Atherosclerosis 204 (2009) 127–135

Contents lists available at ScienceDirect

Atherosclerosis

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

Identification of trichostatin A as a novel transcriptional up-regulator of scavenger receptor BI both in HepG2 and RAW 264.7 cells

Yi Bao[1], Yuan Yang[1], Li Wang, Lei Gao, Wei Jiang, Lifei Wang, Shuyi Si**, Bin Hong*

Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100050, China

ARTICLE INFO

Article history:
Received 8 February 2008
Received in revised form 23 August 2008
Accepted 26 August 2008
Available online 11 September 2008

Keywords:
Scavenger receptor class B type I (SR-BI/CLA-1)
Trichostatin A (TSA)
Transcriptional up-regulator
Reverse cholesterol transport (RCT)

ABSTRACT

Scavenger receptor class B type I (SR-BI) and its human homologue CLA-1 plays an important role in reverse cholesterol transport (RCT). Using a previously established cell-based CLA-1 up-regulator screening assay, one of the positive strains, 04-9179, presented potent activity in elevating CLA-1 transcriptional level. We report here the identification of an active compound 9179A as a known compound trichostatin A (TSA), and its effects on CLA-1/SR-BI expression both in HepG2 human hepatoma cells and RAW 264.7 murine macrophage cells in vitro. The results showed that the mRNA and protein level of CLA-1/SR-BI were significantly up-regulated by 9179A both in HepG2 and RAW 264.7 cells. Corresponding to this, the uptake of DiI-HDL by both cells and the efflux of [^{3}H]cholesterol by RAW 264.7 cells were increased by 9179A in dose-dependent manner. ABCA1 was also increased but SR-A decreased by 9179A in RAW 264.7 cells. Using a combination of reporter assays with various deletion in CLA-1 promoter and electrophoretic mobility shift assay, we demonstrated that −419/−232 bp fragment of the CLA-1 promoter mediated the effects of 9179A (i.e., TSA). Together, these studies identified TSA as a novel up-regulator of CLA-1/SR-BI both in HepG2 and RAW 264.7 cells.

1. Introduction

Atherosclerosis is a progressive disease that is characterized by the accumulation of lipid-rich plaques within the walls of arteries [1]. Despite substantial therapeutic progress resulting from the widespread use of statins, which primarily lower plasma levels of low density lipoprotein (LDL) cholesterol, atherosclerosis is still one of the leading causes of mortality in industrialized and developing nations. Plasma concentrations of HDL cholesterol (HDL-C) are inversely proportional to the risk for atherosclerotic cardiovascular disease. One of the major atheroprotective actions of HDL particles involves the transport of excess cholesterol from peripheral tissues to the liver for excretion, a process known as reverse cholesterol transport (RCT) [2]. HDL-mediated RCT represents a major target for the development of innovative antiatherogenic strategies to reduce the risk of atherosclerotic cardiovascular disease.

Scavenger receptor class B type I (SR-BI) is the first molecularly well-defined HDL receptor in mice [3], and CLA-1 (CD36 and Lysosomal integral membrane protein-II Analogous-1) is the human homologue of the murine SR-BI [4]. Hepatic expression of SR-BI promotes selective uptake of cholesteryl ester from HDL by the liver [5]. SR-BI is also expressed in macrophages and can promote cholesterol efflux to mature HDL in vitro and in vivo [6–8]. Furthermore, hepatic SR-BI could positively regulate the rate of macrophage RCT in vivo [9]. So CLA-1/SR-BI plays a pivotal role both in the initial (cholesterol efflux and removal from the artery wall) and final (selective HDL-C uptake in the liver) phase of RCT which takes place mainly in macrophages and hepatocytes, respectively. The importance of SR-BI in overall HDL-C metabolism and its antiatherogenic activity in vivo has been definitely established by SR-BI gene manipulation in mice [e.g., [7,8,10–12]], and CLA-1/SR-BI receptor has been suggested as a new preventative and/or therapeutic target for atherosclerosis [13,14].

Based on these considerations, a primary high-throughput screening assay was previously established using human hepatoma HepG2 cells stably transfected with a gene construct in which the promoter and regulatory control elements for the CLA-1 gene was fused to a firefly luciferase reporter gene [15]. Using this cell line, 6000 microbial secondary metabolite crude extracts were screened, and the preliminary experiments suggested that the positive strain 04-9179 present potent activity in elevating the CLA-1 transcriptional level. We report here the identification of an active compound 9179A as a known compound trichostatin A (TSA), and

* Corresponding author. Tel.: +86 10 63028003; fax: +86 10 63017302.
** Corresponding author. Tel.: +86 10 63180604; fax: +86 10 63180604.
E-mail addresses: sisyimb@hotmail.com (S. Si), binhong69@hotmail.com (B. Hong).
[1] These authors equally contributed to this study.

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doi:10.1016/j.atherosclerosis.2008.08.041

its effects on CLA-1/SR-BI expression and function both in HepG2 and RAW 264.7 cells. We also investigated the effects of TSA on the expression of other lipoprotein transporter/receptor in RAW 264.7 cells. Finally, we identified the *cis*-elements responsible for the pronounced up-regulation of CLA-1 expression in HepG2 cells by TSA treatment.

2. Materials and methods

2.1. Materials

1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI)-labeled human HDL (DiI-HDL) was purchased from Biomedical Technologies (Stoughton, MA, USA). The mouse monoclonal antibody against human CLA-1 was from BD Biosciences (San Jose, CA, USA). The goat polyclonal antibody against mouse SR-BI was from Novus Biologicals (Littleton, CO, USA). The rabbit polyclonal antibody against amino acids 91–310 of ABCA1 of human origin which was recommended for detection of ABCA1 of mouse, rat and human origin was from Santa Cruz Biotechnology (Santa Cruz, USA). 60 mm dishes and 6-, 24- and 96-well plates for cell culture were obtained from Corning (Corning, MA, USA). Cell culture media and fetal bovine serum (FBS) were purchased from Hyclone (Logan, UT, USA). Trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), sodium butyrate (NaBu) and rosiglitazone (ROS) were purchased from Sigma (St. Louis, MO, USA).

2.2. Cell culture and sample treatment

HepG2, CLAp-LUC HepG2 cells were grown as previously described [15]. RAW 264.7 murine macrophage cells were grown in Dulbecco's modified Eagle's medium (DMEM, high glucose) containing 10% FBS (v/v) at 37 °C in a humidified 5% CO_2 incubator.

2.3. Fermentation of positive strain 04-9179

Positive strain 04-9179, from the Center for Culture Collection of Pharmaceutical Microorganisms (Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, China), was inoculated into a 50 ml flask containing 10 ml of medium I for actinomycetes [15] and incubated for 2 days at 28 °C on a rotary shaker (220 rpm). Then, 5 ml of the first seed culture was transferred to a 500 ml flask containing 100 ml of medium I and incubated for 2 days at 28 °C on a rotary shaker (220 rpm). Of the second seed culture, 100 ml was inoculated into a 5000 ml flask containing 900 ml of medium I and incubated on a rotary shaker (220 rpm) at 28 °C for 4 days.

2.4. Isolation, purification and structure elucidation of 9179A

The fermentation broth of positive strain 04-9179 (3 × 20 L) was centrifuged at 5,000 rpm for 20 min. The supernatants were passed through a 6 × 40 cm column of HP20 macroporous resin (Mitsubishi Chemical, Tokyo, Japan), which was eluted with water, 30% acetone/water, 50% acetone/water, 80% acetone/water, and 100% acetone. The 80% acetone/water fraction containing CLA-1 up-regulatory activity was concentrated to dryness, subjected to a 2 × 100 cm column of the octa decyltrichloro silane (ODS, YMC, Kyoto, Japan), and eluted with two bed volumes of 30% MeOH/water, 60% MeOH/water, and 85% MeOH/water, respectively. The eluates were monitored by analytical high-performance liquid chromatography (HPLC), which was conducted using a VP-ODS column (150 × 4.6 mm, 5 μm, Shimadzu, Kyoto, Japan) with methanol/water/TFA (50:50:0.1) as an eluent at a flow rate of 1 ml/min, UV detection at 352 nm and oven temperature at 40 °C. The fractions containing CLA-1 up-regulatory activity were combined and concentrated to dryness for further purification. The pure active compound 9179A were obtained by purification with semipreparative HPLC (Shim-pack PREP-ODS, 390 × 20 mm, Shimadzu), which was eluted with 55% MeOH/water. The physicochemical properties of 9179A was determined via UV spectrometer (UV-200S, Shimadzu), mass spectrometer (JMS-DX 300, Jeol, Tokyo, Japan), and nuclear magnetic resonance (NMR) spectrometer (XL-400, Varian, Palo Alto, CA, USA). The structure of 9179A was elucidated by means of spectral data of UV, IR, EI-MS, ESI-MS, ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, DEPT and ^{1}H–^{1}H COSY with CD_3OD as the solvent.

2.5. RNA isolation and real time reverse transcriptase (RT)-PCR

HepG2 or RAW 264.7 cells were plated in a 6-well plate in MEM or DMEM media with 10% FBS at 500,000 cells/well and incubated for 24 h. After 24 h treatment with the indicated concentration of 9179A or vehicle (0.1% DMSO) at 37 °C, total cellular RNA was isolated using QuickPrep Total RNA Extraction Kit (GE Healthcare, Piscataway, NJ, USA), and analyzed by agarose gel electrophoresis to check that ribosomal RNAs were intact. RNA samples (4 μg) were reverse transcribed using SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) and random hexamers according to the manufacturer's instructions.

For quantitative real time PCR, SYBR Green PCR Core Reagent (Applied Biosystems, Foster City, CA, USA) was used on an iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad, California, USA). The sequences of the forward and reverse primers used for amplification were 5′-CTG GCA GAA GCG GTG ACT-3′ and 5′-CAG AGC AGT TCA TGG GGA TT-3′ for CLA-1 (the size of the amplicon is 97 bp), 5′-TCC ACT GGC GTC TTC ACC-3′ and 5′-GGC AGA GAT GAT GAC CCT TTT-3′ for human GAPDH (the size of the amplicon is 78 bp), 5′-GCA AGA AGC CAA GCT ATA GGG-3′ and 5′-AAG AAG CGG GGT GTA GGG-3′ for murine SR-BI (the size of the amplicon is 97 bp), 5′-GCC TGC TGG TGG TCA TCT-3′ and 5′-ACC ACG CTG GGG TCA CTA-3′ for murine ABCA1 (the size of the amplicon is 61 bp), 5′-CTT TAC CAG CAA TGA CAA AAG AGA-3′ and 5′-ATT TCA CGG ATT CTG AAC TGC-3′ for murine SR-A (the size of the amplicon is 93 bp), 5′-AGC TTG TCA TCA ACG GGA AG-3′ and 5′-TTT GAT GTT AGT GGG GTC TCG-3′ for murine GAPDH (the size of the amplicon is 62 bp). The PCR reaction conditions were 95 °C for 5 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. A step of 78 °C for 10 s during which fluorescence was measured was included at the end of each cycle. The reactions were subjected to a heat dissociation protocol after the final cycle of PCR to indicate the proper temperature for fluorescence detection. After PCR amplifications, data were analyzed with iQ5 Optical System version 1.1.1442.0 software (Bio-Rad). The threshold cycle (Ct) was calculated from the programme. Serial dilution of the cDNA was subjected to real time PCR. For each transcript, plots of Log_2 (dilution factor) against the Ct values provided an estimate of the efficiency of the amplification. The relative amounts of a particular transcript in different samples were calculated according to the Pfaffl's method [16].

2.6. Analysis for cell surface expression by flow cytometry or Western blot

For the assays, the pretreatment of cells was the same as that for RNA isolation. Then HepG2 cells were trypsinized from the plate, washed and resuspended in 4% paraform fixing solution, incubated overnight at 4 °C. After the fixation, cells were blocked for 15 min at 4 °C in PBS containing 5% FBS. Cells were then incubated with monoclonal antibody to CLA-1 (BD Biosciences, San Jose, CA, USA)