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Oxidized low-density lipoprotein accelerates the destabilization of extracellular-superoxide dismutase mRNA during foam cell formation

Junya Makino, Miyuki Nii, Tetsuro Kamiya *, Hirokazu Hara, Tetsuo Adachi

Department of Biomedical Pharmaceutics, Laboratory of Clinical Pharmaceutics, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu 501-1196, Japan

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ABSTRACT

Extracellular-superoxide dismutase (EC-SOD) is one of the main anti-oxidative enzymes that protect cells against the damaging effects of superoxide. In the present study, we investigated the regulation of EC-SOD expression during the oxidized low density lipoprotein (oxLDL)-induced foam cell formation of THP-1-derived macrophages. The uptake of oxLDL into THP-1-derived macrophages was increased and EC-SOD expression was decreased in a time-dependent manner by oxLDL. Furthermore, EC-SOD suppression by oxLDL was mediated by the binding to scavenger receptors, especially CD36, from the results with siRNA experience. EC-SOD expression is known to be regulated by histone acetylation and binding of the transcription factor Sp1/3 to the EC-SOD promoter region in human cell lines. However, oxLDL did not affect these processes. On the other hand, the stability of EC-SOD mRNA was decreased by oxLDL. Moreover, oxLDL promoted destabilization of ectopically expressed mRNA from EC-SOD or chimeric Cu,Zn-SOD gene with the sequence corresponding to 3′UTR of EC-SOD mRNA, whereas oxLDL had no effect on ectopic mRNA produced from EC-SOD gene lacking the sequence. These results suggested that oxLDL decreased the expression of EC-SOD, which, in turn, accelerated the destabilization of EC-SOD mRNA, leading to weaker protection against oxidative stress and atherosclerosis.

Introduction

Atherosclerosis, which exhibits the features of a chronic inflammatory disease, is characterized by monocytic differentiation into macrophages and foam cell formation. Macrophages are present at all stages of the disease and facilitate the accumulation of cholesterol, which can lead to the formation of atherosclerotic lesions and myocardial infarction [1]. The uptake of low-density lipoprotein (LDL)[1] is a critical process in the development of atherosclerosis, and modified LDL has been detected in atherosclerotic lesions [2]. Oxidized LDL (oxLDL) is recognized by scavenger receptors and taken up in an unrestricted manner by macrophages [3,4]. Thus, the inhibition of oxLDL-induced foam cell formation may contribute to the prevention of atherosclerosis [5].

Superoxide dismutase (SOD) plays a pivotal role against oxidative stress induced by superoxide, in mammals, and its deficiency may lead to various diseases, such as asthma, atherosclerosis, and type 2 diabetes [6–8]. Extracellular-SOD (EC-SOD) is a major SOD isozyme in mammalians that is secreted into the extracellular space and mainly distributed in blood vessel walls by binding to the heparan sulfate proteoglycan in the glycocalyx on cell surfaces [9–11]. The content of EC-SOD is markedly lower than those of the other SOD isozymes in most tissues; however, the presence of a high level of EC-SOD throughout the vessel walls may have an important protective role as an anti-inflammatory factor against superoxide in the vascular system. EC-SOD has been shown to protect arteries from ischemia-induced cytotoxicity and reduce infarct sizes [12–14]. We previously reported that 12-*O*-tetradecanoylphorbol-13-acetate (TPA) up-regulated the expression of EC-SOD during monocytic differentiation in THP-1 cells [15], suggesting that EC-SOD is expressed in THP-1 cells to protect them from superoxide-induced damage and also to maintain the redox balance in the extracellular space. Moreover, the expression of EC-SOD was recently found to be regulated by epigenetic regulation, such as DNA methylation or histone acetylation in the *EC-SOD* promoter region [16–18] and also by the transcription factor Sp1/Sp3, which is known to be involved in the basal and inducible transcription of the murine *EC-SOD*

* Corresponding author. Fax: +81 58 230 8105.
E-mail address: tekamiya@gifu-pu.ac.jp (T. Kamiya).

[1] *Abbreviations used:* ActD, actinomycin D; CD36, cluster of differentiation 36; ChIP, chromatin immunoprecipitation; EC-SOD, extracellular-superoxide dismutase; FCS, fetal calf serum; LDL, low-density lipoprotein; LOX-1, lectin-like oxLDL receptor-1; nLDL, native LDL; oxLDL, oxidized LDL; PBS, phosphate-buffered saline; ROS, reactive oxygen species; RT-PCR, reverse transcriptional-polymerase chain reaction; siRNA, small interfering RNA; SR-A, scavenger receptor-A; SR, scavenger receptor; TBP, TATA box binding protein; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; 18S rRNA, 18S ribosomal RNA; 3′UTR, 3′-untranslated region.

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gene by interactions between a proximal promoter element [19,20].

The overexpression of EC-SOD has been shown to protect against myocardial infarction caused by atherosclerosis in rabbits [21]. On the other hand, the knockout of EC-SOD promoted the expansion of atherosclerosis on transverse aortic constriction in mice [22]. Moreover, the expression of EC-SOD was detected in monocytes/macrophages, and its administration reduced the oxidation of LDL [23]. Macrophage-derived foam cell formation due to the uptake of oxLDL may induce the generation of reactive oxygen species (ROS) such as superoxide or hydrogen peroxide produced by activated NADPH oxidase and decrease resistance to oxidative stress in the pathogenesis of atherosclerosis; therefore, the mechanism underlying the expression of EC-SOD during foam cell formation needs to be elucidated in more detail.

In the present study, we found that oxLDL decreased the expression of EC-SOD in a time-dependent manner during foam cell formation. Moreover, the destabilization of EC-SOD mRNA was induced by the treatment with oxLDL in THP-1-derived macrophages via regulation at the 3′-untranslated region (3′UTR) in EC-SOD mRNA.

Materials and methods

Reagents

TPA and Oil red O were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The cholesterol assay kit was purchased from Bioassay Systems (Hayward, CA, USA). Actinomycin D (ActD) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). *Eco*RI, *Eco*RV, and *Hin*dIII were purchased from Boehringer Mannheim (Indianapolis, IN, USA). Anti-acetyl-histone H3 and H4 rabbit polyclonal antibodies were purchased from Millipore Co. (Billerica, MA, USA). Anti-Sp1 and -Sp3 rabbit polyclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Biotin-conjugated goat anti-rabbit and mouse IgG (H+L) antibodies were purchased from Zymed Laboratories (San Francisco, CA, USA). Lipofectamine® 3000, Lipofectamine® RNAiMAX and TRIzol® were purchased from Invitrogen-Life Technologies (Carlsbad, CA, USA). Silencer® pre-designed small interfering (siRNA) against negative control (Cat. #: AM4611) and CD36 (Cat. #: AM16708) were purchased from Ambion (Austin, TX, USA).

Cell culture

The human leukemic cell line, THP-1 was cultured in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal calf serum (FCS), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained at 37 °C in a humidified 5% CO_2 incubator. To induce the differentiation of THP-1, cells were seeded at 2.0×10^6 -cells/35-mm culture dish and 100 nM TPA was then added. After being subjected to this treatment for 24 h, cells were washed twice with phosphate-buffered saline (PBS) and then incubated with 100 μg/mL oxLDL for the indicated hours.

Purification of LDL and preparation of oxLDL

To prepare LDL, informed consent was obtained from the healthy donors and ethics approval for the study was granted by the Institutional Ethics Committee. LDL (1.019–1.063 g/mL) was isolated from the plasma of healthy donors by sequential ultracentrifugation at 4 °C, and then oxidized by exposure to $CuSO_4$ (10 μM) in PBS at 37 °C for 4 h. OxLDL was extensively dialyzed against 0.3 mM EDTA-saline. The concentration of oxLDL was measured using the BCA protein assay.

Oil red O staining

TPA-induced THP-1-derived macrophages were treated with 100 μg/mL oxLDL for 24, 48, and 72 h. After washing twice with PBS (-), cells were fixed with 10% paraformaldehyde and stained with Oil red O. Oil red O staining was observed under a light microscope.

Intracellular lipid accumulation

THP-1-drived macrophages were treated with 100 μg/mL oxLDL for 24, 48, and 72 h. After collecting cells and washing once with PBS (−), cells were resuspended in 200 μL PBS for ultrasonication. After ultrasonication, the concentration of cellular cholesterol was determined using an EnzyChrom™ cholesterol assay kit (BioAssay systems, Hayward, CA, USA) and normalized with protein concentration according to the protocol provided by the manufacture.

Reverse transcriptional-polymerase chain reaction (RT-PCR) analysis

After cells had been treated, they were lysed in 1 mL TRIzol® reagent. cDNA and RT-PCR were prepared by the methods described in our previous study [24] with minor modifications. The primer sequences used in this study were as follows: extracellular superoxide dismutase (EC-SOD), sense 5′-AGA AAG CTC TCT TGG AGG AG-3′; antisense 5′-ACC GCG AAG TTG CCG AAG TC-3′ (496 bp): scavenger receptors A (SR-A), sense 5′-GCA GTT CTC ATC CCT CTC TCA ATT GGA-3′; antisense 5′-ATT CCC ATG TCC CTG TGG ACT GAG-3′ (330 bp): cluster of differentiation 36 (CD36), sense 5′-TGC CTC TCC AGT TGA AAA CCC-3′; antisense 5′-GCA ACA AAC ATC ACC ACA CCA-3′ (484 bp): lectin-like oxLDL receptor-1 (LOX-1), sense 5′-CCT TGC TCG GAA GCT GAA TG-3′; antisense 5′-CAG CGC CTC GGA CTC-3′ (377 bp): 18S ribosomal RNA (18S rRNA), sense 5′-CGG CTA CCA CAT CCA AGG AA-3′; antisense 5′-GCT GGA ATT ACC GCG GCT-3′ (187 bp). These PCR products were loaded on a 2% (w/v) agarose gel for electrophoresis, and a densitometric analysis of the PCR products was performed with Multi Gauge V3.0 (Fuji Film, Tokyo, Japan).

Histone preparation

The core histone was isolated from THP-1 cells as described below. After the cells had been treated, they were lysed in extraction buffer (0.1 M Tris–HCl, pH 7.5, containing 0.15 M NaCl, 1.5 mM $MgCl_2$, 0.65% NP-40, 10 mM NaF, 1 mM Na_3VO_4, 20 mM β-glycerophosphate, 1 mM DTT, and 1 mM PMSF). After centrifugation at 13,200*g* for 10 s, the pellets were mixed with 0.2 M H_2SO_4 followed by centrifugation at 13,200*g* for 20 min. The supernatant was mixed with trichloroacetic acid and centrifuged at 13,200*g* for 20 min. The pellets were washed with acetone and centrifuged again at 13,200*g* for 5 min. The remaining histone was dissolved in 1× SDS buffer (0.45 M Tris–HCl, pH 8.8 containing 2% SDS, 6% 2-mercaptoethanol, and 0.01% bromophenol blue).

Western blotting for histone

The histone isolated from 5×10^4 cells was boiled for 5 min, separated by SDS–PAGE on 15% (w/v) polyacrylamide gel, and transferred electrophoretically onto PVDF membranes. The membranes were then incubated with the respective specific primary antibodies (1:1000). The blots were incubated with a biotin-conjugated goat anti-rabbit IgG antibody (1:1000) followed by ABC reagents (Vector Laboratories, Inc., Burlingame, CA, USA)