MAJOR ARTICLE

Topical versus Systemic Antimicrobial Therapy for Treating Mildly Infected Diabetic Foot Ulcers: A Randomized, Controlled, Double-Blinded, Multicenter Trial of Pexiganan Cream

Benjamin A. Lipsky,[1,2] Kenneth J. Holroyd,[3] and Michael Zasloff[4]

[1]Veterans Affairs Puget Sound Health Care System and [2]University of Washington School of Medicine, Seattle; [3]Vanderbilt University School of Medicine, Nashville, Tennessee; and [4]Georgetown University School of Medicine, Washington, DC

Background. Topical antimicrobial therapy of infected diabetic foot ulcers can focus on the wound and avoid the adverse effects of systemic anti-infective agents. We compared the efficacy of outpatient treatment using an investigational topical antimicrobial peptide, pexiganan acetate cream, with the efficacy of systemic therapy using an oral fluoroquinolone antibiotic, ofloxacin, for mildly infected diabetic foot ulcers.

Methods. In 2 consecutive, double-blind, controlled trials (study 303 and study 304), we randomized diabetic patients with a mildly infected diabetic foot ulcer to receive the active topical agent or active oral antibiotic, plus a respective inactive placebo. The primary outcome of interest was clinical cure or improvement of the infection. Secondary outcomes included eradication of wound pathogens and wound healing, which was documented by a semiquantitative scoring system.

Results. Overall, 835 patients were randomized; those in each treatment arm were similar with regard to demographic and clinical characteristics. Although study 303 failed to demonstrate equivalence, study 304 and the combined data for the 2 trials demonstrated equivalent results (within the 95% confidence interval) for topical pexiganan and oral ofloxacin in clinical improvement rates (85%–90%), overall microbiological eradication rates (42%–47%), and wound healing rates. The incidence of worsening cellulitis (2%–4%) and amputation (2%–3%) did not differ significantly between treatment arms. Bacterial resistance to ofloxacin emerged in some patients who received ofloxacin, but no significant resistance to pexiganan emerged among patients who received pexiganan.

Conclusions. Topical pexiganan might be an effective alternative to oral antibiotic therapy in treating diabetic patients with a mildly infected foot ulcer, and might reduce the risk of selecting antimicrobial-resistant bacteria.

Clinical trials registration. NCT00563394 and NCT00563433.

Foot infections, a frequent and serious complication of diabetes [1, 2], are among the most common diabetes-related causes of hospitalization and the leading cause of nontraumatic lower limb amputation [3]. Treating a diabetic foot infection (DFI) requires proper wound care and appropriate antibiotic therapy [3]. The antibiotic(s) selected must cover the often-polymicrobial organisms that cause these infections. Clinical trials have demonstrated the effectiveness of various systemic (oral and parenteral) antibiotics in treating DFI, including fluoroquinolones, such as ofloxacin [4–9]. A chosen antibiotic regimen must balance effectiveness against potential adverse effects and the likelihood of emergence of antibiotic resistance.

According to the internationally accepted [2, 10] and verified [11] DFI classification, wound depth is an important determinant of severity. Thus, a mild wound infection (superficial in depth and limited in size) could be amenable to treatment with a topically administered anti-infective agent. Topical treatment has the advantages of avoiding systemic adverse effects, providing increased target site concentration, and allowing the use

Received 29 January 2008; accepted 29 July 2008; electronically published 6 November 2008.

[a] Physicians who participated in the clinical trials are listed at the end of the text.

Reprints or correspondence: Dr. Benjamin A. Lipsky, Veterans Affairs Puget Sound HCS S-111-PCC, 1660 S. Columbian Way, Seattle, WA 98116-1597 (Benjamin.Lipsky@med.va.gov).

Clinical Infectious Diseases 2008; 47:000–000

1058-4838/2008/4712-00XX$15.00
DOI: 10.1086/593185

of agents not available for systemic therapy [12]. An acceptable topical anti-infective agent would need to demonstrate activity against the spectrum of bacteria that are known to cause DFI, and it would need to avoid serious adverse effects, interference with wound healing, or induction of drug resistance.

To date, no topical anti-infective agent has been proven to be effective for treating DFI [13]. Antimicrobial peptides, a class of agents generated by most animals [14], provide a defense against environmental microbes while largely sparing host cells and avoiding the induction of resistant organisms [14–16]. These peptides are active against a wide spectrum of pathogens, including antibiotic-resistant bacteria, and provide an antimicrobial shield that covers and sterilizes the healing wound. Thus, they are promising candidates for the treatment of various skin infections [17]. Pexiganan is a 22–amino acid synthetic analogue of the peptide magainin II [18–21]; it is engineered for potency against many bacteria. In vitro, pexiganan has activity against most clinical bacterial isolates cultured from infected diabetic foot ulcers [18, 19, 22]. We therefore undertook 2 randomized clinical trials to compare the efficacy of topical pexiganan acetate cream with oral ofloxacin.

PATIENTS AND METHODS

Study Design

We conducted 2 outpatient, multicenter, phase 3, double-blind, randomized, parallel group-controlled (active control and double-dummy placebo) studies involving patients with a mildly infected diabetic foot ulcer. Participating sites for the sequential independent studies (designated study 303 and study 304 and carried out in different centers) were in the United States. Magainin Pharmaceuticals sponsored the studies, which were identical in design, except that study 303 initially randomized 100 patients to receive a 2% (rather than 1%) concentration of pexiganan. Study protocols met Declaration of Helsinki and Good Clinical Practices principles and were approved by the institutional review boards of participating centers.

Patients

Men or women aged ⩾18 years who had diabetes mellitus (according to American Diabetes Association definitions) could potentially be enrolled if they had an infected wound below the malleoli that exceeded 0.5 cm^2 in area after appropriate debridement. Wounds had to be full thickness (i.e., through the epidermis and into or through the dermis, but not involving tendon, bone, or joint capsule). Infection was defined by the presence of purulent drainage or ⩾2 of the following: erythema, warmth, pain or tenderness, or edema or induration. The DFI had to be severe enough to require antibiotic therapy, but it had to be amenable to outpatient treatment. Patients were excluded if they had an abscess, extensive gangrene, an imminently limb-threatening infection, evidence of systemic infection (e.g., fever, chills, or hypotension), plain radiograph findings suggestive of osteomyelitis, no palpable dorsalis pedis or posterior tibial pulse or a pedal systolic pressure (by Doppler) of ⩽40 mm Hg on the affected limb, requirement for renal dialysis, need for immunosuppressive medication, or hypersensitivity to either study medication.

Patients were instructed to take 2 tablets (either 200 mg of active ofloxacin [Floxin; Ortho-McNeil] or placebo) orally twice daily and to apply a cream (either active pexiganan acetate or placebo, sufficient to form a dime thick layer) twice daily directly onto the ulcer and to dress the wound with sterile, dry gauze. Patients randomized to treatment with pexiganan received placebo tablets, and those randomized to ofloxacin treatment received placebo cream. Investigators performed appropriate local wound care, including any necessary debridement and pressure off-loading of the infected site, and they obtained wound tissue specimens for aerobic and anaerobic culture at enrollment. Nonstudy systemic or topical anti-infective agents were not allowed after enrollment.

Investigators used a wound scoring system devised for this study, based on the system from Knighton et al. [23] as modified by Pecoraro et al. [24], that measured the wound and assessed signs and symptoms of inflammation or infection. In-

Table 1. Diabetic foot ulcer wound infection score that was used to evaluate an infected wound at each visit during the study.

	Wound infection score			
Parameter	0	1	2	3
Purulent drainage	Absent	…	…	Present
Nonpurulent drainage (serous, sanguinous)	Absent	Mild	Moderate	Severe
Erythema	None	Mild: pink, barely perceptible	Moderate: pale red, defined edges	Severe: red to dark red
Induration	None	Mild	Moderate	Severe
Tenderness (sign)	None	Mild	Moderate	Severe
Pain (symptom)	None	Mild	Moderate	Severe
Local warmth (relative to uninfected contralateral foot)	Same	Mildly increased	Moderately increased	Severely increased

NOTE. Each of the 7 parameters was scored from 0 to 3, then all of the scores were added to generate a total wound infection score.

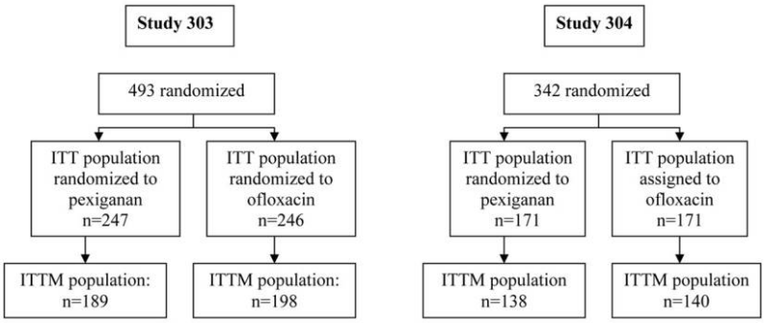

Figure 1. Diagrams of 2 randomized, double-blind controlled trials comparing topical pexiganan cream with oral ofloxacin for the treatment of mildly infected diabetic foot infections. ITT, intention to treat; ITTM, intention to treat microbiological.

vestigators conducted follow-up clinical evaluations (including wound scoring) and obtained wound cultures (when possible) at 3, 10, 14, and 21 days after enrollment; at end of treatment (EOT); and at follow-up (2 weeks after EOT). The minimum treatment course was 14 days, but investigators could extend the treatment for up to 28 days, if necessary. Thus, the EOT visit was 14–28 days after enrollment and the follow-up was 28–42 days after enrollment. For patients who withdrew from the study early, the date of the last recorded clinical evaluation (and wound culture) was deemed to be EOT.

Analysis of Results

The primary outcome was the clinical improvement of infection in response to antimicrobial treatment. Secondary outcomes of note included eradication of the wound pathogens, healing of the ulcer, development of resistance to a study drug, and safety of the treatments.

Clinical outcome. At each visit after enrollment, the investigator graded the clinical response as (1) "infection resolved or cured" (all signs and symptoms of infection resolved), (2) "infection improving" (most, but not all, signs and symptoms of infection improved or resolved), (3) "treatment failure" ($\geqslant$1 signs or symptoms of infection substantially worsening), (4) "unevaluable" (<3 days of study treatment or patient lost to follow-up), or (5) "recurrence" (a previously cured or improved infection showing worsening of signs or symptoms of infection).

Wound assessments. Investigators compiled a "total wound score" that included ratings of signs and symptoms of infection, wound measurements (maximum length, width, and depth), and assessment of granulation tissue. A "wound infection score" (table 1) was semiquantitatively assessed by grading each of 7 parameters with a score of 0–3: (1) purulent drainage, (2) nonpurulent drainage, (3) erythema, (4) induration, (5) tenderness, (6) pain, and (7) local warmth. Investigators measured wound area by planimetry of wound tracings [25] and measured wound depth with a probe.

Microbiological assessments. Investigators cultured samples obtained from the wound at baseline, at day 3, and at all other study visits at which culturable material and signs of

Table 2. Clinical and microbiological outcomes at end of treatment (EOT) and follow-up visits for patients who received either pexiganan or ofloxacin in the intention-to-treat populations.

	Clinical cure or improvement			Microbiological response[a]		
Visit and study(s)	Pexiganan treatment group	Ofloxacin treatment group	Difference (95% CI)	Pexiganan treatment group	Ofloxacin treatment group	Difference (95% CI)
EOT						
303	210/247 (85.0)	224/246 (91.1)	−6.04 (−11.74 to − 0.33)	91/189 (48.1)	94/198 (47.5)	0.68 (−9.29 to 10.63)
304	153/171 (89.5)	153/171 (89.5)	0.00 (−6.51 to 6.51)	63/138 (45.7)	66/140 (47.1)	−1.49 (−13.22 to 10.24)
303 and 304	363/418 (86.8)	377/417 (90.4)	−3.57 (−7.87 to 0.74)	154/327 (47.1)	160/338 (47.3)	−0.25 (−7.84 to 7.35)
Follow-up						
303	186/243 (76.5)	201/240 (83.8)	−7.21 (−14.29 to −0.12)	78/185 (42.2)	90/194 (46.4)	−4.23 (−14.23 to 5.77)
304	134/163 (82.2)	137/163 (84.0)	−1.84 (−9.97 to 6.29)	55/130 (42.3)	62/134 (46.3)	−3.96 (−15.94 to 8.02)
303 and 304	320/406 (78.8)	338/403 (83.9)	−5.05 (−10.41 to 0.31)	133/315 (42.2)	152/328 (46.3)	−4.12 (−11.80 to 3.56)

NOTE. Data are proportion (%) of patients, unless otherwise indicated. Equivalence was demonstrated between pexiganan and ofloxacin if the 95% CI included zero.

[a] Patients in the intention-to-treat population in whom some or all of the initially isolated pathogens were eradicated, in whom there were no new pathogens isolated, and who experienced clinical cure or improvement.

Table 3. The number of individuals with wound pathogens isolated at baseline and the eradication of those pathogens at follow-up in the microbiological intention-to-treat population in studies 303 and 304.

	Pexiganan treatment group		Ofloxacin treatment group	
Pathogen	No. of patients with pathogen isolated at baseline	No. (%) of patients with pathogen eradicated at follow-up	No. of patients with pathogen isolated at baseline	No. (%) of patients with pathogen eradicated at follow-up
Staphylococcus aureus	142	72 (51)	161	97 (60)
Enterococcus faecalis	105	58 (55)	105	67 (64)
Streptococcus agalactiae	59	31 (53)	66	39 (59)
Enterococcus species	26	16 (62)	12	7 (58)
Staphylococcus epidermidis	24	15 (63)	23	16 (70)
Pseudomonas aeruginosa	22	11 (50)	36	21 (58)
Proteus mirabilis	20	12 (60)	21	15 (71)
Escherichia coli	19	15 (79)	16	12 (75)
Streptococcus species	14	11 (79)	12	7 (58)
Xanthomonas maltophilia	14	10 (71)	5	4 (80)
Streptococcus canis	13	6 (46)	17	10 (59)
Enterobacter cloacae	12	9 (75)	15	13 (87)
Enterobacter species	12	10 (83)	5	3 (60)
Serratia marcescens	12	8 (67)	5	4 (80)
Bacteroides fragilis	8	7 (88)	7	5 (71)

infection were present. They obtained specimens using tissue curettage with a sterile scalpel, placed them into transport media, and shipped them to a central laboratory (Corning SciCor) for species identification and antibiotic susceptibility testing. Microbiological responses, comparing results of the initial wound culture with those obtained during post-baseline visits, were scored as "resolved" if all initial pathogens were eradicated or "improving" if at least 1, but not all, of the initial pathogens were eradicated and no additional organisms were isolated. Patients who achieved these 2 responses and who were clinically improved were "microbiological responders." Microbiological nonresponders were considered to have experienced "treatment

Table 4. Baseline wound assessment values and the change from baseline to end of treatment (EOT) and to follow-up for wound assessment variables in the intention-to-treat population.

	Pexiganan treatment group			Ofloxacin treatment group				
		Change from baseline			Change from baseline		*P*	
Study(s) and variable	Baseline value	EOT	Follow-up	Baseline value	EOT	Follow-up	EOT	Follow-up
303								
Total wound score, mean value	25.5	−7.6	−8.5	24.2	−8.0	−8.7	.61	.78
Wound infection score, mean value	7.1	−4.5	−5.1	6.9	−5.0	−5.3	.13	.55
Wound area, median mm^2	131.5	−61.9	67.8	117.3	−58.3	−64.4	.53	.57
Wound depth, median mm	3	−1.0	−1.5	3.0	−1.0	−1.5	.30	.48
304								
Total wound score, mean value	26.2	−8.6	−9.0	25.7	−8.5	−8.9	.92	.87
Wound infection score, mean value	6.9	−4.5	−4.7	7.5	−5.1	−5.2	.11	.24
Wound area, median mm^2	146.9	−63.0	−64.7	160.8	−70.2	−86.4	.17	.03
Wound depth, median mm	3	−1.0	−1.5	3.0	−1.0	−2.0	.42	.58
303 and 304								
Total wound score, mean value	25.8	−8.0	−8.7	24.8	−8.2	−8.7	.75	.91
Wound infection score, mean value	7	−4.5	−5.0	7.2	−5.1	−5.3	.03	.22
Wound area, median mm^2	138	−62.6	67.1	138.7	−65.8	−72.1	.17	.07
Wound depth, median mm	3	−1.0	−1.0	3.0	−1.0	−2.0	.20	.36

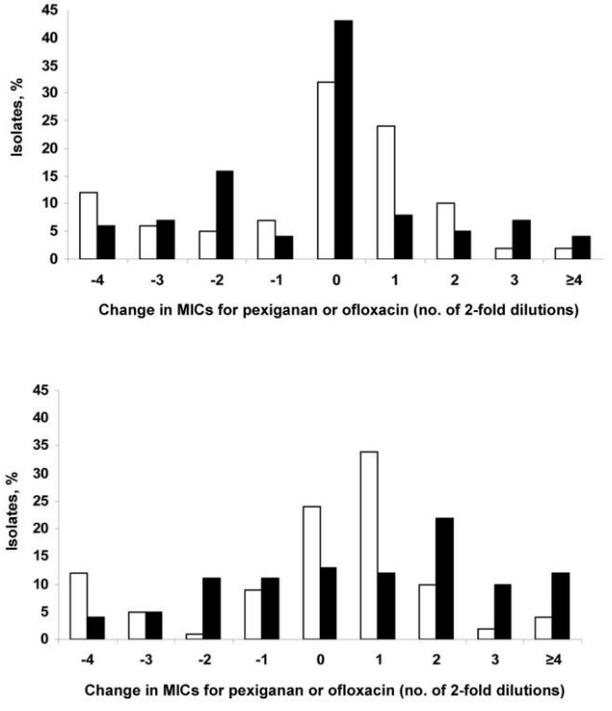

Figure 2. Changes in MIC for pexiganan or ofloxacin for pairs of *Staphylococcus aureus* isolates recovered from subjects before (at baseline visit) and after (at follow-up visit) treatment with pexiganan (24 isolate-pairs) *(top)* or ofloxacin (26 isolate-pairs) *(bottom).* The change in the MIC of the isolates is presented as number of 2-fold dilutions by which MIC differed between values measured for baseline isolates and for isolates recovered at follow-up, tested with pexiganan *(white bars)* and ofloxacin *(black bars).* Statistical significance of the differences in MIC noted for pexiganan, compared with ofloxacin, for isolates recovered from each treatment group were determined using the χ^2 test. $P > .05$, for isolates recovered from patients treated with pexiganan *(top).* $P < .001$, for isolates recovered from patients treated with ofloxacin *(bottom).*

failure" if all original pathogens persisted, to have "colonization" if organisms other than the original pathogens appeared in a setting of clinical resolution, or to have "superinfection" if they experienced clinical failure. Pathogens were any bacteria other than *Corynebacterium*, *Propionibacterium*, or *Bacillus* species, unless one of these 3 species were isolated in pure culture.

Changes in study drug susceptibility. To determine whether the study treatment altered susceptibility of isolated pathogens to either agent, we compared the MIC (by broth dilution) of both study drugs for all bacterial isolates cultured from a wound at baseline that were also recovered at follow-up.

Safety parameters. We actively monitored patients for adverse clinical events at each postbaseline visit. This included questioning patients, examining them for abnormal physical findings, testing for clinical laboratory abnormalities, and (when indicated) repeating radiographic studies.

Statistical Analyses

The trials were designed to be equivalence studies and used US Food and Drug Administration guidelines that have been operative since 1992. To achieve an efficacy of ⩾90% for the better of 2 agents, the 95% CI must cross zero and remain within a lower bound delta of 0.10; for an efficacy of 80%–90%, the lower bound delta must be within 0.15. For adequate statistical power, each study required ⩾142 evaluable patients in each treatment arm, assuming a 90% response rate in ⩾1 group [26–28].

We statistically analyzed the "intention to treat" population, which included all enrolled patients. Patients who experienced treatment failure within 10 days after enrollment were scored as having "treatment failure." Patients who withdrew from the study early were scored as either a "responder" (cured or improved) or a "nonresponder" (treatment failure) on the basis of the last recorded clinical evaluation. For microbiological outcomes, we used the "intention to treat microbiological" population, which was the subset of the intention to treat population who had ⩾1 pathogen isolated in culture of wound samples. Microbiological response was considered "resolved" if initially isolated pathogens were proven (by culture) or presumed (by lack of culturable material) to be eradicated on follow-up.

We compared baseline characteristics between the 2 groups with use of 2 sample t tests for continuous data and either exact probabilities or χ^2 test for discrete data. We analyzed the clinical response of infection to treatment at EOT and follow-up by calculating the 2-tailed 95% CIs between the 2 treatment groups for the difference in the proportion of patients with either resolved or improving infection versus those who experienced treatment failure, were not evaluable, or had an infection recurrence. We analyzed the microbiological response similarly.

RESULTS

From August 1994 through July 1996, study 303 was conducted at 42 centers and involved 493 randomized patients, and study 304 was conducted at 39 centers and involved 342 randomized patients (figure 1). Baseline characteristics of patients randomized to the 2 treatment groups in each of the 2 studies were not statistically different. The median age of enrolled patients was ~60 years; 68% were men, 82% were white (13% were black and 5% were Hispanic), 66% were receiving insulin treatment, 65% had experienced a foot ulcer previously, 22% had a history of foot osteomyelitis, and 42% had undergone foot surgery. The mean duration of antibiotic therapy (with either pexiganan or ofloxacin) was similar for the 2 treatment groups and was ~23 days (median duration, 27 days) in study 303 and 25 days (median duration, 22 days) in study 304.

Table 5. Ulcer-related adverse events that led to patient withdrawal from the study and lower extremity amputations, by treatment group.

Event	No. of patients who experienced adverse event		
	Pexiganan treatment group (n = 418)	Ofloxacin treatment group (n = 417)	Total (n = 835)
Cellulitis	15	7	22
Worsening infection	4	4	8
Osteomyelitis	4	3	7
Lower extremity amputation	11	9	20

NOTE. $P = .36$, by paired t test for difference in frequency of combined adverse events for each group.

Clinical Outcome

Table 2 shows the percentage of individuals who demonstrated a clinical cure or improvement (responders) for the intention to treat populations in studies 303 and 304 (separately and combined) at both EOT and follow-up. The difference in the rates of clinical cure or improvement in study 304 were within the 95% CIs for equivalence at both EOT (89% for both the ofloxacin group and the pexiganan group) and follow-up (84% for the ofloxacin group and 82% for the pexiganan group). In study 303, however, pexiganan did not demonstrate equivalence to ofloxacin either at EOT (rates of clinical cure or improvement were 85% and 91%, respectively) or follow-up (77% and 84%, respectively). For the 2 studies combined, the difference in the rates of clinical cure or improvement for the 2 treatment arms were within the 95% CI for equivalence at EOT (87% for the pexiganan group and 90% for the ofloxacin group) and at follow-up (79% for the pexiganan group and 84% for the ofloxacin group).

Microbiological Outcome

Overall. In the intention to treat population, 80% of patients had at least 1 pathogen isolated from a wound culture. Table 2 shows the overall percentage of patients who had a microbiological response for studies 303 and 304, both separately and combined. The percentages of patients who were microbiological responders in both trials were not significantly different between the ofloxacin and pexiganan arms at both the EOT (~47% for each) and follow-up (46% and 42%, respectively) time points. Rates of microbiological failure at follow-up were low, and similar rates were noted for the pexiganan and ofloxacin groups in studies 303 (8% and 6%, respectively) and 304 (10% and 8%, respectively). The prevalence of colonization and superinfection (the main reasons patients were categorized as microbiological nonresponders) did not differ significantly between treatment groups (19%–24% and 4%–8%, respectively, in studies 303 and 304 for both treatment arms).

Baseline pathogen eradication. Table 3 shows the pathogens that were isolated most frequently from wound samples in the intention to treat microbiological populations of studies 303 and 304 combined and the percentage of patients in whom the isolated pathogens at baseline were eradicated at follow-up. Although eradication rates were somewhat greater for 10 organisms in the ofloxacin-treated population and for 5 organisms in pexiganan-treated populations, none of these differences were statistically significant (2-tailed Fisher's exact test).

Wound Assessments

As table 4 shows, there were no statistically significant differences between the ofloxacin- and pexiganan-treated patients at baseline in the mean total wound score or wound infection score or in median wound area or depth. The wound assessment scores decreased at the EOT visit for all measurements in both studies for both treatment arms, and they decreased further for each measurement at the follow-up visit. The magnitude of the decrease in score was similar for the 2 treatment groups; none of the differences were statistically significant, except for the greater decrease in wound area score at follow-up for patients in study 304 who were receiving ofloxacin, compared with that for patients who were receiving pexiganan.

Changes in Study Drug Susceptibility

Pathogens isolated at baseline were also recovered from the wound at follow-up for 155 patients, including 87 pexiganan-treated patients and 68 ofloxacin-treated patients. We determined whether resistance developed during the course of exposure to each study drug. Figure 2 shows the changes in MICs for pairs of isolates of *Staphylococcus aureus* recovered from patients both before and after treatment with either pexiganan (24 patients) or ofloxacin (26 patients). For isolates from pexiganan-treated patients, there was no significant change in MIC values for pexiganan or ofloxacin ($P > .05$) (figure 2, top), but there was a significant increase in MICs for ofloxacin but not for pexiganan among isolates from ofloxacin-treated patients ($P < .001$) (figure 2, bottom). Similarly, MICs for ofloxacin but

not for pexiganan increased in some persisting isolates of *Streptococcus* and *Enterococcus* species and several gram-negative aerobic species (data not shown) [19].

Safety

The overall incidence and types of systemic and cutaneous adverse events were comparable in the 2 treatment arms of both studies. In study 303, adverse events were experienced by 98 (39.8%) of the pexiganan-treated patients and by 109 (44.3%) of the ofloxacin-treated patients; in study 304, they occurred in 76 (44.4%) of the pexiganan-treated patients and 84 (49.1%) of the ofloxacin-treated patients. Patients who received pexiganan experienced insomnia statistically significantly more frequently than did patients who received pexiganan (25 vs. 3 patients). A total of 37 patients (4.4%) were withdrawn from the studies because of progression of the infected ulcer (i.e., the development of cellulitis, worsening infection, or osteomyelitis), and 20 (2.4%) required some type of lower-extremity amputation. The overall incidence of these adverse outcomes was not statistically significantly different between the 2 treatment arms (table 5).

DISCUSSION

These 2 prospective, randomized studies, with 835 enrolled patients, are, to our knowledge, the largest that have been conducted on the treatment of DFIs. The results support the potential of topical pexiganan as a therapeutic alternative to an orally-administered antibiotic for treating mildly infected diabetic foot ulcers. The primary end point of the studies was clinical resolution (cure or improvement) of wound infection. In study 303, topical pexiganan failed to demonstrate statistical equivalence to oral ofloxacin, but there were no statistically significant differences between the treatments in study 304. We found no differences in population demographic characteristics, study execution, or pexiganan preparations that might explain the outcome disparity between the 2 trials.

Among the secondary end points, wound healing, infection progression, and the number of amputations required were all statistically comparable for the 2 therapies. Overall microbiological response was also statistically equivalent for the 2 treatments, although eradication rates for more pathogens were greater (although not statistically significantly) for ofloxacin-treated patients. Because ofloxacin was administered systemically, it could have more effectively reduced the numbers of susceptible microbes from body surfaces, compared with topical pexiganan applied solely to the wound. Thus, microorganisms from surrounding skin or mucosa could potentially have colonized healing wounds in pexiganan-treated patients. We used a stricter definition of microbiological response than most similar studies; the pathogen eradication rate was similar to those previously reported [4–8]. Our results confirmed results from in vitro studies that demonstrated a low probability of selection of resistance for pexiganan, even after repeated exposure to concentrations below the MIC [15, 18, 19]. Surprisingly, we detected increasing MICs to ofloxacin for several species of organisms after the relatively brief treatment period used in these trials.

Several antimicrobial peptides or analogs are under development, especially for use in treating localized infections, and will probably be important for future anti-infective therapy [29]. Some remaining obstacles for treatment of skin infections (e.g., low tissue penetrability, high production costs, and uncertain cytotoxicity) are addressed by topical administration [17]. Pexiganan is not absorbed intact from open wounds, which makes it most suitable for the treatment of mild, relatively superficially infected ulcers. Unlike many topical antimicrobials [12], pexiganan has not provoked allergic sensitization on the basis of data from ~1300 exposed subjects (M.Z., unpublished data). It has a broad spectrum of activity against the aerobic gram-positive bacteria (including methicillin-resistant *S. aureus*), aerobic gram-negative bacteria, and obligate anaerobes [18, 19, 21, 22] that typically cause DFIs. Topical antimicrobial therapy allows treatment with new antimicrobial agents, avoids potential systemic adverse effects, does not drive resistance to systemic antibiotic agents, and encourages patients to examine the site daily (when applying the treatment). A recent systematic review found only 4 previous studies of topical DFI treatments, 2 of which were available only in conference proceedings [13]. All of these studies used antiseptics, rather than antibiotics, and selected ulcer healing, rather than cure of infection, as the primary end point.

The comparator in these studies was ofloxacin, a relatively broad-spectrum oral agent shown to be effective for treating DFIs [8]. It has largely been replaced by levofloxacin, its L-isomer, which was not available at the time of our study. Fluoroquinolones are among the anti-infective agents currently recommended in authoritative DFI guidelines [2, 9]. Conceivably, for mildly infected foot wounds, adequate wound care alone may be sufficient, but in diabetic patients, many of whom have vascular insufficiency and immunological deficiencies, current guidelines recommend antimicrobial therapy [3, 10].

Our results demonstrate that, for mildly infected diabetic foot ulcers, topical pexiganan was clinically comparable to an oral antibiotic. We believe these data are the first comparing a topical antimicrobial compound with a systemic antibiotic for treating DFIs. These data suggest that, for mildly infected ulcers, topical pexiganan, when accompanied by appropriate wound care, can provide a therapeutic alternative to a broad-spectrum oral antibiotic agent. In addition, topical pexiganan appears to

be safe and may avoid the selection of resistant bacteria that can develop after oral systemic antibiotic therapy.

PARTICIPATING PHYSICIANS

Study 303. Stephen Albert, Veterans Affairs Medical Center, Denver, CO; James C. Beard, Portland Diabetes & Endocrinology Center, Portland, OR; John H. Bowker, Diabetes Research Institute, Miami, FL; Elliot Chaikof, Emory University Hospital, Atlanta, GA; Wayman W. Cheatham, Howard University Hospital, Washington, DC; Philip C. Craven, Infections Limited, Tacoma, WA; Daniel Einhorn, Diabetes Endocrine Associates, San Diego, CA; Gary Edelson and Guy Pupp, Kern Foot Clinic, Warren, MI; W. Travis Ellison, Upstate Clinical Research, Greer, SC; Robert G. Frykberg, Boston Foot Care Group, West Roxbury, MA; Kenneth S. Hershon, North Shore Diabetes and Endocrine Associates, New Hyde Park, NY; Roy Kaplan, East Bay Clinical Trial Center, Concord, CA; A.W. Karchmer, New England Deaconess Hospital, Boston, MA; Carole A. Kauffman and Suzanne F. Bradley, Veterans Affairs Medical Center, Ann Arbor, MI; Miriam Keltz, New York University Medical Center, New York, NY; Carol Kemper, California Infectious Disease Associates, Redwood City, CA; Arthur Krosnick, Joslin Diabetes Center, Princeton, NJ; Steven Krych, Center for Clinical Research, Austin, TX; Lawrence Lavery, Health Science Center at San Antonio, San Antonio, TX; Sam Lerman, Endocrinology and Diabetes Consultants, Hollywood, FL; Bruce Levine, Clinical Research of West Florida, Clearwater, FL; Benjamin Lipsky, Seattle Veterans Affairs Medical Center, Seattle, WA; Peter Lodewick, Birmingham, AL; Michael Makaroun, Veterans Affairs Medical Center, Pittsburgh, PA; Alan Marcus, South Orange County Endocrinology Medical, Laguna Hills, CA; David Margolis, Hospital of the University of Pennsylvania, Philadelphia, PA; Robert D. McInroy, Holy Spirit Hospital Medical Office Building, Camp Hill, PA; Bruce H. Miller, Dermatology Associates, Portland, OR; Jeffrey Page, California College of Podiatric Medicine, San Francisco, CA; Lawrence Parish, Philadelphia, PA; Michael A. Pfeifer, Southern Illinois University School of Medicine, Springfield, IL; James Poindexter, Nucare Clinical Trials, Duluth, GA; Richard A. Pollak, San Antonio Podiatry Associates, San Antonio, TX; Robert Rosen, Salem Research Group, Winston-Salem, NC; Julio Rosenstock, Dallas Diabetes & Endocrine Center, Dallas, TX; Lee J. Sanders, Veterans Affairs Medical Center, Lebanon, PA; Alan Sawchuk, Outpatient Clinical Research Facility, Indianapolis, IN; James Snyder, Southwest Medical Associates, Las Vegas, NV; James S. Tan and Thomas M. File, Jr., Akron Infectious Diseases, Akron, OH; Susan Walker, Martinez Veterans Affairs Center, Martinez, CA; Lowell Scott Weil, Des Plaines, IL; and Gregg Young, Veterans Affairs Medical Center, Salt Lake City, UT.

Study 304. Scott Alter, Miami Heart Research, Miami, Pascual Bidot, Florida Pharmaceutical Research, Tampa, and David Brecher, Florida Pharmaceutical Research, Palm Harbor, FL; Gregory Clark, Scripps Clinic and Research Foundation, La Jolla, CA; John Dawson, Advanced Research Management, Seattle, WA; G. Stephen DeCherney, Medical Research Institute of Delaware, Newark, DE; John Eidt, UAMS Department of Surgery, Little Rock, AR; John Ervin, The Center for Pharmaceutical Research, Kansas City, MO; Stephen Green, Hampton, VA; Jason Hanft, Poinciana Medical Group, South Miami, FL; Kevin Henry, High Point Foot Center, High Point, NC; Donald Hill, Jr., Bay Pines Veterans Affairs Medical Center, Bay Pines, FL; Kirk Jacobson, Oregon Research Group, Eugene, OR; Jeffrey Jensen, The Diabetic Foot and Wound Healing Center, Denver, CO; Terry Jones, J&S Studies, Byran, TX; Joseph Jorizzo, Bowman Gray School of Medicine, Winston-Salem, NC; Charles Kilo, Kilo Clinical Research, St. Louis, MO; Joel Lerner and Michael Lerner, Lerner Podiatry Group, Kenilworth, NJ; Charles Lucas, William Beaumont Hospital, Birmingham, MI; Mary McAnaw, Veterans Affairs Medical Center: Midwest Biomedical Research, Kansas City, MO; Linda Membreno, Camino Medical Group, Sunnyvale Clinic, Sunnyvale, CA; James Mersey, Baltimore, MD; Dennis Mikolich, Omega Medical Research, Providence, RI; Thomas Palmer, Metropolitan Clinic, Portland, OR; Ramon Ramirez, Clireco, Tamarac, FL; Harvey Resnick, R/D Clinical Research, Lake Jackson, TX; Melvin Russell, Southern Drug Research, Tallassee, Angelo Agee, Southern Drug Research, Montgomery, AL; Richard Sachson, Research Institute of Dallas, Dallas, TX; Neil Scheffler and Marc Lipton, American Center for Foot Health Research, Baltimore, MD; Thomas Schnitzer, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL; Lonnie Schwartz, Metroplex Clinical Research Center, Dallas, TX; David Silvers, Drug Research Services, Metairie, LA; David Skrobot, PharmacoTherapy Research, Zanesville, OH; Malcolm Sperling, Edinger Medical Group, Fountain Valley, CA; Dennis Stevens, Veterans Affairs Medical Center, Boise, ID; Anthony Sussman, Memorial Research Center, Savannah, GA; Angeles Valdes, Norwegian American Hospital, Chicago, IL; Franklin Zieve, McGuire Veterans Affairs Medical Center, Richmond, VA; and Christen Zuschke, University of South Alabama Medical Center, Mobile, AL.

Acknowledgments

We thank the employees of Magainin Pharmaceuticals and SmithKline Beecham, who helped develop pexiganan; Michael Silverman, for advice in preparing the manuscript; and Eugene C. Poggio, for reviewing the statistical analyses.

Potential conflicts of interest. At the time these studies were conducted, K.J.H. and M.Z. were employees of, and B.A.L. was a consultant to, Magainin Pharmaceuticals. B.A.L., K.J.H., and M.Z. have served as consultants to MacroChem. B.A.L. has received research grants from and been a consultant and speaker for Ortho McNeil.

Manufacturers' role. Data files were provided to the authors from the former (Magainin Pharmaceuticals) and the future (MacroChem) manufacturers of Pexiganan cream.

References

1. Lavery LA, Armstrong DG, Wunderlich RP, Tredwell J, Boulton AJ. Diabetic foot syndrome: evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. Diabetes Care **2003**; 26:1435–8.
2. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA. Risk factors for foot infections in individuals with diabetes. Diabetes Care **2006**; 29:1288–93.
3. Lipsky BA, Berendt AR, Deery HG, et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis **2004**; 39:885–910.
4. Lipsky BA, Stoutenburgh U. Daptomycin for treating infected diabetic foot ulcers: evidence from a randomized, controlled trial comparing daptomycin with vancomycin or semi-synthetic penicillins for complicated skin and skin-structure infections. J Antimicrob Chemother **2005**; 55:240–5.
5. Lipsky BA, Armstrong DG, Citron DM, Tice AD, Morgenstern DE, Abramson MA. Ertapenem versus piperacillin/tazobactam for diabetic foot infections (SIDESTEP): prospective, randomised, controlled, double-blinded, multicentre trial. Lancet **2005**; 366:1695–703.
6. Lipsky BA, Itani K, Norden C. Treating foot infections in diabetic patients: a randomized, multicenter, open-label trial of linezolid versus ampicillin-sulbactam/amoxicillin-clavulanate. Clin Infect Dis **2004**; 38: 17–24.
7. Lipsky BA, Giordano P, Choudhri S, Song J. Treating diabetic foot infections with sequential intravenous to oral moxifloxacin compared with piperacillin-tazobactam/amoxicillin-clavulanate. J Antimicrob Chemother **2007**; 60:370–6.
8. Lipsky BA, Baker PD, Landon GC, Fernau R. Antibiotic therapy for diabetic foot infections: comparison of two parenteral-to-oral regimens. Clin Infect Dis **1997**; 24:643–8.
9. Rao N, Lipsky BA. Optimising antimicrobial therapy in diabetic foot infections. Drugs **2007**; 67:195–214.
10. Lipsky BA. A report from the international consensus on diagnosing and treating the infected diabetic foot. Diabetes Metab Res Rev **2004**; 20(Suppl 1):S68–77.
11. Lavery LA, Armstrong DG, Murdoch DP, Peters EJ, Lipsky BA. Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. Clin Infect Dis **2007**; 44:562–5.
12. Lio PA, Kaye ET. Topical antibacterial agents. Infect Dis Clin North Am **2004**; 18:717–33, x.
13. Nelson EA, O'Meara S, Golder S, Dalton J, Craig D, Iglesias C. Systematic review of antimicrobial treatments for diabetic foot ulcers. Diabet Med **2006**; 23:348–59.
14. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature **2002**; 415:389–95.
15. Perron GG, Zasloff M, Bell G. Experimental evolution of resistance to an antimicrobial peptide. Proc Biol Sci **2006**; 273:251–6.
16. Zasloff M. Antimicrobial peptides in health and disease. N Engl J Med **2002**; 347:1199–200.
17. Ulvatne H. Antimicrobial peptides: potential use in skin infections. Am J Clin Dermatol **2003**; 4:591–5.
18. Ge Y, MacDonald D, Hait H, Lipsky B, Zasloff M, Holroyd K. Microbiological profile of infected diabetic foot ulcers. Diabet Med **2002**; 19:1032–4.
19. Ge Y, MacDonald D, Henry MM, et al. In vitro susceptibility to pexiganan of bacteria isolated from infected diabetic foot ulcers. Diagn Microbiol Infect Dis **1999**; 35:45–53.
20. Zasloff M. Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci U S A **1987**; 84:5449–53.
21. Lamb HM, Wiseman LR. Pexiganan acetate. Drugs **1998**; 56:1047–52; discussion 53–4.
22. Fuchs PC, Barry AL, Brown SD. In vitro antimicrobial activity of MSI-78, a magainin analog. Antimicrob Agents Chemother **1998**; 42:1213–6.
23. Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler EL. Classification and treatment of chronic nonhealing wounds: successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann Surg **1986**; 204:322–30.
24. Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation: basis for prevention. Diabetes Care **1990**; 13:513–21.
25. Oien RF, Hakansson A, Hansen BU, Bjellerup M. Measuring the size of ulcers by planimetry: a useful method in the clinical setting. J Wound Care **2002**; 11:165–8.
26. Makuch R, Simon R. Sample size requirements for evaluating a conservative therapy. Cancer Treat Rep **1978**; 62:1037–40.
27. Makuch RW, Simon RM. Sample size considerations for non-randomized comparative studies. J Chronic Dis **1980**; 33:175–81.
28. Makuch RW, Simon RM. Sample size requirements for comparing time-to-failure among k treatment groups. J Chronic Dis **1982**; 35: 861–7.
29. Andres E, Dimarcq JL. Cationic antimicrobial peptides: from innate immunity study to drug development: up date [in French]. Med Mal Infect **2007**; 37:194–9.