molecular pharmaceutics

reviews

# Apical Sodium Dependent Bile Acid Transporter (ASBT, SLC10A2): A Potential Prodrug Target

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Received March 3, 2006

**Abstract:** A major hurdle impeding the successful clinical development of drug candidates can be poor intestinal permeability. Low intestinal permeability may be enhanced by a prodrug approach targeting membrane transporters in the small intestine. Transporter specificity, affinity, and capacity are three factors in targeted prodrug design. The human apical sodium dependent bile acid transporter (SLC10A2) belongs to the solute carrier family (SLC) of transporters and is an important carrier protein expressed in the small intestine. In spite of its appearing to be an excellent target for prodrug design, few studies have targeted human apical sodium dependent bile acid transporter (hASBT) to improve oral bioavailability. This review discusses bile acids including their chemistry and their absorptive disposition. Additionally, hASBT-mediated prodrug targeting is discussed, including QSAR, in vitro models for hASBT assay, and the current progress in utilizing hASBT as a drug delivery target.

**Keywords:** Bile acids; prodrugs; hASBT; QSAR; SLC10A2; cell culture; transporters

## Introduction

Membrane transport proteins play important roles in the influx and efflux of various nutrients, metabolic substances, and cell signaling molecules in the cell. Based on the sequencing of the human genome, approximately 500−1200 genes encode transport proteins.[1,2] Transporters tend to be multifunctional and often play vital roles in translocating endogenous substances such as sugars, lipids, amino acids, bile acids, steroids, and hormones across biological membranes; they also influence the disposition and toxicity of drugs.[3−5] Significant progress has been made in the discovery and characterization of transporters, including development of in silico models to predict their interaction with substrates and inhibitors.[6−8] For example, pharmacophore-based models have been proposed for several influx transporters, such as peptide transporter (Pept1), nucleoside transporters (ENT family), OCT family, and the OATP family of proteins.[9−12]

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**10.1021/mp060022d CCC: $33.50** 
Published on Web 05/11/2006

Despite this progress, the design of prodrugs to target transporters appears less developed. An example of a prodrug approach to target an intestinal transporter to enhance oral absorption is valacyclovir.[13] The bioavailability of acyclovir was enhanced 2-fold to 3-fold via the oral administration of its valine ester (valacyclovir), which is a substrate for the small intestinal peptide transporter.[14] However, the historical discovery of drugs whose intestinal absorption is mediated by Pept1 has generally been serendipitous.[9] Two scenarios can be envisioned in the future exploitation of uptake transporters to enhance oral bioavailability. The prodrug approach can involve coupling a drug candidate to a natural substrate for a transporter. The advantage of such an approach is apparently low potential for toxicity, owing to the use of endogenous substrates. The second approach involves what has been referred to as "substrate mimicry", wherein the three-dimensional drug structure resembles natural substrates either by design or by serendipity.[15]

## Bile Acid Transport and Enterohepatic Recirculation

An understanding of bile acid structure and disposition may facilitate prodrug design that targets hASBT. Figure 1 illustrates the general structure of bile acids in humans. R1, R2, and R3 indicate hydroxyl groups while R4 represents a free carboxylic acid (unconjugated bile acids) or glycine or taurine substituent (conjugated bile acids). Table 1 describes 15 native bile acids and the structural differences among these primary and secondary bile acids.

Cholate and chenodeoxycholate, the primary bile acids in humans, are synthesized in hepatocytes from cholesterol. Secondary bile acids are formed via bacterial metabolism in the small intestine. Conjugation of both primary and secondary bile acids occurs in the hepatocyte prior to excretion from the hepatocyte. In humans, cholate and chenodeoxycholate are the primary bile acids. Secondary bile acids are derived from cholate and chenodeoxycholate via bacterial 7-dehydroxylation or 7-epimerization. For example, deoxycholate is derived via 7-dehydroxylation of cholate, while ursodeoxycholate is formed via the epimerization of the 7-α hydroxyl group of chenodeoxycholate.

The principal bile acids in human bile are mainly conjugated cholic acid and chenodeoxycholic acid.[16] Very

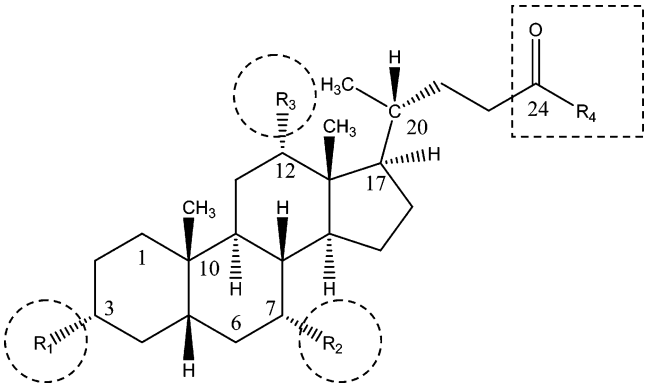


***Figure 1.*** Structure of native bile acids. Bile acids differ in hydroxylation pattern (R1, R2, and R3, indicated by circles) and in amino acid conjugation pattern at the C-24 position (R4, indicated by square). The substituent at R-4 is either a free carboxylic acid or a conjugate of glycine or taurine. The steroidal hydroxyl groups and the C-24 carboxylate represent convenient sites where drugs can be conjugated directly or indirectly via a linker.

***Table 1.*** Bile Acid Nomenclature[a]

| bile acid | $R_2$ (C-7) | $R_3$ (C-12) | $R_4$ (C-24) |
|---|---|---|---|
| Primary Bile Acids | | | |
| cholate | OH | OH | OH |
| glycocholate | | | $NHCH_2COOH$ |
| taurocholate | | | $NH(CH_2)_2SO_3H$ |
| chenodeoxycholate | OH | H | OH |
| glycochenodeoxycholate | | | $NHCH_2COOH$ |
| taurochenodeoxycholate | | | $NH(CH_2)_2SO_3H$ |
| Secondary Bile Acids | | | |
| deoxycholate | H | OH | OH |
| glycodeoxycholate | | | $NHCH_2COOH$ |
| taurodeoxycholate | | | $NH(CH_2)_2SO_3H$ |
| lithocholate | H | H | OH |
| glycolithocholate | | | $NHCH_2COOH$ |
| taurolithocholate | | | $NH(CH_2)_2SO_3H$ |
| ursodeoxycholate | OH (β) | H | OH |
| glycoursodeoxycholate | | | $NHCH_2COOH$ |
| tauroursodeoxycholate | | | $NH(CH_2)_2SO_3H$ |

[a] Primary bile acids are formed in the hepatocyte. Secondary bile acids are derived from primary bile acids. All hydroxyl (OH) groups are in the α-position, except when noted to be in the β-position. Positions $R_2$, $R_3$, and $R_4$ are illustrated in Figure 1.

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small amounts of deoxycholate and ursodeoxycholate conjugates, and trace lithocholate conjugates, are also present. Conjugation increases bile acid polarity and lowers passive transport, such that bile acid reabsorption is controlled by intestinal bile acid transporters. This situation facilitates high intraluminal bile acid concentration to aid lipid absorption. Conjugation also improves bile acid solubility, providing resistance to precipitation in the presence of high calcium ion concentration in the gall bladder.[17]

The enterohepatic recirculation of bile acids is a complex process and involves numerous transport proteins, including the sodium-dependent hepatocyte bile salt uptake system NTCP (SLC10A1); hepatocellular bile salt export pump (BSEP, ABCB11); the apical sodium-dependent bile salt transporter (ASBT, SLC10A2); the organic anion transporting polypeptides OATP-C (SLC21A6), OATP8 (SLC21A8), and OATP-A (SLC21A3); and the multidrug resistance protein MRP3 (ABCC3). ASBT and NTCP are each members of the SLC10 family of solute carrier proteins and require sodium cotransport for their activity. ASBT is expressed on the apical membrane of enterocytes in the terminal ileum and mediates the reabsorption of bile acids from the ileum. In addition to ASBT, additional anion exchange mechanisms operate in the jejunum to reabsorb bile acids.[18] NTCP is expressed in hepatocytes and localized on the basolateral (sinusoidal) domain of hepatocytes to reabsorb bile acids from the portal circulation. ASBT and NTCP serve to transport bile acids from the small intestine into portal circulation and from the portal circulation into the hepatocyte. The bile acid pool in humans is about 3−5 g, resulting in a turnover of 12−18 g of bile acid each day.[16] In spite of this repeated cycling, the loss of bile in the feces is less than 0.5 g per day, reflecting the tremendous capacity and efficiency of hASBT.[19]

Upon their translocation into the enterocytic cytoplasm by hASBT, bile acids bind to the 14 kDa soluble cytoplasmic protein named the ileal bile acid binding protein (iBABP); iBABP is also known as gastrotropin.[20,21] iBABP shuttles bile acids across the cytosol to the basolateral membrane.[19] It has been suggested that iBABP specifically interacts with hASBT, further supporting its role in bile acid transport.[22] Regarding the transport of bile acids across the basolateral membrane of enterocytes, Dawson et al. reported that the heteromeric organic solute transporter Ost$\alpha$−Ost$\beta$ is an ileal basolateral bile acid transporter.[23] Based on expression and transport properties of the transporter complex in various tissues and using hASBT cotransfected cells, Ost$\alpha$−Ost$\beta$ may be the major mechanism for ileal basolateral bile acid transport.[23] Other possible mechanisms across the basolateral membrane include a truncated version of ASBT referred to as t-Asbt[24] and the multidrug resistance-associated protein 3 (MRP3, ABCC3).[19] The role of t-ASBT in basolateral transport was observed only in rats. Meanwhile, the importance of MRP3 is also controversial with conflicting reports about the contribution of MRP3.[19,25]

## Biology of ASBT

ASBT was first cloned in the laboratory of Paul Dawson (Wake Forest University) from hamster ileum; subsequently it has been cloned from rat, mouse, rabbits, and humans.[26−30] ASBT consists of 348 amino acids (347 in rabbits) with an observed molecular weight of 43 kDa, which differs from a predicted molecular weight of 38 kDa due to glycosylation.[28]


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The ASBT gene is localized on chromosome 13q33. It exhibits 35% identity and 63% amino acid sequence similarity with its liver orthologue NTCP.[31] The transporter is electrogenic, coupled with sodium in a 2:1 sodium:bile acid stoichiometry. hASBT presumably functions as a monomer, although some evidence suggests the existence of a dimer as well.[32] hASBT is expressed at high levels in the terminal ileum, renal proximal tubules, and biliary epithelium.[33,34] Inherited mutations in hASBT result in primary bile acid malabsorption syndrome, suggesting that hASBT is the primary mechanism for intestinal reabsorption of bile acids.[28,35]

Structural information on hASBT has been restricted to its primary sequence and membrane topology. While precise information about the substrate binding domains of hASBT are not available yet, its structural and functional determinants have been studied using various biophysical methods.[36−40] These studies have indicated that hASBT exhibits a seven transmembrane (7 TM) topology similar to that of NTCP. Biochemical studies using photo affinity labeling and enzymatic digestion indicate that the substrate-binding domain of hASBT is localized to the seventh transmembrane domain and the C-terminus at 56−67 amino acids.[36,40,41] Further, Zhang et al. have suggested the existence of four distinct binding sites based on a three-dimensional structure of hASBT developed in silico using homology modeling.[37]

## In Vitro Models for Functional Assessment of hASBT-Mediated Transport

The comprehensive characterization of carrier-mediated transport of solutes requires assay methodologies that delineate the contribution of various pathways, including the role of an individual transporter. An additional practical consideration is the throughput capacity of the developed assay. High throughput absorption screens developed for early ADMET screening can be adapted for the rational design and evaluation of prodrugs to target transporters. An example is a cell culture model to study PepT1-mediated transport of peptides and peptide-based drugs.

In the development of cell-based transporter models, two approaches have been utilized. The first approach involves the use of primary or native cell systems that express the transporter of interest. For example, the human colon adenocarcinoma Caco-2 cell line endogenously expresses peptide transporters and has been utilized to evaluate prodrugs aimed at various peptide transporters.[42] A potential limitation of such an approach is the relatively low transporter expression level, along with the expression of other confounding transporters.[43] The second approach involves the use of transiently or stably transfected cell lines to overexpress the transporter. Transfection generally offers high transporter expression, which increases the dynamic range of the assay by limiting influence of passive flux.[44,45]

There is a paucity of cell-based transport assays for hASBT, compared to the several assay models for other, more widely studied transporters, such as Pept1.[43] The development of cell-based assay systems for peptide transporters has aided in the understanding of the substrate requirements of peptide transporters. Such assays have also led to the development of predictive three-dimensional QSAR

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models to rationalize the interaction of substrates and nonsubstrates with the transporter.[46,47] Previously, in vitro cell-based assays for ASBT typically employed nonpolarized cells, such as transiently transfected COS7 cells and stably transfected CHO cells.[33] These models allow for uptake assessment, but are not competent monolayers and do not allow for transepithelial transport. Caco-2 cells have also been used for assessment of hASBT function, but the level of expression is low and not consistent.[48,49] Sun et al. have previously developed a stably transfected hASBT-MDCK model for the study of sorting of hASBT to the apical membrane.[50] However, this model exhibited poor monolayer integrity. Doubly transfected systems with rat Asbt and a basolateral transporter have also been attempted.[25] We have recently developed a stably transfected hASBT-MDCK monolayer assay with suitable monolayer integrity.[51] Such a monolayer model has been beneficial in allowing the measurement of prodrug transport, including quantification of intact prodrug in the receiver compartment. Additionally, the analytical requirements for a monolayer assay are generally less complex since the sample matrix is a protein free buffer compared to cell lysate in case of uptake.

## Bile Acid Derivatives as "Trojan Horses" for Delivery of Therapeutics

Transporter specificity, affinity, and capacity are three critical factors in selecting a transporter for prodrug targeting. Favorable attributes include broad substrate specificity and high affinity for efficient absorption. Transport capacity, dictated both by expression level and by intrinsic transporter capacity or turnover, also impacts extent of absorption enhancement. hASBT is saturable and exhibits high capacity and high affinity for native bile acids, suggesting hASBT to be a potential prodrug target.[51,52]

Interestingly, hASBT has received less emphasis than NTCP, in terms of targeting, perhaps reflecting the importance of the liver as a therapeutic target.[53] NTCP appears to exhibit a broader substrate specificity than hASBT, including the ability to transport a wide range of cholephillic compounds and various drugs.[29] NTCP has been evaluated in previous studies for targeted delivery of therapeutics to the liver.[54] Examples include the targeted delivery of antisense nucleotides, cytostatic drugs, and HMG CoA reductase inhibitors.[54−56] The compound of interest was usually coupled to a native bile acid that is recognized by the transporter. Figure 2 illustrates the structure of potential bile acid derivatives, including sites of drug attachment or integration with bile acid. Drugs can be attached in the C-3 region of the steroidal ring or through the C-24 carboxylate. Additionally, the C-17 region has also been exploited for integrating drug entities.

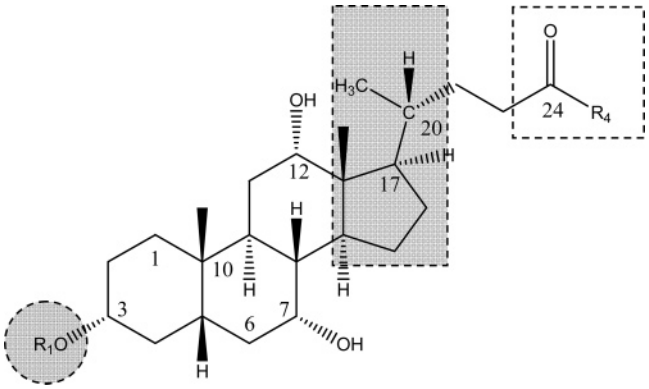


***Figure 2.*** Structure of potential bile acid derivatives. Drugs may be attached/integrated at the C-3 region (shaded circle) or the C-17 region (shaded rectangle) or via the C-24 carboxylate (dashed square). Table 2 lists examples for each of these scenarios.

Compared to NTCP, few studies have targeted ASBT to enhance systemic drug bioavailability. To enhance the transport of renin inhibitory heptapeptide ditekiren, the parent compound was conjugated to either cholic acid or taurocholic acid via a spacer. Although conjugates exhibited hASBT inhibition, they were not transported by hASBT.[57] Similarly, the absorption of the C-24 dipeptide bile acid conjugate cholylglycyltyrosine was low compared to that of taurocholic

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***Table 2.*** Examples of Attempts To Enhance Disposition via Prodrug Derivatives

| position | compound | bile acid | nature of cargo | active transport | ref |
|---|---|---|---|---|---|
| C-3 | ditekiren | cholic acid | peptide | no | 57 |
| | ditekiren | taurocholic acid | peptide | no | 57 |
| | chlorambucil | cholic acid | small molecule | yes[a] | 55, 71 |
| | HR 780 | cholic acid | small molecule | yes[a] | 72 |
| | antisense oligonucleotides | cholic acid | oligonucleotide | no | 56, 73 |
| | naproxen | pyrazole fused cholic acid | small molecules | yes[b] | 60 |
| C-17 | HMG CoA reductase inhibitor | modified cholic acid | hybrid molecule | | 72, 74 |
| C-24 | acyclovir | cholic acid | small molecule | yes | 61 |
| | peptides | cholic acid | peptides | no | 59 |

[a] Assessed through enhanced biliary excretion. [b] Indirect assessment via ion current measurement.

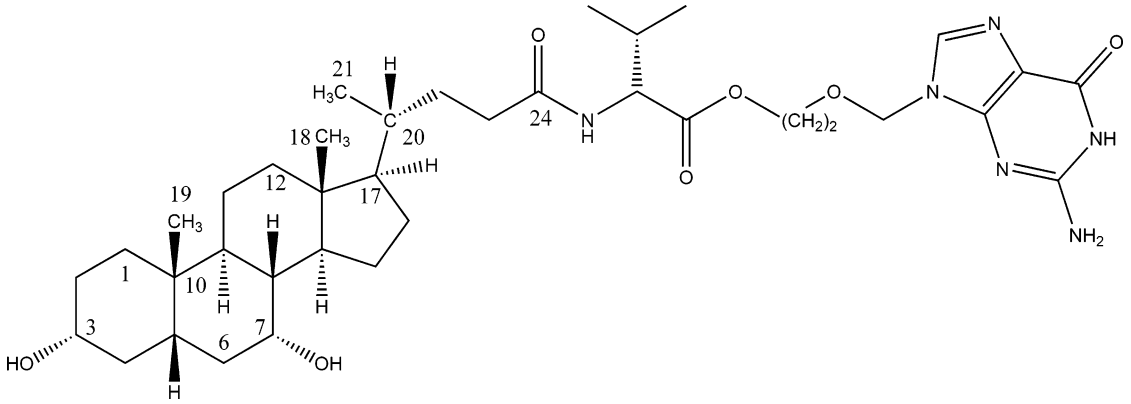


***Figure 3.*** Structure of acyclovir valylchenodeoxycholate. Acyclovir was conjugated to the native bile acid chenodeoxycholate via a valine linker. This prodrug led to increased oral absorption, compared to that of parent molecule administration.

acid.[58] Swaan et al. evaluated the transport of peptides of varying length that were conjugated at the 24-position of cholic acid via an amide linkage.[59] While conjugation of peptides to bile acids did not directly enhance absorption via hASBT-mediated transport, it led to lower metabolic lability.[54,59] Bhat et al. recently evaluated the potential of using steroidal pyrazoles as drug carriers for hASBT.[60] Pyrazole rings were fused to the bile acid skeleton at the C2:C3 carbons, and drug entities were subsequently attached via the pyrazole ring. In general, steroidal pyrazoles exhibited good binding affinity for NTCP, but lower binding affinity for hASBT, suggesting that these modified bile acid analogues are recognized by the bile acid transporters. However, they exhibited poor substrate affinity for hASBT.

Currently, there are very few examples exemplifying enhanced systemic bioavailability of poorly absorbed drugs via prodrug targeting hASBT. Table 2 lists examples where drug has been conjugated to native bile acids to modulate disposition. The only example to our knowledge wherein an unambiguous enhancement in oral bioavailability was attained via prodrug that targeted ASBT is acyclovir valylchenodeoxycholate,[61] which is illustrated in Figure 3. Acyclovir valylchenodeoxycholate is a conjugate of valacyclovir and chenodeoxycholate. Acyclovir is an antiviral drug with an oral bioavailability of only 20% due to low acyclovir intestinal permeability.[62] Acyclovir valylchenodeoxycholate was designed to assess potential for hASBT targeting as against PepT1 targeting. Valacyclovir (L-valine ester prodrug of acyclovir) is a prodrug of acyclovir that has improved the oral bioavailability of acyclovir with human oral bioavailability of 54%.[14,63] Valacyclovir is a substrate for the PepT1 intestinal transporter, with $K_i = 4.08$ mM in PepT1 expressing *Xenopus laevis* oocytes[64] and $K_i = 1.10$ mM in stable lines of CHO/PepT1.[13] Compared to PepT1,

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hASBT has a more favorable micromolar affinity profile.[51,61] Acyclovir valylchenodeoxycholate possesses a favorable affinity for hASBT ($K_i$ = 36 $\mu$M), which was comparable to even the native bile acid cholic acid ($K_i$ = 25 $\mu$M). In rats, acyclovir valylchenodeoxycholate increased acyclovir oral bioavailability 2-fold, based on urinary excretion of acyclovir after oral administration of the acyclovir conjugate versus oral administration of acyclovir. While a 2-fold enhancement in acyclovir bioavailability from 25% to 48% in rats is notable and while hASBT appears to possess better affinity for substrates than does PepT1, the PepT1 prodrug valacyclovir increases acyclovir bioavailability 3-fold to 54% in humans.[65] A possible explanation for the only 2-fold increase in oral acyclovir bioavailability by acyclovir valylchenodeoxycholate, in spite of the prodrug's more favorable in vitro uptake properties, is prodrug hydrolysis in the stomach and proximal intestine. ASBT is located in the distal segment of the small intestine (i.e., ileum), such that a bile acid conjugate must exhibit a degree of hydrolytic stability in order to reach ASBT intact. It should be noted that, in spite of these promising ASBT results, our current understanding of hASBT substrate requirements is limited.

## Structure−Transport Requirements for hASBT

In the absence of a high-resolution crystal structure for hASBT, little is known about the interaction of hASBT with its substrates. There is a general lack of information about the spatial requirements for substrate binding to and transport by hASBT.[40,41,66] Most studies were conducted using ex vivo tissue or organ preparations, or uptake studies using nonpolarized cell culture models.[29,33,67]

Lack and Weiner were the first to describe a basic structure−transport relationship for the intestinal bile acid transport using the rat everted sac model.[67] The Lack model suggests the following generalizations:[68]

(a) Trihydroxy bile acids are better transported than dihydroxy bile acids. No one hydroxyl position on the steroid ring is necessary. Among themselves, the dihydroxy bile acids are equally well transported.

(b) A single negative charge on the side chain is apparently required.

(c) For inhibition, there is an inverse relationship between number of steroid hydroxyl groups and inhibitor affinity.

Lack's model reflects one of the very few translocation-based models to describe the substrate requirements of hASBT. However, the assay system utilized tissue and organ preparations from animals, which do not delineate the contribution of hASBT from other confounding factors, including other transporters, passive transport, and system hydrodynamics.

Recent studies have employed brush border vesicles and cell-based transport assays, which directly measure hASBT interaction with substrate. However, the majority of studies were inhibition studies, reflecting a focus to develop non-absorbable hASBT inhibitors as a means of lowering plasma cholesterol. Kramer et al. described a 3-D pharmacophore model for ASBT with five chemical features: one hydrogen bond donor, one hydrogen bond acceptor, and three hydrophobic features.[69] They suggested the following structure−activity relationship for the native bile acids, which differed from the Lack model:[29]

(a) Two hydroxy groups at position 3, 7, or 12 are optimal whereas the presence of three hydroxy groups decreased affinity.

(b) Vicinal hydroxy groups at positions 6 and 7 or a shift of the 7-hydroxy group to the 6-position significantly decreased the affinity.

(c) One hydroxy group in either position 6 or 7 mediates affinity to the hASBT whereas the concomitant presence of two hydroxyl groups at positions 6 and 7 significantly diminishes the affinity to ASBT.

Dawson and colleagues employed hASBT-COS cells to characterize the transport characteristic of several native bile acids.[33] They reported that glyco-dihydroxy bile acids exhibit the greatest affinity for hASBT, which seems to contradict Lack's claim that trihydroxy bile acids are best transported. Using Caco-2 monolayers, Swaan et al. examined the transport of C-24 bile acid−peptide conjugates. Each provided a negative charge near C-24. They concluded that the C-24 side chain could be at least 14 Å in length to allow for translocation, and that large hydrophobic moieties increase binding to hASBT.[66] They also observed cholic acid to have greater affinity (i.e., lower $K_t$) than taurocholate, which is in contrast to previous studies.[70] In our previous work, prodrugs of acyclovir linked to bile acids via a valine linker were found to have high inhibition potency comparable to that of native bile acids. A negative charge at C-24 was not necessary for the transport of these conjugates.[61]

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Lack's generalizations about hASBT substrates have been found to be debatable, and current studies have provided data that do not support the model. Interstudy comparisons indicate that a reliable relation between bile acid structure and transport parameters (e.g., $J_{max}$ and $K_t$) has not been elucidated, much less Lack's first generalization, concerning trihydroxy bile acids being better transported than dihydroxy bile acids. $K_t$ values vary greatly between studies. We believe that this situation reflects a lack of a comprehensive study of bile acid transport using a method that excludes confounding factors, such as other transporters.

The cloning of hASBT provides an opportunity to develop a selective and sensitive assay system to delineate hASBT substrate requirements. hASBT was stably transfected into MDCK to yield a competent, high-expression, stable assay for hASBT transport and inhibition studies.[51] hASBT-mediated taurocholate permeability across hASBT-MDCK monolayers was almost 25-fold higher with sodium than without sodium where hASBT is not functional. In the presence of sodium, taurocholate and mannitol, permeabilities were $23.0 \times 10^{-6}$ cm/s and $2.60 \times 10^{-6}$ cm/s, respectively, indicating high hASBT functionality and monolayer integrity. Permeability values demonstrated low within day variability. Taurocholate uptake and inhibition kinetic parameters from hASBT-MDCK were similar to those obtained from the hASBT-COS7 model, confirming hASBT functionality in hASBT-MDCK.

This model was used for the systematic elucidation of hASBT-transport and hASBT-inhibition profiles of native bile acids in the absence of any confounding effects.[52] Monohydroxy, dihydroxy, and trihydroxy bile acids were evaluated along with their glycine and taurine conjugates. Observations from this study include the following:

(a) Glycine or taurine conjugation at C-24 enhanced the inhibitory potency of bile acids (i.e., $K_i$). This trend was consistent across all bile acids, including monohydroxy, dihydroxy, and trihydroxy bile acids.

(b) An inverse relationship was observed between number of steroidal hydroxyl groups and inhibitory potency, with monohydroxy bile acids being the most potent inhibitors. Chenodeoxycholate exhibited greater inhibition potency than ursodeoxycholate, suggesting that C-7 α-OH is more favorable than C-7 $\beta$-OH.

(c) Results from transport studies mirrored the trends from inhibition studies. Steroidal hydroxylation had a significant effect on transport affinity (i.e., $K_t$). Fewer hydroxyl groups promoted transport affinity toward hASBT.

## Future Efforts

Future efforts should focus on coupling functional data and biophysical data to promote understanding the differences in transport affinity and inhibition potency of substrates and inhibitors, based upon specific interactions between the substrate and amino acid residues in the transport protein. Studies to date with native bile acids generally indicated that C-24 conjugation enhances hASBT substrate affinity. Future studies will benefit from a better insight into the structural features of the targeting moiety (i.e., bile acid) that favor interaction with hASBT and hold promise for the development of C-24 conjugated prodrugs. Since native conjugated bile acids exhibit a very narrow chemistry space around the C-24 region, the influence of diverse C-24 chemistry space in enhancing or impeding bile acid interaction with hASBT remains a focus of future research. These studies should yield a better understanding of hASBT substrate requirements with application in the design of prodrugs to target hASBT.

## Abbreviations Used

hASBT, human apical sodium-dependent bile acid transporter; SLC, solute carrier family; MDCK, Madin-Darby canine kidney; HBSS, Hanks balanced salt solution; ABL, aqueous boundary layer; TCA, taurocholic acid; CDCA, chenodeoxycholic acid.

**Acknowledgment.** This work was supported by National Institutes of Health Grant DK67530.

MP060022D

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