Inhibition of Human Plasma ApoB-Lipoproteins by Astaxanthine

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The effects of astaxanthine and β-carotene on the oxidation of isolated human plasma apoB-lipoproteins induced by copper ions or hemin/hydrogen peroxide are studied. Astaxanthine inhibits the formation of both primary (diene conjugates) and secondary (thiobarbituric acid-reactive substances) products of lipid peroxidation. Antioxidant activity of astaxanthine is observed in the concentration range 20-100 μg/mg protein of apoB-lipoproteins. The antioxidant activity of astaxanthine is higher than that of β-carotene.

Key Words: *astaxanthine; β-carotene; antioxidants; hemin; apolipoprotein B-containing lipoproteins*

Oxidative modification of atherogenic lipoprotein (LP) particles may accelerate atherosclerosis [13,14]. This prompts the search for new antioxidants capable of protecting atherogenic apoB-LP from oxidation by active oxygen forms and various intermediate radicals.

The naturally occurring derivative of β-carotene astaxanthine (3,3'-dihydroxy-β,β-carotene-4,4'-dion) is present in krill, crustaceans, and salmon muscles and caviar [8]. Astaxanthine protects red yeasts (*Phaffia rhodozyma*) [11] and algae [5,9] from oxidative stress, inhibits lipid peroxidation (LPO) in the mitochondria and erythrocytes, prevents CCl_4- or ^{60}Co-induced LPO in rat liver microsomes [10].

Astaxanthine has a broad spectrum of therapeutic activity: it is effective against xerophthalmia in rats and prevents vitamin A deficiency in some fishes [6], modulates the immune response of human peripheral blood mononuclear cells [7], and influences proliferation of some cell types in mice. However, it is unclear whether astaxanthine is effective against atherosclerosis. In the present study we compared the effectiveness of astaxanthine and β-carotene in the prevention of oxidative destruction of lipids of apoB-lipoproteins isolated from human plasma.

MATERIALS AND METHODS

Plasma (5 mM EDTA) was prepared from blood of healthy donors collected after a 12-h fast. Astaxanthine and β-carotene were conjugated with LP by the method [4]. Astaxanthine or β-carotene in dimethylsulfoxide (<2% v/v) was incubated with plasma for 30 min at 37°C.

ApoB-LP were precipitated by citrate buffer (pH 5.12) in the presence of heparin. The precipitate was washed three times with 5 mM HEPES buffer (pH 7.4) containing 20 mM NaCl, 4 mM $CaCl_2$, and 2 mM $MgCl_2$ and dissolved in 100 mM phosphate-buffered saline (PBS, pH 7.4, 4% NaCl). In some samples, washing was replaced by dialysis against 150 mM PBS (pH 7.4). The antioxidant content in apoB-LP was determined after repeated precipitation with polyethylene glycol (6 kD) [3]. The concentration of astaxanthine in apoB-LP precipitates was measured by extinction at 474 nm ($A^{1\%}_{1\,cm}$ =2100) after extraction with acetone:hexane:water mixture (equal

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volumes). The protein content was determined by the method [2].

Lipoproteins were oxidized with copper ions (20 mM $CuSO_4$ per 0.6 mg protein, 6-h incubation at 37°C) or hemin/H_2O_2 system (1.2 ml incubation mixture contained 0.8 ml 0.1 M PBS, pH 7.4, 0.01 mg apoB-LP, 20 μM hemin in 20 mM NaOH, and 286 or 860 μM H_2O_2, 2 h-incubation at 37°C).

The reaction was stopped by the addition of 50 μl 4 mM Na_2-EDTA and 10 μl 4 mM butylated hydroxytoluol (BHT). The kinetics of the diene conjugate formation was recorded in a Hitachi-557 spectrophotometer at 234 nm. The content of thiobarbituric acid reactive substances (TBARS) was determined as described [2]. Measurements were carried out at 532 nm against control preparation of LP, buffer, BHT, and Na_2-EDTA. The TBARS content was expressed in nM malonic dialdehyde (MDA, $\varepsilon_{532}=1.56\times10^5$ $M^{-1}cm^{-1}$) [15] and calculated per milligram protein apoB-LP.

Na_2-EDTA and thiobarbituric acid were from Serva, astaxanthine was from Hoffman—La Roch, hemin and BHT were from Sigma, other reagents were Russian-manufactured of the highest chemical grade.

RESULTS

More than 80% astaxanthine added to blood plasma was conjugated with apoB-LP, the conjugates being preserved both after dialysis and repeated precipitation. In the studied concentration range almost all astaxanthine bound to isolated LP.

The degree of oxidation of atherogenic LP was assessed by accumulation of primary (diene conjugates) and secondary (TBARS) LPO products. Antioxidant activity of astaxanthine was studied upon oxidation of LP by Cu^{2+} and hemin/H_2O_2. Hemin is a physiologic prooxidant which binds to LP and induces lipid oxidation in the presence of H_2O_2. At a low H_2O_2 concentration (286 μM), astaxanthine (10-60 μg/mg protein) inhibited MDA formation by 30-40% (Fig. 1). At 100 μg/mg protein, astaxanthine almost completely inhibited the formation of MDA and diene conjugates in apoB-LP (Fig. 2, *1*).

An increase in the H_2O_2 concentration to 860 μM led to an opposite effect: the antioxidant activity of astaxanthine decreased considerably probably due to its degradation.

At 20 μM, copper ions oxidized apoB-LP to the same degree as hemin/286 μM H_2O_2. However, in this case astaxanthine inhibited a lower antioxidant activity (Fig. 3). This may be due to certain specific features of LP oxidation in different systems. Since carotinoids react with hydroxyl radical (OH·), which

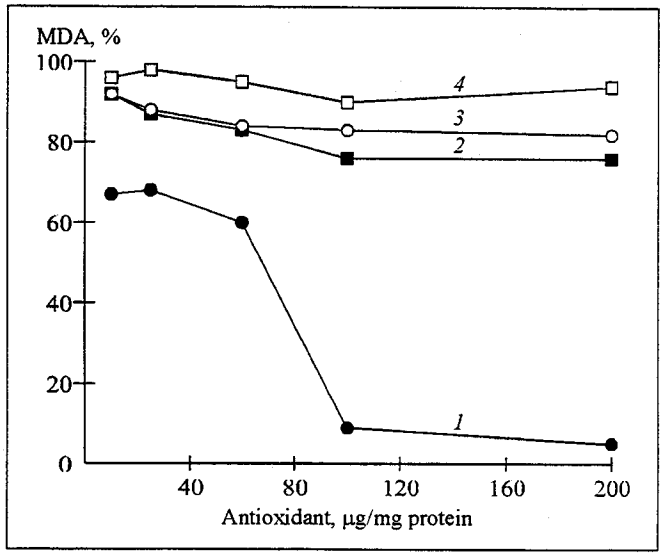

Fig. 1. Oxidation of apoB-LP in hemin/H_2O_2 system in the presence of astaxanthine or β-carotene. Astaxanthine: 286 (*1*) and 860 μM H_2O_2 (*2*); β-carotene: 286 (*3*) and 860 μM H_2O_2 (*4*). The MDA content measured upon oxidation of apoB-LP without the antioxidants is taken as 100%.

can be generated in hemin/H_2O_2 system [8], it is plausible to suggest that the high inhibitory effect of astaxanthine in hemin/H_2O_2 system is due to the localization of hemin and, consequently, of the sites of $OH^{\cdot}$ generation on the surface of an LP particle. This is consistent with the highly effective protection of lipid bilayer by astaxanthine upon initiation of a photosensibilizer-induced oxidation in aqueous phase [12]. Presumably, another mechanism operates upon oxidation of LP by copper or iron released from hemin in the presence of high concentration of H_2O_2, which may account for the low antioxidant activity

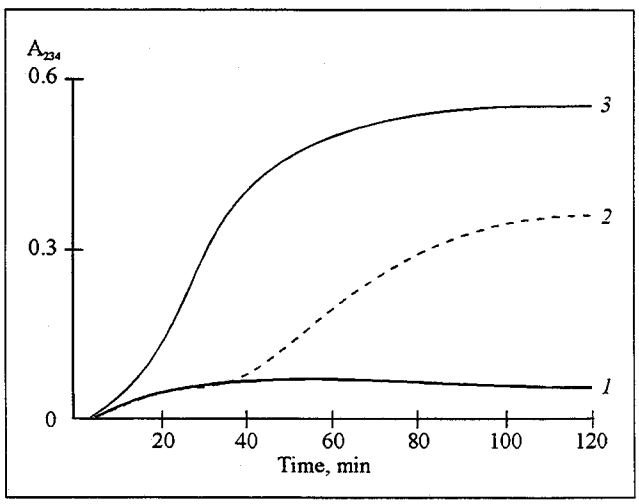

Fig. 2. Kinetics of diene conjugate accumulation upon oxidation of apoB-LP in hemin/286 mM H_2O_2 system. *1*) LP containing 100 μg astaxanthine/mg protein; *2*) LP containing 100 μg β-carotene/mg protein; *3*) LP containing no oxidants.

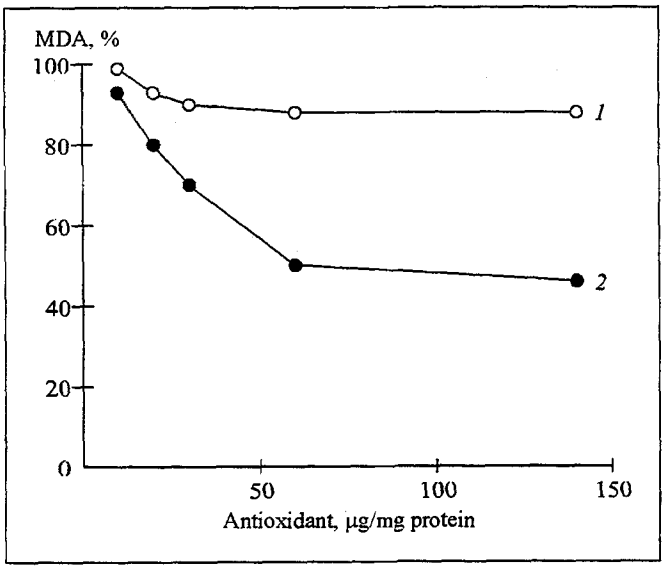

Fig. 3. Effect of β-carotene (*1*) and astaxanthine (*2*) on oxidation of apoB-LP by copper ions (20 μM).

of astaxanthine. It should be noted that oxidation with both copper and iron ions is absolutely non-physiological.

Comparison of the antioxidant activities of astaxanthine and β-carotene showed that β-carotene is a weak inhibitor of MDA formation in both oxidative systems (Fig. 1 and 3). The maximum inhibition of apoB-LP oxidation (20%) with hemin/286 mM H_2O_2 system was observed at 100-200 μg β-carotene/mg LP protein. Prolongation of lag-period was observed at 100 μg β-carotene. These observation agrees with the literature data on the higher antioxidant activity of astaxanthine [16]. However, antioxidant activity of carotinoids varies in different oxidative systems and depends on the presence of other antioxidants in an LP particle [4].

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