LDL oxidation: therapeutic perspectives

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Abstract

The peroxidation step of lipid transformation is considered to be essential in the pathogenesis of atherosclerosis. Although data concerning the mechanisms by which lipid peroxidation occurs in vivo are scarce, several lines of evidence suggest that some endogenous and exogenous compounds with antioxidant activity could have some beneficial effects in the prevention of atherosclerosis. Ascorbic acid (vitamin C) and α-tocopherol (vitamin E) act as the most important hydrophilic and lipophilic antioxidants, respectively in vivo. Accordingly, animal and human studies suggest that these compounds may have some preventive effect against the development of clinical coronary heart disease. Many plant phenols and flavonoids may be important dietary antioxidants and it has been speculated that these compounds in red wine or in the Mediterranean diet could explain the ‘French paradox’. Several studies show that antioxidants such as probucol and butylated hydroxytoluene can inhibit development of atherosclerotic lesions in Watanabe and cholesterol-fed rabbits. Some drugs such as β-blockers, calcium antagonists, hypolipidemic drugs, appear to have at least in vitro antioxidant effects but the clinical relevance of these properties remains unknown. Moreover, some interventions aimed to decrease the LDL-oxidative susceptibility have not been shown to attenuate atherogenesis when cholesterol levels remain markedly elevated. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Cholesterol accumulation in the arteries is now considered as the direct cause of atherosclerosis. However, oxidation of lipoproteins, particularly of LDL, appears to be of prime importance in initiating the atherogenic process. Thus, if reduction of serum cholesterol level is an essential target, attempts to inhibit or to reduce the oxidation process appear to be quite justified. Studies are now available to get an insight into the relationship between antioxidant properties and prevention of atherosclerosis. It is the aim of this paper to make a survey of compounds that could have some clinical interest.

To assess the degree of lipid oxidation, several techniques are available but it appears that decreased susceptibility of LDL to oxidation seems well related to the presence of the disease [1,2], its severity [3] and its progression [4], at least for coronary heart disease (CHD). In this technique, the isolated LDL are submitted to oxidation by different means and the oxidizability of LDL is estimated by measuring the time before the oxidation products become detectable (the lag time), the rate of oxidation and the maximum amount of oxidation products [5].

As far as natural antioxidants are concerned, vitamin E has been shown to have protective effects against LDL oxidation, but an intake of at least 300-400 mg per day is necessary: in healthy men, the lag phase is significantly increased only when the dosage of vitamin E is higher than 400 mg per day [6]. On the other hand, the intake of p carotene [7] does not increase the lag phase, while vitamin C [8] tends to preserve vitamin E levels during oxidation stress. Concerning the influence of these natural compounds on atherosclerosis, some randomized prevention trials are available. Among those that can be considered as primary prevention trials (Table 1), it can be seen that p carotene either
<table>
<thead>
<tr>
<th>Trials</th>
<th>Study participants</th>
<th>Follow-up period (years)</th>
<th>Antioxidant (daily dose)</th>
<th>Outcomes</th>
<th>Relative risk (95% CI)</th>
<th>Other effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin cancer prevention 1990</td>
<td>1805 patients with a history of skin cancer</td>
<td>8.2</td>
<td>β-carotene 50 mg</td>
<td>Cardiovascular deaths</td>
<td>+15% (NS)</td>
<td>Total mortality -9% (S)</td>
</tr>
<tr>
<td>Lixian studies 1993</td>
<td>29584 Middle-aged adults</td>
<td>5.3</td>
<td>Vit. C 125 mg, β-carotene 15 mg+Vit. E 30 mg+I3enium</td>
<td>Cerebrovascular mortality</td>
<td>+4% (NS)</td>
<td></td>
</tr>
<tr>
<td>ATBC study 1994</td>
<td>29133 Men 50–69 years, smokers</td>
<td>6.1</td>
<td>Vit. E 50 mg, β-carotene 20 mg</td>
<td>Cardiovascular death</td>
<td>-2% (NS)</td>
<td>Hemorrhagic stroke +49% (S), Total mortality +9% (S)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Angina</td>
<td>-3% (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cardiovascular death</td>
<td>+11% (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Angina</td>
<td>+13% (NS)</td>
<td></td>
</tr>
<tr>
<td>Physicians' health study 1996</td>
<td>22071 male physicians 40–84 years</td>
<td>12</td>
<td>β-carotene 50 mg on alternative days</td>
<td>All important cardiovascular events</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Caret 1996</td>
<td>18314 Smokers, former smokers, workers exposed to asbestos</td>
<td>4</td>
<td>β-carotene 30 mg + Vit. A 25,000 UI</td>
<td>Cardiovascular deaths</td>
<td>+26% (NS)</td>
<td>Lung cancer +28% (S)</td>
</tr>
</tbody>
</table>

*Primary prevention trials.*
Table 2
Randomized trials of antioxidants in cardiovascular diseases

<table>
<thead>
<tr>
<th>Trials</th>
<th>Study participants</th>
<th>Follow-up period</th>
<th>Antioxidant (daily dose)</th>
<th>Outcomes</th>
<th>Relative risk (d. CI)</th>
<th>Other effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaziano et al. (1990) [55]</td>
<td>Men with angina, 333</td>
<td>5 years</td>
<td>(a)-carotene 50 mg on alternate days</td>
<td>Myocardial infarction, stroke, CV death</td>
<td>-51</td>
<td></td>
</tr>
<tr>
<td>Demuio et al. (1996) [49]</td>
<td>Patients having angioplasty, 100</td>
<td>4 months</td>
<td>Vitamin E 1200 UI</td>
<td>Restenosis</td>
<td>-30 (P=0.061)</td>
<td></td>
</tr>
<tr>
<td>Chaos (1996) [49]</td>
<td>Patients with angiographically proven CHD, 2002</td>
<td>510 days</td>
<td>Vitamin E 800 UI, Vitamin E 400 UI</td>
<td>Cardiovascular death</td>
<td>-47 (S)</td>
<td>Cardiovascular death (NS)</td>
</tr>
</tbody>
</table>

Secondary prevention trials

alone or associated with vitamin E has no beneficial effect on CHD, while it increases the risk of lung cancer in smokers and total mortality. No sufficient data are available for vitamin C. With vitamin E at low dosage, there is no beneficial effect on CHD while an increased risk of hemorrhagic stroke has been observed. In a subgroup of patients who were smokers and had a previous myocardial infarction, there were significantly more cardiovascular deaths in those who had received combined \(a\)-tocopherol and \(p\) carotene or \(p\) carotene alone [9]. In secondary prevention trials (Table 2), vitamin E, at a high dosage, has been shown to reduce the incidence of restenosis and to reduce the incidence of nonfatal myocardial infarction. Only one trial used \(a\)-carotene: at low dosage and on alternate days, it had a beneficial effect on CHD events, but the decrease was not significant.

Thus, data are consistent with the suggestion that only vitamin E, at least at a dosage \(> 400 \text{ mg per day, could have a beneficial effect. Beyond its antioxidant effect, vitamin E could also act through its action on platelets [10], coagulation factors [11], smooth muscle cells [12] and monocyte adhesion [13].}

The French Paradox emphasizes the fact that France, and to lesser degree Switzerland, have a strikingly low rate of CHD when compared with neighbouring countries with comparable dietary intake and particularly with comparable dairy fat intake [14]. One explanation is that France has a typical Mediterranean-type diet which is characterized by a high level of vitamin E, vitamin C, \(w-3\) fatty acids, oleic acid, flavonoids and garlic. Moreover, we know that the typical French diet usually includes alcohol and wine. We have shown that garlic given as six tablets a day-each of them containing \(190 \text{ mg of dried garlic- to 12 healthy subjects for 2 months leads to a 10.5% reduction in total cholesterol and a 15% reduction in triglyceride concentration (Heller, unpublished data). A previous work made by (helps and Harris [11]) had shown that the susceptibility if apo B containing lipoproteins to oxidation was significantly decreased by 40% in healthy volunteers. In our work, the LDL resistance to copper oxidation tended also to increase but the difference, although nearly significant, was very small and of doubtful clinical significance. Although alcohol cari have some beneficial effects on HDL or platelet aggregation and fibrinolysis, in itself it has prooxidant activity. However, beverages containing alcohol could have more additional effects. Indeed, CRIQUI [16] showed, in an epidemiological analysis of 21 developed countries that wine more than alcohol intake was inversely correlated with CHD. Actually, wine contains > 100 phenolic compounds known to have antioxidant effects and Frankel has shown that phenolic compounds extracted from Californian red wine cari inhibit the copper induced LDL oxidation more than vitamin E [17]. Moreover, there is a significant relationship between the phenolic content of beverages and the capacity of these beverages to inhibit LDL oxidation. The phenolic content is strikingly higher in red wine than in white wine or beers [18]. White wine has no effect or sometimes increases the LDL susceptibility to oxidation, while red wine has been found to protect LDL against oxidation in vitro and sometimes in vivo. Differences between studies cari be related to the technique used and to the wine region [19-22].

Most hypolipidemic drugs used today has been shown to have antioxidant activity. Probucol cari prevent or retard the development of atherosclerosis in several animal species, although in humans, the PQRS study showed that it has no effect on femoral atherosclerosis. The antioxidant activity of probucol has been clearly demonstrated in vitro and in vivo. That its antioxidant activity could influence the process of atherogenesis is suggested by three facts. First, when control WHHL rabbits are compared with WHHL rabbits, with serum levels of cholesterol made similar to those treated by probucol and WHHL rabbits treated by probucol. the extent of lesion formation is reduced significantly in rabbits receiving probucol.
Table 3
WHHL rabbits and antioxidant therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Progression of atherosclerotic involvement (%)</th>
<th>Plasma total cholesterol (mg/dl)</th>
<th>Resistance to LDL oxidation</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probucol</td>
<td>-74</td>
<td>444</td>
<td>—</td>
<td>Nagano (1992)</td>
</tr>
<tr>
<td>Probucol</td>
<td>-70</td>
<td>560</td>
<td>x 15</td>
<td>Pol Mao (1991)</td>
</tr>
<tr>
<td>Probucol</td>
<td>-65</td>
<td>671</td>
<td>—</td>
<td>Carew (1987)</td>
</tr>
<tr>
<td>Probucol</td>
<td>-54</td>
<td>345</td>
<td>—</td>
<td>Nagdo (1992)</td>
</tr>
<tr>
<td>Probucol</td>
<td>-50</td>
<td>-581</td>
<td>x 8.8</td>
<td>Fruebis (1994)</td>
</tr>
<tr>
<td>MDL-29, 3 1</td>
<td>-35</td>
<td>731</td>
<td>x 5.2</td>
<td>Fruebis (1994)</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>-32</td>
<td>618</td>
<td>—</td>
<td>Williams (1992)</td>
</tr>
<tr>
<td>Vit. E</td>
<td>-32</td>
<td>344</td>
<td>X2</td>
<td>Fruebis (1994)</td>
</tr>
<tr>
<td>BMI 150639</td>
<td>0</td>
<td>-745</td>
<td>X 3.3</td>
<td>Kleinvel (1995)</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0</td>
<td>917</td>
<td>X 2-4</td>
<td>Kleinvel (1995)</td>
</tr>
<tr>
<td>Vit. E = Oleic acid</td>
<td>-0</td>
<td>983</td>
<td>X 2-4</td>
<td>[23]</td>
</tr>
</tbody>
</table>

[23]. Secondly, in cholesterol-fed monkeys, the extent of the lesions is reduced by probucol and the extent of the reduction is significantly inversely correlated with the increase of the lag time [24]. Thirdly, a probucol analogue and other compounds, which do not affect cholesterol levels but have antioxidant activity also reduce significantly the lesions in WHHL rabbits [25].

However, when studies conducted in WHHL rabbits treated by hypolipidemic drugs or antioxidants are classified according to the degree of the reduction in atherosclerotic involvement (Table 3), it can be seen that a higher reduction can be observed when the resistance to LDL oxidation is increased, but on the other hand, a high reduction rate is also associated with lower plasma total cholesterol concentration. Thus, it is unlikely that compounds with antioxidant activity will have significant effects in patients with very high levels of cholesterol, at least if used without the association of hypolipidemic drugs.

Statins have been recently recognized as useful drugs for primary and secondary prevention of CHD. Their action is clearly related to their potent hypocholesterolemic action. Nevertheless, other interesting properties have been recently recognized including an increased resistance to LDL oxidation. When analyzing the susceptibility of LDL to copper-induced oxidation in hypercholesterolemic subjects, both pravastatin and simvastatin decrease the rate and the total amount of dienes produced during the reaction with no difference between drugs [26]. In hypercholesterolemic patients, fluvastatin might have the most potent effect among statins [27]. Recently, we have analyzed the action of atorvastatin in patients with mixed hyperlipidemia. The drug was given to eight patients at a daily dosage of 10-20 mg for 2 months. The lag time tended to increase and the maximum production of dienes tended to decrease. However, the difference was small and not significant (Heller, unpublished data).

Very few data concerning the fibrates are available. In hypercholesterolemic patients, it has been shown that bezafibrate is more active than pravastatin in reducing the susceptibility of LDL oxidation [28]. Moreover, in diabetics, De Leeuw and Van Gaal have found that fenofibrate, but not pravastatin or simvastatin, can reduce the oxidizibility of LDL and of VLDL [29].

β adrenergic blocking agents have also been shown to have beneficial effect on atherosclerosis. In animals, they retard the atherogenic process and in humans, β-blockers are effective particularly in secondary prevention, as they reduce all cause and coronary mortality by 20%. Several mechanisms of action have been suggested including an antioxidant action. All β-blockers have in vitro antioxidant activity which appears to be related to their degree of lipophilicity. In patients with CHD, Croft and coworkers showed that, while the lag time in patients with CHD is not significantly different from controls, in patients with CHD who are taking β-blockers, the lag time is higher than that observed in patients who are not taking β-blockers [30]. When LDL are oxidized in vitro by copper or by macrophages, carvedilol, the most lipophilic β-blocker appears more potent than pindolol, labetolol, atenolol and propranolol and this is confirmed in vivo [31].

ACE inhibitors have been shown to have a beneficial effect in atherosclerosis. They reduce the progression of the disease in animals. In patients with myocardial infarction, their use has been associated with a reduced
recurrence of myocardial infarction of +25%. These beneficial effects of ACE inhibitors have been related to several mechanisms of action, but here too, an antioxidant activity against LDL oxidation has been demonstrated. In vitro, the lag time was found to be clearly increased by the presence of captopril at concentrations close to those that can be achieved therapeutically with large doses. A similar effect is observed with N-acetylcycteine which contains like captopril, a sulphhydril group. In the same study, quinapril, which lacks the sulphhydril group, had no antioxidant activity [32]. In vivo, Aviram and coworkers have shown that the propensity of LDL to oxidation is increased in patients with hypertension and is positively correlated with the blood pressure. Giving captopril or enalapril for 3 weeks decreases the oxidizibility of LDL. That suggests that the sulphhydril group, which is absent in enalapril, does not have any influence on the resistance of LDL oxidation [33]. Actually, the same group gave data suggesting that the antioxidant activity might be related to the decreased production of angiotensin-II (A-II) as A-II appears to increase the LDL oxidation by macrophages [34]. Losartan, which is a specific inhibitor of AT-1 receptors of angiotensin-II, would have the same action, but in our preliminary study no significant change was observed in patients with hypertension receiving this drug (Heller and Descamps, unpublished data).

Calcium antagonists inhibit the development of atherosclerosis in most animals. In humans, these drugs seem to prevent the development of new coronary lesions, although their effect on myocardial infarction is equivocal. All these calcium antagonists are potent antioxidants in vitro and this property is probably related to their interaction with the lipid bilayer of the membranes. Lacidipine has the highest degree of interaction with the membrane [35]. We have found that lacidipine inhibits the LDL oxidation produced by several oxidants. The inhibition is dose related and observed at concentrations found in patients treated with this drug (Heller and Descamps, unpublished data).

Prospective observational studies have suggested that postmenopausal women who take oestrogens have a lower rate of cardiovascular events than untreated women. Several mechanisms have been suggested including an antioxidant action. In vitro, estradiol protects the LDL against oxidation. The effect is dose-related. Estradiol is more potent than estriol, estrone and testosterone [36] and the effect is dependent on the conversion of estradiol into fatty acid esters [37]. Very recently, it has been shown that in postmenopausal women, transdermal oestriadiol, alone or in combination with medroxyprogesterone acetate, significantly increases the lag time of LDL oxidation, while conjugated oestrogens have no effect [38].

Troglitazone has been shown to be effective as a hypoglycemic agent by reducing the insulin resistance and by altering the hepatic glucose metabolism. Its structure is somewhat similar to vitamin E. In the presence of troglitazone, the lag phase is increased when LDL are oxidized by copper [39]. The antioxidant action can be observed also in vivo as, when the drug is given at a dosage of 400 mg per day to healthy subjects, the lag phase increased already by 1 week, while in subjects receiving placebo, the lag phase is not significantly changed.

Dipyridamole inhibits platelet aggregation and induces vasodiation. It has been proved to delay the progression of peripheral occlusion arterial disease and recently in the ESPS [40], it has been shown that dipyridamole can decrease the occurrence of ischemic events. Dipyridamole has a potent antioxidant action in vitro: the lag phase is increased by dipyridamole and the increase is significantly correlated with the concentration of the drug. It appears to be superior in vitro to probucol and vitamin E (Descamps and Heller, unpublished data).

2. Conclusions

Several drugs and compounds have been shown to have an antioxidant effect on LDL. However, firstly, it is still unproved that this biological effect plays a significant role in retarding the atherogenic process. Secondly, among these compounds, the use of vitamin E at a dosage of at least 400 mg pet day, is the most promising. Vitamin E does not seem to have serious toxicity, although it could have a pro-oxidant effect in some conditions, particularly in situations of mild oxidation in the absence of water-soluble antioxidants [41]. Clearly, we need more trials with vitamin E. Thirdly, imbalance between production of reactive oxygen species and antioxidant defenses can occur very easily in vivo. This situation of oxidation stress can significantly contribute to the development of atherosclerosis and must be kept in control. If antioxidants could be very useful, reducing the production of oxidants, for example by stopping tobacco smoking, might remain one of the most useful therapeutic agents for preventing atherosclerosis. The cholesterol controversy lasted for many years before we accepted that plasma cholesterol levels were important in CHD. It would be not very surprising if the 'antioxidant controversy' were to last at least as long.
References


