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Regulation of apolipoprotein A-I gene expression by the histamine H1 receptor: Requirement for NF-κB

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ABSTRACT

Aims: Earlier it had been found by us that apolipoprotein A-I (apo A-I) is suppressed by histamine in HepG2 cells. Histamine has been shown to regulate NF-κB activity, though not in hepatocytes. Therefore we examined the role of the histamine receptors and NF-κB in histamine-mediated apo A-I gene expression in HepG2 liver cells.

Main methods: The effect of histamine on histamine H1 receptor expression, and NF-κB p65 and p50 subunits was examined by Western blot. Histamine H1 receptor involvement was examined by loss-of-function (via siRNA) and gain-of-function studies overexpressing the histamine H1 receptor. The requirement for the p65 subunit of NF-κB for histamines effect was elucidated by loss-of-function studies (siRNA). Finally, the effect of histamine on NF-κB binding to the apo A-I gene promoter was examined by chromatin immunoprecipitation.

Key findings: Treatment of HepG2 cells with histamine had no effect on histamine H1 receptor expression. However, treatment with histamine increased NF-κB p65 and p50 subunit expression significantly. At low levels, the exogenous histamine H1 receptor plasmid suppressed apo A-I gene promoter activity while addition of higher levels of plasmid DNA actually increased apo A-I gene promoter activity. Inhibition of NF-κB activity with SN50 prevented histamine from repressing apo A-I promoter activity as did silencing p65 expression via siRNA. Finally, treatment with histamine increased binding of the p65 subunit of NF-κB to the apo A-I gene promoter.

Significance: Histamine suppresses apo A-I gene expression in hepatocytes via the histamine H1 receptor by elevating NF-κB expression and binding to the apo A-I promoter.

1. Introduction

Atherosclerotic cardiovascular disease (ASCD) is the leading cause of morbidity and mortality in the United States [1]. Currently, 3-hydroxy-3-methyl-glutaryl- (HMG) coenzyme A reductase inhibitors (statins) targeting low-density lipoprotein cholesterol (LDL-C) are the cornerstone for treatment of ASCD, though significant residual risk remains [2]. One important element of prevention may be therapies targeting high-density lipoprotein cholesterol (HDL-C), the levels of which are inversely correlated with ASCD [3, 4]. This has been examined in several clinical trials with drugs that enhance HDL-C levels, including niacin [5, 6] and cholesteryl ester transfer protein (CETP) inhibitors [7, 8]. However, these studies, with the exception of one, have failed in attaining their primary endpoints and several were halted for their futility. Importantly, several genetic studies have also failed to demonstrate that individuals with high HDL-C levels are protected from developing ASCD [9, 10] prompted a revision of the "HDL hypothesis" to consider HDL functionality instead. One approach to counter this is to develop drugs that target de novo apolipoprotein A-I (apo A-I) synthesis [11, 12]. About 70% of plasma apo A-I is derived from the liver while 30% is from the small intestine. Apo A-I gene transcription is regulated by several factors including hormones such as cortisol and retinoids, fatty acids including several endogenous peroxisome proliferator receptor α (PPARα) ligands, and some pro-inflammatory mediators such as tumor necrosis factor α (TNF α) and interleukin-1β (IL-1β) [13]. While the former stimuli induce apo A-I gene expression, TNF α and IL-1β suppress apo A-I gene transcription by interfering with PPARα function [13]. Newly synthesized nascent HDL-C has been shown to be the preferred substrate for cholesterol efflux from macrophages onto HDL-C or apo A-I by the ATP binding cassette protein A1

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(ABCA1) expressed in macrophages [14], the first step in the process of reverse cholesterol transport. Both fibrates and statins have been shown to induce apo A-I expression by stimulating PPARα [15, 16]. Another drug that targets apo A-I gene transcription, RVX-208 [17], is currently in clinical trials though initial results have been somewhat disappointing [18, 19]. Furthermore, fibrates, statins, and RVX-208 have only modest effects on HDL-C levels in vivo. Therefore, there is a strong need to understand and identify novel regulators of apo A-I gene expression.

Recent studies in our laboratory demonstrated that several histamine H1 receptor antagonists induce apo A-I gene expression while histamine itself represses it [20]. In humans, there are three histamine receptor genes (H1, H2, and H3) that code for G-protein-coupled receptors (GPCR's) which bind histamine with high-affinity and stimulate various intracellular signaling pathways [21]. H1 and H2 histamine receptors are expressed in hepatocytes [22], however there are only a few reports regarding their potential roles in modulating hepatic function. Rokita et al. [23] demonstrated that histamine was essential in priming murine hepatocytes to express genes involved in the acute phase response when treated with interleukin 6 (IL-6). The response required the histamine H1 receptor but not the histamine H2 receptor. Histamine was also shown to increase IL-6 binding in CESS B cell lymphoma cells, but decreased IL-6 binding in HepG2 hepatocytes and U937 monocytoid cells [24]. Interestingly, increased IL-6 binding in the B-cell lymphoma cells was dependent on the histamine H1 receptor, while the histamine H2 receptor was essential for the effects of histamine in HepG2 cells as well as the monocytoid cells. A third histamine H1 receptor antagonist/serotonin receptor antagonist, cyproheptadine, was recently shown to induce cell cycle arrest of HepG2 and Huh-7 hepatocytes, in part by inducing expression of the cell cycle regulators HMG-box transcription factor 1, p16, p21, and p27 [25]. It is also important to point out that mast cell activation is heavily influenced by local factors and that pathways that modulate mast cell activity in the liver and atherosclerotic plaque should be examined independently. Though mast cells and histamine have been shown to have a potentially important role in the early and late stages of atherosclerosis within the atherosclerotic plaque, especially in plaque destabilization [26, 27], there is little evidence in the literature to suggest that histamine has effects on other major risk factors for ASCD, including lipoprotein, cholesterol, and triglyceride levels. Fexofenadine was shown however to increase HDL-C levels in rats while histamine reduced plasma HDL-C levels and suppressed LDL-C receptor expression [28]. Further support for a role of histamine in promoting atherogenesis came from a study in apoE$^{-/-}$ mice in which antihistamines were shown to inhibit atherosclerosis in mice fed a high-fat diet for three months [29]. These studies clearly provide support for the idea that histamine may have an underappreciated role in promoting atherosclerosis.

Since we recently reported that various antihistamines induce apo A-I gene expression [20], we wanted to examine the role of the histamine receptors in regulating apo A-I gene expression. In the presence of histamine, the H1 histamine receptor stimulates several intracellular signaling pathways, including nuclear factor-κB (NF-κB) activity [30, 31]. Since NF-κB has been shown to repress apo A-I gene expression in lipopolysaccharide-treated hepatocytes [32], we wished to determine whether NF-κB is involved in regulating apo A-I gene expression in HepG2 hepatoma derived cells.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagles medium (DMEM) and penicillin and streptomycin solution was purchased from BioWhittaker (Walkersville, MD). Fetal bovine serum (FBS) and newborn calf serum (NCS) were purchased from Hyclone (Logan, UT). Lipofectamine was purchased from Invitrogen (Gaithersburg, MD) and plasmid preparation kits were purchased from Qiagen (Germantown, MD). Human tumor necrosis factor α (TNF α) was purchased from R&D Systems (Minneapolis, MN). The NF-κB inhibitor SN50 was purchased from Cayman Chemical (Ann Arbor, MI). Synthetic DNA oligonucleotides for use as chromatin immunoprecipitation (ChIP) primers were purchased from Invitrogen. An antibody and a siRNA specific for the human histamine H1 receptor, as well as siRNA for the p65 subunit of NF-κB, and a control siRNA were purchased from Santa Cruz Biotechnology (Dallas, TX). Antibodies for human NF-κB p50 and p65 subunits were purchased from Millipore/EMD (Billerica, MA) while an antibody specific for β-actin was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO). Appropriate secondary antibodies conjugated to horseradish peroxidase were purchased from Southern Biotech (Birmingham, AL). Anti-apo A-I and anti-albumin antibodies were purchased from Calbiochem (San Diego, CA) and Rockland Immunochemical (Limerick, PA), respectively. The SimpleChip Enzymatic Chromatin IP kit was purchased from Cell Signaling Technology (Danvers, MA). Reagents for performing enhanced chemiluminescence (ECL) were obtained from Pierce Biotechnology (Rockford, IL). All other materials were obtained either from Thermo-Fisher Scientific (Pittsburg, PA) or Sigma-Aldrich.

2.2. HepG2 cell culture

HepG2 cells were obtained from American Type Culture Collection (Manassas, VA) and were maintained in DMEM containing 10% FBS, 10 μg/ml streptomycin, and 10 units/ml penicillin. The cells were maintained in a humidified incubator at 37 °C and 5% CO_2.

2.3. Plasmids and transient transfection

An expression plasmid for the histamine H1 receptor was purchased from Bloomsburg University cDNA Resource Center (Bloomsburg, PA) while the plasmid pNF-κB.3×.Luc was purchased from Addgene (Cambridge, MA). The plasmid p474.A1.CAT contains the full-length apo A-I gene promoter driving chloramphenicol acetyltransferase (CAT) expression [20]. HepG2 cells were transfected with each plasmid as well as the plasmid pCMV.SPORT.β-gal (to normalize transfection efficiency) and treated as indicated in each figure. Luciferase activity, CAT activity, and β-galactosidase activity were then measured as described [33–35].

2.4. Western blotting

Cells were treated as described in each figure and lysed in a solution containing 1% sodium dodecylsulfate (SDS), 50 mM tris-(hydroxymethyl)-aminomethane-Cl (Tris-Cl) (pH 8.0), and 150 mM NaCl and insoluble material removed by centrifugation at 16000 × *g* for 10 min at 4 °C. The concentration of protein in each sample was measured using the bicinchoninic acid (BCA assay) [36] and 50 μg of protein was fractionated by electrophoresis on a 10% SDS-polyacrylamide gel [37]. After transfer to Immobilon-P PVDF membranes, the blots were blocked in 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 0.05% Tween 20 (Tris-buffered saline Tween 20, TBST) containing 10% NCS for 2-h at room temperature. Antibodies were diluted as recommended by the manufacturer in TBS containing 10% NCS and incubated with the membranes overnight at 4 °C. After washing with TBST, the membranes were incubated with the appropriate secondary antibodies dilute 1:4000 in TBS/10% NCS for 1-h at room temperature. After washing with TBST, binding was detected by ECL, the films were scanned, and the signal quantified with Image J. The blots were then stripped following the membrane manufacturer's directions and incubated with the antibody to β-actin. Expression of each protein measured was then normalized to β-actin content.