Effect of Vitamin E Supplementation on Platelet Thromboxane A_2 Production in Type I Diabetic Patients

Double-Blind Crossover Trial

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Vitamin E deficiency is associated with increased platelet aggregation, which can be normalized through vitamin E supplementation. In diabetes, increased platelet thromboxane A_2 (TXA_2) production is correlated with decreased platelet vitamin E content. We therefore investigated the effect of 400 mg DL-α-tocopherol acetate daily for 4 wk on ADP- and collagen-induced platelet aggregation and platelet TXA_2 production in 22 type I (insulin-dependent) diabetic patients without macroangiopathy and with no or only minimal microangiopathy by a double-blind placebo-controlled crossover study. Platelet aggregation was induced in platelet-rich plasma by two or three different concentrations of ADP and collagen. TXA_2 was measured by the stable spontaneous breakdown product thromboxane B_2 by a specific radioimmunoassay. Whereas metabolic control remained unchanged during the study period, platelet TXA_2 production was significantly ($P < .05$ and $P < .01$) reduced at each ADP concentration and at two of three collagen concentrations. Because increased TXA_2 production of diabetic platelets is thought to play an important pathogenetic role in diabetic angiopathy, we conclude that vitamin E treatment could be beneficial with respect to platelet–vessel-wall interaction and thus might be promising for the prevention of diabetic angiopathy. *Diabetes* 37:1260–64, 1988

Several studies have shown that platelets from patients with diabetes mellitus exhibit an increased sensitivity to platelet-aggregating agents (1–3) and produce increased amounts (4–8) of thromboxane A_2 (TXA_2), a vasoconstrictor and potent stimulator of platelet aggregation (9). It has also been suggested that platelet activation plays a role in the pathogenesis of diabetic macroangiopathy and microangiopathy (10–12).

In 1982, Karpen et al. (13) reported a decreased platelet vitamin E content and an increased platelet TXA_2 release in streptozocin-induced diabetic rats compared with those of platelets from matched controls. When these diabetic rats were given supplementary vitamin E in their diet, platelet vitamin E levels increased, and platelet TXA_2 production returned to normal levels (13). The same group also reported a decrease in vitamin E content in platelets obtained from insulin-dependent (type I) diabetic patients and a significant negative linear correlation between intraplatelet concentrations of this vitamin and TXA_2 production (14).

Before this study, it was not known whether supplementation with vitamin E could have a beneficial effect on platelet behavior in patients with type I diabetes. Our aim was to examine this possibility in a group of type I diabetic patients with a double-blind, controlled crossover trial of vitamin E treatment.

MATERIALS AND METHODS

Patients and control subjects. Twenty-two type I diabetic patients [10 men, 12 women; aged 23.5 ± 1.3 yr; duration of disease 8.4 ± 1.2 yr; body mass index 23.8 ± 0.6 kg/m^2; median HbA_{1c} 6.8% (range 6.2–8.1), upper normal range 6%] who had previously attended a diabetes treatment and teaching program (8–48 mo before the study) to improve metabolic control as described earlier (15) were examined during a routine outpatient visit at our clinic. Patients were excluded from the study if they had hypertension (systolic blood pressure >160 mmHg, diastolic blood pressure >90 mmHg); signs of macroangiopathy, as determined by clinical examination, medical history, and ECG; or a history of severe hypoglycemic attacks; or if they were taking any medication other than insulin [macrovascular disease (16) and hypoglycemia (17) have previously been shown to be associated with platelet activation and increased TXA_2 release]. The extent of microangiopathy was assessed by fluorescein an-

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giography to evaluate and grade diabetic retinopathy (5 patients showed minimal changes, 17 showed no changes) and by estimation of the 24-h excretion of albumin to detect an early stage of nephropathy [median albuminuria 8.5 μg/min (range 6–13.4 μg/min); normal <20 μg/min]. No patient had proteinuria, and plasma creatinine concentration was within the normal range. Twelve carefully sex- and age-matched volunteers (6 men, 6 women; aged 24.8 ± 1.6 yr) from our laboratory and medical staff served as control subjects.

Collection of blood samples and methodology. The patients rested for at least 30 min, and then blood was taken from an antecubital vein with minimal occlusion. Routine blood chemistry was performed by an automatic analyzer (Parallel Analyzer, American Monitor System, Indianapolis, IN), percentage of HbA_{1c} was measured by high-pressure liquid chromatography (Diamat, Toyasoda, Tokyo; upper normal range 6.0%), peripheral platelet counts were estimated by an automatic routine counter (Coulter Electronics, Hialeah, FL), and urinary albumin excretion was estimated by radioimmunoassay (Pharmacia, Uppsala, Sweden). Plasma vitamin E content was measured by high-performance liquid chromatography (18).

Platelet aggregation was performed within 30 min of blood collection. Sodium citrate (3.8%) was used as an anticoagulant (1:9 citrate to blood). Aggregation tests were performed in platelet-rich plasma (PRP) in a Born-type aggregometer (Upchurch, Leicester, UK). Platelet aggregation was induced with two or three different concentrations of ADP (Sigma Chemie, Taufkirchen, FRG) and collagen (Collagen Reagent Horm, Hormon-Chemie, Munich, FRG). To assess the first phase of aggregation (19), the percentage of the maximum first-wave increase in light transmission was evaluated ($\%\Delta T_{max}$). To assess disaggregation and/or release (19), the change of light transmission 4 min after addition of either ADP or collagen to the PRP was determined ($\%\Delta T + 4$). Four minutes after addition of either ADP or collagen, 500 μl of PRP was diluted with 600 μl of absolute alcohol and immediately frozen for further thromboxane B_2 (TXB_2) analysis.

TXB_2, the spontaneous breakdown product of TXA_2, was measured by a specific radioimmunoassay (New England Nuclear, Boston, MA) as described earlier (20).

Study design. Vitamin E was given according to a double-blind, placebo-controlled crossover protocol. The patients received 400 mg DL-α-tocopherol acetate (Ephynal, Hoffmann-LaRoche, Basel) or placebo daily. They received each treatment for 4 wk. This vitamin E dose has been shown to increase α-tocopherol content of platelets from healthy volunteers by ~50% (21) and is considered to cause no side effects (22). Clinical and laboratory investigations were carried out before the study, at the crossover point of the study 4 wk later, and after another 4 wk at the end of the study.

Presentation of results and statistical analysis. Data distribution was analyzed by a verified computer program (SAS). For normal distributions, results are given as means ± SE; otherwise they are given as median and the range between lower and upper quartiles. Statistical analysis was performed with paired and unpaired Student's *t* tests, if appropriate, or nonparametric tests: the Wilcoxon test for paired data and the Mann-Whitney test for unpaired data (23).

RESULTS

Except for the percentage of HbA_{1c} and blood glucose, patients and control subjects showed no significant differences for clinical and metabolic parameters, i.e., plasma cholesterol, plasma triglycerides, body mass index, blood pressure, and peripheral platelet count.

No significant differences were found between diabetic patients and control subjects with respect to the maximum first-wave platelet aggregation (ΔT_{max}) and disaggregation ($\Delta T + 4$) after stimulation with different concentrations of ADP and collagen (Table 1).

Table 2 shows ADP- and collagen-induced platelet TXA_2 production in patients and control subjects. TXA_2 release was substantially and significantly increased in diabetic patients after stimulation with each concentration of ADP ($P < .01$) and collagen ($P < .05$ and $P < .01$). Correlation analysis showed no significant correlation between TXA_2 formation and plasma lipids, blood glucose, or HbA_{1c} in the diabetic and control groups.

In the 22 diabetic patients, no significant change in blood glucose, HbA_{1c}, body weight, serum total cholesterol, platelet count, or blood pressure could be seen after either the vitamin E or the placebo period in comparison with the prestudy values. In each study pattern (vitamin E then placebo, and placebo then vitamin E), similar values for the clinical and metabolic findings, vitamin E plasma levels, platelet aggregation tests, and TXB_2 production were observed after each vitamin E and placebo period, thus excluding any carryover effect. Plasma vitamin E levels increased in each patient by ≥30% and almost doubled in the whole group (prestudy 23.2 ± 1.2, active treatment 43.2 ± 2.4, placebo 23.7 ± 1.1 μM α-tocopherol; $P < .001$), reflecting an adequate dosage regimen and patient compliance.

Figure 1 shows ADP- and collagen-induced platelet aggregation expressed as a percentage of maximal change of light transmission (ΔT_{max}) and as a change in light transmission 4 min after induced aggregation ($\Delta T + 4$) to assess reversibility and/or disaggregation. Medians of ΔT_{max} and

TABLE 1
ADP- and collagen-induced maximum first-wave aggregation and disaggregation in type I diabetic patients and healthy control subjects

	n	ΔT_{max} (%)	$\Delta T + 4$ (%)
1.6 μmol ADP			
Diabetic	16	38 (36–41)	31 (29–32)
Control	12	36 (34–41)	30 (28–32)
8 μmol ADP			
Diabetic	17	69 (61–71)	69 (61–71)
Control	12	68 (59–70)	67 (59–70)
40 μmol ADP			
Diabetic	20	71 (69–74)	71 (69–74)
Control	12	70 (67–73)	70 (67–72)
0.15 μg collagen			
Diabetic	11	61 (32–70)	61 (30–70)
Control	12	57 (38–63)	56 (32–63)
0.25 μg collagen			
Diabetic	17	67 (42–72)	67 (38–72)
Control	12	65 (40–70)	63 (40–69)
0.50 μg collagen			
Diabetic	20	70 (67–74)	70 (67–74)
Control	12	69 (66–74)	69 (66–73)

Values in parentheses are ranges.

TABLE 2
Platelet thromboxane B_2 production on stimulation with different concentrations of ADP and collagen in diabetic patients and healthy control subjects

	Diabetic patients		Control subjects		
	n	Thromboxane B_2 (ng/10^9 platelets)	*n*	Thromboxane B_2 (ng/10^9 platelets)	*P*
ADP (μmol)					
1.6	16	16 (5.1–22)	12	6.2 (3.4–10.5)	<.01
8	17	148 (92–190)	12	23 (12–38)	<.01
40	20	160 (108–250)	12	77 (62–98)	<.01
Collagen (μg)					
0.15	11	135 (76–182)	12	46 (38–64)	<.05
0.25	17	142 (98–190)	12	87 (58–115)	<.01
0.50	20	233 (148–312)	12	132 (92–156)	<.01

Values in parentheses are ranges.

ΔT + 4 induced by ADP and the lowest and highest concentration of collagen did not show a significant change after vitamin E treatment compared with prestudy and placebo values. In contrast, at 0.25 μg collagen, both ΔT_{max} and ΔT + 4 were significantly ($P < .05$) decreased after vitamin E treatment.

Figure 2 shows ADP-induced TXA_2 formation at three different concentrations. The median values in the vitamin E group were significantly lower than those found before the study ($P < .05$) and after placebo administration ($P < .01$ and $P < .05$), and were similar to those found in healthy controls.

Figure 3 shows platelet TXA_2 release stimulated by the addition of different concentrations of collagen. Except for addition of 0.15 μg collagen, for which only 11 patients were examined, vitamin E treatment resulted in a significant decrease of platelet TXA_2 production compared with prestudy or placebo values, and approached values found in healthy control subjects.

DISCUSSION

Our data show clearly that treatment of type I diabetic patients with vitamin E resulted in a significant reduction of increased platelet TXA_2 release after challenge with either

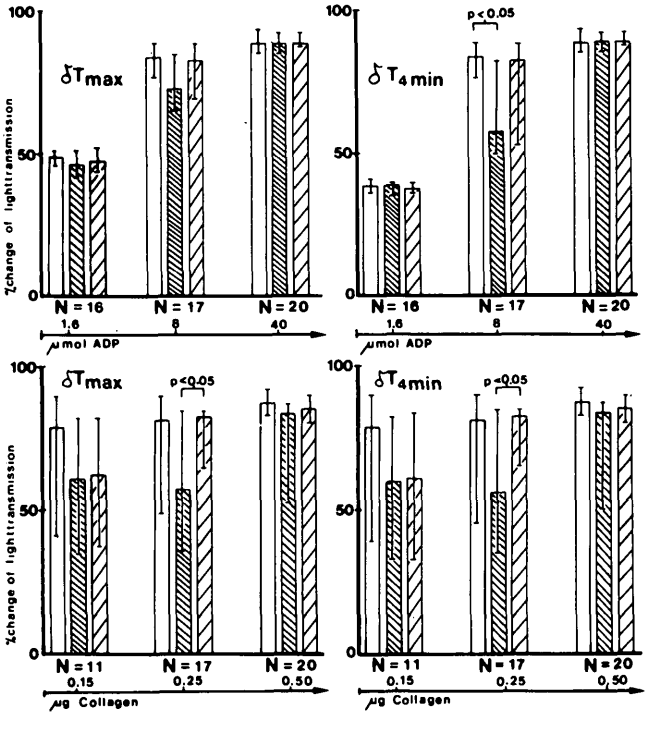

FIG. 1. ADP- and collagen-induced maximum (ΔT_{max}) and 4-min (ΔT_{4min}) platelet aggregation before study (*open bars*) and after vitamin E (*closely hatched bars*) and placebo (*widely hatched bars*) periods. Results are medians; *vertical lines* indicate lower and upper quartiles.

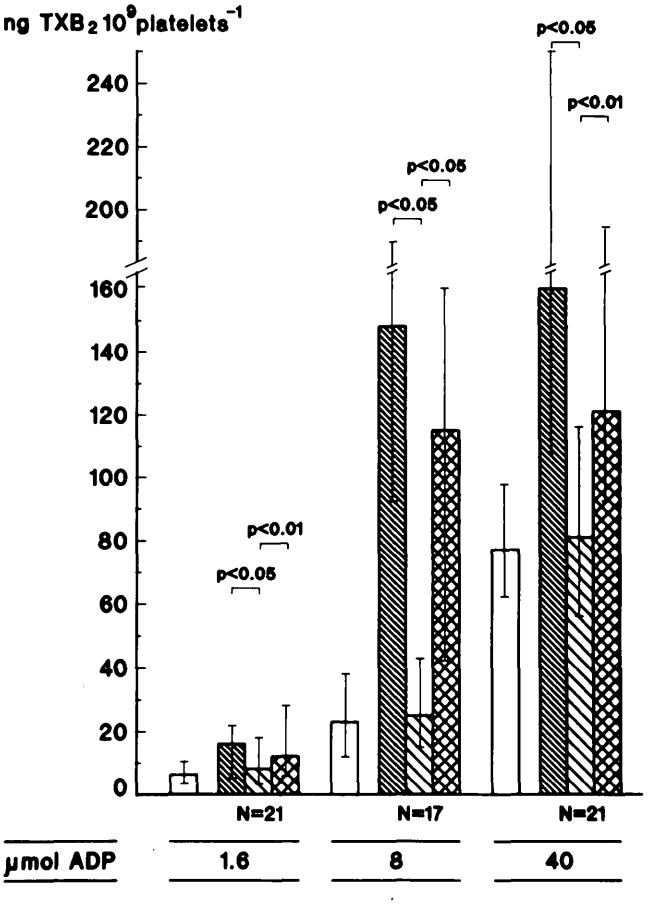

FIG. 2. ADP-induced thromboxane B_2 (TXB_2) formation before study (*closely hatched bars*) and after vitamin E (*widely hatched bars*) and placebo (*crosshatched bars*) periods. Results are medians; *vertical lines* indicate lower and upper quartiles. *Open bars*, controls.

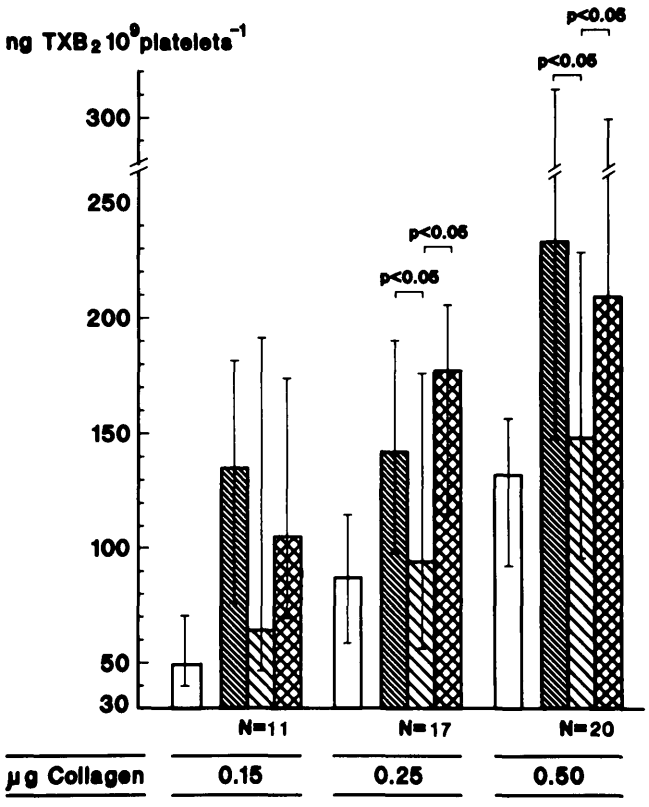

FIG. 3. Collagen-induced thromboxane B_2 (TXB_2) formation before study (*closely hatched bars*) and after vitamin E (*widely hatched bars*) and placebo (*crosshatched bars*) periods. Results are medians; *vertical lines* indicate lower and upper quartiles. *Open bars*, controls.

collagen or ADP. Reduction in platelet aggregation, which was not significantly increased compared with that of healthy control subjects, was less impressive and was significant only at one concentration of collagen. It has been previously observed that the inhibition of TXA_2 production is occasionally a more sensitive index of the inhibition of platelet function than aggregation itself (20,24). Therefore, the potential clinical benefit of vitamin E supplementation with this dosage may be decreased. Whether a more prolonged course of treatment or a higher dose of vitamin E would have resulted in a more consistent inhibition of platelet function is worth further investigation.

The fact that the inhibition of platelet TXA_2 release occurred in the absence of a significant alteration in glucose homeostasis suggests that the effect of vitamin E does not depend on the quality of diabetic control. This is important because the quality of diabetic control and its improvement have been shown to affect platelet function (25,26). Furthermore, patients with type I diabetes and near normoglycemia may have enhanced malondialdehyde formation, an index of TXA_2 production (27).

It has been demonstrated that platelets from type I diabetic patients (14) and diabetic rats (13) have diminished vitamin E content and that vitamin E treatment of the diabetic rat leads to a normalization of platelet activity (13). Our data now provide the information that vitamin E therapy achieves the same results in humans. We deliberately excluded patients with macrovascular disease (16,28), hyperlipidemia (29–31), or recurrent hypoglycemia (32,33), and those on drugs other than insulin (21,34), because these variables have been shown to affect platelet function.

The effect of vitamin E on platelet aggregation has also been shown in conditions not related to diabetes, both in vitro (35) and in vivo (36). Vitamin E deficiency is associated with marked platelet hyperaggregability (37). The administration of vitamin E to nonalcoholic men during ethanol oxidation (38) also resulted in diminished TXA_2 production by platelets. The mechanism underlying this inhibitory effect of vitamin E on TXA_2 may be mediated through a reduction of arachidonic acid release by membrane phospholipids. This may be due to direct or indirect inhibition of phospholipase A_2 in the cell membrane (39).

Because it is possible that platelet hyperaggregability and enhanced TXA_2 release play a role in the pathogenesis of diabetic macroangiopathy and microangiopathy, the administration of vitamin E may provide a novel therapeutic approach to this condition. Indeed, severe vitamin E deficiency caused by abetalipoproteinemia is known to be associated with a type of retinopathy (40), and this retinopathy has recently been shown to be prevented by treatment with large doses of vitamin E (41).

In summary, we have found that vitamin E supplementation significantly reduced increased TXA_2 formation in type I diabetic patients.

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