



# Taurocholate or Glycocholate – which is the more appropriate Substrate for Assessing Hepatic Bile Salt Transport?

Erica Deibert, Jonathan Jackson, Joy Mitchell, Kirsten Amaral, Rafal Witek, Stephen Ferguson and Jasminder Sahi ADME/Tox Division of Life Technologies, Durham NC USA

## ABSTRACT

**Purpose:** Interactions with the hepatic bile salt transporters NTCP and BSEP can contribute to cholestasis and hypercholesterolemia. The initial studies to evaluate uptake and biliary excretion via these transporters were developed by Brouwer and LeCluyse (1998) in primary sandwich cultured rat hepatocytes, using the bile salt taurocholate as a substrate. Since then, a majority of *in vitro* biliary transporter studies have been conducted with taurocholate for preclinical and human extrapolations. While taurine conjugated bile acids (e.g. taurocholate) are the major bile salts in rat, human plasma predominantly contains glycine-conjugated bile acids (e.g. glycocholate). To identify the appropriate bile salt for *in vitro* transporter evaluations, we compared the substrate and inhibition potential of taurocholate and glycocholate in human and rat *in vitro* systems. **Methods:** Mechanistic studies were conducted using primary rat and human hepatocytes in suspension and in primary culture. Hepatocytes were prepared from healthy human liver resections and from male Sprague Dawley rats and used freshly isolated or after cryopreservation. The oil spin method was used to compare transporter-mediated uptake of taurocholate and glycocholate in suspension hepatocytes. Uptake was also conducted after 24 hours in primary culture on collagen coated plates. Additional hepatocytes were overlaid with Geltrex® Matrix (Invitrogen) and used at 5-7 days in culture to assess uptake and biliary efflux of the bile salts. Membrane vesicles over-expressed with the human and rat BSEP transporter (Genomembrane) were also used. **Results:** Time linearity studies with taurocholate and glycocholate indicate linear uptake at 5 minutes incubation for the rat and human hepatocytes in culture and a 1 minute incubation time for suspension hepatocytes. In 24-hour plated hepatocytes, the Km for taurocholate uptake was similar: 13.3 µM in human and 22.1 µM in rat; while the Km for glycocholate demonstrated differences between the two species: 23.1 µM (human) and 166.8 µM (rat). Using sandwich hepatocytes with intact bile canaliculi, we demonstrated a biliary excretion index (BEI) for taurocholate of 82.5% in rat and 49.6% in human hepatocytes. The BEI for glycocholate was 77.5% for human and 53.4% for rat hepatocytes. As a whole, in these two culture systems, glycocholate is a more efficient substrate in human vs. rat hepatocytes and taurocholate is a stronger substrate in rat hepatocytes. BSEP vesicles mimicked these trends. Inhibition studies were less conclusive and further evaluations are being conducted to understand differences if any, using a wider range of inhibitors in both species. **Conclusions:** In preliminary evaluations, glycocholate is a stronger substrate for human hepatic bile salt uptake and efflux transporters while taurocholate is the appropriate substrate for assessing rat biliary transport. Mechanistic studies to further understand differences between these two bile salts in rat and human hepatocytes are currently being conducted. Our data thus far indicates that serious consideration should be given to using glycocholate for *in vitro* studies aimed at studying human bile salt transporters.

## INTRODUCTION

Hepatic toxicity is a major factor for discontinuing the development of compounds in pharmaceutical preclinical development or Phase I clinical trials. Some compounds demonstrate liver toxicity while being tested in experimental animals suggesting potential adverse clinical liver effects. In contrast, other compounds demonstrate only minor or no signs of hepatotoxicity in the animal species tested, yet cause an increase in serum bile salts and/or liver enzymes in more than 10% of humans during early clinical trials. Typically, drugs that result in hepatotoxicity are preferentially eliminated from the body via the biliary pathway. We and others have hypothesized that hepatotoxicity in humans taking these drugs may be associated with drug-mediated inhibition of active canalicular transport of bile components including, but not limited to, bile acids (1,2). These drugs are likely substrates for active liver transporter-mediated uptake (NTCP) and efflux (BSEP) into the bile canaliculi. The transporters that participate in biliary drug elimination also transport endogenous bile components. This results in the potential for mutual inhibition of drug and bile acids efflux from the liver, resulting in an increase in drug and bile acids retained in the liver over time. Compounds with greater inhibition of bile acid transport have a higher risk of being hepatotoxic.

A majority of studies conducted to evaluate bile salt inhibition mediated hepatotoxicity *in vitro*, use primary hepatocytes or membrane vesicles over-expressed with the bile salt efflux transporters BSEP. Typically, a competition assay between the drug and the bile acid taurocholate is used to test whether canalicular efflux of taurocholate can be inhibited in a concentration-dependent manner and whether this inhibition would correlate with clinical hepatotoxicity. However, while taurocholate is the major bile salt in the rat, glycine conjugated bile salts (e.g. glycocholate) and their metabolites (e.g. glycochenodeoxycholic) are the predominant bile salts in humans<sup>3</sup>. In this study, 14 bile acids were quantitated and glycochenodeoxycholic acid was the most abundant, followed by glycocholic acid. Taurocholic acid was one of the relatively minor bile salts. We hypothesized that glycocholic acid should be the appropriate bile acid for *in vitro* studies that are conducted to predict clinical cholestasis and have conducted extensive comparisons of these two bile acids using *in vitro* models.

## MATERIALS AND METHODS

**Transporter-Mediated Uptake:** Uptake and inhibition of uptake was evaluated in suspension and primary cultured hepatocytes by standard methodologies<sup>4</sup>. **Biliary Excretion**™ This was evaluated in hepatocytes after 5-7 days in culture using standard methodologies<sup>1</sup>. **BSEP Vesicles:** Mechanistic studies to evaluate the efflux and inhibition potential of inhibitors were evaluated using Genomembrane membrane vesicles as described before (5).

## RESULTS

Fig 1. Hepatocyte Transporters

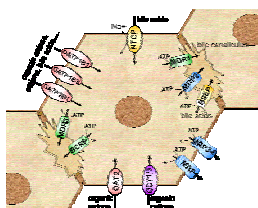


Table 1. Relative Concentrations of Bile Salts in Healthy Human Subjects

Bile Salt	In order of abundance:
GCDCa	0.696 µM
GCA	0.232 µM
TCDCa	0.228 µM
CDCA	0.200 µM
DCA	0.129 µM
UDCA	0.118 µM
CA	0.111 µM
GDCa	0.085 µM
TCA	0.070 µM
TDCA	0.019 µM
LCA	0.010 µM
TUDCA	0.006 µM
GLCA	0.006 µM
TLCA	0.0005 µM
total	1.911 µM

Tagliacozzi et al., 2003

Fig 2. Bile Acid Uptake in Plated Human & Rat Hepatocytes

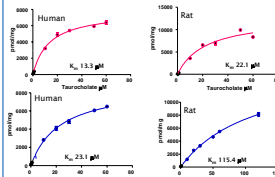


Fig 3. Bile Acid Uptake in Suspension Human & Rat Hepatocytes

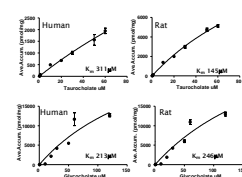


Fig 4. Inhibition of Bile Salt Uptake in Suspension Hepatocytes by Cyclosporin A, Ritonovir & Troglitazone

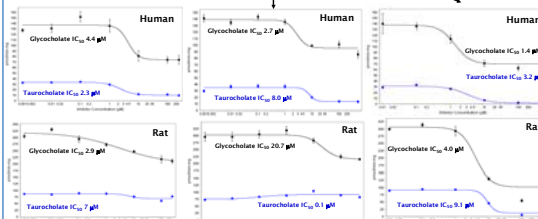


Fig 5. Biliary Clearance in Human & Rat Sandwich Cultured Hepatocytes

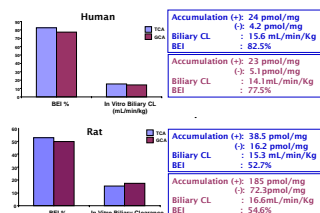
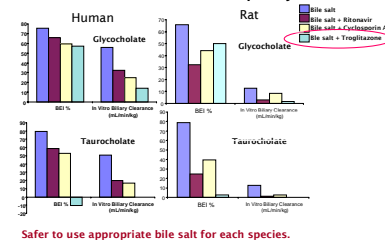


Figure 6. Effect of inhibitors on Biliary Clearance in Human Sandwich Cultured Hepatocytes



Safer to use appropriate bile salt for each species.

Figure 7. Uptake of Bile Salts in Human & Rat BSEP Vesicles

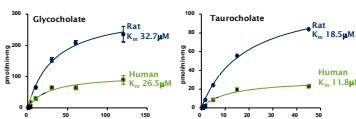
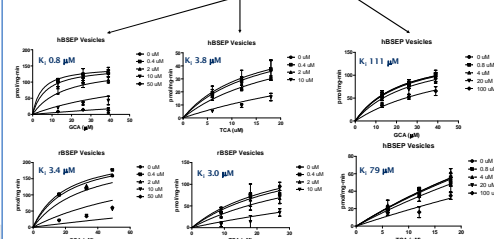


Figure 8. Inhibition of Bile Salt Uptake in Human & Rat BSEP Vesicles with Cyclosporin A and Troglitazone



## CONCLUSIONS

- Clinical studies indicate that chenodeoxycholic acid is the major circulating bile salt in human plasma, followed by glycocholic acid.
- Taurocholic acid is a relatively minor bile salt in humans and the major bile acid in rat
- In vitro* uptake studies in suspension and plated human hepatocytes reveal equivalent uptake for both bile acid.
- In vitro* uptake studies in suspension and plated rat hepatocytes indicate taurocholic acid is a better substrate than glycocholic acid.
- B-clear studies demonstrate equivalent biliary excretion in human hepatocytes with the two bile acids.
- BSEP membrane vesicles studies reveal similar uptake trends. Inhibition needs to be repeated.
- Inhibition studies using the B-clear model indicate different profiles using troglitazone as the inhibitor, indicating that to predict human inhibition of bile salts, the species – appropriate bile salt should be used

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