positive urine pregnancy test result within 24 hours before study entry, were otherwise known to be pregnant, or were breastfeeding or planned to become pregnant during the time of the study were excluded, as were patients with liver disease (total bilirubin level >5 times the upper limit of normal), those with neutropenia (absolute neutrophil count <500 cells/mm³), and those with known hypersensitivity to chlorine or quinolones. Patients receiving glucocorticoid regimens (>5 mg of prednisone per day or equivalent) and those receiving adjuvant therapy with hyperbaric oxygen or topical formulations containing growth factors (ie, platelet-derived growth factor gel), antimicrobials (ie, bacitracin and mupirocin), enzymatic debriders (ie, Accuzyme; HealthPoint, Ltd, Ft Worth, TX), or granulation promoters (ie, Regranex, Systagenix Wound Management, London, England) were also excluded from the study, as were those with disorders of immune function (human immunodeficiency virus or chronic granulomatous disease). Any patient with a medical condition that, in the investigator's

opinion, would require dose modification of levofloxacin to less than 750 mg/d (ie, renal disease requiring dialysis) or who had received an investigational agent within 1 month before the baseline evaluation also was excluded. Table 1 summarizes the inclusion and exclusion criteria.

Assessments

Patients who agreed to participate in this study underwent baseline medical history, physical examination, wound assessment, and screening tests, including hematology, blood chemistry, urinalysis, electrocardiography, and, in women, urine pregnancy testing. Radiographs of the study foot were taken (≥ 2 images) at baseline (visit 1) and at the test-of-cure visit solely for the purpose of ruling out osteomyelitis. For patients who had more than one qualifying ulcer, the largest one was used for study treatment and wound assessment. Wound measurements, photographs, and cultures (aerobic, anaerobic, and yeast) with susceptibility testing were obtained after debridement.

Patients were evaluated on day 3 \pm 1 day (visit 2), at the end of treatment (10 days \pm 1 day), and 14 days after completion of therapy (test of cure = end of treatment + 14 days \pm 1 day). These evaluations consisted of a clinical wound assessment and photographs to determine the clinical response to therapy. For visit 2 and end of treatment, if a microbiologic sample was obtained at visit 1 with a confirmed baseline pathogen and the patient was clinically evaluable, then the patient was considered microbiologically evaluable at that visit. Clinical laboratory tests and electrocardiography were repeated at the end of therapy. Safety and wound-healing end points also were evaluated at end of treatment and test of cure. The study was completed on day 28.

Treatments

Eligible patients were randomly assigned to receive appropriate wound care and one of the following treatments for 10 days \pm 1 day: 1) topical Microcyn Rx monotherapy once per day; 2) topical sterile nonbacteriostatic saline plus oral levofloxacin, 750 mg once per day; or 3) topical Microcyn Rx plus oral levofloxacin at the same dose. Wound cleaning and coverage was performed once a day with 30 mL of either Microcyn Rx or saline. Sterile gauze was saturated with approximately 25 mL of Microcyn Rx or saline, and the excess solution was wrung out.

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
<ul style="list-style-type: none">• Males and females >18 years of age with diabetes mellitus (type 1 or type 2) who have a foot infection.• Presence of an infected, nonischemic diabetic foot ulcer that involves skin and deeper soft tissue as stratified by Infectious Diseases Society of America guidelines and the University of Texas Classification/1B.¹⁸• Foot ulcers presumed to be of bacterial etiology and that are anticipated to be cured within 10 days of oral antibiotic therapy.• Foot ulcers located in the plantar, dorsal, or malleolar area.• Ulcers 1–9 cm² in size.• Accessible infection site for culture.• Ankle-brachial index by Doppler ≥ 0.8 or transcutaneous oxygen pressure ≥ 30 mm Hg.• Adequate circulation to the foot as evidenced by a palpable pulse on the study foot (either dorsalis pedis or posterior tibial artery).• Willing and able to give informed consent.• Willing to comply with the requirements for participation in the study.	<ul style="list-style-type: none">• Previous antibiotic treatment received for >24 h within 72 h of study entry unless the pathogen showed drug resistance or the treatment failed (defined as no clinical improvement after 3 days of treatment).• Necrotizing fasciitis, deep abscesses in the soft tissue, sinus tracts, gas gangrene, or infected burns.• Superinfected eczema or other chronic medical conditions (ie, atopic dermatitis) where inflammation may be prominent for an extended period even after successful bacterial eradication.• Ulcer located on the stump of an amputated extremity.• Ulcer due to a nondiabetic etiology (arterial insufficiency, venous stasis, radiation, trauma, rheumatoid arthritis, vasculitis, collagen vascular disease, nondiabetic etiologies).• Infections complicated by the presence of prosthetic materials, such as central venous catheters, permanent cardiac pacemaker battery packs, those involving joint replacement prostheses, etc.• Osteomyelitis.• Females of childbearing potential who are unable to take adequate contraceptive precautions, have a positive urine pregnancy test result within 24 h before study entry, are otherwise known to be pregnant, or are breastfeeding or plan to become pregnant during the study.• Liver disease, with total bilirubin >5 times the upper limit of normal; known to have neutropenia (absolute neutrophil count <500 cells/mm³).• Hypersensitivity to chlorine or quinolones.• Need for any additional concomitant systemic antibacterial agent other than the study drug(s).• Concomitant glucocorticoid doses or regimens that may compromise evaluation of the study drug (>5 mg of prednisone a day or an equivalent).• Adjuvant therapy with hyperbaric oxygen or topical formulations containing growth factors (ie, platelet-derived growth factor gel), antimicrobials (ie, bacitracin, mupirocin), enzymatic debriders (ie, Accuzyme), or promoters of granulation (ie, Regranex).• A history of diseases of immune function (human immunodeficiency virus, chronic granulomatous disease).• Any medical condition that, in the investigator's opinion, will require dose modification of levofloxacin to <750 mg/d.• Received an investigational agent ≤ 1 month before the baseline evaluation.

Working from the inside out, the wound was scrubbed gently to remove drainage and exudates. Once the wound bed was prepared, another sterile 2 × 2-inch gauze pad was saturated with an additional 5 mL of Microcyn Rx or saline, and the excess solution was wrung out. Enough of the soaked gauze was applied to fill, but not tightly pack, the wound. The wound was covered with an occlusive dressing after each dressing change. At the end-of-treatment visit, all of the wounds were cleaned and dressed using only 30 mL of sterile saline daily until the test-of-cure visit. A dry dressing also was permitted if the wound was closed. All of the treatments were recorded daily in a patient diary. Off-loading, if necessary, was achieved with fixed ankle boots or healing sandals, as indicated by the investigator. Debridement procedures were limited to three for the duration of the study.

Clinical Efficacy

Clinical efficacy was evaluated using the investigator assessment of one of the following “clinical outcome” criteria at visits 3 and 4 relative to the baseline assessment of wound condition:

1. *Cure*: Resolution of all signs and symptoms, including the presence of culturable exudates, warmth, erythema, induration, tenderness, pain, swelling, and a healing wound (as determined by the investigator) after 5 or more days of treatment.
2. *Improvement*: Resolution of at least two signs as described for cure after at least 5 days of treatment.
3. *Failure*: Persistence or progression of baseline clinical signs and symptoms of infection after at least 3 days of therapy requiring a switch to an antibiotic other than levofloxacin.
4. *Indeterminate*: Circumstances preclude classification.

The primary clinical efficacy end point was clinical success, defined as an outcome of either cure or improvement. For a patient to be considered a clinical success, he or she could not have received additional nonstudy drugs as antimicrobial therapy (topical, systemic, or antimicrobial dressings), undergone surgical intervention (debridement was not considered a surgical intervention for this study), or developed osteomyelitis. In addition, all presenting signs and symptoms of infection must

have resolved without the appearance of any new ones.

Wound measurements were made using a ruler and photographic analysis. Electronic data from the digital photographs were used for the data summaries. Investigators were trained to take the photographs at approximately a 1-foot distance from the lesion. The wound area was then calculated from the photograph by an independent third party using validated software (PictZar version 4.02; BioVisual Technologies LLC, Elmwood Park, New Jersey).

Microbiological Response

Microbiologic response was assessed at visit 2 and, as the microbiologic end point, at visit 3. Patients were classified by microbiologic response as follows:

Eradication: Elimination of the causative organism(s) from the same site during or on completion of therapy.

Presumed eradication: Where a post-therapy culture was not obtained because there was no culturable material and there is an adequate clinical response.

Persistence: Failure to eradicate the original causative organism at all post-baseline time points from sites previously cultured, regardless of whether signs and symptoms of infection are present.

Relapse: Reappearance of the original causative organism from the original site of infection after a post-baseline culture has been negative.

Superinfection: Development of a new infection during the study that is attributable to a new pathogen that was not recognized as the original causative organism.

Colonization: Positive culture yielding a bacterial strain other than the primary causative isolate and not associated with fever or other signs and symptoms of infection.

Contaminants: Presence of coagulase-negative *Staphylococci* and *Corynebacterium* spp as the sole isolate from an appropriately obtained specimen.

Safety

Safety was assessed on the basis of 1) physical examination findings, 2) vital sign measurements, 3) clinical laboratory test results, and 4) adverse events. Patients who received at least one dose of study drug were included in the safety assessment.

Statistical Analysis

Randomization was accomplished at each study site by using a manual system with envelopes containing group designations opened sequentially and was stratified by site. The study was terminated after at least 15 patients were clinically and microbiologically evaluable per study arm. Because this study was not statistically powered, the target of more than 15 evaluable patients per arm was not deemed necessary to show proof of concept. There were no safety issues influencing the decision to terminate the study early.

The primary analysis was comparison of the clinical success rates in the Microcyn Rx group and the saline solution plus levofloxacin group at visit 3 using the intention-to-treat sample. The intention-to-treat sample consisted of all randomized patients who received at least one dose of study drug and provided any on-treatment data. Logistic regression was used for the comparison, and the logistic model included terms for treatment and duration of wound at baseline. Estimates and confidence intervals were calculated for the clinical success rates for each treatment group and for the treatment odds ratios. For patients who were in the intention-to-treat sample but had a missing result for clinical outcome at visit 3, the result was considered a failure.

The same analysis was used to compare the clinical success rates of the Microcyn Rx group and the saline solution plus levofloxacin group at visit 4 and at either visit 3 or 4 and the Microcyn Rx group and the Microcyn Rx plus levofloxacin group at visit 3, visit 4, and either visit 3 or 4. For patients who were in the intention-to-treat sample but had a missing result for clinical outcome at visit 4, the result was considered a failure at visit 4. For patients who were in the intention-to-treat sample but with a missing result for clinical outcome at both visits 3 and 4, the result was considered a failure at visit 3 or 4.

The numbers and percentage of clinical successes were summarized by treatment at three time points: visit 3, visit 4, and either visit 3 or 4. Clinical outcome was also listed. All of these analyses were performed using the intention-to-treat sample.

Results

Patient Sample

Eighty-one patients were enrolled, of which 67 were randomized. Of these, 66 were analyzed in the intention-to-treat sample, and 59 (88.1%) completed

the study. The number of patients discontinuing the study was slightly higher in the Microcyn Rx plus levofloxacin group ($n = 4$; 16.0%) than in the Microcyn Rx alone and saline plus levofloxacin groups ($n = 2$; 9.5% each). The most common reasons for withdrawal were non-drug-related adverse events and loss to follow-up ($n = 3$; 37.5% each). The enrollment, treatment, and analysis of patients are demonstrated in Figure 1.

Baseline characteristics of the 67 randomized patients, including age and body mass index, were similar across the three treatment groups (Table 2). The mean age was 57.2 years. Most patients were white (89.6%) and male (73.1%). The mean weight was 98.36 kg, mean height was 176.63 cm, and mean body mass index (calculated as weight in kilograms divided by height in meters squared) was 31.39. Medical history, diabetes history, and diabetic foot ulcer history were generally similar in the three treatment groups. Ulcer location was also similar; most ulcers (82.1%) were located on the sole of the foot.

Clinical Efficacy

In the intention-to-treat group, the overall clinical success rate (cure or improvement) was highest in the Microcyn Rx alone group at visits 3 and 4 (Table 3). In the intention-to-treat sample at visit 3, the clinical success rate, defined as patients achieving cure or improvement, was higher in the Microcyn Rx alone group (75.0%) than in the saline plus levofloxacin group (57.1%) or in the Microcyn Rx plus levofloxacin group (64.0%). Results at visit 4 were similar: 75.0% for the Microcyn Rx alone group, 52.4% for the saline plus levofloxacin group, and 72.0% for the Microcyn Rx plus levofloxacin group. The differences in clinical success rates among the three treatment groups were not statistically significant at any visit; however, Microcyn Rx alone seemed to have an effect on clinical success that was comparable with that of saline plus levofloxacin. A Kaplan-Meier plot showing time to clinical success is shown in Figure 2.

In the clinically evaluable study population, the clinical success rate at visit 4 (test of cure) for patients treated with Microcyn alone was 93.3% compared with 56.3% for levofloxacin plus saline-treated patients (Fig. 3). This study was not statistically powered, but the high clinical success rate (93.3%) and the P value of 0.033 suggest that the difference is meaningfully positive for Microcyn-treated patients. Also, for this set of data, the 95% confidence interval for the Microcyn-only arm

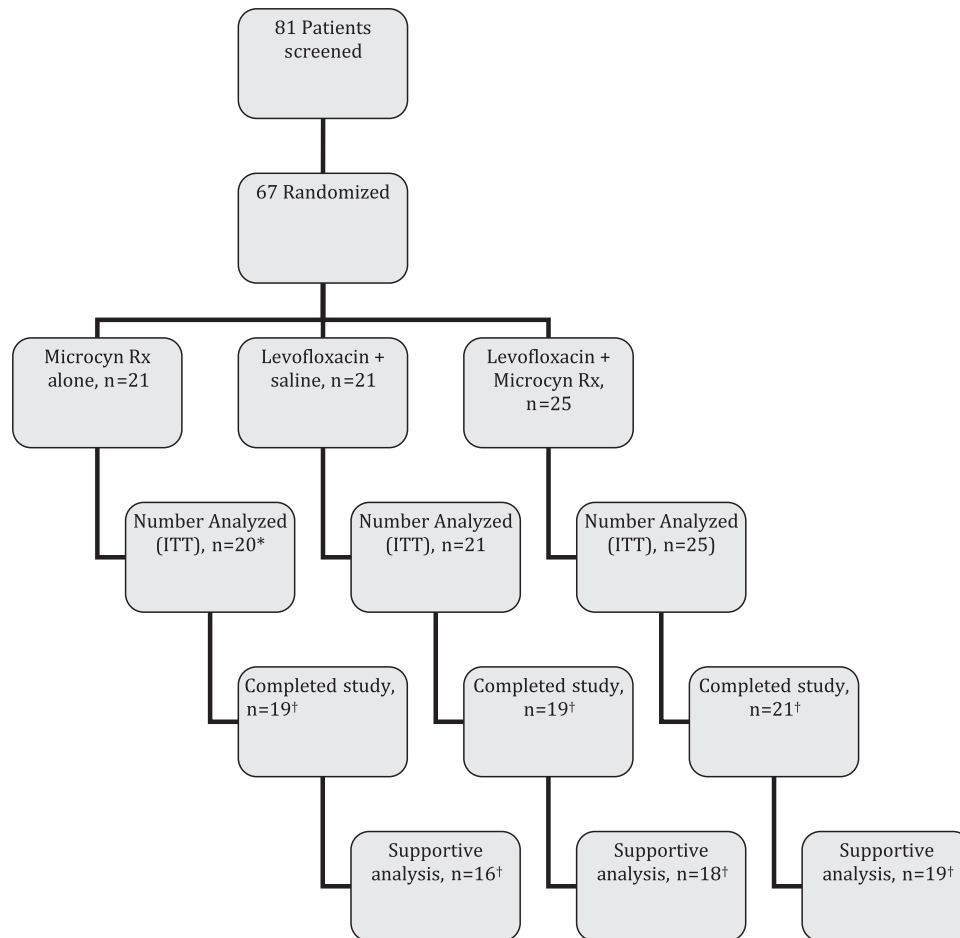


Figure 1. Consort diagram demonstrating the enrollment and evaluation of patients in this study. *Number analyzed in the intention-to-treat (ITT) Microcyn Rx alone group was 20 of 21 because one patient was removed after receiving the study drug because he or she did not provide any on-treatment data. †Microcyn Rx: two patients discontinued (one protocol violation and one voluntarily withdrew); levofloxacin + saline: two patients discontinued (1 adverse event and 1 lost to follow-up); and levofloxacin + Microcyn Rx: four patients discontinued (two adverse events and two lost to follow-up). ‡The supportive analysis was a secondary analysis that evaluated only patients who had baseline wound culture results.

ranged from 80.7% to 100.0%, and that for the levofloxacin and saline arm ranged from 31.9% to 80.6%; the confidence intervals do not overlap, thus indicating a favorable clinical success rate for Microcyn compared with levofloxacin. At visit 3 (end of treatment), the clinical success rate for patients treated with Microcyn alone was 77.8% compared with 61.1% for the levofloxacin group.

Microbiologic Efficacy

The per-patient and per-pathogen microbiologic response was better in the saline plus levofloxacin and Microcyn Rx plus levofloxacin groups than in the Microcyn Rx alone group. Overall, more patients in the groups receiving levofloxacin were classified

as microbiologic cures than in the Microcyn Rx alone group; however, no statistically significant differences among the treatment groups were observed.

Clinical outcome was not necessarily predictive of microbiologic outcome. The largest disparity between clinical outcome and microbiologic response was seen in the Microcyn Rx alone group compared with the saline plus levofloxacin and Microcyn Rx plus levofloxacin groups. In the Microcyn Rx alone group, the clinical success rate was consistently higher than the microbiologic response rate.

Favorable microbiologic response at visit 2 for all pathogens was more common in the Microcyn Rx plus levofloxacin group, followed by the saline plus

Table 2. Demographics and Baseline Characteristics of the Randomized Sample

	Microcyn Rx (n = 21)	Saline + Levofloxacin (n = 21)	Microcyn Rx + Levofloxacin (n = 25)	Total (N = 67)
Age (years)				
Mean (SD)	55.4 (12.81)	56.5 (12.21)	59.2 (12.94)	57.2 (12.59)
Range	35–82	35–79	27–81	27–82
Sex (No. [%])				
Male	16 (76.2)	16 (76.2)	17 (68.0)	49 (73.1)
Female	5 (23.8)	5 (23.8)	8 (32.0)	18 (26.9)
Race (No. [%])				
White	20 (95.2)	18 (85.7)	22 (88.0)	60 (89.6)
Black	1 (4.8)	2 (9.5)	3 (12.0)	6 (9.0)
Other	0	1 (4.8)	0	1 (1.5)
Ethnicity (No. [%])				
Hispanic or Latino	3 (14.3)	2 (9.5)	4 (16.0)	9 (13.4)
Not Hispanic or Latino	18 (85.7)	19 (90.5)	21 (84.0)	58 (86.6)
Body mass index ^a				
No.	21	21	25	66
Mean (SD)	32.56 (5.936)	31.68 (5.928)	30.11 (6.388)	31.39 (6.096)
Range	25.0–49.2	19.4–42.7	20.8–43.3	19.4–49.2

Note: Percentages are based on the number of randomized patients in each treatment group; values in boldface represent totals.

^aBody mass index was calculated as weight in kilograms divided by height in meters squared.

levofloxacin group and the Microcyn Rx alone group. This was also true at visit 3, suggesting that combination therapy with Microcyn Rx plus levofloxacin resulted in eradication of more pathogens.

The clinical success rate per baseline pathogen was similar among the treatment groups at visit 2. At visit 3, the ratio between clinical success and baseline pathogens overall was highest in the Microcyn Rx alone group (80.0% [24 of 30]) compared with the saline plus levofloxacin group (63.6% [21 of 33]) and the Microcyn Rx plus levofloxacin group (57.7% [15 of 26]). Microcyn Rx alone maintained the highest clinical response rate at visit 4 (89.3% [25 of 28]), followed by saline plus levofloxacin (58.6% [17 of 29]) and Microcyn Rx plus levofloxacin (80.0% [20 of 25]). These results suggest that Microcyn Rx alone was able to reduce clinical signs and symptoms despite the presence of pathogens in the wound. A summary of clinical outcomes is presented in Table 3.

For methicillin-resistant *Staphylococcus aureus*, the favorable microbiologic response was higher in the Microcyn Rx alone and Microcyn Rx plus levofloxacin groups than in the saline plus levofloxacin group, as expected. Clinical success rates for methicillin-resistant *Staphylococcus aureus* at visit 2 were similar for all treatment groups; however, at visits 3 and 4, the highest clinical success rates for

methicillin-resistant *Staphylococcus aureus* were in the groups receiving Microcyn Rx. For Microcyn Rx alone at visit 2, the best correlation between clinical success and microbiologic response was for methicillin-resistant *Staphylococcus aureus*. This finding suggests that Microcyn Rx was able to eradicate methicillin-resistant *Staphylococcus aureus* and reduce clinical signs and symptoms in wounds with this pathogen.

Wound-Healing Efficacy

The effect on wound healing was similar for all three groups at visit 3 but became more pronounced in the Microcyn Rx plus levofloxacin group, followed by the saline plus levofloxacin group at visit 4, based on wound area from digital photograph data. However, these results are inconclusive because all of the wound measurements were highly variable. A different study design will be necessary for the evaluation of wound healing as a primary end point.

Adverse Events

A total of 32 adverse events were reported by 23 of 67 patients (34.3%) (Table 4). The number of events reported was similar across all of the treatment

Table 3. Summary and Comparison of Clinical Success Rates^a by Visit (ITT Sample)

	Microcyn Rx Alone (n = 20)	Saline + Levofloxacin (n = 21)	Microcyn Rx + Levofloxacin (n = 25)
Visit 3			
Clinical success (No. [%])	15 (75.0)	12 (57.1)	16 (64.0)
Cure (No. [%])	6 (30.0)	7 (33.3)	9 (36.0)
Improvement (No. [%])	9 (45.0)	5 (23.8)	7 (28.0)
Clinical success rate (% [95% CI])	75.0 (56.0–94.0)	57.1 (36.0–78.3)	64.0 (45.2–82.8)
<i>P</i> value for comparison of clinical success rates ^b		0.211	0.422
Treatment odds ratio (95% CI) ^c		2.4 (0.6–9.1)	1.7 (0.5–6.4)
Visit 4			
Clinical success (No. [%])	15 (75.0)	11 (52.4)	18 (72.0)
Cure (No. [%])	11 (55.0)	6 (28.6)	11 (44.0)
Improvement (No. [%])	4 (20.0)	5 (23.8)	7 (28.0)
Clinical success rate (% [95% CI])	75.0 (56.0–94.0)	52.4 (31.0–73.7)	72.0 (54.4–89.6)
<i>P</i> value for comparison of clinical success rate ^d		0.129	0.818
Treatment odds ratio (95% CI) ^e		2.8 (0.7–10.7)	1.2 (0.3–4.5)

Note: Percentages are based on the number of ITT patients in each treatment group.

Abbreviations: CI, confidence interval; ITT, intention-to-treat.

^aClinical success is defined as a clinical outcome of either cure or improvement.

^bThe *P* value is from the logistic model, with whether clinical success was obtained as the response variable and treatment and duration of wound at baseline as explanatory variables. The *P* value under the saline plus levofloxacin column is for the comparison of treatment between the Microcyn Rx alone group and the saline plus levofloxacin group. The *P* value under the Microcyn Rx plus levofloxacin column is for comparison of treatment between the Microcyn Rx alone group and the Microcyn Rx plus levofloxacin group.

^cThe odds ratio under the saline plus levofloxacin column is the odds ratio between the Microcyn Rx alone group and the saline plus levofloxacin group. The odds ratio under the Microcyn Rx plus levofloxacin column is the odds ratio between the Microcyn Rx alone group and the Microcyn Rx plus levofloxacin group.

^dThe primary analysis is between the Microcyn Rx alone group and the saline plus levofloxacin group at visit 3.

groups. None of the adverse events was reported by 5% or more of patients. The most frequently reported adverse events were infections and infestations (17.9%) and skin and subcutaneous disorders (6%), which are not unexpected complications for this patient population. The number of events was similar across all of the treatment groups.

Three adverse events were considered possibly or definitely related to the study drug, and all were reported in the Microcyn Rx plus levofloxacin group. The two possibly related events were mild amnesia that continued and mild stomach discomfort that resolved without treatment. The definitely related event was a mild burning sensation at the site of the wound that resolved in 9 days without treatment.

Most adverse events were considered mild or moderate. Six serious adverse events were reported for six patients, one event each. These consisted of hospitalization for infection (four events) and for cellulitis (two events). None of the six serious adverse events was considered related to the study drug. Study treatment was discontinued because of

three of the serious adverse events. There were no clinically significant abnormalities observed in clinical laboratory evaluations, vital sign measurements, and physical examination findings.

In summary, few patients experienced adverse events, and the events that occurred were not unexpected for this patient population. No apparent treatment-related trends were observed. There were no deaths and no drug-related serious adverse events.

Discussion

Based on the data presented herein, it is clear that mildly infected diabetic foot ulcers respond clinically and microbiologically to topical treatment with Microcyn Rx. Furthermore, the differences between study participants treated with oral levofloxacin and those treated with topical Microcyn Rx were statistically nonsignificant. The adverse events associated with topical Microcyn Rx were minimal and involved only one patient, who reported a burning sensation that resolved uneventfully with-

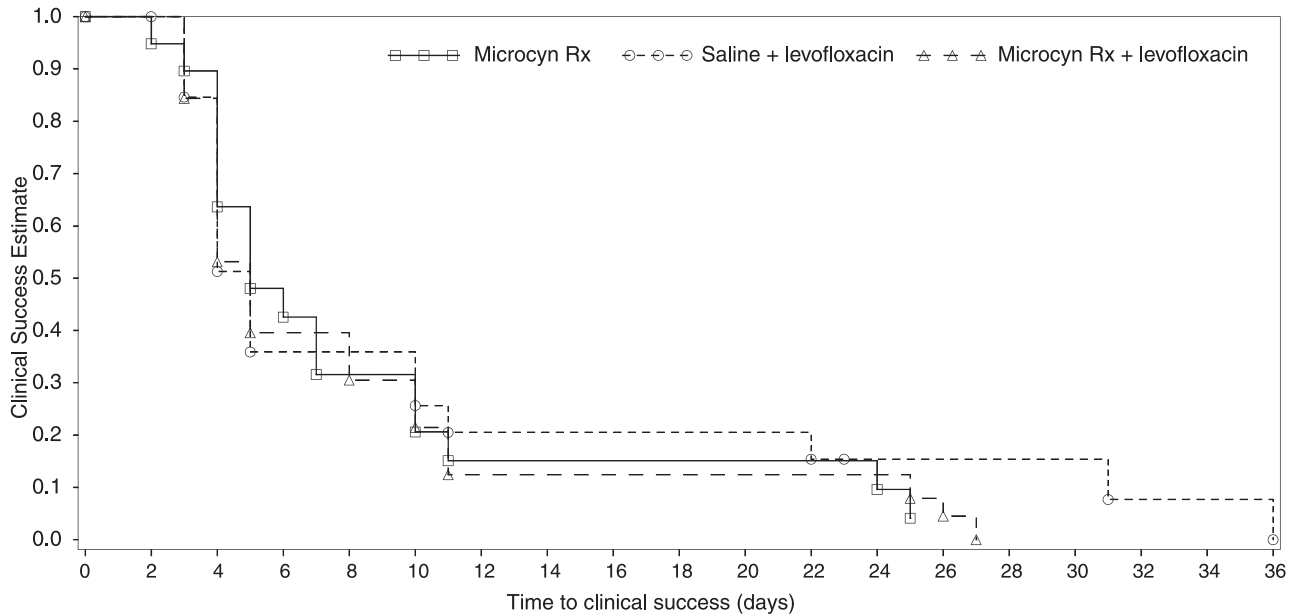


Figure 2. Kaplan-Meier plot of time to clinical success. Time to clinical success (days) is equal to the date of clinical success minus the first dose date plus 1.

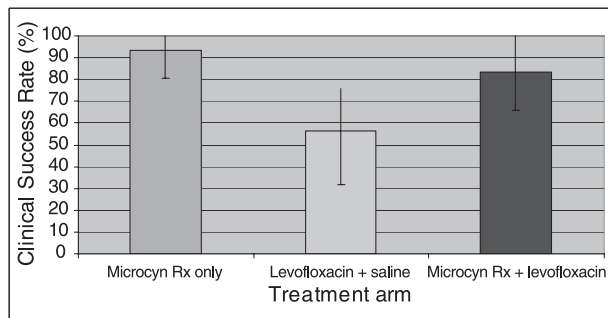


Figure 3. Relative clinical success rates by treatment arm. Error bars represent 95% confidence intervals.

out further intervention. Other complications, such as the incidence of worsening infection, were on par with this study population and were not believed to be related to either the oral or topical antibiotic treatment.

Previous studies have been conducted using electrolyzed solution. The largest trial¹⁶ of super-oxidized (electrolyzed) water in infected foot ulcers included 218 patients with a mean duration of diabetes of 17.4 years who were assigned alternately to Microcyn Rx or povidone-iodine with daily dressing changes. Patients with absent pulses in the foot, transcutaneous oxygen tension less than 50 mm Hg, and greater than 50% stenosis were referred

Table 4. Adverse Events

	Microcyn Rx (n = 21)	Saline + Levofloxacin (n = 21)	Microcyn Rx + Levofloxacin (n = 25)	Total (N = 67)
Patients with ≥ 1 adverse event (No. [%])	7 (33.3)	7 (33.3)	9 (36.0)	23 (34.3)
Relation to study drug (No. [%])				
Definitely not	6 (28.6)	5 (23.8)	5 (20.0)	16 (23.9)
Probably not	1 (4.8)	2 (9.5)	1 (4.0)	4 (6.0)
Possible	0	0	2 (8.0)	2 (3.0)
Probable	0	0	0	0
Definite	0	0	1 (4.0)	1 (1.5)
Severity (No. [%])				
Mild	4 (19)	2 (9.5)	5 (20.0)	11 (16.4)
Moderate	2 (9.5)	3 (14.3)	3 (12.0)	8 (11.9)
Severe	1 (4.8)	2 (9.5)	1 (4.0)	4 (6.0)

for angioplasty before treatment. Approximately half of the patients in both groups had peripheral vascular disease, and more than 80% had neuropathy. All of the patients underwent debridement and received oral or parenteral antibiotics during topical treatment. The outcome measures were reduction in bacterial load, healing time, and incidence of skin reactions at the time of elective operation (conservative or minor or major amputation).

At the time of elective surgery, 97 of the 110 patients (88.2%) assigned to Microcyn Rx had no bacteria recoverable from the ulcer compared with 74 of the 108 patients (68.5%) treated with povidone-iodine ($P < .001$, Fisher exact test). Only 13 bacterial strains could be recovered at that time from patients in the Microcyn Rx group versus 43 in the povidone-iodine group. The number of patients having conservative surgery, minor amputations, and major amputations were 60 (54%), 45 (42%), and 5 (5%), respectively, in the Microcyn Rx group and 47 (44%), 51 (47%), and 10 (9%), respectively, in the povidone-iodine group. The investigators could not determine whether the likelihood of less extensive surgery was a consequence of the type of topical treatment administered. The median healing time after surgery was 43 days for Microcyn Rx and 55 days for povidone-iodine ($P < .0001$). The odds ratio for a successful outcome was 3.4 (95% confidence interval, 1.7–7.0) for treatment with Microcyn Rx. There was no difference in the rates of postoperative wound dehiscence and recurrence of ulceration. No patients treated with Microcyn Rx experienced a local reaction, whereas 16.7% of those treated with povidone-iodine had skin rash or some other adverse event.

In a single-blind study from Pakistan,¹⁴ 100 diabetic patients with foot ulcers were randomized to treatment with either superoxidized water or physiologic saline applied in soaked gauze twice daily. Statistically significant improvements in length of stay, downgrading of wound category, and healing time occurred in the group that received superoxidized water. The authors noted that if the benefits of superoxidized water are confirmed, it could provide an economical alternative to commonly used antiseptics.

A team of investigators in Mexico randomized 45 patients with diabetic foot ulcers to local treatment with neutral superoxidized water or conventional disinfectant.¹⁵ The two groups were well matched for duration of diabetes, obesity, fasting serum glucose concentration, ulcer duration, severity of lesions, and infecting organism. All patients treated with super-

oxidized water achieved elimination of wound odor compared with only 25% of those treated with the conventional disinfectant. The extent of cellulitis diminished in 80.9% of those treated with superoxidized water compared with only 43.7% of those treated with conventional disinfectant ($P < .01$), and granulation tissue was seen in 90.4% vs 62.5% ($P < .01$). Only a third of patients treated with superoxidized water showed evidence of tissue toxicity compared with 94% of those treated with the conventional disinfectant ($P < .01$).

In a study available only as an abstract,¹⁷ Italian researchers randomized 40 patients into two groups. All of the patients received daily ceftriaxone, teicoplanin, and metronidazole; equal numbers received povidone-iodine- or Microcyn Rx-soaked gauze twice a day. There were striking reductions in the clinical signs of infection (requiring a mean of 5.4 days in the Microcyn Rx group and 7.9 days in the povidone-iodine group), odor (requiring 2 days vs 19 days to control), and days of hospitalization (8.2 [range, 7–10] days in the Microcyn Rx group and 12.3 [range, 8–19] days in the povidone-iodine group). There were no significant differences in wound pain, measured by a visual analog scale, or normalization of the white blood cell count.

Chittoria et al⁶ used superoxidized water in 20 patients with no control arm. In eight patients, complete wound healing was achieved, and in the remaining 12 patients, the treatment reduced infection and promoted granulation, permitting definitive surgery.

Finally, Goretti et al¹³ examined the effect of Microcyn Rx in 18 diabetic patients with wide (mean \pm SD, 25.8 \pm 10.4 cm²; mean duration, 95.7 days) postoperative infected ulcers. Dressings saturated with Microcyn Rx were applied daily. The historical control group (mean \pm SD lesion diameter, 20.2 \pm 12.3 cm²; mean duration, 78.3 days) had been treated with povidone-iodine. Surgical debridement and antibiotics were used in all of the patients, with off-loading when necessary. Patients were observed to complete healing. The primary end points were time to healing, proportion of patients achieving healing by 6 months, and number of adverse events. The secondary end points were duration of antibiotic use, number of new or recurrent infections, and number of new secondary operations.

Minor amputations were significantly more common in patients treated with povidone-iodine, who also had a slower healing time (212.3 days versus 144.6 days for the Microcyn Rx group; $P = .004$). In

the Microcyn Rx group, 87.5% of patients had healed lesions at 6 months compared with 51.4% of the povidone-iodine group ($P = .008$). Patients treated with Microcyn Rx required antibiotics for a shorter time (mean \pm SD, 74.7 ± 32.1 days versus 129.6 ± 54.4 days; $P = .0137$) and had fewer reinfections (four versus nine patients; $P = .002$) and less need for repeated debridements (6 versus 16; $P = .001$).

The results of the present open-label study support the growing body of literature that Microcyn Rx topical wound care solution is an effective adjunct in the treatment of diabetic foot ulcers. The clinical success rate was higher in the Microcyn Rx group than in the other two groups at end of treatment and test of cure, although the results were not statistically significant. As might be expected, the use of levofloxacin with Microcyn Rx was associated with eradication of more pathogens than with Microcyn Rx alone or levofloxacin plus saline. The combination of Microcyn Rx plus levofloxacin led to a reduction in the number of concomitant drugs that patients needed, implying less risk of adverse effects and lower treatment costs. Further clinical trials will be needed to extend these results and help clarify the most appropriate candidates for this new therapeutic modality.

Although the rate of wound closure was not a primary end point in this study, and the period of observation (14 days) was too short to expect to see meaningful improvement in the time to closure, or the percentage of wounds that close, previous studies have indicated that treatment with this superoxidized solution results in a statistically significant increase in the percentage of wounds achieving closure and a measurable decrease in the time to closure compared with patients treated with povidone-iodine on the wound surface.¹³ Intuitively, if we can decrease the bioburden of the wound bed, wound closure rates should improve. We anticipate that future studies will be conducted to demonstrate the precise effect that treatment with a superoxidized solution will have on diabetic wound closure.

Acknowledgments: The Members of the Oculus Collaborative Study Group are Walter F. D'Costa, DPM, Allan B. Grossman, DPM, Jeffrey L. Jensen, DPM, Robert W. Mendicino, DPM, Gerit Mulder, DPM, Michael Palladino, DPM, Bret M. Ribotsky, DPM, Steven Royall, DPM, Stephen Schleicher, MD, Darin R. Serletic, DPM, and J. Neal Sharpe, MD.

Financial Disclosure: This project was supported by a research grant from Oculus Innovative Sciences.

Conflict of Interest: None reported.

References

1. LIPSKY BA, BERENDT AR, DEERY HG, ET AL: Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* **39**: 885, 2004.
2. NELSON EA, O'MEARA S, GOLDER S, ET AL: Systematic review of antimicrobial treatments for diabetic foot ulcers. *Diabet Med* **23**: 348, 2006.
3. GOLDENHEIM PD: An appraisal of povidone-iodine and wound healing. *Postgrad Med J* **69** (suppl 3): S97, 1993.
4. GUTIERREZ AA: The science behind stable, super-oxidized water: exploring the various applications of super-oxidized solutions. *Wounds Compend Clin Res Pract* **18** (suppl): 7, 2006.
5. NAGAMATSU Y, CHEN KK, TAJIMA K, ET AL: Durability of bactericidal activity in electrolyzed neutral water by storage. *Dent Mater J* **21**: 93, 2002.
6. CHITTORIA RK, YOOTLA M, SAMPATRAO LM, ET AL: The role of super oxidized solution in the management of diabetic food ulcer: our experience. *Nepal Med Coll J* **9**: 125, 2007.
7. NAKAE H, INABA H: Effectiveness of hydrolyzed water irrigation in a burn-wound infection model. *J Trauma* **49**: 511, 2000.
8. TANAKA H, HIRAKATA Y, KAKU M, ET AL: Antimicrobial activity of superoxidized water. *J Hosp Infect* **34**: 43, 1996.
9. OHNO H, HIGASHIDATE M, YOKOSUKA T: Mediastinal irrigation with superoxidized water after open-heart surgery: the safety and pitfalls of cardiovascular surgical application. *Surg Today* **30**: 1055, 2000.
10. WOLVOS TA: Advanced wound care with stable super-oxidized water: a look at how combination therapy can optimize wound healing. *Wounds Compend Clin Res Pract* **18** (suppl): 11, 2006.
11. ALLIE DE: Super-oxidized Microcyn technology in lower-extremity wounds. *Wounds Compend Clin Res Pract* **18** (suppl): 3, 2006.
12. DALLA-PAOLA L, BROCCO E, SENESI A, ET AL: Use of Microcyn Rx, a new antiseptic agent for the treatment of diabetic foot ulcers. *J Wound Healing* **2**: 201, 2005.
13. GORETTI C, MAZZURCO S, AMBROSINI-NOBILI L, ET AL: Clinical outcomes of wide postsurgical lesions in the infected diabetic foot managed with 2 different local treatment regimes compared using a quasi-experimental study design: a preliminary communication. *Lower Extrem Wounds* **6**: 22, 2007.
14. HADI SF, KHALIQ T, BILAL N, ET AL: Treating infected diabetic wounds with superoxidized water as anti-septic agent: a preliminary experience. *J Coll Phys Surg Pak* **17**: 740, 2007.
15. MARTINEZ-DE JESUS FR, RAMOS-DE LA MEDINA A, REMES-TROCHE JM, ET AL: Efficacy and safety of neutral pH superoxidised solution in severe diabetic foot infections. *Int Wound J* **4**: 353, 2007.
16. DALLA-PAOLA L, BROCCO E, SENESI A, ET AL: Super-oxidized solution (SOS) therapy for infected diabetic foot ulcers. *Wounds Compend Clin Res Pract* **18**: 262, 2006.
17. RICCI E, ASTOLFI S, CASSINO R: Clinical results about an

antimicrobial solution (Microcyn Rx® Wound Care) in the treatment of infected chronic wounds. Paper presented at: European Wound Management Association, May 3, 2007; Glasgow, England.

18. ARMSTRONG DG, LAVERY LA, HARKLESS LB: Validation of a diabetic wound classification system: the contribution of depth, infection, and ischemia to risk of amputation. *Diabet Care* **21**: 855, 1998.