



The Effect of α -Lipoic Acid on the Neurovascular Reflex Arc in Patients with Diabetic Neuropathy Assessed by Capillary Microscopy

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Patients with diabetic polyneuropathy are known to have an impaired neurovascular reflex arc compared to healthy controls. This is seen in a delayed decrease in microcirculation of the ipsilateral hand after cooling of the contralateral hand. The aim of this pilot study was to investigate whether intravenous α -lipoic acid (ALA) (Thioctacid, Asta Medica) therapy might be able to improve this impaired neurovascular reflex arc in patients with diabetic neuropathy. In addition, clinical effects were evaluated with the aid of the neuropathy symptom score (NSS) and the neuropathy disability score (NDS). Ten patients with diabetes mellitus and polyneuropathy (5 females, 5 males, 2 smokers, 5 IDDM, 5 NIDDM, body mass index $26.1 \pm 1.0 \text{ kg/m}^2$, age 58.3 ± 9.5 years, diabetes duration 15.7 ± 11.2 years, Hb A_{1c} $6.8 \pm 0.3\%$) were investigated by nail-fold capillaroscopy after contralateral cooling before and after intravenous therapy with 600 mg α -lipoic acid per day over 3 weeks. Cardiac autonomic neuropathy was excluded by beat-to-beat variation analysis. Symptoms of diabetic neuropathy were evaluated before and after therapy with the aid of the NSS and NDS. Capillary blood cell velocity (CBV) of the hand was determined before, during, and for the following 30 min after cooling (3 min at 15°C) of the contralateral hand. Blood pressure, heart rate, and local skin temperature were monitored at 2-min intervals. ALA therapy

resulted in a significant improvement of the microcirculatory response to cooling, as seen by an immediate decrease in CBV of 12.3% ($P < 0.02$ vs before treatment), which was absent before therapy. Blood pressure, heart rate, and local skin temperature were not different between investigations. There was a significant improvement of the NSS after therapy (5.4 ± 1.1 vs 8.6 ± 1.1 points, $P < 0.01$). These results demonstrate that intravenous therapy with ALA has a positive influence on the impaired neurovascular reflex arc in patients with diabetic neuropathy. © 1999 Academic Press

Key Words: α -lipoic acid; capillary blood cell velocity; contralateral cooling test; diabetic neuropathy; nail-fold capillaroscopy.

INTRODUCTION

Diabetic neuropathy is a common complication in patients with diabetes mellitus. The prevalence of diabetic neuropathy is influenced by diabetes duration, age of the patients, and metabolic control (DCCT Research Group, 1993; Young *et al.*, 1993).

Until now there have been a variety of open questions with respect to the exact pathogenesis of diabetic neuropathy. While it is undoubted that chronic hyper-

glycemia is the initial cause of diabetic neuropathy, it is speculated that metabolic or microvascular disorders or a combination of both play the major role in the further development (Greene *et al.*, 1992; Luft, 1996). Several research groups have pointed out that oxidative stress plays an important part in the pathogenesis of diabetic neuropathy (Packer, 1993). It has been shown that elevated free oxygen radicals affect nerve function. Thus, previous studies demonstrated that a reduced oxygen free radical scavenging activity occurs in patients with diabetes mellitus compared to healthy controls (Cameron *et al.*, 1994; Packer, 1993). ALA acts as an antioxidant increasing the oxygen free radical scavenging activity in patients with diabetic neuropathy and it is speculated that nerve dysfunction is improved by this effect (Nagamatsu *et al.*, 1995; Packer, 1993).

A- δ -fibers and C-fibers are responsible for the conduction of thermal perception. A- δ -fibers are myelinated and connected to cold receptors. The nerve bundles are crossing in the spinal cord to the contralateral side and run within the tractus spinothalamicus to the thalamus and finally end in the cerebral cortex (Ziegler *et al.*, 1988; Zimmermann, 1987).

Previous studies have demonstrated that nerve blood flow is impaired in patients with diabetic neuropathy in comparison to healthy controls (Kohriyama *et al.*, 1995). This might lead to a disturbed nerve function as well. Prior studies of our group demonstrated that in diabetic patients the neurovascular reflex arc is impaired. Thus, contralateral cooling which leads to a rapid decrease in capillary blood cell velocity (CBV) of the ipsilateral side in healthy controls is lacking in patients with diabetic neuropathy (Haak *et al.*, 1998a).

Videocapillaroscopy is a well-established *in vivo* method of investigating the CBV (Bollinger and Fagrell, 1990; Fagrell *et al.*, 1977). In combination with dynamic tests it is also possible to detect distinct changes in microcirculation (Creutzig and Caspary, 1994).

Cooling of contra- or ipsilateral parts of the body in combination with videocapillaroscopy is used as a function test for nerve or microcirculatory disorders (Bartelink *et al.*, 1993; Suichies *et al.*, 1992).

The aim of the present study was to evaluate

whether ALA therapy is able to improve the impaired neurovascular reflex arc that is seen in patients with diabetes mellitus and peripheral polyneuropathy. In addition, we investigated potential beneficial effects of ALA on the neuropathy symptom score (NSS) and the neuropathy disability score (NDS) in patients with diabetic neuropathy.

MATERIALS AND METHODS

Subjects

Ten patients with diabetes mellitus and peripheral diabetic neuropathy were investigated. Five of these patients were females. Two patients were smokers. The body mass index amounted 26.1 ± 1.0 (kg/m²). The mean age was 58.3 ± 9.5 years and the mean diabetes duration was 15.7 ± 11.2 years. Five of the patients were insulin-independent and 5 of them were non-insulin-dependent. The mean hemoglobin A_{1c} was $6.8 \pm 0.3\%$.

Neurological Score

Physical examination including neurological examination was evaluated before and after treatment with α -lipoic acid. The NDS and NSS according to Young were used to evaluate clinical symptoms of diabetic peripheral neuropathy (Young *et al.*, 1993). Peripheral diabetic neuropathy has been diagnosed by moderate signs (6–8 points in the NDS) with or without symptoms or mild signs (3–5 points in the NDS) with moderate symptoms (5–6 points in the NSS). The ankle jerk reflex was tested as well as the vibration perception threshold on the foot (great toe, sole, dorsum, medial malleolus, mid and head of tibia). For the latter we used a 128-Hz scaled tuning fork (Biothesiometer, Bio-Medical Instrument Co., Newbury, OH). A vibration perception threshold value less than 6/8 of the scale was defined as pathologic. The extent of temperature discrimination (identical sites as described before, sole excepted) was quantified with a thermit device (Axon Neuroscreen, small-fiber, Düsseldorf, Germany) which allows us to determine the range of the temper-

ature discrimination by a stepwise reduction of the temperature difference. Temperature discrimination was defined as abnormal if the ability to distinguish warmth from cold required more than 4°C.

Autonomic neuropathy was excluded in all patients by measurement of heart rate variability at rest and during deep breathing (Proscicard Analyzer, Proscicard, Germany).

Study Protocol

After a run-in period of 30 min at rest in an air-conditioned room (temperature 22–24°C), patients were investigated in a sitting position with the hand at heart level. The skin temperature in the observed area was continuously recorded with a digital electronic thermistor (Digimed H 11, ttw, Waldkirch, Germany) that was mounted proximal to the nail fold of the investigated finger and maintained between 27.3 and 30.5°C. Patients had been requested to refrain from smoking and from drinking caffeinated beverages for at least 12 h prior to the investigation. Besides insulin, patients did not take any drugs which might influence capillary perfusion.

Patients were studied before and after they had received 600 mg α -lipoic acid (Thioctacid, ASTA Medica, Germany) intravenously per day over 3 weeks. Skin microcirculation of the fourth finger of the left hand was investigated by nail-fold videocapillaroscopy as described below. Capillary blood cell velocity was monitored before and for the following 30 min after a 3-min cooling of the contralateral hand in a waterbath at 15°C. Blood pressure and heart rate were determined at 2-min intervals during the investigation using an automatic sphygmomanometer (NAIS, Matsushita Germany, Düsseldorf).

Water Bath

For the cooling of the contralateral hand a temperature-regulated water bath was used. A thermostat ensured that potential changes of the water temperature due to the warmth of the patient's hand were less than 1.0°C.

Videocapillaroscopy

Nail-fold capillaries of the finger were visualized on a TV monitor (Panasonic, FT-2900, Hamburg, Germany) by a microscope (Nikon, OPTIPHOT-2, Düsseldorf, Germany) on which a video camera (FK-6990 IQ/S, Pieper GmbH, Düsseldorf, Germany) was mounted. The whole investigation was recorded on video (S-VHS Panasonic AG-7355, Aalen, Germany) for the subsequent analysis of capillary blood cell velocity. Using a 20/0.4 oil objective (Nikon, Düsseldorf, Germany) the overall magnification was 1500-fold. Only capillaries of the last row of the nail fold with a straight arteriolar limb were selected. It was ensured that identical capillaries of each patient were examined.

The CBV was measured with the aid of a computerized analysis system using the videophotometric, temporal cross-correlation technique (CapiFlow, Lorenz Electronics, Sulzbach, Germany). The system calculates the blood cell velocity by the dual-window technique. For that technique the computer generates two photometric windows. The interwindow distance and the sizes of the windows were adjusted to the size of different capillaries. From the interwindow distance and the passage time of blood cells through both windows, the system is able to calculate CBV. The measurements are repeated six times per second and given as mean values of these measurements. Artifacts are recognized by the system from low correlations between the single measurements and eliminated.

Biochemical Parameters

Before the investigation blood samples were taken from a cubital vein. Blood glucose was determined by the hexokinase method on an ACP 5040 autoanalyzer (Eppendorf, Hamburg, Germany). Glycosylated hemoglobin A_{1c} was determined by high-pressure liquid chromatography (DIAMAT, Bio-Rad). Hematocrit and mean platelet volume were measured by an automatic blood count analyzer (S+IV, Fa. Coulter, Krefeld). Total cholesterol and triglycerides were measured enzymatically. Fibrinogen was derived as a side parameter of the determination of thromboplastin time.

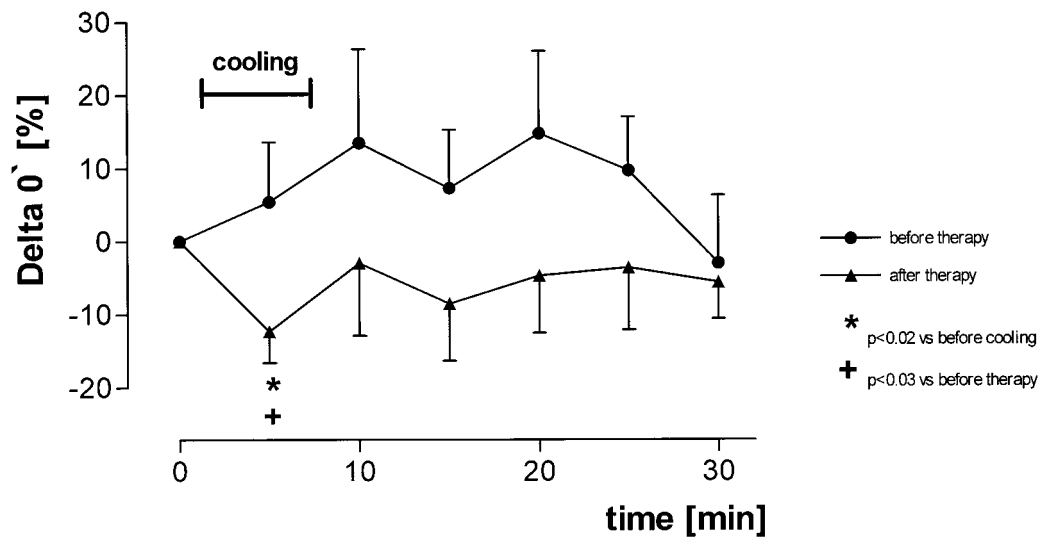


FIG. 1. Changing in capillary blood cell velocity (CBV) in patients with diabetic neuropathy before, during, and after contralateral cooling before and after therapy with α -lipoic acid. Values are given as means \pm SEM.

Statistical Analysis

All data are given as means \pm standard deviation of the mean. The Wilcoxon's signed rank test for paired and unpaired data was used to evaluate statistical significance. A P value less than 0.05 was considered significant.

RESULTS

Before therapy with ALA all patients showed a disturbed neurovascular reflex arc, as seen by an absent reaction in the ipsilateral hand's CBV immediately after contralateral cold exposure (delta0 5.4 \pm 8.2% vs before cooling) (Fig. 1).

After 3 weeks of therapy with ALA the response to the contralateral cooling test was improved, as seen by a significant immediate reduction in CBV after cooling (delta0 -12.3 \pm 4.3%, $P < 0.02$ vs before cooling, $P < 0.03$ vs before therapy) (Fig. 1).

Hemodynamic parameters and local skin temperature remained unchanged throughout the study (Table 1).

Serum triglycerides and fibrinogen levels were elevated above the normal range, but they remained

unchanged by therapy with ALA. There was also no significant difference in hemoglobin A_{1c}, hematocrit, and mean platelet volume before and after therapy with ALA (Table 1). The judgment of the neurological score achieved 6.4 \pm 0.5 points in the NDS and 8.6 \pm 1.1 points in the NSS before therapy.

Concerning the clinical effectiveness, there was a significant reduction in the NSS (5.4 \pm 1.1 vs 8.6 \pm 1.1 points, $P < 0.01$) after therapy with ALA, while the NDS remained unchanged throughout the study.

TABLE 1

Serum Fibrinogen, Triglycerides, Cholesterol, Hemoglobin A_{1c}, Mean Platelet Volume, and Hematocrit in Patients with Diabetic Neuropathy before and after Therapy with α -Lipoic Acid

	Before therapy	After therapy
Systolic blood pressure (mm Hg)	153 \pm 5	142 \pm 4
Diastolic blood pressure (mm Hg)	84 \pm 3	82 \pm 2
Local skin temperature ($^{\circ}$ C)	28 \pm 0.3	28 \pm 0.3
Hemoglobin A _{1c} (%)	6.8 \pm 0.3	6.7 \pm 0.3
Serum fibrinogen (mg/dl)	454 \pm 37	425 \pm 32
Serum cholesterol (mg/dl)	224 \pm 13	226 \pm 15
Serum triglycerides (mg/dl)	249 \pm 58	263 \pm 60
Mean platelet volume (fl)	10.3 \pm 0.3	10.3 \pm 0.3
Hematocrit (%)	41.7 \pm 1.1	41.9 \pm 1.3

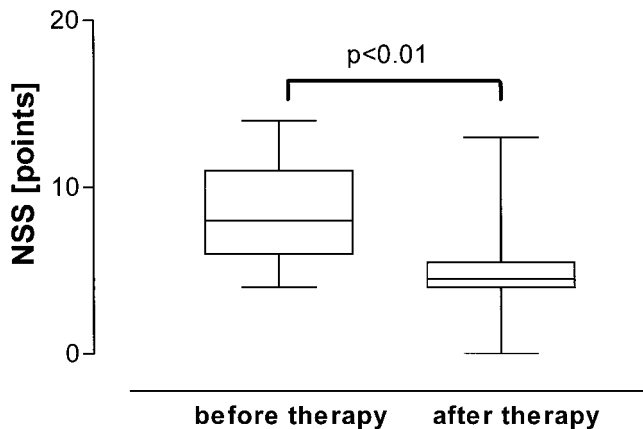


FIG. 2. Neuropathy symptom scores (NSS) in 10 patients with diabetic neuropathy before and after therapy with α -lipoic acid. Box plot values of NSS showing median values and the 10th, 25th, 75th, and 90th percentiles.

DISCUSSION

Previous studies gave evidence that nerve microcirculation is impaired in patients with diabetic neuropathy. This dysfunction in microcirculation in nutritional as well as in thermal capillaries is most likely due to an impairment in nerve blood flow (Haak *et al.*, 1998a; Kohriyama *et al.*, 1995). These pathological findings lead to the introduction of the contralateral cooling test for the diagnosis of diabetic nerve dysfunction. In healthy volunteers contralateral cooling leads to a decrease in CBV of the ipsilateral hand. In contrast, a lack of reaction to contralateral cold exposure in patients with diabetic neuropathy is a sign that the neurovascular reflex arc is impaired in these patients.

Until now, there has been no evidence as to whether the impaired neurovascular reflex arc might be influenced by a therapeutic intervention. The results of the present study showed that indeed, a therapeutic intervention is able to improve this dysfunction in patients with diabetic neuropathy. After intravenous therapy with ALA, contralateral cooling resulted in a significant decrease in CBV, with a rapid restoration of CBV which was absent in these patients before therapy.

The reason for the beneficial effect on nerve function appears to be an improvement in nerve perfusion by treatment with ALA. It has been demonstrated that

small fibers, e.g., A- δ -fibers, are impaired in the early stage of diabetic neuropathy. A- δ -fibers are combined with cold receptors and are responsible for nerve perfusion (Ziegler *et al.*, 1988). Probably ALA therapy is able to restore the function of small fibers, as seen in an amelioration to the cold function test.

It has been indicated that oxygen free radicals are responsible for damage in microvessels and contribute to a reduction of reperfusion (Schmelzer *et al.*, 1989). ALA is a low molecular weight substance which is able to cross the blood-brain barrier (Packer *et al.*, 1997). ALA exerts its action conjointly with α -tocopherol or acts as a metal chelator (Grunert, 1960; Kagan *et al.*, 1992; Sigel and Prijs, 1978; Suzuki *et al.*, 1991). This antioxidant, which has structural similarities to the vitamin biotin, is able to increase free radical defenses (Luft, 1996; Packer, 1993; Zemleni *et al.*, 1997). Therefore, a reduction in free oxidative radicals might contribute to an amelioration of nerve blood flow and lead to improved nerve function. Nagamatsu demonstrated this effect of ALA on nerve blood flow in diabetic rats. A reduced oxidative stress from improving oxygen free radical scavenging activity is suggested to be the mechanism of ALA action in this model (Nagamatsu *et al.*, 1995).

Parameters with effects on hemorheology, such as serum fibrinogen, are known to influence microcirculation as well (Haak *et al.*, 1998b). In our study, serum fibrinogen levels were elevated to the normal range but they did not alter during the study. Thus, in this study, serum fibrinogen levels cannot explain changes in microcirculation.

Furthermore, neither serum triglycerides, nor serum cholesterol, hematocrit, mean platelet volume, or the hemodynamic parameters blood pressure and heart rate were changed throughout the study. Therefore, hemorheology is unlikely to play a role in the effects of ALA on microcirculation.

With regard to its clinical relevance, the present study demonstrated a significant improvement in neuropathic symptoms (NSS), which is in line with the results in the ALADIN study (Ziegler *et al.*, 1995). In this study the total symptom score (TSS) was improved after a 3-week intravenous treatment with 600 and 1200 mg ALA. The higher concentration was not

more effective in ameliorating the TSS but an enhanced rate of adverse side effects was documented.

In the underlying investigation, the neuropathy disability score (NDS) as a second scoring system for neuropathological disorders remained unchanged by treatment with ALA. This is in agreement with the findings of Ziegler and co-workers. They did not find a significant difference in NDS either, although there was a significant amelioration in subjective neurological symptoms, e.g., burning, paresthesia, numbness, and pain, from ALA therapy in the ALADIN study (Ziegler *et al.*, 1995). Probably the duration of the treatment was too short to demonstrate a significant improvement in the NDS. Sachse, who investigated 10 patients with diabetes mellitus with 300 mg ALA orally per day, could not find any amelioration in neuropathic symptoms (Sachse and Willms, 1980). However, the group of patients was small and the dose of treatment was low in comparison to the doses used nowadays.

The effects observed in the NSS and in microcirculation are not based on an improvement in metabolic control because glycemic control was sufficient ($Hb A_{1c} < 7\%$) throughout the study. It was demonstrated that capillary blood cell velocity might also be influenced by the diameter of the capillaries and the skin temperature. In this study it was ensured that the same capillaries in the last row of the nail fold, with equal diameters were investigated before and after therapy with ALA. In addition, local skin temperature was kept within the range of 27.3 and 30.5°C, which is known to have no influence on microcirculation (Jung *et al.*, 1987).

In conclusion, this investigation is the first that demonstrates that ALA is able to improve nerve function, as seen by an improved neurovascular reflex arc in patients with diabetic neuropathy. We presume that ALA reduces free oxygen radicals and by this improves nerve perfusion. In particular, ALA could be a useful therapeutic agent in combating nerve dysfunction in patients with diabetic neuropathy. Further long-term studies with a larger number of patients are required to confirm these preliminary findings.

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