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REGULAR ARTICLE

Platelet–stimulating effects of oxidized LDL are not attributable to toxic properties of the lipoproteins

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Abstract

One prominent feature of oxidized LDL (OxLDL) is their ability to activate human platelets and effects of OxLDL on platelet function have been shown to depend on the chemical mechanisms that form the basis for the oxidation process.
In this regard, the possibility that the observed platelet–stimulating properties of OxLDL might be a direct consequence of cytotoxic effects mediated by these lipoproteins merits further investigation, as experimental strategies to overcome the toxic effects of OxLDL towards a variety of different cell types did not yield conclusive results.
In the present work, we show that copper–oxidized LDL mediate severe toxic effects towards a macrophage cell line (decrease in both the number of adherent cells and the amount of incorporated tritiated thymidine, induction of apoptosis and subsequent loss of membrane integrity) – effects that are presumably attributable to products emerging from lipid peroxidation. When added to resting human platelets, copper oxidized LDL stimulate platelets but are not able to trigger an aggregation response on their own.

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; OxLDL, oxidized LDL; hyp–OxLDL, hypochlorite–oxidized LDL; Cu–OxLDL, copper–oxidized LDL; EDTA, ethylenediaminetetraacetic acid; f.c., final concentration; PBS, phosphate–buffered saline; apoB, apolipoprotein B–100; ADP, adenosine 5′–diphosphate; TBARS, thiobarbituric acid–reactive substances; REM, relative electrophoretic mobility; PRP, platelet–rich plasma.

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In contrast, hypochlorite–oxidized LDL are able to trigger platelet aggregation, but do not mediate toxic effects towards nucleated cells. Even in the absence of exogenous antioxidants, these lipoproteins mediate cytostatic effects but do not negatively affect cell viability.
As a conclusion, platelet–activating effects of oxidatively modified LDL are not attributable to toxic properties of the lipoproteins and this finding might expand possibilities for therapeutical intervention.

Introduction

Atherosclerotic vascular disease and its complications represent the leading cause of death in Western countries. High plasma levels of low density lipoproteins (LDL) are an established risk–factor for the development of both atherosclerosis and (athero)thrombosis. Today it is generally accepted that LDL gain their (full) atherogenic potential upon oxidative modification [1] and the presence of oxidized LDL (OxLDL) has been confirmed in vivo [2].

In the course of oxidative modification, LDL lose their binding specificity for the classical LDL–receptor and the term "OxLDL" is primarily characterized by the ability of the lipoproteins to bind to certain receptors with broad ligand specificity, the so–called scavenger receptors [1].

LDL can be readily oxidized by a variety of different means and the last years have shown significant effort in identifying some of the mechanisms that are responsible for LDL oxidation in vivo [3,4]. Lipid peroxidation is commonly assumed to represent the initial step in LDL oxidation, resulting in the formation of a large number of bioactive lipid mediators. Some of these – depending on their reactivity – are able to react with the protein moiety of LDL and thereby change receptor specificity of the lipoproteins from the LDL (apoB)–receptor to scavenger receptor(s).

OxLDL were initially identified by strong cytotoxic effects of these lipoproteins [5]. Toxicity has been shown to reside in the lipid phase of the lipoproteins [5] and lipid (hydro)peroxides and their breakdown products have been identified as the main toxins [6]. A significant part of these cytotoxic effects results from rather unspecific interaction between reactive groups within the lipid mediators and the cell membrane. Toxic effects of OxLDL show some sensitivity to the action of antioxidants [7] and high density liopoproteins [8]. Toxic effects are intensified by binding of OxLDL to the cell membrane and subsequent internalisation of the lipoproteins [9].

OxLDL have gained scientific interest mainly by their involvement in the development of atherosclerosis and atherothrombosis. By acting on a number of different cells, they are able to decrease synthesis and/or bioavailability of nitric oxide and to mediate chemotactic and proliferative effects (reviewed in [1]). Furthermore, the interaction of OxLDL with human blood platelets leads to cellular activation and platelet aggregation that is sensitive to the action of antioxidants and seems to depend on binding of OxLDL to (yet unidentified) receptors in the platelet membrane [10]. As these characteristics (at least in part) also hold true for toxic effects mediated by OxLDL and as toxicity is considered an integral property of oxidized LDL, platelet aggregation induced by OxLDL might be a direct consequence of OxLDL–mediated toxicity.

In light of the rather unspecific mode of action by which some lipid peroxidation products mediate their deleterious effects, the identification of toxicity as the mechanism underlying platelet aggregation induced by OxLDL might have consequences on the general strategies for therapeutical and/or preventional intervention.

Therefore, it was the aim of this study to test the hypothesis if OxLDL–mediated platelet–aggregation is related to lipoprotein–imparted toxic effects.

Materials and methods

Phosphate buffered saline (PBS) and fetal calf serum were obtained from BioWhittaker Europe (Verviers, Belgium). Cell culture plastic ware was from Greiner (Frickenhausen, Germany). ^{3}H–thymidine was purchased from MP Biomedicals Europe (Illkirch, France), propidium iodide was from Alexis (Lausen, Switzerland). Dulbecco's Modified Eagle's Medium (DMEM), antibiotics and NaOCl were from Sigma–Aldrich (Vienna, Austria). NaOCl was standardized at 290 nm using a molar extinction coefficient of 350 M^{-1} cm^{-1}. Osmic acid, Epon 812 and other reagents for electron microscopy were from Fluka Chemie (Buchs, Switzerland). 0.45 μm filter units were purchased from Millipore (Vienna, Austria), 0.22 μm filter units for sterile filtration of lipoproteins were from Iwaki (Bertoni GmbH, Vienna, Austria). PE–labeled antibody directed against CD62 was purchased from Becton Dickinson (Becton Dickinson Austria, Vienna, Austria).

Isolation of human platelets

Freshly drawn blood was anticoagulated with 1/10 volume of 3.8% (w/v) trisodium citrate and centrifuged immediately at 120 ×*g* for 20 min to yield platelet rich plasma. Platelets were isolated by gel filtration of platelet rich plasma as originally described [11] in Tyrode buffer without Ca^{2+} (137 mM of NaCl,