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Effect of α-lipoic acid on the progression of endothelial cell damage and albuminuria in patients with diabetes mellitus: an exploratory study

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Abstract

Oxidative stress plays a central role in the pathogenesis and progression of late microangiopathic complications (diabetic nephropathy) in diabetes mellitus. Previous studies suggested that treatment of diabetic patients with the antioxidant α-lipoic acid reduce oxidative stress and urinary albumin excretion. In this prospective, open and non-randomized study, the effect of α-lipoic acid on the progression of endothelial cell damage and the course of diabetic nephropathy, as assessed by measurement of plasma thrombomodulin and urinary albumin concentration (UAC), was evaluated in 84 patients with diabetes mellitus over 18 months. Forty-nine patients (34 with Type 1 diabetes, 15 with Type 2 diabetes) had no antioxidant treatment and served as a control group. Thirty-five patients (20 with Type 1 diabetes, 15 with Type 2 diabetes) were treated with 600 mg α-lipoic acid per day. Only patients with an urinary albumin concentration < 200 mg/l were included into the study. After 18 months of follow up, the plasma thrombomodulin level increased from 35.9 ± 9.5 to 39.7 ± 9.9 ng/ml ($P < 0.05$) in the control group. In the α-lipoic acid treated group the plasma thrombomodulin level decreased from 37.5 ± 16.2 to 30.9 ± 14.5 ng/ml ($P < 0.01$). The UAC increased in patients without α-lipoic acid treatment from 21.2 ± 29.5 to 36.9 ± 60.6 ng/l ($P < 0.05$), but was unchanged with α-lipoic acid. It is postulated that the significant decrease in plasma thrombomodulin and failure of UAC to increase observed in the α-lipoic acid treated group is due to antioxidative effects of α-lipoic acid, and if so that oxidative stress plays a central role in the pathogenesis of diabetic nephropathy. Furthermore, progression of the disease might be inhibited by antioxidant drugs. A placebo-controlled study is needed.

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1. Introduction

Physiological and pathophysiological processes lead to the generation of reactive oxygen-species such as free radicals, non-radical oxygen derivates and lipid- and carbohydrate-derived carbonyl-compounds (oxidative stress). Oxidative stress is widely thought to play a crucial role in the pathogenesis and progression of late micro- and macroangiopathic complications in diabetes mellitus [1–13]. Oxidative stress may lead to endothelial cell damage and vascular dysfunction by various different mechanisms [14–36]. Oxidative stress leads to the formation of advanced glycation end products (AGE-proteins) [4,24,25] and activation of the nuclear factor kappa B (NF-κB) [26]. It has been shown recently that oxidative stress is related to the underlying metabolic disorder. It occurs before the manifestation of late diabetic complications become evident. This is consistent with the idea that oxidative stress is an early event in the pathology of diabetes and its complications [37]. Furthermore, it has been demonstrated that AGE-proteins, which can induce oxidative stress by themselves [26,38], occur in early stages of diabetes before complications become evident [39].

Several studies suggested that antioxidant therapy might be beneficial in diabetic patients [40–47]. Hence, it seems likely that a substance shown to reduce oxidative stress dependent NF-κB activation in vivo [7,8,48] will reduce progression of endothelial cell damage and albuminuria in people with diabetes. α-Lipoic acid is an antioxidant known to block oxidative stress in patients with diabetes mellitus irrespective of glycaemic control and the presence of diabetic complications [45,46]. In the present prospective, open, non-randomized pilot study, 84 patients with diabetes mellitus were monitored over a period of 18 months. Increase of plasma thrombomodulin as a marker of endothelial cell damage [6–13], and the progression of UAC, was evaluated in 49 patients without α-lipoic acid treatment and 35 patients treated with 600 mg/day α-lipoic acid.

2. Methods

2.1. Patients

The study was approved by the ethical committee of the Department of Medicine, University of Heidelberg, Germany. Only patients giving their informed consent were included.

A total of 84 Europid patients with Type 1 or Type 2 diabetes and normoalbuminuria or microalbuminuria were included in this prospective open, non-randomized pilot study. Forty-nine patients without α-lipoic acid treatment and 35 patients treated with α-lipoic acid (600 mg/day) were followed for 18 months. Patients were allocated either to the α-lipoic acid treated group or to the control group as they attended the outpatient clinic. Participation in this study did not affect glucose-lowering or antihypertensive therapy. All patients had stable blood glucose control and blood pressure control and had been instructed in following a stable diet. Protein restriction was not prescribed.

Clinical and laboratory data of the patients at entry into the study are shown in Table 1. At study entry the two groups differed significantly ($P < 0.05$) with respect to retinopathy, neuropathy and creatinine (Table 1).

Other drug treatment of the patients showed no notable differences between the groups (Table 3). Diagnosis of diabetic nephropathy was based on the UAC in a morning spot urine (normal 0–20 mg/l; microalbuminuria 21–200 mg/l; macroalbuminuria >200 mg/l). Patients with macroalbuminuria or creatinine >1.4 mg/dl at study entry were excluded, as plasma thrombomodulin is increased with reduced renal function [49].

The diagnosis of diabetic retinopathy was based on the examination by experienced ophthalmologists after dilation of the pupils. Diagnosis of diabetic neuropathy was based on clinical assessment and measurement of vibration perception threshold. Diabetic macroangiopathic complications were diagnosed according to clinical findings, ECG, doppler sonography and/or

angiography. Glycaemic control was determined by periodical measurement of the HbA_{1c}. All patients self-measured blood glucose concentration daily.

Blood pressure was measured after 5 min rest in a sitting position using a standard manometer. Mean blood pressure was calculated as diastolic blood pressure plus 1/3 of the pulse pressure. Patients were classified as hypertensive if diastolic blood pressure was >95 mmHg or systolic blood pressure >140 mmHg on at least two occasions, or if antihypertensive treatment was necessary to achieve these blood pressure levels.

Clinical and laboratory data were collected during the regular clinic visits of the patients, who usually attended the Diabetes Centre four times a year.

Table 1
Baseline data of the 84 patients with diabetes mellitus included into the study and allocated to two groups according to the use of α-lipoic acid[a]

	Control	α-lipoic acid	*P*
(*n*)	49	35	
Age (year)	49 ± 15	54 ± 13	NS
Male/female (*n*)	21/28	20/15	NS
Duration of diabetes (year)	18 ± 11	21 ± 10	NS
Type 1/2 (*n*)	34/15	20/15	NS
Smokers (*n*)	9	8	NS
BMI (kg/m2)	24.7 ± 3.3	25.6 ± 3.0	NS
Nephropathy (*n*)	14	11	NS
Retinopathy (*n*)	17	22	0.01
Neuropathy (*n*)	7	35	<0.005
Macrovascular complications (*n*)	6	7	NS
HbA1c (%)	8.4 ± 1.5	8.4 ± 1.1	NS
Oral glucose lowering (*n*)	9	7	NS
Insulin treatment (*n*)	47	31	NS
Conventional (*n*)	20	12	NS
Intensive (*n*)	27	19	NS
Anti-hypertensive drugs (*n*)	15	12	NS
Albuminuria (mg/dl)	21 ± 30	29 ± 43	NS
Thrombomodulin (ng/ml)	36 ± 10	38 ± 16	NS
HDL cholesterol (mg/dl)	55 ± 15	54 ± 15	NS
Systolic BP (mmHg)	132 ± 19	126 ± 16	NS
Diastolic BP (mmHg)	76 ± 9	77 ± 8	NS
Mean BP (mmHg)	96 ± 13	93 ± 9	NS
Creatinine (mg/dl)	0.82 ± 0.16	0.92 ± 0.2	0.02

[a] Mean ± SD.

2.2. Measurements

For plasma thrombomodulin determination, plasma was prepared from blood samples using a standard protocol. The samples were stored at −80°C for not more than 1 month. Repeated analyses showed stable thrombomodulin levels over 6 months at −80°C. Plasma thrombomodulin was determined by an immunoenzymatic assay (Asserachrom Thrombomodulin, Diagnostica Stago, Asnières-sur-Seine, France) [50,51], normal range in our laboratory 20–30 ng/ml. Intra- and inter-assay CV's were 6 and 16%, respectively [7–9,45,46].

HbA_{1c} was measured by high performance liquid chromatography (DiamatTM, Bio-Rad, München, Germany), top end of normal range: 6.1% (DCCT standardized version). Plasma lipids, creatinine and albuminuria were determined by the Central Laboratory of the Medical Clinic of the University of Heidelberg using standard methods. Urinary albumin excretion (UAC) (morning spot urine, three samples) was determined by using an immunonephelometric method (BNA, Behringwerke, Marburg, Germany).

2.3. Statistical analyses

Statistical analyses was performed using the SAS 6.12 (SAS Institute, Heidelberg, Germany). For descriptive purposes mean ± SD are given. The χ^2-test and McNemar's test were performed to compare categorical data. The Wilcoxon test and the Mann–Whitney *U*-test were used to compare quantitative data.

3. Results

At baseline plasma thrombomodulin and UAC did not differ significantly between the groups and HbA_{1c} and systolic blood pressure were similar (Table 1).

Table 2
Comparison of clinical status within the control and α-lipoic acid groups, at baseline and end of study

	Control			α-lipoic acid		
	Baseline	18 months	*P*	Baseline	18 months	*P*
n	49	49		35	35	
BMI (kg/m^2)	24.7 ± 3.3	24.9 ± 3.3	NS	25.6 ± 3.0	25.7 ± 3.1	NS
Hypertension (*n*)	14	16	NS	11	11	NS
Insulin treatment (*n*)	47	47	NS	25	32	NS
Nephropathy (*n*)	14	15	NS	11	11	NS
Retinopathy (*n*)	17	19	NS	22	23	NS
Neuropathy (*n*)	7	10	NS	35	32	NS
Macrovascular complications (*n*)	6	6	NS	9	9	NS
HbA1c (%)	8.4 ± 1.5	8.8 ± 1.5	0.03	8.4 ± 1.1	8.5 ± 1.2	NS
Systolic BP (mmHg)	132 ± 19	142 ± 19	0.0007	126 ± 16	133 ± 20	NS
Diastolic BP (mmHg)	76 ± 9	80 ± 9	0.0076	77 ± 8	76 ± 0	NS
Mean BP (mmHg)	96 ± 13	100 ± 12	0.0002	93 ± 9	95 ± 11	NS
Triglyceride (mg/dl)	105 ± 79	114 ± 86	NS	131 ± 160	124 ± 176	NS
Total cholesterol (mg/dl)	192 ± 34	192 ± 31	NS	201 ± 39	197 ± 43	NS
HDL cholesterol (mg/dl)	55 ± 15	53 ± 13	NS	54 ± 15	51 ± 14	0.04
Creatinine (mg/dl)	0.82 ± 0.16	0.87 ± 0.17	NS	0.92 ± 0.2	0.9 ± 0.2	NS
Albuminuria (mg/l)	21 ± 30	37 ± 61	0.032	29 ± 43	15 ± 11	NS
Thrombomodulin (ng/ml)	35.9 ± 9.4	39.7 ± 9.9	0.01	37.5 ± 16.2	30.9 ± 14.5	<0.001

After 18 months, 55% of the patients in the control group (27/49 patients) showed an increase, 10% no change and 35% (17) a decrease of UAC. In the α-lipoic acid treated group 46% (16/35) showed an increase, 11% (4) no change and 43% (15) a decrease of the UAC. In the control group 69% of the patients (34/49) showed an increase, 2% (1) no change and 29% (14) a decrease of plasma thrombomodulin. In contrast, in the α-lipoic acid treated group 23% of the patients (8/35) showed an increase, while 77% (27) showed a decrease of plasma thrombomodulin after 18 months.

After 18 months, a significant increase in UAC (from 21 ± 30 to 37 ± 61 mg/l, $P = 0.032$) and plasma thrombomodulin (from 36 ± 9 to 40 ± 10 ng/ml, $P = 0.01$) was observed in the control group (Table 2). Significant increases were also found in systolic, diastolic and mean blood pressure, and in HbA_{1c}, but other changes were not statistically significant. In the α-lipoic acid treated group, a significant decrease was seen in plasma thrombomodulin (from 38 ± 16 to 31 ± 15 ng/ml, $P < 0.001$) and HDL-cholesterol (from 54 ± 15 to 51 ± 14 mg/dl $P = 0.04$). UAC was unchanged (28.8 ± 42.7 to 14.5 ± 10.5 mg/l (NS)). The decrease of plasma thrombomodulin in patients treated with α-lipoic acid was statistically significant at 6 months (from 38 ± 16 to 34 ± 14 ng/ml, $P = 0.01$). No significant changes in blood pressure and HbA_{1c} could be seen in the α-lipoic acid group.

Statistical analyses of measured variables with possible effects on the course of albuminuria and thrombomodulin concentration at study end showed no statistically significant differences with respect to HbA_{1c}, HDL cholesterol, total cholesterol, triglyceride, creatinine, systolic BP, diastolic BP, and mean BP.

4. Discussion

The aim of the present study was to evaluate under the setting of a normal outpatient diabetes clinic whether treatment of diabetic patients with the antioxidant α-lipoic acid might decrease the progression of the endothelial cell damage marker thrombomodulin and of diabetic nephropathy (determined as urine albumin concentration) in

patients with diabetes mellitus. The data indicate that albuminuria was unchanged and plasma thrombomodulin decreased in the α-lipoic acid treated group, while both increased in the control group. A bias due to the non-placebo-controlled study design cannot be excluded, even though both groups were clinically similar at study entry.

After 18 months a significant increase in HbA_{1c} and blood pressure was seen in the control group and not in the α-lipoic acid treated group. However, there were no significant differences between the two groups with respect to $HbA_{1c,}$ measures of blood pressure, creatinine, HDL-cholesterol, total cholesterol and triglycerides at study end. That reduces the likelihood that the increase in HbA_{1c} and blood pressure in the control group is responsible for the increase in plasma thrombomodulin and urinary albumin excretion, while it cannot be excluded. It seems possible, that the increase of plasma thrombomodulin and UAC in the control group is partly due to insufficient control of blood glucose and blood pressure in the control group. However, there was a significant decrease in plasma thrombomodulin (UAC decreased, but statistically not significant) in the α-lipoic acid group during the follow up of 18 months without significant changes in blood pressure or $HbA_{1c,}$. Therefore, the significant reduction of plasma thrombomodulin and the in trend reduction in UAC observed in the α-lipoic acid treated group appear to reflect at least in part beneficial effects of α-lipoic acid treatment. During the course of the study, concomitant treatment was similar in both groups (Table 3), so the effects observed cannot be attributed to any other therapy.

Indeed the present study suggests that α-lipoic acid might improve urinary albumin excretion and plasma thrombomodulin levels without normalizing glucose metabolism. This is not in contradiction to the UKPDS and DCCT results, but it simply provides evidence that antioxidant treatment has beneficial effects in the presence of hyperglycaemia as suggested recently by others [47].

In contrast to the control group, all patients of the experimental group had neuropathy and α-lipoic acid has been in particular investigated for management of this [43,44]. Since neuropathy is associated with albuminuria [52–55], it would appear that the difference between groups with respect to neuropathy did not favour the α-lipoic acid group.

A mixture of Type 1 and 2 diabetic patients were studied and the magnitude of the effect of α-lipoic-acid might differ with different type of diabetes type, but the pathogenesis of microvascular complications in Type 1 and 2 diabetes are believed to be similar. Furthermore, oxidative stress parallels the development of complications in Type 1 and 2 diabetes. Further studies are necessary to investigate a possibly differential effect in Type 1 and Type 2 diabetes.

Table 3
List of concomitant drug treatment of the patients allocated to the α-lipoic acid group or the control group at study entry and after 18 months

	Control group		α-lipoic-acid group	
	Baseline	18 months	Baseline	18 months
n	49	49	35	35
ACEI[a]	4	5	3	4
Aspirin	6	6	12	15
Diuretics	7	8	6	7
Diltiazem/verapamil	4	4	1	1
Nifedipine	4	4	4	4
β-adrenergic blocker	2	4	0	0

[a] ACEI, angiotensin converting enzyme inhibitors.

The main purpose of this study was to use α-lipoic acid as a tool for supporting the concept of oxidative-stress-mediated nephropathy in a clinical setting. Previous studies have shown that oxidative stress occurs prior to diabetic complications [35,37], indicating that oxidative stress might indeed be a core mediator of tissue damage. Furthermore, diabetic nephropathy is associated with increased markers of oxidative stress [56] and correlates with mononuclear NF-κB activation [57]. NF-κB activation might be central to causation, since it controls genes such as leukocyte adhesion molecules, cytokines or endothelin-1, known to be involved in cellular reaction in nephropathy. For example, elevated levels of vascular adhesion molecule-1 (VCAM-1) have been found in diabetic nephropathy [58] and can be suppressed by antioxidant therapy [59]. Increased oxidative stress has been described in various glomerular diseases [60] and has also been proposed as the basis for the pathogenesis of diabetic nephropathy [61,62], suggesting the susceptibility of the kidney to oxidative stress to be an important factor at early stages in the development of diabetic nephropathy [56]. Similar evidence has shown that renal tubular damage occurs in parallel with endothelial cell damage and oxidative stress in patients with diabetes mellitus before diabetic nephropathy becomes clinically evident [35,47].

In the present study, antioxidant therapy resulted in biological effects similar to the prevention of glomerular dysfunction in diabetic rats by vitamin E [40] or reduction of streptotozin induced nephropathy by taurine [41]. Furthermore, prevention of interactions of advanced glycation end products (AGE-proteins) with its receptor (RAGE) does not only block NF-κB in vitro [63], but also the increased permeability in diabetic animals [64]. It remains unclear whether the putative effect of α-lipoic acid is by blocking NF-κB activation by indirect routes such as reducing AGE-formation [65], inhibition of aldose reductase [66], decreasing the cellular NADH/NAD ratio [67], or changes in glucose uptake and utilisation [68,69]. Furthermore, the decrease in UAC could well be related to an antioxidant dependent protection of the renal glucosaminoglycans from antioxidant injury [36].

Based on in vitro data, the animal studies, and the clinical study presented here, it is likely that oxidative stress plays indeed a central role in the pathogenesis of diabetic nephropathy. A placebo-controlled study of α-lipoic acid, including around 250 patients is now needed.

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