FEBS Letters 582 (2008) 3893–3898

Regulatory role of thioredoxin in homocysteine-induced monocyte chemoattractant protein-1 secretion in monocytes/macrophages

Jing Dai[a,1,2], Xiaofei Wang[a,1], Juan Feng[a], Wei Kong[a], Qingbo Xu[b], Xun Shen[c], Xian Wang[a,*]

[a] *Department of Physiology and Pathophysiology, School of Basic Medical Science, Peking University, Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Beijing 100083, P.R. China*
[b] *Cardiovascular Division, The James Black Centre, Kings College London, London SE5 9NU, UK*
[c] *Institute of Biophysics, Chinese Academy of Sciences, Beijing, P.R. China*

Received 7 October 2008; accepted 17 October 2008

Available online 29 October 2008

Edited by Stuart Ferguson

Abstract **We have previously shown that homocysteine (Hcy) can induce monocyte chemoattractant protein-1 (MCP-1) secretion via reactive oxygen species (ROS) in human monocytes. Here, we show that Hcy upregulates expression of an important antioxidative protein, thioredoxin (Trx), via NADPH oxidase in human monocytes in vitro. The increase of Trx expression and activity inhibited Hcy-induced ROS production and MCP-1 secretion. Of note, 2-week hyperhomocysteinemia (HHcy) ApoE$^{-/-}$ mice showed accelerated lesion formation and parallel lower Trx expression in macrophages than ApoE$^{-/-}$ mice, suggesting that HHcy-induced sustained oxidative stress in vivo might account for impaired Trx and hence increased ROS production and MCP-1 secretion from macrophages, and subsequently accelerated atherogenesis.**

Keywords: Atherosclerosis; Chemokine; Antioxidation; Macrophage; Thioredoxin

1. Introduction

Atherosclerosis is the leading cause of cardiovascular morbidity and mortality in the world. Hyperhomocysteinemia (HHcy) has been recognized as an independent risk factor for this disease for more than 30 years [1]. Despite studies reporting that diet-induced mild to moderate HHcy accelerates atherosclerotic lesion development in ApoE$^{-/-}$ mice [2], the mechanisms are still not completely understood.

Recent studies have elucidated that the development of atherosclerosis involves reactive oxygen species (ROS)-induced oxidative stress and inflammation [3]. During the early stage of atherogenesis, monocytes and T cells are recruited to the subendothelium. The cell recruitment is mainly regulated by adhesion molecules and chemokines. One of the most important chemokines is monocyte chemoattractant protein-1 (MCP-1). Gu et al. [4] reported that the ablation of MCP-1 greatly decreased the lesion size in atherosclerotic LDLR$^{-/-}$ mice. We have previously demonstrated that homocysteine (Hcy) enhances vascular inflammation by promoting the expression and secretion of MCP-1 and IL-8 in human monocytes in vitro, and ROS may mediate the Hcy-induced MCP-1 expression [5].

Human thioredoxin (Trx) is a ubiquitously expressed multifunctional protein crucial for the regulation of transcription factors, modulation of immune response, and especially for antioxidative defense [6]. Trx reduces the oxidized form of Trx peroxidase, which then scavenges ROS, such as H_2O_2 [7], and acts as a redox regulator of signaling molecules and transcription factors. Trx could maintain a reduced intracellular microenvironment by reducing protein disulfides under oxidative stress, thereby mediating the cellular response to redox state [8].

We aimed to determine the role of Trx in Hcy-induced MCP-1 secretion from monocytes both in vitro and in vivo. Our data show that Hcy elevated Trx protein in a NADPH oxidase-dependent manner in human monocytes. Overexpression of Trx inhibited Hcy-induced ROS production as well as MCP-1 secretion which might delay atherogenesis. Interestingly, Trx expression was significantly decreased after long-term HHcy stimulation in early stage of ApoE$^{-/-}$ mice, which might hence accelerate atherogenesis.

2. Materials and methods

2.1. Cell culture

Primary human monocytes were isolated from blood of healthy donors as described previously [9]. Murine peritoneal macrophages were isolated by flushing the peritoneal cavity with ice-cold PBS containing 10% FBS and 1 mM EDTA. Human monocytes (THP-1) and type II alveolar epithelial cells (A549) were obtained from ATCC. For experiments, THP-1 cells were differentiated for 24 h with 10 nM PMA. THP-1 cells were grown in RMPI-1640 containing 10% FBS. A549 cells were cultured in DMEM (Hyclone, Logan, UT) supplemented with 10% FBS.

2.2. Preparation of cell extracts and Western blotting

Cells extracts were prepared and separated by 12% SDS–PAGE, and then transferred onto a nitrocellulose membrane as described previously [9]. Membranes were immunoblotted with anti-Trx antibody (1:2000) (Santa Cruz Biotechnology, Santa Cruz, CA) and then were incubated with IRDye™-conjugated secondary antibody for 1 h. The immunofluorescence band was detected by use of the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

*Corresponding author. Fax: +86 10 82801443.
E-mail address: xwang@bjmu.edu.cn (X. Wang).

[1] J. Dai and X. Wang contributed equally to this work.

[2] Present address: Cardiovascular Division, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA.

Abbreviations: Trx, thioredoxin; MCP-1, monocyte chemoattractant protein-1; ROS, reactive oxygen species; Hcy, homocysteine; HHcy, hyperhomocysteinemia

0014-5793/$34.00
doi:10.1016/j.febslet.2008.10.030

2.3. Plasmid transfection

A549 cells were transfected with 1.5 μg pcDNA3.1-Trx expression plasmid or pcDNA3.1 plasmid for 24 h by use of cationic polymer transfection reagent (JetPEI, France) prior to measure ROS generation or for western blotting.

2.4. Measurement of ROS generation

The generation of ROS in A549 cells was detected by horseradish peroxidase-catalysed chemiluminescence in a light-tight box with a BPCL Ultra-weak luminescence analyzer (Beijing, China) at 37 °C as described in Ref. [10]. Transfection of A549 cells was performed as previously described, with 10 μg/ml horseradish peroxidase, 0.5 mM luminol, and 100 μM Hcy in a total volume of 1 ml.

2.5. Measurement of MCP-1 protein secretion

Cultured human monocytes were treated with Hcy and some were pretreated with 1 μM sodium selenite for 24 h, then the supernatant was harvested 3 days later. MCP-1 concentration was determined on ELISA (R&D Systems, Minneapolis, MN) [9].

2.6. Animals and tissue sample preparations

Female ApoE$^{-/-}$ mice, 6 weeks old, were fed normal mouse chow and water with or without 1.8 g/L DL-Hcy added ($n = 8$ mice) for 2 weeks. Mice were anesthetized, then blood was drawn and serum harvested for determination of plasma Hcy and lipids. Subsequently, peritoneal macrophages were isolated for quantitative real-time PCR analysis. After being removed, the heart was cut transversely. Cryostat

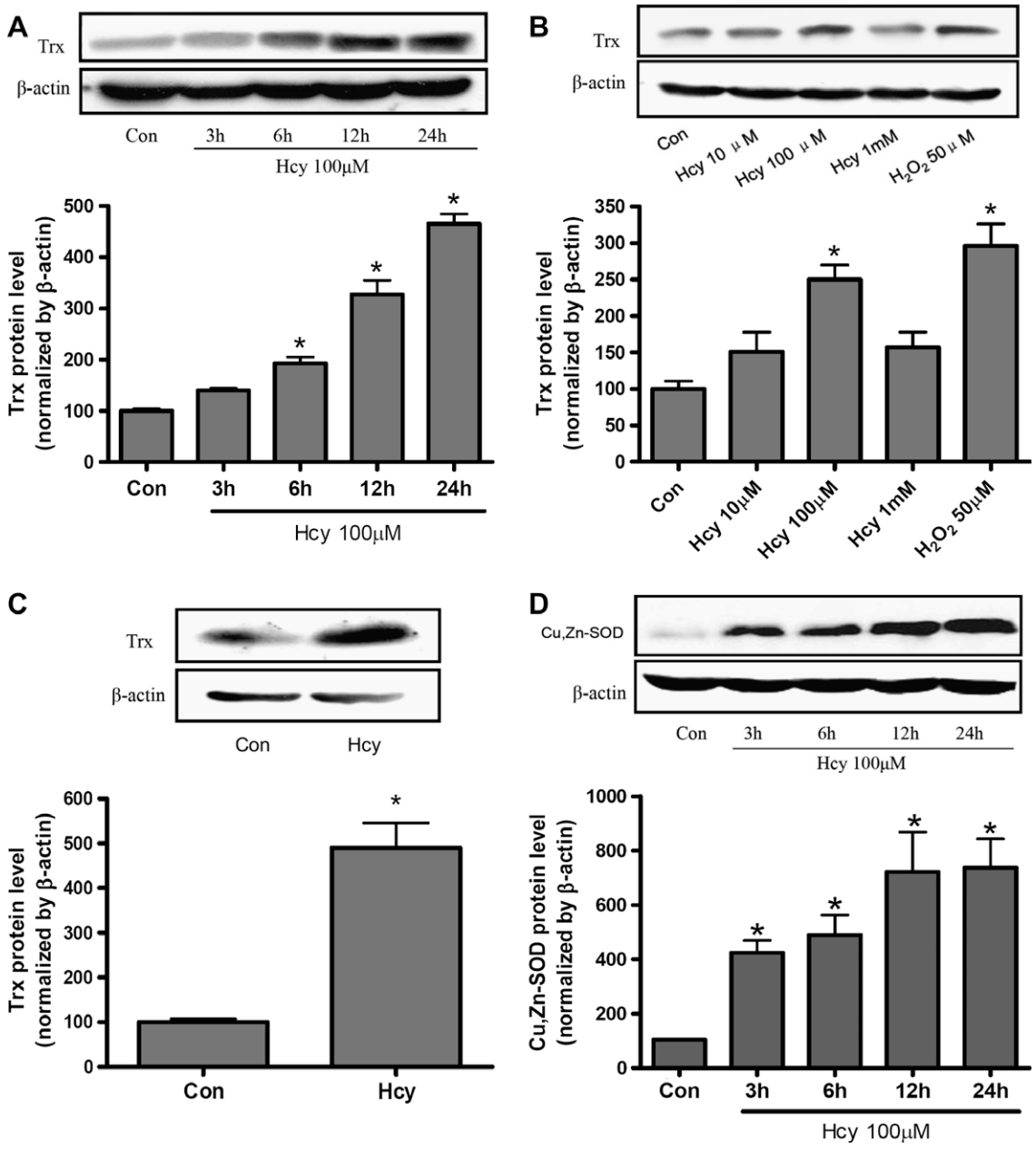

Fig. 1. Effect of Hcy on Trx and Cu, Zn-SOD protein levels in monocytes. Western blot of Trx protein expression in differentiated THP-1 cells incubated with Hcy for various times, (A) and various concentrations or H_2O_2 as positive control, (B); primary cultured human monocytes incubated in the absence or presence of Hcy (100 μM) for 12 h, (C); and Cu, Zn-SOD protein expression in differentiated THP-1 cells incubated with Hcy for various times (D). $^*P < 0.05$ vs. control (Con), $n = 3$.