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The effect of insulin and sulodexide (Vessel Due F) on diabetic foot syndrome Pilot study in elderly patients

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Abstract

To assess the efficacy of insulin plus sulodexide (a mixture of 80% heparin-like substances and 20% dermatan sulphate) on diabetic ulcers, and its influence on foot skin microcirculation and diabetic neuropathy. Two groups of diabetic patients, suffering from severe neuropathy and ulceration, were randomly assigned to insulin (I) plus sulodexide (S) ($n=12$) or insulin plus placebo (P) ($n=6$) therapy, for 10 weeks. Laser Doppler assessment of foot skin flow (LDF), at rest and 30 or 60 s after arterial occlusion, and nerve conduction tests (sensorial evoked and motoric conduction potentials) have been evaluated in both groups. Postischaemic flow was 2.5 times shorter in ulcerated vs. non-ulcerated feet in diabetic patients. A significant increase in flows after 30 and 60 s ischaemia was detected in both groups at the end of therapy (IS group, ulcerated foot, LDF = 60 s: from 99.1 ± 14.3 to 218.6 ± 28.6 PU, $P<.001$. IP group = from 110.5 ± 13.0 to 164.8 ± 15.4 PU, $P<.05$). The length of reactive hyperaemia was higher in IS vs. IP group (IS: from 30.3 ± 2.9 to 43.9 ± 2.2 s, $P<.001$; IP: from 28.7 ± 3.0 to 33.3 ± 3.3 s, ns). Ninety-two percent of ulcers heals in a mean time of 46.4 days (IS group) vs. 83% and 63.0 days, respectively, in IP group. Nerve conduction studies have not demonstrated within- and between-group differences. Sulodexide and insulin improve the postischaemic skin flow in ulcerated feet, without affecting nerve conduction tests. The effect of sulodexide results additive to insulin; it is clinically relevant, in the view of the possibility of reducing the time needed to completely heal ulcers. The ultimate validation of these preliminary results requires extensive trials. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Diabetic foot syndrome; Peripheral diabetic neuropathy; Glycosaminoglycans

Diabetes is the leading cause of lower-extremity amputation, particularly in the elderly: 50% of diabetics develop foot ulcers in late stages of their lifetime (MMWR, 1998; Sanders, 1994) and one every five patients with foot ulcer result in amputation. In the Medicare population (Minnesota, 1993–1995) one-half of diabetes-related amputation occurs in older people (MMWR, 1998), as well as in European population (Trautner, Haastert, Giani, & Berger, 1996).

The estimated cost of diabetic foot complications [peripheral occlusive arterial disease (POAD), peripheral neuropathy, foot ulcer, leg amputation] accounts for over 30% of total cost of diabetes management, particularly in the inpatient setting (Reiber, Lipsky, & Gibbons, 1998).

It is well ascertained that preventive measures (Boulton, Meneses, & Ennis, 1999) and adequate podiatric medical care (Sowell, Mangel, Kilczewski, & Normington, 1999) decrease the amputation rate to one-half.

Prevention is the "key to treatment" (Sanders, 1994), and it is based on risk factor detection and correction, foot

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protection against external forces (Boulton, 1998), standard treatment of foot ulcers (Margolis, Kantor, & Berlin, 1999). However, the clinical course of foot ulcer depends upon metabolic control of diabetes (Shaw & Boulton, 1997) and on POAD and neuropathy evolution, usually leading to inadequate self-regulation of the capillary network (Arora et al., 1998; Cameron & Cotter, 1997).

The aim of this study is to assess if sulodexide (Vessel Due F), an antithrombotic drug with a proved therapeutic action on POAD (Gaddi, Galetti, Illuminati, & Nascetti, 1996), is effective in foot ulcer healing. The rationale for testing sulodexide is also based on recent data on its effect on glycosaminoglycans turnover within the vessel wall (Gambaro, Skrha, & Ceriello, 1998) and on plasma viscosity (Lunetta & Salanitri, 1992).

1. Research design and methods

1.1. Patients

Eighteen inpatients with persisting diabetic foot syndrome and monolateral foot ulcers were recruited (Table 1). Patients with very poor controlled diabetes (often not compliant to diet and drug), with genetic hyperlipoproteinemias, systemic diseases and malignancies, with initial gangrene of foot, were excluded from the study.

1.2. Study design and treatments

This pilot study was designed by a double blind protocol which required, after a 5-day run-in phase for insulin (I) dose adjustment, that patients be randomly allocated in a 2 to 1 fashion to sulodexide (S) or placebo (P) administration. Sulodexide (Vessel Due F, Alfa Wassermann, Bologna, Italy) group (IS, $n = 12$) received 600 LRV (one vial) daily for 15 days, followed by 250 LSU (1 caps) bid for 2 months. Placebo group (IP, $n = 6$) received identical vials and capsules. All patients complied with a sugar-free, fibre-rich, isocaloric diet (carbohydrates 55%, protein 22%, and lipid 28% of total calories) (American Diabetes Association, 1992). Ulcer treatment consisted in weight alleviation, targeted antibiotics, and daily local dressings. Diet and local treatments were identical in both study groups. No other drugs were administered during the whole study period. Patients gave informed consent to participate to the study. Lab and instrumental tests were performed before sulodexide therapy and at the end of the study. A questionnaire for diet and drug adherence was also collected at the end of the study.

Table 1
Case report characteristics

	IS group	IP group
Sample size (n)	12	6
Diabetes Type 1 (n, %)	2 (16.6)	2 (33.3)
Diabetes Type 2 (n, %)	10 (83.4)	4 (66.7)
Males	8 (66.7)	3 (50.0)
Females	4 (33.3)	3 (50.0)
Age (x, S.D.)	52.6 (8.5)	57.2 (11.1)
Time from onset of diabetes (x, S.D.)	16.2 (9.9)	13.3 (11.3)
BMI (x, S.D.)	29.5 (5.3)	28.2 (6.5)
GHb (x, S.D.)	8.5 (1.8)	6.9 (1.3)
Retinopathy (n, %)	9 (75.0)	4 (66.7)
Proliferative retinopathy (n, %)	3 (25.0)	2 (33.3)
Nephropathy (n, %)	9 (75.0)	2 (33.3)
Neuropathy (n, %)	12 (100.0)	6 (100.0)
Smoking habits (n, %)	4 (33.3)	4 (66.7)
Hypertension (n, %)	5 (41.2)	4 (66.7)
Hyperlipoproteinemia (n, %)	5 (41.2)	5 (83.3)

IS = Insulin plus sulodexide group; IP = insulin plus placebo group. Values are given as average (x) and standard deviation (S.D.).

1.3. Capillary flow assessment

Skin capillary flow at rest and after transitory artery occlusion was evaluated to detect alterations in capillary self-regulation properties (Fagrell, 1995).

Following 30 min resting at room temperature (21°C) and 5 min in clinostatic position, the flow was measured by a 401 Angled probe electrode (Periflux 4001, Master Laser Doppler flow-meter). The skin electrodes stabilisation and the evaluation of flow changes during reactive were performed by Perisoft software (Perimed); flows were expressed in arbitrary units (PU); data were standardised by subtraction of zero values, i.e. values during complete occlusion of artery inflow (Ludwig, Caspary, & Creutzig, 1996; Tooke, Ostergren, & Fagrell, 1983; Walmsley & Wiles, 1990).

Rest flow was assessed in six points on the dorsal foot surfaces (three distal and three proximal) both in ulcerated and non-ulcerated feet; results are presented as the mean value of rest flow (rLDF) out of the six points.

Post-occlusion flows were measured by electrodes allocated on foot dorsal surface, proximally, between first and second metatarsal, 5 min after rest flow continuous registration: a tourniquet was been pressurised up to 220–240 mm Hg for 30 s, and "zero flow" values were registered. After occlusion, peak hyperaemic flow (pLDF, in PU), its time of onset [tpLDF, in seconds (s)], hyperaemia duration (HD, s) were measured. The percentage of blood flow change (% LDF) was calculated as (pLDF − rLDF)/rLDF. Ten minutes later, after flow stabilisation, a 60-s occlusion was similarly performed.

1.4. Neuropathy assessment

Nerve conduction tests were performed by stimulating/registering surface electrodes, by a Shapire 2 ME equipment (Medelec, UK). Sensory evoked potentials (SEP) were recorded with ring electrodes by orthodrome method, measuring sensory threshold, sensory evoked response amplitude, response latency, and conduction velocity

(Ohgaki et al., 1998) on the right ulna, right median, and both sural nerves.

Motoric potentials (MP) were registered on right ulna, right median, right fibular, and left tibial nerves, by assessing motor evoked response amplitude, distal response latency, F-wave, and conduction velocity (Ohgaki et al., 1998). For all tests, skin temperature was monitored and maintained in the 31–33°C range.

1.5. Statistics

Because of the not normal distribution of variables and the small number of patients, nonparametric tests were used. Comparison across ulcerated with non-ulcerated feet and between-groups analysis was based on Mann–Whitney *U* test. Within-groups comparison (initial and endpoint results of lab tests performed on each patient) was performed by Friedman test.

2. Results

2.1. Homogeneity checks, compliance, and side effects

The baseline values of age, time of onset of diabetes, BMI, and GHb are quite similar (Table 1). The two groups are also matching for frequency of diabetes complications ($\chi^2 = 1.07$, $P > .1$) and of risk factor frequency (smoking, hypertension, and hyperlipoproteinemias; ($\chi^2 = 0.074$, $P > .1$).

All patients regularly took sulodexide or placebo and insulin and diet. No complaints or adverse events were reported at the end of the study.

2.2. Microcirculatory flow at rest and after reactive hyperaemia

Mean values of rLDF are similar in non-ulcerated and ulcerated feet (pooled values, respectively, 12,4 vs. 12.1 PU, $P > .1$ at $t = 0$ and 12.2 vs. 12.8 PU, $P > .1$, at $t = +8$; group's values: see Table 2). No changes of rLDF are observed in IP or IS groups at time +8 weeks.

Maximal flow after 30 s occlusion (pLDF) is five times higher than rLDF in ulcerated feet (14 in non-ulcerated). The pLDF (Table 2) values increased significantly in both groups, since HD values increase were significant ($P < .05$) only in the IS group (Fig. 1).

In the IS group, the pLDF and HD values at the endpoint (pLDF = 170 PU, HD = 31 s) are similar to the values registered in non-ulcerated feet (pLDF = 159–173 PU, HD = 31–34 s). A 60-s ischaemia causes a greater hyperaemic reaction, particularly in non-ulcerated feet: 18 times higher than at rest values and 1.4 vs. 30 s occlusion. (8.7 and 1.7, respectively, for ulcerated feet). A marked increase of both pLDF (Table 2) and HD (Fig. 1) has been detected in IS group (within-group statistic: pLDF +120%, $P < .001$; HD +43%, $P < .01$; between-groups: pLDF +33%, $P < .05$; HD +26%, $P < .05$). The pLDF final values are 218.6 in the IS group. Insulin alone seems to be insufficient to improve the non-ulcerated feet values, the HD values (Fig. 1) and pLDF (165 PU at the end of the study vs. 213–227 PU).

2.3. Ulcer healing rate

In the IS group, 92% of foot ulcers present a complete restitutio ad integrum, with a mean time for ulcer disappearance of 46.4 (±5.2) days. In the IP group, 83% of

Table 2
Skin microcirculatory flows at rest and after 30 or 60 s ischaemia, in ulcerated and non-ulcerated feet, in IS and IP groups

	$t=0$					$t=+8$						P value between	
	n	mean	S.E.M.	min	max	mean	S.E.M.	min	max	Δ%	P value within	$t=0$	$t=8$
Rest flow													
Ulcerated foot, IS group	12	12.3	0.9	7.7	16.1	12.1	0.7	7.7	15.9	−2.4	ns	ns	ns
Ulcerated foot, IP group	6	11.6	1.3	7.6	15.2	12.3	1.1	9.3	16.2	6.3	ns		
Non-ulcerated foot, IS group	12	12.4	1.0	7.4	17.2	12.8	0.9	7.6	17.6	3.4	ns	ns	ns
Non-ulcerated foot, IP group	6	12.6	0.9	9.6	15.1	13.0	1.0	9.6	15.9	3.2	ns		
Reactive hyperaemia 30 s													
Ulcerated foot, IS group	12	77.1	23.1	15	170	170.1	18.9	25	268	120.6	<.001	ns	ns
Ulcerated foot, IP group	6	51.7	15.2	25	111	147.0	16.2	89	197	184.5	<.01		
Non-ulcerated foot, IS group	12	159.2	22.3	58	244	173.9	15.7	74	255	9.2	ns	ns	ns
Non-ulcerated foot, IP group	6	170.3	15.5	128	196	164.1	14.6	127	212	−3.7	ns		
Reactive hyperaemia 60 s													
Ulcerated foot, IS group	12	99.1	14.3	52	186	218.6	28.6	52	401	120.5	<.001	ns	<.05
Ulcerated foot, IP group	6	110.5	13.0	74	153	164.8	15.4	111	212	49.1	<.01		
Non-ulcerated foot, IS group	12	226.0	15.2	116	293	226.9	13.1	136	274	0.4	ns	ns	ns
Non-ulcerated foot, IP group	6	225.5	18.9	174	283	213.5	21.0	149	274	−5.3	ns		

Probability values in the last column (between-group statistics) refer to comparison at the baseline ($t = 0$, left) and at the end of the study (right).

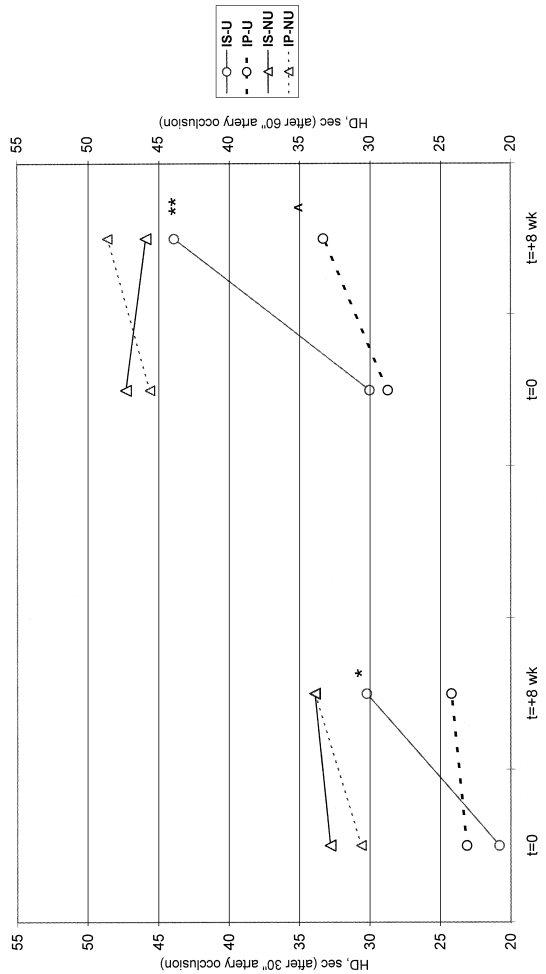

Fig. 1. Duration of reactive hyperaemia (HD) after arterial occlusion for 30 s (right) or 60 s (left) in diabetics with (U) and without (NU) foot ulcers, before and after therapy. $*P<.05$, within-group; $**P<.01$ within-group; $\wedge P<.05$ between-groups.

ulcers heal in a mean time of 63 (±8.5) days (95 IC for difference: −36.7+3.47, $t=1.75$, $df=16$, $P=.09$).

2.4. SEP and motoric conduction tests

Electrophysiological tests confirm the severity of neuropathy at the beginning of the study, with a marked involvement of both SEP and MP in all the districts investigated, without differences between the two groups. Despite the large number of variable investigated, no statistically significant (or clinically relevant) effects were found at the end of therapy, both in IP and IS groups (Table 3).

2.5. Conclusions

Sulodexide was extensively investigated in diabetic nephropathy (established effect on macro- and microalbuminuria) (Solini, Carraro, Barzon, & Crepaldi, 1994) and in POAD (claudication improvement) (Crepaldi et al., 1990; Gaddi et al., 1996). Present data support a favourable effect on skin microcirculation in diabetic foot syndrome. In fact:

(a) In ulcerated feet, postocclusive (30 s) hyperaemia increases significantly in IS and IP groups; after 60 s ischaemia, pLDF and HD are significantly higher ($P=.013$) in the IS group than in insulin alone;
(b) The outcome of ulcers is very favourable in both groups;
(c) Sulodexide does not affect nerve conduction tests, and rest flow, pLDF and HD in non-ulcerated feet.

The absence of differences between ulcerated and healed feet suggests that rest flow is not a useful parameter in diabetic foot syndrome: literature results are not consistent

Table 3
Baseline and endpoint results of nerve conduction test in IP and IS groups

Response parameter	IP group $t=0$	IP group $t=+8$	IS group $t=0$	IS group $t=+8$	Reference values
Sensory amplitude of right median nerve (μV)	6.5	6.6	6.7	6.7	>16.0
Conduction velocity of right median nerve (m/s)	36.2	37.0	37.8	38.0	>49.8
Sensory amplitude of right ulna nerve (μV)	3.6	3.8	4.6	4.8	>15.0
Conduction velocity of right ulna nerve (m/s)	33.5	34.5	23.3	24.5	>44.5
Sensory amplitude of right sural nerve (μV)	0.37	0.46	0.39	0.44	>16.0
Conduction velocity of right sural nerve (m/s)	3.1	3.6	3.1	3.4	>40.0
Sensory amplitude of left sural nerve (μV)	0.40	0.50	0.48	0.55	>16.0
Conduction velocity of left sural nerve (m/s)	23.0	22.0	23.0	23.0	>40.0
Motoric amplitude of right median nerve (mV)	6.7	6.6	5.7	5.6	>5.0
Conduction velocity of right median nerve (m/s)	45.1	45	45.1	46.0	>49.6
Motoric amplitude of right ulna nerve (mV)	4.3	4.2	4.3	4.8	>5.0
Conduction velocity of right ulna nerve (motoric) (m/s)	47.8	48.2	47.8	48.2	>51.0
Motoric amplitude of right tibial nerve (mV)	1.9	1.2	0.95	1.0	>5.0
Conduction velocity of right tibial nerve (m/s)	23.5	24.7	25.5	26.7	>43.0
Motoric amplitude of right fibular nerve (mV)	1.8	1.2	0.80	1.0	>4.0
Conduction velocity of right fibular nerve (m/s)	26.0	25.2	27.7	28.0	>44.0

No significant variations were found in either within- or between-group statistical analysis.

for lower rLDF values in ulcerated feet, since the usefulness of pLDF in monitoring diabetic foot syndrome has been confirmed (Arora et al., 1998; Rayman, Malik, Sharma, & Day, 1995; Tooke et al., 1983).

Pharmacological improvement of foot microcirculation has been attempted with several drugs, as adrenergic or calcium-channel blockers, ACE-inhibitors, endothelin antagonists, nitrates, gamma-linolenic acid, pentoxifylline, with contrasting results (Cameron & Cotter, 1993, 1997; Keen, 1993). The rationale of these studies is that the microcirculation alteration of type 2 diabetes is also a pathogenic factor for diabetic neuropathy (Tesfaye, Malik, & Ward, 1994) and vice versa.

However, our results have demonstrated an outstanding effect of insulin and sulodexide on postischaemic flow, without functional improvement of neuropathy, despite the high sensibility of nerve conduction tests (Ohgaki et al., 1998; Walmsley & Wiles, 1990). In our opinion, this finding is explained by the action of sulodexide on blood rheological properties, thus removing mainly the "vascular component" of ulcers.

In diabetic foot syndrome, sulodexide can improve the beneficial effect of diabetes control (Diabetes Control and Complication Trial Research Group, 1993) by lowering plasma fibrinogen (Gaddi et al., 1996; Harenberg, 1998) and blood viscosity (Lunetta & Salanitri, 1992), and by direct action on endothelial cells, fibrinolysis (Gambaro et al., 1998) and on microthrombosis at postcapillary level (Corbu, Predoi, & Goicea, 1996). Furthermore, sulodexide promotes VLDL-triglycerides hydrolysis due to endothelial lipoprotein lipase activation (Harenberg, 1998) in hypertriglyceridemias and mixed hyperlipoproteinemias, often associated with type 1 and type 2 diabetes.

Recently, some authors have pointed out that the presence of undersulfated glycosaminoglycan chains has a pivotal role in the pathogenesis of both proteinuria (for reduction of anionic charges in the glomerular basal membrane) and of micro and macroangiopathy (Gambaro & Van der Woude, 2000). Moreover, in the Steno hypothesis (Deckert, Feldt-Rasmussen, Borch-Johnsen, & Kofoed-Enevoldsen, 1989), a primitive dysregulation of glycosaminoglycan production by several cell types (endothelial, mesengial, etc.) can lead both to development of angiopathy and proteinuria. This justifies a possible therapeutic effect of glycosaminoglycans on both diabetic neuropathy and angiopathy, also based on restoration of anionic charge in glomerules, on down-regulation of proteases, and on the restoration of synthesis of mesengial matrix (Gambaro & Van der Woude, 2000). However, apart from all the "possible" action mechanisms of sulodexide, our preliminary study supports the idea that attempts to improve microcirculation by drugs are feasible and advisable, despite some discouraging results of the past.

Diabetes management is a worldwide problem. The frequency of major diabetes complication is very high (coronary heart disease, POAD, neuropathy, nephropathy) and they are often combined in the same patient. Several recent guidelines point out that preventive measure should be always associated to therapy aimed at lowering blood glucose and glycation processes (Boulton, 1998; Reiber et al., 1998; Sanders, 1994; Trautner et al., 1996). From this point of view, present results suggest that sulodexide, active on diabetic nephropathy and on POAD, has an additive effect to diabetes control by insulin and by diet, and may represent a first choice "insulin complement" in diabetic foot syndrome therapy.

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