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International Journal of Lower Extremity Wounds 2013 12: 100
DOI: 10.1177/1534734613490506

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What is This?
Clinical Research

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 being aggravating 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 a careful monitoring of the healing process. Therefore, we examined 8 patients with MRSA-infected diabetic foot syndrome Wagner classification grades 2 or 3 (corresponding to the Texas classification stage 2 and 3) during antibiotic treatment with daptomycin. We documented the wound size and obtained samples of wound secretion for analyses of pro-inflammatory interleukin-6 (IL-6), protease (matrix metalloproteinase-9 [MMP-9]), and antiprotease activity (metalloproteinase inhibitor 1 [TIMP-1]). During the course of anti-MRSA therapy, a decrease in the concentration of local IL-6 within the first 3 days followed by a drop of MMP-9 and an increase of TIMP-1 was observed. Finally, a reduction of wound size could be documented. The present data show that efficient antimicrobial treatment with daptomycin leads to a number of beneficial processes at the molecular level of wound healing in MRSA-infected diabetic foot ulcers.

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

In diabetic foot patients, antibiotic treatment depends on the seriousness of the infection. Oral antibiotics with Staphylococcus-effective chemotherapy drugs are being used for moderate infections and sequential treatment and/or intravenous administration of a broad-spectrum antibiotic in the event of a serious infection. The treatment must be specified once the results of the microbiological tests are available, thus ultimately shortening the treatment period. So-called problem pathogens are increasingly becoming a clinical burden.1,3 Observed in recent years, the rate of lesions already contaminated/infected at the time of first contact with methicillin-resistant Staphylococcus aureus (MRSA) has been steadily increasing. Traditional MRSA-effective antibiotics, 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 patients with diabetes.3 In addition, more recently developed substances such as oxazolidinone (linezolid) are frequently not taken into account since neuropathological side effects and bone marrow suppression have been observed.5,6 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 is one of the more recent glycopeptides (cyclical lipopeptide) developed in the past, effective against gram-positive bacteria, which has been clinically tested for the treatment of complicated skin and soft tissue infections.7 Due to its effective range and taking the profile of side effects into account, daptomycin appears to be a supplementary treatment for the patient group with MRSA-infected diabetic foot syndrome.

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Apart from the current (approval) studies, studies of molecular and immunological phenomena in diabetic foot syndrome are of interest. It is well known that an untreated 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 pro-inflammatory mediators in the wound region, excessive concentrations of proteases and an acceleration in the degradation of their inhibitors have also been observed. The influence of antibiotic treatment on these effects can only be speculated.

Therefore, the present case series investigated the effects of sufficient treatment with antibiotics on the cytokine and protease profile in patients with MRSA-infected diabetic foot lesions.

### Materials and Methods

#### Patients/Foot Documentation/Medication

Patients with diabetes mellitus type 1 and 2 suffering from MRSA-infected diabetic foot syndrome Wagner classification grade 2 or 3 were included. Grades 2 and 3 of the Wagner classification, regularly used in Germany, correspond to the internationally accepted Texas classification. Patient inclusion and exclusion criteria are given in Table 1.

To check criteria for inclusion and exclusion, a physical examination according to a standard protocol was performed. Additionally, medical history and previous and concomitant treatments were documented.

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 Diabetes Association. Wound size was given in mm³ 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/kg body weight) for a maximum of 14 days.

#### Microbiology/Success of Therapy

Microbiological smears were taken at day 0 according to inclusion criteria and at each visit monitoring 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 chemical laboratory tests and the safe progress 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 given, since these parameters were not in aim of the study.

#### Proteases/Pro-Inflammatory Cytokines

Commercially available ELISA kits were used to detect matrix metalloproteinase-9 (MMP-9), metallopeptidase inhibitor 1 (TIMP-1) ELISAs (Amersham Pharmacia Biotech, Little Chalfont, UK), and interleukin-6 (IL-6; DPC Bieman, Los Angeles, CA) in wound fluid/extraction with the tests being carried out in accordance to 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, Barenstein, Germany) was placed onto the lesion after rinsing the surface of the wound 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 wound fluid was preserved in 0.5 mL of an alcoholic extraction buffer (Tris HCl

### Table 1. Inclusion and Exclusion Criteria for Patients.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
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<tr>
<td>Male and female test subjects between the ages of 18 and 80 years</td>
<td>Known oversensitivity to daptomycin</td>
</tr>
<tr>
<td>Patients suffering from diabetes mellitus type 1 or 2 and diabetic foot syndrome Wagner classification grade 2 or 3</td>
<td>A consumptive underlying disease</td>
</tr>
<tr>
<td>Microbiological proof of MRSA</td>
<td>Problems with blood coagulation</td>
</tr>
<tr>
<td>A positive appraisal received from the ethics committee of Magdeburg University Hospital</td>
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Abbreviation: MRSA, methicillin-resistant Staphylococcus aureus.
150 mM, NaCl 50 mM, polyethylene laureylether 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 a standard deviation of 19.3 and 1.5 pg/mL 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 [mm³]) over time. From day 14 onwards, there is a change in the sense of a reduction in ulcer size. Microbiological eradication was achieved in 8/8 patients at the end of treatment (day 14).

Profile of Pro-Inflammatory IL-6 and Proteases During Therapy

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

Discussion

With regard to the impact of MRSA compared to methicillin-sensitive S aureus (MSSA) in diabetic foot ulcers, the association to increased frequency of treatment failure, longer time of ulcer healing, and higher risk of lower limb amputation was proven by different studies. Therefore, maximizing positive outcome for serious MRSA infection in diabetic foot ulcers requires an aggressive treatment approach and a careful monitoring of the healing process. The present pilot study investigating effects of daptomycin in MRSA-infected diabetic foot syndrome demonstrates an optimal eradication rate combined by an ulcer size reduction during the observational period of 21 days. On the molecular level of wound healing, a reduction of local pro-inflammatory IL-6 within the first 3 days of therapy, a decrease of MMP-9 after 14 days, paralleled by an increase of antiprotease TIMP-1 was observed reflecting 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. In this regard, no gold standard exists as to which sampling technique could be referred. However, aspiration techniques that accumulate wound fluid under an occlusive film dressing over time is the most common technique used. This technique requires high logistic effort and is unlikely to work in daily clinical routine. In a recent study by Schmohl et al, a novel method of superficial sampling and processing of wound fluid using nylon flocked swabs was described. 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 but 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% of vancomycin used as the comparator. However, in this 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%.\textsuperscript{22}

As shown in Figure 2A, the eradication of MRSA in the present study could be monitored by a rapid decrease of pro-inflammatory IL-6 within the first 3 days of therapy. In this line, we have recently demonstrated that IL-6 evaluated

![Graphs showing the course of pro-inflammatory IL-6, MMP-9, and TIMP-1 during therapy with daptomycin in diabetic foot infections.](image-url)
from wound fluid is capable of predicting high bacterial load, a polymicrobial infection, or an infection with *Pseudomonas* spp. and/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-α also reflects the extension of the inflamed area, since both cytokines were independently predicted by the ulcer size. In our opinion, local measurement of pro-inflammatory markers in chronic ulcers is more sensitive compared to serum markers like C-reactive protein for monitoring the inflammatory process and therapy success, respectively. However, in a recent work by Dinh and coworkers, nonhealing of diabetic ulcers was strongly associated with a preexisting, systemic pro-inflammatory status including increased serum concentrations of MMP-9. In this view, monitoring of systemic inflammation may help identify diabetic patients at risk for nonhealing ulcers.

Prior to the reduction of the 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 2B and C). As shown by us and many other investigators, MMP-8 and MMP-9 are the predominant collagenase present in normal wound healing, and the overexpression and activation of these proteinases may be involved in the pathogenesis of nonhealing chronic leg ulcers. 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, both cell types being important for the inflammatory response to bacterial infection. In addition, excessive collagenolytic activity particular in diabetic feet may be possible because of the reduced levels of antiprotease TIMP-1. For an effective healing process, the proteinase/antiproteinase relation must be balanced.

Taken together, the present data have shown that efficient anti-MRSA treatment with daptomycin led to a sequel of processes on molecular level of wound healing: in a first step, microbial eradication and anti-inflammation occurs reflected by a decrease of local IL-6, followed by a drop of MMP-9 and an increase of antiprotease activity. As a result of balancing the protease/antiprotease relation, wound size reduction could be observed.

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 therefore only the character of a case series without investigating 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, particularly in diabetic patients with osteomyelitis related to foot ulcers. Consequently, more comprehensive clinical studies appear to be clearly justified on the basis of these preliminary data, particularly in patients with diabetic foot infections with problem bacteria such as MRSA.

**Declaration of Conflicting Interests**

The author(s) 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.

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