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Apoptosis induced by oxidized lipids is associated with up-regulation of p66Shc in intestinal Caco-2 cells: protective effects of phenolic compounds

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Abstract

In this study, we investigated the alterations of the redox balance induced by the lipid fraction of oxLDL in Caco-2 intestinal cells, and the effects of tyrosol and protocatechuic acid, two dietary phenolic compounds. We found that oxidized lipids extracted from oxLDL (LipE) induced oxidative stress by determining, 6 h after treatment, ROS overproduction (about a 100% and a 43% increase of O_2^- and H_2O_2 production, respectively, $P<.05$: LipE vs. control) and, 12 h after treatment, GSH depletion (about a 26% decrease, $P<.05$: LipE vs. control), and by impairing the activities of superoxide dismutase, catalase and glutathione peroxidase. In response to the induced oxidative stress, we observed significant overexpression of glutathione peroxidase (6 h after treatment: $P<.05$), glutathione reductase and γ-glutamylcysteine synthetase (12 h after treatment: $P<.05$). Notably, when GSH depletion occurred, p66Shc protein expression increased by about 300% with respect to control ($P<.001$; LipE vs. control). These effects were fully counteracted by dietary phenolics which inhibited ROS overproduction and GSH consumption, rendered the reactive transcription of glutathione-associated enzymes unnecessary and blocked the intracellular signals leading to the overexpression and rearrangement of p66Shc signalling molecule. Altogether, these results suggest that the impairment of the antioxidant system hijacks intestinal cells towards an apoptotic-prone phenotype via the activation of p66Shc molecule. They also propose a reappraisal of dietary polyphenols as intestinal protecting agents, indicating the antiapoptotic effect as a further mechanism of action of these antioxidant compounds.

Keywords: Apoptosis; oxLDL; Oxidized lipids; Caco-2; Glutathione; p66Shc; Polyphenols

1. Introduction

In the gastrointestinal tract, prooxidants such as endogenous and exogenous lipid peroxides have been demonstrated to impact intestinal integrity. In fact, cellular and molecular events involved in degenerative pathological processes leading to intestinal disorders have been associated with redox alterations. Being the interface between the organism and its luminal environment, the intestine is constantly challenged by diet-derived oxidants as well as by endogenously generated reactive species or oxidants. In particular, a high intake of dietary polyunsaturated fatty acids can contribute to the luminal accumulation of lipid hydroperoxides, and the subsequent lipid peroxidation can induce oxidative stress and redox imbalance, contributing to the development of gut pathologies, such as inflammation and cancer [1,2]. To preserve cellular integrity and tissue homeostasis, the intestine possesses several defence

Abbreviations: CAT, catalase; DHE, dihydroethidium; DHR 123, dihydrorhodamine 123; GPx, glutathione peroxidase; GSSGred, glutathione reductase; γ-GCS, γ-glutamylcysteine synthetase; GSH, glutathione; LipE, lipid extract from oxLDL; MBC, monochlorobimane; oxLDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; SOD, superoxide-dismutase.

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mechanisms such as the ability to maintain high antioxidant levels (glutathione, tocopherol and ascorbic acid), to up-regulate the antioxidant enzyme system (glutathione peroxidase, glutathione reductase and superoxide dismutase) and to induce cell death by apoptosis in order to dispose of injured or spent cells.

There is an increasing interest in the mechanisms of response of the intestinal epithelium to oxidative stress and in the capability of nutritional antioxidants to strengthen endogenous antioxidant defences [3,4]. Among diet antioxidants, polyphenols, naturally occurring in vegetables, fruits and plant-derived beverages such as tea, red wine and extra virgin olive oil, are the most abundant ones. They can contribute to the prevention of several oxidative stress-associated diseases, characterized by inflammatory injuries, including injury of the intestine [5–8]. Polyphenols exert their protective action as reducing agents, but increasing evidence exists that they may improve antioxidant defences through the induction of antioxidant and phase II enzymes [9,10]. Moreover, several other protective effects have been recently described such as antiviral, antimicrobial, anti-inflammatory and anticarcinogenic effects, as well as the ability to interact with cell receptors or to modulate certain signalling pathways [11].

Low-density lipoproteins undergoing oxidative modification (oxLDL) generate a mixture of compounds with cytotoxic activity, i.e., lipid hydroperoxides, aldehydes and oxysterols. OxLDL with their oxidized lipid component could thus represent a suitable "physiological" model to study the effects of oxidized lipids in inducing intracellular oxidative stress. It is well known that the interaction of oxLDL oxidant products with cells and tissues results in oxidative imbalance and leads to cell death by apoptosis [12,13], but the mechanism is still unclear. We have previously demonstrated that oxLDL induces apoptosis in intestinal Caco-2 cells via the intrinsic pathway, i.e., the mitochondrial pathway [14,15]. Since the Caco-2 cell line retains many of the morphological and enzymatic features typical of normal human enterocytes [16], it is largely used as a model system for evaluating the effects of normal dietary constituents as well as additives, contaminants, toxicants, oxidants and drugs [17,18].

The aim of the present work was to investigate (i) the redox imbalance underlying apoptosis induced by oxidized lipids in Caco-2 cells in terms of expression and/or activity of the main actors of cell homeostasis, i.e., reduced glutathione (GSH), superoxide-dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSSGred) and γ-glutamylcysteine synthetase (γ-GCS); (ii) the ability of two extra virgin olive oil phenolic compounds, namely, tyrosol and protocatechuic acid, to counteract the pro-oxidant subcellular effects of oxidized lipids; and (iii) the intracellular behavior — i.e., expression and localization — of p66Shc, an oxidative stress sensor protein recently identified as an important cytoplasmic signal transducer that regulates the apoptotic response to oxidative stress [19].

2. Materials and methods

2.1. LDL isolation and oxidation

LDL (1.019–1.063 g/ml) was prepared from freshly isolated pooled plasma from healthy human donors by density gradient ultracentrifugation and then oxidized with 5 μM $CuSO_4$ for 18 h at 37°C as reported elsewhere [20].

2.2. Lipid extraction from native and oxidized LDL

Lipids were extracted from native LDL (nLDL) and oxLDL with chloroform/methanol mixture (2:1 vol/vol) containing 5 μg/ml butylated hydroxytoluene [21]. The organic phase was dried under nitrogen and the content of extracts was determined by microgravimetry. The lipid residue was dissolved in ethanol and added to culture medium (0.2 mg LDL protein equivalent/ml culture medium).

2.3. Caco-2 cell culture and experimental procedure and treatments

Caco-2 cells (European Collection of Cell Culture, Salisbury, UK) were cultured as previously reported [14]. On culture Day 5, when cells are at the initial step of their differentiation process, the medium with serum was replaced by DMEM containing 2% Ultroser G (a lipoprotein-free serum substitute). Then the cells were exposed to 0.2 mg protein/ml of nLDL or oxLDL with and without phenolic compounds. All the experiments performed at 6, 12, 18, 24 and 48 h after exposure to oxLDL or lipid extract from oxLDL (LipE) included, as controls, (i) untreated cells, (ii) cells treated with nLDL and (iii) cells treated with the lipid extract from nLDL (nLipE). Because the results obtained in oxLDL-treated cells and in LipE-exposed cells were completely overlapping, we report only the results obtained with lipid extracts. In the same vein, as controls, only the results obtained from untreated cells are shown. In the experiments to evaluate the antioxidant activity of extra virgin olive oil phenols, 0.5 mM tyrosol or 0.25 mM protocatechuic acid concentrations were chosen on the basis of preliminary experiments performed to evaluate the minimal effective concentrations. The phenolic compounds were added 2 h before oxLDL or LipE treatment and were maintained in the medium throughout the exposure to oxidized lipids.

2.4. Cytofluorimetric measurements of intracellular ROS and GSH levels

To evaluate O_2^- and H_2O_2 intracellular production, cells (5×10^5) were incubated in 490 μl of Hanks' balanced salt solution (HBSS, pH 7.4) with 1 μM dihydroethidium (DHE, Molecular Probes) or 10 μM dihydrorhodamine 123 (DHR 123, Molecular Probes), respectively, in polypropylene test tubes for 5 min at 37°C [22]. Because fluorescent oxidation products are produced in metabolically active cells only, both DHR 123 and DHE can also be used as viability indicators. Intracellular GSH content was assessed by using monochlorobimane (MBC, Molecular Probes) as previously