Intermittent intravenous urokinase for critical limb ischemia in diabetic foot ulceration

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Summary
Patients with diabetic foot ulceration and critical limb ischemia have a high risk of major amputation, especially if limbs cannot be revascularized. Urokinase is effective in improving microcirculation in critical limb ischemia and might improve outcomes. There are no data on the efficacy and safety of urokinase treatment (survival free of major amputation, ulcer healing and the rate of minor and major bleeding). Therefore, we aimed to investigate the effect of urokinase treatment in a phase II clinical trial. We performed an open, prospective, non-controlled, multicenter phase II cohort study in 77 type-2 diabetic patients with critical limb ischemia and diabetic foot ulceration. Patients had no surgical or endovascular treatment option based on interdisciplinary consensus. Urokinase (1 Mio IU if plasma fibrinogen ≥2.5 g/l, 0.5 Mio IU if fibrinogen < 2.5 g/l) was administered for 21 days as an intravenous infusion over 30 minutes. Each patient was followed up for 12 months. Treatment for a median of 21 days resulted in 33% of patients being alive, having no major amputation and completely healed ulcers after 12 months. Total survival rate was 84.6%, amputation-free survival 69.2% and rate of major amputation 21.1%. Eighty-two percent of patients experienced at least once a complete ulcer healing within the course of study. Three serious adverse events were urokinase-related. Urokinase treatment in diabetic patients with critical limb ischemia appears to be effective, feasible and safe. Although this calls for a larger, randomized and controlled trial, the results are highly relevant for clinical practice to prevent these patients from receiving major amputation due to diabetic foot syndrome.

Keywords
Urokinase, critical limb ischemia, diabetic foot syndrome, peripheral arterial disease, revascularization

Introduction
The diabetic foot syndrome (DFS) is a multifactorial disease originating from neurogenic, vascular, haemorheologic, mechanic and metabolic causes complicated by a reduced immune response and a propensity for infections. An estimated 15% of people with diabetes will develop foot ulceration during their lifetime, and 14% to 24% of people with a foot ulcer will require an amputation (1). It is the commonest cause of non-traumatic lower extremity amputations in the world.

In type 2 diabetic patients with angiopathic or angioneuropathic foot syndrome, revascularization is less often feasible and patients are prone to develop complications because of mostly peripheral vascular lesions (2). Despite current standards of care with antibiotic treatment of infections, endovascular revascularization, application of vasodilators, improvement of blood sugar control and immobilisation patients have a high risk of major amputation (3, 4).

It has been shown in previous trials that urokinase is effective in improving the microcirculation in diabetic patients with peripheral arterial disease (PAD) stages III and IV Fontaine (CLI) or Rutherford stage 4–6 (5–8). In these studies it was shown, that diabetic patients seem to profit more from the rheologic treatment of PAD than non-diabetic patients (9). Overall however,
data on the effect of intravenous urokinase treatment in patients with CLI and diabetic foot lesions are mostly retrospective in small patient cohorts and this was the reason to set up this prospective, multicenter, cohort-study.

The following three questions were addressed in the current study: 1) What is the long-term effect of urokinase treatment (total survival, survival rate free of major amputation, rate of complete ulcer healing)?, 2) Overall mortality, rate of major amputation, and the development of new ischemic ulcers?, 3) Frequency of minor and major bleeding and other adverse events? Point estimates were to be generated for subsequent controlled clinical trials.

Patients and methods

This was an open, prospective, non-controlled multicenter phase II study conducted at four centers for diabetology and vascular medicine in Germany. The protocol was approved by the local ethics committee. Written informed consent was obtained. It was conducted in accordance with the requirements of the German Medicines Law, the Declaration of Helsinki and Good Clinical Practice.

Patient inclusion criteria

Patients (age ≥18 years) with angi- or angioneuropathic diabetic foot lesions (angiographic exclusion of neuropathic diabetic foot lesions without angiopathy), critical limb ischemia, and no surgical or endovascular treatment option were enrolled into the study. A critical limb ischemia was assumed when the Ankle-Brachial-Index (ABI) was < 0.4 (no uncontrolled hypertension) and / or toe occlusive pressure was < 30 mmHg and / or pulse-volume curve silent or partially pulsatile only and / or acral light plethysmographs of all residing toes silent or partially pulsatile only and / or the CW Doppler-signal of the arteria tibialis posterior and dorsalis pedis showed a collateral form (the ascending part of the signal is not steeper than the descending part). Ischemic etiology of foot lesions was verified by angiography. The assessment of endovascular treatment options was made in a multidisciplinary consensus between angiologists (vascular physicians), vascular surgeons, and Interventionalists from angiographic findings. Structured wound care was provided in every patient according to international recommendations, including wound debridement and moist wound dressings (4). Growth factors were not used. Only patients in whom a basic treatment with antibiotics, tight blood sugar control and wound debridement did not initiate wound healing for a week and who did not have previous major amputation were included. Plasma fibrinogen level had to exceed 4.0 g/l. Diabetic foot lesions were classified according to Wagner-Armstrong (10).

Patient exclusion criteria

Exclusion criteria were feasibility of vascular surgery or angioplasty, prior treatment of the current ulceration with urokinase, creatinine > 180 µM, any kind of cerebral event less than three months before inclusion into the study, proliferative retinopathy (not remediated), uncontrolled hypertension, hemorrhagic diathesis, gastrointestinal bleeding, need for oral anticoagulation, mental disorders, pregnancy, or participation in another study.

History, clinical examination, and current status of ulceration and amputation was noticed before starting treatment with urokinase and thereafter every four weeks during follow-up examinations up to 12 months (based on recommendations of the Basel Group [11]) (Table 1).

Study medication

Urokinase was administered daily as an intravenous infusion over 30 minutes (21 applications). This procedure had provided favourable results on a case-by-case basis with no bleeding and no changes in coagulation parameters (7). During the study, patients with a plasma fibrinogen ≥2.5 g/l received 1 Mio IU urokinase (< 2.5 g/l received 0.5 Mio IU urokinase). Treatment with urokinase was stopped if ulcer healing occurred before 21 doses had been administered and if plasma fibrinogen concentration dropped to < 1.6 g/l. It was continued after rising up to ≥2 g/l. After minor amputation, modification of the urokinase dose was

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<th>Patient Inclusion Criteria</th>
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<th>Study Medication</th>
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<tr>
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</table>
allowed after assessing the bleeding risk and in accordance with the coordinating investigator. Current treatment with insulin, oral antidiabetic agents, antibiotics and inhibitors of platelet aggregation and others were continued during the study period without any restrictions. During the treatment period with urokinase, oral anticoagulation had to be discontinued. Concomitant treatment with heparin had to be adjusted to the activated partial thromboplastin time (aPTT) with a target value 1.0 to 1.5 ULN. Unfractionated heparin could be replaced by low-molecular-weight heparin that was allowed only in prophylactic or half therapeutic dosage.

**Outcome measures**
The primary outcome was the number of patients being alive, having no major amputation and healed ulceration at the 12 months follow-up. Secondary outcome measures were total survival, major amputation rate, survival free of major amputation, and rate of complete ulcer healing. Major amputation was defined as an amputation above the ankle, minor amputation as an amputation with preservation of the heel. Minor amputation was not reported as an outcome but as a treatment modality to achieve definitive ulcer healing and to avoid major amputation. Safety outcomes were minor and major bleeding or other adverse events. Major bleeding was defined as overt bleeding associated with a decline in haemoglobin of > 2 g/l, with transfusion of more than two units of packed red blood cells, or bleeding into a critical organ (e.g. cerebral, fundus of the eye) bleeding with need for surgical revision, or bleeding followed by death. Minor bleedings were defined as all other bleeding complications.

**Statistical methods**
According to the predefined statistical analysis plan, the statistical analysis was performed descriptively and was interpreted in an explorative way. Categorical data were presented with frequencies and percentages. For continuous data medians, ranges, means, and standard deviations were calculated. Cumulative incidences and survival rates were estimated using Kaplan-Meier methods to take into account any censoring. Statistical analysis was based on the Full-Analysis-Set analysed according to intention-to-treat. The statistical software system SAS version 9.1.3 was used for performing the calculations.

**Results**
Over the course of five years 77 patients were enrolled into the study (Fig. 1). A total of 76 patients were analyzed for the assessment of safety (1 patient died before starting urokinase treatment) and 75 patients for the assessment of efficacy (1 further patient was lost to follow-up). The median number of urokinase applications was 21 [min. 1, 25% quantile (Q1) 17, 75% quantile (Q3) 21, max. 22]. Baseline characteristics are displayed in Table 2. Patients had a mean age of 67.7 ± 9.7 years, 80% of which were male (n=60) and all had type-2-diabetes mellitus. All patients received antibiotic treatment. In 72 patients foot ulcers were classified as angioneuropathic (n=72), and three patients had angiopathic foot ulcers (4%).

**Primary outcome**
At 12-month follow-up-visit 25 patients [33%; 95% confidence interval (CI) 22.9; 45.2] were alive, had no major amputation and had completely healed ulcers (Fig. 2). The respective patient numbers at 3, 6, and 9 months were 10 [13%; 95%CI 6.6; 23.2], 14 [19%; 95%CI 10.6; 29.3], and 17 [23%; 95%CI 13.8; 33.8], respectively.

**Secondary outcomes**
Sixty-four patients were alive, 11 had died. The 12-month survival rate was 84.6% (95%CI 73.8; 91.1). The Kaplan-Meier curve (Fig. 3A) illustrates that 55% of the events (n=6) occurred during the first three months and five events in the six months thereafter (46%). Fifty-three patients survived without major amputation leading to a 12-month rate of 69.2% (95%CI 57.0;
Most of the events occurred during the first three months of follow up (n=10, 46%) and until month 6 (n=11, 50%). Most of the major amputations were performed within the first six months (n=12), and only two thereafter. The cumulative incidence is shown in Figure 3C. At 12 months 21.1% (95%CI 13.0; 33.1) had received a major amputation.

It took a median of 9.2 months [95%CI 5.9; 11.3] for a complete healing of all ulcers. This was the case in 38 of all patients (50.7%) leading to a 12-month rate of 82.0% [95%CI 66.4; 93.2] of patients experiencing a complete ulcer healing at least once within course of study (Fig. 3D).

**Bleeding and other adverse events**

Regarding safety of urokinase therapy, the data of 76 patients were analyzed (Fig. 1). Treatment was terminated prematurely in 18 patients (23.7%). One patient underwent major amputation during treatment with urokinase so that the study endpoint was reached for this patient and treatment terminated. One patient died during a minor amputation procedure from right heart failure.

Eleven patients experienced adverse events (AEs). Five of the AEs were severe and three of them were possibly related to treatment with urokinase – one cerebral bleeding, one hypotension (after urokinase treatment, complete resolution and further 6 uncomplicated urokinase infusions thereafter), and one bleeding into both calves (on day 16 after 15 days of urokinase treatment, spontaneous resolution of hematoma). Severe AEs without relation to treatment were one myocardial infarction without ST-elevation and one septic shock. Seven patients experienced non-severe AEs. Other reasons for discontinuation are displayed in Table 3.

**Discussion**

The probability to salvage an affected limb in patients with diabetic foot ulceration who have concomitant CLI is low. The present study demonstrates that urokinase treatment for a median of 21 days in patients with type-2-diabetes, diabetic foot lesions and critical limb ischemia resulted in 33% of patients being alive, having no major amputation and completely healed ulcers.
Weck et al. Urokinase in critical limb ischemia after 12 months. Furthermore, total survival after 12 months was 84.6% and amputation-free survival 69.2%, and the rate of major amputation 21.1%; 82.0% of patients at risk had healed ulcers at least once within course of study. Three out of 11 AEs were urokinase treatment-related.

Endpoints in DFS and CLI
Studies to document the efficacy of new pharmacologic treatment options are difficult in both DFS and CLI, partly because valid primary endpoints are poorly defined. DFS and CLI differ in the underlying pathophysiology that, essentially, endpoint studies have to be stratified into diabetic and non-diabetic patients. The endpoint suggested by the European Medicines Agency’s CPMP guidelines that is ulcer healing after six months, appears not appropriate (12). Accordingly, in the recent TALISMAN study, this endpoint was negative while the endpoint amputation-free survival was significantly different between treatment groups (13). Given these inconsistencies, we chose as the primary endpoint the rate of patients who, at 12 months, were alive, had both legs, and healed ulcerations. In addition, we recorded a variety of clinical endpoints, including survival free of major amputation, to cover all aspects of CLI in DFS patients.

Morbidity and mortality
Results of the different studies on the effect of a targeted intervention on outcomes are difficult to evaluate partially because of the varying severity of the disease at baseline. This fact is also a major difficulty when giving an overview about the results of previous clinical studies in Table 2 because of the wide variety of baseline characteristics and severities of ischemia. A differential prognosis has been documented in an analysis of 20 clinical trials with 6,118 patients by Wolfe and Wyatt (14). Twenty-six percent of patients with critical limb ischemia died after one year compared with 5% of age and sex-matched controls. Twenty-seven percent of patients in the low-risk group (pain at rest and/or ankle pressure > 40 mmHg) were not amputated within a follow-up of

**Figure 3: Secondary outcomes.** A) Total survival; B) Survival without major amputation. CI, confidence interval; C) Cumulative incidence of major amputation; D) Time to complete healing of all ulcers. CI, confidence interval.
one year. On the contrary, 95% of patients in the high-risk group (tissue loss and/or ankle pressure < 40 mmHg) received an amputation within one year of being treated conservatively. However, no data were presented for diabetic patients. The outcome of diabetic patients with critical limb ischemia has been reported, however, in four trials with mortality rates of 19% up to 80% and amputation rates of 15% up to 83% after one year (Table 4) (15–18).

In the recently published BASIL trial, a surgery-first and angioplasty-first treatment strategy were compared for CLI in a prospectively randomised study (19). In this highly selected patient population, in which only about 30% of the patients with CLI qualified for randomisation and which was not stratified for the presence of diabetes, neither approach was superior to the other. Rates of mortality were 22% (surgery) and 17% (angioplasty), respectively (15% in the present trial). Amputation-free survival at one year was 68% (surgery) and 71% (angioplasty) and compared well with the rate of 69.2% in the present trial.

Four randomized, placebo-controlled trials have investigated the role of pharmacotherapy in diabetic patients unsuitable for revascularization or surgical intervention using alprostadil (PGE1), prostacyclin (PGI2) and the low-molecular-weight heparin dalteparin (20–23), but only a subset has reported data on mortality. Particularly good results were seen in the dalteparin study that evaluated its effect on healing of chronic foot ulcers in diabetic patients with peripheral arterial occlusive disease compared with placebo (23). Seventy-eight patients with ischemic foot ulceration of more than two months duration, ulcer stage I and II according to the Wagner classification, and toe/arm index ≤0.6 were included. The good results reported in that study seem to be a consequence of the inclusion of ulceration according to the lower Wagner stages and a toe/arm index far from severe ischemia that indicates a better circulation than patients with critical ischemia.

A comparison between the results from the present cohort study and the results of these previous placebo-controlled studies is difficult due to the great methodical discrepancies such as study design and patient selection (low vs. high risk). The mortality rate in the present study in diabetic patients having received urokinase treatment is low (15.4% over 12 months) as compared to mortality rates reported in the literature, as is the rate of major amputation (21.1%) (15–18). Thirty-three percent of patients in this study even met the ultimate treatment goal of freedom from amputation and residual ulcers for at least one year. Although neither controlled, randomized nor blinded, the present study delivers a strong rationale for the use of urokinase in this patient population.

Pathophysiologic rationale

The reasons for the low event rates in the present study are largely speculative but may have been the result of a tailored pharmacotherapy targeting at a specific pathophysiology of DFS and CLI in diabetic patients. High fibrinogen levels, entry criterion for the present study was a plasma fibrinogen level of 24.0 g/l, have been associated with an increase in cardiovascular risk (24) and are known to rise as an acute-phase protein in acutely infected DFS. Fibrinogen is a crucial determinant of plasma viscosity and is partially responsible for a worsening of microvascular blood flow. A decrease in fibrinogen concentration in turn with lysis of microthrombi in the microvasculature has been discussed to be responsible for the effect of urokinase therapy (25). Subsequently microvascular blood flow is improved in the long-term, in particular within the nutritive skin capillaries.

AEs and bleeding complications

Out of 76 patients treated with urokinase (adjusted dose according to fibrinogen levels) three patients experienced a serious AE with a possible relation to urokinase treatment. One patient had cerebral bleeding (1.3%), one further patient had bleeding into both lower legs (1.3%), and one patient transient hypotension (1.3%). This is comparable with the data known from the summary of product characteristic (SPC) of urokinase. Furthermore, we have observed no complications from urokinase therapy (especially no bleeding complications and no changes in coagulation parameters) in a prior case-control study in 12 patients with acute angioneuropathic DFS (7). Documenting mostly multimorbid, elderly patients with diabetes, these numbers appear remarkably low to support a further study on the efficacy of urokinase in the treatment of diabetic patients with CLI and foot ulcers.

Limitations

Limitations of the present prospective phase II study are that it has no comparator arm, is not randomized and that recruitment took quite long. Hence all disadvantages of non-randomized single-armed outcome studies are possibly present. On the other
Table 4: Mortality rates, rate of amputation, and ulcer healing in diabetic foot syndrome (DFS).

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>N</th>
<th>Diabetes (N)</th>
<th>Critical limb ischemia</th>
<th>Follow-up (months)</th>
<th>Mortality (%)</th>
<th>Major amputation (%)</th>
<th>Ulcer healing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volaco A. 2004 (18)</td>
<td>98</td>
<td>98</td>
<td>n.k.***</td>
<td>6</td>
<td>21%</td>
<td>19%</td>
<td>35%</td>
</tr>
<tr>
<td>Faglia E. 2001 (17)</td>
<td>115</td>
<td>115</td>
<td>31%</td>
<td>12</td>
<td>19%</td>
<td>27%</td>
<td>n.k.</td>
</tr>
<tr>
<td>Holstein PE 1999 (16)</td>
<td>23</td>
<td>108</td>
<td>75%</td>
<td>12</td>
<td>80%</td>
<td>25%</td>
<td>15%</td>
</tr>
<tr>
<td>Krawzak HW 1989 (26)</td>
<td>15</td>
<td>15</td>
<td>n.k.</td>
<td>end of Tx</td>
<td>7%</td>
<td>53%</td>
<td>n.k.</td>
</tr>
<tr>
<td>Fratezi AC 1995 (15)</td>
<td>37</td>
<td>37</td>
<td>54%</td>
<td>12</td>
<td>26%</td>
<td>57%</td>
<td>n.k.</td>
</tr>
<tr>
<td>Stiegler H 1992 (27)</td>
<td>37</td>
<td>73</td>
<td>0</td>
<td>6</td>
<td>n.k.</td>
<td>8%</td>
<td>n.k.</td>
</tr>
<tr>
<td>Brock FE 1990 (21)</td>
<td>53</td>
<td>109</td>
<td>n.k.</td>
<td>11</td>
<td>n.k.</td>
<td>10%</td>
<td>23%***</td>
</tr>
<tr>
<td>Norgren L 1990 (22)</td>
<td>103</td>
<td>33</td>
<td>Partially, no % given</td>
<td>6</td>
<td>15%</td>
<td>43%</td>
<td>25%***</td>
</tr>
<tr>
<td>Kalani M 2003 (23)</td>
<td>87</td>
<td>87</td>
<td>0</td>
<td>6</td>
<td>7%</td>
<td>9%</td>
<td>21%</td>
</tr>
<tr>
<td>BASIL trial (19)</td>
<td>228</td>
<td>95</td>
<td>n.k.</td>
<td>12 (up to 60)</td>
<td>22%</td>
<td>32%***</td>
<td>29%***</td>
</tr>
<tr>
<td>Present study</td>
<td>75</td>
<td>75</td>
<td>100%</td>
<td>12</td>
<td>15%</td>
<td>21%</td>
<td>82%</td>
</tr>
</tbody>
</table>

Compilation of different studies on clinical trials in diabetic foot syndrome. Comparison is limited because of the wide variety of patient characteristics and different degrees of ischemia at baseline. * At least partial healing; ** combination of major amputation or death; *** not known. Tx = Treatment.

Conclusions

Urokinase treatment in diabetic patients with DFS and critical limb ischemia is feasible and safe and appears effective. Comparing the high rate of amputation-free survival with healed ulcers in our study against the low rates reported in the literature, it seems reasonable, beyond calling for a larger, randomized and controlled trial, to encourage the use of urokinase in clinical practice to prevent these patients from dying or receiving major amputation with DFS.

Participating centers

The study was conducted by clinical centers in Dresden (SS, HR), Freital and Kreischa (MW) and Karlsbad (HL).

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