Vitamin E prevents extensive lipid peroxidation in patients with hypertension

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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]

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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.

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

Table 1. Characteristics of subjects studied

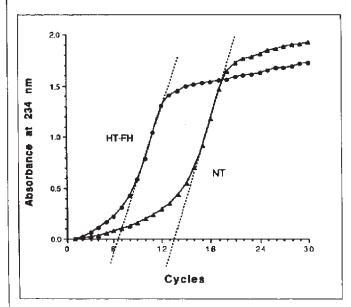|	Normotensives	Hypertensives
Subjects (n)	31	16
Females (n)	23	8
Males (n)	8	8
Age (years)	47±15	52±10
Blood pressure (mmHg)		
systolic	117±16	149±18*
diastolic	76±10	97±11**

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

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

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

well as pregnant subjects, were excluded from the study. Subject characteristics are shown in Table 1.

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 xg 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.[3]

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.[32] 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 ± 33 minutes 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.[12] 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. □

References

1 Kannel WB, Sorlie P. Hypertension in Framingham. In: Paul O, ed. *Epidemiology and control of hypertension.* New York: Stratton International Medical Book Cooperation, 1975: 553-92.
2 Allemann Y, Weidmann P. Cardiovascular, metabolic and hormonal dysregulation in normotensive offspring of essential hypertensive parents. *J Hypertens* 1995; 13: 163-73.
3 Pierdomenico SD, Constantini F, Bucci A, De Cesare D, Cuccurullo F, Mezzetti A. Low-density lipoprotein oxidation and vitamins E and C in sustained and white-coat *hypertension. Hypertension* 1998; 31: 621-6.
4 Bracht C, Locher R, Suter P, Vetter W. LDL-Oxidation bei essentieller Hypertonie. *Kardiovaskuläre Medizin* 1997; 1: 54-8.
5 Witztum JL. The oxidation hypothesis of atherosclerosis. *Lancet* 1994; 344: 793-5.
6 Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoproteins that increases its atherogenicity. *N Engl J Med* 1989; 320: 1196-7.

7 Grundy SM. Oxidized LDL and atherogenesis: relation to risk factors for coronary heart disease. *Clin Cardiol* 1993;16: (4 Suppl 1): I3-5.

8 Witztum JL, Steinberg D. Role of oxidized low-density lipoprotein in atherogenesis. *J Clin Invest* 1991; 88: 1785-92.

9 Veterans Administration Cooperative Study Group on Antihypertensive Agents. Effects of treatment on morbidity in hypertension. II. Results in patients with diastolic blood pressure averaging 90 through 114 mmHg. *JAMA* 1970; 213: 1143-52.

10 Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. The fifth report of the Joint National Committee on detection, evaluation and treatment of high blood pressure (JNC V). *Arch Intern Med* 1993; 153: 154-83.

11 Devaraj S, Jialal I. The effects of alpha-tocopherol on critical cells in atherogenesis. *Curr Opin Lipidol* 1998; 9: 11-5.

12 Odeh RM, Cornich LA. Natural antioxidants for the prevention of atherosclerosis. *Pharmacotherapy* 1995; 15: 647-59.

13 Kinlay S, Fang JC, Hikita H *et al.* Plasma alpha-tocopherol and coronary endothelium-dependent vasodilator function. *Circulation* 1999; 100: 219-21.

14 Swain RA, Kaplan Machlis B. Therapeutic uses of vitamin E in prevention of atherosclerosis. *Altern Med Rev* 1999; 4: 414-23.

15 Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 1988; 260: 1917-21.

16 Odeh RM, Cornish LA. Natural antioxidants for the prevention of atherosclerosis. *Pharmacotherapy* 1995; 15: 648-59.

17 Pryor WA. Vitamin E and heart disease: basic science to clinical intervention trials. *Free Radic Biol Med* 2000; 28: 141-64.

18 Pezeshk A, Derick Dalhouse A. Vitamin E, membrane fluidity and blood pressure in hypertensive and normotensive rats. *Life Sci* 2000; 1: 1881-9.

19 Keaney JF, Simon DI, Freedman JE. Vitamin E and vascular homeostasis: implications for atherosclerosis. *FASEB J* 1999; 13: 965-75.

20 Mottram P, Shige H, Nestel P. Vitamin E improves arterial compliance in middle-aged men and women. *Atherosclerosis* 1999; 145: 399-404.

21 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.

22 Esterbauer H, Striegl G, Puhl H, Rotheneder M. Contiuous monitoring of *in vitro* oxidation of human low-density lipoprotein. *Free Rad Res Commun* 1989; 67-75.

23 Pyörälä K. Interpopulation correlations between serum cholesterol level and the occurrence of coronary heart disease.

Eur Heart J 1987, 8: 23-30.

24 O' Leary VJ, Tilling L, Fleetwood G, Stone D, Darley-Usmar V. The resistance of low-density lipoprotein to oxidation promoted by copper and its use as an index of antioxidant therapy. *Atherosclerosis* 1996; 119: 169-79.

25 Digiesi V, Oliviero C, Gianno V *et al.* Reactive metabolites of oxygen, lipid peroxidation, total antioxidant capacity and vitamin E in essential hypertension. *Clin Ter* 1997; 148: 515-9.

26 Maggi E, Marchesi R, Ravetta V, Falaschi F, Finardi G, Bellomo G. Low-density lipoprotein oxidation in essential hypertension. *J Hypertens* 1993; 11: 1103-11.

27 Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low-density lipoproteins in normal humans. *J Lipid Res* 1982; 23: 97-104.

28 Shimano H, Yamada N, Ishibashi S *et al.* Oxidation-labile subfraction of human plasma low-density lipoprotein isolated by ion-exchange chromatography. *J Lipid Res* 1991; 32: 763-73.

29 Jun-Jun W, Xiao-Zhuan L, Yi-Yi Z, Lu-Yan L. Correlation between susceptibility of LDL subfractions to *in vitro* oxidation and *in vivo* oxidised LDL. *Clin Biochem* 2000; 3: 71-3.

30 Vasankari T, Ahotuba M, Toikka J *et al.* Oxidized LDL and thickness of carotid intima-media are associated with coronary atherosclerosis in middle-aged men with lower levels of oxidized LDL with statin therapy. *Atherosclerosis* 2001; 155: 403-12.

31 Lengfelder W. Antioxidative vitamin: Prävention von kardiovaskulären Erkrankungen. *Dtsch Med Wschr* 1998; 123: 1014-7.

32 Steinberg D. Clinical trial of antioxidants in atherosclerosis: are we doing the right thing? *Lancet* 1995; 346: 36-8.

33 Bunout D. Therapeutic potential of vitamin E in heart disease. *Expert Opin Investig Drugs* 2000; 9: 2629-35.

34 Brown AA, Hu FB. Dietary modulation of endothelial function: implications for cardiovascular disease. *Am J Clin Nutr* 2001; 73: 673-86.

35 Mosca L, Rubenfire M, Mandel C *et al.* Antioxidant nutrient supplementation reduces the susceptibility of low-density lipoprotein to oxidation in patients with coronary artery disease. *J Am Coll Cardiol* 1997; 30: 392-9.

36 Rimm EB, Stampfer MJ. Antioxidants for vascular disease. *Med Clin North Am* 2000; 84: 239-49.

37 Croft KD, Dimmitt SB, Moulton C, Beilin LJ. Low-density lipoprotein composition and oxidizability in coronary artery disease apparent favourable effect of beta-blockers. *Atherosclerosis* 1992; 97: 123-30.

38 Lupo E, Locher R, de Graaf J, Vetter W. *In vitro* antioxidant activity of calcium antagonists against LDL oxdition compared with alpha-tocopherol. *Biochem Biophys Res Commun* 1994; 203: 1803-8.