S- 5-5 Lipid Peroxidation, Vitamin E, and Cardiovascular Disease

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I. INTRODUCTION

As early as in 1978 Nordøy et al. [1] discovered that human low density proteins (LDL) inhibit the generation of an unstable anti-aggregatory principle by cultured human endothelial cells. A year later Henriksen et al. [2] described that a damage to cultured endothelial cells by LDL did not occur either in presence of high density lipoproteins (HDL) or when a modification of the procedure for preparation of LDL was introduced. This modification consisted of the introduction of an antioxidant, butylated hydroxytoluene (BHT), at all stages of the adopted preparative procedure for LDL [1]. We confirmed that BHT reduced a thiobarbituric acid-reactive substance (TBARS) in LDL from 9 to below 1 nmoles/mg LDL. Moreover, we demonstrated that BHT-treated LDL did not inhibit the generation of prostacyclin (PGI₂) by rat aortic slices, whereas oxidized LDL were powerful inhibitors of PGI₂ formation by arterial wall [3,4]. We also found that bovine coronary arterial strip pretreated with oxidized LDL hardly transformed arachidonic acid to PGI₂. These findings prompted us to put forward a hypothesis that LDL are carriers for lipid peroxides [3] and thus inhibit PGI₂ synthase in arterial endothelial cells [5].

Thus, in vitro experiments pointed to a difference between native and oxidized LDL (n-LDL and ox-LDL) in their actions on biosynthesis of PGI₂ by arterial walls. Unlike n-LDL, ox-LDL inhibited release of PGI₂ from arteries.

There is also growing evidence that ox-LDL might inhibit the generation of another vasodilatory product of endothelium, namely "endothelium-derived relaxing factor" (EDRF) [6,7].

II. LIPID PEROXIDES, PGI₂, AND THE EFFECT OF VITAMIN E

The effect of lipid peroxides on the production of prostacyclin in vivo has been studied in an experimental model.

Feeding an atherogenic diet to 25 rabbits for a week resulted in elevation of plasma lipid peroxides (as measured by thiobarbituric acid reactive substances - TBARS - in plasma) and a 90% decrease in arterial generation of prostacyclin. Enrichment of the atherogenic diet with 100 mg vitamin E daily prevented the increase in plasma lipid peroxides and protected the prostacyclin generating system in arteries (Table 1). Thus, in hyperlipoproteinemias vitamin E corrects certain abnormalities of lipid metabolism which might predispose to atherosclerosis [8]. Moreover, plasma lipid peroxide levels correlated inversely with arterial capacity to generate PGI₂ ($r=0.8$, $p<0.01$).

TABLE 1

The effect of vitamin E on lipid peroxides and generation of PGI₂ by rabbit arterial slices (mean $\pm$ S.E.M.)

Group of animals	TBARS (nmol MDA/ml)	PGI ₂ (ng/mg wet tissue)
I Standard diet n = 8	0.82 $\pm$ 0.12	1.50 $\pm$ 0.08
II Standard diet + vitamin E n = 5	0.79 $\pm$ 0.03	1.89 $\pm$ 0.25
III Atherogenic diet n = 6	1.47 $\pm$ 0.11*	0.16 $\pm$ 0.05*
IV Atherogenic diet + vitamin E n = 6	0.53 $\pm$ 0.07°	0.84 $\pm$ 0.14*°

* = $p < 0.05$ when group III or IV were compared with group I

° = $p < 0.05$ when group IV was compared with group III.

III. LIPID PEROXIDES, CARDIOVASCULAR DISEASE, AND THE EFFECT OF VITAMIN E

Our human studies included a placebo controlled trial in which 27 healthy volunteers and 58 patients with hyperlipoproteinemias types II and IV suffering also from ischemic heart disease, peripheral vascular disease, or both received orally vitamin E at doses of 300 mg or 600 mg daily for 14 days [9]. Serum tocopherol levels increased by 2-2.5 fold in healthy subjects and in patients. In patients with hyperlipoproteinemias, vitamin E effectively depressed elevated concentrations of plasma lipid peroxides (measured as TBARS) down to levels of healthy subjects. This effect of vitamin E was most pronounced in patients with high levels of plasma lipid peroxides whose serum antioxidant activity was weakened (Table 2). In these patients, a mild platelet suppressant effect of vitamin E (600 mg) was observed.

TABLE 2

Effects of vitamin E (for 2 weeks) on plasma lipid peroxides in healthy subjects and patients with hyperlipoproteinemias and cardiovascular diseases

	Controls		Patients	
	Before	After vit.E 300 mg	Before	After vit.E 300 mg
Serum tocopherol (ng/ml)	17 $\pm$ 0.8	30 $\pm$ 2.6°	36 $\pm$ 3	77 $\pm$ 6.7°
Lipid peroxides (nmol MDA/ml)	1.4 $\pm$ 0.1	1.0 $\pm$ 0.1	1.9 $\pm$ 0.1*	1.0 $\pm$ 0.1°
	600 mg		600 mg	
Serum tocopherol (ng/ml)	14.5 $\pm$ 0.1	30 $\pm$ 2.3°	25 $\pm$ 2.2	51 $\pm$ 4°
Plasma lipid peroxides (nmol MDA/ml)	1.0 $\pm$ 0.1	1.0 $\pm$ 0.2	1.7 $\pm$ 0.1*	1.0 $\pm$ 0.2°

* = $p < 0.01$ as compared to healthy subjects

° = $p < 0.001$ as compared to results before treatment.

We further studied indices of lipid peroxidation in plasma of 30 patients with hyperlipidemias type II or IV and ischemic heart disease or peripheral arterial disease and compared them with those of 19 healthy subjects [10]. Lipid peroxides were this time measured both iodometrically, as hydroperoxides (Hicks [11] - with own modification) and as TBARS. Both methods have shown significantly higher levels of lipid peroxides in patients (10.05 ± 0.58 vs. 5.55 ± 0.37 nmol/ml, $p < 0.01$ - for hydroperoxides and 3.15 ± 0.15 vs. 2.24 ± 0.1 nmoles MDA/ml, $p < 0.01$ - for TBARS, respectively). However, there was no correlation between concentrations of hydroperoxides and TBARS possibly because the two methods assessed different breakdown products of lipid peroxidation. Vitamin E administered in a dose of 300 mg for 4 to 6 weeks to 6 of those patients depressed elevated hydroperoxides by 33% and TBARS by 44% on average.

IV. DISCUSSION

It has been recently proposed that formation of oxidized LDL takes place in vivo and is responsible for the initiation of atherosclerosis [12]. Free radicals play a major role in the process of lipid peroxidation. Human serum is equipped with several mechanisms scavenging those radicals. One of them is vitamin E - a major lipid-soluble, chain-breaking antioxidant.

Our previous work [3,4] and recent results from laboratory animals show that vitamin E protects from damage the PGI₂ synthesizing capacity of arterial walls. On the other hand, increased lipid oxidation occurs in patients with hyperlipoproteinemias and clinical evidence of vascular complications of atherosclerosis as shown by the increased levels of lipid peroxides. This phenomenon can also be reversed by the dietary supplementation with vitamin E. In other situations known to increase the risk of developing ischemic heart disease, like smoking, the increase in oxidative stress on plasma lipoproteins has been noted [13], and prevented by vitamin E administration [13,14]. Moreover, plasma vitamin E was independently and inversely related to the risk of angina pectoris [15].

It is the possible that lipid peroxides might not only initiate the process of atherosclerosis but also - by inhibiting the production of vasodilatory and anti-platelet PGI₂, and possibly EDRF - play a major role in its vascular complications. However, it still remains to be proven that administration of vitamin E or other oxidants will be able to help preventing the development of cardiovascular diseases.

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