Inhibition of Oxidative Modification of Low Density Lipoprotein by Antioxidants

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Summary Oxidation of LDL induced by free radicals proceeds by a chain mechanism to give phosphatidylcholine hydroperoxides and cholesteryl ester hydroperoxides as the major primary products. In addition, apolipoprotein B100 is also oxidized. Various antioxidants suppress the oxidative modification of LDL. Water-soluble radical-scavenging antioxidants such as vitamin C and uric acid act as the first defense to suppress the chain initiation. Lipophilic radical-scavenging antioxidants in LDL such as vitamin E and ubiquinol scavenge radicals attacking from outside and also within the LDL. The overall importance and potency of antioxidants depend not only on chemical reactivity but also on the physical factors such as location and mobility at the microenvironment in LDL.

Key Words free radical, oxidation, LDL, antioxidant, vitamin E, ubiquinol, vitamin C

INTRODUCTION

An increasing amount of experimental and epidemiological evidence implicates the involvement of free radicals in the pathogenesis of various diseases (1). Among others, the importance of low-density lipoprotein (LDL) oxidation in atherogenesis has received much attention (2, 3). The oxidatively modified LDL is taken up via a scavenger receptor of macrophages (4–7). This internalization by macrophages is not under control of cholesterol-dependent down regulation. By the resultant accumulation of cholesterol, the macrophages develop into cells with the appearance of foam cells (8–10). The finding of massive accumulation of foam cells in early atherosclerotic lesions (9, 11) supports the hypothesis that the unlimited uptake of the oxidatively modified LDL by macrophages is involved in the development of atherosclerosis in vivo.

Aerobic organisms are protected against oxidative stress by an array of defense systems (12). As shown in Fig. 1, the preventive antioxidants such as peroxidases and metal chelating proteins suppress the generation of free radicals which initiate the oxidative damage. Radical-scavenging antioxidants such as
Fig. 1. Defense systems by antioxidants against oxidative damage in vivo.

vitamin C and vitamin E suppress the chain initiation and/or break the chain propagation. The repair and de novo enzymes such as phospholipases and transferases repair the damage and reconstitute the membranes and DNA. Furthermore, so called adaptation function induces the specific antioxidant and transfers it to the right site when required. These antioxidants function not independently but cooperatively or even synergistically.

OXIDATION IN BLOOD AND THEIR INHIBITION

Blood contains a number of versatile antioxidants which protect lipoproteins, blood cells and endothelial cells from oxidative damage. Ingold and his colleagues (13) measured the total radical-trapping capacity of antioxidants in human plasma and found that their contributions were 35–65% from urate, 0–24% from ascorbate, 5–10% from vitamin E, and 10–50% from plasma proteins. According to Stocker et al. (14) the antioxidants reacted with aqueous peroxyl radicals in the order of ascorbate > albumin-bound bilirubin > urate. Vitamin C acts as the primary defense against the aqueous peroxyl radicals in the whole blood (15) or plasma (16), and is consumed most rapidly.

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The relative importance of radical-scavenging antioxidants is determined not only by their inherent chemical reactivities and concentrations but also by their location and the reactivity and location of the attacking radicals. There are both hydrophilic and lipophilic radical-scavenging antioxidants in the blood. As mentioned above, vitamin C is a potent radical-scavenging antioxidant and its concentration in the plasma is relatively high, 30–150 nmol/ml. Uric acid is also an important radical-scavenging antioxidant (17, 18) and its concentration in the plasma is even higher, 160–450 nmol/ml. These antioxidants scavenge aqueous radicals before the radicals attack lipoproteins and blood cells. However, they can not scavenge lipophilic radicals efficiently within the lipophilic compartment. For example, neither vitamin C (19) nor uric acid (20) can scavenge radicals within the liposomal membranes. Also as illustrated in Fig. 2, plasma can suppress the oxidation of liposomal membranes induced by aqueous radicals but it could not suppress the oxidation initiated by lipophilic radicals generated in the liposomal membranes. Similar findings were observed for LDL (21). These findings suggest that the water-soluble radical-scavenging antioxidants can inhibit the chain initiation by aqueous radicals, but that they can not break the chain propagation taking place within the LDL. This also implies that the lipophilic antioxidants are required in LDL to effectively suppress its oxidative modification.

The activity of attacking radicals is also important in determining the role of the antioxidants. The more reactive the attacking radicals become, the less...
selective it is, and, in such a case, the concentration of antioxidants may become a more important factor than its reactivity toward the radical. Hence, for example, uric acid may well play a more important role than vitamin C against hydroxyl radicals in the plasma.

OXIDATION OF LDL AND ITS INHIBITION

LDL has a heterogeneous structure: phosphatidylcholine (PC) and free cholesterol compose an outer monolayer, while cholesteryl ester and triglyceride form a core. A large protein termed apolipoprotein B-100 is embedded in the outer layer. The oxidation of LDL induced by free radicals proceeds by a chain mechanism and gives cholesteryl ester hydroperoxides and phosphatidylcholine hydroperoxides as the major primary products (21). Conjugated diene and thiobarbituric acid reactive substances (TBARS) are also formed (3, 22). In addition to lipids, apoprotein B is also oxidized and both crosslinking and cleavage take place (3). Furthermore, the negative charge of the protein is also increased (23, 24). Figure 3 shows an example of the oxidation of human LDL induced by cupric chloride. The addition of cupric chloride to the aqueous suspensions of LDL initiates the oxidation and conjugated diene, cholesteryl ester hydroperoxides, phosphatidylcholine hydroperoxides, and TBARS were formed. The increase in relative electrophoretic mobility, that is, the negative charge of apoprotein B, was also observed. The endogenous vitamin E was consumed linearly with time and when it was depleted, oxidation proceeded rapidly.

![Fig. 3. Oxidation of human LDL (0.25 mg protein/ml) induced by 2 μM CuCl₂ at 37°C under air in phosphate buffered saline, pH 7.4. Oxygen uptake (●) was measured with an oxygen electrode, and formation of phosphatidylcholine hydroperoxide (●) and cholesteryl ester hydroperoxide (○) and consumption of endogenous α-tocopherol (▲) (initial concentration: 1.1 μM) were followed with HPLC. Conjugated diene (+), thiobarbituric acid reactive substances (TBARS, △) and relative electrophoretic mobility (REM, ■) were measured as described in the literature (21, 37). J. Nutr. Sci. Vitaminol.](image-url)
Table 1. Lipophilic radical-scavenging antioxidants in human LDL (3)

| Antioxidants         | nmol/mg LDL | mol/mol LDL |
|----------------------|-------------|-------------|
| Ubiquinol-10         | 0.18        | 0.10        |
| α-tocopherol         | 11.58       | 6.37        |
| γ-tocopherol         | 0.93        | 0.51        |
| β-carotene           | 0.53        | 0.29        |
| α-carotene           | 0.22        | 0.12        |
| Lycopene             | 0.29        | 0.16        |
| Cryptoxanthin        | 0.25        | 0.14        |
| Cantaxanthin         | 0.04        | 0.02        |
| Lutein + zeaxanthin  | 0.07        | 0.04        |
| Phytolene            | 0.09        | 0.05        |

Probably, the water-soluble radical-scavenging antioxidants act as the primary defense against LDL oxidation. However, as mentioned above, if the radicals are formed initially within LDL or once the radicals attack the lipids, then the lipophilic radical-scavenging antioxidants are responsible for the inhibition of oxidative modification. LDL contains several lipophilic radical-scavenging antioxidants (Table 1) (3). α-Tocopherol is by far the most abundant and most active antioxidant in LDL. Ubiquinol is contained much less than α-tocopherol, only 0.1 molecule on average per 1 LDL particle, and its reactivity toward the peroxyl radical is smaller than α-tocopherol (25). However, ubiquinol-10 was depleted much faster than α-tocopherol in the oxidation of LDL induced by aqueous or lipophilic peroxyl radicals (26) or copper (27). This may arise from the direct scavenging of oxygen radicals by ubiquinol, its autoxidation and/or the reduction of the vitamin E radical by ubiquinol. It is unlikely that ubiquinol scavenges oxygen radicals much faster than α-tocopherol. It may be noteworthy that, although the rate of depletion of ubiquinol is much faster than that of α-tocopherol, the absolute amount of the α-tocopherol reacting can be larger than that of ubiquinol.

As shown in Table 1, α-tocopherol is the most abundant, and chemically most active, antioxidant in LDL. Esterbauer and his colleagues found that the oxidation of polyunsaturated lipids in LDL occurred only after a significant drop in vitamin E (28), and Jessup et al. (29) showed that oxidative modification of LDL by cultured macrophages or Cu(II) ions did not occur unless it was depleted of its vitamin E. Oral supplementation with α-tocopherol increased both the vitamin E content of LDL and its resistance to oxidation (30) and also the supplementation of culture media with vitamin E prevented oxidative modification of LDL by cells (6). The epidemiological studies also show that the incidence of ischemic heart disease mortality inversely correlates with the level of plasma vitamin E (31). On the other hand, the content of vitamin E was
not fully correlated with the oxidizability or resistance of the LDL (22). Gebicki et al. (32) studied the oxidation of LDL induced by γ-irradiation and observed no correlation between the initial levels of vitamin E in LDL and its oxidizability.

We found that α-tocopherol did not suppress the oxidation of LDL as efficiently as it inhibited the oxidation of methyl linoleate in homogeneous organic solution. We have previously found by a study using a spin probe that the efficiency of scavenging radicals by α-tocopherol in the liposomal membrane decreases as the radical penetrates deeper into the interior of the membrane (33). Similar results were observed with LDL. The spin probes, N-oxyl-4, 4-dimethyloxazolidine derivatives of stearic acid, having a nitroxide group at different positions of stearic acid incorporated into human LDL acted as an antioxidant and they were consumed as the oxidation proceeded. It was spared by endogenous α-tocopherol and antioxidant added exogenously. The spin probe 5-NS, where the nitroxide group was attached at the fifth carbon from the surface was spared efficiently by α-tocopherol, but 16-NS, where the nitroxide group was attached at the 16th carbon was not spared appreciably by α-tocopherol. These findings suggest that the vertical mobility of α-tocopherol is restricted, which makes its antioxidant activity in LDL smaller than that in a homogeneous solution.

Carotenoids are weak radical-scavenging antioxidants and they are consumed after most of the ubiquinol and vitamin E are depleted (34). The location and function of carotenoids in LDL have not been fully elucidated yet.

Probucol is well-known as a drug which prevents atherogenesis by acting as an antioxidant and suppressing the oxidative modification of LDL, in addition to its recognized effects of lowering cholesterol levels (35). Probucol has much

Fig. 4. Consumption of antioxidant (IH) during the oxidation of soybean PC (2.80 mM) liposomes induced by (A) water-soluble radical initiator AAPH (1.0 mM) and (B) lipophilic radical initiator AMVN (0.5 mM) at 37°C in air. α-Tocopherol (△, 2.5 μm) and ubiquinol (□, 3.0 μm) were incorporated into liposomal membranes, while ascorbic acid (○, 10 μm) was added into the aqueous phase. The consumption of antioxidant and formation (●) of phosphatidylcholine hydroperoxides were followed by HPLC.

_J. Nutr. Sci. Vitaminol._
smaller chemical reactivity toward oxygen radicals than \( \alpha \)-tocopherol but it suppresses the oxidation of LDL better than \( \alpha \)-tocopherol (36, 37). These findings suggest that the antioxidant potency against LDL oxidative modification is dependent more on physical factors than on the chemical reactivity of the antioxidant.

As mentioned above, the radical-scavenging antioxidants act in vivo not individually but rather cooperatively or even synergistically with each other. For example, it is known that vitamin C and ubiquinol reduce the vitamin E radical to regenerate vitamin E and also to inhibit the chain initiation by the vitamin E radical (38). As shown in Fig. 4, the relative importance of vitamin C and ubiquinol depends on the site of initial radical generation.

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