Vitamin E and Atherosclerosis: An Overview


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1. INTRODUCTION

In the last decade a new attractive theory on the pathogenesis of atherosclerosis has been developed which is mainly based on the finding that vascular cells (endothelial cells, smooth muscle cells, monocyte-macrophages) can oxidatively modify LDL to a form (oLDL) which leads to formation of lipid-laden foam cells and exhibits cytotoxic and chemotactic properties. Most of the properties of cell modified LDL can be reproduced by exposing LDL to copper ions or certain agents producing free radicals (for review see 1, 2). Oxidative modification of LDL is principally a lipid peroxidation process by which the polyunsaturated fatty acids (PUFAS) contained in the LDL lipids are oxidized to lipid hydroperoxides which in secondary and tertiary metal ion catalyzed reactions break down to a great variety of products including reactive aldehydes such as hexanal, MDA or 4-hydroxynonenal (HNE). Several lines of research suggest that reactive lipid peroxidation products (e.g. aldehydes) derivatize free amino groups in the apolipoprotein B and thereby create new epitopes which are recognized by the macrophage scavenger receptor and ultimately responsible for the uncontrolled degradation of oLDL by these cells (2-4).

Immunohistochemical methods showed that MDA or HNE conjugated LDL is indeed present in lesions of WHHL rabbits and autoantibodies against such aldehyde-altered LDL are contained in serum of human and rabbits (5). There are various other findings that support the hypothesis that lipid peroxidation plays a role in atherosclerosis. Atherosclerotic lesions of human aorta contain lipid peroxides (6) and the LDL which can be extracted from the arterial wall exhibits chemical (increased REM, decreased PUFAs) and biological (uptake by macrophages) properties similar to in vitro oxidized LDL (7). It has repeatedly been reported that plasma of atherosclerotic patients contains increased levels of TBARS, an index for oxidized lipids. A analysis of the different lipoproteins indicate that TBARS are mainly contained in VLDL and LDL (8). Intravenous injection of lipid hydroperoxides causes in rabbits atherosclerotic like symptoms (9). Many lipid peroxidation products are highly cytotoxic and if present in plasma could provoke formation of lesions of the endothelial layer of the vascular system and thus enhance infiltration of LDL, platelet aggregation, release of growth factors, disturbance of eicosanoid homeostasis and accumulation of inflammatory cells (for review see 10).
If formation of atherogenic oLDL is the consequence of lipid peroxidation, then chain-breaking natural or synthetic antioxidants should be important denominators in the protection of LDL against peroxidative processes initiated by cells or transition metal ions.

2. POLYUNSATURATED FATTY ACIDS AND ANTIOXIDANTS IN LDL

LDL has an average molecular weight of 2.5 million and consists of a central core of 1600 molecules cholesterylester and 170 molecules triglycerides, the core is surrounded by a monolayer of about 700 molecules phospholipids and 600 molecules free cholesterol. The total number of fatty acids in an LDL particle is about 3000 and roughly half of them are PUFAs (Table 1). Protections of these PUFAs against oxidation is conferred by a great variety of lipophilic antioxidants, by far the major one is α-tocopherol, its average content is about 6 mol/mol LDL. The other substances with potential antioxidant activity are present in amounts of only about 1/20 to 1/300 of α-tocopherol (Table 1) (2,11-13).

Table 1

Fatty acids and antioxidants in native LDL.
This is an updated version of tables published previously (2,11-12) with a larger number of subjects (n).

<table>
<thead>
<tr>
<th></th>
<th>nmol/mg LDL protein</th>
<th>mol/mol LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ±SD (n)</td>
<td>mean</td>
</tr>
<tr>
<td>palmitic acid</td>
<td>1260 ±375 (19)</td>
<td>693</td>
</tr>
<tr>
<td>palmitoleic acid</td>
<td>80 ±44 (19)</td>
<td>44</td>
</tr>
<tr>
<td>stearic acid</td>
<td>260 ±118 (19)</td>
<td>143</td>
</tr>
<tr>
<td>oleic acid</td>
<td>825 ±298 (19)</td>
<td>454</td>
</tr>
<tr>
<td>linoleic acid</td>
<td>2000 ±541 (31)</td>
<td>1100</td>
</tr>
<tr>
<td>arachidonic acid</td>
<td>278 ±100 (31)</td>
<td>153</td>
</tr>
<tr>
<td>docosahexaenoic acid</td>
<td>53 ±31 (15)</td>
<td>29</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>11.58 ±3.34 (87)</td>
<td>6.37</td>
</tr>
<tr>
<td>gamma-tocopherol</td>
<td>0.93 ±0.36 (88)</td>
<td>0.51</td>
</tr>
<tr>
<td>β-carotene</td>
<td>0.53 ±0.47 (122)</td>
<td>0.29</td>
</tr>
<tr>
<td>α-carotene</td>
<td>0.22 ±0.25 (28)</td>
<td>0.12</td>
</tr>
<tr>
<td>lycopene</td>
<td>0.29 ±0.20 (136)</td>
<td>0.16</td>
</tr>
<tr>
<td>cryptoxanthin</td>
<td>0.25 ±0.23 (114)</td>
<td>0.14</td>
</tr>
<tr>
<td>cantaxanthin</td>
<td>0.04 ±0.07 (53)</td>
<td>0.02</td>
</tr>
<tr>
<td>lutein + zeaxanthin</td>
<td>0.07 ±0.05 (113)</td>
<td>0.04</td>
</tr>
<tr>
<td>phytofluene</td>
<td>0.09 ±0.05 (10)</td>
<td>0.05</td>
</tr>
<tr>
<td>ubiquinol 10</td>
<td>0.18 ±0.18 (7)</td>
<td>0.10</td>
</tr>
<tr>
<td>total PUFAS (mean)</td>
<td>2332</td>
<td>1283</td>
</tr>
<tr>
<td>total antioxidants (mean)</td>
<td>14.2</td>
<td>7.8</td>
</tr>
</tbody>
</table>
3. EFFECT OF $\alpha$-TOCOPHEROL ON THE OXIDATION RESISTANCE OF LDL

Oxidation of LDL in vitro with macrophages (14) or Cu$^{++}$-ions (2,11-13) is proceeded by a lag-phase, during which the endogenous antioxidants contained in LDL are consumed with $\alpha$-tocopherol as the first and $\beta$-carotene as the last one. When the LDL is depleted from all antioxidants, lipid peroxidation rapidly accelerates (propagation-phase) and all the PUFAs are quickly oxidized. This sequence of events suggest that the lag-phase and hence the oxidation resistance of LDL is mainly determined by its antioxidant content. The lag-phase can readily be determined by recording the diene vs. time profile as shown in Fig. 1.

To dissociate the effect of vitamin E, from the protective effect of the other antioxidants, LDL from given donors was loaded with $\alpha$-tocopherol, by incubating plasma samples with increasing (100-1000 $\mu$M) concentrations of $\alpha$-tocopherol prior to the isolation of LDL. By that, the $\alpha$-tocopherol of a given LDL could be increased several fold, whilst all other properties of the LDL were likely not affected (12,13). The oxidation resistance of such $\alpha$-tocopherol loaded LDL sample increased always strict linearly ($r^2=0.96$) with the $\alpha$-tocopherol content according to the equation $y=kx+a$, where $y$ is the lag-phase in minutes and $x$ is mol $\alpha$-tocopherol/mol LDL, $k$ is an efficiency constant of $\alpha$-tocopherol and $a$ is a vitamin E independent variable (Fig. 1).

Fig. 1: Determination of the efficacy of $\alpha$-tocopherol to increase the oxidation resistance of LDL.

The LDL samples with different $\alpha$-tocopherol contents were prepared by adding increasing concentrations of $\alpha$-tocopherol to plasma samples of donor A prior to isolation of LDL. The $\alpha$-tocopherol content of isolated LDL was determined by HPLC. LDL (0.25 mg/ml equal 0.1 $\mu$M) in oxygen saturated PBS was oxidized with 1.67 $\mu$M Cu$^{++}$, the curves show the diene vs. time profile.

For donor A the relationship between lag-phase ($y$) and $\alpha$-tocopherol ($x$) is: $y=5.41x + 6.48$ ($r^2=96$).
The determination of the values for the constants $k$ and $a$ in different subjects revealed a very strong individual variation with a mean value $\pm$ SD for $k=4.39 \pm 3.05$ (range 0.7 to 17), and a mean $\pm$ SD value for $a=35.99 \pm 35.86$ minutes (range minus 68.6 to plus 108.6 minutes) (13 and unpublished). This suggests strong individual differences in the protective effects of vitamin E, persons with a large $k$ value are good responders whereas those with a low value respond only marginally. This relationship found for LDL loaded in vitro with vitamin E is also valid for oral vitamin E intake (15). Daily doses of 150, 225, 800 or 1200 IU RRR-$\alpha$-tocopherol over three weeks increased the LDL $\alpha$-tocopherol content to 138 $\pm$ 12, 158 $\pm$ 32, 144 $\pm$ 12 and 215 $\pm$ 47% compared to the baseline values (=100%) and in parallel the oxidation resistance (= lag-phase) increased to 118 $\pm$ 17, 156 $\pm$ 22, 135 $\pm$ 23 and 175 $\pm$ 21% compared to the baseline (=100%). The increase of the lag-phase was linearly correlated ($y=kx+a$) with the $\alpha$-tocopherol content with $r^2$ 0.56 to 0.95. Mino et al. (16) found a strict linear correlation between the lag-phase (T inhibition) and the $\alpha$-tocopherol content of LDL and HDL with AAPH induced oxidation.

The $\alpha$-tocopherol content of a given LDL is, due to the strong individual variation of $k$ and $a$, per se not predictive for the oxidation resistance. However, based on a large number of different LDL samples with different $\alpha$-tocopherol contents (baseline values, supplemented in vitro or by oral intake) for which the oxidation resistance was determined, a statistical prediction is possible (13). We now find a statistical correlation between lag-phase ($y$) and LDL $\alpha$-tocopherol content in mol/mol LDL ($x$) the equation: $y=2.94x+52.4$, $r^2=0.46$, $p<0.001$, $n=206$. In the statistical average with 6.88 mol vitamin E/mol LDL (Table 1) the lag-phase should therefore be 72 min, this agrees very well with the experimentally determined value of 67.5 $\pm$ 15.1 (n=76). Statistically, vitamin E ($\alpha$- + gamma-tocopherol) contributes about 30% to the lag-phase, whereas 70% is due to the vitamin E independent variable. However, it should be emphasized again that some subjects LDL (with low or negative $a$ value) solely depend on the protection by $\alpha$-tocopherol.

4. ANTIATHEROGENIC ACTIVITY OF VITAMIN E AND OTHER ANTIOXIDANTS

The therapeutic potential of vitamin E in treatment of atherosclerosis was recently extensively reviewed by Janero (17). Epidemiological studies in the European population revealed a highly significant inverse correlation between the incidence of ischemic heart disease mortality and the level of plasma $\alpha$-tocopherol (18,19), low levels of $\alpha$-tocopherol and ascorbate appear to be a risk factor in early angina pectoris (20). Gaziano et al. (21) presented preliminary findings from the USA physician study, a group of patients receiving 50mg $\beta$-carotene every second day showed significant reduction of major coronary and vascular events. A number of animal studies were performed until 1982 to test the antiatherosclerotic effect of dietary vitamin E (reviewed in 17), some reported that vitamin E did not reduce diet-induced lesions, whereas other reported a protective effect.

Verlangieri et al. (22) very recently published that vitamin E significantly reduces symptoms of atherosclerosis in primates fed an atherogenic cholesterol diet. Chronic deficiency of vitamin E and C is associated with atherosclerotic like lesion in rodents, pigs and primates (reviewed in 18,19). Vitamin E supplementation of diabetic rats prevents oxidation of LDL in vivo and its conversion to a cytotoxic form (23). A
number studies deal with probucol, a widely used drug for treatment of hypercholesterolemia in men. Animal experiments with WHHL rabbits showed that probucol reduces the extent of lesions to a much larger extent than expected from its cholesterol lowering effect, most likely due to its antioxidant activity (24). LDL isolated from probucol treated rabbits or humans is highly resistant to in vitro oxidation by macrophages or transition metal ions (25). In vitro experiments show that vitamin E and possible other natural or synthetic antioxidants might prevent initiation and progression of atherosclerosis also by effects not related to their chain-breaking antioxidant activity in LDL. For example, physiological concentrations on vitamin E inhibit proliferation of smooth muscle cells (26), provides general protection to endothelial cells and inhibits lipoxygenase activities (reviewed in 17). Much more basic biochemical studies are required to understand the potential benefits of antioxidants in prevention or treatment of atherosclerosis and other diseases.

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5. REFERENCES


