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Sulfated Oxysterol, 25HC3S, is a Potent Regulator of Lipid Metabolism in Human Hepatocytes

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Abstract

Recently, a novel oxysterol, 5-cholesten-3 β , 25-diol 3-sulfate (25HC3S) was identified in primary rat hepatocytes following overexpression of the cholesterol transport protein, StarD1. This oxysterol was also detected in human liver nuclei. In the present study, 25HC3S was chemically synthesized. Addition of 25HC3S (6 μ M) to human hepatocytes markedly inhibited cholesterol biosynthesis. Quantitative RT-PCR and Western blot analysis showed that 25HC3S strongly decreased HMG-CoA reductase mRNA and protein levels. Coincidentally, 25HC3S inhibited the activation of sterol regulatory element binding proteins (SREBPs), suggesting that inhibition of cholesterol biosynthesis occurred via blocking SREBP-1 activation, and subsequently inhibiting the expression of HMG CoA reductase. 25HC3S decreased SREBP-1 mRNA levels and inhibited the expression of target genes encoding acetyl CoA carboxylase-1 (ACC-1) and fatty acid synthase (FAS). In contrast, 25-hydroxycholesterol increased SREBP1 and FAS mRNA levels in primary human hepatocytes. The results imply that 25HC3S is a potent regulator of SREBPs mediated lipid metabolism.

Keywords

cholesterol and lipids metabolism; HMG-CoA Reductase; bile acid synthesis; acetyl CoA carboxylase-1; fatty acid synthase; SREBPs; mitochondria; 5-cholesten-3 β ; 25-diol 3-sulfate

Introduction

The “acidic” pathway of bile acid biosynthesis is initiated by the mitochondrial enzyme, sterol 27-hydroxylase (CYP27A1) (1). Oxysterol intermediates of the “acidic” pathway such as 27-hydroxycholesterol and 25-hydroxycholesterol have been shown to be regulators of cholesterol homeostasis (2–8). These oxysterols regulate expression of many genes encoding enzymes

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involved in cholesterol and lipid metabolism (3;9;10). Increased CYP27A1 activities in peripheral tissues can down-regulate cholesterol biosynthesis (6). However, the relationship between the CYP27A1 activity and intracellular cholesterol metabolism is unknown. It is possible that the regulatory oxysterols generated by the CYP27A1 pathway inhibit cholesterol biosynthesis, and increasing the expression of cholesterol transporters, such as ABCA1 and ABCG8, subsequently enhancing the cellular efflux of cholesterol *via* activation of liver oxysterol receptor, LXR.

Previous reports showed that overexpression of the gene encoding the steroidogenic acute regulatory protein (StarD1), a protein which facilitates cholesterol delivery into mitochondria, dramatically increases cholesterol catabolism to bile acids both in primary hepatocytes in culture and *in vivo* (11;12). These findings suggested that cholesterol delivery to the mitochondria, where the enzyme CYP27A1 is localized, is the rate-determining step for bile acid synthesis via the “acidic” pathway. Subsequently, StarD1 was detected in hepatocytes (13). Overexpression of the gene encoding StarD1 *in vivo* not only increased bile acid synthesis to the same level as overexpression of CYP7A1, but also produced a similar composition of bile acids in bile (12). Recently, a novel sulfated oxysterol, 5-cholesten-3 β , 25-diol 3-sulfate (sulfated 25-hydroxycholesterol, 25HC3S) was found and characterized in mitochondria and nuclei of primary rat hepatocytes following overexpression of StarD1. This oxysterol was subsequently found to be present in human liver nuclei (14). In addition, this oxysterol could be synthesized by hydroxysteroid sulfotransferase (SULT2B1b) during the incubation of cholesterol with mitochondrial and cytosol fractions in the presence of 3'-phosphoadenosyl 5'-phosphosulfate (PAPS) (40). These observations suggest that the presence of this compound may have a physiological significance. However, the function of 25HC3S remains unknown.

Recent report showed that overexpression of SULT2B1b inactivates oxysterol-LXR signaling in several cultured mammalian cell lines but does not alter receptor response to the nonsterol agonist (T0901317) (15). Furthermore, triple-knockout mice deficient in the biosynthesis of three oxysterol ligands of LXRs, 24S-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol, respond to dietary T0901317 by inducing LXR targeting genes in liver but show impaired responses to dietary cholesterol (15). The results suggested that oxysterols are *in vivo* ligands for LXR and sulfation of oxysterols inactivates the LXR signaling activity (15).

In the present study, we present evidence that 25HC3S decreases expressions of HMG-CoA reductase, acetyl CoA carboxylase-1 (ACC-1), and fatty acid synthase (FAS) via inhibiting SREBP-1 expression and activation while 25-hydroxycholesterol increases SREBP-1 and FAS expression in primary human hepatocytes (PHH). The results suggest that 25HC3S may play an important role in the maintenance of intracellular cholesterol and lipid homeostasis in hepatocytes.

Materials and Methods

Materials

Cell culture reagents and supplies were purchased from GIBCO BRL (Grand Island, NY); [^{14}C]Cholesterol and [^3H]25-hydroxycholesterol from New England Nuclear (Boston, MA). [^{14}C]27-OH Cholesterol was prepared as previously described (16). HepG2 cells were obtained from American Type Culture Collection (Rockville, MD). The reagents for real time RT-PCR were from AB Applied Biosystems (Warrington WA1 4 SR, UK). The chemicals used in this research were obtained from Sigma Chemical Co. (St. Louis, MO) or Bio-Rad Laboratories (Hercules, CA). Polyclonal rabbit antibodies against SREBP1, SREBP-2 and HMG-CoA reductase were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All solvents were obtained from Fisher (Fair Lawn, NJ) unless otherwise indicated. The enhanced chemiluminescence (ECL) reagents were purchased from Amersham Biosciences (Piscataway,

NJ). The testosterone and 27-hydroxycholesterol were obtained from Research Plus Inc. (Bayonne, NJ). LK6 20 × 20 cm thin layer chromatography (TLC) plates were purchased from Whatman Inc. (Clifton, NJ).

Chemical synthesis of 5-cholesten-3 β , 25-diol 3-sulfate

A mixture of 25-hydroxycholesterol (402 mg, 1 mmol) and triethylamine-sulfur trioxide pyridine complex (160 mg, 1 mmol) in 5 ml of chloroform was stirred at 25°C for 7 days as previously described (17) with modification. After the solvent was evaporated at reduced pressure, products were purified by HPLC using a silica gel column and methylene chloride and methanol (5%) as mobile phase. The product was purified by reverse phase HPLC using C18 column as a white powder. The structure of the product was characterized by mass spectrum (MS) and nuclear magnetic resonance (NMR) spectroscopy analysis. The purity was determined by MS and HPLC.

Mass spectral analysis

The synthesized compound was analyzed by a MDS Sciex ABI 4000 Triple Quadrupole Mass Spectrometer (MDS Sciex, Toronto, Canada) with a Turbo IonSpray ionization (ESI) source. The mass spectrometer was operated in negative ion modes and data were acquired using full scan mode as previously described (14).

Proton nuclear magnetic resonance spectroscopy

Samples were prepared for ^1H NMR analysis as described previously (18). Briefly, each sample, 1 mg, was dissolved in 0.5 ml of D_2O and lyophilized to remove exchangeable protons. The residue was dissolved in 0.5 ml of dimethyl sulfoxide- $d_6/\text{D}_2\text{O}$ (98:2, v/v). NMR spectra were obtained on spectrometers operating at ^1H frequencies of 300 MHz.

Cell culture

Hepatoma cells line (HepG2) cells were grown in MEM containing non-essential amino acids, 30 mM NaHCO_3 , 10% FBS, 1 mM L-glutamine, 1 mM sodium pyruvate and 1% Pen/Strep and incubated at 37°C in 5% CO_2 . When cells reached ~90% confluence, the oxysterols in DMSO or in ethanol (final concentration, 0.1%) and/or [$1\text{-}^{14}\text{C}$]acetate for cholesterol synthesis was added. Microsomal and cytosolic fractions were isolated from broken cells as described previously (19).

Primary human hepatocytes (PHH) were purchased from an NIH-approved facility (Liver Tissue Procurement Distribution System, Univ. of Minnesota). Cells were obtained from a random sampling of males and females, 18–69 yr of age. Experiments were performed as cells became available to corroborate findings in experiments conducted in HepG2 cells as previously described (20).

For study of HMG CoA expression regulation, HepG2 or PHH were cultured in the media as described above in the presence or absence of mevinolin (50 μM) and mevalonate (0.5 μM). After cultured for 48 hrs, oxysterols were added and cultured for another 6 hrs, the cells were harvested for determining mRNA and protein levels.

Determination of cholesterol biosynthesis by TLC and HPLC

After incubation of HepG2 cells in media containing different concentrations of 25HC3S as indicated for 6 hrs, cells in 60 mm dishes were given 3 ml of the same fresh medium containing 5 μCi of [$1\text{-}^{14}\text{C}$]acetate. After 2 hr incubation at 37°C, the media was removed and the cells were washed twice with phosphate-buffered saline (PBS), harvested with rubber police as described, and collected in microcentrifuge tubes. The cells were sedimented by centrifugation

and the pellets were washed three times by resuspension and sedimentation. Subcellular fractions, microsomal, cytosol, and nuclear, were isolated as previously described (19). The cellular or subcellular pellets were resuspended in 0.3 ml of PBS. To each sample, 1.5 μ g of testosterone was added as an internal standard. The total lipids were extracted and separated by adding 3 volumes of chloroform:methanol (1:1). [14 C]Cholesterol and hydroxycholesterols were isolated into chloroform phase and separated on TLC (toluene:acetyl acetate, 2/3, v/v). [1- 14 C]acetate derivatives were visualized by Image Reader, Fujifilm BAS-1800 II.

For analysis of unlabeled sterol products, the extracted lipids were incubated with 2 units of cholesterol oxidase at 37°C for 20 min. The oxidation reaction was terminated by adding 1.5 ml of methanol followed by 0.5 ml of saturated KCl. The sterols were extracted twice using 3 ml of hexane. The hexane phase was collected and evaporated under a stream of nitrogen. The residues were dissolved in mobile phase solvents for HPLC analysis as previously described (8).

[1- 14 C]Acetate derivatives in the chloroform phase were analyzed by HPLC on an Ultrasphere Silica column (5 μ \times 4.6 mm \times 25 cm; Backman, USA) using HP Series 1100 solvent delivery system (Hewlett Packard) at 1.3 ml/min flow rate. The column was equilibrated and run in a solvent system of hexane:isopropanol:glacial acetic acid (965:25:10, v/v/v), as the mobile phase. The effluents were collected every 0.5 min (0.65 ml per fraction) except as indicated. The counts in [14 C]acetate derivatives were determined by Scintillation Counting. The column was calibrated with [14 C]cholesterol, [3 H]25-hydroxycholesterol, and [14 C]27-hydroxycholesterol.

Determination of mRNA levels of SREBPs and its targeting genes by real-time RT-PCR

Total RNA was isolated from HepG2 or PHH using SV Total RNA Isolation Kit (Promega). Two μ g of total RNA was used for first-strand cDNA synthesis as recommended by manufacturer (Invitrogen). Real-time PCR was performed using SYBR Green on ABI 7500 Fast Real-Time PCR System (Applied Biosystems). The final reaction mixture contained 5~10 ng of cDNA, 100 nM of each primer, 10 μ l of 2 \times SYBR $^{\circledR}$ Green PCR Master Mix (Applied Biosystems), and RNase-free water to complete the reaction mixture volume to 20 μ l. All reactions were performed in triplicate. The PCR was carried out for 40 cycles of 95°C for 15 s and 60°C for 1 min. The fluorescence was read during the reaction, allowing a continuous monitoring of the amount of PCR product. The data was normalized to internal control- β -actin or GAPDH mRNA. The sequences of primers are as shown in Supplemental Materials.

Western blot analysis

Microsomal fractions were isolated as previously described (19). Microsomal or total extracted proteins from the treated cells were separated on a 7.5% SDS-polyacrylamide denaturing gel. Following SDS-PAGE proteins were electrophoretically transferred to Immobilon-P (PVDF) membranes (Millipore). The membranes were then blocked at 25°C for 60 minutes in blocking buffer (PBS, pH 7.4, 0.1% Tween, 5% non-fat dry milk). Proteins were then incubated at 4°C for overnight with a rabbit polyclonal IgG against human SREBP1, SREBP-2, or HMG-CoA reductase. After washing with PBS, pH 7.4, containing 0.05% of Tween 20, goat anti-rabbit IgG-horse-radish peroxidase conjugate, 1:2500, in washing solution was added and incubated for 60 minutes. Protein bands were detected using the Amersham ECL plus Kit. Positive bands were quantitated by the Advanced Image Data Analyzer (Aida Inc., Straubenhardt, Germany).

Statistics

Data are reported as the mean \pm standard deviation. Where indicated, data were subjected to *t*-test analysis and determined to be significantly different if $p < 0.05$.

Results

Chemical synthesis of the nuclear oxysterol, 5-cholesten-3 β , 25-diol 3-Sulfate

To study its role in cellular lipid homeostasis, 25HC3S was chemically synthesized and purified as shown in Supplement Materials. MS analysis of the synthesized compound shows the same molecular mass ion, m/z 481 as the “authentic” nuclear oxysterol (14), and the purified product was not contaminated by the starting material, 25-hydroxycholesterol, m/z 401. ^1H NMR analysis shows that the proton resonance at C3 with multiple small (1.5 Hz) splits near 3.65 ppm in the spectrum of 25-hydroxycholesterol (starting material) is shifted to 4.20 ppm in the product spectrum, which confirms that a HSO_3^- group is added at the C3 position of 25-hydroxycholesterol (Fig 1 of Supplement Materials). The results indicate that the synthesized molecule is 5-cholesten-3 β , 25-diol 3-sulphate (25HC3S).

25HC3S inhibits cholesterol biosynthesis

To examine the effects of 25HC3S on cholesterol biosynthesis, the rates of cholesterol synthesis were determined. The effects of 25HC3S on cholesterol biosynthesis were summarized in Fig. 1. After addition of 25HC3S, the cells were cultured for 6 hrs and with $[1-^{14}\text{C}]$ acetate for an additional 2 hrs, total lipids were extracted and partitioned. TLC analysis shows that $[^{14}\text{C}]$ cholesterol ester, $[^{14}\text{C}]$ cholesterol, and $[^{14}\text{C}]$ 25-hydroxycholesterol were synthesized but $[^{14}\text{C}]$ 27-hydroxycholesterol was not detected (Fig. 1A). Free $[^{14}\text{C}]$ cholesterol was found significantly decreased following addition of 25HC3S while the other labeled sterols did not significantly change in HepG2 and PHH. The decrease was concentration dependent (Fig 1A). The decreased amounts of $[^{14}\text{C}]$ cholesterol bands on the TLC were confirmed by HPLC analysis as shown in Fig 1B. The results are summarized in Fig. 1C.

It was observed that no 25-hydroxycholesterol could be detected in chloroform phase extracted from the cells treated with 25HC3S up to 25 μM (36 μg) as shown Supplemental Materials. In contrast, concentration dependent levels of 25-hydroxycholesterol could be detected in the cells treated with 25-hydroxycholesterol as shown in figure 2 of Supplemental Materials.

25HC3S inhibits cholesterol biosynthesis by decreasing HMG-CoA reductase mRNA levels

To investigate how 25HC3S inhibits cholesterol biosynthesis, total RNA were isolated from the treated HepG2 cells or PHH. The mRNA levels of HMG-CoA reductase were determined by real time RT-PCR. As shown in Fig. 2, decreases in HMG-CoA reductase mRNA levels following the addition of 25HC3S to the cells in culture were concentration (Fig. 2A) and time (Fig. 2B) dependent.

25HC3S inhibits HMG-CoA reductase expression by inhibition of both SREBP1 activation and expression in hepatocytes

To study whether SREBP regulatory system is involved in the inhibition of HMG-CoA reductase mRNA levels and inhibition of cholesterol biosynthesis by 25HC3S, HepG2 or PHH were cultured in the presence of 50 μM of mevinolin and 0.5 μM of mevalonate. Under these conditions, HMG-CoA reductase expression is upregulated. Total cellular protein was extracted from the cells treated with 25HC3S or 25-hydroxycholesterol at different concentrations for 6 hrs. The precursor and mature forms of SREBPs were determined by Western blot analysis using specific antibodies (21;22). As expected, decreases of the mature forms of SREBP-1 ($p < 0.001$) and SREBP-2, and increases ($p < 0.001$) of the precursor form of SREBP-1 following addition of 25HC3S were concentration dependent (Figs. 3A, C, and D). However, 25-hydroxycholesterol increased SREBP-1 protein levels by 4-fold while mature form did not change significantly (Fig. 3B, C and D). It was observed that the activation of SREBP1 was much more sensitive to the treatment with 25HC3S than SREBP-2. The inhibition

of SREBP-1 and SREBP-2 activation is consistent with the decrease in HMG-CoA reductase mRNA. Thus, 25HC3S inhibits the activation of SREBP-1 and SREBP-2, and subsequently inhibit the expression HMG-CoA reductase.

To further confirm 25HC3S functions in a different manner from 25-hydroxycholesterol, expressions of SREBP-1 and its targeting genes were determined in PHH as shown in Fig 4. At 6 μ M, cultured for 6 hrs, addition of 25HC3S decreased SREBP1 mRNA by 50%, acetyl CoA carboxylase-1 (ACC-1) and fat acid synthase (FAS) by 60% (Fig. 6A). In contrast with 25HC3S and consistent with protein levels, 25-hydroxycholesterol increased SREBP1 mRNA levels by 2 folds and slightly increased FAS (Fig. 4A). The regulation of these genes was concentration dependent (Fig. 4B). Similar results were also observed in primary rat hepatocytes (data not shown).

Discussion

The 5-cholesten-3 β , 25-diol 3-sulfate (25HC3S) unlike 25-hydroxycholesterol inhibits SREBP-1 expression and activation. Although there are many oxysterols reported to be LXR ligands and regulate LXR targeting gene expression (23), detail mechanism of the regulation is unclear. The identification of liver oxysterol receptors (LXRs) as regulators of lipid metabolism has been taken as strong evidence that oxysterols mediate intracellular lipids homeostasis. LXRs bind with high affinity to selective oxysterols, which are generally believed to be the most important activators for these receptors (24–29). However, administration of synthetic LXR ligands triggers induction of the lipogenic pathway, and elevates plasma triglyceride levels via induction of SREBP-1, a direct target of LXR (30). From a drug development standpoint, the most desirable compound would appear to be one that is a strong inducer of the elimination of lipids, yet lacks activity on the SREBP-1 and fatty acid synthase (FAS) promoters. The present results show that this hydrophilic 25HC3S, unlike 25-hydroxycholesterol, plays unique roles in cholesterol and lipids metabolism at both transcriptional and posttranslational levels: decreased SREBP-1 mRNA levels, inhibited SREBPs activations, and inhibited expression of SREBP-1 targeting genes, HMG-CoA reductase, ACC-1, and FAS. However, 25-Hydroxycholesterol increases SREBP-1 expression. The finding of addition of 25-hydroxycholesterol increased SREBP-1 expression is consistent with the results of administration of synthetic LXR ligands in vivo, which triggers induction of the hyperlipidemia via SREBP-1 (30). After 25-hydroxycholesterol is sulfated by SULT2B1b (40), the product, 25HC3S, decreases SREBP-1 expression and inhibits its activation by acting on the opposite direction against the substrate. Thus, 25HC3S may serve as a promising molecule for the therapy of hyperlipidemia.

Both 25-hydroxycholesterol and 25HC3S inhibit cholesterol biosynthesis but through different mechanism. 25HC3S inhibits cholesterol synthesis by inhibiting SREBP-1 expression and its activation. However, 25-hydroxycholesterol inhibits the biosynthesis by increasing HMG CoA reductase fast degradation (ubiquitination) and decreasing SREBP-2 activation (3;10). Meanwhile, 25-hydroxycholesterol is able to increase SREBP-1 expression as LXR ligand (31), which is consistent with the present data that 25-hydroxycholesterol increases SREBP-1 mRNA levels (Fig. 4). 25HC3S may inhibit SREBP-1 expression by inactivating 25-hydroxycholesterol-induced LXR signaling. Recent report that overexpression SULT2B1b impairs oxysterol-induced LXR signaling (15) supports this hypothesis. Thus, the pair of the molecules, 25-hydroxycholesterol and 25HC3S, may play an important role in maintenance of intracellular lipid metabolism.

The sulfated oxysterol, 25HC3S, inhibits the cholesterol biosynthesis via decreasing mRNA levels and inhibiting the activation of SREBP-1, and subsequently inhibits the expression of HMG-CoA reductase, ACC-1, and FAS. Based on the microarray data from transgenic and

knockout mice, both of SREBP-1 and SREBP-2 activation stimulate HMG-CoA reductase (32). SREBP-1 also strongly stimulates fatty acid synthase but SREBP-2 does not (32). Our results show that 25HC3S is much more potent in inhibiting SREBP-1 activation than SREBP-2. We believe that 25HC3S directly inhibits both SREBP-1 activation and subsequently inhibits HMG-CoA reductase resulting in the decline of cholesterol biosynthesis. However, Acyl CoA:cholesterol acyltransferase (ACAT) and CYP27A1 for the synthesis of cholesterol ester and 25HC are not SREBP targeting genes (32), which support the result that 25HC3S does not affect the synthesis of cholesterol ester and 25HC (Fig. 1). SREBPs play important roles not only in cholesterol synthesis and fatty acid metabolism but also many important biological events (33–39). It will be interesting to investigate whether these oxysterols are also involved in these events.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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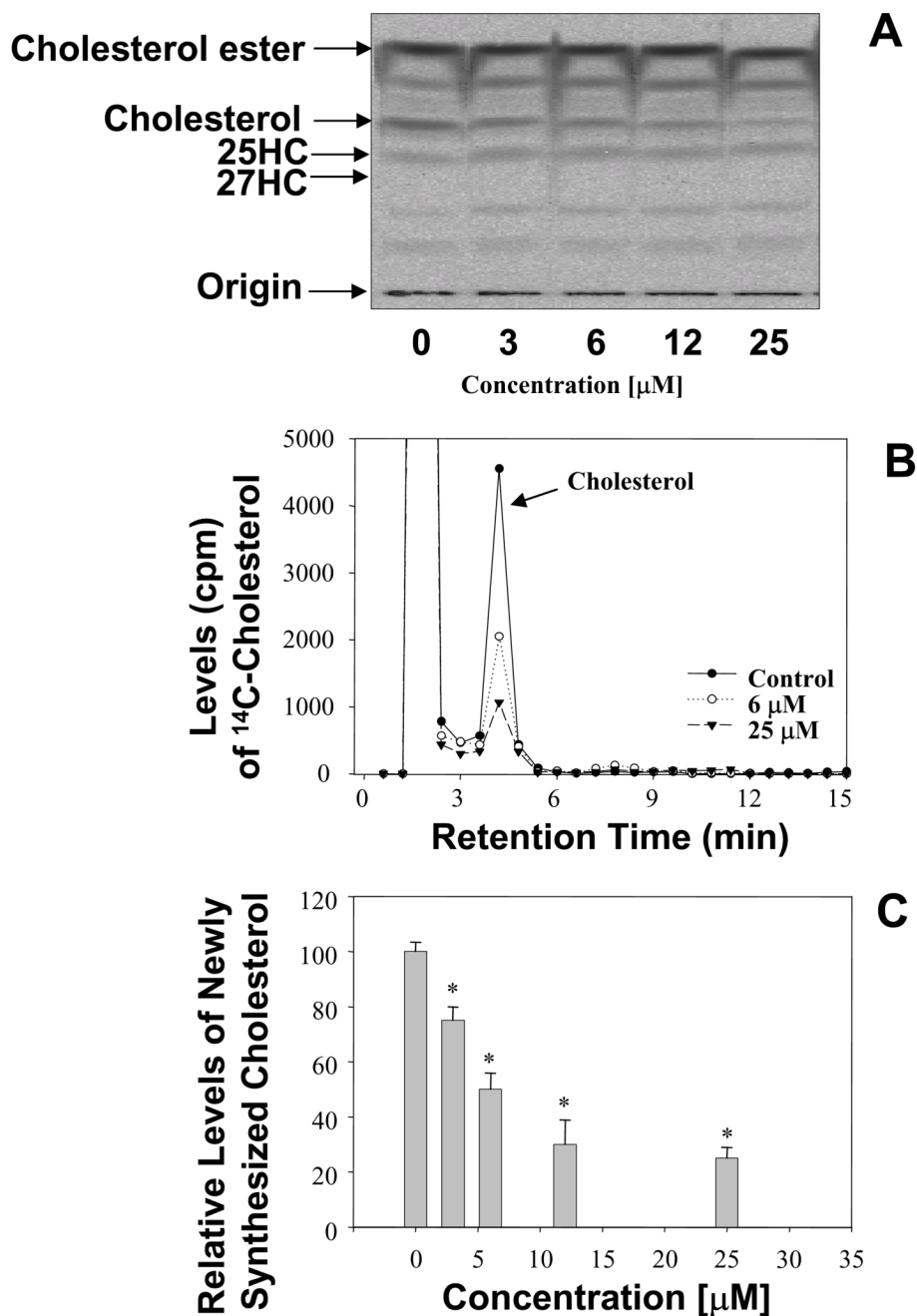


Fig. 1. TLC and HPLC analysis of the newly synthesized [^{14}C]cholesterol

After incubation of the 25HC3S-treated cells with [$1\text{-}^{14}\text{C}$]acetate, the total neutral lipids were extracted. TLC analysis of the [^{14}C]-acetate derivatives extracted from the equivalent of 5×10^6 cells per each lane is shown in Panel A; HPLC analysis of the [^{14}C]-acetate derivatives, Panel B. The column was calibrated by standards [^{14}C]cholesterol, [^{14}C]27-hydroxycholesterol, and [^3H]25-hydroxycholesterol. A summary of three experiments of HPLC analysis, Panel C. Each bar represents the mean of three experiments \pm standard deviation. The symbol * represents significant difference ($p < 0.01$).

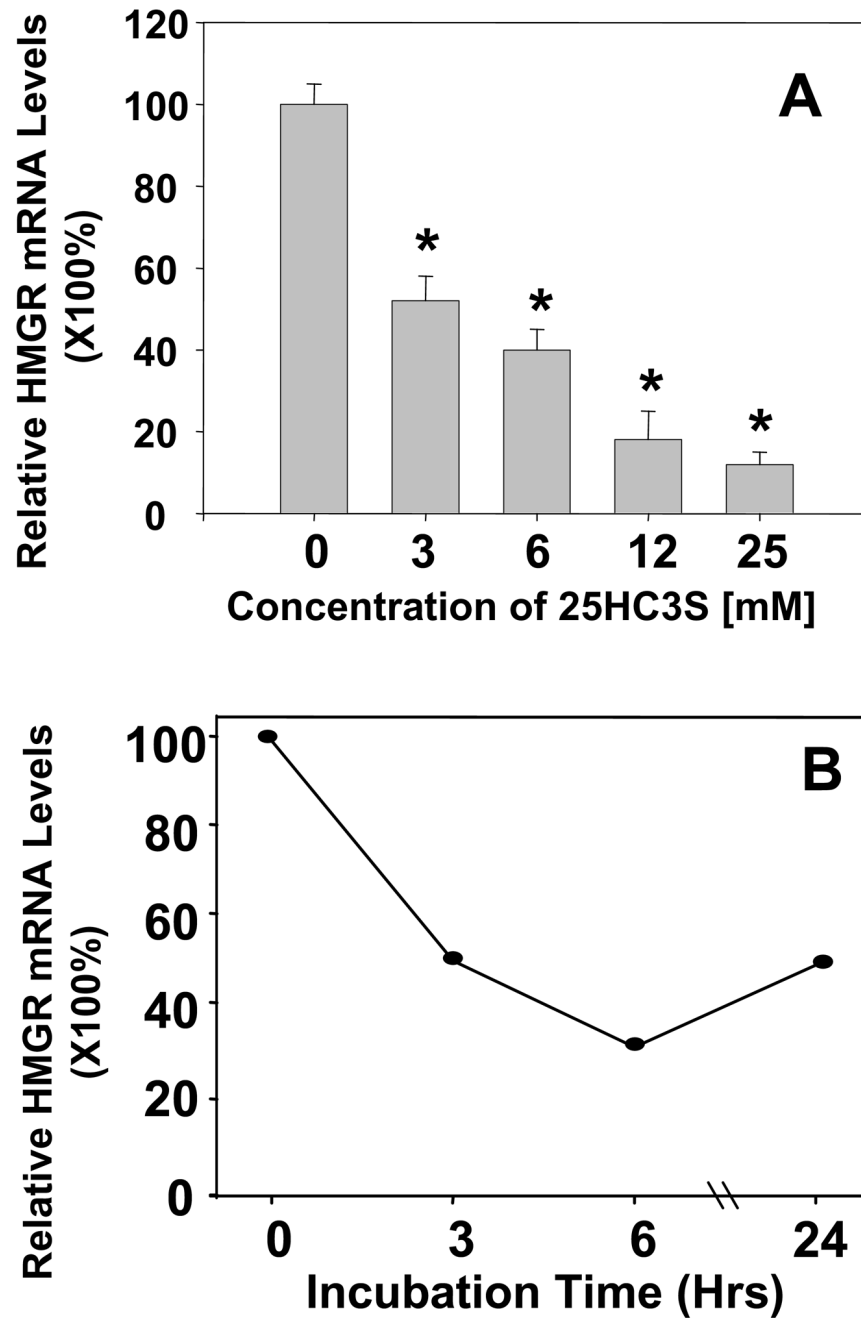


Fig. 2. 25HC3S regulates HMGR mRNA Expression

Real time RT-PCR analysis of HMG-CoA reductase mRNA levels in HepG2 cells is shown in Panels A and B. The expression levels were normalized to GAPDH. The mRNA levels in the cells treated with 25HC3S at different concentrations as indicated are shown in Panel A (each value represents mean of three separate experiments \pm standard deviation), and for the different times, Panel B. Each value represents mean of three experiments \pm standard deviation. The symbol, *, represents significant difference ($p < 0.05$).

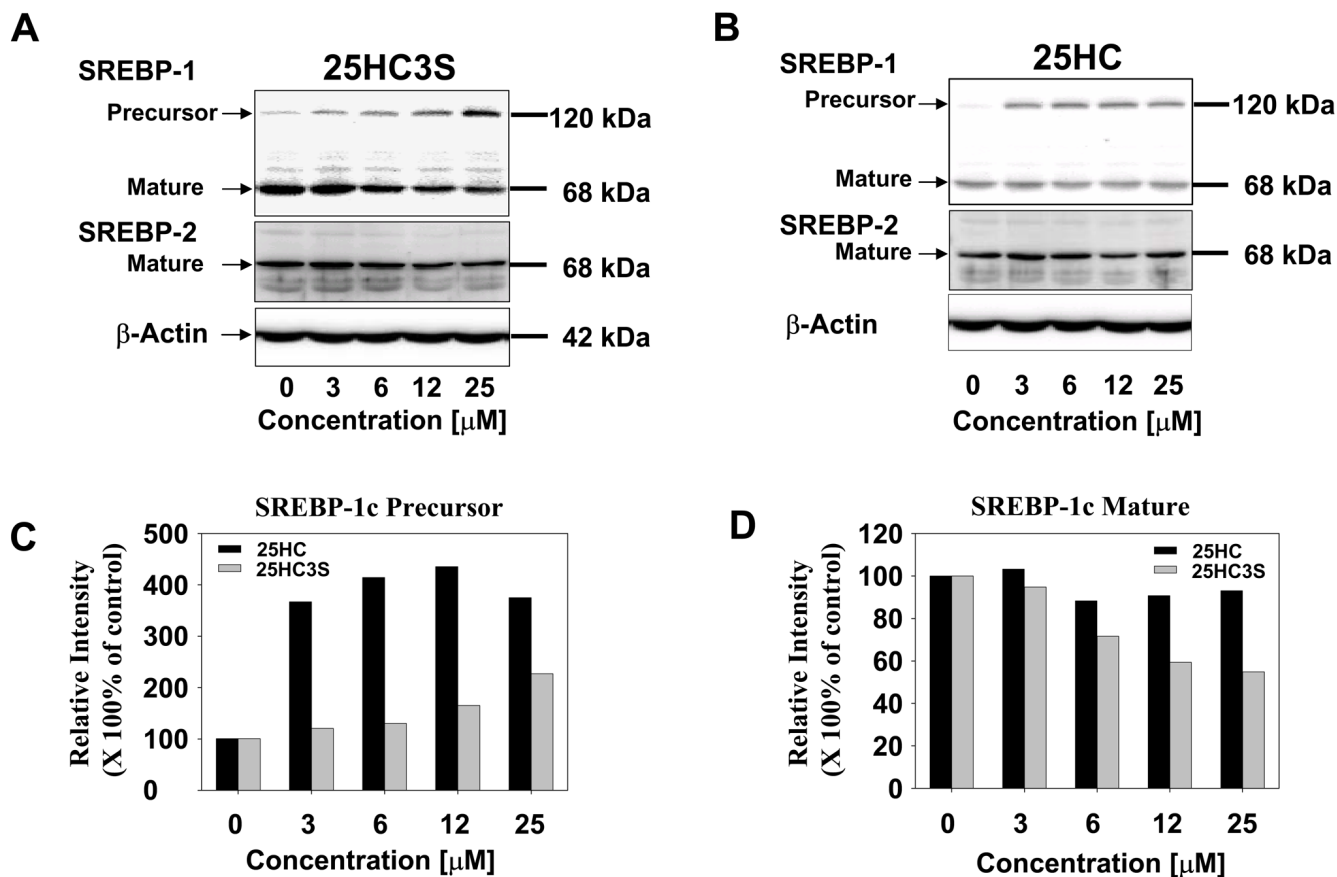


Fig. 3. Analysis of SREBPs expression and activation following 25HC3S treatment in HepG2 cells Western blot analysis of SREBP1 and SREBP-2 in HepG2 cells cultured in 10% of LPDS and treated with different concentrations of 25HC3S (Panels **A**) or 25-hydroxycholesterol (Panels **B**) in the presence of mevinolin (50 μ M) and mevalonate (0.5 μ M). The data represent a typical result from one of three independent experiments. The extracted protein (50 μ g) was loaded onto each lane for each condition as indicated. Each positive band was quantified by the Image Data Analyzer. A summary of SREBP1 precursor levels is shown in Panel **C** and mature form levels in Panel **D**. The data represent mean of three independent experiments \pm standard derivation. The symbol, *, represents significant difference ($p < 0.05$).

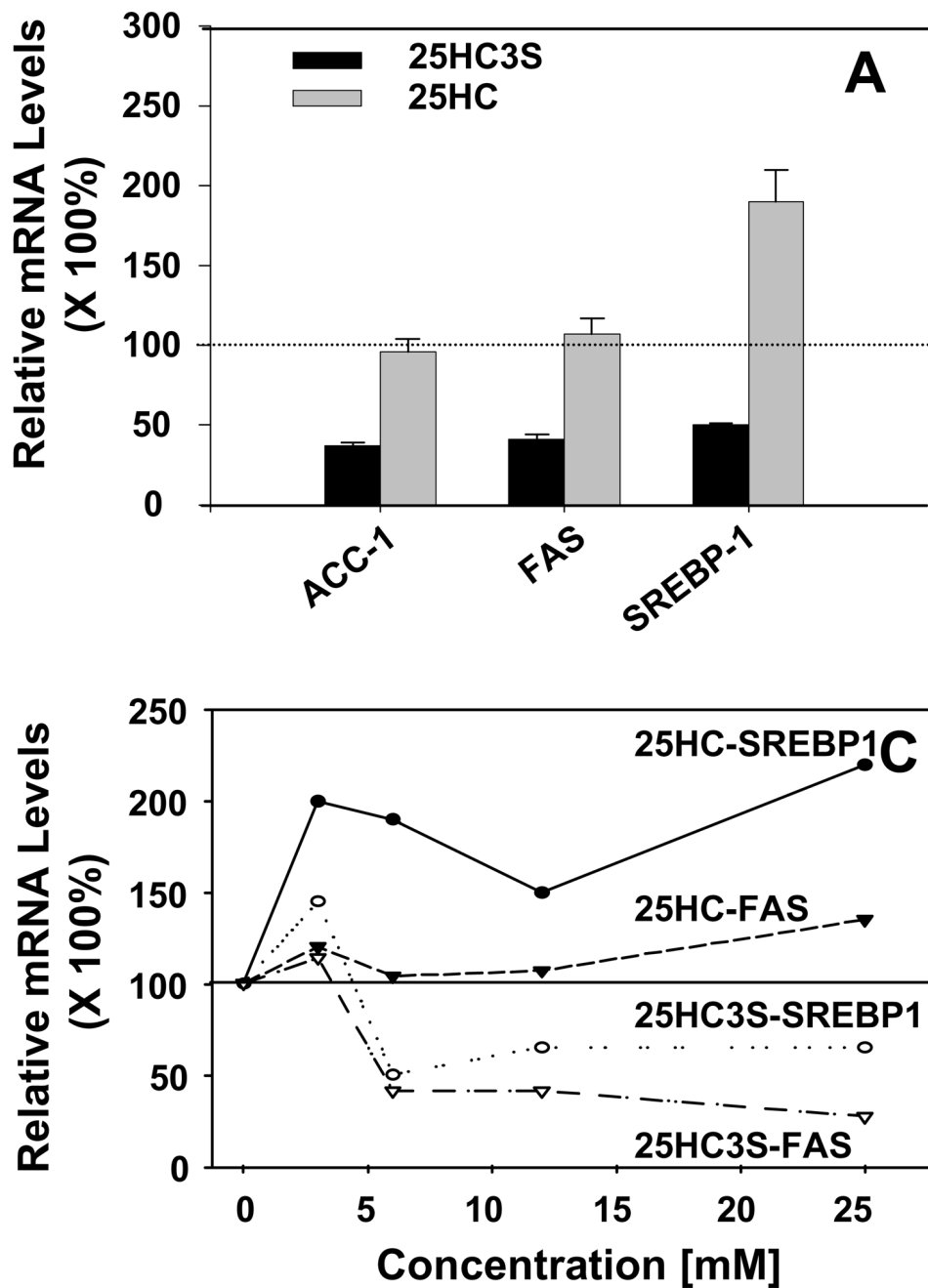


Fig. 4. Real time RT-PCR analysis of expression of SREBP-1 targeting genes

At 6 hr following addition of 6 μ M of 25HC3S or 25-hydroxycholesterol to PHH culture (Panel A), or different concentration of 25HC3S or 25-hydroxycholesterol (Panel B), effects of 25HC3S and 25-hydroxycholesterol on FAS and SREBP1 expression were determined by real time RT-PCR. The expression levels were normalized to GAPDH. The values represent means \pm SD (n =3).

Table 1

Primer sets for real time RT-PCR

Gene Name	Gene Bank #	Forward Sequence	Reverse Sequence
hGAPDH	NM_002046	CAATGACCCCTTCATTGACC	TTGATTTTGGAGGGATCTCG
hHMGR	NM_000859	GTCATTCCAGCCAAGGTGT	GGGACCACTTGCTCCATTA
hFAS	NM_004104	TGTGGACATGGTCACGGAC	GGCATCAAACCTAGACAGGTC
hACC1	NM_198834	TCGCTTTGGGGAAATAAAGTG	ACCACCTACGGATAGACCGC
hSREBP1	NM_004176	CAGCCCCACTTCATCAAGG	ACTGTTGCCAAGATGGTTCCG
hSREBP-2	NM_004599	AACGGTCATTCACCCAGGTC	GGCTGAAGAATAGGAGTTGCC