Note

Effect of L-Ascorbic Acid on the Oxidative Modification of Apolipoprotein E in Human Very-Low-Density Lipoprotein

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(Received April 17, 2003)

Summary Antioxidant activity of L-ascorbic acid (AsA) against oxidative modification of apolipoprotein (apo) E in human very-low-density lipoprotein (VLDL) was investigated. The VLDL oxidation induced by peroxy radicals and peroxyxynitrite led to lipid peroxidation and oxidative modification of apoE. The binding activity of apoE to heparin was decreased by the oxidative modification. AsA (200, 500, and 1,000 μM) inhibited both the lipid peroxidation and the oxidative modification of apoE. These results suggest that AsA prevents the biological function of apoE in VLDL from the oxidation by free radicals.

Key Words apolipoprotein E, L-ascorbic acid, oxidation, radicals, very-low-density lipoprotein

A number of studies have suggested that the oxidation of low-density lipoprotein (LDL) may contribute to the onset of atherosclerosis in humans (1–4). In the previous paper, Mohr and Stocker (5) have described the mechanism for lipid peroxidation of very-low-density lipoproteins (VLDL), a precursor of LDL. We have also referred to the mechanism of the oxidative modification of apolipoprotein (apo) E (6), which regulates VLDL metabolism by acting as the ligands for the LDL receptor and glycosaminoglycan along the LDL receptor-related protein pathway (7, 8).

L-Ascorbic acid (AsA) is recognized to be a potential water-soluble antioxidant existing in human blood (9). In this paper, we study the effects of AsA on the lipid peroxidation and the oxidative modification of apoE in VLDL in vitro.

3-Morpholiniosydnonimine (SIN-1), a peroxyxynitrite generator (10), was purchased from Dojindo Laboratories (Kumamoto, Japan). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH), a peroxy radical generator, and AsA were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of reagent grade. VLDL ($d < 1.006 \text{ g/mL}$) was centrifugally separated from the fresh plasma withdrawn 12 h after fasting from a healthy volunteer with fully informed consent (11). VLDL (100 μg protein) in Tris-HCl-buffered saline (TBS; pH 7.4) was oxidized by AAPH (5 mM) or SIN-1 (1 mM) at 37°C in the presence

of AsA. Cholesteryl ester hydroperoxides (CE-OOH) were determined by UV measurement with a high-performance liquid chromatograph (HPLC; Hitachi, Japan) as described previously (12). ApoE in VLDL was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% acrylamide slab gel (13), followed by Western blotting with goat anti-apoE antisera (Chemicon International, Temecula, CA, USA) as the primary antibody and peroxidase conjugated rabbit anti-goat IgG as the secondary antibody (14). For evaluation of the heparin binding activity of apoE, VLDL previously oxidized for 12 h was incubated with 25 mg of heparin Sepharose CL-6B (Amersham Pharmacia Biotech Co., England) for 3 h at 4°C, and then was washed 10 times with 1 mL of TBS containing 0.05% Tween 20. ApoE dissociable from heparin-Sepharose with an SDS-containing eluent was further subjected to SDS-PAGE and characterized by immunoblotting in the manner above-mentioned.

Figure 1 shows the effect of AsA on lipid peroxidation in VLDL. CE-OOH accumulated during VLDL oxidation by AAPH (A) or SIN-1 (B) in the absence of AsA. The concentration of AsA in human blood plasma has been reported to range from 30 to 150 μM (9). In both oxidation systems, the accumulation of CE-OOH was suppressed by AsA at a physiological concentration or more of 200, 500, and 1,000 μM . Figure 2 shows the effect of AsA on the oxidative modification of apoE in VLDL. After the oxidation of VLDL for 12 h by AAPH (A) or SIN-1 (B) in the absence of AsA, apoE was detected as a smear band (lane 2), whereas intact apoE was detected at 34 kDa (lane 1) by immunoblot analysis. The oxidative modification of apoE in VLDL by AAPH (A) or SIN-1 (B) was moderately avoided by the coexistence of AsA at concentrations of 200, 500, and 1,000 μM (lanes 3–5).

The binding sites of apoE to the LDL receptor or hep-

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Abbreviations: apo, apolipoprotein; AsA, L-ascorbic acid; AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; CE-OOH, cholesteryl ester hydroperoxides; LDL, low-density lipoprotein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS, Tris-HCl-buffered saline; VLDL, very-low-density lipoprotein.

Fig. 1. Effect of L-ascorbic acid (AsA) on lipid peroxidation in human plasma very-low-density lipoprotein (VLDL). VLDL (100 μg of protein) was oxidized by 5 mM 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (A) and by 1 mM 3-morpholinosydnnonimide (SIN-1) (B) in 500 μL of Tris-HCl-buffered saline (pH 7.4) at 37°C. AsA was added to VLDL before the oxidation. Cholesteryl ester hydroperoxides were determined by reversed-phase high-performance liquid chromatography. The concentration of AsA was as follows: none (●), 200 μM (△), 500 μM (□), 1,000 μM (▽).

Fig. 2. Effect of AsA on oxidative modification of apoE in VLDL. VLDL was oxidized over a period of 12 h by AAPH (A) or by SIN-1 (B) as described in Fig. 1. The oxidized VLDL samples were subjected to immunoblot analysis using the anti-apoE antibody. Lane 1, intact VLDL; lane 2, VLDL oxidized in the absence of AsA; lane 3, VLDL oxidized in the presence of 200 μM AsA; lane 4, VLDL oxidized in the presence of 500 μM AsA; lane 5, VLDL oxidized in the presence of 1,000 μM AsA.

Fig. 3. Heparin-binding activity of apoE in oxidized VLDL. VLDL previously oxidized for 12 h was incubated with 50 mg of heparin-immobilized Sepharose CL-6B for 3 h at 4°C. The heparin-binding fraction was subjected to immunoblot analysis. Lane 1, intact VLDL; lane 2, VLDL oxidized by AAPH in the absence of AsA; lane 3, VLDL oxidized by AAPH in the presence of 1,000 μM AsA; lane 4, VLDL oxidized by SIN-1 in the absence of AsA; lane 5, VLDL oxidized by SIN-1 in the presence of 1,000 μM AsA.

arin in glycosaminoglycans are mainly due to the residues of basic amino acids, which contribute to the uptake of VLDL by hepatocytes (15, 16). Kato et al. (17) have reported that the lysine residue of apolipoprotein in LDL is reacts easily with lipid hydroperoxides. As shown in Fig. 3, intact apoE in VLDL is responsible for the heparin-binding activity (lane 1). In this connection, the heparin-binding activity of apoE was decreased by AAPH-induced (lane 2) or somewhat SIN-1-induced (lane 4) VLDL oxidation for 12 h. This fact suggests that the lysine residue of apoE might be modified by CE-OOH arisen from VLDL oxidation (18, 19). It seems likely that the oxidative modification of apoE impairs VLDL metabolism and accumulates oxidized VLDL in the circulatory system. In practice, AsA at 1,000 μ M disturbed a lowering of the heparin-binding activity of apoE in VLDL oxidized by AAPH (lane 3) or SIN-1 (lane 5).

Our data suggest that AsA existing in blood serves as a preventive against free radical-induced oxidation of apoE in VLDL.

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