Lack of effect of vitamin E on L-arginine-responsive endothelial dysfunction in patients with mild hypercholesterolaemia and coronary artery disease

P. J. CHOWIENCZYK, B. J. KNEALE*, S. E. BRETT, G. PAGANGAT, B. S. JENKINS* and J. M. RITTER
Department of Clinical Pharmacology, UMDS, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, U.K., *Department of Cardiology, UMDS, Guy's and St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, U.K., and †The Free Radical Research Group, UMDS, Guy's Hospital, London SE1 9RT, U.K.

(Received 27 June/22 August 1997; accepted 1 September 1997)

1. Dietary supplementation with vitamin E reduces ischaemic events in patients with established coronary artery disease and improves endothelial function in cholesterol-fed rabbits. We examined whether such dietary supplementation with vitamin E improves endothelial function in patients with mild hypercholesterolaemia and coronary artery disease.

2. Twenty patients (total cholesterol 6.8 ± 1.1 mmol/l, mean ± SD) with angiographically documented coronary artery disease were randomly allocated to receive placebo (n = 10) or vitamin E, 400 i.u. daily, (n = 10) for 8 weeks. Endothelium-dependent and independent vasodilatation within forearm vasculature was assessed by brachial artery infusion of acetylcholine (co-infused with saline vehicle and L-arginine) and nitroprusside before and after supplementation.

3. Plasma concentrations of vitamin E increased from 32.9 ± 3.8 to 69.1 ± 11.8 pmol/l (means ± SE) in the vitamin E-supplemented group (P < 0.01) but did not change significantly in the placebo group. Lipid profiles remained similar before and after supplementation in both groups. Forearm blood flow responses to acetylcholine (7.5 and 15 μg/min) and nitroprusside (3 and 10 μg/min) were similar before and after supplementation in both groups. Acute intra-arterial administration of L-arginine (10 mg/min) augmented the response to acetylcholine (15 μg/min) in both groups before and after supplementation to a similar degree (mean augmentation: 60 ± 18%, P < 0.01).

4. Acute administration of L-arginine reverses endothelial dysfunction in forearm vasculature of patients with mild hypercholesterolaemia and coronary artery disease but supplementation with vitamin E (400 i.u. daily) for 8 weeks does not reverse L-arginine-responsive endothelial dysfunction.

INTRODUCTION

Generalized endothelial dysfunction characterized by impaired responses to endothelium-dependent vasodilators is a universal finding in atherosclerosis and occurs at the earliest stages in its development [1]. In hypercholesterolaemia it is not restricted to the coronary circulation but is also present in other vascular beds such as that of the forearm [2]. Studies in vitro show that low-density lipoprotein (LDL) particles have potent inhibitory effects on endothelium-dependent relaxation [3]. In patients with coronary artery disease (CAD) reduction of LDL-cholesterol both reduces cardiovascular mortality [4] and improves endothelial function [5]. The reduction in cardiovascular events occurs more quickly than can be explained by regression of atherosclerotic plaque [6] and it has been proposed that improved endothelial function, which occurs rapidly [7, 8], may increase myocardial perfusion and stabilize atherosclerotic plaque [5]. Studies in rabbits show that vitamin E is also capable of restoring endothelial function in hypercholesterolaemia [9, 10]. In man, epidemiological evidence suggests that dietary supplementation with vitamin E prevents CAD [11, 12], and the recent Cambridge antioxidant study (CHAOS [13]) demonstrated that supplementation with vitamin E (400–800 i.u. daily) reduces cardiovascular events in patients with established CAD. The present study was undertaken to determine whether such clinical benefit, which again occurs too quickly to be accounted for by regression of atheroma, is likely to be mediated through an improvement in endothelial function. We assessed endothelial function in forearm vasculature of hypercholesterolaemic patients with CAD by measuring vasodilator responses to brachial artery infusion of acetylcholine and nitroprusside before and after supplementation with vitamin E or placebo. Acetylcholine acts, in part, through stimulating release of endothelium-derived nitric oxide.
(NO) [14, 15], whereas nitroprusside is an endothelium-independent vasodilator which acts via the same effector mechanism as NO. Acute administration of L-arginine, the precursor of NO, has been shown to restore endothelial function in some studies [16, 17]. A subsidiary aim, therefore, was to determine whether acute administration of L-arginine would improve endothelial function in these patients.

METHODS

Subjects

Twenty patients (2 women), aged 39 to 71 years, with stable CAD and total cholesterol greater than 5.0 mmol/l who were not taking lipid-lowering drugs were recruited from a cardiovascular prevention clinic at Guy’s and St Thomas’ Hospital. All patients had CAD confirmed on angiography with a stenosis >60% in at least one coronary artery. All were asymptomatic at the time of the study and none had suffered a myocardial infarction in the preceding 3 months. No patient had clinical or radiological signs of heart failure and all had ejection fractions estimated by echocardiography to be greater than 40%. Mean blood pressure was 135±21/77±11 mmHg. All patients were taking aspirin (75–150 mg/day). Other drug therapy included β-blockers (9/20), nitrates (5/20), calcium-antagonists (8/20) and angiotensin-converting enzyme inhibitors (3/20). Drug therapy did not change in the 2 months before or during the study.

Study design

Patients received either vitamin E (all-rac-α-tocopherol acetate, 400 mg daily) or placebo for 8 weeks in a randomized double-blind parallel-group study design. All patients were given standardized dietary advice. Compliance was assessed by tablet count on the day of the last visit and was equally high in both groups. Endothelial function in resistance vessels of forearm vasculature was assessed by measuring blood flow responses to acetylcholine alone and with L-arginine before and after supplementation. Endothelial function, lipid profiles and plasma concentrations of vitamin E were measured before and on completion of the 8-week period of dietary supplementation. The study was approved by the West Lambeth Health Authority Research Ethics Committee and all patients gave written informed consent.

Blood flow studies

Blood flow studies were performed in a quiet clinical laboratory (temperature controlled to 24–26°C during each study) in the morning after a light breakfast. Forearm blood flow was measured in both arms using venous occlusion plethysmography with strain gauges [18], electrically calibrated [19]. Collecting cuff pressure was 40 mmHg and wrist cuff occlusion pressure was 180 mmHg. A 27-gauge needle was inserted into the left brachial artery under sterile conditions using <1 ml of 1% lignocaine hydrochloride to provide local anaesthesia. Drugs were dissolved in saline (0.9% NaCl) and saline or drug solution infused at a rate of 1.0 ml/min by constant-rate infusion pumps. Basal blood flow was recorded after a 15-min infusion of saline. Two cumulative doses of sodium nitroprusside (3 and 10 μg/min, each dose for 6 min) were then infused. After return of blood flow to baseline and repeat basal blood flow measurements, two cumulative doses of acetylcholine chloride (7.5 and 15 μg/min, each dose for 6 min) were infused. Blood flow was then again allowed to return to baseline after which L-arginine (10 mg/min) was infused alone (for 6 min) and then co-infused during a second similar cumulative dose infusion of acetylcholine (7.5 and 15 μg/min, each dose for 6 min). These doses of acetylcholine were chosen because at higher doses the proportion of the response mediated through the L-arginine/NO pathway appears to diminish with acetylcholine acting through an NO-independent mechanism [20]. Forearm blood flow was measured during the last 3 min of each infusion period. Flows were recorded for 10 s in every 15 s and the mean of the last five measurements in each recording period was used for analysis. Blood flow was measured in units of ml per min per 100 ml of forearm volume [18]. Blood pressure was measured supine in triplicate using a Dinamap vital signs monitor (Dinamap model 1846 SX, Critikon, Florida, U.S.A.) on completion of forearm blood flow measurements.

Plasma vitamin E concentration and lipid measurements

Fasting venous blood samples were taken at the beginning and on completion of the 8-week period of dietary supplementation. Plasma concentrations of vitamin E were determined by normal-phase HPLC [21]. Lipid profiles were obtained using standard enzymic methods. High-density lipoprotein-cholesterol was measured after precipitation of apoprotein B-containing lipoproteins using dextran sulphate/magnesium chloride.

Drugs

Vitamin E and sodium nitroprusside were obtained from Roche (Basle, Switzerland), acetylcholine chloride from Coopervision (Southampton, U.K.) and L-arginine from Torbay pharmacy (Torbay, U.K.).
Vitamin E and endothelial function

Data analysis and statistical methods

Descriptive statistics are summarized as means ± SD. Unless otherwise stated results are expressed as means ± SE. Analysis of variance for repeated measures was used to test for differences in vasodilator responses between patients receiving placebo and vitamin E.

RESULTS

Lipid profiles and plasma concentrations of Vitamin E

Lipid profiles and plasma concentrations of vitamin E before and after supplementation are shown in Table 1. Serum concentrations of total cholesterol, High-density lipoprotein-cholesterol and triacylglycerols before and after supplementation did not differ significantly in either placebo or vitamin E-supplemented groups. Plasma concentrations of vitamin E increased from 32.9±3.8 to 69.1±11.8 μmol/l in the vitamin E-supplemented group (P<0.01). Plasma concentrations of vitamin E did not change significantly in the placebo group.

Blood flow studies

During the blood flow studies the flow of blood in the non-cannulated control arm did not change significantly in response to drug infusions in the contralateral arm, indicating that, at the doses used, these drugs had no systemic effects. Blood flow in the cannulated arm during infusion of nitroprusside, acetylcholine alone and acetylcholine with L-arginine is shown in Table 2. Mean blood flow responses to both doses of each agonist are shown in Fig. 1. Before supplementation nitroprusside (3 and 10 μg/min) increased blood flow from 1.9±0.2 to 6.3±0.6 and 9.1±0.8 ml min⁻¹ 100 ml⁻¹ (means ± SE, for all subjects). Acetylcholine (7.5 and 15 μg/min) increased blood flow from 1.9±0.2 to 4.2±0.5 and 4.9±0.7 ml min⁻¹ 100 ml⁻¹. L-Arginine alone had no significant effect on basal blood flow but when co-infused with acetylcholine it significantly augmented responses to acetylcholine (P<0.01, Fig. 1) in both groups before and after supplementation to a similar degree. The mean augmentation for the higher dose of acetylcholine (15 μg/min) was 60±18%. Augmentation of the acetylcholine response by L-arginine remained significant in the vitamin E group after supplementation (51±13%, P<0.05). Blood flow responses to nitroprusside, acetylcholine alone and acetylcholine with L-arginine did not differ significantly before and after supplementation in either the placebo or vitamin E-supplemented groups.

DISCUSSION

Studies in cholesterol-fed rabbits have demonstrated effects of vitamin E on endothelial function

Table 1. Lipid profiles and concentrations of vitamin E in plasma before and after supplementation with placebo or vitamin E. Values for triacylglycerols are medians, all others are means ± SE. *Significantly greater than before supplementation, P<0.001. HDL, high-density lipoprotein.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vitamin E</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.6±0.2</td>
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<td>HDL-cholesterol (mmol/l)</td>
<td>0.8±0.1</td>
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<td>Triacylglycerols (mmol/l)</td>
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<td>Plasma vitamin E (μmol/l)</td>
<td>42.6±3.0</td>
<td>43.5±2.8</td>
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Table 2. Forearm blood flow (ml min⁻¹ 100 ml⁻¹) during infusion of nitroprusside, acetylcholine alone and acetylcholine with L-arginine before and after supplementation with placebo or vitamin E. All values are means ± SE. *Blood flow during infusion of acetylcholine with L-arginine significantly greater than during infusion of acetylcholine alone, P<0.01 by analysis of variance.

<table>
<thead>
<tr>
<th>Dose (μg/min)</th>
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<th>Vitamin E</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Saline</td>
<td>2.0±0.2</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>L-Arginine ½</td>
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<td>1.8±0.2</td>
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<tr>
<td>Nitroprusside</td>
<td>6.8±0.8</td>
<td>7.2±0.7</td>
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<tr>
<td>Nitroprusside</td>
<td>9.6±1.0</td>
<td>11.3±1.3</td>
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<tr>
<td>Acetylcholine</td>
<td>4.1±0.7</td>
<td>2.8±0.4</td>
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<tr>
<td>Acetylcholine</td>
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<tr>
<td>Acetylcholine/L-arginine</td>
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<td>5.9±1.7*</td>
</tr>
<tr>
<td>Acetylcholine/L-arginine</td>
<td>6.7±1.2*</td>
<td>6.4±1.1*</td>
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*Dose of L-arginine = 10 mg/min.
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Fig. 1. Mean forearm blood flow (±SE) during infusion of saline, L-arginine (L-arg, 10 mg/min), acetylcholine (ACh, 7.5 and 15 μg/min) co-infused with saline, acetylcholine co-infused with L-arginine and nitroprusside (NP, 3 and 10 μg/min) before (open bars) and after (shaded bars) supplementation with placebo or vitamin E (400 i.u. daily for 8 weeks). Blood flow values during drug infusion are the mean for both doses of drug. *Blood flow during infusion of ACh + L-arg significantly greater than during infusion of ACh alone, P < 0.01 by analysis of variance.

[9, 10, 22]. Vitamin E may influence endothelial function in hypercholesterolaemia in a number of ways. Hypercholesterolaemia increases endothelial superoxide anion (O₂⁻) production [23] which may result in inactivation of endothelium-derived NO through the formation of peroxynitrite. Vitamin E may inhibit this reaction [24]. Oxidation of lipids in endothelial cell membranes may lead to disruption of receptor-operated signalling events in the NO pathway [25] or altered activity of endothelial constitutive NO synthase (eNOS) [26, 27]. The 'chain breaking' antioxidant effects of vitamin E may prevent the effects of increased oxidative stress on cell membranes [28]. Vitamin E reduces the susceptibility of LDL to oxidation [29] and thus might inhibit the formation of oxidized LDL, which is particularly potent in inhibiting endothelium-dependent relaxation [30].

At moderate doses vitamin E reverses endothelial dysfunction in cholesterol-fed rabbits [9, 10] but a deleterious effect of vitamin E on endothelial function has been observed at high doses [22]. It is possible that at high doses and under certain conditions of increased oxidative stress vitamin E may act as a pro-oxidant. The dose of vitamin E used is therefore critical. In the present study we examined a dose of 400 i.u. daily, the dose used in the CHAOS trial in which clinical benefits of supplementation with vitamin E were demonstrated [13]. We examined effects of vitamin E on endothelial function in hypercholesterolaemic patients who had developed CAD, reasoning that this group would be most likely to benefit from vitamin E. Despite increasing plasma concentrations of vitamin E by 120% (a similar increase to that seen in the vitamin E-supplemented group in the CHAOS trial), vitamin E supplementation did not augment endothelium-dependent vasodilatation by acetylcholine in forearm vasculature. This was not because endothelial function was irreversibly impaired since acute administration of L-arginine significantly improved acetylcholine-induced vasodilatation. The main limitation of our study is the relatively small sample size. However, the study had more than 80% power (α = 0.05) to detect an increase in the blood flow response to acetylcholine of 50% or more. This is less than the increase seen with lipid-lowering therapy in similar patients [8] and also less than the effect of acute administration of L-arginine in the present study. Our study suggests, therefore, that in hypercholesterolaemic men with CAD, vitamin E does not produce the marked improvement in endothelial function seen in some animal models of hypercholesterolaemia [9, 10] where effects of vitamin E are similar to those seen with acute administration of L-arginine [31, 32]. Our findings are consistent with those observed by others in hypercholesterolaemic patients with [33] and without [34, 35] clinical evidence of CAD. Although we reasoned, by extrapolation from animal models, that hypercholesterolaemic patients would be most likely to benefit from vitamin E supplementation, this may not be the case. In the human coronary circulation the antioxidant probucol produces beneficial effects when used in combination with aggressive lipid-lowering therapy, improving endothelial function to a greater extent than cholesterol-lowering therapy alone [36]. This may be because small, dense LDL, likely to be present in high concentrations in patients with CAD [37, 38], is depleted of antioxidants [39].
We measured endothelial function in forearm resistance vessels and therefore we cannot exclude the possibility that vitamin E produced an improvement in endothelial function in other vascular beds and in particular within coronary vasculature. By inhibiting formation of oxidized LDL within atherosclerotic lesions effects of vitamin E may be limited to those vessels affected by atherosclerotic disease. However, with lipid-lowering interventions, normalization of endothelial function is seen both in coronary and peripheral vascular beds [5, 7, 8]. The beneficial effects of vitamin E seen in the CHAOS study may be due to effects other than an improvement in endothelial function such as the prevention of plaque enlargement and/or improved plaque stability [13]. A reduction in platelet adhesion and aggregation and inhibition of vitamin K-dependent clotting factors are other possible mechanisms [40].

Previous studies in hypercholesterolaemia have demonstrated an improvement in endothelial function after acute administration of L-arginine [16, 17]. The present study shows that this is also the case in mildly hypercholesterolaemic patients with CAD. The mechanism by which L-arginine acts is controversial. The concentration of L-arginine within endothelial cells [41] is such as to make substrate limitation of NO production by eNOS unlikely. Incubation of cultured endothelial cells with LDL may increase formation of O$_2^-$ from eNOS as a result of the uncoupling of electron transport within the enzyme [42]. This uncoupling of eNOS may be reversed by high concentrations of L-arginine [42]. Chronic administration of L-arginine has been shown to improve flow-mediated dilation of the brachial artery (a phenomenon thought to be mediated by endothelium-derived NO) in young adults with hypercholesterolaemia [43]. The possibility that chronic administration of L-arginine improves endothelial function in patients with CAD needs to be examined.

ACKNOWLEDGMENT

This work was supported by the British Heart Foundation.

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have different inhibitory effects on endothelium-derived relaxing factor in rabbit aorta. Br J Pharmacol 1990; 100: 21–6.
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