Cholesterol modulates human intestinal sodium-dependent bile acid transporter

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Alrefai, Waddah A., Zaheer Sarwar, Sangeeta Tyagi, Seema Saksena, Pradeep K. Dudeja, and Ravinder K. Gill. Cholesterol modulates human intestinal sodium-dependent bile acid transporter. Am J Physiol Gastrointest Liver Physiol 288: G978–G985, 2005. First published December 16, 2004; doi:10.1152/ajpgi.00379.2004.—Bile acids are efficiently absorbed from the intestinal lumen via the ileal apical sodium-dependent bile acid transporter (ASBT). ASBT function is essential for maintenance of cholesterol homeostasis in the body. The molecular mechanisms of the direct effect of cholesterol on human ASBT function and expression are not entirely understood. The present studies were undertaken to establish a suitable in vitro experimental model to study human ASBT function and its regulation by cholesterol. Luminal membrane bile acid transport was evaluated by the measurement of sodium-dependent [3H]-taurocholate (H-TC) uptake in human intestinal Caco-2 cell monolayers. The relative abundance of human ASBT (hASBT) mRNA was determined by real-time PCR. Transient transfection and luciferase assay techniques were employed to assess hASBT promoter activity. Caco-2 cell line was found to represent a suitable model to study hASBT function and regulation. 25-Hydroxycholesterol (25-HCH; 2.5 μg/ml for 24 h) significantly inhibited Na+-dependent H-TC uptake in Caco-2 cells. This inhibition was associated with a 50% decrease in the Vmax of the transporter with no significant changes in the apparent Km of the transporter. The inhibition in hASBT activity was associated with reduction in both the level of hASBT mRNA and its promoter activity. Our data show the inhibition of hASBT function and expression by 25-HCH in Caco-2 cells. These data provide novel evidence for the direct regulation of human ASBT function by cholesterol and suggest that this phenomenon may play a central role in cholesterol homeostasis.

human apical sodium-dependent bile acid transporter; human intestinal bile acid absorption; oxysterols; transcriptional regulation

Bile acids are synthesized in the liver from cholesterol and are secreted into the intestinal lumen, where they promote the digestion and the absorption of dietary lipids (18). The majority of the secreted bile acids is reabsorbed in the distal ileum, whereas only ~10% are excreted (16). After the absorption from the intestine, bile acids are recirculated back to the liver, where they are removed from the portal blood by the hepatocytes to complete their enterohepatic circulation (3). The circulation of bile acids is maintained by the coordinated function of an array of transporters that are distributed along the hepatocytes and the enterocytes (38).

In the intestine, the active absorption of bile acids represents the major route of their intestinal reclamation and is restricted to ileum (16, 23). Active intestinal bile acid absorption is initiated by their transport across the apical membrane of the enterocytes via apical sodium-dependent bile acid transporter (ASBT) (16). ASBT plays a pivotal role in bile acid retrieval from the intestine, and mutations in its gene lead to a genetic disorder, primary bile acid malabsorption (PBAM), characterized by intestinal bile acid malabsorption, diarrhea, and low plasma cholesterol (29). Also, because ASBT represents the first step in intestinal bile acid absorption, its function was suggested to be the major determinant of the size of bile acid pool in the enterohepatic circulation (23). This fact has been experimentally proven, at least in mice, by the recent studies of Dawson et al. (12), demonstrating the elimination of enterohepatic circulation of bile acids in ASBT knockout mice. Previous studies (10, 48) have shown that bile acids in the enterohepatic circulation exert a negative feedback effect on their hepatic biosynthesis from cholesterol via the activation of Farnesoid X receptor nuclear receptor. Therefore, the size of circulating pool of bile acids is an essential factor influencing lipid and cholesterol homeostasis (18, 30).

It is well established that blocking the intestinal absorption of bile acids results in low levels of plasma cholesterol (18). Furthermore, recent studies (5, 20, 33, 41) demonstrated that pharmacological inhibitors of ASBT also cause similar effect on the plasma cholesterol. These observations strongly suggest the involvement of ASBT function in maintaining cholesterol homeostasis in the body (23, 28). However, the molecular mechanism(s) underlying the coordination between intestinal ASBT function and cholesterol metabolism are still not well defined. The modulation of hepatic bile acid synthesis and transport by high levels of cholesterol has been extensively studied in the past (30–32, 46, 47). However, the direct effect of cholesterol on human intestinal human ASBT (hASBT) function and expression and the molecular basis of such modulation have not been investigated.

Previous studies investigating the effect of high dietary cholesterol have shown upregulation of ASBT mRNA in rabbit (49) but downregulation in mice (42), and the molecular mechanisms underlying these changes were not delineated. With regard to understanding the molecular mechanisms of ASBT regulation, it appears that in vivo studies and the use of animal models pose a challenge, given the complexity of the response mechanisms and the involvement of compensatory pathways. Thus an in vitro cellular model is warranted to directly delineate the molecular mechanisms of the cholesterol effect on hASBT function and expression. Human intestinal Caco-2 cell line has been previously used as an intestinal model to investigate the function and regulation of several electrolyte and nutrient transport processes (2, 15, 34, 35, 43). Therefore, the current studies were undertaken to examine whether Caco-2 cells represent a suitable in vitro cellular model to study the function and expression of human ASBT and to examine its regulation by cholesterol.

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Our data demonstrated that Caco-2 cell line could serve as an excellent experimental in vitro model to investigate hASBT function and regulation. Furthermore, we have shown that hASBT function was significantly inhibited by 24 h incubation with cholesterol derivative 25-hydrocholesterol (25-HCH) via decreasing the \( V_{\text{max}} \) of the transporter with no significant changes in the apparent \( K_m \). The observed inhibition of \(^3\)H-labeled taurocholic acid (\(^3\)H-TC) uptake by 25-HCH occurred, at least partially, at the transcriptional level via a reduction in hASBT promoter activity in Caco-2 cells.

**MATERIALS AND METHODS**

**Cell culture.** Caco-2 cells were obtained from ATCC and were grown routinely in T-75-cm\(^2\) plastic flasks at 37\(^\circ\)C in a 5% CO\(_2\)-95% air environment. The culture medium consisted of high-glucose DMEM, 20% FBS, 100 IU/ml penicillin, and 100 \( \mu \)g/ml streptomycin. For the uptake experiments, cells from passages between 25 and 50 were plated in 24-well Falcon plates (treated by vacuum gas plasma, Becton-Dickinson, Franklin Lakes, NJ) at a density of \( 2 \times 10^4 \) cells/cm\(^2\) and were fed with fresh incubation media every alternate day. Confluent monolayers were then used for the transport experiments at day 10 postplating. Oxyostyler: 25-, 22-, and 24-HCH were obtained from Sigma (St. Louis, MO) or from Steraloids (Steraloid, Newport, RI). To study the effect of oxysterols, cells were incubated with the indicated oxysterol for 24 h in media containing 1% FBS to minimize the effects of cholesterol and other lipids found in the lipoproteins of the fetal bovine serum, whereas control cells were treated with ethanol alone (vehicle).

**TC uptake.** Na\(^+\)-dependent TC transport in Caco-2 cells was assessed as previously described with minor modifications (40). Briefly, cells were equilibrated at room temperature for 20 min at the time of the experiment and were then washed and incubated for 15 min at 25\(^\circ\)C with buffer containing (in mM) 110 NaCl (with sodium) or choline chloride (without sodium), 4 KCl, 1 MgSO\(_4\), 1 CaCl\(_2\), 50 mannitol, and 10 HEPES, pH 7.4. The cells were then washed and incubated with the same buffer containing the indicated concentration of TC along with 1 \( \mu \)Ci/ml of \(^3\)H-TC (Perkin-Elmer, Boston, MA) for the designated period of time. To terminate the transport process, cells were washed twice with ice-cold PBS. Finally, the cells were solubilized with 0.5 N NaOH for at least 4 h. The protein concentration was measured by the method of Bradford (6), and the radioactivity was counted by Packard liquid-scintillation analyzer, Tri-CARB 1600-TR (Packard Instrument, Downers Grove, IL). Because the 5-min time point was in the linear range of the uptake of \(^3\)H-TC, the uptake was measured at 5 min and was expressed as picomole per milligram protein per 5 min. For the kinetic experiments, the uptake values were analyzed for simple Michaelis-Menten kinetics using a nonlinear regression data analysis from a computerized model (GraphPad, PRISM, San Diego, CA).

**RNA extraction and real-time RT-PCR analysis.** Total RNA was prepared from Caco-2 cells treated as indicated using Absolutely RNA RT-PCR Miniprep Kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. Equal amounts of RNA from both treated and control samples were reverse transcribed and amplified in a one-step reaction using Brilliant SYBR Green QRT-PCR Master Mix Kit (Stratagene). Real-time PCR was performed using Mx3000 (Stratagene). hASBT was amplified with gene-specific primers (sense primer: 5'-GGGGTACCCGCCATCAAC-TACAAGCTTGCTAG-3'; primer-3: 5'-GGGTACCCCTTTCTATTT-GAAAGGAAAAATGGGAG-3'). The sequence of the reverse primer contained site for SacI enzyme and is: 5'--AAGAGCTCGTGTCGTGCTGCAAGCTAC-3'. The amplifications were performed using proof reading Elongase enzyme mix (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. PCR products were then digested with \( KpnI \) and SacI enzymes and subcloned into luciferase reporter gene vector, pGKL-2 basic (Promega). The fidelity of the constructs were then confirmed by sequencing, and plasmids were prepared for transfection using a kit from Qiagen (Valencia, CA).

**Transient transfection and luciferase assay.** For transfection studies, Caco-2 cells (1.5 \times 10\(^5\)) were seeded into 24-well plates and cotransfected 24 h later (80–90% confluent) with one of the hASBT promoter-luciferase constructs and pCMV\(\beta\), \( \beta \)-galactosidase mammalian expression vector (BD Biosciences Clontech, Palo Alto, CA), using Lipofectamine-2000 reagent (Invitrogen). The latter plasmid served as an internal control for transfection efficiency. A total of 3 \( \mu \)g DNA/well, at a ratio of five to one for experimental vs. pCMV\(\beta\), was used for each transfection. After 48 h, cells were washed with PBS and lysed using a kit from Promega. The activities of both firefly luciferase and \( \beta \)-galactosidase were measured by luminometer according to the manufacturer’s instruction using kits from Promega and Clontech, respectively. The promoter activity was expressed as a ratio of luciferase to \( \beta \)-galactosidase activity in each sample.

**Statistical analysis.** Results are expressed as means \( \pm \) SE. Student’s \( t \)-test was used in statistical analysis. \( P < 0.05 \) or less was considered statistically significant.

**RESULTS**

\(^3\)H-TC transport in Caco-2 cells. Previous studies (28) have demonstrated the expression of hASBT mRNA in Caco-2 cell line. However, the functional features of hASBT in Caco-2 cells are not well established. Therefore, in an attempt to establish an intestinal epithelial in vitro cellular model for studying the function and regulation of human intestinal sodium-dependent bile acid transporter (ASBT), the uptake of \(^3\)H-TC in well-differentiated, postconfluent Caco-2 cells was examined. As shown in Fig. 1A, uptake of \(^3\)H-TC (10 \( \mu \)M) was significantly higher at each time point in the presence of sodium chloride compared with the presence of choline chloride. TC uptake was linear as a function of time up to 10 min in the presence but not in the absence of sodium in Caco-2 cells. \(^3\)H-TC uptake also demonstrated saturating kinetics (\( V_{\text{max}} = 159 \pm 15 \) pmol \( \cdot \)mg protein\(^{-1} \cdot \)min\(^{-1}\), \( K_m = 20 \pm 8 \) \( \mu \)M) in the presence of increasing concentrations (5–150 \( \mu \)M) of TC (Fig. 1B). This indicates the involvement of a carrier-mediated process for sodium-dependent TC uptake in Caco-2 cells. These data demonstrate the presence of functional ASBT in Caco-2 cells and indicate that these cells represent a suitable model to study the regulation of ASBT in the human intestinal epithelium.

**Effect of oxysterols on TC uptake in Caco-2 cells.** The regulation of hASBT function by cholesterol in Caco-2 cells sent the difference between the threshold cycle of amplification (Ct) of treated and control RNA for hASBT and \( \beta \)-actin, respectively.

**Plasmid construction.** Three different fragments from the promoter region of hASBT were amplified utilizing human genomic DNA (Promega, Madison, WI) and gene specific primers based on previously published sequence (21). Three different forward primers and a reverse primer were used in the PCR reactions to amplify different fragments representing 5’ deletions of the hASBT promoter. Forward primers contained an internal site for \( KpnI \) restriction enzyme and their sequences are: primer-1: 5'-GGGGTACCCCTTTCTATTT-GAAAGGAAAAATGGGAG-3'; primer-2: 5'-GGGTACCCCTATTGCTAG-3'; primer-3: 5'-GGGTACCCCTTTCTATTT-GAAAGGAAAAATGGGAG-3'. The sequence of the reverse primer contained site for SacI enzyme and is: 5'--AAGAGCTCGTGTCGTGCTGCAAGCTAC-3'.
was investigated. The first set of experiments was performed to assess the effect of various oxysterols (oxygenated cholesterol derivatives) on Na\textsuperscript{+}/H\textsuperscript{+}-dependent 3H-TC uptake in Caco-2 cells. As shown in Fig. 2, TC uptake was significantly inhibited by 24-h exposure to 2.5 \( \mu \text{g/ml} \) concentration of 24(S), 22(R), or 25-HCH. Also, data depicted in Fig. 2 illustrated that the maximal inhibition of TC uptake occurred in response to incubation with 25-HCH. These results clearly show that hASBT is inhibited by different cholesterol derivatives and that 25-HCH was the most potent inhibitor of TC uptake in Caco-2 cells.

Characterization of the effect of 25-HCH on Na\textsuperscript{+}-dependent 3H-TC uptake. As a first step to understanding the mechanism by which incubation with 25-HCH decreases TC uptake, we evaluated its effect on the kinetic parameters of Na\textsuperscript{+}-dependent TC uptake in Caco-2 cells. As shown in Fig. 4, 24-h incubation with 2.5 \( \mu \text{g/ml} \) concentration of 25-HCH significantly decreased the \( V_{\text{max}} \) (23 \( \pm \) 8 vs. 48 \( \pm \) 8 pmol\textsuperscript{-}mg\textsuperscript{-}1 \( \pm \) 5 min\textsuperscript{-}1).

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Fig. 2. 25-Hydroxycholesterol (HCH) is a potent inhibitor of TC uptake. Postconfluent Caco-2 cells were incubated with 2.5 \( \mu \text{g/ml} \) of 22-, 24-, and 25-HCH in media containing 1% FBS for 24 h. 3H-TC uptake (10 \( \mu \text{M} \)) was then assessed for 5 min as described in A. Results are presented as %control and represent means \( \pm \) SE obtained from 3 separate experiments. *\( P < 0.05 \) compared with control (cells incubated with ethanol alone).

Fig. 3. Dose-dependent inhibition of TC uptake by 25-HCH. Postconfluent Caco-2 cells were treated with different concentrations (0.5–5 \( \mu \text{g/ml} \)) of 25-HCH for 24 h in media containing 1% FBS and sodium-dependent 3H-TC uptake (10 \( \mu \text{M} \)) was measured (A). B: sodium-dependent 3H-TC uptake (10 \( \mu \text{M} \)) was measured in postconfluent Caco-2 cells after incubation with 2.5 \( \mu \text{g/ml} \) of 25-HCH in 1% FBS-containing media for the indicated period of time. Data are expressed as %control and represent 6 uptake determinations performed on 3 separate occasions. *\( P < 0.05 \) compared with control.

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Fig. 1. 3H-taurocholic acid (TC) uptake in Caco-2 Cells. Five- to seven-day postconfluent Caco-2 cells were washed and then incubated at 25°C with HEPES-buffered uptake solution, pH 7.4, containing 110 mM of NaCl (●) or choline chloride (○) along with 10 \( \mu \text{M} \) 3H-TC for the indicated period of time (A). Results represent means \( \pm \) SE of 6–9 determinations from 3 separate experiments and are expressed as pmol/mg protein. B: 3H-TC uptake was measured for 5 min in the presence of increasing concentrations of the substrate TC. Sodium-dependent 3H-TC uptake was expressed as pmol\textsuperscript{-}mg protein\textsuperscript{-}1 \( \pm \) 5 min\textsuperscript{-}1 and represented the difference in the uptake values in presence and the absence of sodium. Representative Michaelis-Menten plot of experiments performed on 3 different occasions is shown.
protein\(^{-1}\cdot5\text{min}^{-1}\)) of TC uptake compared with incubation with vehicle alone (cells treated with ethanol) with no significant changes in the \(K_m\) for TC.

**Effect of 25-HCH on the level of hASBT mRNA in Caco-2 cells.** Incubation with 25-HCH decreased the \(V_{\text{max}}\) of Na\(^+\)-dependent TC uptake in Caco-2 cells suggesting an alteration in the expression of hASBT. Therefore, we investigated the relative abundance of hASBT mRNA in Caco-2 cells in response to 24 h incubation with 25-HCH. hASBT mRNA level was quantified by real-time PCR relative to the level of \(\beta\)-actin mRNA as an internal control. As shown in Fig. 5, there was a significant decrease in the relative abundance of ASBT mRNA in response to 5 \(\mu\)g/ml of 25-HCH parallel to a reduction in the \(V_{\text{max}}\) of TC uptake. These data clearly indicate that 25-HCH downregulates both the expression and the function of hASBT in Caco-2 cells.

**Effect of 25-HCH on the promoter activity of hASBT in Caco-2 cells.** Because the level of hASBT mRNA was altered in response to 25-HCH, we next examined the effect of 25-HCH on the transcriptional rate of hASBT gene in Caco-2 cells. We amplified a fragment of hASBT promoter, based on a previously published sequence (21), flanking a region between \(-1410\) and \(+592\) of ASBT gene by PCR using human genomic DNA (+1 is considered as the transcription initiation site). hASBT promoter fragment was then subcloned into the promoterless vector pGL2 in frame with luciferase cDNA and designated as pASBT-1. Furthermore, we engineered two constructs representing progressive 5\(^{-}\)-deletions of pASBT-1 promoter fragment (designated as pASBT-2 and pASBT-3). To examine the activity of the three promoter vectors of hASBT in Caco-2 cells, hASBT promoter constructs were transiently cotransfected into Caco-2 cells along with \(\beta\)-gal mammalian expression vector as internal control. The relative promoter activity was expressed as a ratio of luciferase to \(\beta\)-gal activity in each sample to adjust for the transfection efficiency. As shown in Fig. 6, ASBT promoter constructs demonstrated high relative luciferase activity in Caco-2 cells showing \(-12\)- to 20-fold increase compared with cells transfected with pGL2 empty vector alone. Additionally, Fig. 6 shows that the 5\(^{-}\)-untranslated region (5\(^{-}\)-UTR) of the ASBT gene (+1/+592) representing a noncoding sequence demonstrated the highest relative promoter activity.

To examine the effect of 25-HCH on the transcriptional activity of ASBT gene, Caco-2 cells were transiently transfected with pGL2 construct pASBT-1 that contains the \(-1410/\ +592\) fragment of hASBT promoter and then incubated with different concentrations of 25-HCH for 24 h. As depicted in Fig. 7A, 25-HCH significantly reduced the relative activity of hASBT promoter in a dose-dependent manner with the maximal inhibition at 5 \(\mu\)g/ml concentration. These results clearly show the modulation of ASBT gene transcription by 25-HCH.

**Identification of 25-HCH-response element on ASBT promoter.** Further experiments were performed to locate the region of hASBT promoter involved in its modulation by 25-HCH. Caco-2 cells were transiently transfected with the three constructs (\(-1410/\ +592\), \(-1010/\ +592\), and \(+1/+592\)) representing the 5\(^{-}\)-deletions of ASBT promoter and then treated with 5 \(\mu\)g/ml concentration of 25-HCH for 24 h. Figure 7B demonstrates that 25-HCH almost equally inhibited the activities of the three constructs indicating the presence of 25-HCH-response element in the minimal promoter region that represents the 5\(^{-}\)-UTR of ASBT gene.

**DISCUSSION**

Ileal ASBT plays a central role in bile acid and cholesterol metabolism (23). Previous studies (42, 49) have examined the effect of high dietary cholesterol on the expression of ASBT in different animal models. The results of these studies, however, demonstrated differences between the species with respect to the regulation of ASBT mRNA in response to high cholesterol. Interestingly, animal species such as mouse and rat, which show resistance to the development of hypercholesterolemia,
had decreased or unaltered levels of ASBT mRNA (42, 49), whereas ASBT mRNA expression was increased in species that attain high plasma cholesterol in response to cholesterol feeding, such as rabbit (49). One limitation of the studies in animal models is that these data are not conclusive with respect to whether the observed effects of cholesterol are a result of a direct regulation by cholesterol or they are secondary to alterations in the size of bile acid pool and subsequent changes in their intestinal flow. Therefore, an in vitro model is necessary to investigate the direct effects of cholesterol on ASBT expression, function, and regulation.

Studies from our laboratory and others (2, 15, 34, 35, 43) have extensively used human colonic adenocarcinoma, Caco-2, cell line as an in vitro model to study various intestinal and colonic electrolyte and nutrient transport processes. Although Caco-2 cells are of cancer origin, previous studies demonstrated that they exhibit several parameters of differentiation in at least 2–5 days postconfluence e.g., formation of domes, development of well-defined brush-border membrane possessing microvilli, and the expression of alkaline phosphatase enzyme (7, 36). Also, human postconfluent Caco-2 cells develop a phenotype similar to that of human small intestinal epithelial cells (8). Studies on the suitability of Caco-2 cell line as a model to study sodium-dependent bile acid transport are controversial. For example, earlier study by Hidalgo et al. (17) indicated that the major component of transepithelial bile acid transport in Caco-2 cells was Na\(^{+}\)-independent. However, Chandler et al. (9) later demonstrated the presence of Na\(^{+}\)-dependent cotransport process in Caco-2 cells. These discrepancies in the results might have been observed due to either variability in subclones of Caco-2 cells and/or different culture conditions. We herein demonstrated the existence of sodium-dependent \(^{3}H\)-TC uptake in well-differentiated postconfluent Caco-2 cells with characteristics of carrier-mediated process similar to those of ASBT reported in previous studies (33–35). For instance, the \(K_m\) of 20 \(\mu M\) of hASBT for TC described in the current study is comparable with 12 \(\mu M\) for hASBT transfected in monkey kidney CHO cells (44) and 33 \(\mu M\) for hamster ASBT in COS cells (45), respectively, and to \(K_m\) of 37 \(\mu M\) for TC demonstrated in human ileal brush-border membrane vesicles (4).

The effect of oxysterols on hASBT function in Caco-2 cells was next examined. Oxysterols are naturally occurring intermediates of cholesterol metabolism that exert a wide range of biological effects in various tissues and cell types (37). Oxysterols are involved in the regulation of cholesterol metabolism by several pathways including the activation of the orphan
nuclear receptor, liver X-receptor (LXR) (26), downregulation of SREBP (19) and binding to oxysterol-binding protein (37). Also, 25-HC has been recently shown to reduce the expression of ileal bile acid binding protein, a protein responsible for the transcellular transport of bile acids from apical to basolateral membrane of intestinal epithelial cells, in human intestinal Caco-2 cells (50). Our results demonstrated that sodium-dependent \(^3\)H-TC uptake in Caco-2 cells was significantly inhibited in response to incubation with cholesterol derivatives and that the maximal inhibition occurred in response to 25- but not 22- and 24-HCH. Previous studies (37) have shown that 24-HCH but not 25-HCH is a potent activator of LXR nuclear receptor. Also, Jung et al. (21) recently showed that hASBT promoter is not directly responsive to LXR activation. In lieu of these previous results, our studies would indicate that the effect of 25-HCH on hASBT function is likely to be independent of the direct effects of LXR. Further studies are needed to investigate the possible involvement of other molecular pathways sensitive to 25-HCH in the regulation of hASBT. It should be mentioned that high plasma cholesterol is also observed in liver diseases and cholangiopathies that are managed by bile acid-based therapy (25, 39). ASBT was found to be expressed in the cholangiocytes (1). However, it is not known yet whether its expression in these cells is also modulated by cholesterol.

The decrease in hASBT function by 25-HCH in Caco-2 cells occurred in a time- and dose-dependent manner with a maximal inhibition after 12 h of incubation with a concentration of 2.5 \(\mu\)g/ml (~6.2 \(\mu\)M). This concentration is within the range of previously used concentrations (5–25 \(\mu\)M) to investigate the effect of 25-HCH on the expression of various genes (13, 14, 24). Because hASBT function was inhibited by 12 but not 1 or 4 h incubation with 25-HCH, the observed changes are likely to result from changes in hASBT expression. This notion was further supported by the reduction in the \(V_{\text{max}}\) of the transporter in response to incubation with 25-HCH. In parallel to the decrease in the \(V_{\text{max}}\) of the transporter, real-time PCR studies demonstrated that the abundance of hASBT mRNA was also reduced by incubation with 25-HCH in Caco-2 cells. This decrease in the level of hASBT mRNA may be a result of a reduction in the hASBT mRNA stability and/or it may be a consequence to an inhibition in the promoter activity of hASBT in Caco-2 cells. Our finding that activities of hASBT promoter constructs were also inhibited by incubation with 25-HCH in Caco-2 cells clearly demonstrated that the cholesterol derivative decreased the expression and the function of hASBT, at least partially, via a reduction in its transcriptional rate.

25-HCH-induced inhibition occurred in promoter construct containing only the 5’-UTR indicating that the 5’-UTR region of the hASBT gene harbors the response element involved in the inhibition of hASBT expression by 25-HCH in Caco-2 cells. The 5’-UTR of hASBT gene also demonstrated high basal activity in Caco-2 cells. This finding is consistent with recent studies of Jung et al. (21) and is in agreement with the presence of multiple binding sites for hepatic nuclear factor-\(\alpha \)1 in the 5’-UTR that are essential for the basal transcriptional activity of ASBT promoter. Interestingly, this region of the gene has been recently shown to contain the cis-response elements that also mediate the negative feedback effect of bile acids on hASBT expression (28).

Our studies suggest that the modulation of hASBT by cholesterol could be an adaptive response to high cholesterol, similar to that observed in mice (42) but not rabbit (49). In this scenario, the function and the expression of intestinal hASBT will be reduced in response to high cholesterol leading to an inhibition in intestinal absorption of bile acids and a subsequent reduction in the size of their circulating pool. This, in turn, will limit the negative feedback effect of bile acids on their hepatic biosynthesis from cholesterol and, therefore, enhance cholesterol catabolism and lower its plasma level. It should be noted, however, that there are variations in humans in responses to diet enriched with cholesterol, because some will develop hypercholesterolemia and others are resistant (22, 27). In this regard, one would argue for the presence of regulatory molecules present in Caco-2 cells and some individuals that will respond to high cholesterol and will lead to an inhibition in hASBT function and expression, subsequently conferring resistance to the development of hypercholesterolemia. The differential expression of such regulatory pathways between different human subjects might underlie the discrepancy in their responses to high-cholesterol diet and the development of hypercholesterolemia. Delineation of the molecular mechanism(s) by which 25-HCH regulates hASBT is, therefore, of great significance, because such pathways would be the target of pharmacological upregulation as a modality for the treatment of cholesterol-related disorders. This argument emphasizes the importance of our studies by providing an excellent model to elucidate the molecular regulation of hASBT by cholesterol.

In summary, using Caco-2 cells as an experimental model of the human intestinal epithelium, the current studies, for the first time, demonstrated the downregulation of hASBT function and expression by 25-HCH. Future studies should focus on detailed analysis of the molecular mechanisms including the role of transcription factors, e.g., SREBP in the observed inhibition of hASBT. These studies will expand our knowledge with respect to the molecular mechanisms that govern the modulation of human hASBT in response to high cholesterol and will advance the therapeutic modalities for the management of cholesterol-related disorders such as atherosclerosis.

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REFERENCES

INHIBITION OF hASBT BY 25-HYDROXYCHOLESTEROL


