Chemico-Biological Interactions xxx (2016) 1–9

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Arsenic trioxide suppresses liver X receptor β and enhances cholesteryl ester transfer protein expression without affecting the liver X receptor α in HepG2 cells

Tain-Junn Cheng [a, b], Shu-Wen Lin [c], Chih-Wei Chen [b, d], How-Ran Guo [c, e, **], Ying-Jang Wang [c, f, g, *]

[a] Department of Neurology and Occupational Medicine, Chi Mei Medical Center, 901 Zhonghua Road, Yongkang Dist., Tainan 710, Taiwan
[b] Department of Occupational Safety and Health/Institute of Industrial Safety and Disaster Prevention, College of Sustainable Environment, Chia Nan University of Pharmacy and Science, 60 Sec. 1, Erren Road, Rende Dist., Tainan 71710, Taiwan
[c] Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan
[d] Division of Neurosurgery, Department of Surgery, Chi Mei Medical Center, 901 Zhonghua Road, Yongkang Dist., Tainan 710, Taiwan
[e] Department of Occupational and Environmental Medicine, National Cheng Kung University Hospital, 138 Sheng-Li Road, Tainan 704, Taiwan
[f] Department of Biomedical and Informatics, Asia University, 500 Lioufeng Road, Wufeng, Taichung, 41354, Taiwan
[g] Department of Medical Research, China Medical University Hospital, China Medical University, 500 Lioufeng Road, Wufeng, Taichung, 41354, Taiwan

ARTICLE INFO

Article history:
Received 29 March 2016
Received in revised form
21 August 2016
Accepted 9 September 2016
Available online xxx

Keywords:
Arsenic
Atherosclerosis
Liver X receptor
Cholesteryl ester transfer protein
Sterol regulatory element-binding protein
Reverse cholesterol transport

ABSTRACT

Chronic arsenic exposure is associated with cerebrovascular disease and the formation of atherosclerotic lesions. Our previous study demonstrated that arsenic trioxide (ATO) exposure was associated with atherosclerotic lesion formation through alterations in lipid metabolism in the reverse cholesterol transport process. In mouse livers, the expression of the liver X receptor β (LXR-β) and the cholesteryl ester transfer protein (CETP) was suppressed without any changes to the lipid profile. The aim of this study was to elucidate whether ATO contributes to atherosclerotic lesions by suppressing LXR-β and CETP levels in hepatocytes. HepG2 cells, human hepatocytes, were exposed to different ATO concentrations *in vitro*. Cell viability was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay. The liver X receptor α (LXR-α), LXR-β, sterol regulatory element-binding protein-1c (SREBP-1c) and CETP protein levels were measured by Western blotting, and their mRNA levels were measured by real-time PCR. Cholesterol efflux was analyzed by flow cytometry.

The results showed ATO inhibited LXR-β mRNA and protein levels with a subsequent decrease in SREBP-1c protein levels and reduced cholesterol efflux from HepG2 cells into the extracellular space without influencing LXR-α mRNA and protein levels. CETP protein levels of HepG2 cells were significantly elevated under arsenic exposure. Transfection of LXR-β shRNA did not change CETP protein levels, implying that there is no cross-talk between LXR-β and CETP. In conclusion, arsenic not only inhibits LXR-β and SREBP-1c mRNA and protein levels but also independently increases CETP protein levels in HepG2 cells.

1. Introduction

Arsenic is a natural element abundant in the environment. Excessive arsenic exposure can cause chronic diseases such as cancers, cardiovascular disease (CAD), peripheral vascular disease (such as blackfoot disease; BFD) and cerebrovascular diseases (CVD), among others [1,2]. In a previous epidemiology study, we found that chronic arsenic exposure was associated with an increased risk of CVD mortality [1]. Atherosclerosis is the most common cause of CAD and CVD, and we propose that arsenic

* Corresponding author. Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan.
** Corresponding author. Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan.
E-mail addresses: hrguo@mail.ncku.edu.tw (H.-R. Guo), yjwang@mail.ncku.edu.tw (Y.-J. Wang).

http://dx.doi.org/10.1016/j.cbi.2016.09.009
0009-2797/

Abbreviations

22R-OHC	22R-hydroxylcholesterol
9-cis-RA	9-cis retinoic acid
ATO	arsenic trioxide
ABCA1	ATP-binding cassette transporters A1
BFD	blackfoot disease
CAD	cardiovascular disease
CETP	cholesteryl ester transfer protein
CETP-1	cholesteryl ester transfer protein-1
CVD	cerebrovascular diseases
ELISA	enzyme-linked immunosorbent assay
FACS	Fluorescence Activated Cell Sorting
HEPES	N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein-cholesterol
LXR	liver X receptors
LXR-α	liver X receptor α
LXR-β	liver X receptor β
LXREs	LXR responsive elements
MEM	minimum essential medium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NaOH	sodium hydroxide
NBD	7-nitrobenz-2-oxa-1,3-diazol-4-yl
qPCR	real-time quantitative polymerase chain reaction
RCT	reverse cholesterol transport
RNAi	RNA interference
RXR	retinoid X receptor
SREBP-1	sterol regulatory element-binding protein-1

exposure leading to CVD is caused by atherosclerotic lesion formation.

It is well known that cholesterol plays an important role in atherosclerotic lesion formation. Biotransformation processes among different types of lipids and cholesterols can also affect atherosclerotic lesion formation. One such process is reverse cholesterol transport (RCT) in which cholesterol is transported back to the liver from the cells in the periphery of the body. The first step of RCT is 'cholesterol efflux', where high-density lipoprotein (HDL) removes cholesterol from lipid-laden cells into the plasma and then delivers it to the hepatocytes in the liver. During this transportation, cholesteryl ester transfer protein (CETP) transfers cholesterol from HDL-C to apolipoprotein B-containing lipoproteins to become low-density lipoprotein (LDL)-C. In hepatocytes, at least two pathways take up cholesterol from the plasma to complete RCT. One pathway is a LDL receptor-mediated process and the other is a HDL receptor-mediated process [3]. After entering the hepatocyte, cholesterol can be secreted into bile, undergo conversion to bile acids [4] or be secreted again by the hepatocyte [5]. The RCT process has an important antithrombotic effect because it removes cholesterol from the intima of vasculature [4]. The major role of RCT is cholesterol efflux from cells, and it is controlled by the liver X receptors (LXRs). LXRs were first found in the liver [5] and are a nuclear receptor superfamily [6]. LXR-α is highly expressed in the liver, the intestines, the kidneys and adipose tissue, and LXR-β is found in nearly all tissues [7]. Therefore, the RCT process is also likely occurring in hepatocytes.

RCT is regulated primarily by LXRs. These receptors function as "cholesterol sensors". LXR agonists can be endogenous oxysterols such as 22(R)-hydroxycholesterol, 24(S)-25-epoxycholesterol and other non-steroidal synthetic LXR agonists. Both LXR-α and LXR-β bind to the retinoid X receptor (RXR) to form LXR responsive elements (LXREs), which then increase the transcription of target genes. As a result, the serum HDL-C levels increase and atherogenesis decreases [4]. LXREs also regulate sterol regulatory element-binding protein-1 (SREBP-1) mRNA expression [8]. SREBP-1 is abundant in the liver and can regulate the lipogenesis of free fatty acids and triglycerides. Schuster et al. [9] conversely demonstrated that LXR depletion impaired the cholesterol efflux pathway in macrophages by affecting the gene expression of ATP-binding cassette transporters A1 (ABCA1) and ABCG1, in turn developing cholesterol overload in the macrophages of the spleen, lungs and arterial walls.

During transportation of HDL-C to the liver, CETP can transfer cholesterol from HDL-C to become LDL-C. Therefore, CETP plays an important role in determining the serum level of LDL-C. When CETP activity increases, the serum level of LDL-C increases, and atherosclerotic lesion formation is enhanced. Okamoto et al. [10] demonstrated that reducing CETP activity by applying CETP inhibitors could elevate HDL-C levels and significantly reduce atherosclerosis in rats. Human studies demonstrated that a genetic CETP deficiency could elevate HDL-C and subsequently inhibit atherosclerosis, decreasing the risk of CAD [11]. CETP activity is increased by a high cholesterol, high fat diet [12], and its expression is regulated by LXR-α [13].

Many possible mechanisms are proposed for how arsenic induces atherosclerosis by affecting lipid metabolism [14–18]. Simeonova et al. [19] observed atherosclerotic lesion formation in rats under a high cholesterol diet after 24 weeks of arsenic exposure in the form of sodium arsenite (20–100 μg/ml in drinking water). An epidemiological study on the residents of the Lang-Yang Basin in Taiwan demonstrated the joint effects of apolipoprotein E and monocyte chemoattractant protein-1 on the LDL-C inflammatory reaction in the progression of atherosclerosis following chronic arsenic exposure [20]. Our previous study found that arsenic exposure could accelerate aortic atherosclerotic lesion formation by modifying the function of RCT in hypertensive rats, especially when they were exposed to a high cholesterol diet early in life, through suppression of LXR-β and CETP-1 in the liver [21]. However, the parallel changes to LXR-β and CETP resulting in atherosclerotic lesion formation after arsenic exposure are not compatible with the aforementioned effects of LXR and CETP on atherosclerotic lesion formation. Therefore, we hypothesized that LXR could interfere with CETP expression after arsenic exposure.

In this *in vitro* study, we evaluated the effects of arsenic trioxide on RCT-related proteins in HepG2 hepatocytes by measuring the mRNA levels of LXR-α, LXR- β, and SREBP-1c using real-time quantitative PCR (qPCR) and subsequent LXR-α, LXR- β, SREBP-1c and CETP protein levels by Western blotting. We found that ATO inhibited LXR-β and SREBP-1c mRNA and protein levels, enhanced CETP protein level without influencing the mRNA or protein levels of LXR-α (Fig. 1). There were no interactions found between LXR-β and CETP with ATO exposure. These two results contribute to the idea of atherosclerotic lesion formation after arsenic exposure.

2. Materials and methods

2.1. Cell culture and arsenic treatment

HepG2 cells (a human hepatocellular carcinoma cell line) were

Please cite this article in press as: T.-J. Cheng, et al., Arsenic trioxide suppresses liver X receptor β and enhances cholesteryl ester transfer protein expression without affecting the liver X receptor α in HepG2 cells, Chemico-Biological Interactions (2016), http://dx.doi.org/10.1016/j.cbi.2016.09.009