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Interaction of Native Bile Acids with Human Apical Sodium Dependent Bile Acid Transporter (hASBT): Influence of Steroidal Hydroxylation Pattern and C-24 Conjugation

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Abstract

Purpose—The human Apical Sodium-dependent Bile Acid Transporter (hASBT) is a potential target for drug delivery, but an understanding of hASBT substrate requirements is lacking. The objective of this study was to characterize hASBT interaction with its native substrates, bile acids, including an evaluation of C-24 conjugation and steroidal hydroxylation on transport affinity and inhibition potency.

Methods—Transport and inhibition kinetics of 15 bile acids were evaluated (cholate, chenodeoxycholate, deoxycholate, ursodeoxycholate, and lithocholate, including their glycine and taurine conjugates) using an hASBT-Madin-Darby canine kidney (MDCK) monolayer assay. Samples were analyzed via LC-MS or LC-MS-MS.

Results—C-24 conjugation improved the inhibitory potency of all native bile acids. There was an inverse association between number of steroidal hydroxyl groups and inhibitory potency. Glycolithocholate and tauroolithocholate were the most potent inhibitors. Results from transport studies followed trends from inhibition studies. Conjugated dihydroxy and monohydroxy bile acids exhibited the highest hASBT-mediated transport (i.e. lower K_t and higher J_{max}). Across the 15 bile acids, K_t generally followed K_i. Additionally, J_{max} correlated with K_i, where greater inhibition potency was associated with higher transport capacity.

Conclusion—C-24 conjugation and steroidal hydroxylation pattern modulated native bile acid interaction with hASBT, with C-24 effect dominating steroidal hydroxylation effect. Results indicate that bile acid binding to hASBT may be the rate limiting step in the apical transport of bile acids.

INTRODUCTION

The human ileal bile acid transporter, also known as hASBT (SLC10A2), is a key component in the enterohepatic recirculation of bile acids, as well as a target for enhanced oral drug absorption (1,2). Structural information on hASBT has been restricted to its primary sequence and membrane topology (3). In the absence of a high resolution crystal structure for hASBT, little is known about the interaction of hASBT with its substrates. As a result, the substrate requirements for hASBT are not well developed.

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Lack and Weiner first described a structure-transport relationship for intestinal bile acid transport using the rat everted sac model (4). These and subsequent observations were based on isolated tissue and organs preparations from animals, where bile acid transport was confounded with other intestinal and hepatic transporters. Evaluation of the literature shows lack of agreement across studies from different research groups regarding hASBT substrate requirements, which appears to reflect the lack of a preferred assay system (5-7). Additionally, native bile acids lack a chromophore, yielding analytical challenges (e.g. lack of sensitivity).

The general lack of information about hASBT substrate requirements has impeded efforts towards rational prodrug design targeting hASBT (8). Toward this end, a stably transfected hASBT-MDCK monolayer assay was recently developed to evaluate hASBT-mediated transport of solutes (9). Additionally, methods have been developed to avoid bias in kinetic estimates (e.g. K_i , K_t) due to system hydrodynamics (10). These methods are applied here to characterize hASBT interaction with its native substrates, bile acids, including an evaluation of C-24 conjugation and steroidal hydroxylation pattern on transport affinity and inhibition potency.

Native bile acids are the physiological substrates for hASBT and therefore represent an important set of compounds to probe hASBT substrate requirements. Native bile acids vary in steroidal hydroxylation pattern and C-24 conjugation pattern. Figure 1 illustrates the general structure of native bile acids and highlights R1, R2, R3, and R4. Table I describes 15 native bile acids and their differences in hydroxylation (R1, R2, and R3) and C-24 conjugation (R4).

The overall goal of this study was to systematically evaluate the interaction of native bile acids with hASBT and identify chemical features of bile acids that promote interaction with hASBT. Three major objectives are pursued in this study. The first objective was to evaluate effect of steroidal hydroxylation pattern and C-24 conjugation on the inhibition potency of native bile acids (i.e. K_i). The second objective investigated the effect of steroidal hydroxylation and C-24 conjugation on hASBT-mediated transport kinetics (i.e. K_t and J_{max}). Bile acid structure requirements for hASBT inhibition may differ from requirements for hASBT-mediated transport. Consequently, the third objective was to compare hASBT-inhibition potency versus kinetic parameters of hASBT-mediated transport.

METHODS

Materials

Geneticin, fetal bovine serum, trypsin, and DMEM were purchased from Invitrogen Corporation (Carlsbad, CA). [^3H]-taurocholic acid, [^3H]-cholic acid, [^{14}C]-chenodeoxycholic acid, [^{14}C]-lithocholic acid and [^{14}C]-mannitol were purchased from American Radiolabeled Chemicals (St. Louis, MO). Glycolithocholic acid, Glycoursodeoxycholic acid and tauroursodeoxycholic acid were obtained from Calbiochem (San Diego, CA). All other chemicals were obtained from Sigma Chemical (St. Louis, MO) or TCI America (Portland, OR).

Cell Culture

Stably transfected hASBT-MDCK cells were cultured as described previously (9). Briefly, cells were grown at 37°C, 90% relative humidity, and 5% CO_2 atmosphere, and fed every 2 days. Media comprised DMEM supplemented with 10% FBS, 50 U/mL penicillin, and 50 $\mu\text{g}/\text{mL}$ streptomycin. Geneticin was used at 1 mg/mL to maintain selection pressure. Cells were passaged every 4 days or after reaching 90% confluence.

Transport across hASBT Monolayers

Transport studies were performed to yield kinetic data that relates to the binding and subsequent translocation across the membrane. hASBT-MDCK cells were grown on polyester Transwells, as described earlier (9). Briefly, cells were seeded at a density of 0.75 million cells/cm² on polyester Transwells (Corning; Corning, NY, 0.4 μ pore size, 1cm²) and grown under conditions described above. Cells were washed thrice with HBSS or modified HBSS prior to transport study and studies were carried out at 37°C at 100 rpm using an orbital shaker. Transport buffer consisted of either Hank's Balanced Salts Solution (HBSS) which contains 137 mM NaCl or a sodium-free, modified HBSS where sodium chloride was replaced with 137 mM tetraethylammonium chloride. Since bile acid transport is sodium-dependent, studies using sodium-free buffer allowed for the measurement of passive transport of bile acids.

Kinetics of hASBT-mediated transport was assessed from transport studies conducted at different donor concentrations of each native bile acid. In all studies, [¹⁴C]-mannitol was used to monitor monolayer integrity in parallel wells. Mannitol permeability was always less than 2.5×10^{-6} cm/sec in each individual well. The apical and basolateral volumes were 0.5 ml and 1.5 ml, respectively. Samples were quantified using LC-MS or LC-MS-MS and liquid scintillation counter as described later.

To confirm the absence of OATP-mediated transport across hASBT-MDCK cells, taurocholate transport was measured in the absence and presence of the OATP substrate bromosulphalein (BSP). No differences were observed in taurocholate transport in the presence and absence of 100 μ M BSP (data not shown).

Inhibition Studies

hASBT-MDCK cells were seeded at a density of 1.5 million cells/well in 12 well plates (Corning; Corning, NY) and induced with 10 mM sodium butyrate for 12-15 h at 37°C prior to study on day 4. Kinetics of hASBT-mediated taurocholate uptake was assessed from uptake studies (n = 3) at differing donor concentrations (1-200 μ M spiked with [³H]-taurocholate) (9). Studies were performed using HBSS with 10mM HEPES (pH 6.8) at 37°C and 50 rpm for 10 min. The donor solution was removed and the cells were washed three times with chilled sodium free buffer. Cells were lysed using 0.25 ml of 1 N NaOH and neutralized with 0.25 ml of 1 N HCl. Cell lysate was then counted for associated radioactivity using a liquid scintillation counter (Beckman Instruments; Fullerton, CA).

To characterize hASBT binding requirements, cis-inhibition studies of taurocholate uptake were carried out using individual native bile acids at various concentrations (0-100 μ M). Cells were exposed to donor solution containing taurocholate and inhibitor for 10 min. The donor solution was removed and the cells were washed three times with chilled sodium free buffer. Cells were lysed and lysate counted for associated radioactivity, as described above. Inhibition data were analyzed in terms of inhibition constant K_i , as described below.

Analytical Methods

Bile acids were analyzed by means of LC-MS or LC-MS-MS on an Agilent HPLC system, equipped with an autosampler (CTC Analytics, Switzerland) and Applied Biosystems Sciex API4000-Qtrap mass spectrometer. The column was a Phenomenex Luna C18 50 $\times$ 2 mm 3 μ , heated to 40°C. Flow rate was 0.7 mL/min, and mobile phase contained a gradient of 1–80% acetonitrile in ammonium acetate (pH=6.8). Detection was achieved under negative or positive ion electrospray tandem mass spectrometry using [M-H]⁻ peak for all the native bile acids, as [M-H]⁻ peak provided the greatest sensitivity. Radiolabeled bile acids and mannitol were quantified using a Beckman liquid scintillation counter model LS 5801 (Beckman Instruments; Fullerton, CA), in Econosafe scintillation cocktail.

Kinetic Analysis

Flux data was analyzed as described earlier (10). Briefly, flux data was fitted to a previously developed modified Michaelis-Menten model that takes into account aqueous boundary layer resistance (eqn 1). Data from sodium-free studies were fitted to a passive transport model (eqn 2).

$$J = \frac{P_{ABL} \cdot \left(\frac{J_{\max}}{K_t + S} + P_p \right) \cdot S}{P_{ABL} + \frac{J_{\max}}{K_t + S} + P_p} \quad (1)$$

$$J = \frac{P_{ABL} \cdot P_p \cdot S}{P_{ABL} + P_p} \quad (2)$$

where J is taurocholate flux, J_{max} and K_t are the Michaelis-Menten constants for hASBT-mediated transport, S is taurocholate (i.e. substrate) concentration, P_p is the passive taurocholate permeability coefficient, and P_{ABL} is the aqueous boundary layer (ABL) permeability. In eqn 1 and 2, P_{ABL} was 70 × 10⁻⁶ cm/sec (10). Equations 1 and 2 were applied simultaneously to with sodium and sodium-free flux data to estimate K_t, J_{max}, and P_p. A pooled data analysis approach was used to model flux data from multiple studies. The pooled approach entailed fitting eqn 1 and 2 to flux data pooled from multiple experiments, to yield a single K_t estimate across multiple studies; J_{max} and P_p were unique for each study.

Taurocholate flux was measured in each study to serve as a normalizing approach to account for variation in hASBT expression levels between studies. Taurocholate J_{max} ranged between 0.000065 to 0.001 nmoles/sec/cm² across studies. hASBT expression levels can be expected to directly affect J_{max} estimates. To correct for variation in hASBT expression levels across studies, J_{max} of each bile acid was normalized against taurocholate J_{max} from the same study, yielding a normalized J_{max} for the bile acid of interest. Normalized J_{max} that exceed a value of one indicate J_{max} exceeded the J_{max} of taurocholate. Normalized J_{max} less than one indicate that J_{max} was lower than taurocholate J_{max}.

The following competitive inhibition model was applied to cis inhibition studies of taurocholate uptake by individual bile acids:

$$J_{ABL} = \frac{P_{ABL} \cdot \left(\frac{J_{\max}}{K_t \left(1 + \frac{I}{K_i} \right) + S} + P_p \right) \cdot S}{P_{ABL} + \left(\frac{J_{\max}}{K_t \left(1 + \frac{I}{K_i} \right) + S} \right) + P_p} \quad (3)$$

where I is the concentration of inhibitor (i.e. inhibitory bile acid) and S is the concentration of taurocholate (i.e. 0.5 or 5 μM). In applying eqn 3, only K_i was estimated. The other three parameters (i.e. J_{max}, K_t, and P_p) were estimated from taurocholate uptake studies without inhibitor using eqn 1 and 2.

Statistical Analysis

Non-linear curve fitting was performed using WinNonlin 4.1 (Pharsight, Mountain View, CA). Results were analyzed using Student's *t*-test and ANOVA. A *p*-value of less than 0.05 was considered significant. SEM of a ratio was calculated using the delta method (11).

RESULTS

Inhibition Studies

Each bile acid inhibited taurocholate uptake into hASBT-MDCK in a concentration dependent fashion. For example, Fig. 2 illustrates the inhibition profile of tauroursodeoxycholate (TUDCA). Similar profiles were observed for all other native bile acids. Glycine and taurine conjugates of lithocholate exhibited the greatest inhibitory potency with *K_i* estimates of about 0.5 μ M. Ursodeoxycholate and cholate were the least potent compounds with *K_i* estimates of approximately 20 μ M.

Table II lists the *K_i* values for the unconjugated, glycine conjugate, and taurine conjugate of five parent bile acids. These five parent bile acids differ in steroidal hydroxylation pattern, although each possesses a C-3 α -OH. Cholate is a trihydroxy bile acid. Ursodeoxycholate, deoxycholate, and chenodeoxycholate are dihydroxy bile acids. Lithocholate is a monohydroxy bile acid.

For each parent bile acid, glycine or taurine conjugation at C-24 enhanced inhibition potency (i.e. lowered *K_i*). For cholate, glycine and taurine conjugation resulted in a two-fold and three-fold enhancement in inhibitory potency, respectively. Similarly, for the dihydroxy bile acids chenodeoxycholate and deoxycholate, glycine and taurine conjugates enhanced potency two-fold. Ursodeoxycholate exhibited the greatest sensitivity to conjugation, with glycine and taurine conjugates providing 7-fold and 22-fold enhancement in inhibition potency, respectively. Conjugates of lithocholate exhibited 4-fold greater inhibition potency than unconjugated lithocholate. In general, glycine and taurine enhanced inhibitory potency of parent bile acids to similar extents.

Inhibitory potency generally exhibited an inverse relationship with number of hydroxyl groups, with the dihydroxy and monohydroxy bile acids exhibiting greater potency than trihydroxy bile acids. Across unconjugated bile acids, chenodeoxycholate and lithocholate exhibited the greatest inhibitory potency with *K_i* value of 2 μ M. Cholate and ursodeoxycholate exhibited the lowest inhibitory potency with *K_i* values of 17 and 22 μ M, respectively. The dihydroxy bile acids deoxycholate, chenodeoxycholate, and ursodeoxycholate differed with respect to the location and orientation of the second hydroxyl group (Table I). Among these three dihydroxy bile acids, chenodeoxycholate exhibited the greatest potency (*K_i* = 2 μ M). Its C-7 β -OH epimer, ursodeoxycholate, yielded a *K_i* of 22 μ M. Deoxycholate exhibited a modestly lower potency (*K_i* = 8 μ M) and possesses the second hydroxyl group at C-12. Steroidal hydroxylation pattern also influenced C-24 conjugated bile acids and mirrored the effect observed in unconjugated bile acids. Among conjugates, monohydroxy bile acids exhibited the greatest potency, followed by dihydroxy and trihydroxy bile acids. Glycine and taurine conjugates of lithocholate exhibited the greatest potency with *K_i* estimates of about 0.5 μ M. The dihydroxy bile acid conjugates generally exhibited potency that were within about 3-fold of each other (i.e. between 1 and 3 μ M). In general, steroidal hydroxylation effects in conjugated bile acids were less prominent compared to their effects in unconjugated bile acids. This pattern indicates that C-24 conjugation dominated hydroxylation effects in determining inhibitory potency. This pattern is most evident for ursodeoxycholate (*K_i* = 22 μ M), where taurine conjugation enhances potency 22-fold (*K_i* = 0.82 μ M).

Transport Studies

Each of the 15 native bile acids can be expected to exhibit hASBT-mediated transport. For example, Fig. 3 illustrates the concentration dependent transport of glycodeoxycholate across hASBT-MDCK monolayers. Glycodeoxycholate transport was mediated largely by hASBT, with an approximately 20-fold greater flux from sodium-containing studies than from sodium-free studies. Similar profiles were obtained for all conjugated bile acids and cholate, such that 11 of the 15 bile acids exhibited significant hASBT-mediated transport. The unconjugated forms of the three dihydroxy bile acids and the monohydroxy bile acid lithocholate did not show apparent hASBT-mediated transport, perhaps due to their high passive permeability (12).

Table III lists the transport parameters of five parent bile acids and their glycine and taurine conjugates. Kinetic parameters include normalized J_{max} , K_t , and P_p . K_t represents the Michaelis-Menten parameter for hASBT. Normalized J_{max} is J_{max} , relative to taurocholate J_{max} . For example a normalized J_{max} of 2.0 indicates a J_{max} that is two-fold the taurocholate J_{max} . P_p refers to passive permeability. In Table III, unconjugated dihydroxy and monohydroxy bile acids exhibited high passive permeability which appears to mask hASBT-mediated flux. Hence, K_t and J_{max} were not measurable for these four bile acids.

Figure 4 illustrates the effect of C-24 conjugation on hASBT-mediated transport of cholate, which was the only unconjugated bile acid to exhibit measurable hASBT-mediated transport. In panel A of Fig. 4, glycine and taurine conjugation generally enhanced affinity (i.e. lowered K_t). Taurocholate exhibited the greatest affinity with $K_t = 4.39 \mu\text{M}$, which is approximately 3.5-fold lower than unconjugated cholate ($K_t = 15.1 \mu\text{M}$). This trend is qualitatively similar to inhibition data (Table II). In panel B of Fig. 4, conjugation did not impact J_{max} in a consistent fashion relative to unconjugated cholate, although J_{max} was generally higher for conjugates.

Across the various bile acids, affinities of glycine conjugates were generally the same as the affinities of taurine conjugates, except for cholate conjugates. Hence, while C-24 conjugation enhanced affinity, neither conjugate was more effective. Normalized J_{max} estimates were essentially the same across both glycine and taurine conjugates.

In comparing the effects of steroidal hydroxylation pattern, an inverse relationship was observed between transport affinity (K_t) and the number of steroidal hydroxyl groups (Table III). Dihydroxy and monohydroxy bile acids generally exhibited greater affinity than trihydroxy bile acids. Conjugates of dihydroxy bile acids deoxycholate and chenodeoxycholate exhibited very high affinity, with K_t estimates less than $1 \mu\text{M}$. Similarly, glycine conjugate of lithocholate also exhibited high affinity with K_t less than $1 \mu\text{M}$. Ursodeoxycholate conjugates exhibited a 10-fold poorer affinity than its epimer chenodeoxycholate, indicating the C-7 β -OH is less favorable than the C-7 α -OH. Normalized J_{max} exhibited a modest inverse association with the number of steroidal hydroxyl groups. Cholate conjugates showed normalized J_{max} values of about 1.0, while normalized J_{max} of the dihydroxy and monohydroxy bile acids exhibited normalized J_{max} about over 4-fold higher. In summary, dihydroxy and monohydroxy conjugates exhibited both greater affinity and higher J_{max} compared to trihydroxy conjugates.

Comparison of inhibition kinetics versus transport kinetics

hASBT inhibition requirements can be expected to differ from hASBT-mediated transport requirements. For example, a compound may be a better inhibitor than substrate. Figure 5 compares inhibitory potency (K_i) against transport affinity (K_t) for each bile acid. Overall, all bile acids were potent inhibitors, with K_i ranging from about 0.5 to $15 \mu\text{M}$, and potent substrates, with K_t ranging from about 0.1 to about $15 \mu\text{M}$. Qualitatively, most bile acids

yielded K_i and K_t values that were similar to one another (i.e. within 5-fold). Two bile acids were more potent as inhibitors than as substrates (i.e. K_i lower than K_t). These two bile acids were tauroolithocholate (TLCA) and tauroursodeoxycholate (TUDCA) and fall below the line of unity in Fig. 5. Each is a taurine conjugate with a α -OH at only C-3 position. Meanwhile, taurochenodeoxycholate (TCDCA) and glycolithocholate (GLCA) exhibited K_t values that were more than 5-fold lower than K_i estimates (i.e. points above the line of unity in Fig. 5). Both taurochenodeoxycholate (TCDCA) and glycolithocholate (GLCA) exhibited K_i less than 1.0 μ M, and also exhibited K_t less than 1.0 μ M; hence, there was qualitative agreement between K_i and K_t values for taurochenodeoxycholate (TCDCA) and glycolithocholate (GLCA).

Figure 6 compares normalized J_{max} to inhibitory potency. There was an inverse association between K_i and normalized J_{max} indicating that, among the studied native bile acids, potent inhibitors generally exhibit a greater J_{max} .

DISCUSSION

The scope of the present study was to characterize hASBT-mediated transport and inhibition characteristics of native bile acids. Current understanding of hASBT substrate requirements is based on studies prior to the cloning of human ASBT and did not lead to a unified model. These studies suffered from various limitations including confounding effects of hepatic transporters and unstirred water layer effects (6). Recent studies have employed cell based models, included hASBT transfected systems, to delineate hASBT substrate requirements (13-17). Dawson and colleagues developed the hASBT-COS system and characterized several bile acids (13). Glyco-dihydroxy bile acids exhibited the greatest transport affinity for hASBT, in apparent contrast to the prevailing hypothesis that trihydroxy bile acids are best transported (4). Using Caco-2 monolayers, Swaan et al. examined the transport of several C-24 bile acid-peptide conjugates (17,18) and concluded that the C-24 side chain could be at least 14 Å in length and still allow for translocation; large hydrophobic moieties increased binding to hASBT. Cholic acid exhibited a greater affinity (i.e. lower K_t) than taurocholate (15), in contrast to results from Dawson and colleagues (13). The inconsistency of results across studies has led to a poor understanding of the substrate requirements for hASBT, even after the cloning of hASBT. Further, no study has attempted to systematically characterize the interaction of native bile acids with hASBT via both inhibition studies and transport studies.

The current study uses an hASBT-MDCK monolayer model that over-expresses hASBT, thus avoiding confounding effects of other transporters (9). Additionally, aqueous boundary layer effects can be accommodated, yielding K_t estimates in the μ M range, in contrast to previous studies where aqueous boundary layer influences led to biased K_t estimates (6). Since both inhibition and transport studies were performed, the present study compares the associations between inhibition potency, substrate potency, and transport capacity. No previous study measures these kinetic parameters and investigates these associations for hASBT, or apparently for any transporter.

Interestingly, the most developed pharmacophore for ASBT was developed using a chemical library that did not include bile acids (19). Rather, the library was largely composed of non-bile acid analogues designed to serve as cholesterol-lowering agents via ASBT inhibition. The assay system employed rabbit ASBT. The resulting 3-D pharmacophore model for ASBT identified five chemical features: one hydrogen bond donor, one hydrogen bond acceptor, and three hydrophobic features. The pharmacophore concerns ASBT inhibition, as data for the model was inhibition data and not transport data. It is also interesting to note that taurocholate did not map all the features of the pharmacophore (19).

Results in the present study indicate that C-24 conjugation enhanced the inhibitory potency of bile acids (i.e. K_i). This trend was consistent across all bile acids, including trihydroxy, dihydroxy and monohydroxy bile acids. This report is the first comprehensive attempt to evaluate C-24 conjugation effects across each mono, di- and tri- hydroxy bile acids. An inverse relationship was observed between number of hydroxyl groups and inhibitory potency, with monohydroxy bile acids being the most potent inhibitors. Across dihydroxy bile acids, chenodeoxycholate exhibited greater potency than deoxycholate and ursodeoxycholate. This pattern indicates that C-7 α -OH is more favorable than C-12 α -OH, which is more favorable than C-7 β -OH. C-24 conjugation generally dominated steroidal hydroxylation effects.

Results from transport studies mirrored these trends from inhibition studies. C-24 glycine or taurine conjugation generally enhanced transport affinity in case of cholic acid. Unconjugated dihydroxy and monohydroxy bile acids exhibited high passive permeability and did not exhibit measurable hASBT-mediated transport. Steroidal hydroxylation had a significant effect on transport affinity (i.e. K_t). Fewer hydroxyl groups promoted bile acid interaction with hASBT. The hydroxylation pattern influenced both K_t as well as the J_{max} . Cholate and its conjugates exhibited higher K_t and lower J_{max} , while chenodeoxycholate conjugates exhibited lower K_t and higher J_{max} . However, overall, all native bile acids showed high affinity for hASBT, as K_t values were on the order of 1 to 15 μ M, rather than 1 mM as reported earlier (5).

Translocation of substrate by a transporter is a complex process. Minimally, translocation requires (a) binding of substrate to transporter, (b) vectorial translocation of substrate via change in transporter conformation, (c) dissociation of substrate, and (d) restoration of transporter to binding conformation. This description is a simplistic one, particularly for transporters such as hASBT, which co-transporters sodium. Based upon the high capacity and high affinity of hASBT-mediated transport of bile acids, we speculate that bile acid binding to hASBT may be the rate limiting step in the apical transport of bile acids. In Fig. 6, binding, as assessed by K_i , appears to determine J_{max} , suggesting binding is the rate limiting step in the apical transport of bile acids. This hypothesis would suggest that vectorial translocation of substrate, dissociation of substrate, and restoration of transporter are not rate limiting. A future aim will be the consideration of biophysical data in concert with transport/inhibition kinetic data to better understand hASBT functioning.

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List of Abbreviations

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
GCDCA	glycochenodeoxycholic acid
TCDCa	taurochenodeoxycholic acid
CDCA	chenodeoxycholic acid
TDCA	taurodeoxycholic acid
GDCA	glycodeoxycholic acid

TCA	taurocholic acid
GCA	glycocholic acid
DCA	deoxycholic acid
CA	cholic acid
UDCA	ursodeoxycholic acid

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Figure 1. Structure of native bile acids. Bile acids differ in hydroxylation pattern (R₁, R₂, and R₃, as indicated by circles) and in amino acid conjugation pattern at C-24 position (R₄, as indicated by square). Substituent at R-4 is either a free carboxylic acid or conjugate of glycine or taurine.

Figure 2. Concentration-dependent inhibition of taurocholate uptake into hASBT-MDCK monolayers by tauroursodeoxycholate. Cis-inhibition studies of taurocholate uptake (0.5 μM) were carried out at varying concentrations of individual native bile acids. Closed circles indicate observed data points. Solid line indicates model fit. Taurocholate uptake into hASBT-MDCK cells was reduced significantly by tauroursodeoxycholate. Qualitatively similar profiles were observed for each native bile acid. Each point is the mean $\pm$ SEM of at least three measurements.

Figure 3.

Concentration-dependent transport of glycodeoxycholate across hASBT-MDCK monolayers. Glycodeoxycholate flux was measured at varying donor concentrations (1-50 µM) in the presence and absence of sodium to delineate hASBT-mediated and passive components of flux. Filled circles represent flux data in the presence of sodium. Open circles represent passive flux (i.e. absence of sodium). The solid lines represent model fits. In the presence of sodium, glycodeoxycholate transport was significantly larger compared to transport in the absence of sodium. Similar profiles were obtained for other conjugated bile acids and cholic acid. Unconjugated dihydroxy and monohydroxy bile acids exhibited high passive flux, such that active flux was not discerned. Each point is the mean $\pm$ SEM of at least three measurements.

Panel A

Panel B

Figure 4.

Effect of C-24 conjugation on Kt and Jmax of cholate and its conjugates. Panel A illustrates effect on Kt. Panel B illustrates effect on Jmax. In panel A, conjugation at C-24 improved the affinity relative to cholic acid, with taurine conjugates exhibiting the greatest affinity. This conjugation effect was similar to inhibition results (Fig. 3). In panel B, C-24 conjugation had no patterned effect on Jmax. Each point is the mean $\pm$ SEM of at least three measurements.

Figure 5.

Comparison of K_i and K_t estimates for native bile acids. Qualitatively, most bile acids yielded K_i and K_t values that were similar to one another (i.e. within 5-fold similar). Four bile acids yielded K_i and K_t estimates that differed by more than 5-fold, although were within 20-fold of each other. Tauroolithocholate (TLCA) and tauroursodeoxycholate (TUDCA) yielded K_i that were over 5-fold more potent than K_t . Meanwhile, taurochenodeoxycholate (TCDCA) and glycolithocholate (GLCA) yielded K_t that were over 5-fold more potent than K_i . Each point is the mean $\pm$ SEM of at least three measurements.

Figure 6. Relationship between K_i and J_{max} . Higher capacity (i.e. J_{max}) was associated with greater inhibitory potency. Each point is the mean $\pm$ SEM of at least three measurements.

Table I

Bile acid nomenclature. All hydroxyl (-OH) groups are in the α -position, except when noted to be in the β -position. Figure 1 illustrates the structure of bile acids

Bile acid	R ₁ (C-3)	R ₂ (C-7)	R ₃ (C-12)	R ₄ (C-24)
Cholate (CA)	-OH	-OH	-OH	-OH
Chenodeoxycholate (CDCA)	-OH	-OH	-H	-OH
Deoxycholate (DCA)	-OH	-H	-OH	-OH
Lithocholate (LCA)	-OH	-H	-H	-OH
Ursodeoxycholate (UDCA)	-OH	-OH (β)	-H	-OH
Glycocholate (GCA)	-OH	-OH	-OH	-NHCH ₂ COOH
Taurocholate (TCA)	-OH	-OH	-OH	-NH(CH ₂) ₂ SO ₃ H
Glychenodeoxycholate (GCDCA)	-OH	-OH	-H	-NHCH ₂ COOH
Taurochenodeoxycholate (TCDCA)	-OH	-OH	-H	-NH(CH ₂) ₂ SO ₃ H
Glycodeoxycholate (GDCA)	-OH	-H	-OH	-NHCH ₂ COOH
Taurodeoxycholate (TDCA)	-OH	-H	-OH	-NH(CH ₂) ₂ SO ₃ H
Glycolithocholate (GLCA)	-OH	-H	-H	-NHCH ₂ COOH
Taurolithocholate (TLCA)	-OH	-H	-H	-NH(CH ₂) ₂ SO ₃ H
Glycoursodeoxycholate (GUDCA)	-OH	-OH (β)	-H	-NHCH ₂ COOH
Tauroursodeoxycholate (TUDCA)	-OH	-OH (β)	-H	-NH(CH ₂) ₂ SO ₃ H

Table II

Effect of C-24 conjugation steroidal hydroxylation pattern on inhibition potency of native bile acids. Values in parenthesis indicate SEM (n=3).

Hydroxylation Pattern	Parent Bile acid	Ki value (μM)		
		Unconjugated	Glycine	Taurine
Trihydroxy (3 α , 7 α , 12 α)	Cholate	17.7 (± 1.4)	7.53 (± 0.44)	4.96 (± 0.17)
Dihydroxy (3 α , 12 α)	Deoxycholate	8.31 (± 0.77)	3.22 (± 0.26)	3.19 (± 0.16)
Dihydroxy (3 α , 7 α)	Chenodeoxycholate	1.94 (± 0.17)	0.992 (± 0.089)	0.933 (± 0.096)
Dihydroxy (3 α , 7 β)	Ursodeoxycholate	22.6 (± 3.0)	3.41 (± 0.21)	0.819 (± 0.046)
Monohydroxy (3 α)	Lithocholate	2.10 (± 0.22)	0.427 (± 0.017)	0.50 (± 0.052)

Table III

Kinetic estimates of hASBT-mediated transport of unconjugated and glycine/taurine conjugates of bile acids. Values in parenthesis indicate SEM (n=3).

Parent Bile Acid	C-24 Conjugate	Normalized Jmax	Kt (μM)	Pp (cm/sec) × 10 ⁶
Cholate	Unconjugated	0.714 (0.028)	15.1(1.4)	0.962 (0.005)
	Glycine	1.52 (0.24)	11.0 (1.9)	0.220 (0.157)
	Taurine	1.00 (0.18)	4.39 (0.29)	1.15 (0.11)
Ursodeoxycholate	Unconjugated ^a	nm	nm	21.2 (0.9)
	Glycine	2.26 (0.36)	11.5 (2.0)	1.01 (0.13)
	Taurine	2.21 (0.68)	10.3 (4.0)	1.00 (6.32)
Deoxycholate	Unconjugated ^b	nm	nm	23.8 (0.4)
	Glycine	3.49 (0.47)	1.10 (0.26)	0.520 (0.162)
	Taurine	1.75 (0.11)	0.497 (0.147)	2.47 (0.14)
Chenodeoxycholate	Unconjugated ^c	nm	nm	20.7 (0.7)
	Glycine	4.32 (0.58)	0.662 (0.337)	0.414 (0.201)
	Taurine	3.47 (0.46)	<0.1	0.923 (0.013)
Lithocholate	Unconjugated ^d	nm	nm	14.2 (0.3)
	Glycine	4.05 (0.64)	<0.1	0.865 (0.780)
	Taurine	8.50 (2.11)	8.43 (3.10)	0.020 (0.263)

nm = not measurable due to high passive permeability

^a Permeability of ursodeoxycholate in the presence of sodium was $23.8(0.3) \times 10^{-6}$ cm/sec.

^b Permeability of deoxycholate in the presence of sodium was $30.9(0.2) \times 10^{-6}$ cm/sec.

^c Permeability of chenodeoxycholate in the presence of sodium was $23.9(0.4) \times 10^{-6}$ cm/sec.

^d Permeability of lithocholate in the presence of sodium was $12.4(0.3) \times 10^{-6}$ cm/sec.