Free Radical Biology and Medicine 60 (2013) 272–281

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

High-density lipoprotein nitration and chlorination catalyzed by myeloperoxidase impair its effect of promoting endothelial repair

Bing Pan [a,1], Baoqi Yu [a,1], Hui Ren [a], Belinda Willard [b], Ling Pan [c], Lingyun Zu [d], Xiaoli Shen [c], Yijing Ma [a], Xiuli Li [e], Chenguang Niu [a], Jinge Kong [a], Siyu Kang [a], Y. Eugene Chen [f], Subramaniam Pennathur [f], Lemin Zheng [a,*]

[a] *The Institute of Cardiovascular Sciences and Institute of Systems Biomedicine, School of Basic Medical Sciences; Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education; and Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides, Ministry of Health, Peking University Health Science Center, Beijing 100191, China*

[b] *Proteomics Laboratory, Cleveland Clinic, Cleveland, OH 44195, USA*

[c] *Fujian Key Laboratory of Cardiovascular Disease, Fujian Provincial Hospital, Fuzhou 350001, China*

[d] *Department of Cardiovascular Medicine, Peking University Third Hospital, Beijing 100191, China*

[e] *Department of Gynecology and Obstetrics, Chinese PLA General Hospital, Beijing 100853, China*

[f] *Department of Medicine, University of Michigan, Ann Arbor, MI 48109, USA*

ARTICLE INFO

Article history:
Received 13 May 2012
Received in revised form
30 January 2013
Accepted 7 February 2013
Available online 14 February 2013

Keywords:
HDL
MPO
Oxidation
Endothelial cell
Migration
Free radicals

ABSTRACT

High-density lipoprotein (HDL) plays a key role in protecting against atherosclerosis. In cardiovascular disease, HDL can be nitrated and chlorinated by myeloperoxidase (MPO). In this study, we discovered that MPO-oxidized HDL is dysfunctional in promoting endothelial repair compared to normal HDL. Proliferation assay, wound healing, and transwell migration experiments showed that MPO-oxidized HDL was associated with a reduced stimulation of endothelial cell (EC) proliferation and migration. In addition, we found that Akt and ERK1/2 phosphorylation in ECs was significantly lower when ECs were incubated with oxidized HDL compared with normal HDL. To further determine whether oxidized HDL diminished EC migration through the PI3K/Akt and MEK/ERK pathways, we performed experiments with inhibitors of both these pathways. The transwell experiments performed in the presence of these inhibitors showed that the migration capacity was reduced and the differences observed between normal HDL and oxidized HDL were diminished. Furthermore, to study the effects of oxidized HDL on endothelial cells in vivo, we performed a carotid artery electric injury model on nude mice injected with either normal or oxidized HDL. Oxidized HDL inhibited reendothelialization compared to normal HDL in vivo. These findings implicate a key role for MPO-oxidized HDL in the pathogenesis of cardiovascular disease.

© 2013 Elsevier Inc. All rights reserved.

Introduction

Numerous lines of evidence indicate that the levels of circulating high-density lipoprotein (HDL)[2] and the major HDL protein apolipoprotein A-I (apoA-I) have an inverse correlation with the risk of atherosclerosis and coronary heart disease [1,2]. In addition, clinical trials also demonstrated that elevation of HDL decreases the incidence of cardiovascular disease [3]. HDL protects against cardiovascular disease (CVD) mainly by reverse cholesterol transport, transferring cholesterol from peripheral tissues and delivering it to the liver for final excretion in bile and to steroidogenic organs. In addition, HDL has other important properties including anti-inflammatory [4,5], antioxidative [6], antithrombotic [7], and antiapoptotic [8] properties; increased endothelial cell (EC) nitric oxide synthase [9]; enhanced endothelial progenitor cell (EPC)-mediated vasculoprotection [10]; and stimulation of endothelial cell proliferation and migration [11].

Myeloperoxidase (MPO), a member of the heme peroxidase superfamily, is a potential participant in the promotion and propagation of atherosclerosis. In vivo, HDL is a target for site-specific modification by MPO-derived oxidants in the artery wall with consequent functional inactivation. HDL isolated from atherosclerotic lesions contains numerous MPO-modified peptides, including site-specific oxidative modifications by reactive chlorinating and

Abbreviations: apoA-I, apolipoprotein A-I; Akt, protein kinase B; CVD, cardiovascular disease; Cl-HDL, oxidation of HDL with apoA-I chloro-Tyr; EC, endothelial cell; EPC, endothelial progenitor cell; ERK, extracellular regulated protein kinase; HDL, high-density lipoprotein; HUVEC, human umbilical vein endothelial cell; MEK, MAPK/ERK kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NO_2-HDL, oxidation of HDL with apoA-I nitro-Tyr; SR-BI, scavenger receptor class B type I.

* Corresponding author.
E-mail addresses: zhengl@bjmu.edu.cn, leminzhengl@gmail.com (L. Zheng).

[1] These authors contributed equally to this work.

0891-5849/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.freeradbiomed.2013.02.004

nitrating species [12,13]. Recent studies have suggested that oxidized HDL may become dysfunctional and lose its cardioprotective effects as a result of changing its protein composition. Oxidized HDL can lose of the ability to remove cellular cholesterol through the ATP-binding cassette A1 and G1 (ABCA1 and ABCG1) pathways [14]. ApoA-I is one of the major proteins of HDL, and its cholesterol acceptor activity is inversely correlated with apoA-I nitro- and chlorotyrosine (Tyr) levels, whether in endogenous plasma or with MPO-mediated modification in vitro [12,15]. Interestingly, the 3-nitrotyrosine level in HDL derived from patients with cardiovascular disease is twice that of HDL derived from healthy subjects, and this has left some investigators to speculate that the level of nitrated HDL may be a marker for clinically significant vascular disease [16]. Remarkably, the degree of site-specific modification of apoA-I and enrichment of apoA-I with nitro-Tyr and chloro-Tyr are each associated with a reduction in the ability of apoA-I to promote ABCA1-dependent cholesterol efflux in vitro and in vivo [12,13,15]. Recently, it has been demonstrated that HDL interacts with SR-BI and activates the small G protein Rac via Src kinase, phosphoinositol 3-kinase (PI3K), Akt, and ERK, which stimulates the rapid initial stimulation of lamellipodia formation, an indicator of cell migration [17]. In vivo, carotid artery reendothelialization after perivascular electric injury is diminished in apoA-I-deficient and hence HDL-deficient mice, and reconstitution of apoA-I expression rescues normal reendothelialization. Furthermore, reendothelialization is also impaired in SR-BI-deficient mice [17].

In this study, MPO catalyzed the oxidation of HDL resulting in both apoA-I Tyr nitration (NO_2-HDL) and Tyr chlorination (Cl-HDL) in vitro. We determined the effects of oxidized HDL (NO_2-HDL and Cl-HDL) on the capacity of human umbilical vein endothelial cells (HUVECs) to stimulate proliferation and migration and then compared these results with the effect of normal HDL. In addition, we investigated whether these phenomena induced by oxidized HDL are through the PI3K/Akt and MEK/ERK pathways. Finally, we demonstrated the in vivo relevance of these pathways utilizing the carotid artery electric injury mouse model in which oxidized HDL reduced reendothelialization in vivo.

Materials and methods

Isolation of HDL

Fresh, fasting plasma was separated by centrifugation from peripheral blood obtained from healthy subjects (referred to as HDL). The study protocol was approved by the local ethics committee. HDL was isolated from fresh plasma by ultracentrifugation ($d = 1.063$ to 1.21 g/ml), dialyzed against 3 × 1 L of endotoxin-free phosphate-buffered saline (10 mM, pH 7.0, 5.77 mM Na_2HPO_4, 4.23 mM NaH_2PO_4) with 100 μM diethylenetriamine pentaacetic acid (DTPA; Sigma, USA), sterilized with a 0.22-μm filter, stored in sealed tubes at 4 °C in the dark, and used within 2 months. The concentrations of HDL used in this study were based on apoA-I content of HDL.

HDL nitration and chlorination catalyzed by MPO in vitro

HDL nitration and chlorination were carried out in 60 mM sodium phosphate buffer, pH 7.0, containing 100 μM DTPA, 500 μg/ml HDL protein, 57 nM MPO, and either 1 mM $NaNO_2$ (for the nitration reactions and produced NO_2-HDL) or 100 mM NaCl (for the chlorination reactions and produced Cl-HDL). MPO was added at the start and subsequently hydrogen peroxide was added to initiate reactions. Additions of 25 μM hydrogen peroxide were made at 25-min intervals at 37 °C a total of four times (final concentration 100 μM). Then, 2 mM L-methionine was added to quench the generated HOCl for 25 min. Finally, after MPO modification, HDL was dialyzed against 3×1 L of endotoxin-free phosphate-buffered saline (10 mM, pH 7.0, 5.77 mM Na_2HPO_4, 4.23 mM NaH_2PO_4). UV detection at 240 nM revealed that there is no detectable H_2O_2 after this procedure [13]. Then, Cl-HDL and NO_2-HDL were sterilized with a 0.22-μm filter and stored at 4 °C in the dark.

Protein digestion and peptide analysis

The protein bands were digested according to an in-gel digestion procedure [18]. Briefly, the bands were cut from the gel and washed in 50% ethanol/5% acetic acid, alkylated with iodoacetamide, and reduced with dithiothreitol. The digestions were carried out with either a modified, sequencing-grade trypsin (Promega, Madison, WI, USA) or overnight at room temperature. The peptides were extracted from the gel, evaporated to dryness, and reconstituted in 1% acetic acid for analysis.

The mass spectrometry experiments were performed on an LTQ linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with a nano-electrospray source. The source is operated at a flow rate of 250 nl/min at an operating voltage of ~2.5 kV. The proteolytic peptides were analyzed by reverse-phase HPLC using an Eksigent nano-1D HPLC system (Dublin, CA, USA) with a self-packed 9-cm×75-μm Phenomenex Jupiter C18 column. The peptides were eluted using a 2–70% acetonitrile/0.1% formic acid gradient. The digests were analyzed in a data-dependent manner as previously described [18].

Initial protein modification analysis was carried out by searching the liquid chromatography–tandem mass spectrometry (LC–MS/MS) data specifically against the sequence of apoA-I using the program Sequest bundled into Proteome Discoverer 1.3 (Thermo Scientific, San Jose, CA, USA). Positively identified modifications were required to have Sequest Xcorr scores greater than 2.0 for $[M+2H]^{+2}$ ions and 2.5 for $[M+3H]^{+3}$ ions and these spectra were also subjected to manual inspection.

The quantitation of the modified peptides was carried out using the native reference peptide method [19,20]. This method used a selected reaction monitoring (SRM) experiment to acquire the full MS/MS spectrum for each ion of interest along with an unmodified reference peptide from the protein of interest. The formation of specific fragment ions along with the chromatographic elution times is used to construct chromatograms used in the quantitative analysis. The peak areas for each peptide were determined and normalized to the peak areas of the reference peptides.

Delipidation of HDL and ox-HDL

Normal HDL, Cl-HDL, and NO_2-HDL were delipidated as described previously [21]. Briefly, HDL, Cl-HDL, and NO_2-HDL were subjected to organic extraction with a mixture of butanol and diisopropyl ether (v/v, 40:60) for 30 min at room temperature. The resultant mixture was centrifuged at 2000 rpm for 5 min to separate the aqueous and organic phases. The delipidated HDL (d-HDL containing the protein components) in the aqueous phase was further sterilized and stored at 4 °C.

Isolation of HUVECs and cell culture

HUVECs were isolated by collagenase digestion of umbilical veins from fresh cords [22]. Briefly, the vein was rinsed three times with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer after perfusion of the vein with a metal gauge. Endothelial cells were digested from the vein walls by collagenase type IA (Sigma; 100 U/ml) at 37 °C for 15 min. The reaction was

Download English Version:

https://daneshyari.com/en/article/8271604

Download Persian Version:

https://daneshyari.com/article/8271604

Daneshyari.com