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Alpha-Lipoic Acid for the Prevention of Diabetic Macular Edema

Christos Haritoglou[a] Joachim Gerss[b] Hans P. Hammes[c] Anselm Kampik[a]
Michael W. Ulbig[a] for the RETIPON Study Group[1]

[a]Department of Ophthalmology, Ludwig Maximilian University, Munich, [b]Institute of Biostatistics and Clinical Research, University of Münster, Münster, and [c]5th Medical Department, University Hospital Mannheim, Mannheim, Germany

Key Words
Diabetic macular edema · Insulin-dependent diabetes · α-Lipoic acid · Type II diabetes mellitus

Abstract
Introduction: To evaluate the effect of α-lipoic acid (ALA) on the occurrence of diabetic macular edema. ***Methods:*** Randomized, double-blind, placebo-controlled, multicenter, multinational study. Patients were randomized to the treatment group with 600 mg ALA per day or the placebo group. Every 6 months stereo fundus photographs, HbA1c levels, and an ophthalmological examination were documented. The primary endpoint was the occurrence of clinically significant macular edema (CSME) within a follow-up period of 2 years. ***Results:*** We randomized 235 patients with type II diabetes mellitus into the treatment group (mean age 58.0 years) and 232 into the placebo group (mean age 57.9 years). Mean HbA1c level was 8.1, with no significant differences between the treatment (mean 8.2, SD ± 1.35) and placebo groups (mean 8.1, SD ± 1.29). HbA1c values remained constant over time. In the treatment and placebo groups, 84 and 86 patients (35.7 and 37.1%) had insulin-dependent diabetes mellitus (IDDM) with a median duration of diabetes of 9.3 versus 9.0 years in the placebo group. Visual acuity remained unchanged during the entire trial. Concerning the primary endpoint, the study provided a negative result, i.e. 26/235 patients in the treatment group and 30/232 patients in the placebo group developed CSME. Confirmatory intention-to-treat analysis of the primary endpoint revealed no statistically significant difference between groups (log-rank test, p = 0.7108, HR = 0.9057 with CI = 0.5355–1.5317). Median follow-up was identical (2.00 years). ***Conclusions:*** A daily dosage of 600 mg ALA does not prevent the occurrence of CSME in IDDM patients.

Introduction

Diabetic retinopathy including macular edema is the most frequent cause of blindness in Western industrialized nations [1–3]. Prevention of visual loss depends on timely detection of diabetic retinopathy and instant-laser treatment. As a timely and close glycemic control with near-normal HbA1c levels and blood pressure below

C.H. and J.G. contributed equally to this work.

[1] See Appendix 1 for participating centers and investigators.

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Christos Haritoglou, MD
Department of Ophthalmology, Ludwig Maximilian University
Mathildenstrasse 8
DE–80336 Munich (Germany)
Tel. +49 895 160 3084, E-Mail christos.haritoglou@med.uni-muenchen.de

130/80 mm Hg cannot always be achieved, medical therapy to prevent the occurrence and progression of sight-threatening complications remains a challenge.

Retinal damage induced by diabetic macular edema is due to vascular leakage and nonperfusion [4]. Sustained hyperglycemia affects several vasoactive factors, notably vascular endothelial growth factor, protein kinase C and angiotensin II, all of which are interrelated and may influence the development of structural and functional changes in diabetic retinopathy [4–9]. The upregulation of vascular endothelial growth factor is associated with a breakdown of the blood-retinal barrier and increased vascular permeability resulting in retinal edema [9, 10]. High glucose levels in endothelial cells are associated with a mitochondrial overproduction of reactive oxygen species, which inactivate glyceraldehyde-3-phosphate dehydrogenase [11]. This process plays a role in the pathogenesis of endothelial damage [12]. Therefore, antioxidants appear to be a therapeutic approach. α-Lipoic acid (ALA) differs in several aspects from other antioxidants [13–15] as it distributes to mitochondria, has a very low redox potential, recycles other cellular antioxidant redox pairs (such as ascorbate) and is regenerated by hyperglycemia and nonessential fatty acid-induced nicotinamide adenine dinucleotide phosphate (via pyruvate dehydrogenase), linking the antioxidant activity to the degree of increased metabolic flux [16].

The potential of ALA in preventing microvascular damage has been demonstrated in animals [17]. ALA has been used for years in the long-term treatment of chronic sensorial disorders in diabetic polyneuropathy at a dosage of 600 mg/day. We raised the hypothesis that a beneficial effect may also be seen in diabetic retinopathy and in the prevention of clinically significant macular edema (CSME) [18–24].

Table 1. Exclusion criteria

Ophthalmic exclusion criteria
– Severe nonproliferative or proliferative diabetic retinopathy
– Macular edema
– Eye diseases interfering with the examinations of the fundus such as preretinal hemorrhage, cataract, vitreous hemorrhage
– Amblyopia
– Best corrected visual acuity ≤0.5
– Glaucoma
– Patients with cataract surgery within a period of 3 months
– Other relevant retinal diseases
– Unauthorized interventional therapy of diabetic retinopathy (e.g. laser, kryocoagulation, vitrectomy)
General exclusion criteria
– Chronic administration of ALA or for more than 5 successive days during the last 12 months
– Known intolerance/hypersensitivity to ALA
– Type I diabetes mellitus
– Poor metabolic control with HbA1c >10.5%/dl
– Late sequelae of diabetes with organic manifestation (e.g. dialysis in cases of renal insufficiency, history of kidney transplantation, creatinine >1.6 mg/dl)
– Poorly controlled arterial hypertension (systolic blood pressure >160 mm Hg and/or diastolic blood pressure >95 mm Hg)
– Severe disturbances in lipid metabolism (triglycerides >500 mg/dl or total cholesterol >320 mg/dl)
– Unauthorized concomitant medications, defined as any medicine with a potential interaction with ALA or with the effect of ALA, were excluded as concomitant medications. These included aldose reductase inhibitors, substances promoting blood flow, anticoagulants apart from acetylsalicylic acid ≤500 mg/day and short-term treatment of diseases with the normal dose of acetylsalicylic acid, chronically and systemically administered corticosteroids, hormonal contraceptives
– Malignancies or life-threatening diseases
– Drug or alcohol abuse
– Blood donation or blood loss greater than 500 ml within the last 3 months
– Pregnancy or breast-feeding
– Participation in a clinical trial within the last 30 days

Methods

Study Design

The RETIPON Study is a phase III, randomized, multicenter, multinational, double-blind, placebo-controlled, comparative trial to evaluate a potential effect of ALA in prolonging the time interval between the diagnosis of mild to moderate diabetic retinopathy at enrolment and the development of CSME in high-risk insulin-dependent diabetes mellitus (IDDM) patients.

The study was approved by the local ethics committee.

*Inclusion and Exclusion Criteria (*table 1)

We included outpatients, male or female, with diagnosed adult-onset IDDM, aged 45–68 years, showing mild to moderate nonproliferative diabetic retinopathy (NPDR) according to the classification of the ETDRS protocol [25] in at least one eye and presenting with microalbuminuria >30 mg/l as diagnosed with a urine dipstick microalbumin test (Micro-Bumintest®, Bayer®, Leverkusen, Germany). These patients are known to bear a high risk for microvascular complications such as CSME. Patients were insulin dependent or non-insulin dependent. Written informed consent had to be given.

Treatment Modalities and Randomization

The 600-mg dosage of ALA was established for treatment of peripheral polyneuropathy. The chemical name of the investigational drug is 1,2-dithiolane-3-valeric acid. The trial medication

was prepared in compliance with Good Manufacturing Practice (GMP), packed and supplied by Bausch & Lomb, Berlin, Germany).

The trial medication had been granted marketing authorization and was newly packed in blister packs and relabeled as 600-mg tablets. The placebo and the drug tablets were similarly packed and labeled, and had an identical appearance and taste.

The number of tablets dispensed at the visits was recorded and subjects were instructed to return any unused medication at the next visit. All returned medications were counted to determine the actual number of tablets taken. Noncompliance was defined as an intake of less than 80% of the medication between visits.

Eligible patients accomplished a 4-week compliance test phase starting with visit 1. In case of at least 80% compliance, they were randomized at visit 2 (day 0) to receive either ALA 600-mg tablets once daily or placebo for a 24-month period. After inclusion, patients were followed at 6-month intervals.

A block design was used for randomization. Random numbers were allocated in ascending order corresponding to the order in which subjects were included after the examination on visit 2. Each patient was allocated to either active treatment or placebo using a stratified randomization within the investigation center as well as the following strata: 1 = HbA1c <9%, IDDM; 2 = HbA1c <9%, non-IDDM (NIDDM); 3 = HbA1c ≥9%, IDDM, and 4 = HbA1c ≥9%, NIDDM.

Date and time of randomization (i.e. the time point at which the current random number was allocated) were recorded for each subject. The balance of randomization was approved.

The randomization code for the trial investigational medicinal product was provided to the investigator in separate sealed envelopes (emergency envelope) labeled with trial and randomization code numbers. The breaking of the treatment code was strictly forbidden except in the event of a medical emergency. The sponsor had to be immediately notified and the reason for breaking the code documented in the subject's medical records and on the case report form. The investigator assessed the relationship of the adverse event to the trial investigational medicinal product before the treatment code was broken.

Medical Review and Physical Examination

Blood samples for laboratory tests obtained at visit 1, and 3–6 included RBC, WBC, platelet count, hemoglobin, hematocrit, GOT, GPT, γ-GT, creatinine, urea, uric acid, HbA1c, fasting blood glucose, total cholesterol, triglycerides. Morning urine was checked by a dipstick test for glucose, total protein, and bilirubin at visit 1 on-site. Laboratory tests were performed in a single central laboratory for all centers (MDS Pharma Services, Hamburg, Germany). Clinically relevant findings were documented in the case report form as adverse events and treated accordingly. All clinically relevant abnormal laboratory results were to be followed up until they returned to normal (i.e. to baseline), stabilized or could be explained otherwise.

At the initial visit, the patient's body weight, height, blood pressure and concomitant medication were recorded. In addition, a complete medical examination was performed. Systolic and diastolic blood pressure and pulse rate were measured at visit 1 and visits 3–6 after a 10-min rest in the supine position and recorded in the case report form. All blood pressure measurements were made on both arms using a sphygmomanometer with an appropriate cuff size for the individual subject and the mean value was calculated.

Ophthalmic Examination and Method of Macular Edema Detection

At each visit, i.e. every 6 months, patients underwent bilateral eye examination including best corrected visual acuity using the Early Treatment Diabetic Retinopathy Study (ETDRS) charts, slit lamp examination, intraocular pressure, and stereoscopic biomicroscopy. Seven-field stereo fundus photographs (in color 30°) according to the criteria of the ETDRS protocol [25] were taken. The quality of the photographs was assessed immediately upon receipt by the senior author (M.W.U.). In cases of insufficient quality, the respective center was asked to submit a new set of photographs. Fluorescein angiography at the beginning and end of the study was optional and evaluated according to the ETDRS protocol [26]. Adverse events whether or not considered to be caused by the study medication had to be reported.

Macular edema was diagnosed on stereoscopic fundus photographs according to the ETDRS protocol [26]. The fundus photographs were always evaluated by two masked examiners at the RETIPON Reading Center in Munich, Germany, under the guidance of the senior author (M.W.U.). Both graders had to concur on the final assessment and potential differences in the grading were discussed by the graders. Graders were all certified to ETDRS standards. The same applied for all participating photographers in the study centers. Optical coherence tomography (OCT) scans were neither adequate nor possible. Most participating centers had no OCT device, and OCT scans are more useful to measure changes in preexisting macular thickening.

Primary and Secondary Endpoints

Detection of CSME in at least one eye was defined as the primary endpoint. Both eyes were graded at each visit. Following detection of CSME in the central reading center, its occurrence was immediately reported and the patient was appropriately treated by laser and excluded from further follow-up.

Secondary objectives were the development of neovascularization elsewhere or at the disk, changes in severity of diabetic retinopathy according to the ETDRS severity scale [25], the course of best corrected visual acuity, and contrast sensitivity as well as cataract formation measured by the Lens Opacity Classification System II.

Statistical Analysis

The statistical evaluation was based on a group sequential design with one interim and a final analysis. Using the O'Brien-Fleming approach and the Lan-De Mets α-spending function methodology in the primary analysis, the single significance levels for an overall level of $\alpha = 0.05$ are $\alpha_1 = 0.0054$ for the interim analysis and $\alpha_2 = 0.0492$ for the final analysis. This report presents the results of the final analysis.

As in the O'Brien-Fleming approach, the final analysis keeps close to the overall significance level and has approximately the same power as a fixed-sample design, a fixed-sample approach was applied for sample size and power calculation. The effect size estimation was based on expert ratings from experienced ophthalmologists. It was assumed that we had a 62% event-free survival in the placebo group after 2 years and a 75% event-free survival in the treatment group. Given these assumptions and

Table 2. Summary of baseline characteristics

	ALA	Placebo	Total	p value
Randomized, n	235	232	467	
Demographic characteristics				
Sex				
Male, n	101 (43.0%)	94 (40.5%)	195 (41.8%)	0.5897
Female, n	134 (57.0%)	138 (59.5%)	272 (58.2%)	
Age, years				
Mean ± SD	58.0 ± 6.11	57.9 ± 6.30	57.9 ± 6.20	0.8556
Range	44–68	45–69	44–69	
Baseline characteristics				
Degree of retinopathy				
Microaneurysms only, n	17 (7.2%)	14 (6.0%)	31 (6.6%)	
Mild NPDR, n	188 (80.0%)	196 (84.5%)	384 (82.2%)	
Moderate NPDR, n	28 (11.9%)	22 (9.5%)	50 (10.7%)	0.4342
Moderately severe NPDR, n	1 (0.4%)	0	1 (0.2%)	
Severe NPDR, n	1 (0.4%)	0	1 (0.2%)	
Type of treatment for DM				
IDDM, n	84 (35.7%)	86 (37.1%)	170 (36.4%)	0.7662
NIDDM, n	151 (64.3%)	146 (62.9%)	297 (63.6%)	
Stratum 1, HbA1c <9%, IDDM, n	53 (22.6%)	56 (24.1%)	109 (23.3%)	
Stratum 2, HbA1c <9%, NIDDM, n	110 (46.8%)	107 (46.1%)	217 (46.5%)	0.9821
Stratum 3, HbA1c ≥9%, IDDM, n	31 (13.2%)	30 (12.9%)	61 (13.1%)	
Stratum 4, HbA1c ≥9%, NIDDM, n	41 (17.4%)	39 (16.8%)	80 (17.1%)	
Median duration of DM, years	9.3	9.0	9.1	0.7348
Mean HbA1c (SD, range), %	8.2 (1.35, 3.8–10.5)	8.1 (1.29, 5.3–10.5)	8.1 (1.32, 3.8–10.5)	0.4087
Study discontinuation				
Total (at least one of the below reasons), n	39 (16.6%)	29 (12.5%)	68 (14.6%)	0.2096
HbA1c >10.5% at two consecutive regular follow-up visits, n	6 (2.6%)	6 (2.6%)	12 (2.6%)	
Inability to comply with the study medication, n	1 (0.4%)	0	1 (0.2%)	
Inability to comply with the visit schedule and/or lost of follow-up, n	7 (3.0%)	8 (3.4%)	15 (3.2%)	
Malignant or other life-threatening diseases, n	2 (0.9%)	2 (0.9%)	4 (0.9%)	
Necessity of not permitted concomitant medication, n	0	1 (0.4%)	1 (0.2%)	
Unacceptable adverse events, n	4 (1.7%)	2 (0.9%)	6 (1.3%)	
Withdrawal of consent, n	19 (8.1%)	10 (4.3%)	29 (6.2%)	

accounting for a 20% rate of nonevaluable patients, a required sample size of n = 260 recruited patients per group was calculated, providing 80% power of significantly detecting treatment differences in the primary analysis. Finally, slightly fewer patients have actually been recruited. Recalculation of the power, however, showed that it still was acceptable, amounting to 75%.

The primary endpoint was evaluated by a two-sided log-rank test, comparing the two treatment groups in an intention-to-treat approach (ITT). This particular hypothesis test provides confirmatory statistical evidence and constitutes the primary study result. Further supplementary analyses of the primary endpoint were performed, including Kaplan-Meier plots, a corresponding per-protocol analysis, and confidence interval (CI) estimation of the hazards ratio (HR) between the two treatment groups. Proportional hazard assumptions were verified using the Grambsch-Thernau residual-based test. Secondary endpoints were evaluated, comparing means and proportions of the parameters given above between treatment groups with Student's t test and the χ^2 test, respectively. Bonferroni adjustment was applied to prevent type I error enhancement due to multiplicity.

Beyond analyses of primary and secondary endpoints, exploratory analyses were performed. The homogeneity of the treatment groups was checked with regard to demographic and relevant pretreatment data. Further exploratory analyses comprised evaluation of safety data, subgroup analyses, and multivariate analyses of the time to CSME development.

Statistical analyses were performed using SAS software (Version 9.1.3 for Windows, SAS Institute Inc., Cary, N.C., USA). In

general, descriptive analyses were performed, describing quantitative variables by number of values, number of missing values, mean, standard deviation (SD), minimum, median and maximum (or median and interquartile range if appropriate). Qualitative variables were described by absolute and relative frequencies of incidence or absolute and relative frequencies of variable categories. Moreover, inductive statistical analyses were performed, applying appropriate significance tests and calculating CIs. Missing values were not replaced.

Withdrawal

All subjects were free to withdraw from this trial at any time, for any reason, specified or unspecified and without impact on their future treatment. Reasons for withdrawal are summarized in table 1.

Results

Baseline Characteristics (table 2) *and Main Efficacy Results*

All outcome assessments were performed by masked examiners. Of all 467 patients, 235 were randomized into the treatment group and 232 into the placebo group. In the treatment group, 101 patients (43.0%) were male and 134 female (57.0%), versus 94 (40.5%) and 138 (59.5%) in the placebo group. Mean age was 58.0 years in the treatment group and 57.9 years in the placebo group. Best corrected visual acuity over all patients measured with ETDRS letters (both eyes) was 108.9 in the treatment group and 106.9 in the placebo group at visit 1 ($p = 0.0991$) and remained unchanged until the final visit (107.9 vs. 104.6, $p = 0.1464$).

Mean HbA1c level was 8.1 (±1.32 SD), with no significant differences seen following randomization at study entry when comparing the treatment group and placebo group. HbA1c values remained constant over time in all randomization strata. In the treatment and placebo groups, 84 (35.7%) versus 86 (37.1%) patients had IDDM and median duration of diabetes was 9.3 versus 9.0 years, respectively. For rates and reasons for study discontinuation, see table 2.

At baseline, the degree of retinopathy did not significantly differ between treatment groups ($p = 0.4342$). Subsequently, the degree of retinopathy deteriorated in the placebo group with borderline significance ($p = 0.0708$) whereas it remained constant over time in the treatment group. On visit 3, significant differences in the degree of retinopathy emerged between the two groups ($p = 0.0311$). On visits 4, 5 and 6, however, the degree of retinopathy did not show any differences between groups anymore. A more detailed description of the participants, randomization and reasons of withdrawal is shown in figure 1.

Comorbidity and Need for Medication Emerging during Therapy (table 3)

Apart from IDDM, the most common concomitant conditions were hypertension (62.5%), coronary artery disease (8.6%), ischemic cardiomyopathy (7.5%), and dyslipidemia (7.1%). Other conditions were documented in less than 6% of the ITT population. In total, 463 patients treated with an ITT approach (99.1%) were taking a concomitant medication during the trial. Most prevalent concomitant medications, except those used for the treatment of diabetes, were enalapril maleate (27.4%), indapamide (19.3%), and acetylsalicylic acid (12.2%).

Primary Endpoint

Concerning the primary endpoint, i.e. the cumulative CSME probability, the study provided a negative result: within the total observational period, 26/235 patients of the treatment group and 30/232 patients of the placebo group developed CSME. Confirmatory ITT analysis of the primary endpoint revealed no statistically significant difference between the two groups (log-rank test, $p = 0.7108$, HR = 0.9057 with CI = 0.5355–1.5317). Median follow-up was identical in the two groups (2.00 years).

Univariate Predictors of CSME Development (table 4)

Looking at all randomized patients (n = 467), 411 patients did not develop CSME during the follow-up, while CSME was observed in 56 patients. Gender, HbA1c, body weight, body mass index, arterial hypertension, duration of diabetes, age and degree of retinopathy were no significant predictors of CSME development. Diabetes treatment (IDDM vs. NIDDM) turned out to be the only baseline covariate with a significant predictive capacity for CSME development (HR = 2.025, CI = 1.199–3.421, $p = 0.0083$).

Multivariate Analysis of CSME Development

The time to CSME development was modeled in a multivariate approach using the baseline covariates gender, diabetes treatment, HbA1c, body weight, body mass index, arterial hypertension, duration of diabetes, age, degree of retinopathy and treatment with ALA versus placebo. Except for diabetes treatment, none of the covariates proved to significantly influence CSME development. In the full model including all above covariates, the HR of IDDM versus NIDDM was 2.691 (CI = 1.469–4.929,

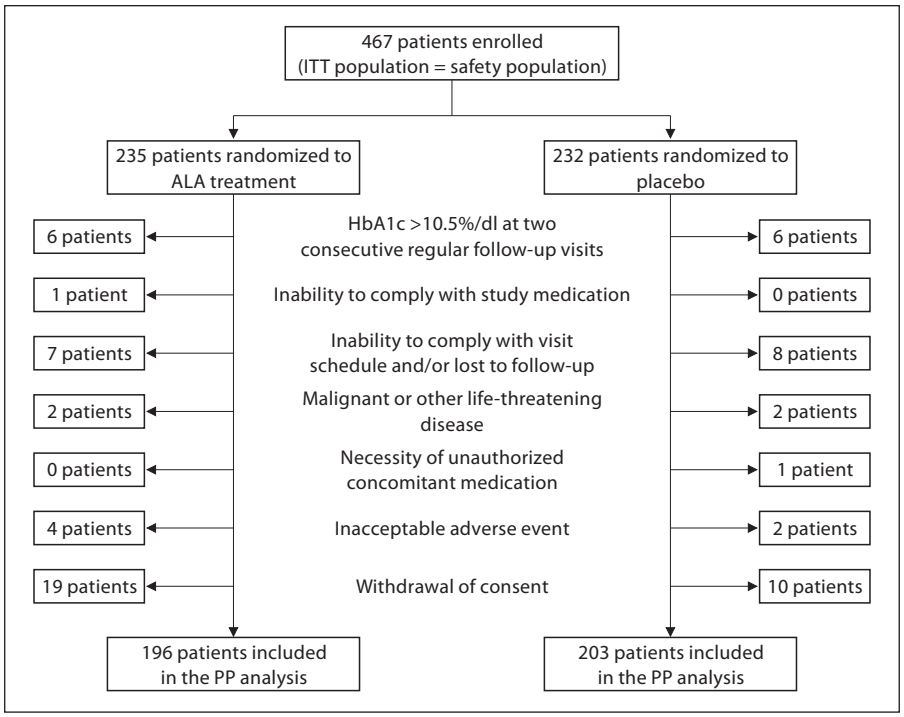

Fig. 1. Consort flow chart of the RETIPON Study. PP = Per protocol.

Table 3. List of cardiovascular risk factors, concomitant diseases and medications

	ALA (n = 235)	Placebo (n = 232)
Risk factors for cardiovascular disease		
Baseline characteristics: blood pressure category high, n	208 (88.5%)	196 (84.5%)
Medical history: hypertension, n	146 (62.1%)	146 (62.9%)
Baseline characteristics: current smoker, n	29 (12.3%)	33 (14.2%)
Disorder of lipid metabolism, n	168 (71.5%)	172 (74.1%)
Baseline characteristics: weight ≥ 80 kg, n	115 (48.9%)	119 (51.3%)
Most frequent findings of medical history		
Metabolism and nutrition disorders, n	235 (100.0%)	232 (100.0%)
Nervous system disorders, n	16 (6.8%)	19 (8.2%)
Cardiac disorders, n	59 (25.1%)	65 (28.0%)
Vascular disorders, n	150 (63.8%)	149 (64.2%)
Eye disorders, n	40 (17.0%)	40 (17.2%)
Most prevalent concomitant medications		
Agents acting on the renin-angiotensin system, n	131 (55.7%)	136 (58.6%)
Antithrombotic agents, n	30 (12.8%)	35 (15.1%)
β-Blocking agents, n	43 (18.3%)	60 (25.9%)
Calcium channel blockers, n	56 (23.8%)	48 (20.7%)
Cardiac therapy, n	54 (23.0%)	59 (25.4%)
Diuretics, n	53 (22.6%)	65 (28.0%)
Drugs used in diabetes, n	234 (99.6%)	223 (96.1%)
Serum lipid-reducing agents, n	32 (13.6%)	36 (15.5%)

Concomitant medication such as acetylsalicylic acid (up to 500 mg/day), antihypertensives and diuretics were permitted as long as such treatments had been applied for at least 3 months, were to be continued for the duration of the clinical trial, and the patient met the inclusion criteria.

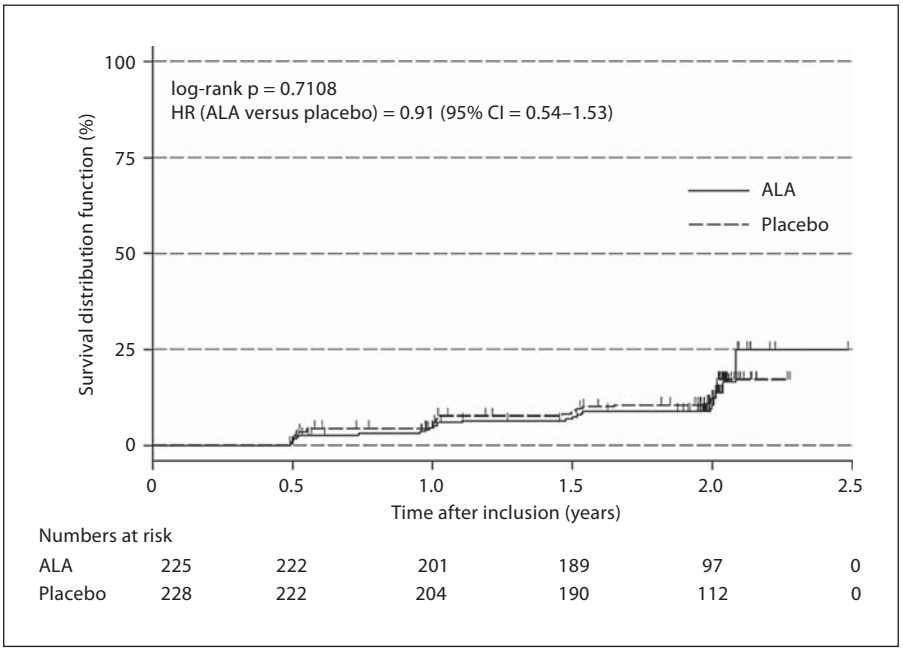

Fig. 2. Confirmatory ITT analyses of primary endpoint. The cumulative probability of developing CSME did not significantly differ between the treatment and the placebo group (log-rank test, p = 0.7108, HR = 0.9057 with CI = 0.5355–1.5317). During the study period, 26/235 patients in the treatment group and 30/232 patients in the placebo group developed CSME.

Table 4. Univariate predictors of CSME development

Potential predictors	Univariate HR	CI	p value
Males versus females	1.288	0.761–2.178	0.3461
HbA1c ≥9% versus <9%	1.045	0.591–1.848	0.8802
Body weight ≥80 kg versus <80 kg	0.865	0.511–1.462	0.5870
Body mass index ≥30 versus <30	0.673	0.377–1.203	0.1816
Systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg versus systolic BP <140 mm Hg and diastolic BP <90 mm Hg	0.965	0.564–1.651	0.8974
Annual increase in the duration of diabetes	0.995	0.957–1.035	0.8076
Annual increase in age	1.008	0.965–1.052	0.7199
Mild diabetic retinopathy versus microaneurysms only	1.182	0.367–3.811	0.7791
At least moderate diabetic retinopathy versus microaneurysms only	2.172	0.598–7.892	0.2389
IDDM versus NIDDM	2.025	1.199–3.421	0.0083

p = 0.0013). IDDM increases the risk of developing CSME by a factor of 2.691 compared to NIDDM. The only relevant covariates, diabetes treatment and HbA1c (i.e. the covariates determining the randomization strata), and (randomized) treatment with ALA versus placebo were included in a reduced model. The reduced model allows for estimation of the adjusted treatment effect that results if the possible confounders, diabetes treatment and HbA1c, are kept constant. The adjusted HR of ALA treatment versus placebo amounts to 0.911 (CI = 0.539–1.541, p = 0.7276), indicating that active treatment does not significantly influence the time to CSME development. In further model-based analyses, it was investigated whether ALA treatment possibly shows interaction effects with baseline covariates. Interaction tests did not yield any positive results, indicating that the treatment effect was independent of baseline covariates, i.e. there was a null effect.

Table 5. Subgroup analysis of time to CSME development

	Patients		HR	95% CI	p value
	ALA	placebo			
Stratum 1	53	56	1.3522	0.5842–3.1301	0.4811
Stratum 2	110	107	0.9570	0.3679–2.4893	0.9282
Stratum 3	31	30	0.7389	0.1653–3.3031	0.6921
Stratum 4	41	39	0.4227	0.1092–1.6355	0.2122
Male sex	101	94	1.3272	0.6096–2.8898	0.4758
Female sex	134	138	0.6395	0.3040–1.3451	0.2386
Body weight <80 kg	120	113	0.5748	0.2735–1.2082	0.1440
Body weight ≥80 kg	115	119	1.5204	0.6976–3.3139	0.2919
BMI <30	151	144	0.9419	0.5062–1.7527	0.8501
BMI ≥30	84	88	0.8286	0.3084–2.2268	0.7094
Systolic BP <140 mm Hg and diastolic BP <90 mm Hg	100	84	0.7750	0.3342–1.7973	0.5526
Systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg	135	148	0.9988	0.5091–1.9594	0.9972
Baseline degree of retinopathy					
Microaneurysms only	17	14	1.7096	0.1550–18.8565	0.6615
Mild NPDR	188	196	0.7167	0.3888–1.3212	0.2858
Moderate, moderately severe or severe NPDR	30	22	1.5270	0.3816–6.1105	0.5496

Subgroups are determined by randomization strata (i.e., diabetes treatment and HbA1c), gender, body weight, body mass index, and hypertension. The univariate HR of ALA treatment versus placebo is estimated in each subgroup, supplemented by the 95% CI and a Wald χ^2 significance test.

Subgroup Analyses (table 5)

Exploratory post-hoc analyses were performed in order to investigate whether ALA treatment might have a positive impact on the time to CSME development in certain subgroups of patients. In none of the subgroups did a significant treatment effect emerge. Moreover, no systematic pattern became apparent that might possibly have indicated a slight trend towards a beneficial treatment effect, e.g. in 'high-risk' subgroups of patients.

Safety Results

During the trial, 109 (46%) subjects of the ALA group and 112 (48%) subjects of the placebo group reported TEAEs. Most frequently occurring events were infections, metabolism and nutrition disorders, vascular disorders, cardiac disorders and nervous system disorders.

Comparing the ALA and the placebo group, TESAEs were reported by 9% versus 10% of subjects. TEAEs in 10 versus 4 subjects were considered to be related to the trial treatment; 11 subjects in both groups reported events of severe intensity, 4 versus 2 terminated the trial prematurely due to TEAEs, death (SAEs) occurred in 2 versus 3 subjects.

Conclusions

Diabetes increases oxidative stress, which is postulated to play an important role in the development of its microvascular complications [27], such as retinopathy and macular edema, a potentially sight-threatening condition. In rats, ALA has been shown to prevent microvascular damage by normalizing pathways downstream of mitochondrial overproduction of reactive oxygen species and to preserve pericyte coverage of retinal capillaries, which may provide additional endothelial protection [16]. Others described beneficial effects on diabetic retinopathy via inhibition of accumulation of oxidatively modified DNA and nitrotyrosine [17]. Therefore, we assumed that supplementation with antioxidants such as ALA as an adjunct therapy may help to reduce the risk of visual loss in diabetic humans with NPDR.

For the present trial, we deliberately selected a high-risk population of IDDM patients with NPDR and microalbuminuria as especially the latter factor is known as a strong predictor of the development of microvascular and macrovascular complications [28] and assumed that this population may be suitable to detect potential protec-

tive effects of our study medication. The Wisconsin data on the risk of developing macular edema [29] were based on HbA1c levels beyond 10%. In our study, we aimed at an average HbA1c level of 8%. However, we observed that ALA at the dose of 600 mg/day provided no significant protective effect on the occurrence of CSME. Differences in the level of retinopathy as seen during the first follow-up visits can be neglected, as all patients included presented with very mild NPDR and low retinopathy levels. Mild changes in the retinopathy level as seen in the placebo group have no clinical relevance either. No differences between the placebo and the treatment group concerning retinopathy levels were noted later on during the trial.

A number of clinical trials, such as the ALADIN (I, II, and III), SYDNEY (I and II), and ORPIL, using ALA have been undertaken in diabetic patients to treat symptomatic diabetic peripheral neuropathy [30–36] using oral ALA supplements from 600 up to 1,800 mg/day. ALA was administered both intravenously at doses of 600–1,200 mg/day for up to 3 weeks, sometimes combined with prolonged administration of additional oral medications [30–37]. Some of these reports described less symptoms of peripheral neuropathy [30, 34–36], while others noted no clinically meaningful effect compared with placebo [32]. The fact that some positive results have been reported in diabetic neuropathy following ALA treatment but not in the prevention of diabetic macular edema may point to different pathomechanisms, and suggests that neuroglial damage is not an early event in diabetic macular edema formation while the breakdown of the blood-retinal barrier is the predominant causative mechanism. An improvement in insulin sensitivity in IDDM patients following the oral administration of ALA was also described [33].

The observations described in the present clinical trial on human diabetic patients stand in contrast to previously reported findings in rats [16, 17]. However, in both animal studies, much higher ALA concentrations of 60 mg/kg [16] and 400 mg/kg body weight were administered over a period of 30 versus 44 weeks. One may conclude that the dosage of 600 mg/day in an adult (7.5 mg/kg body weight in an 80-kg adult) as in our study may not have been high enough to exert a permanent effect on the human retina. No upper limit for ALA intake in humans has been established yet. In contrast, safety levels have been described for animals with pronounced species-dependent differences, showing that rats appear to be far more tolerant than many other species, such as dogs (LD_{50} for dogs 400–500 mg/kg [36] vs. >2,000 mg/kg for rats [37]). Some groups reported that an oral dose of 600 mg once daily appeared to provide the optimum risk-to-benefit ratio [38]. Our daily dosage of 600 mg/day was within the range of ALA dosages reported in the literature and could be considered safe.

One may suggest that 2 years' ALA administration was too short. In published clinical trials, the duration of oral ALA supplementation at a dose of 600 mg/day ranged from 4 weeks [33] to 2 years (combined with an initial intravenous injection of 600 mg for 3 days in the study by Reljanovic et al. [31]. In similar ophthalmological trials, such as the CALDIRET study [39], a follow-up of 5 years was eventually found to be too long because the final result was predictable already after 2 years.

One may criticize that imaging techniques, such as OCT, were not part of the protocol of this trial. This is due to the fact that OCT was not available at all study centers, especially in Eastern Europe. However, according to the ETDRS protocol, the gold standard for the first detection of macular edema is stereoscopic photography, as done in this trial.

In summary, antioxidants such as ALA have become popular in different medical fields. While a positive effect in the treatment of diabetic patients using the dosage of 600 mg/day appears to be beneficial for neuropathy, no protective effect regarding the development of macular edema, another microvascular complication in diabetes, was seen in high-risk IDDM patients using the same dosage. Therefore, regular ophthalmological surveillance and interdisciplinary cooperation remain mandatory.

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Disclosure Statement

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Appendix 1

Centers and principal investigators participating in the clinical trial (28 centers in 5 countries)

Country	Center and principal investigator
Germany	Eye Disease Clinic, Greifswald (Principal Investigator: Prof. Stefan Clemens). University Eye Clinic, Leipzig (Principal Investigator: Prof. Sebastian Wolf).
Poland	Medical Academy I Ophthalmology Department, Lublin (Principal Investigator: Prof. Zbigniew Zagórski). Medical Academy, Ophthalmology Department & Clinic, I Physician's Faculty, Warsaw (Principal Investigator: Prof. Tadeusz Kęcik). Ophthalmology Office, Katowice (Principal Investigator: Dr. Henryk Kozioł). Provincial Hospital Podkarpacki Named Jana Pawła II, Ophthalmology Department, Krosno (Principal Investigator: Dr. Antoni Bąk). Ophthalmology Department & Clinic by Silesian Medical Academy in Katowice, Bytom (Principal Investigator: Prof. Stefan Pojda). Ophthalmology Office Krzysztof Dzięgielewski s.c., Łódź (Principal Investigator: Prof. Jerzy Nawrocki).
Ukraine	Medical Academy of Postgraduate Education, Department of Ophthalmology, Eye Microsurgery Center, Kyiv (Principal Investigator: Prof. Nikolaj M. Sergienko). Filatov Institute of Eye Diseases and Tissue Therapy of Academy of Medical Sciences of Ukraine, Odessa (Principal Investigator: Prof. Natalia Pasechnikova).
Romania	Opticontact and Occulus Center, Bucharest (Principal Investigator: Dr. Ozana Manuela Cernica). Ophthalmologic private practice, Brasov (Principal Investigator: Dr. Stefan Sisak). Clinical County Hospital No. 1 – Ophthalmology, Timisoara (Principal Investigator: Dr. Valerica Augustin Ivanescu). Central Military Hospital, Bucharest (Principal Investigator: Prof. Benone Cârstocea). Diabetics and Nutrition Diseases Institute, Bucharest (Principal Investigator: Dr. Simona Barsan). University Clinic Hospital Sfantul Spiridon, Clinic of Ophthalmology, Iasi (Principal Investigator: Prof. Dr. Dorin Chiselita). Cluj County Hospital, Cluj (Principal Investigator: Prof. Dr. Mihai Calugaru).
Russia	Endocrinological Scientific Center, Moscow (Principal Investigator: Prof. Marina Shestakova). Chair of Endocrinology and Diabetology of the Russian State University, City Clinical Hospital No. 1, Moscow (Principal Investigator: Prof. Irina Demidova). City Multi Field Hospital No. 2, Endocrinology Department, St. Petersburg (Principal Investigator: Dr. Alsou Zalevskaya). Chair of Endocrinology of Novosibirsk Medical Academy, District Clinical Hospital, Novosibirsk (Principal Investigator: Prof. Irina Bondar). Chair of Endocrinology and Diabetology of the Russian Medical Academy for Advanced Medical Studies, Railway Clinical Hospital, Moscow (Principal Investigator: Prof. Alexandre Ametov). Chair of Ophthalmology of the Russian State Medical University, City Clinical Hospital No. 15, Moscow (Principal Investigator: Prof. Eugeniy Egorov). Chair of Endocrinology and Diabetology of EPGE Setchenov MMA, City Clinical Hospital No. 67, Moscow (Principal Investigator: Prof. Mihail Balabolkin).

Study centers of the RETIPON Study distributed into Western and Eastern European centers.

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