Lipid Peroxidation and Antioxidant Vitamins C and E in Hypertensive Patients

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Summary

Lipid peroxidation is a free radical process which is implicated in the formation of atherosclerosis. Vitamins C and E are important natural antioxidants which inhibit lipid peroxidation and a high intake of these vitamins, particularly vitamin E, is related to a reduced incidence of ischaemic heart disease. Hypertension is an independent risk factor for atherosclerosis and its relationship to antioxidant status is undetermined. In this study, we investigated free radical activity by measuring plasma malondialdehyde (MDA) using high-performance liquid chromatography (HPLC), vitamin C status measured as plasma ascorbic acid and vitamin E status measured as plasma lipid standardized α-tocopherol and erythrocyte α-tocopherol. We compared 28 patients with essential hypertension to 31 healthy subjects. Results showed that in comparison with the healthy subjects, the hypertensive patients had significantly higher plasma MDA levels (0.95 ± 0.28 vs 0.69 ± 0.21 μmol/l, mean ± SD, $p<0.001$) and significantly lower levels of plasma ascorbic acid (34.83 ± 12.88 vs 51.76 ± 13.34 μmol/L, $p<0.01$). In addition, erythrocyte α-tocopherol concentration, which may reflect vitamin E protection in cell membranes, was significantly lower in hypertensive patients when compared with the normotensive controls (3.87 ± 0.53 vs 4.82 ± 1.01 μmol/l, $p<0.001$), although plasma α-tocopherol levels were similar in the two groups (25.07 ± 10.45 vs 23.96 ± 6.07 μmol/l). Our results suggest that hypertensive patients may have increased lipid peroxidation and reduced protection from vitamins C and E. This may contribute to the propensity in such patients to develop atherosclerosis.

Introduction

A free radical is any chemical species (molecule or atom) with one or more unpaired electrons. It is now accepted that such species are extremely reactive and may cause injury to a variety of tissues by their action on a variety of molecules including lipoprotein, DNA and proteins[1]. Lipid peroxidation is a free radical reaction in which a free radical, such as the active oxygen radical, reacts with polyunsaturated fatty acids (PUFAs). This results in the formation of lipid peroxides, for example, malondialdehyde (MDA), the intermediate lipid peroxyl radicals which may attack adjacent fatty acid side chains, yielding a lipid hydroperoxide and a new PUFA radical, thus initiating a free radical chain reaction[2]. The oxidative modification of low-density lipoprotein is considered to play a causal role in the pathogenesis of atherosclerosis[3]. Circulating MDA concentrations are elevated in patients with atherosclerosis and diabetes and this is considered to indirectly reflect *in vivo* free radical activity[4].

Aerobic metabolically generated free radicals such as oxygen radicals and hydrogen peroxide are constantly produced in the human body and there is normally a balance between production of free radicals and natural antioxidant defences of which vitamins C and E are two examples. Vitamin C is a reducing agent (electron donor) and scavenges superoxide, peroxide and hydroxyl radicals[5]. Vitamin E may concentrate within the phospholipid bilayer of cell membranes and react with superoxide and lipid peroxyl radicals, thereby interrupting the chain reaction of lipid peroxidation and preventing tissue damage[6]. However, as plasma tocopherol is readily influenced by the plasma lipid concentration and does not reflect the content of vitamin E in cell membranes, erythrocyte α-tocopherol may be a better indicator of vitamin E nutritional status[7].

Epidemiological studies have repeatedly identified an important and independent risk relation between high blood pressure and coronary heart disease[8]. It has also been reported that there was lower dietary intake of vitamin C, lower blood vitamin C level, and elevated free radical generation in hypertensive patients while the studies regarding vitamin E status were controversial[9,10]. In this study, we investigated lipid peroxidation, vitamins C and E status in 28 patients with essential hypertension and the results were compared with those in 31 sex- and age-matched healthy subjects.

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Patients and Methods

A total of 28 patients with essential hypertension was recruited from our out-patient clinic, BP > 160/95 mmHg. The control group comprised 31 normotensive subjects recruited from College staff and healthy blood donors. Both groups were free of pharmacologic vitamin supplementation. The clinical characteristics of

TABLE I
Summary of clinical characteristics of control and hypertensive subjects.

	Normotensive (no = 31)	*Hypertensive (no = 28)*
Sex (female/male)	14/17	13/15
Age (year)	50.6±12.7	58.3±19.5
No. of smokers (% of total)	6 (19)	4 (14)
Daily cigarettes consumption	4.2	2.6
Duration of hypertension (year)	0	13.1±15.6
Current complications:		
[1]PVD	0	2
[2]LVH	0	1
[3]IHD	0	5
Hyperlipidaemia	0	2
Diabetes	0	2
Obesity	2	1
*Antihypertensive drug therapy:		
Diuretics	0	9
ß-blockers	0	9
Calcium antagonists	0	4
ACE inhibitors	0	11

*Eight patients were on two or more antihypertensive drugs and 2 patients were not on antihypertensive drug.
[1]PVD: peripheral vascular disease
[2]LVH: left ventricular hypertrophy
[3]IHD: ischaemic heart disease

normotensive and hypertensive subjects are summarised in Table I.

Venous blood samples were drawn from subjects with EDTA as anticoagulant after 12 hours overnight fasting. The analysis of blood samples started within 1 hr of taking the samples to avoid further free radical generation and loss of antioxidant vitamins. The laboratory procedures are described briefly as follows:

High-performance liquid chromatography (HPLC):

HPLC was employed to measure vitamin E as α-tocopherol in plasma and red cells and lipid peroxides in plasma as MDA. A Shimadzu (Shimadzu, Kyoto, Japan) LC - 6A pump was fitted with a SPD - 6 AV spectrophotometric detector, an ODS 25 cm x 4.6 mm C-18 column and a 50 µl loop. The peak area was measured by a Shimadzu C - R3A microcomputerized data processor.

Plasma MDA measurement:

The method was described by Wong et al[11]. Plasma lipid peroxides were hydrolysed with thiobarbituric acid (TBA). MDA was separated from interfering chromogens and quantified spectrophotometrically at 532 nm. The concentrations of plasma lipid peroxides were determined by reference to a calibration curve prepared by assays of 1,1,3,3,-tetraethoxypropane from Sigma.

Plasma α-tocopherol:

The method used was that described by Dieber-Rotheneder et al[12]. Plasma α-tocopherol was extracted with n-hexane and analysed by HPLC. A spectrophotometric detector with wavelenth of 292 nm and 98% methanol as mobile phase were used throughout for all the α-tocopherol measurements. Plasma α-tocopherol concentrations were standardized to plasma total cholesterol values[13].

Red cell α-tocopherol:

The method used was that described by Sierra et al[14]. α-tocopherol in washed red cells was extracted with n-hexane and determined by HPLC.

Ascorbic acid:

Plasma ascorbic acid concentrations were measured after derivatization with 2,4-dinitrophenylhydrozine by spectrophotometry according to the method of Omaye et al[15].

Statistical analysis:

Data was analysed by using paired Student's *t*-test and linear regression analysis. In all comparisons, two-tailed $p < 0.05$ was considered significant. Results are expressed as means ± SD.

Results

As shown in Table II, plasma MDA concentrations of patients with hypertension were significantly greater than controls. Plasma ascorbic acid levels were significantly lower in hypertensive patients in comparison with controls and the hypertensive subjects also had lower red cell α-tocopherol concentrations, although plasma α-tocopherol values were similar in two groups.

Overall there was a weak but significant correlation between plasma lipid standardized α-tocopherol and red cell α-tocopherol ($r=0.384$, $p < 0.01$) values. There were no significant correlations either between plasma MDA and ascorbic acid ($r = -0.22$, $p > 0.1$) or α-tocopherol ($r = 0.07$, $p > 0.5$) concentrations. Red cell a-tocopherol values were not correlated to plasma ascorbic acid levels ($r = 0.15$, $p > 0.2$). In hypertensive patients, plasma MDA concentrations were not related to either antihypertensive drug therapy or current complications.

Discussion

Polyunsaturated fatty acids contained in lipids are particularly susceptible to peroxidation[1]. Several studies have suggested that lipid peroxides may be important in the development of atherosclerosis and elevated levels of circulating lipid peroxides, expressed as MDA, have been found in patients with atherosclerosis, diabetes (particularly in patients with angiopathy), hyperlipidaemia and acutely after myocardial infarction and stroke[4,16,17].

TABLE II
Results of plasma MDA, ascorbic acid and α-tocopherol and erythrocyte α-tocopherol in hypertensive patient and control

	Control	*Patient*
	µmol/l, mean±SD	
MDA	0.69±0.21	**0.95±0.28
Plasma ascorbic acid	51.76±13.34	*34.83±12.88
Plasma α-tocopherol	23.96±6.07	25.07±10.45
Erythrocyte α-tocopherol	4.82±1.01	**3.87±0.53

*p<0.01. **p<0.001

The MDA-TBA reaction has been used widely to assess lipid peroxides but the conventional fluorometric method lacks specificity. In the present study, the utilization of HPLC effectively excluded the interfering chromogens thereby improving the specificity of the assay[18]. Our data shows that plasma MDA is raised in patients with essential hypertension, indicating elevated free radical activity and possibly an increased risk of developing atherosclerosis.

A number of epidemiological studies have demonstrated that a high consumption of vitamins C and E is inversely related to the incidence of atherosclerosis and its complications[1,19]. Vitamin C acts as a free radical scavenger and reduces the quantity of free radicals in plasma thereby causing a decreased free radical diffusion from plasma into lipid phases including cell membranes and low density lipoproteins[5]. Vitamin E is the major chain-breaking antioxidant in lipoproteins and the only effective antioxidant in cell membranes. Although vitamin E is present in low concentration in red cells, freshly isolated erythrocytes from healthy subjects do not contain any significant amount of lipid peroxides[7]. When vitamin E reacts with free radicals, it is oxidized to a tocopheroxyl radical and loses its antioxidant function. Vitamin C can regenerate this oxidized form of vitamin E in either red cell membrane[20] and LDL[7] by directly reducing tocopheroxyl radicals. Thus the two vitamins may act synergistically to prevent free radical cell damage[5,6].

Vitamin E status is commonly assessed by measuring the concentration of α-tocopherol in plasma. However, erythrocyte α-tocopherol levels are believed to reflect vitamin E nutritional status more precisely. Our study describes that in hypertensive patients, apart from a lower plasma vitamin C level, there is a weakened antioxidant defence in cell membranes, expressed as low red cell vitamin E, although plasma vitamin E levels were similar in the two groups. In agreement with previous investigations[9,21,22], we considered that a reduced vitamin C level in hypertensive subjects may be caused by lower dietary vitamin C intake and, may be at least partly, responsible for an elevated free radical activity (expressed as increased plasma MDA concentrations). The consumption of vitamin E in cellular membranes may increase to trap the increased free radicals diffused from plasma but its regeneration is less effective due to the low vitamin C level. Therefore dietary supplementation with vitamin C, or combined with vitamin E, may increase the resistance to free radical damage and consequently reduce the risk for atherosclerosis.

In conclusion, our results suggest that in hypertensive patients, increased lipid peroxidation with reduced protection from vitamins C and E may contribute to the propensity of such patients to develop atherosclerosis. Dietary supplementation with antioxidant vitamins may have the potential to prevent the development of atherosclerosis.

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