Effect of Daptomycin on Local Interleukin-6, Matrix Metalloproteinase-9, and Metallopeptidase Inhibitor 1 in Patients With MRSA-Infected Diabetic Foot

Andreas Ambrosch, MD¹,², Daniel Halevy, MD³, Boushra Fwity, MD², Thomas Brin, MD², and Ralf Lobmann, MD³

Abstract
Infection is a major cause of the diabetic foot syndrome that is promoted by the increased burden of multiresistant germs like methicillin-resistant Staphylococcus aureus (MRSA). Maximizing positive outcome for serious MRSA infections requires an aggressive treatment approach and careful monitoring of the healing process. Therefore, we examined 8 patients with MRSA-infected diabetic foot syndrome of Wagner classification grade 2 or 3 (corresponding to the Texas classification stage 2 or 3) during antibiotic treatment with daptomycin. We documented the wound size and obtained samples of wound secretion for analyses of proinflammatory interleukin-6 (IL-6), protease (matrix metalloproteinase-9 [MMP-9]), and antiprotease (metallopeptidase inhibitor 1 [TIMP-1]) activity. During the course of anti-MRSA therapy, we observed a decrease in the concentration of local IL-6 within the first 3 days followed by a decrease of MMP-9 and an increase of TIMP-1. Finally, a reduction of wound size was documented. The present data show that efficient antimicrobial treatment with daptomycin has a number of beneficial effects on wound healing at the molecular level in MRSA-infected diabetic foot ulcers.

Keywords
daptomycin, MMP-9, TIMP-1, MRSA, diabetic foot

In patients with diabetic foot, the antibiotic treatment depends on the seriousness of the infection. Oral antibiotics that are effective against Staphylococcus are used for moderate infections and sequential treatment, and intravenous administration of a broad-spectrum antibiotic is used for serious infections. The treatment must be specified once the results of microbiological tests are available, thus ultimately shortening the treatment period. So-called problem pathogens are increasingly becoming a clinical burden.⁴,⁵ Observed in recent years, the rate of lesions already contaminated or infected at the time of first contact with methicillin-resistant Staphylococcus aureus (MRSA) has been steadily increasing. Antibiotics that traditionally have been effective against MRSA, such as glycopeptides (vancomycin), show a gradual decrease in sensitivity (so-called vancomycin creep of the minimum inhibitory concentration), leading to side effects that are inconvenient for diabetic patients.⁶ Newer substances such as oxazolidinone (linezolid) frequently are not considered, given their potential neuropathological side effects and bone marrow suppression.⁷ In addition, the time period of administration is limited, which, in the event of diabetic foot syndrome, would be quite frequently associated with several weeks of antibiosis, including a change of the substance.

Daptomycin, one of the more recent glycopeptides (cyclical lipopeptide), is effective against gram-positive bacteria and has been clinically tested for the treatment of complicated skin and soft tissue infections.⁷ Due to its effective range and favorable profile of side effects, daptomycin appears to be useful as a supplementary treatment for patients with MRSA-infected diabetic foot syndrome.

¹Institute of Laboratory Medicine, Microbiology and Hygiene, Hospital Barmherzige Brüder, Regensburg, Germany
²Institute of Laboratory Medicine and Microbiology, St. Joseph Hospital, Bremerhaven, Germany
³Department of Endocrinology, Diabetology and Geriatrics, Stuttgart–Bürgerhospital, Stuttgart, Germany

Corresponding Author:
Ralf Lobmann, Medical Clinic 3, Department of Endocrinology, Diabetology and Geriatrics, Klinikum Stuttgart–Bürgerhospital, Tunzhofer Straße 14-16, Stuttgart, 70191, Germany.
Email: r.lobmann@klinikum-stuttgart.de
Studies of molecular and immunological phenomena in diabetic foot syndrome are of interest. It is well known that diabetic foot syndrome is the result of a chronic wound-healing dysfunction that results in an imbalance in the molecular mechanisms of wound healing. Apart from the infection, which leads to the induction of proinflammatory mediators in the wound region, excessive concentrations of proteases and an acceleration in the degradation of their inhibitors have been observed. The influence of antibiotic treatment on these effects can only be speculated upon.

This observational study thus investigated the effects of sufficient antibiotic treatment on the cytokine and protease profile in patients with MRSA-infected diabetic foot lesions.

Material and Methods

Patients, Foot Documentation, and Medication

Patient inclusion and exclusion criteria are given in Table 1. To determine patients’ suitability for inclusion, a physical examination was performed according to a standard protocol. Medical history and previous and concomitant treatments were documented as well.

Diabetic foot lesions were documented at each of the possible visits (days 0, 3, 5, 7, 14, 21, and 28) using the official standardized wound information sheet from the Diabetic Foot Working Group of the German Diabetic Society. Wound size was given in cubic millimeters calculated from depth (mm), width (mm), and length (mm) of the wound.

According to the study protocol, all patients obtained daptomycin (daily dose 4-6 mg per kilogram of body weight) for a maximum of 14 days.

Microbiology and Success of Therapy

Microbiological smears were taken at day 0 according to inclusion criteria and at each visit to monitor the microbiological eradication rate. Success of therapy by daptomycin was defined as absence of MRSA at the end of treatment.

Chemical Laboratory Tests

Blood samples were taken at each visit for the purposes of performing chemical laboratory tests and ensuring safe progression of the therapy. At baseline (day 0), mean concentrations ± standard deviation were 128 ± 67 µmol/L for creatinine, 10.3 ± 3.7 × 10³ µL for white blood cell count, 98 ± 102 mg/dL for C-reactive protein, 7.94 ± 3.22 mmol/L for glucose, and 8.2% ± 2.5% for HbA1c. Further values obtained during the course of therapy are not presented here, since these parameters were not in aim of the study.

Proteases and Proinflammatory Cytokines

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to detect matrix metalloproteinase-9 (MMP-9), metallopeptidase inhibitor 1 (TIMP-1) (Amersham Pharmacia Biotech, Little Chalfont, UK), and interleukin-6 (IL-6) (DPC Bierman, München, Germany) in wound fluid/extraction; tests were carried out in accordance with the corresponding protocols.

All standards and samples were probed in duplicate and the concentrations for each sample were calculated, starting from the standard curve, as ng/mL or pg/mL.

Sampling of Wound Fluid by a Paper Strip Method

According to a modified Schirmer assay procedure, a strip of filter paper (6 × 40 mm; Munktell, Bärenstein, Germany) was placed onto the lesion after the surface of the wound was rinsed with sterile NaCl. The filter strip soaked up wound fluid by capillary action for 2 minutes. Subsequently, the paper strip containing up to 90 µL of wound fluid was preserved in 0.5 mL of an alcohol extraction buffer (Tris HCL 150 mM, NaCL 50 mM, Polyoxyethylen(23)laurylether 0.05%) and frozen at −20°C prior to analyses. For each wound, 2 paper strips were used and subsequently analyzed for cytokines and proteases.

In recent dilution experiments with a defined stock of IL-6 (data not shown), the extraction resulted in a recovery between 60% and 70%. In addition, linear dilution experiments were performed, and standard deviations of 19.3 and 1.5 pg/mL, respectively, were calculated from serial measurement of extractions from different concentrations of IL-6 stock (62.8 pg/mL and 10.7 pg/mL).

Results

Progression of Wound Healing and MRSA Eradication During Therapy With Daptomycin

Figure 1 shows the average value for the wound size (depth × width × length in cubic millimeters) over time. From day 14 onward, there was a reduction in ulcer size.

Microbiological
The International Journal of Lower Extremity Wounds 13(1)

eradication was achieved in 8 of 8 patients at the end of treatment (day 14).

Profile of Proinflammatory IL-6 and Proteases During Therapy

As already described, sampling of wound secretion was obtained during antibiotic treatment, using the paper strip method, with the concentration of proinflammatory IL-6 and protease being determined after extraction. Despite significant interindividual variability in the concentrations, significant reductions in IL-6 and MMP-9 were detected during the course of treatment paralleled by an increase in the antiprotease TIMP-1 (Figure 2).

Discussion

With regard to the impact of MRSA compared with methicillin-sensitive *S* aureus (MSSA) in diabetic foot ulcers, different studies have shown that MRSA is associated with increased frequency of treatment failure, longer time of ulcer healing, and higher risk of lower limb amputation. Therefore, maximizing positive outcomes for serious MRSA infection in diabetic foot ulcers requires an aggressive treatment approach and careful monitoring of the healing process. The present pilot study investigating the effects of daptomycin in MRSA-infected diabetic foot syndrome demonstrates an optimal eradication rate combined with ulcer size reduction during the observational period of 21 days. On the molecular level of wound healing, a reduction of local proinflammatory IL-6 within the first 3 days of therapy and a decrease of MMP-9 after 14 days paralleled by an increase of antiprotease TIMP-1 were observed. This reflects a positive effect of antimicrobial treatment on the healing process.

As mentioned above, in the present study wound fluid collection was performed by a paper strip–based method. No gold standard exists regarding sampling technique. However, aspiration of wound fluid accumulated under an occlusive film dressing over time is the most commonly used technique. This technique requires high logistic effort and is unlikely to work in daily clinical routine. In a recent study, Schmohl et al described a novel method of superficial sampling and processing of wound fluid using nylon-flocked swabs. By this technique, wound fluid was collected after sharp debridement and hemostasis, and a median sample volume of 40 µL could be obtained. In dilution experiments, a recovery in control samples between 25% and 100% depending on the parameter analyzed was described, which was comparable to our experience in diluting samples with a defined stock of IL-6. In contrast, no invasive sharp debridement before sampling was needed, only rinsing with sterile NaCl.

With regard to daptomycin therapy, a 100% microbial eradication at the end of treatment was achieved in the present small study population. It is known from recent studies that daptomycin is active against staphylococci including MRSA and other gram-positive bacteria. Resistance to daptomycin is uncommon but can be induced by serial passage in increasing concentrations of the antimicrobial. In patients with infected diabetic ulcers, daptomycin was shown to have a clinical success rate of 66%, similar to the 70% rate for vancomycin used as the comparator. However, in that study, the patient group with MRSA was also too small to draw any conclusions about the relative efficacy of daptomycin against this pathogen. In a randomized trial in nondiabetic patients, the clinical success rate in complicated skin and skin-structure infections with MRSA was 77%.

As shown in Figure 2A, the eradication of MRSA in the present study could be monitored by a rapid decrease of proinflammatory IL-6 within the first 3 days of therapy. Along this line, we have recently demonstrated that IL-6 evaluated from wound fluid is capable of predicting high bacterial load, a polymicrobial infection, or an infection with *Pseudomonas* spp or *S* aureus. However, among all these factors, a mixed infection seems to be the most significant trigger for local IL-6. Furthermore, IL-6 as well as local tumor necrosis factor alpha (TNFα) reflects the extension of the inflamed area, since both cytokines were independently predicted by the ulcer size. In our opinion, local measurement of proinflammatory markers in chronic ulcers is more sensitive than measurement of serum markers like C-reactive protein in monitoring the inflammatory process and therapy success. However, in a recent work by Dinh and coworkers, nonhealing of diabetic ulcers was strongly associated with a preexisting, systemic proinflammatory status including increased serum concentrations of MMP-9.
In this view, monitoring of systemic inflammation may help to identify diabetic patients at risk for nonhealing ulcers.

Prior to the reduction of ulcer size observed after day 14 in the present data, a decrease of proteases (MMP-9) and an increase of antiproteases (TIMP-1) was documented during efficient daptomycin therapy (Figure 2, B and C). As shown by us and many other investigators, MMP-8 and MMP-9 are the predominant collagenases present in normal wound healing, and the overexpression and activation of these proteinases may be involved in the pathogenesis of nonhealing chronic leg ulcers.24-27 The mechanism of increased MMP-9 in diabetes is uncertain. It is likely to be related to increased inflammation because MMP-9 is expressed mainly by neutrophils and macrophages, and both cell types are important for the inflammatory response to bacterial infection.28 In addition, excessive collagenolytic activity, particularly in diabetic feet, may be possible because of the reduced levels of antiprotease TIMP-1. For an effective healing process, the relation of proteinase and antiproteinase must be balanced.

The present data have shown that efficient anti-MRSA treatment with daptomycin led to a sequence of processes at the molecular level of wound healing: Microbial eradication and anti-inflammation occurred first, reflected by a decrease of local IL-6; this was followed by a decrease of MMP-9 and an increase of antiproteinase activity. As a result of balancing the relation of proteinase and antiproteinase, wound size was reduced.

Despite the pilot character of the present study, several limitations deserve consideration. The present study includes only 8 patients with diabetic foot lesions and has the character of an observational study without a control group. Furthermore, MRSA eradication was proven by superficial swab analyses. In this context, deep invasive diagnostic sampling could be considered for more sensitive testing, particularly in diabetic patients with osteomyelitis related to foot ulcers.29 More comprehensive clinical studies appear to be justified on the basis of these preliminary data, particularly in patients with diabetic foot infections caused by bacteria such MRSA.

Declaration of Conflicting Interests
The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by a nonrestricted grant obtained from Novartis.
References