Vitamin E prevents extensive lipid peroxidation in patients with hypertension

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ABSTRACT

Oxidative modification of low-density lipoprotein (LDL) increases atherogenic potential to induce the accumulation of lipids and cells in the vascular wall. Previous studies reveal that hypertensive patients have a higher susceptibility to LDL oxidation. As animal models indicate that vitamin E protects LDL from oxidation, here we study the influence of vitamin E on the resistance of LDL to oxidation (lag time) in 47 subjects (31 normotensive, 16 hypertensive) before and after oral administration of vitamin E (400 IE) daily for two months. LDL was isolated and oxidised by incubation with copper ions. The time course of oxidation was measured by continuous photometric monitoring of diene formation at 234 nm. At the beginning of this study, normotensive subjects showed a lag time of 108±26 minutes and hypertensive patients a lag time of 85±24 minutes ($P<0.05$). Vitamin E caused a significant increase in the lag time in both groups: normotensive subjects 128±33, hypertensives patients 114±27 minutes ($P<0.01$). At completion of the study, lag times in both groups were similar (P=not significant). The data presented here suggests that vitamin E protects against the increased risk of vascular disease in patients with hypertension by reducing the susceptibility to oxidative modification of LDL. Vitamin E may therefore act as an inhibitor of atherogenesis.

KEY WORDS: Atherosclerosis. Hypertension. Vitamin E.

Introduction

Hypertension is a risk factor for atherogenesis.[1] The increased risk of cardiovascular disease in hypertensive patients correlates with blood pressure and may be related to other factors.[2] Studies show an association between hypertension and the oxidation of low-density lipoprotein (LDL), and particularly the fact that its susceptibility to oxidation is greater in patients with essential hypertension than in normotensive subjects.[3,4] It is suggested that oxidative modification of LDL could promote and accelerate the development of atherosclerosis.[5,6] Animal experiments reveal that oxidative modification of LDL is a crucial early step in the pathogenesis of atherosclerosis.[6,7]

When LDL is chemically modified, an uncontrolled uptake of oxidised LDL by the scavenger receptors in macrophages occurs. As a consequence, they dedifferentiate into foam cells that accumulate in the arterial wall, forming early sclerotic lesions.[8]

Randomised control studies indicate the benefit of safe and effective blood pressure (BP) reduction methods.[9] Optimal preventive management should be multifaceted, with reduction of saturated fat and cholesterol intake, restriction of salt and alcohol consumption, weight control, increased physical activity, smoking cessation and eventually antihypertensive medication considered as treatment options.[9,10]

Several studies show that vitamin E decreases LDL-oxidation.[11,12] Additionally, vitamin E may enhance endothelial function by preserving nitric oxide activity.[13] These factors suggest an antiatherogenic effect.[14] Assessment to determine whether or not vitamin E deficiency produces an additional risk factor in the development of vascular disease is still in progress;[15] however, observational data suggests that patients who have risk factors for the development of atherosclerotic vascular diseases benefit from antioxidant supplementation.[16,17]

Short-term oral high-dose antioxidant therapy in hypertensive animals and patients reduces BP, possibly by increasing endothelium-dependent vasodilation.[13,18] Furthermore, vitamin E inhibits smooth muscle proliferation, platelet aggregation and improves arterial compliance.[19,20] Thus, vitamin E may act to inhibit atherogenesis in hypertensive patients, and antioxidants are now proposed as an adjunct to antihypertensive therapy.

The aim of the present study is to evaluate the influence of vitamin E on LDL oxidation in hypertensive patients and whether or not it decreases the risk of cardiovascular disease in hypertensive patients.

Materials and methods

A total of 47 subjects (31 normotensives, 16 hypertensives) were investigated with full informed consent. The protocol was approved by the ethics committee on the use of human subjects in clinical investigations of the University Hospital, Zurich. Hypertensive patients, with BP >140/90 mmHg, were recruited in the division of hypertension at the hospital. The normotensive group (controls) comprised nurses, doctors and some volunteers from a sports club. The

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Table 1. Characteristics of subjects studied

	Normotensives	Hypertensives
Subjects *(n)*	31	16
Females *(n)*	23	8
Males *(n)*	8	8
Age (years)	47±15	52±10
Blood pressure (mmHg)		
systolic	117±16	149±18*
diastolic	76±10	97±11**

*=P<0.01; **=P<0.001

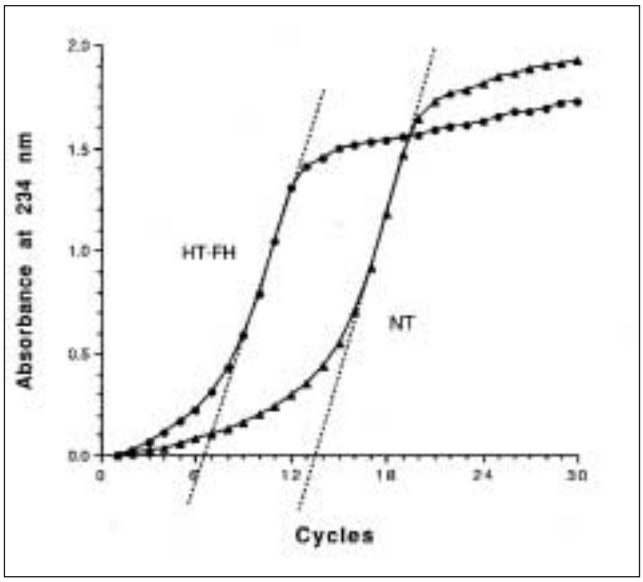

Fig.1. Oxidation curve of LDL in a normotensive subject (NT) and in a hypertensive patient (HT). The lag time corresponds to the intercept on the x-axis given by the tangent of the curve and expresses the antioxidative capacity and oxidative resistance to LDL oxidation with copper. Oxidation measurements were taken every 10 min (one cycle: 10 min).

subjects were divided into two groups depending on BP, according to the joint national committee for detection, evaluation and treatment of high BP.

Hypertensive patients were treated according to clinical practice with antihypertensive medication: three patients received β-blockers, two patients were administered diuretics with calcium antagonists or β-blockers and four patients took diuretics, β-blockers and calcium antagonists or angiotensin-converting enzyme (ACE) inhibitors. All patients were receiving the antihypertensive treatment prior to being enrolled in the trial.

Subjects with diabetes mellitus, hypercholesterolaemia, impaired renal function or other concomitant diseases, as well as pregnant subjects, were excluded from the study. Subject characteristics are shown in Table 1.

Table 2. Summary of results

	Normotensives	Hypertensives
Cholesterol (mmol/L)	5.5±1.1	5.0±1.1
LDL-cholesterol (mmol/L)	3.2±1.1	3.0±1.0
VE pre-pplication (mg/L)	11.2±2.9	14.0±5.4
VE post-pplication (mg/L)	17.9±5.9	20.5±9.1
Lag time, pre-VE (min)	108±26	85±24
Lag time, post-VE (min)	128±33**	114±27**

pre-VE= before vitamin E; post-VE= after vitamin E
*=P<0.05 versus normotensives;
**=P<0.05 versus before vitamin E

The subjects took 400 IE (268 mg; Antistress SA, Rapperswil, Switzerland) of natural vitamin E daily with breakfast for two months, otherwise their diet did not change. An intravenous blood sample was taken (between 8.00 am and 10.00 am) at the beginning and end of the study period. Total cholesterol, LDL and vitamin E were determined by standard laboratory techniques. LDL oxidation was measured photometrically.

Results are expressed as means ± standard deviation (SD). Differences between the control group and the study group were analysed using the unpaired *t*-test.

Determination of LDL oxidation

Venous blood (10 mL) was collected into Vacutainer tubes containing EDTA. Plasma was recovered by centrifugation for 10 min at 1000 x*g* at 4°C and stored at –70°C. Thirty-six hours prior to the determination of oxidation susceptability, LDL was isolated by ultracentrifugation and purified by dialysis at 4°C in the dark with three changes of 1.5L 0.15 mol/L NaCl (pH 7.4) within 24 h to remove EDTA. The LDL-protein content was determined using the Lowry method.[21]

In order to measure LDL oxidation kinetics, 175 µg LDL was placed in a quartz cuvette containing 1 mL phosphate-buffered saline (PBS) and 1.67 mmol/L copper.[22] LDL oxidation was monitored by the change in spectrophotometric absorbance (*A*; 234 nm) at 22°C. Assay variance was 10%. Initial *A* was taken as a baseline and any change was recorded every 10 min for 30 cycles.

In vitro oxidation of LDL was induced with copper. Oxidation kinetics typically showed three distinct periods: an antioxidative, a propagation and a decomposition phase. The first phase was characterised by the consumption of endogenous LDL antioxidants. Once the antioxidants were consumed, the unsaturated fatty acids in LDL were rapidly oxidised in an autocatalytic process (propagation phase). In the decomposition phase, lipids and polypeptides broke

down to different end-products (e.g. various aldehydes). The lag phase was expressed as the intercept given by the tangent of the slope of the *A* curve in the propagation phase with the baseline (Figure 1). A longer lag phase indicated reduced susceptibility of the LDL particle to oxidation and signified increased antioxidative resistance of LDL.[22]

Results

The results are summarised in Table 2. There were no differences in cholesterol and LDL-cholesterol levels between the groups before and after the study period. Furthermore, oral administration of vitamin E (400 IE/daily) did not significantly increase the concentration of the antioxidant in the blood.

At the beginning of the study, the mean values for resistance of LDL to oxidation in normotensive subjects and hypertensive patients were 108±26 mins and 85±24 min, respectively ($P<0.05$). Vitamin E produced a significant increase in this parameter in both groups: normotensive patients increased to 128±33minutes and hypertensive patients increased to 114±27 minutes ($P<0.01$). Thus, at the end of the study period, no significant difference between the lag phases in normotensive subjects and hypertensive patients was demonstrated.

Discussion

Atherosclerosis is the leading cause of morbidity and mortality in industrialised countries. Hypertension and LDL, specifically oxidised LDL, are risk factors for atherogenesis and cardiovascular disease.[23] Oxidative modification of LDL increases its uptake in macrophages by scavenger receptors, leading to their increased accumulation in the arterial intima that, in turn, contributes to accelerated atherogenesis.[4,22]

Oxidation of LDL is a lipid peroxidation process: the polyunsaturated fatty acids of LDL are successively degraded to different products. It is possible to measure the oxidation of LDL, which is strongly catalysed by metal ions,[24] *in vitro* by continuously monitoring increasing *A* at 234 nm. The oxidative susceptibility of LDL is increased when combined with cardiovascular risk factors.[11] Generally, measurements of the susceptibility of LDL to oxidation may be used to assess the risk of atherogenesis.

Patients with hypertension may have increased lipid peroxidation.[25,26] The lag time is significantly shorter in patients with hypertension independent of BP, as in normotensive subjects.[4] One explanation for the differences in oxidative susceptibility may be the distribution of LDL subfractions. There is evidence of a genetic influence on the LDL subfraction patterns, which vary in chemical composition, density, size, metabolic properties and possibly atherogenic potential.[27,28]

The three LDL subfractions isolated by density ultracentrifugation differ in their susceptibility to lipid peroxidation *in vitro*, indicating that dense LDL and light LDL are less well protected against oxidation than is very light LDL.[29,30] Hypertensive patients may have a preponderance of small, dense LDL particles;[4] a phenomenon associated with an atherogenic lipoprotein profile and a three-fold increased risk of cardiovascular disease.

Prevention of LDL oxidation may be an effective strategy to prevent or slow the process of vascular disease. A lipophilic antioxidant, such as vitamin E, protects polyunsaturated fatty acids against oxidation and thus prevents the beginning of the oxidation cascade.[22,31] Therefore, this specific substance may exert its greatest effect on early atherosclerotic lesions.[32] However, the vascular endothelium is the primary site of dysfunction in cardiovascular disease.

Supplementation with antioxidants in some animal models has been shown to protect the vascular endothelium from oxidised LDL-mediated dysfunction and genesis of atherosclerosis,[33,34] and oral applications of antioxidants reduce the susceptibility of LDL oxidation in patients with coronary artery disease.[35,36] However, susceptibility to LDL oxidation depends on polyunsaturated fatty acid and vitamin E content.

Results of the present study demonstrated that vitamin E decreases LDL susceptibility to oxidation in both normotensive subjects and hypertensive patients. Prior to natural vitamin E supplementation for two months, there was a significant difference in lag phase between the two groups. At the end of the two-month period, however, no significant difference could be demonstrated.

A possible limitation of the present study could be that hypertensive patients were treated according to clinical practice with antihypertensive medication. Calcium antagonists, β-blockers and ACE inhibitors are known to increase LDL resistance to oxidation.[37,38] However, antihypertensive therapy prevents the oxidation of LDL rather than decreases lag times of LDL oxidation.

Our data suggests a protective effect for antioxidative treatment with vitamin E on the increased risk of vascular disease caused by a reduction in the oxidative modification of LDL. Thus, as LDL oxidation plays a key role in the development of atherogenesis, antioxidants may act as potential antiatherogenic medications. □

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