Effects of Vitamin E Supplementation on F2-Isoprostane and Thromboxane Biosynthesis in Healthy Cigarette Smokers

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Background—Increased formation of 8-iso-prostaglandin (PG) F2α and thromboxane (TX) A2, potent agonists of platelet and vascular thromboxane (TH)/PGH2 receptors, has been detected in cigarette smokers. We performed a randomized, double-blind, placebo-controlled study of the effects of vitamin E (300, 600, and 1200 mg/d, each dose for 3 consecutive weeks) on 8-iso-PGF2α and TXA2 biosynthesis in 46 moderate cigarette smokers.

Methods and Results—Urinary immunoactive 8-iso-PGF2α and 11-dehydro-TXB2, plasma vitamin E, and serum TXB2 were measured by previously validated techniques. Baseline urinary 8-iso-PGF2α and 11-dehydro-TXB2 excretion averaged 241±78 and 430±293 pg/mg creatinine, respectively. Urinary 8-iso-PGF2α was significantly correlated with 11-dehydro-TXB2 (r=0.360, n=138, P<0.0001). Baseline plasma vitamin E levels averaged 20.6±4.9 μmol/L and were inversely correlated with urinary 11-dehydro-TXB2 (r=−0.304, P=0.039) but not with 8-iso-PGF2α (r=−0.227, P=0.129). Vitamin E supplementation caused a dose-dependent increase in its plasma levels that reached a plateau at 600 mg (42.3±11.2 μmol/L, P<0.001). This was not associated with any statistically significant change in urinary 8-iso-PGF2α or 11-dehydro-TXB2 excretion.

Conclusions—Supplementation with pharmacological doses of vitamin E has no detectable effects on lipid peroxidation and thromboxane biosynthesis in vivo in healthy subjects with a mild degree of oxidant stress. These findings are consistent with the hypothesis that the basal rate of lipid peroxidation is a major determinant of the response to vitamin E supplementation and have implications for the use of vitamin E in healthy subjects as well as for the design and interpretation of clinical trials of antioxidant intervention. (Circulation. 2000;102:539-545.)

Key Words: prostaglandins ■ thromboxane ■ vitamins ■ smoking ■ lipids

**V**itamin E (α-tocopherol) is thought to have a role in the prevention of atherosclerosis through inhibition of oxidative modifications of LDLs.1,2 Although observational studies suggest an inverse correlation between the intake of antioxidants, such as vitamin E and β-carotene, and the incidence of coronary heart disease, clinical trials of antioxidant supplementation in patients with ischemic heart disease have yielded apparently conflicting results.3-5 This may reflect, at least in part, the variable dose and duration of vitamin E supplementation as well as the oxidant/antioxidant balance of the different patient populations entered into the trials. Moreover, the inconsistency of trial results probably reflects the inadequacy of traditional indices of lipid peroxidation in guiding the selection of the appropriate dose of vitamin E to be tested in humans.6

A novel analytical approach to quantify the antioxidant effect of vitamin E in vivo is provided by measurements of F2-isoprostanes in plasma and urine. F2-isoprostanes are formed nonenzymatically through free radical–catalyzed attack on esterified arachidonic acid, followed by their enzymatic release from cellular or lipoprotein phospholipids (reviewed in References 7, 8, and 9). F2-isoprostanes circulate in plasma at low concentrations and are excreted in urine. 8-iso-Prostaglandin (PG) F2α (also referred to as iPF2α-III19) is an abundant F2-isoprostane formed in vivo in humans.7-9 This compound is of particular interest because it induces vasoconstriction11 and modulates the function of human platelets12,13 through the interaction with receptors that are distinct from but closely related to thromboxane (TX) A2/PGH2 receptors.

Enhanced formation of F2-isoprostanes has been reported in association with several cardiovascular risk factors, including hypercholesterolemia,14,15 diabetes mellitus,16 and cigarette smoking,17,18 that are characterized by increased lipid
peroxidation in response to complex metabolic abnormalities or various constituents of cigarette smoke.

In both hypercholesterolemic14 and diabetic16 patients, 2-week supplementation with pharmacological doses of vitamin E (600 mg/d) was associated with normalization of enhanced F2-isoprostane formation. Similar findings were reported in apolipoprotein E–deficient mice with a long-term dosing regimen of vitamin E.19

In contrast, the short-term administration of vitamin E (100 and 800 IU/d for 5 days) to healthy chronic smokers failed to suppress urinary 8-iso-PGF2α excretion, and administration of vitamin C (2 g/d), alone or in combination with vitamin E, only partially reduced isoprostane excretion (by ~20% to 30%).18 Factors that may account for this apparent discrepancy include the small sample size (5 to 7 subjects), short duration of vitamin E supplementation (5 days), and lack of placebo control in this study.18 Alternatively, the different rates of lipid peroxidation associated with cigarette smoking versus hypercholesterolemia and diabetes mellitus might represent an important determinant of the variable effects of vitamin E supplementation in these settings. Resolving this apparent discrepancy might have implications for the interpretation of the results of recently completed clinical trials of antioxidant intervention, such as GISSI-Prevenzione4 and the Heart Outcomes Prevention Evaluation Study.5 Thus, we designed a randomized, placebo-controlled, double-blind dose-finding study of the effects of vitamin E supplementation on lipid peroxidation in moderate cigarette smokers with vitamin E levels; then, subjects were randomized to receive placebo or vitamin E 300, 600, or 1200 mg/d for 3 consecutive weeks; after

Methods

Clinical Study Design

Forty-eight apparently healthy volunteers (30 male and 18 female subjects, 20 to 47 years old) who had smoked 15 to 30 cigarettes per day during the previous 2 years were recruited to participate in a randomized, double-blind, placebo-controlled trial of vitamin E (dl-a-tocopheryl acetate; Bayer SpA) supplementation. None of the subjects were taking any medication, including vitamins. During the first week of treatment because of the onset of viral illness, samples were collected at 11 PM to 7 AM and at 10 AM, and 1-mL aliquots of each urine sample on Sep-Pak C18 cartridges (Waters Associates) and elution with chloroform/isopropanol (85/15, vol/vol). Plasma vitamin E was measured by reversed-phase high-performance liquid chromatography.25

Statistical Analysis

With a sample size of 12 subjects per arm, the study had an 85% power (1 – β) to detect a 35% difference in urinary 8-iso-PGF2α between placebo and vitamin E, with α = 0.05. Statistical comparisons were made by ANOVA, and significant differences between treatments were determined by the Student-Newman-Keuls test. The Pearson coefficient (r) was calculated to quantify the direction and magnitude of correlation between variables, and linear regression was used to find the line that best predicts y from x. All data were expressed as mean±SD. A probability value of P<0.05 was assumed to be statistically significant.

Results

The baseline measurements obtained in the 46 healthy cigarette smokers who completed the study are detailed in the Table. Only minor differences were noted in blood lipid levels among the different groups.

Urinary cotinine excretion, a sensitive marker of exposure to cigarette smoking,26 averaged 2394±1454 ng/mg creatinine (n=138) throughout the study and was comparable in the 4 treatment groups at baseline. As shown in Figure 1, the administration of vitamin E did not affect the urinary excretion of cotinine to any statistically significant extent, nor was any appreciable time-related change measured in the placebo arm of the study.

Baseline plasma levels of vitamin E averaged 20.6±4.9 μmol/L (n=46) and were comparable in the 4 treatment groups (Table). As shown in Figure 2, vitamin E supplementation was associated with dose-dependent increases in its plasma levels that reached a plateau at 600 mg (placebo, 21.9±7.7 μmol/L; and vitamin E 300 mg/d, 33±9.2 μmol/L; 600 mg/d, 42.3±11.2 μmol/L; and 1200 mg/d, 42.7±15.3 μmol/L; P<0.001 versus baseline and versus placebo) and returned to pretreatment levels during the washout period of the study. A comparison of vitamin E
concentrations measured repeatedly during the 3 weeks of supplementation clearly demonstrates achievement of steady-state plasma levels during the treatment period of the study (Figure 2).

Baseline urinary 8-iso-PGF$_{2\alpha}$ averaged 241±78 pg/mg creatinine (n=46) and was not significantly different among the 4 treatment groups (Table). In the placebo-treated subjects, urinary 8-iso-PGF$_{2\alpha}$ excretion, assessed throughout the 9 weeks of the study, averaged 233±130 pg/mg creatinine (n=107) (intrasubject coefficient of variation, 33±11%, n=12). No statistically significant correlation was found between the excretion rate of 8-iso-PGF$_{2\alpha}$ and plasma levels of vitamin E evaluated at baseline (r = −0.227, n=46, P=0.129; Figure 3A). Increased availability of vitamin E, produced by supplementation with 300, 600, and 1200 mg/d for 3 weeks, was not associated with any statistically significant change in urinary 8-iso-PGF$_{2\alpha}$ excretion (Figure 4). The urinary excretion of 8-iso-PGF$_{2\alpha}$ and plasma levels of vitamin E measured throughout the 9 weeks of the study showed no statistically significant correlation (r = −0.065, n=138, P=0.452).

We also evaluated the potential impact of vitamin E supplementation on the maximal biosynthetic capacity of circulating platelets, as reflected by measurements of TXB$_2$ production during whole-blood clotting, 20 as well as on the actual rate of TXA$_2$ biosynthesis in vivo, as reflected by the urinary excretion of its major metabolite, 11-dehydro-TXB$_2$. 21 Serum TXB$_2$ averaged 564±88 ng/mL (n=46) at baseline and was not affected by supplementation with vitamin E up to 1200 mg/d to any statistically significant extent (data not shown). Urinary 11-dehydro-TXB$_2$ averaged 430±293 pg/mg creatinine (n=46) at baseline. As shown in Figure 3B, urinary 11-dehydro-TXB$_2$ excretion rates correlated inversely with plasma vitamin E levels (r = −0.304, n=46, P=0.039). A trend for baseline urinary 11-dehydro-TXB$_2$ to correlate with urinary 8-iso-PGF$_{2\alpha}$ was apparent, but this relationship failed to attain conventional statistical significance (r = 0.285, n=46, P=0.054; Figure 5A). However,
the Pearson correlation coefficient for urinary excretion rates of 11-dehydro-TXB\(_2\) and 8-iso-PGF\(_{2\alpha}\), measured throughout the 9 weeks of the study, was statistically significant (\(r=0.360, n=138, P<0.0001; \)Figure 5B). 11-dehydro-TXB\(_2\) excretion remained substantially unchanged during vitamin E supplementation (Figure 6).

**Discussion**

The measurement of F\(_2\)-isoprostane formation in vivo is currently accepted as a useful tool for identifying populations that may have enhanced rates of lipid peroxidation.\(^{27}\) In fact, elevated levels of F\(_2\)-isoprostanes have been observed in plasma and urine of animals and humans under a wide variety of conditions of enhanced oxidative stress.\(^{14–19}\) The finding of increased levels of F\(_2\)-isoprostanes both in the circulation and in the urine of persons who smoke\(^{17,18}\) is consistent with the notion that cigarette smoke contains a large number of oxidants and free radicals that could directly initiate and propagate the process of lipid peroxidation.\(^{28}\)

As reported by Reilly et al.,\(^{18}\) a dose-response relationship exists between the number of cigarettes smoked and the urinary excretion of 8-iso-PGF\(_{2\alpha}\). Thus, in moderate (15 to 30 cigarettes per day) and heavy (>30 cigarettes per day) smokers, the urinary excretion of 8-iso-PGF\(_{2\alpha}\) averaged \(\approx 290\) and \(550\) pg/mg creatinine, respectively. In the moderate chronic smokers participating in the present study, the urinary excretion...
Enhanced lipid peroxidation in cigarette smokers may be related to antioxidant depletion that could be restored by antioxidant supplementation. In a short-term study, the administration of vitamin C (2 g/d for 5 consecutive days) alone or in combination with vitamin E (800 IU/d) to chronic cigarette smokers resulted in a statistically significant (20% to 30%) decline in F$_2$-isoprostane excretion. In contrast, supplementation with vitamin E alone, 100 IU/d (in moderate smokers) or 800 IU/d (in heavy smokers), failed to significantly affect the urinary excretion of 8-iso-PGF$_{2\alpha}$. Failure of vitamin E to suppress isoprostane biosynthesis in this study might be related to the doses administered, the small sample size, or the short duration of the study. Thus, using 8-iso-PGF$_{2\alpha}$ excretion as the primary end point of the study, we set out to investigate the time- and dose-dependence of the antioxidant effects of vitamin E supplementation in chronic cigarette smokers.

The administration of vitamin E caused a dose-dependent increase in its plasma levels that reached steady state during the 3-week treatment study. A maximum 2-fold increase was detected during the administration of 600 mg/d of vitamin E. We have previously described a similar 2-fold increase in plasma vitamin E concentrations by supplementation with 600 mg/d in patients with type IIa hypercholesterolemia and in patients with type II diabetes mellitus. This is in agreement with previous studies showing that large doses of supplemental vitamin E do not increase circulating vitamin E concentrations more than 3-fold. This is probably not due to limitation of vitamin E absorption but rather to the fact that newly absorbed vitamin E in part replaces α-tocopherol in circulating lipoproteins. In fact, >50% of the variation in plasma α-tocopherol is explained by plasma cholesterol and triacylglycerol concentrations.

Supplementation with pharmacological doses of vitamin E up to 1200 mg/d was not associated with any detectable change in urinary 8-iso-PGF$_{2\alpha}$ excretion in the present study. Several factors might contribute to this negative finding. These include (1) a type II error due to inadequate sample size, (2) noncompliance with the study medication, and (3) lack of specificity of the analytical signal. In fact, given the intraindividual variability of urinary 8-iso-PGF$_{2\alpha}$ excretion on repeated sampling, our study had 85% power to detect a 35% change in this biochemical end point with $\alpha=0.05$. Detection of a smaller change in F$_2$-isoprostane formation would require a much larger sample size and would probably be meaningless. Noncompliance with the study medication seems unlikely in view of the consistent changes in vitamin E levels (Figure 2). The specificity of the radioimmunological measurement of 8-iso-PGF$_{2\alpha}$ might be questioned. However, the assay was previously validated by comparison with gas chromatography/mass spectrometry and has been used extensively to demonstrate changes in F$_2$-isoprostane formation in studies of similar sample size and duration in the setting of hypercholesterolemia, non-insulin-dependent diabetes mellitus, and cystic fibrosis. The daily administration of 600 mg of vitamin E for 2 weeks to hypercholesterolemic and diabetic patients was associated with statistically significant reductions in 8-iso-PGF$_{2\alpha}$ excretion by 58% and 37%, respectively (References 14 and 16 and Figure 7A). The same dose of vitamin E as administered to patients with cystic fibrosis significantly (by 42%) reduced the excretion rate of 8-iso-PGF$_{2\alpha}$ (Figure 7A). Taken together, these data suggest that the same pharmacological dose of vitamin E may have variable antioxidant effects in different patient populations characterized by variable rates of lipid peroxidation. Moreover, the finding of a linear correlation between the basal rate of lipid peroxidation and the slope of changes in this index of lipid peroxidation as a function of changes in plasma vitamin E associated with short-term dosing with 600 mg/d in different clinical settings (Figure 7B) is consistent with the hypothesis that the basal rate of lipid peroxidation is a major determinant of the response to vitamin E supplementation.

Further evidence for unchanged levels of biologically active isoprostanes during vitamin E supplementation in the present study can be found in the lack of detectable changes in the rate of TXA$_2$ biosynthesis in vivo. F$_2$-isoprostanes and other biologically active isoeicosanoids can amplify the platelet response to other agonists in vitro. Consistent
with this concept, persistent changes in the rate of \( F_2 \)-isoprostane formation, as detected in patients with metabolic disorders,14,16,18,19 are associated with concordant changes in the rate of \( TXA_2 \) biosynthesis in vivo. Thus, we have suggested that \( F_2 \)-isoprostane formation may provide an important biochemical link between oxidant stress and platelet activation in these settings.14,16 A similar link is apparent in the present study, although the correlation between urinary excretion rates of 8-iso-PGF\(_{2\alpha}\) and 11-dehydro-TXB\(_2\) is much weaker (Figure 5) than in earlier studies of hypercholesterolemia14 and diabetes mellitus.16 The failure of vitamin E supplementation to reduce thromboxane metabolite excretion in healthy cigarette smokers in the present study provides important support to our contention.14,16 That vitamin E may blunt \( F_2 \)-isoprostane–mediated amplification of platelet activation in other clinical settings, such as those depicted in Figure 7, rather than exerting a direct antiplatelet effect.

The overall picture emerging from a series of studies,14,16,18,19 as well as the present study, of vitamin E supplementation using the in vivo formation of \( F_2 \)-isoprostanes as the primary biochemical end point suggests that the effect of vitamin E on lipid peroxidation cannot be equated to that of a conventional drug blocking an enzyme or receptor in a reproducible fashion in the vast majority of patients exposed to treatment. Most likely, both the mechanism(s) responsible for enhanced oxidant stress and the rate of lipid peroxidation are important determinants of the antioxidant effects of vitamin E supplementation. This hypothesis may help in interpreting the conflicting and largely disappointing results of recently completed trials of vitamin E supplementation in patients with ischemic heart disease.3–5 Any protective effect of antioxidant intervention that is readily apparent in the setting of genetically determined enhanced lipid peroxidation19 is likely to be diluted by inclusion of a large proportion of patients with low levels of lipid peroxidation because of dietary habits (such as in the GISSI-Prevenzione Study, carried out in the setting of a largely Mediterranean diet6) or lack of metabolic abnormalities associated with oxidant stress.

In conclusion, the present findings may have practical implications for the use of vitamin E supplements for cardiovascular prevention in the general population. Moreover, they suggest the need to reevaluate the response of potential target populations based on noninvasive measurements of \( F_2 \)-isoprostane formation as a rational basis for a new trial design.

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