Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 29 (2016) 21–26

Journal of Nutritional Biochemistry

Astaxanthin prevents and reverses the activation of mouse primary hepatic stellate cells

Yue Yang[a], Minkyung Bae[a], Bohkyung Kim[a], Young-Ki Park[a], Sung I. Koo[a], Ji-Young Lee[a,b,*]

[a]Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA
[b]Institute of Systems Genomics, University of Connecticut, Storrs, CT 06269, USA

Received 18 May 2015; received in revised form 27 October 2015; accepted 6 November 2015

Abstract

Activation of hepatic stellate cells (HSCs) is a critical step that leads to the development of liver fibrosis. We showed that astaxanthin (ASTX), a xanthophyll carotenoid, displays antifibrogenic effects in LX-2 cells, a human HSC cell line. In this study, we further determined the effect of ASTX on HSC activation and inactivation using primary HSCs from C57BL/6J mice. Quiescent and activated HSCs were incubated with ASTX (25 μM) at different stages of activation. ASTX prevented the activation of quiescent HSCs, as evidenced by the presence of intracellular lipid droplets and reduction of α-smooth muscle actin, an HSC activation marker. Also, ASTX reverted activated HSCs to a quiescent phenotype with the reappearance of lipid droplets with a concomitant increase in lecithin retinol acyltransferase mRNA. Cellular accumulation of reactive oxygen species was significantly reduced by ASTX, which was attributable to a decrease in NADPH oxidase 2 expression. The antifibrogenic effect of ASTX was independent of nuclear erythroid 2-related factor 2 as it was observed in HSCs from wild-type and *Nrf2*$^{-/-}$ mice. In conclusion, ASTX inhibits HSC activation and reverts activated HSCs to a quiescent state. Further investigation is warranted to determine if ASTX effectively prevents the development of liver fibrosis.
© 2015 Elsevier Inc. All rights reserved.

Keywords: Astaxanthin; Liver fibrosis; Hepatic stellate cells; Reversal of hepatic stellate cell activation; NASH; Carotenoids

1. Introduction

Nonalcoholic fatty liver disease is the most common cause of chronic liver diseases in the developed countries [1]. Fatty liver is susceptible to oxidative stress and inflammation, resulting in the progression of simple steatosis to nonalcoholic steatohepatitis (NASH) [2]. In response to oxidative stress, hepatocyte death and inflammation, the liver initiates a wound-healing response by producing extracellular matrix (ECM) [3]. Excessive deposition of ECM leads to liver fibrosis. Although fibrosis is a reversible process, it can slowly advance to cirrhosis, which is considered irreversible and disrupts normal liver functions [4].

Hepatic stellate cells (HSCs) are primarily responsible for ECM production and fibrosis development in the liver [5]. In the normal liver, quiescent HSCs (qHSCs) are present in the space of Disse and contain cytoplasmic lipid droplets mainly consisting of retinyl esters [6]. In response to injury, qHSCs transdifferentiate to myofibroblast-like cells, which highly express α-smooth muscle actin (α-SMA), a myofibroblast marker, and ECM proteins, e.g., procollagen type I, alpha 1 (Col1A1). The activated HSCs (aHSCs) are highly proliferative and migrate to the sites of injury. Recent studies have suggested that HSC activation is a reversible process [7]. When stimulants, such as oxidants or fibrogenic cytokines, are removed, aHSCs are either eliminated through apoptosis or reverted to an inactive phenotype, i.e., inactivated HSCs (iHSCs), which share common features with qHSCs but differ in their sensitivity to fibrogenic insults [7].

Astaxanthin (ASTX) is a xanthophyll carotenoid that is abundant in red marine animals, such as salmon and shrimp [8]. It is known as a potent antioxidant [9]. Our previous study demonstrated that ASTX prevents fibrogenic responses and accumulation of reactive oxygen species (ROS) induced by transforming growth factor β1 (TGFβ1), a fibrogenic cytokine, in LX-2 cells, a human HSC cell line [10]. LX-2 cells are commonly used as an HSC cell model, but they exhibit a minimal characteristics of a quiescent phenotype [11]. Therefore, the present study used primary mouse HSCs, which are in a quiescent state and can be transdifferentiated to aHSCs in culture, to determine the antifibrogenic effect of ASTX.

2. Materials and methods

2.1. Cell culture

Mouse primary HSCs were isolated from C57BL/6J, nuclear erythroid 2-related factor 2 (NRF2) knockout (*Nrf2*$^{-/-}$) and wild-type mice using a pronase/collagenase

Abbreviations: ADRP, adipocyte differentiation-related protein; α-SMA, α-smooth muscle actin; ASTX, astaxanthin; Col1A1, procollagen type I, alpha 1; ECM, extracellular matrix; GCLc, glutamate-cysteine ligase catalytic subunit; HSC, hepatic stellate cell; aHSC, activated HSC; iHSC, inactivated HSC; qHSC, quiescent HSC; LRAT, lecithin retinol acyltransferase; NASH, nonalcoholic steatohepatitis; NRF2, nuclear erythroid 2-related factor 2; qRT-PCR, quantitative real-time PCR; ROS, reactive oxygen species; SREBP1c, sterol regulatory element binding protein 1c; tBHP, *tert*-butyl hydrogen peroxide; TGFβ1, transforming growth factor β1.

* Corresponding author. Institute of Systems Genomics, University of Connecticut, Storrs, CT 06269, USA. Tel.: +1-860-486-1827; fax: +1-860-486-3674.
E-mail address: ji-young.lee@uconn.edu (J.-Y. Lee).

http://dx.doi.org/10.1016/j.jnutbio.2015.11.005
0955-2863/© 2015 Elsevier Inc. All rights reserved.

digestion method as previously described [10]. The cells were then cultured on untreated petri dishes (BD Falcon, Franklin Lakes, NJ), which is a well-established condition for HSC activation *in vitro*[12], and were maintained in a low-glucose DMEM medium supplemented with 10% FBS, 4 mM L-glutamine and P/S (100 U/100 μg per milliliter). Cells were cultured in a 37°C humidified cell culture chamber with 5% CO_2. All cell culture supplies were purchased from HyClone unless stated otherwise (Thermo Scientific, Logan, UT).

2.2. ASTX treatment

ASTX, provided by Fuji Chemical Industry Co., Ltd. (Toyama, Japan), was solubilized in DMSO as previously described [10]. After plated on an uncoated dish, mouse qHSCs were incubated with 25 μM ASTX during activation. aHSCs were prepared by culturing qHSCs on an uncoated dish for 6 days and were incubated with 25 μM ASTX for 2 or 4 more days. Also, cells were incubated with 3 μM *tert*-butyl hydrogen peroxide (tBHP, Thermo Scientific) for 12 h with or without 25 μM ASTX. Medium containing ASTX was replenished daily and DMSO vehicle control was run in parallel. Hereafter, "Xd+Yd" indicates that HSCs were activated for X days followed by ASTX treatment for Y days. Experimental scheme is shown in Fig. 1.

2.3. Quantitative real-time PCR (qRT-PCR)

Total RNA extraction, cDNA synthesis and qRT-PCR were conducted using a Bio-Rad CFX96 Real-Time system (Bio-Rad, Hercules, CA) as previously described [13,14]. Primer sequences will be available upon request.

2.4. Western blot analysis

Whole cell lysates and human tissue protein extracts were prepared, western blot analysis was performed as previously described [14] using antibodies against α-SMA (Sigma, St. Louis, MO) and β-tubulin (Santa Cruz) was used as a loading control.

2.5. ROS measurement

Mouse primary qHSCs were seeded on an uncoated black 24-well μ-Plate (Ibidi, Verona, WI). At day 6 of activation, cellular ROS levels were measured using dichlorofluorescin (Sigma-Aldrich, St. Louis, MO) as previously described [15]. The cells were then fixed with 4% formaldehyde for 10 min and incubated with 0.1% CellTag 700 stain (Li-COR, Lincoln, NE), a fluorescent stain, for cell number normalization, for 1 h. Fluorescent signals were obtained by an Odyssey CLx Infrared Imaging System (Li-COR) and a Li-COR Image Studio software. Data were expressed as relative fluorescent intensity divided by total cell stain signal.

2.6. Statistical analysis

One-way analysis of variance with Newman Keul's pairwise comparison or unpaired *t* test was used to determine significant differences between groups by GraphPad Prism6 (GraphPad Software, La Jolla, CA). *P* values less than .05 were considered significant and all values were presented as mean±S.E.M.

3. Results

3.1. ASTX inhibited the activation of qHSCs

To investigate the inhibitory effect of ASTX on the activation of qHSCs, mouse primary HSCs were cultured on plastic substratum for 6 days in the absence or presence of ASTX. The expression of α-SMA was markedly induced during activation in controls, whereas ASTX significantly decreased their mRNA (Fig. 2A). Protein levels of α-SMA were also noticeably decreased when HSCs were treated with ASTX for 2 days during activation (Fig. 2B). At days 2 and 4, no noticeable differences in cell morphology were observed between control and ASTX-treated cells. However, at day 6, control cells displayed stretched, myofibroblast-like appearance with few cytoplasmic lipid droplets whereas the cells treated with ASTX for 2–6 days displayed more lipid droplets than controls (Fig. 3).

3.2. ASTX reverted aHSCs to iHSCs

To examine if ASTX can revert aHSCs to iHSCs, aHSCs were incubated with ASTX for 2 or 4 days. While control aHSCs displayed a myofibroblast-like appearance, there was reappearance of cytoplasmic lipid droplets in the ASTX-treated HSCs (Fig. 4A). Furthermore, ASTX significantly decreased α-SMA and Col1A1 mRNA (Fig. 4B) and α-SMA protein (Fig. 4C). As lipid droplets reappeared when aHSCs were incubated with ASTX, we examined the ability of ASTX to alter the expression of genes that facilitate lipid droplet formation. ASTX significantly decreased the mRNA levels of sterol regulatory element binding protein 1c (SREBP1c), a transcriptional factor critical for lipogenesis [16], but markedly increased lecithin retinol acyltransferase (LRAT) mRNA, an enzyme that esterifies retinol to retinyl esters (Fig. 4D). There was no significant difference in mRNA levels of adipocyte differentiation-related protein (ADRP), a lipid droplet surface protein [17], between control and ASTX-treated cells.

3.3. ASTX prevented cellular ROS accumulation independent of NRF2 in HSCs

As ROS can activate HSCs [18,19], we evaluated if ASTX can reduce cellular ROS levels. ASTX treatment for 6 days during the activation of mouse primary HSC significantly lowered cellular ROS levels (Fig. 5A). NADPH oxidases NOX2 and NOX4 have been shown to produce ROS in HSCs, activating the cells [20,21]. When ASTX was added at day 4 for 2 days during HSC activation, ASTX significantly increased NOX4 mRNA but decreased NOX2 mRNA (Fig. 5B).

The activation of NRF2, a transcription factor for endogenous antioxidant defense, has been suggested to ameliorate liver fibrosis [22]. Also, we previously observed that ASTX induced the hepatic expression of NRF2 and its target antioxidant genes *in vivo*[23]. Therefore, we determined if the inhibitory action of ASTX in HSC activation is mediated via NRF2. When HSCs isolated from wild-type and *Nrf2*$^{-/-}$ mice were activated for 6 days, α-SMA mRNA was not significantly different between HSCs from wild-type and *Nrf2*$^{-/-}$ mice, while there was a significantly lower Col1A1 in *Nrf2*$^{-/-}$ HSCs

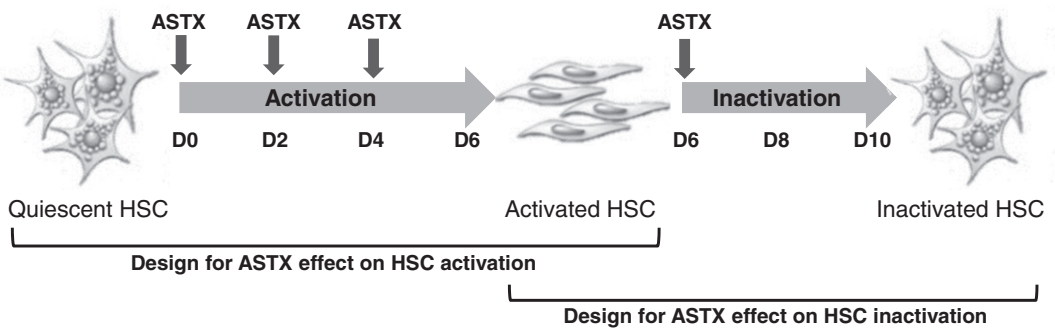

Fig. 1. Experimental scheme for HSC activation and ASTX treatment. Mouse primary HSCs were activated on a plastic surface for 6 days and ASTX was added during the activation at D0, D2 and D4 until D6 (Design for ASTX effect on HSC activation). aHSCs (at D6 with no prior exposure to ASTX during activation) were also treated with ASTX for 2 or 4 additional days to address the effect of ASTX on inactivation of aHSCs (Design for ASTX effect on HSC inactivation).

Download English Version:

https://daneshyari.com/en/article/1989579

Download Persian Version:

https://daneshyari.com/article/1989579

Daneshyari.com