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Sulfation of 25-hydroxycholesterol by SULT2B1b decreases cellular lipids via the LXR/SREBP-1c signaling pathway in human aortic endothelial cells

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Abstract

Objective—25-hydroxycholesterol (25HC) and its sulfated metabolite, 25-hydroxycholesterol-3-sulfate (25HC3S), regulate certain aspects of lipid metabolism in opposite ways. Hence, the enzyme for the biosynthesis of 25HC3S, oxysterol sulfotransferase (SULT2B1b), may play a crucial role in regulating lipid metabolism. We evaluate the effect of 25HC sulfation on lipid metabolism by overexpressing the gene encoding SULT2B1b in human aortic endothelial cells (HAECs) in culture.

Methods and Results—The human SULT2B1b gene was successfully overexpressed in HAECs following infection using a recombinant adenovirus. HPLC analysis demonstrated that more than 50% of ³H-25HC was sulfated in 24 hrs following overexpression of the SULT2B1b gene. In the presence of 25HC, SULT2B1b overexpression significantly decreased mRNA and protein levels of LXR, ABCA1, SREBP-1c, ACC-1, and FAS, which are key regulators of lipid biosynthesis and transport; and subsequently reduced cellular lipid levels. Overexpression of the gene encoding SULT2B1b gave similar results as adding exogenous 25HC3S. However, in the absence of 25HC or in the presence of T0901317, synthetic liver oxysterol receptor (LXR) agonist, SULT2B1b overexpression had no effect on the regulation of key genes involved in lipid metabolism.

Conclusions—Our data indicate that sulfation of 25HC by SULT2B1b plays an important role in the maintenance of intracellular lipid homeostasis via the LXR/SREBP-1c signaling pathway in HAECs.

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Keywords

cytosolic sulfotransferase (SULT2B1b); 25-hydroxycholesterol (25HC); 25-hydroxycholesterol-3-sulfate (25HC3S); oxysterol sulfation; lipid metabolism

Introduction

Vascular endothelial cells (VECs) form a barrier between the vessel wall and lipoproteins and lipids in blood, playing a critical role in the maintenance of vascular integrity. VEC dysfunction is fundamental to the pathogenesis of atherosclerosis and related cardiovascular diseases^{1, 2}. Although there may be several drivers of EC dysfunction, the accumulation of intracellular lipids inclusive of triglycerides, cholesterol, and free fatty acids has emerged as a key pathophysiological factor³⁻⁵. The endothelium of aortic vessels is one of the earliest locations for occurrence of human atherosclerosis. Therefore, decreasing intracellular lipid levels in human aortic endothelial cells may be key for preventing or reversing atherosclerosis.

Nuclear receptors are ligand-activated transcription factors that regulate the expression of target genes affecting processes as diverse as reproduction, development, and general metabolism⁶. A number of nuclear receptors, such as those for oxysterols (liver oxysterol receptor, LXR), bile acids (farnesoid X receptor FXR), retinoic acids (retinoid X receptor, RXR), and peroxisome proliferation activator receptors (PPARs) function as sensors of intracellular cholesterol and lipid levels⁷; eliciting gene expression important to the maintenance of cellular lipid homeostasis⁸. Sterol regulatory element binding proteins (SREBPs) are a family of transcription factors that have been established as key regulators of cholesterol and fatty acid synthesis by directly activating the expression of more than 32 genes involved in the regulation of lipid metabolism^{9, 10}. In the liver, the nuclear receptor, LXR, has been shown to regulate SREBP-1c expression¹¹. Administration of synthetic LXR ligands to mice triggers induction of the lipogenic pathway and elevates plasma triglyceride levels via SREBP-1c^{12, 13}. However, the regulation of LXR activity remains incompletely understood.

Recently, we identified an oxysterol, 25-hydroxycholesterol-3 sulfate (25HC3S), that accumulates in hepatocyte nuclei following overexpression of the mitochondrial cholesterol delivery protein, StarD1¹⁴⁻¹⁶. This oxysterol is synthesized from its precursor, 25HC, by SULT2B1b¹⁷. It has been reported that overexpression of SULT2B1b inactivates the response of LXR α to 25HC, and inhibits LXR target gene expression, including SREBP-1c and ABCA1¹⁸. It has been hypothesized that the oxysterol sulfation is an inactivation processing¹⁸. However, addition of exogenous 25HC3S to primary human hepatocytes and THP-1 derived macrophages decreases SREBP-1/2 expression and blocks the activation of SREBP-1c; suppresses the expression of key enzymes, including 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), acetyl-CoA carboxylase-1 (ACC-1), and fatty acid synthase (FAS) involved in lipid metabolism; and subsequently decreases cholesterol and neutral lipid levels¹⁹⁻²¹. These results indicate that the sulfated oxysterol may act as LXR antagonist rather than only an inactive form of LXR ligand. Therefore, whether endogenous oxysterol sulfation regulates lipid metabolism has yet to be answered.

In the present study, we evaluated the effects of 25HC sulfation on lipid metabolism and its possible mechanism by overexpression of SULT2B1b in HAECs. The results indicate that 25HC sulfation dramatically decreases intracellular lipid levels via inhibiting the LXR-SREBPs signaling pathway. These findings provide physiologic evidence that sulfation of

25HC plays an important role in the maintenance of lipid homeostasis, and may carry therapeutic value for the prevention of atherosclerosis.

Materials and Methods

Cell culture reagents and supplies were purchased from American Type Culture Collection (Manassas, VA). The reagents for real-time RT-PCR were obtained from AB Applied Biosystems (Warrington, UK). Primary antibodies against human LXR α/β , SULT2B1, SREBP-1, SREBP-2, FAS, ACC1, Lamin B1, and β -actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against human ATP-binding cassette transporter A1 (ABCA1) and HMGR were from Abcam (Cambridge, MA). Secondary antibodies against rabbit and mouse IgG were obtained from Kirkegaard & Perry Laboratories (Guildford, UK). LXR agonist T0901317 was from New Cayman Chemical (Ann Arbor, MI). ^{14}C -Acetic acid and ^3H -25-hydroxycholesterol were from New England Nuclear (Boston, MA). Free cholesterol and total cholesterol assays, and NEFA-HR (2) assay kits for free fatty acid were from Wako Bioproducts (Richmond, VA). Infinity triglyceride assay kit was purchased from Thermo Electron (Arlington, TX). The recombinant adenovirus encoding human SULT2B1b (Ad-SULT2B1b) and control adenovirus (Ad-Control) were prepared as previously described^{22, 23}. The solvents were obtained from Fisher (Fair Lawn, NJ), and all other reagents were from Sigma-Aldrich Chemical Co (St. Louis, MO) unless otherwise mentioned.

Cell culture

Primary human aortic endothelial cells (HAECs) obtained from American Type Culture Collection (Manassas, VA) were maintained according to the manufacturer's instructions in vascular cell basal medium (PCS-100-030) supplemented with endothelial cell growth kit-BBE (PCS-100-040), creating a complete cell culture which contains 0.2% bovine brain extract (BBE), 5ng/ml human recombinant epidermal growth factor, 10mM L-glutamine, 0.75units/ml of heparin sulfate, 1u/ml of hydrocortisone, 50 ug/ml of ascorbic acid, and 2% of fetal bovine serum. The cells were incubated at 37°C in a humidified atmosphere of 5% CO₂. At 70% to 90% confluence, cells were subcultured by using trypsin 0.025%-EDTA 0.01% (GBICO) to detach cells from their substrate and were used for all the experiments between passages 5 and 9.

Infection of HAECs with SULT2B1b recombinant adenoviruses

HAECs were infected with recombinant adenovirus encoding Ad-CMV-SULT2B1b at a multiplicity (MOI) of 5, 10, or 20 plaque forming units/cell (pfu/cell) for 48h as previously described²³. The control adenovirus (Con) and no-virus-addition (NA) were used as controls. The recombinant adenoviruses encoding Ad-SULT2B1b were prepared as described in supplemental data.

Determination of SULT2B1b specific activities

The specific activities of SULT2B1b were determined by [^3H]-25-hydroxycholesterol (^3H -25HC) and HPLC assays as previously described²⁴. Briefly, the rate of 25HC sulfation was determined by addition of [^3H]-25HC (1 μCi) to the culture of HAECs after adenovirus infection. The media and cells were harvested at 2 hrs or 24 hrs. Conversion of [^3H]-25HC to [^3H]-methanol/water-soluble products was determined by scintillation counting after Folch extraction with chloroform/methanol (2:1, v/v) from cells or cultured media¹⁴. The rates of 25HC3S biosynthesis following adenovirus infection were calculated as the ratio of [^3H]-methanol/water-soluble counts to the sum of chloroform/methanol/water-soluble

counts. To confirm the product of 25HC sulfation, the extracted samples were analyzed by HPLC, ^3H -25HC and ^3H -25HC3S were used as standard controls.

Determination of intracellular total cholesterol, free cholesterol, free fatty acids, and triglycerides

The intracellular lipids were measured as previously described²⁰. Briefly, after infection with Ad-SULT2B1b for 24 hrs and treatment with 25HC for another 48 hrs, total lipids in HAECs were extracted by addition of 10 ml of chloroform-methanol (2:1, v/v) mixture. The extract, 0.5 ml, was evaporated to dryness and dissolved in 100 μl of isopropanol containing 10% Triton X-100 for cholesterol assay, isopropanol only for triglyceride assay, or NEFA solution (0.5 g of EDTA- Na_2 , 2 g of Triton X-100, 0.76 ml of 1N NaOH, and 0.5 g of sodium azide/L of H_2O , pH 6.5) for free fatty acid assay. The intracellular total and free cholesterol, triglyceride, and free fatty acid assays were performed according to the manufacturer's instructions.

Determination of newly synthetic lipids in HAECs by TLC

HAECs were infected and treated with 25HC as described above. [^{14}C]-Acetic acid (5 μCi) was added into the culture. After incubation at 37°C for 2 hrs, the total lipids in HAECs were extracted with 10 volumes of chloroform-methanol (2:1, v/v), and separated by thin layer chromatography (TLC) on a silica-gel plate. The plate was developed in a mixture of petroleum ether-diethyl ether-glacial acid 82:18:1 (v/v/v) as described previously²⁵. [^{14}C]-Acetate derivatives were visualized by Image Reader, Fujifilm BAS-1800 II. Then the bands for cholesterol ester, triglycerides and free cholesterol were scraped, and [^{14}C] radioactivity was measured by liquid-scintillation counting. The data was normalized for cellular protein.

Determination of gene expression involved in lipids metabolism

After infection and treatment with 25HC or 25HC3S, nuclear protein and cytosol were extracted according to the manufacturer's instructions. Twenty μg nuclear extracts or fifty μg cytosolic proteins were loaded on 10% SDS-PAGE for detection of the specific proteins, including LXR, ABCA1, SREBP-1, SREBP-2, ACC-1, FAS, and HMGR, using Lamin B1 and β -actin as loading control for nuclear and cytoplasmic protein respectively. Western blot analysis was performed as previously described²³.

Total RNA was extracted using SV Total RNA Isolation Kit (Promega, Wisconsin, WI) according to the supplier's instruction. The relative mRNA levels were measured by real-time RT-PCR as previously described²³. The cells infected with Ad-control and no addition were used as negative controls, and the data were normalized to GAPDH. The primer sets used in these assays are shown in Supplemental Table 1.

Statistic analysis

Data are expressed as means $\pm$ standard deviation (SD) and evaluated by Independent-Samples T Test or ANOVA analysis. A value of $P < 0.05$ was considered statistically significant.

Results

Overexpression of the gene encoding SULT2B1b in HAECs by a recombinant adenovirus

Western blot and real-time PCR analysis showed that infection of HAECs with recombinant adenovirus encoding SULT2B1b gene at a multiplicity of 5, 10, 20 pfu/cell for 48 hrs

produced high SULT2B1b mRNA (3000~5000 fold) and protein levels (150~200 fold) with no evidence of cell toxicity as shown in Suppl. Fig.1A-C. SULT2B1b protein and mRNA levels were dramatically increased in a time-dependent manner (0, 6, 12, 24, 48h) following infection with Ad-SULT2B1b in HAECs as shown in Suppl. Fig.1D-F.

Determination of SULT2B1b activities in HAECs by HPLC analysis

HAECs were infected with Ad-Control or Ad-SULT2B1b at MOI of 10 for 24h. [^3H]-25HC or [^3H]-25HC3S (1 μCi) was added into the serum-free media. The data in Fig.1A shows the relative percent of counts in media at the different time points. More than 70% of 25HC or 25HC3S entered cells within 24 hrs. In contrast, less than 50% was found in cells following SULT2B1b overexpression. Fig.1B shows the rates of sulfation by calculating the ratio of the counts in the chloroform phase and methanol/water phase after Folch partition. About 40% of the [^3H]-25HC in the cells infected with Ad-SULT2B1b (MOI=10) was sulfated at 2 hrs, more than 50% was sulfated at 24 hrs, while only 1~2% of [^3H]-25HC was sulfated in the cells with Ad-Control or no infection.

To characterize the product of 25HC sulfation, the cells with Ad-Control or Ad-SULT2B1b infection in the presence of [^3H]-25HC were analyzed by HPLC after extraction with chloroform-methanol (2:1). Compared with the standard of [^3H]-25HC and [^3H]-25HC3S as shown in Fig.1C-D, the main product of 25HC sulfation was 25HC3S. Consistent with Fig. 1B, HPLC analysis also showed that more than 50% of [^3H]-25HC was sulfated to [^3H]-25HC3S in the cells infected with Ad-SULT2B1b, while only 1~2% of [^3H]-25HC was sulfated in the cells with Ad-Control infection as shown in Fig.1E-F.

Effect of 25HC sulfation on intracellular lipid levels

HAECs were infected with Ad-Control or Ad-SULT2B1b and treated with 25HC or 25HC3S as described above. To examine the effects on intracellular lipid levels, total neutral lipids were extracted by chloroform-methanol. Quantitative analysis showed that cells with SULT2B1b overexpression in the presence of 25HC decreased intracellular triglycerides by 20~30%, free fatty acids by 70~80%, total cholesterol by 30~40%, and free cholesterol 10~20%, compared with the cells infected with Ad-Control or no infection as shown in Fig. 2A-D.

To determine the effect of 25HC sulfation on newly synthesized lipids, [^{14}C]-Acetic acid was added to cells after infection in the presence of 25HC. The cells were collected at 2 hrs and the total lipids were extracted as described above. TLC analysis showed that the intracellular lipids (cholesterol ester, triglycerides and free cholesterol) in the cells with SULT2B1b overexpression were dramatically decreased, compared with the cells infected with Ad-Control (Fig.2E-F).

Effect of 25HC sulfation on LXR activity and its target gene expression

HAECs were infected with Ad-control or Ad-SULT2B1b for 24 hrs, and treated with 25HC or 25HC3S (3 μM) for another 6 hrs for mRNA and 24 hrs for protein analysis. The protein and mRNA levels of LXR, its target genes (SREBP-1c and ABCA1), and SREBP-2 were detected by Western Blot and real-time PCR respectively. The levels of LXR and SREBPs mature proteins in the cells infected with Ad-SULT2B1b at different MOI (5, 10, or 20) significantly decreased in the nuclear extracts, SREBP-1 precursor and ABCA1 also decreased in cytosol, while SREBP-2 precursor did not change as shown in Fig.3A-C. Consistent with protein levels, mRNA levels of LXR, SREBP-1c and ABCA1 in the cells with SULT2B1b overexpression decreased by >20%, >60%, and >80%, respectively, as shown in Fig.3D-F. The mRNA level of SREBP-2 did not change between the cells infected with Ad-Control or Ad-SULT2B1b in the presence of 25HC, when compared with the cells

with no 25HC, both of them decreased by >30% (Fig.3G). As a positive control, treatment with 25HC3S decreased the mRNA levels of LXR, SREBP-1, ABCA1 and SREBP-2 as shown in Fig.3D-G.

Effect of 25HC sulfation on SREBP target genes

HAECs were infected and treated as described above. Total protein levels of SREBPs target genes, including ACC-1, FAS, and HMG-CoAR in the cells infected with Ad-SULT2B1b significantly decreased, compared with the cells infected with Ad-Control as shown in Fig. 4A-B. Consistently, the mRNA levels of SREBP-1 target genes (ACC-1 and FAS) were significantly decreased in the cells with overexpression of SULT2B1b in the presence of 25HC as shown in Fig.4C-F. However, the mRNA levels of SREBP-2 target genes (HMG-CoAR and LDLR) decreased in the cells infected with Ad-Control or Ad-SULT2B1b in the presence of 25HC when compared with the cells with no 25HC, but there was no significant difference between the cells with Ad-Control and Ad-SULT2B1b infection.

Effect of SULT2B1b on lipids metabolism was depended on the presence of 25HC

To determine that the effect of SULT2B1b on lipids metabolism was depended on 25HC, we compared the effect of SULT2B1b in HAECs after infection in the absence or presence of 25HC or T0901317. The results showed that intracellular TG and TC and the mRNA and protein levels of SREBP-1 were decreased after SULT2B1b overexpression only in the presence of 25HC, but there was no effect in the absence of 25HC or in the presence of T0901317, which was synthetic LXR agonist (Fig.5A-E). To better evaluate the biological effect of SULT2B1b on lipid metabolism, we determined LXR, SREBP-1, SREBP-2 and FAS protein expressions in HepG2 cells with small increases in SULT2B1b protein levels (Suppl. Fig3A-D). The results were consistent with those in HAECs (Fig.3A, Fig.4A, and Fig.5A-B).

To better understand the effect and mechanism of SULT2B1b on lipids metabolism, we performed the loss-of-function studies by using siRNA-SULT2B1b in HepG2 cells, which had relative high levels of SULT2B1b expression¹⁷. SULT2B1b gene was successfully knocked down after transfecting siRNA-SULT2B1b for 24~48 hrs as shown in Suppl. Fig. 2A-B. The mRNA levels of genes involved in lipid metabolism and lipid levels (including TG and TC) in the absence or presence 25HC or T0901317 were performed. In the cells with siRNA-SULT2B1b infection, 25HC increased intracellular triglycerides and total cholesterol. 25HC also increased mRNA levels of SREBP-1, FAS and ACC1 compared with those in the cells transfected with negative siRNA control. There was no significant change in the absence of 25HC or in the presence of T0901317 (Suppl. Fig2C-F).

Discussion

It has been reported that human oxysterol sulfotransferase, SULT2B1b, can sulfate oxysterols and overexpression of SULT2B1b inactivates oxysterol signaling^{18, 26, 27}. Cells with high expression of SULT2B1b are significantly more resistant to the cytotoxic effect of 7-ketocholesterol, and overexpression of SULT2B1b protects from 7-ketocholesterol-induced loss of cell viability²⁶. Furthermore, overexpression of SULT2B1b inactivates LXR signaling induced by oxysterols in several cultured mammalian cell lines, but does not alter receptor response to the non-sterol agonist¹⁸. Thus, it was hypothesized that oxysterol sulfation is an inactivation process. However, the role of oxysterol sulfation in lipid metabolism had not been investigated before the discovery of 25HC3S in hepatocyte nuclei¹⁶. Addition of 25HC3S into cultured macrophages or hepatocytes inhibited cholesterol and triglyceride biosynthesis and decreases intracellular lipid levels^{19, 20}. These

results suggest that 25HC3S is a regulatory molecule rather than just an inactive form of 25-hydroxycholesterol, which may play an important role in lipid metabolism.

SULT2B1b is responsible for sulfation of 25-hydroxycholesterol (25HC) to 25-hydroxycholesterol-3-sulfate (25HC3S)^{17, 19}. Both 25HC and 25HC3S regulate lipid metabolism, but with opposite effects on lipid biosynthesis²⁰. 25HC is a LXR ligand, which increases SREBP-1c, ABCA1, ACC1, and FAS expression and subsequently increases intracellular lipid levels including TG, TC, FC and FFA. However, 25HC3S decreases intracellular lipid levels by inhibiting LXR/SREBP-1c signaling pathway^{17, 20}. The results suggest that oxysterol sulfation by SULT2B1b plays an important role in the control of lipid metabolism. In the current study, we found that SULT2B1b is also expressed in human primary aorta endothelial cells (HAECs) at low level. Interestingly, after the same amount of [³H]-25HC was added into the culture, total counts in the cells with SULT2B1b overexpression were much lower than the other cells with Ad-Control or no infection after 2 hrs when 25HC3S was being activated [Fig.1A-B], which may partly explain why intracellular lipid levels decreased after SULT2B1b overexpression with 25HC. SULT2B1b overexpression decreased intracellular lipid levels in the presence of 25HC which was coincident with the formation of 25HC3S [Fig.1B]. 25HC3S has been reported to decrease intracellular lipids via LXR/SREBP-1c signaling pathway^{19, 20}.

Previous reports described that overexpression of SULT2B1b, which sulfates oxysterols, is responsible for impairing LXR signaling in cultured cells¹⁸. In the present study, we have shown that decreases in intracellular lipid levels by SULT2B1b overexpression happen through 25HC sulfation and 25HC3S formation. We compared the effect of authentic ligand 25HC with that of synthetic agonist, T0901317, in the cells with SULT2B1b overexpression. The results showed that SULT2B1b overexpression decreased the intracellular lipid levels and SREBP-1 expression only in the presence of 25HC, but had no effect in the absence of 25HC or in the presence of T0901317. Furthermore, we performed the loss-of-function studies by using siRNA-SULT2B1b in HepG2 cells. In the presence of 25HC, total triglycerides and cholesterol levels increased as well as the expression of SREBP-1, FAS and ACC1 in siRNA-SULT2B1b transfected cells as compared to controls. These results are consistent with our previous studies²¹. All these data indicate that decreases in intracellular lipid levels are via 25HC sulfation rather than by a direct effect of SULT2B1b protein.

To confirm the mechanism of decreasing the intracellular lipid levels in HAECs following SULT2B1b overexpression in the presence of 25HC, we determined the protein and mRNA expression of the genes involved in lipids metabolism. The results showed that compared with the cells infected with Ad-Control, the expression of LXR α and its target genes ABCA1 SREBP-1, ACC1, and FAS were dramatically decreased in the cells with SULT2B1b overexpression. It is noted that ABCA1 is known to mediate efflux of cellular lipids and decrease intracellular lipid levels^{28, 29}, however, SREBP-1 and its target genes (ACC1 and FAS) are involved in fatty acid synthesis and increase intracellular lipid levels^{9, 10, 30, 31}. The decreases in lipid levels in HAECs by SULT2B1b overexpression in the presence of 25HC suggest that LXR/SREBP-1 lipid synthetic pathway is dominant in this process. Therefore, we detected the newly synthesized lipids in HAECs after SULT2B1b overexpression with 25HC using [¹⁴C]-acetic acid by TLC, and found that the newly synthesized lipids, including cholesterol esters and triglycerides, dramatically decreased [Fig. 2E].

In summary, according to previous studies and our data, we believe that the sulfated 25HC, 25HC3S, acts as a competitive antagonist and have a negative effect on the LXR/SREBP-1c signaling pathway, and for the first time to provide more physiological evidence that the

endogenous 25HC3S generated by overexpression of SULT2B1b in HAECs inhibits cholesterol, free fatty acids and triglycerides biosynthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Determination of SULT2B1b overexpression on the metabolism of 25HC in HAECs.

HAECs were infected with Ad-Control or Ad-Sult2B1b at MOI = 10 pfu/cell for 24h, then the medium was changed to serum-free media containing $1\mu\text{Ci}$ ^3H -25HC or ^3H -25HC3S.

(A) The relative percent of counts in the media were detected at the different times as indicated. (B) The efficiency of 25HC sulfation in the cells was measured after folch partitioning with chloroform-methanol (2:1, v/v). (C and D) The standard controls of ^3H -25HC and ^3H -25HC3S were analyzed by HPLC. (E and F) HPLC analysis of the cells with Ad-control or Ad-SULT2B1b infection in the presence of ^3H -25HC. The data represent one of three separate experiments. * $P < 0.05$ vs. 25HC or Ad-Sult 2h; ** $P < 0.01$ vs. 25HC; ## $P < 0.01$ vs. Ad-Con.

Figure 2. Effect of SULT2B1b overexpression on lipids metabolism in the presence of 25HC in HAECs. HAECs were infected with Ad-control or Ad-SULT2B1b at the indicated MOI and treated with 25HC (3μM) for another 48h. Total intracellular neutral lipids were extracted by adding 10 volumes of chloroform/methanol mixture (2:1, v/v). (A-D) Intracellular triglycerides (TG), free fatty acids (FFA), total cholesterol (TC) and free cholesterol (FC) were analyzed as described in Methods. (E and F) TLC analysis of the newly synthesized lipids by using ^{14}C -acetic acid and quantification by liquid-scintillation counting. The data represent one of two experiments performed in duplicate. * $P < 0.05$ vs. Con, ** $P < 0.01$ vs. Con, # $P < 0.05$ vs. 25HC, ## $P < 0.01$ vs. 25HC.

Figure 3.

Effect of SULT2B1b overexpression on LXR and its target genes. (A) Western blot analysis of nuclear extracts and cytosol proteins with specific antibodies against LXRα, ABCA1, SREBP-1 and SREBP-2 after infection with adenovirus in the presence of 25HC. (B-E) Real-time PCR analysis of LXRα, ABCA1, SREBP-1c and SREBP-2 mRNA levels, respectively. The data of western blot represent one of three separate experiments; and the mRNA data represent one of two experiments performed in duplicate. * $P < 0.05$ vs. Con, ** $P < 0.01$ vs. Con, # $P < 0.05$ vs. 25HC, ## $P < 0.01$ vs. 25HC, & $P < 0.05$ vs. Ethanol, && $P < 0.01$ vs. Ethanol.

Figure 4.

Effect of SULT2B1b overexpression on SREBPs target genes. (A) Western blot analysis of total proteins in HAECs with specific antibodies against ACC1, FAS, and HMGR after infection with adenovirus in the presence of 25HC. (B-E) Real-time PCR analysis of ACC1, FAS, HMGR, and LDLR mRNA levels. The data of western blot represent one of three separate experiments; and the mRNA data represent one of two experiments performed in duplicate. * $P < 0.05$ vs. Con, ** $P < 0.01$ vs. Con, # $P < 0.05$ vs. 25HC, ## $P < 0.01$ vs. 25HC, & $P < 0.05$ vs. Ethanol, && $P < 0.01$ vs. Ethanol.

Figure 5.

Effect of SUL2B1b overexpression depended on the presence of 25HC in HAECs. HAECs were infected with Ad-Control or Ad-SUL2B1b in the presence or absence of 25HC (3μM) or with T0901317 (1μM) for another 24h. (A-B) Western blot and real-time PCR analysis of SREBP-1 protein and mRNA expression. (C-D) Intracellular triglycerides and total cholesterol were analyzed as described in Methods. The data of western blot represent one of three separate experiments; and the data of mRNA and lipids represent one of two experiments performed in duplicate. * $P < 0.05$ vs. Con, # $P < 0.05$ vs. NA, ## $P < 0.01$ vs. NA.