American Journal of Hematology 60:242–244 (1999)

Vitamin E (α-Tocopherol) Does Not Inhibit Platelet Stimulation by Oxidized Low Density Lipoprotein In Vitro

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Platelet-rich plasma were treated with increasing concentrations of vitamin E (α-tocopherol). Washed platelets were exposed to oxidized low density lipoprotein (LDL) and examined by aggregometry and electron microscopy. The treatment of washed platelets by oxidized LDL induced morphological signs of activation like pseudopodia formation and an increase in light transmission. α-Tocopherol in a range of 0.001–1.0 mmol had no inhibiting influences on platelet activation by oxidized LDL. These results indicate that the free radical scavenger vitamin E cannot directly inhibit platelet activation by oxidized LDL. It may be supposed that platelet activation by oxidized LDL does not occur in a radical-dependent mechanism. Am. J. Hematol. 60:242–244, 1999. © 1999 Wiley-Liss, Inc.

Key words: platelet; oxidized LDL; vitamin E; antioxidants

INTRODUCTION

The oxidation of low density lipoprotein (LDL) is thought to play a major role in the process of atherogenesis. Oxidized LDL promotes foam cell formation, endothelial cytodamage, and platelet activation [1–4]. It is well established that α-tocopherol, biologically the most active form of vitamin E, inhibits the oxidation of LDL. Besides this protection of LDL from oxidation, vitamin E is also known to reduce platelet activation. Supplementation of volunteers with vitamin E decreases platelet adhesiveness and aggregation in response to ADP and arachidonic acid [5,6].

The aim of the present study was to determine whether incubations of platelet-rich plasma (PRP) with increasing concentrations of α-tocopherol influence platelet stimulation by oxidized LDL or arachidonic acid.

MATERIALS AND METHODS

Oxidized LDL was prepared as described previously [1]. Briefly, LDL was isolated from normal human plasma by discontinuous density gradient ultracentrifugation in a density range of 1.019–1.063 g/ml and dialyzed against phosphate buffered saline (pH 7.4). LDL was oxidized by exposure to cupric ions. Oxidative modification was characterized by the measurement of the thiobarbituric acid reactive substance, using malondialdehyde as a standard, which quantifies the peroxidation of arachidonic acid. Concentrations of lipoproteins were given as their protein content as determined using BSA as a standard.

Blood samples were drawn from healthy volunteers by venipuncture and PRP was obtained by centrifugation. α-Tocopherol was added to PRP according to Freedman et al. [7]. 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, or 1.0 mM α-tocopherol was dissolved in 95% ethanol and added as 1% vol/vol for 30 min at 37°C. No tocopherol but only ethanol was used in control experiments. PRP was pelleted by centrifugation and washed as previously described [8]. Washed human platelets were treated with arachidonic acid (50 μmol) (Sigma, Germany) or oxidized LDL (20, 40, or 60 μg/ml) for 10 min at 37°C [1]. The stimulation was revealed by aggregometry and electron microscopy. The tests were performed in a double channel aggregometer. At the end of the experiments, all platelet samples were fixed in suspension by adding equal volumes of 2.5% glutaraldehyde in cacodylate buffer (pH 7.4) for 30 min. The fixed platelets were prepared for electron microscopy using routine methods.

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Received for publication 22 August 1998; Accepted 7 October 1998

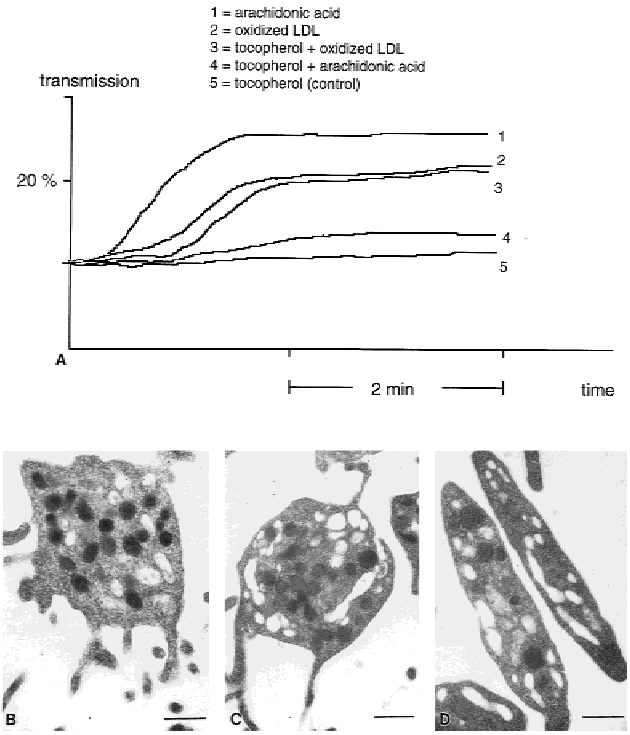

Fig. 1. A: Aggregometer traces of platelet suspensions. Oxidized LDL (40 µg/ml) and arachidonic acid (50 µmol) lead to an increase in light transmission. α-Tocopherol inhibit the activation by arachidonic acid but not the stimulation by oxidized LDL. B: Treatment of platelet suspension with oxidized LDL (40 µg/ml for 10 min) induces shape change and pseudopodia formation. Bar: 0.25 µm. C: PRP incubated with 0.5 mM α-tocopherol, 30 min (dissolved in 96% ethanol and added as 1% vol/vol). After washing procedure, oxidized LDL (40 µg/ml) induces shape change and pseudopodia formation. Bar: 0.25 µm. D: PRP incubated with 1.0 mM α-tocopherol, 30 min (dissolved in 96% ethanol and added as 1% vol/vol). Tocopherol inhibits the activation of arachidonic acid (50 µmol). Bar: 0.25 µm.

Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM 109 transmission electron microscopy. All experiments were repeated at least five times.

RESULTS

The treatment of platelet suspension by oxidized LDL induces a change from discoidal to spherical shape (Fig. 1B). Aggregometer traces of platelet suspensions demonstrate that oxidized LDL leads to an increase in light transmission indicating shape change and degranulation of platelets (Fig. 1A). Preincubation of platelets with tocopherol in a range of 0.001–1.0 mmol had no inhibiting influences on platelet activation by oxidized LDL (Fig. 1A,C). When platelets were treated only with α-tocopherol or ethanol (controls) no stimulation was detectable (Fig. 1A).

Treating of platelets with arachidonic acid (50 µmol) induced an increase in light transmission (Fig. 1A). In contrast to our observations with oxidized LDL, preincubation of platelets with vitamin E (0.5 and 1.0 mmol) prior to adding arachidonic acid resulted in a disappearance of activation (Fig. 1A, D).

DISCUSSION

α-Tocopherol is known to reduce platelet adhesiveness and aggregation, whereas oxidized LDL induces the platelet activation [1,5,6,9]. A recent in vitro study demonstrated that the inhibition of platelet aggregation by α-tocopherol was associated with the stimulation of platelet protein kinase C (PKC) [7]. Treatment of PRP with α-tocopherol produced a significant inhibition of aggregation by PKC-dependent agonists like arachidonic acid. Using the same test conditions, we found that α-tocopherol in a range of 0.001–1.0 mmol does not inhibit platelet activation by oxidized LDL in vitro. Oxidized LDL in the presence or absence of vitamin E causes pseudopodia formation and a change from discoidal to spherical shape. In contrast, concentrations of 0.5 and 1.0 mmol α-tocopherol prevent an arachidonic acid-mediated platelet stimulation. Since arachidonic acid is a PKC-dependent agonist and α-tocopherol has been shown to inhibit PKC stimulation [7], it may be supposed that platelet activation by oxidized LDL does not occur in a PKC-dependent mechanism. Oxidized LDL inhibits the Ca^{2+}-ATPase activity of purified platelet plasma membrane and causes an increase in cytoplasmatic calcium [10]. In summary, the data suggest that oxidized LDL may initiate platelet stimulation by an agonist-induced increase in cytosolic Ca^{2+}. Since the free radical scavenger vitamin E have no inhibiting effects on oxidized LDL activation, this process seems not to be radical-mediated.

ACKNOWLEDGMENTS

We thank Ms. P. Hassmann, Ms. D. Aschhoff, and Ms. L. Greune for expert technical assistance.

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