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Effects of aldehydes on CD36 expression

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Abstract

Introduction: During the oil frying process lipid peroxidation compounds are formed. These products can modulate gene expression and alter cellular behaviour. The cellular uptake of oxidized LDL, a key step in the development of atherosclerosis, is mediated by the CD36 scavenger receptor, whose expression is down-regulated by α-tocopherol.

Objective: To determine the effects of water-soluble aldehydes, obtained from thermally oxidized sunflower oil on the expression of CD36 scavenger receptor in human monocytes (THP-1 cells). We also wanted to study the effects of α-tocopherol on CD36 expression in the presence of water-soluble aldehydes.

Materials and Methods: Sunflower oil was heated in a frying pan, at 180–200°C for 40 min, water-soluble aldehydes were isolated, and the content of thiobarbituric acid reacting substances (TBARS) was determined. THP-1 monocytes were cultured in RPMI medium during 24 h and incubated with increasing concentrations of the water-soluble aldehydes (ranging from 0.05 to 1 μM) and with or without 50 μM of α-tocopherol. In parallel, THP-1 cells were cultured with the same volume of an extract obtained from non-oxidized oil or distilled water. The CD36 expression at the cell surface was studied with fluorescence-activated cell sorting (FACS).

Results: Monocytes incubated in a medium containing water-soluble aldehydes, showed a dose dependent increase in the expression of the CD36 protein on the cell surface, compared to with the control groups. When the cells were treated simultaneously with 50 μM of α-tocopherol a significant reduction in the expression of the CD36 protein was observed.

Conclusion: Water-soluble aldehydes, extracted from thermally oxidized culinary oil, increase the expression of CD36. This effect is partially decreased by the presence of α-tocopherol.

Keywords: *Atherosclerosis, scavenger receptors, CD36, aldehydes, oxidized oils*

Introduction

CD36 is a multifunctional cellular receptor with broad relevance in the pathogenesis of atherosclerosis. This scavenger receptor recognises oxidized forms of LDL (oxLDL) [1,2] and mediates lipid accumulation and macrophages foam cells formation [2,3]. It is up-regulated in lipid-rich atheroma, but perhaps the most compelling data on the role of CD36 in atherosclerosis are from studies with CD36-deficient mice, which show a 70–80% reduction in aortic lesion size [4]. *In vitro* experiments demonstrate that macrophages from CD36-deficient mice take up different forms of oxLDL poorly and are resistant to foam cell formation, providing a mechanism for the athero-protection observed in CD36 null mice [3,4].

In macrophages, CD36 expression is up-regulated by the content of oxLDL and lipid peroxidation products, such as 9- and 13-hydroxyoctadecadienoic acid (9-, and 13-HODE) [5–7], or aldehydes [8,9]. This effect could

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be associated with increased expression and activity of nuclear receptors such as peroxisome proliferator-activated receptor γ (PPAR-γ), a receptor that participates in lipid and carbohydrate metabolism [7].

Subjection of glycerol-bound polyunsaturated fatty acids (PUFA) present in culinary oils to episodes of thermal stressing according to standard frying/cooking practice gives rise to lipid oxidation products such as α, β-unsaturated aldehydes, compounds which are highly reactive and can give rise to cellular damage [10]. Indeed, it has been shown that the lipid aldehydes generated during the heating process can be absorbed and be toxic [10]. We have previously shown that the administration of these compounds to pregnant rats increases the rate of congenital malformations [11]. Moreover, these compounds could be incorporated into the LDL, making these particles more susceptible to oxidation and therefore more atherogenic [10].

Some anti-atherogenic agents, such as vitamin E, prevent LDL oxidation. Some of the effects of vitamin E, however, can be attributed to other properties of the compound that are not directly related to its antioxidant properties [12]. In human aortic smooth muscle cells (SMCs), α-tocopherol inhibits oxLDL uptake by a mechanism involving down-regulation of CD36 mRNA and protein expression [13]. Therefore, the beneficial effect of α-tocopherol on atherogenesis, can be explained, at least in part, by its effect of lowering the uptake of oxidized lipoproteins, with consequent reduction of foam cell formation or by reducing the risk of its development [13].

In the present study, we assessed the effect of aldehydes, extracted from heated oil, on expression of CD36 scavenger receptor. We show that the presence of heated oil aldehydes, lead to an increase expression of CD36 at the surface of THP-1 cells and we further demonstrate the efficacy of α-tocopherol in inhibiting CD36 expression.

Materials and methods

Aldehydes preparation

Sunflower oil was heated in a frying pan, at 180–200°C for 40 min. In order to perform experiments in a cell culture model, only the water-soluble aldehydes were isolated by mixing equal volume of oil with distillate water during 2 h. The content of thiobarbituric acid reacting substances (TBARS) was determined following the method described by Wong et al.

Cell lines, culture and treatments

Human THP-1 monocytes (ATCC - TIB-202) were cultured in RPMI/10% FCS, 2 mM L-glutamine, 1.0 mM sodium pyruvate and 4.5 g/l glucose. THP-1 cells (10^6 per plate) were plated 24 h before incubation during 24 h with increasing concentrations of the water-soluble aldehydes (ranging from 0.05 to 0.8 μM, higher doses led to cell death) and with α-tocopherol (50 μM) diluted in ethanol or equal volume of ethanol. In parallel, THP-1 cells were cultured with the same volume of an extract obtained from non-oxidized oil or distilled water.

Flow cytometric analysis

After incubations with antibodies, 10^6 cells per sample were washed twice with PBS. Background fluorescence was determined with unstained cells. A filter transmitting at 530 nm (30 nm bandwidth) was used to collect the green fluorescence.

The CD36 expression was studied with fluorescence-activated cell sorting (FACS) using a monoclonal anti-human CD36-FITC antibody (Ancell) diluted in PBS/1%BSA. A minimum of 10,000 cells per sample was analyzed; data were acquired and analyzed using the Cellquest software (FACSscan, Becton & Dickinson).

DNA fragmentation

To evaluate the existence of DNA laddering typical for apoptosis, THP-1 cells were treated with increasing concentrations of water-soluble aldehydes (0.05–0.8 μM) for 24 h. DNA was extracted from cells using the apoptotic DNA ladder kit (Roche Molecular Biochemicals). DNA samples were resolved on agarose gels following the manufacturer's protocol. DNA extracted from U937 apoptotic cells (treated with 4 μg/ml camptothecin for 3 h) provided in the kit was evaluated with samples as a positive control of apoptosis.

Statistical analysis

The values are expressed as the mean ± standard deviation as indicated in the text. For FACS results, the median fluorescence intensity was determined and the mean ± standard deviation calculated. Student's *t*-test was used to analyze the significant differences between two conditions. A P value of less than 0.05 was taken as significant. The analysis was performed using the SPSS program (11.5 for Windows).

Results

THP-1 cells, after 24 h of incubation with increasing concentrations of water-soluble aldehydes ranging from 0.05 to 0.8 μM showed an increased expression of the CD36 receptor on the cell surface, in a concentration dependent manner (Figure 1).

Cells treated with the highest concentration (0.8 μM) show a different profile, which is probably

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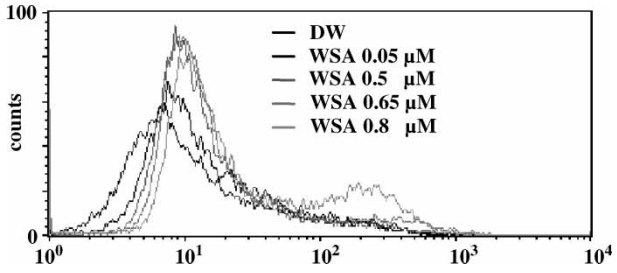

Figure 1. Expression of CD36 scavenger receptor on THP-1 monocytes after treatment with aldehydes or distillate water (DW). THP-1 cells were treated during 24 h with increasing concentration of water-soluble aldehydes (WSA) obtained from thermally oxidized sunflower oil, and CD36 expression analyzed by FACS. Results are representative of 6 independent experiments with essentially similar results.

due to a toxic effect, as it was described previously with high concentrations of different aldehydes up to 50 μM [8]. Therefore, we have studied the effects of increasing concentrations of aldehydes on cell death by nucleosome laddering. DNA extracted from cells incubated for 24 h with the four concentrations of water-soluble aldehydes did not present the characteristic laddering pattern of an apoptotic state (Figure 2).

After this first experiment we choose the 0.5 μM concentration for further study because the clearly visible change on CD36 expression without any toxic effect on THP-1 cells. When cells were treated with non-oxidized oil extract, no differences were observed on the profile when compare with the group treated with distillate water, being the mean of fluorescence intensity 23.4 vs. 23.9, respectively, whereas clear and statistically significant differences ($p < 0.05$) were shown after treatment with 0.5 μM of water-soluble aldehydes (mean 49.5). These results show that the increase of surface expression of the CD36 scavenger receptor is the result of the water-soluble aldehydes generated after oil frying.

The administration of α-tocopherol (50 μM), in addition water-soluble aldehydes, produced a decrease in the fluorescence intensity, from 49.5 to 27.6 ($p < 0.08$), when compared with the group treated with 0.5 μM of water-soluble aldehydes plus the vehicle of the α-tocopherol (ethanol 0.1%) (Figure 3). The incubation with α-tocopherol did not have any effect in the group treated with non-oxidized oil extract when compared to the control.

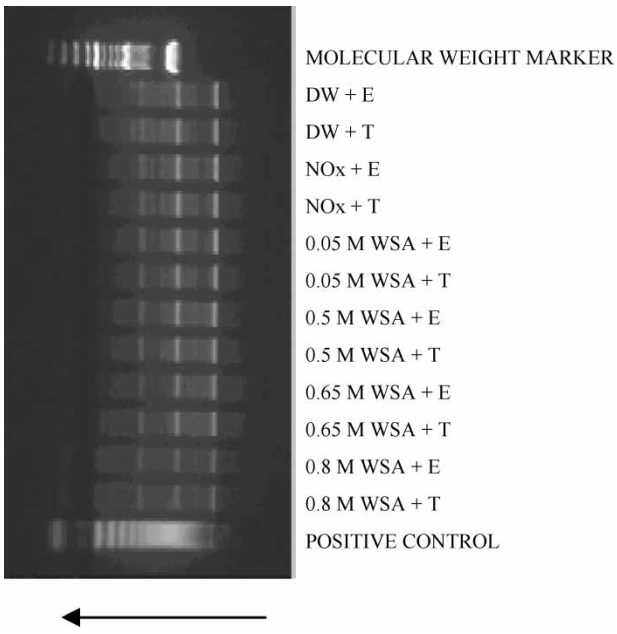

Figure 2. Apoptosis of THP-1 cells after treatment with aldehydes. DNA laddering in THP-1 incubated with increasing concentrations of water-soluble aldehydes (WSA) (0.05–0.8 μM), non-oxidised oil extract (NOx), distilled water (DW), co-treated with α-tocopherol (*T*, 50 μM) or its vehicle, ethanol (*E*, 0.1%).

Discussion

Thermal stressing of culinary oils and fats according to standard frying practices (up to 180°C) gives rise to oxygen radical-mediated autoxidation of PUFA's therein, primarily generating conjugated hydroperoxydiene species. These react further to produce aldehydes via a process which involves the β-scission of preformed alkoxyl radicals [14,15]. Such aldehydes species (n-alkanals and *trans*-2-alkenals) have the capacity to exert a variety of toxic effects in view of their extremely high reactivity with critical molecules (DNA base adducts, proteins such as LDL, peptides, free amino acids and endogenous thiols such as glutathione). These toxicological hazards depend on the rate and extend of their absorption to the circulation. Grootveld et al. have demonstrated that in rats, reactive *trans*-2-alkenal, which are end products of the lipid peroxidation process, are indeed readily absorbed from the gut to the systemic circulation *in vivo* [10].

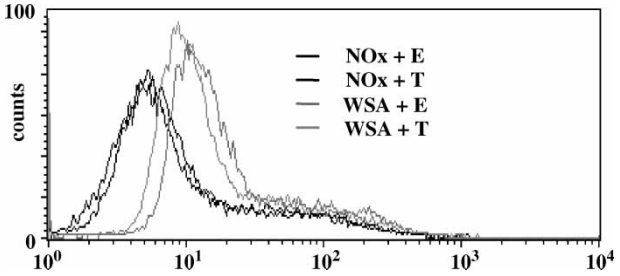

Figure 3. Effect of α-tocopherol on CD36 scavenger receptor expression after treatment with aldehydes. THP-1 cells treated during 24 h with non-oxidized oil extract (NOx) and water-soluble aldehydes (WSA) (0.5 μM), with α-tocopherol (*T*, 50 μM), or equal volume of the vehicle of α-tocopherol, ethanol (*E*, 0.1%). CD36 expression was analyzed by FACS. Results are representative of 6 independent experiments with essentially similar results.

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Aldehydes, either in gastrointestinal tract or in the blood stream, could react with other lipids, including LDL fatty acids, making these particles more susceptible to oxidation and therefore more atherogenic. Indeed, HNE, a major aldehydic product arising from the autoxidation of PUFAs, reacts with selected amino acids of ApoB, rendering it susceptible to uptake by the macrophage scavenger receptor [16]. ApoB lysine residues are readily derivatized by aldehydic products, and since a critical number of these modified residues are required for the recognition of oxLDL by the scavenger receptors, its uptake by macrophages with an increasing level of such compounds [16]. Therefore, after their *in vivo* absorption, the aldehydes present in thermally, oxidatively stressed culinary oils ingested in the diet will have the capacity to directly affect structural modifications of the ApoB component of LDL, a process which induces the generation of foam cells from macrophages.

Alternatively to this mechanism, aldehydes could have a regulatory effect on gene expression. For instance, α,β-unsaturated aldehydes can act through the antioxidant response element (ARE) to readily induce the phase II metabolic enzyme, glutathione S-transferase (GST), the primary enzyme responsible for their elimination [17]. Our results show that the incubation of monocytes (THP-1 cells) with different concentrations of water-soluble aldehydes, extracted from thermally oxidized culinary oil, increased the expression of CD36 in a concentration dependent manner. This effect was partially decreased by the presence of α-tocopherol.

The class B scavenger receptor, CD36, binds modified low density lipoprotein (oxLDL). It is found in atherosclerotic lesions, and is up-regulated by oxLDL, therefore, playing an important role in the pathogenesis of atherosclerosis [5–7]. In fact, the CD36-apoE double-null animals showed a 77% reduced lesion area in the aorta when compared to apoE null mice [4]. Inhibition of CD36 scavenger receptor expression should lead to reduced oxLDL uptake and decreased foam cells formation.

Similar compounds have been described to modulate the expression of the scavenger receptors. For instance, 9 and 13-HODE, which are components of oxLDL are able to induce CD36 gene expression through a mechanism involving peroxisome proliferator-activated receptor γ (PPAR-γ) activation. It can be speculated that the aldehydes up-regulated CD36 receptor, serving as agonist of PPAR-γ like 9- and 13-HODE [6,7].

Another possible mechanism could be through the induction of the Nrf2 signalling pathway. Using Nrf2 deficient mouse macrophages, Mann et al. recently established that Nrf2 partially regulates CD36 expression in response to oxLDL and HNE [18]. These results implicate Nrf2 as a second important transcription factor involved in the induction of the scavenger receptor CD36 in atherosclerosis [19]. Supporting this notion, Nrf2/small Maf heterodimers have been described as putative important coactivators of the ARE-mediated induction of GST [20,21].

When cells are co-treated with α-tocopherol, in parallel to water-soluble aldehydes, a reduction of CD36 expression was observed. It is well described that α-tocopherol inhibits oxLDL uptake by a mechanism involving downregulation of CD36 mRNA and protein expression [13]. Therefore, the beneficial effects of α-tocopherol against atherosclerosis can be explained, at least in part by its effect of lowering the uptake of oxidized lipoproteins, with consequent reduction of foam cell formation.

In summary, our findings indicate that differences in cooking practices may be related to development of the atherosclerotic process. If the over-expression of CD36 scavenger receptor is linked to the development of atherosclerosis, the toxic effect induced by lipid peroxidation products (aldehydes) could be partially prevented with the administration of α-tocopherol. Further experiments are needed to clarify, in detail, the action of these compounds on the mentioned pathways.

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