Effects of iloprost (a prostacyclin analogue) on the endothelial dysfunction and foot ulcers in diabetic patients with peripheral arterial disease

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Abstract
Objectives: To asses the efficiency of iloprost (an analogue of prostacyclin) infusion on endothelial functions and amputation rate in diabetic foot ulcers with complicated macroangiopathy. **Material and Methods:** Sixty (36 men / 24 women) type 2 diabetic patients (61.8 ± 9.7 years, mean ±SD) with diabetic foot ulcer and peripheral arterial occlusive disease, stage III or more by Wagner classification, and 15 (9 male/ 6 female) healthy controls (60.7 ± 9.1 years, mean ±SD) were enrolled in the study. Thirty patients (group I) had iloprost infusion (0.5-2 ng/kg/min for 6 h) for 10 consecutive days. Endothelial functions were determined by brachial arterial flow mediated dilation (FMD) method at stage 0 (basal), 10th and 30th days. Group II patients (n=30) were treated in the same manner as group I except iloprost treatment constituting a patient control group. **Results:** Group I patients showed a significant improvement in the endothelial functions at 10th day, and 30th day (p=0.002) in respect to group II . There were no differences between group I and group II regarding the hospitalization period and amputation rates. Iloprost was well tolerated. Three patients had adverse reactions such as maculo-papular skin eruptions, itching, hypotension and dyspnea due to iloprost infusion; one completed the treatment and 2 had to discontinue the iloprost infusion. **Conclusion:** Ten-day iloprost infusion therapy to patients with diabetic foot ulcers seems to be efficient in the improvement of endothelial function, but, despite our positive clinical observation, this improvement does not affect the outcome of the amputation rates at 30 days follow up period.

Key words: *Diabetes mellitus, Endothelium function, Foot ulcers, Prostacyclin,*

Introduction

Diabetic foot is one of the important causes of morbidity in diabetes mellitus.[1,2] It is caused by ulcerations due to neuropathic and ischemic changes, which are frequently complicated by infections.[1,2] Foot ulcers occur in 5% of the diabetic population and up to 3% will have a lower limb amputation.[3] The presence or absence of infection and/or ischemia, footwear and pressure relief, and overall glycemic control influence the healing of ulcers.[4]

The first-line treatment for occlusive disease at the stage of chronic severe ischaemia consists of revascularization by means of surgery or transluminal angioplasty. However, such revascularization cannot be carried out in 39% of general cases[5] and in particular in diabetic patients, who constitute about 45% of all lower extremity amputations cases.[6]

Endothelial dysfunction is thought to be an important factor in the development of atherosclerosis, hypertension, and heart failure. Over the past decade, a noninvasive technique has evolved to evaluate flow-mediated vasodilatation (FMD), an endothelium-dependent function in the brachial artery.[7,8] This stimulus provokes the endothelium to release nitric oxide (NO) with subsequent vasodilatation that can be imaged and quantified as an index of vasomotor function. This technique is attractive because it is noninvasive and allows repeated measurements. However, despite its widespread use, there are technical and interpretive limitations.

Iloprost, a novel analogue of prostacyclin (PGI2), with similar potent vasodilating and anti-platelet properties but with less hypotensive action, has been shown to exert beneficial effects when infused in patients affected by ischemic vascular disease.[9] Improvement in walking distance and decrease in major amputation rates were observed in both diabetic and non-diabetic subjects following treatment with iloprost.[10]

This study was designed to examine the effects of 10 days iloprost administration on endothelial functions and the foot amputation rates in diabetic patients with ischemic foot ulcers.

Material and Methods

This prospective, randomized, controlled clinical study included 60 patients with type 2 diabetes mellitus and severe peripheral ischemic foot ulcer unsuitable for revascularization hospitalized for treatment at our University Endocrinology and Metabolism Clinic between June 2004 and October 2006. Most of the patients were referred from other regional hospitals. The remainder were individually recruited. After clinical examination and investigation of the patients, they were informed about the

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Table 1: Characteristics of the study patients

Variables	Group I	Group II	*P* value
Number (gender)	30 (18 M /12 F)	30 (18 M /12 F)	NS
Age (mean ±SD), years	60.5 ± 9.1	63.1 ± 9.2	NS
Duration of diabetes (year)	14.53 ± 8.12	14.10 ± 7.26	NS
Diet and OAD (n)	11	16	NS
Diet and insulin (n)	10	13	NS
Fasting blood glucose (mg/dl)	236.7 ± 105.5	232.8 ± 103.4	NS
HbA1c (%)	10.4 ± 2.1	10.8 ± 2.3	NS
Retinopathy (n)	29	30	NS
Nephropathy (n)	28	30	NS
Neuropathy (n)	30	30	NS
CHD (n)	7	13	0.085
Smoking history (packs/ year)	22.53 ± 28.52	19.83 ± 19.23	NS
Age of foot ulcer (day)	69.83 ± 69.16	68.67 ± 35.46	NS
Osteomyelitis (n)	16	16	NS
Wagner (stage)	3.40 ± 0.89	3.40 ± 0.89	NS

OAD. oral antidiabetic drug; M. Male; F. Female; CHD. Coronary heart disease, NS-nonsignificant

study, and their written informed consent was taken. The local ethical committee approved the protocol of the study.

The study population (Table 1) consisted of 36 men and 24 women, mean age 61.8 ± 9.1 years (range: 38 to 83 years). Wagner (grade) classification system[11] was applied to classify foot ulcers. Ulcer size, appearance, clinical evidence of infection, ischemia, and neuropathy at presentation were recorded, and patients were followed up until the ulcers healed.

The patients were randomly divided into two study groups. Group I (n=30, 18 male/12 female, age: 60.5 ± 9.1 years) patients were administered iloprost infusion for 10 days , in addition to routine treatment strategies. Group II (n=30, 18 male/12 female, age: 63.1 ± 9.2 years) patients were only treated with our routine therapy and constituted a patient control group. Group III (n=15, 9 male/ 6 female, age: 60.7 ± 8.7 years) included non-diabetic healthy subjects of comparable age with the diabetic study subjects. Group III subjects were only assessed for pretreatment comparison of endothelial function with diabetic subjects characterized by endothelial dysfunction.

Serum biochemistry (fasting blood glucose, lipid profile, BUN, creatinine, uric acid, AST, ALT, ALP, LDH, Na, K, Ca, P) and hematological parameters (CBC, erythrocyte sedimentation rate, high sensitive C-reactive protein (CRP), protein C and S, antithrombin III, von Willebrand factor, prothrombin time, homocysteine and fibrinogen level) before (basal) and following the iloprost treatment at 10^{th} and 30^{th} days were obtained.

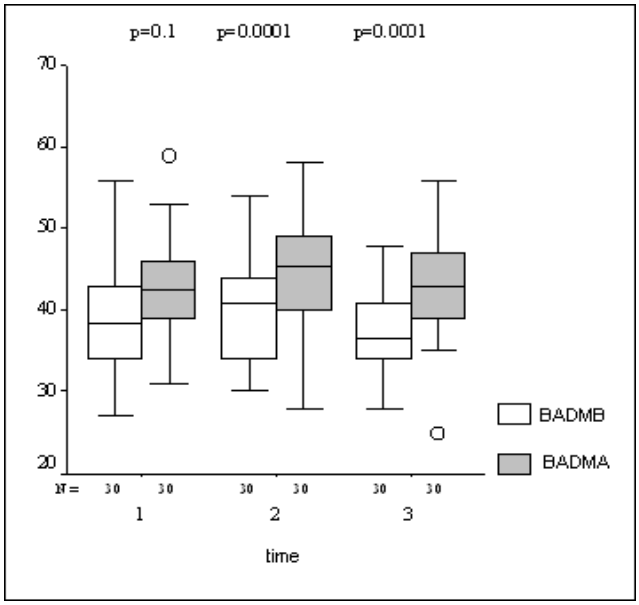

Fig 1: FMD measurements of group I (iloprost treated) patients.
BADMB. Brachial artery diameter measurements (before FMD).
BADMA. Brachial artery diameter measurements (after FMD).

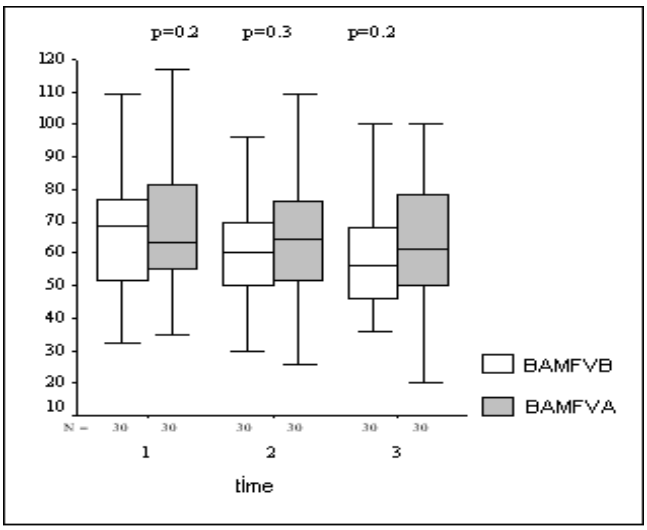

Fig 2: FMD- brachial artery flow velocity of group I (iloprost treated) patients.
BAMFVB. Brachial artery max flow velocty before FMD.
BAMFVA. Brachial artery max flow velocty after FMD**.**

The past medical history, duration and type of diabetes, drugs used for diabetes and concomitant disease, smoking habits, chronic complications of diabetes including neuropathy, nephropathy, retinopathy, coronary heart disease were also evaluated. Following the ilomedin therapy, duration of hospitalization (till healing and discharge), adverse reactions, antibiotic usage, endothelial function (by FMD), performed number and levels of amputation (minor; distal metatarsal and phalanx and major; proximal to distal metatarsal) were followed up and registered.

Table 2: FMD measurements of group I patients: 1st (basal), 2nd (at day 10), and 3rd (at day 30) following the iloprost treatment.

Variables	1st (Basal) measurement	2nd measurement	3rd measurement	*P* 1 vs. 2	*P* 1 vs. 3
Change in the diameter of brachial artery (mm) and % of vasodilatation	-3.0 ± 4.4 (% 7,5)	-3.7 ± 3.2 (% 9)	-6.3 ± 3.1 (% 17)	0.51	0.002
The maximum flow velocity difference of the brachial artery (cm/s)	-0,6 ± 10,8	3,2 ± 9,5	3,6 ± 9,0	0.3	0.2

All patients were initially examined and graded for foot ulcer. Blood and wound cultures, the depth of wound and existence of osteomyelitis by direct X-ray graphs or magnetic resonance imaging (MRI) of the affected limb, Doppler ultrasound and/or magnetic MRI angiography were performed. After these evaluations, appropriate antibiotics, daily wound dressing and debridements, were applied to all patients in addition to adjusted diabetic diets and intensive insulin therapy (four times a day) given.

Iloprost (Ilomedin-20^{R}, Schering), a stable analogue of prostacyclin was administered to the patients with a dose of 0.5 to 2 ng/kg/min over 6 h infusion for 10 consecutive days. Dose range was increased to maximum dosage that the patients could tolerate and continued. Those patients who had septic shock, renal and liver failure, decompensated heart failure, acute or subacute coronary syndromes, active peptic ulcer, acute cerebral hemorrhage, using anticoagulant drug and a known contraindication to iloprost were excluded from the study.

Assessment of endothelial function by Flow Mediated Dilatation (FMD) was performed blindly by the same physician who was an experienced specialist in radiology and Doppler ultrasound systems. Ultrasound system (Acuson- Aspen Mountain View, CA) was equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler imaging. Image resolution was enhanced with a broad band (multiple-frequency: 6 to 11 MHz) linear array transducer.

By using B-mode imaging, baseline brachial artery rest images were obtained just above the antecubital fossa in the longitudinal axis. By magnifying this part of the brachial artery, at first basal brachial artery diameters and maximum flow velocities were obtained. Then, a sphygmomanometry cuff was first placed proximal to the forearm and the cuff was inflated to at least 50 mmHg above systolic pressure to occlude arterial inflow for 5 minutes. After cuff deflation, endothelial induced FMD was determined by measuring the brachial artery diameters.[12] Endothelial derived vasodilatation was assessed quantitatively by comparing the change between the first basal brachial artery diameter and that of the diameter of the brachial artery 60 seconds after the cuff deflation.

Before doppler ultrasound examination subjects were at fasting state for at least 8 h before the study, and they waited for 30 minutes in a quiet, temperature-controlled room before the measurements. All vasoactive medications were withheld. In addition, subjects were not allowed to exercise, ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4 to 6 h before the study.

Statistical analysis

SPSS 14.0 for Windows (Statistical Package for Social Science) software package program was used for statistical analysis. Variables were tested for normality of data distribution by Shapiro Wilks test. In the analysis of continuous variables showing normal distribution, student's *t*-test, one-way ANOVA, paired *t*-test, and ANOVA for Repeated Measures Analyses tests were used. Mann Whitney U test, Kruskall Wallis test and Friedman tests were used for variables showing abnormal distribution. Chi-square test was used for analysis of categorical variables. Values of $P < 0.05$ were regarded as statistically significant. Data are expressed as mean ± SD.

Results

Blood glucose levels of the patients were significantly decreased by intensive insulin in the clinic (Basal vs. end; 236.7 ± 105.5 mg/dl vs. 163.8 ± 36.3 mg/dl; $P = 0.004$).

Serum biochemical and hematological parameters including glucose, BUN, creatinine, serum electrolytes, AST, ALT, apolipoprotein B, Lip(a), WBC, ESR, CRP, homocysteine, fibrinogen, protein C, protein S, vWF and anti-thrombin III levels (data not shown) after therapy were not found to be different as compared to their pretreatment levels (base vs. end; P= 0.110). The hospital stay duration was not different between group I and group II (mean 48.1 ± 22.8 days vs. 54.6 ± 34.6 days; P=0.390).

Basal FMD measurements of 60 diabetic patients and their comparison with that of group III (controls) were significantly different (for the brachial artery diameter: 7.5% vs. 14.9%, p=0.02, and for max flow velocity (cm/s), -0.6 ± 10.8 vs. 7.0 ± 7.2, P= 0.03). The brachial artery FMD measurements of pre and post iloprost administration and their comparisons are shown in Table 2, and Figures 1and2. As can be seen from Table 2 the brachial artery diameter changes in response to vasodilation increased significantly (p=0.002) on the 30th day of measurements as compared with baseline examination in patients who underwent therapy with iloprost.

Amputation rates in group I patients (iloprost treated) were found to be as follows; 12 minor amputations, 13 major amputations, and 5 patients healed without amputation.

Amputation rates in group II were as follows; 12 minor amputations, 17 major amputations, and 1 patient healed without amputation. There was no statistically significant difference between the two groups in amputation rates (P = 0.097).

During the study period, iloprost was well tolerated. Adverse reactions due to iloprost were seen in 3 patients with maculo-papular skin lesions, itching, dyspnea, tachycardia, headache, and hypotension. One of these three patients had completed the iloprost therapy, but in the remaining two patients, the therapy had to be discontinued with their consent. Thirty patients completed the study. The other usual side effects of prostaglandins - such as headache, flushes, dry mouth, burning sensation, tingling sensation, tiredness, nausea and painful irritation of the infused vein were either reversible or could be made tolerable by reducing the flow rate of the infusion.

Discussion

In recent years, iloprost has been used in certain clinical conditions, especially peripheral occlusive arterial diseases such as Buerger'disease and atherosclerosis in diabetic or non diabetic patients and efficiency of iloprost infusion (from 3 weeks to 8 weeks) on the clinical outcomes were found to be effective in the control of ischemic pain symptoms, analgesic usage, reduction of trophic lesions, walking distance, and in the long-term (at first year) amputation rate.[13-19] There are not enough controlled studies on the effects of iloprost therapy on the treatment of diabetic foot ulcers. Furthermore, the main problem related with the reported studies are difficulties in making comparison between the studies because of different study designs including differences in iloprost infusion rates and duration, patients' profile and metabolic control parameters.

Iloprost is currently administered through intravenous infusion for 6h/day for 28 days at a maximal dose of 2 ng/kg/min. Recent studies have shown that iloprost can be safe and effective in patients with critical limb ischemia when administered with a shorter schedule of treatment (for 7 days at a maximal dose of 1.5 ng/kg/ min).[19,20] In this study considering the treatment cost, we used iloprost for 10 days arbitrarily.

We found that ten days of iloprost infusion therapy improved the endothelial function significantly at 10^{th} and 30^{th} days following the therapy in patients with diabetic foot ulcers affected by critical limb ischemia. As is generally accepted, an increase in the diameter of brachial artery by FMD method equal to or more than 10% shows normal persistent endothelial functions.[12] Endothelial dysfunction is a common finding complicated with atherosclerosis among diabetic patients. At baseline prior to iloprost therapy state, we showed the presence of endothelial dysfunction by FMD method in our diabetic patients as compared with nondiabetic control subjects (7.5% vs. 14.9%, p=0.02). Following ten days of iloprost therapy, FMD measurements in group I patients showed a 9% increase in the diameter of brachial artery, and interestingly FMD measurements obtained on the 30^{th} day (17%) were better than that of the 10^{th} day. These findings show that a 10-day iloprost infusion can improve the endothelial dysfunction persisting until 30 days after discontuning the iloprost infusion among diabetic patients with foot ulcers. Consistent with our findings is a similar long-term effect persisting 6 or 12 months following the iloprost infusion that were reported in certain studies.[15,17] However, we had to follow our patients over 30 days because we had to make a decision on the wound status and amputation needs of the patients. In this regard, we had enough time to see the progress of wound healing. However, most of the studies on iloprost therapy reported in the literature were different from our study by design, since they generally used iloprost in patients with peripheral arterial occlusive disease with ischemic symptoms such as pain, claudication in a long time-period. However, all of our study patients were in partial clinical emergency status because their Wagner wound classification grade was advanced (>3), and wounds were complicated with infection and local tissue gangrene.

The primary endpoint of this study was to see the efficiency of iloprost on the amputation rate among the patients with diabetic foot. Of 30 patients in group I, 12 had minor amputations, 13 had major amputations. In group II, 12 had minor amputations, 17 had major amputations. Although the clinical observation regarding the effects of iloprost infusion on the ischemic symptoms of the patients and wound healing was positive, the amputation rate was not statistically different between group I and group II patients (p=0.097).

Several factors could have affected the outcome of the present study, specially the amputation rate. Nearly all the patients were referred from other hospitals, as we are a reference centre, so our patients were usually septic and had late presentations after initiation of the wounds (mean admittance to our clinic was approximately 70 days). Many of the patients had smoking habits, the older age of the patients, and the complicating infections of the wounds were contributing factors that influenced the amputation rates. Studies restricted to evaluate the ilomedin efficiency in diabetic patients with foot ulcers without smoking history and wound infection (dry ischemia) should be addressed. Therefore, we consider these factors as responsible factors for amputation rate: 1) high grade (>3) Wagner stage, 2) suppurative bacterial infections complicating the wound healing and 3) ten days of iloprost infusions may not be long enough to improve the amputation rates.

During the study period, consistent with the reported studies, iloprost was well tolerated by our patients, we only encountered three patients with adverse reactions including maculo-papular skin lesions, itching, dyspnea, tachycardia, headache, and hypotension. One of the three patients completed the iloprost therapy, but in two patients the treatment had to be discontinued. The other usual side effects of prostaglandins - such as headaches, flushes, dry mouth, burning sensation, tingling sensation, tiredness, nausea and painful irritation of the infused vein were either reversible or could be made tolerable by reducing the flow rate of the infusion.

Regarding measurements of serum biochemical and hematological parameters, we did not find any significant differences between these values before and after iloprost therapy, but blood glucose levels were significantly decreased and were controlled better than that of the basal measurements. This finding was a result of our intensive insulin therapy during the study, because all patients had been on conventional insulin or OAD therapy before the study for their glucose control. We also did not find any difference in the duration of hospitalization period between group I and group II patients.

Limitations of the study

Several limitations, which could possibly explain the failure of the 10-day iloprost infusion to reduce amputation rate, despite improvement of endothelial function in our patients with diabetic foot ulcers should be mentioned:

1) The short period of treatment with iloprost, which was not enough to achieve the goal of reduction in amputation rate; 2) Grade of the disease was higher and infection was more severe compared to other studies. 3) The FMD method may not be the appropriate technique to predict positive outcomes of treatment with iloprost.

Conclusion

The results of this study suggest that 10-day treatment with iloprost improved the endothelial functions with persistence of the effect up to one month after treatment in patients with diabetic foot ulcers complicated with macroangiopathy. However, iloprost infusion did not prevent amputation in our patients. Further studies should be addressed to study longer treatment periods with iloprost in cases with early presentation of disease.

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