Clinical Care/Education/Nutrition/Psychosocial Research
BRIEF REPORT

Effect of α-Lipoic Acid on Platelet Reactivity in Type 1 Diabetic Patients

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OBJECTIVE—Type 1 diabetes is associated with increased platelet reactivity. We investigated whether α-lipoic acid (ALA) has any effect on platelet reactivity in these patients.

RESEARCH DESIGN AND METHODS—We randomly assigned 51 type 1 diabetic patients to ALA (600 mg once daily) or placebo for 5 weeks. Platelet reactivity was evaluated by the PFA-100 method and by measuring CD41 and CD62 platelet expression. C-reactive protein (CRP) and 8-iso-prostaglandin F2α serum levels also were measured.

RESULTS—Baseline variables were similar in the two groups. After treatment, closure time was longer ($P = 0.006$) and CD62P platelet expression was lower, both before ($P = 0.002$) and after ($P = 0.009$) ADP stimulation in the ALA group compared with the placebo group. CRP and 8-iso-prostaglandin F2α levels showed no differences between the two groups.

CONCLUSIONS—Our data show that ALA reduces measures of platelet reactivity ex vivo in type 1 diabetic patients, independently of antioxidant or anti-inflammatory effects.

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Type 1 diabetes is associated with the increased risk of cardiovascular disease (1). Higher platelet reactivity has been reported in diabetic patients (2,3). α-Lipoic acid (ALA) acts as a cofactor in multienzyme complexes, including pyruvate dehydrogenase and branched-chain ketoacid dehydrogenase, and is licensed for treatment of symptomatic diabetic neuropathy (4,5). Recent studies have suggested that ALA has anti-inflammatory and antioxidant proprieties (6–8) that might eventually improve platelet function.

RESEARCH DESIGN AND METHODS—We performed a randomized, double-blind, placebo-controlled pilot study to assess whether ALA has any effects on platelet reactivity, oxidative stress, and inflammation in type 1 diabetic patients. We enrolled 51 type 1 diabetic patients without any evidence of cardiovascular

disease on full noninvasive clinical investigation, which included the maximal exercise stress test to exclude ischemic heart disease. Patients taking antiplatelet drugs were excluded. After obtaining informed written consent, patients were randomly assigned to receive ALA (600 mg once daily; $n = 26$) (4,5) or placebo ($n = 25$) for 5 weeks. The study complied with the Declaration of Helsinki and was approved by the institutional review board of our institution.

Blood samples were drawn at baseline and after treatment in the early morning, before insulin administration, and in a fasting state. Blood was collected in lithium fluoride for plasma glucose estimation, lithium heparin for lipid fraction analysis, and EDTA for hemochrome evaluation. Three milliliters of blood were collected into a tube filled with a 0.129 mol/L (3.8%) trisodium citrate solution (vacuette; Greiner Bio-One, Monroe, NC) and assayed within

1 h. An aliquot of 800 μL was used to assess platelet reactivity to collagen/ADP by the PFA-100 method (Dade Behring, Milan, Italy), in which a shorter closure time indicates a greater platelet aggregability (9,10). Another aliquot was used within 10 min of collection to prepare samples for flow cytometry analyses (FACScan; Becton Dickinson). Blood aliquots were incubated with ADP (final concentration 10^{-7} mol/L) and with saturating concentrations of the fluorescein isothiocyanate–conjugated CD41 (GP IIb) and phycoethrin-conjugated CD62 (P-selectin) monoclonal antibodies (Becton Dickinson, Milan, Italy) (11,12).

Serum C-reactive protein (CRP) was measured by immunonephelometric assay (N Latex CRP mono; Dade Behring, Manburg, Germany). Serum 8-iso-prostaglandin-F2α levels were measured by a competitive enzyme-linked immunoassay (OxiSelect 8-iso-prostaglandin F2α; Cell Biolabs, San Diego, CA) (13), with the results expressed as natural logarithm of values.

Statistical analysis
Continuous and categorical variables were compared by the unpaired t test and Fisher exact test, respectively. Within-group changes were assessed by the paired t test. SPSS version 17.0 statistical software was used for analysis. Data are reported as means ± SD. A $P < 0.05$ was required for statistical significance.

RESULTS—There were no significant differences between the two groups in age, sex, and in the main clinical and laboratory parameters (Supplementary Table 1). Drug therapy also was similar in the two groups and remained unchanged during the period of the study.

Data of platelet reactivity are summarized in Table 1. Before treatment, there were no differences between the two groups in closure time value and flow cytometry variables. After 5 weeks of treatment, closure time was longer in the ALA group compared with the placebo group ($P = 0.006$) as a result of a significant prolongation, compared with the pretreatment value, in the former ($P < 0.001$) but not in the latter group.

CD41 platelet expression did not differ between the two groups at baseline and after ADP stimulation, both before and after

Table 1—*Main results of outcome variables*

	Pretreatment			Posttreatment			
	ALA group	Placebo group	P	ALA group	Placebo group	P	P for changes*
Closure time (ms)	94 ± 14.4	98 ± 15.4	0.43	107 ± 11.8†	96 ± 15.2	0.006	<0.001
CD41 (MFI)							
Baseline	199 ± 78.2	191 ± 58.5	0.72	184 ± 70.3	200 ± 53.1	0.38	<0.001
ADP	229 ± 94.9	229 ± 73.4	0.82	208 ± 78.8	238 ± 68.8	0.15	<0.001
CD62P (MFI)							
Baseline	21 ± 4.5	22 ± 3.9	0.75	19 ± 5.0†	23 ± 4.2	0.002	<0.001
ADP	26 ± 4.8	26 ± 4.5	0.61	24 ± 5.2†	28 ± 4.8	0.009	<0.001
CRP (mg/dL)	1.0 ± 0.5	1.1 ± 0.8	0.99	2.0 ± 1.0	2.2 ± 1.5	0.71	0.40
8-Iso-PG F2α (lnUI/L)	10 ± 1.0	11 ± 1.3	0.65	11 ± 2.0	11 ± 1.03	0.85	0.19

Data are means ± SD. 8-Iso-PG F2 α, 8-iso-prostaglandin F2 α. MFI, mean fluorescence intensity. *P for between-group comparisons of percentage changes of variables after treatment compared with pretreatment (see Supplementary Table 2 for values). †P values <0.001 for comparisons vs. pretreatment values within the group.

treatment; however, there was a significant difference in CD41 changes, both at baseline and in response to ADP, between the two groups as a result of a significant reduction in the ALA group but not in the placebo group (Supplementary Table 2). CD62P platelet expression, on the other hand, was significantly lower in the ALA group compared with the placebo group, both before (P = 0.002) and after (P = 0.009) ADP stimulation, also showing a significant reduction after treatment in the ALA group ($P < 0.001$ for all) but not in the placebo group (Supplementary Table 2). CRP and serum 8-iso-prostaglandin F2α levels did not differ between the two groups before and after treatment, showing no changes, compared with pretreatment values, in either group.

CONCLUSIONS—Our study shows, for the first time, that 5-week treatment with ALA reduced platelet activation in type 1 diabetic subjects. We failed, however, to find any effect on oxidative state, as assessed by changes in 8-iso-prostaglandin F2α plasma levels after 5 weeks of treatment, suggesting that the effect on platelet function of ALA did not depend on oxidative stress. Why we failed to find evidence of the antioxidant effect of ALA is not clear. 8-Iso-prostaglandin F2α might not have been the ideal oxidative stress marker; alternatively, ALA might not have significant effects on the oxidative state in vivo, in contrast with the evidence acquired in vitro (6–8). We also did not observe any significant effect by ALA on CRP levels, suggesting that its effect on platelet function also was not related to a reduction of inflammation. Thus, the mechanisms of the antiplatelet property of ALA remain to be elucidated. Of note, a possible direct inhibitory effect

on platelet function by ALA, through cyclo-oxygenase-1 inhibition (14), recently has been reported. In conclusion, our data show that ALA may have significant antiplatelet effects; additional studies, however, are needed to elucidate the potential clinical benefit of ALA treatment.

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