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Pregnane X receptor-agonists down-regulate hepatic ATP-binding cassette transporter A1 and scavenger receptor class B type I

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Abstract

Pregnane X receptor (PXR) is the molecular target for a wide variety of endogenous and xenobiotic compounds. It regulates the expression of genes central to the detoxification (cytochrome P-450 enzymes) and excretion (xenobiotic transporters) of potentially harmful compounds. The aim of the present investigation was to determine the role of PXR in regulation of high-density lipoprotein (HDL) cholesterol metabolism by studying its impact on ATP-binding cassette transporter A1 (ABCA1) and scavenger receptor class B type I (SR-BI) expression in hepatocytes. ABCA1 and SR-BI are major factors in the exchange of cholesterol between cells and HDL. Expression analyses were performed using Western blotting and quantitative real time RT-PCR. Luciferase reporter gene assays were used to measure promoter activities. Total cholesterol was measured enzymatically after lipid extraction (Folch's method). The expression of ABCA1 and SR-BI was inhibited by the PXR activators rifampicin and lithocholic acid (LCA) in HepG2 cells and pregnenolone 16α-carbonitrile (PCN) in primary rat hepatocytes. Thus, PXR appears to be a regulator of hepatic cholesterol transport by inhibiting genes central to cholesterol uptake (SR-BI) and efflux (ABCA1).

Keywords: ABCA1; SR-BI; PXR; Cholesterol; Liver

HDL plays a key role in maintaining cholesterol homeostasis. Excess peripheral cholesterol is transported to the liver by HDL [1]. The exchange of cholesterol between HDL and cells is dependent on specific receptors and cholesterol transporters. Two well-known factors in this regard are SR-BI and ABCA1. SR-BI was cloned in 1994 and shown to be the receptor mediating selective uptake of HDL-cholesterol into liver, adrenals, testes, and ovaries [2–4], and cholesterol efflux from macrophages and other peripheral cells [5,6]. The human ABCA1, cloned in 2000 [7], is abundantly expressed in the liver and peripheral macrophages among other tissues. It mediates the efflux of cellular phospholipids and cholesterol to lipid-poor apoA-I, producing nascent HDL particles [8–15]. Thus, both SR-BI and ABCA1 play important roles in the determination of the plasma HDL levels.

Cholesterol delivered to the hepatocytes can be secreted directly into the bile, used for synthesis of new lipoproteins (very low-density lipoprotein), exported back to the blood to produce nascent HDL particles (via ABCA1) ([14] and reviewed in [15]), or converted to bile acids. The first and rate-limiting enzyme in the synthesis of bile acids is cholesterol 7β-hydroxylase (CYP7A1).

Pregnane X receptor (PXR) and its human homolog steroid and xenobiotic receptor (SXR, termed human PXR in this paper) are nuclear xenobiotic receptors. The human PXR was cloned in 1998 and was shown to be expressed primarily in liver and intestine [16].

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PXR is activated by a wide variety of compounds. In addition to rifampicin and the anti-glucocorticoid PCN, endogenous steroids (e.g., progestins, estrogens, and corticosteroids) [16–19] and xenobiotic compounds (e.g., nifedipine, clotrimazole, and dexamethasone) [20–22] are among compounds that activate PXR. Upon activation, PXR heterodimerizes with retinoid X receptor (RXR) and regulates the expression of genes central to breakdown and removal of potentially harmful compounds from the liver and intestine [23–26]. In addition, PXR has been shown to be involved in bile acid homeostasis. Lithocholic acid (LCA) is a hydrophobic secondary bile acid that is formed in the intestine by dehydroxylation of the primary bile acid chenodeoxycholic acid. This secondary bile acid is a potent cholestatic agent and can cause histological liver damage and other pathological changes [27]. It has been reported that LCA is an activator of mouse and human PXR [22,26,28]. Activated PXR has been shown to reduce the expression and activity of CYP7A1 [28–33] and thereby preventing toxic bile acids from accumulating in the hepatocytes.

Since cholesterol transport and bile acid production generally are co-ordinately regulated, it is of interest to determine if PXR is involved in the regulation of cholesterol transport in hepatocytes. In this paper, we show that PXR negatively regulates the expression of SR-BI and ABCA1. PXR therefore seems to be involved in the regulation of both uptake and efflux of HDL-cholesterol in the liver.

Materials and methods

Plasmids. Expression plasmids for human RXRα (RXRα-pCMV) and β-galactosidase-pSV were kindly provided by Dr. H. Nebb (Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway). Human PXR-pSG5 was a kind gift from Dr. B. Goodwin (Nuclear Receptor Discovery Research, Glaxo-SmithKline, Five Moore Drive, Research Triangle Park, NC 27709, USA). The human ABCA1 (−928/+101 bp) and the human SR-BI (−1200/−126 bp) promoter-luciferase reporter constructs were kind gifts from Dr. P. Costet (INSERM U 539, Centre de Recherche en Nutrition Humaine de Nantes, France) and Dr. H.H. Hobbs (Department of Molecular Genetics, University of Texas, USA), respectively. The plasmids were sequenced to verify DNA sequence fidelity by Cybergene AB (Sweden) and GATC Biotech AG (Germany).

Isolation and culturing of rat primary liver cells. Rats used in the experiments were treated according to established Guidelines for the Use of Experimental Animals. Primary rat hepatocytes were isolated from adult Wistar rats as described elsewhere [34]. After isolation, cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 100 U penicillin, 100 μg/ml streptomycin (Bio Whittaker, Europe), and 3% fetal bovine serum (Sigma) at 37 °C, 5% CO_2. After 3–4 h, cells were stimulated with the given concentrations of PCN (Sigma), LCA (Sigma) or vehicle (ethanol) in their respective medium containing 0.5% charcoal-treated fetal bovine serum. Lipoprotein-depleted serum was prepared as described [35].

Cell cultures. Human hepatoma cells (HepG2) were grown in DMEM supplemented with 2 mM L-glutamine, 100 U penicillin, 100 μg/ml streptomycin, 1× non-essential amino acids (Bio Whittaker, Europe), and 5% fetal bovine serum (Sigma) (medium A) at 37 °C, 5% CO_2. After reaching 70% confluence, cells were stimulated with the given concentrations of rifampicin (Sigma), LCA (Sigma) or vehicle (methanol or ethanol, respectively) in their respective medium containing 0.5% charcoal-treated fetal bovine serum (medium B).

Western blotting. Primary rat hepatocytes or HepG2 cells were washed twice with phosphate-buffered saline prior to lysis in non-reducing SDS lysis buffer (20 mM Hepes, 300 mM NaCl, 0.2 mM EDTA, 1.5 mM $MgCl_2$, and 1% Triton X-100) containing 2% phenylmethylsulfonyl fluoride. Protein concentrations were determined by BCA protein assay (Pierce) and 25 μg of cell lysates was subjected to SDS–polyacrylamide gel electrophoresis on 7.5% polyacrylamide gels. Proteins were transferred onto nitrocellulose membranes (Millipore, USA) and pre-incubated with 5% skim milk phosphate-buffered saline–0.1% Tween to prevent unspecific binding. Monoclonal rat ABCA1 antibody kindly provided by Dr. K. Ueda (Lab. Cell. Biochem., Div. Applied Life Science; Kyoto University, Japan) or polyclonal rabbit SR-BI antibody (Novus Biologicals, Littleton, USA) was diluted in 5% skim milk phosphate-buffered saline–0.1% Tween and interacting anti-rat HRP-IgG (Sigma) or anti-rabbit HRP-IgG (Amersham Biosciences) was detected by chemiluminescence ECL blot detection system (Amersham Biosciences). The blots were stripped (0.8% mercaptoethanol in phosphate-buffered saline–0.1% Tween at 50 °C for 30 min) and re-hybridized with mouse-anti-β actin (clone AC-74, Sigma) and anti-mouse HRP-IgG (Amersham Biosciences). Immunoreactive SR-BI or ABCA1 bands were quantified using a Gel Doc 1000 scanner (Bio-Rad) and normalized to the intensity of the immunoreactive β-actin bands.

Quantitative real time PCR. Total RNA was isolated from rat primary hepatocytes or HepG2 using Veragene RNA Cell Kit (Gentra Systems) according to the manufacturer's instructions. Four micrograms of total RNA was reverse transcribed using SuperScript II RNase H^- Reverse Transcriptase (Invitrogen) and oligo $dT_{(15)}$ primers (DNA Technology A/S, Denmark). The cDNA was used as template for quantitative real time RT-PCR on a LightCycler (Roche) using SYBR Green I technology (Roche). All primers used were amplified in the same PCR conditions using 60 °C as the annealing temperature. The primers used were designed such that they do not amplify from genomic DNA. This was tested by using RNA samples reverse transcribed without the enzyme which was more than 10 Cp-values different than RNA samples reverse transcribed using the enzyme. The primers were designed by the Primer 3 Output program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and are listed in Table 1.

Transient transfection. 300,000 HepG2 cells were cultured in six-well plates the day before transfection. The cells were transiently transfected with 0.3 μg promoter-pGL3 reporter construct using FuGENE 6 Transfection Reagent according to the manufacturer (Roche). Transfection efficiency was assessed measuring the activity of co-expressed β-galactosidase (0.5 μg). In some experiments, 0.3 μg human PXR-pSG5 and 0.3 μg human RXRα-pCMV were co-transfected. Sixteen hours post-transfectionally, medium A was replaced with medium B with the given concentration of rifampicin (Sigma), LCA (Sigma) or vehicle (methanol or ethanol, respectively) for 24 h. The cells were washed twice with phosphate-buffered saline and lysed with Reporter lysis buffer (Promega). Luciferase activity and β-galactosidase activity were measured according to manufacturer's instructions. Luciferase activities were normalized to β-galactosidase activities to account for any variation in transfection efficiency.

Cholesterol measurements. A lipid soluble fraction was isolated from HepG2 cells or primary rat hepatocytes by Folch's method. Total and free cholesterol components were assayed by the commercial kit Cholestérol RTU based on the cholesterol oxidase method [36] according to the manufacturer's instructions (Kits BioMérieux, Marcy l'Etoile, France).