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Methods to Evaluate Biliary Excretion of Drugs in Humans: an Updated Review

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Abstract

Determining the biliary clearance of drugs in humans is very challenging because bile in not readily accessible due to the anatomy of the hepatobiliary tract. The collection of bile usually is limited to post-surgical patients with underlying hepatobiliary disease. In healthy subjects, feces typically are used as a surrogate to quantify the amount of drug excreted via non-urinary pathways. Nevertheless, it is very important to characterize hepatobiliary elimination because this is a potential site of drug interactions that might result in significant alterations in systemic or hepatic exposure. In addition to the determination of *in vivo* biliary clearance values of drugs, the availability of *in vitro* models that can predict the extent of biliary excretion of drugs in humans may be a powerful tool in the pre-clinical stages of drug development. In this review, recent advances in the most commonly used *in vivo* methods to estimate biliary excretion of drugs in humans are outlined. Additionally, *in vitro* models that can be employed to investigate the molecular processes involved in biliary excretion are discussed to present an updated picture of the new tools and techniques that are available to study the complex processes involved in hepatic drug transport.

Keywords

bile; excretion; biliary clearance; hepatic transport; hepatobiliary transport

INTRODUCTION

With the advent of combinatorial chemistry, an increasing number of new chemical entities that are more lipophilic and characterized by high molecular weight are being synthesized and screened during the drug discovery process.[1] Drugs with these physicochemical characteristics often are associated with significant hepatic metabolism and/or excretion via the biliary route.[2,3] The threshold molecular weight for drugs and metabolites to be excreted preferentially into bile varies between species, ranging from ~235 in rats up to 500–600 in humans. Biliary excretion of compounds can significantly impact the systemic exposure, pharmacological effect and toxicity of certain drugs. Drugs excreted into bile often undergo some degree of reabsorption along the gastrointestinal tract (e.g., mycophenolic acid[4], warfarin[5], and digoxin[3]). Enterohepatic recycling is also a very important physiological process for bile salt homeostasis. In humans, the intestinal reabsorption of bile salts is very efficient; more than 95% of the bile salt pool is reabsorbed in the distal ileum.[6,7] So far, limited *in vivo* information has been available regarding the extent of biliary elimination of drugs and metabolites in humans, primarily due to the difficulty in obtaining bile samples from healthy human subjects. An accurate measure of biliary clearance and of the extent of biliary excretion of compounds

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would be extremely valuable in evaluating the contribution of biliary clearance to total systemic clearance, elucidating potential mechanisms of hepatobiliary toxicity, predicting drug-drug interactions, and identifying enterohepatic versus enteroenteric recirculation. Several clinical studies have shown that drug-drug interactions can result in changes in drug absorption due to inhibition of transport proteins [increased exposure to talinolol after P-glycoprotein (P-gp, gene symbol *ABCB1*) inhibition[8], or inhibition of intestinal secretion resulting in improved therapeutic efficacy of paclitaxel[9]]. Important modifications in the pharmacokinetics/pharmacodynamics of digoxin, another P-gp substrate, have been observed with co-administration of other P-gp substrates/inhibitors, which are thought to cause interactions in intestinal[10], biliary[11,12] and/or renal[13,14] levels. However, drug-drug interactions influencing biliary clearance of compounds have not been investigated systematically due to technical difficulties in performing these experiments in humans. The inhibition of hepatobiliary transport processes can affect the ability of the liver to extract bile salts from the bloodstream or, potentially more importantly, can affect the metabolism and biliary excretion of bile salts. Although mechanisms of hepatotoxicity are believed to be multifactorial, it has been demonstrated that inhibition of bile salt biliary excretion by drugs (e.g., troglitazone[15] and bosentan[16]) can affect hepatocyte viability due to the accumulation and direct toxicity of detergent-like bile salts.[17]

The contribution of biliary excretion to the overall disposition and pharmacological effect of some drugs has been underestimated due to the lack of methodologies to accurately quantify the amount of drug subjected to enterohepatic recirculation, and a poor understanding of the processes involved in hepatic excretion and reabsorption of drugs from the canalicular space, bile ducts and intestine. Several animal models, primarily rodent, have been used to study genetic diseases affecting biliary clearance and drug-drug interactions. Mouse and rat isolated perfused liver experiments and bile duct cannulated animals are useful tools for studying biliary disposition of drugs in the early phases of drug development. The availability of rodent models genetically deficient in specific transport proteins has improved understanding of the complex molecular processes involved in excretion of endogenous and exogenous compounds into bile.[18–22] However, accumulating evidence suggests that significant inter-species differences exist in the function and regulation of transport proteins, in addition to the already thoroughly characterized species differences in metabolism; these findings complicate the direct extrapolation of animal data to humans.[23–25] Enterohepatic recirculation of drugs has been studied extensively in experimental animals where cannulation of the bile duct allows for interruption of bile flow into the intestine. The importance of enterohepatic recirculation also has been investigated with a similar study design in humans. The pharmacokinetic profiles of drugs have been compared in patients with bile duct obstruction, before and after removal of nasobiliary drainage. This design allows complete collection of bile and interruption of enterohepatic recirculation while the drainage is in place.[26] However, since the subjects studied in this type of experimental design are affected by hepatobiliary disease, it is not possible to account for changes in the hepatobiliary tract that might have followed the resolution of bile duct obstruction.

Mechanisms for Excretion of Endogenous and Exogenous Compounds into Bile

Several recent reviews have been published on the transporters involved in hepatobiliary disposition of drugs and bile salts.[27–31] The liver (Figure 1) plays a central role in the biotransformation and removal of xenobiotics and endogenous compounds from the blood. While the majority of small, lipophilic compounds enter the hepatocyte from the Space of Disse on the basolateral membrane by simple passive diffusion, more polar and bulky molecules require transport systems to cross the sinusoidal membrane (Figure 2 A). Once inside the hepatocyte, compounds can be transported into bile either unchanged or as more hydrophilic metabolites after phase I and/or phase II biotransformations, or can be excreted into blood by

basolateral transport proteins (Figure 2 B). The presence of several transport proteins on the canalicular domain of hepatocytes results in the excretion of solutes into the bile canaliculi, which are dilated intercellular spaces. One of the many functions of the liver is the synthesis of bile acids from cholesterol[32]. The major bile acids present in human bile are cholic and chenodeoxycholic acids conjugated with glycine and taurine.[33] Bile salts are excreted into bile by the bile salt export pump (BSEP/*ABCB11*, Figure 2 B). Bile salts facilitate the absorption of nutrients in the intestine by emulsifying lipophilic molecules due to their surfactant characteristics.[34] Once this function is served, bile salts are very efficiently reabsorbed into the cells of the distal ileum by the apical sodium-dependent bile salt transport protein (ASBT/IBAT/*SLC10A2*[35]) and excreted across the basolateral membrane into portal blood by the recently identified organic solute transport proteins OST α and β.[36,37] In addition to bile salt transport, hepatic transport proteins facilitate excretion of end-products of metabolism, such as bilirubin and/or xenobiotics, into bile for subsequent removal via the intestine, and thus act as an important clearance mechanism.

Bile Formation and Excretion

Bile formation includes uptake of bile components from the sinusoidal blood and secretion of these substances into the canalicular space. Water and other small ions also diffuse into bile due to the differential osmotic pressure between bile and the hepatocyte cytoplasm. Subsequently, bile is drained into the biliary tree along the liver lobules and reaches the hepatic ducts. In humans, a fraction of this hepatic bile is secreted continuously into the duodenum while the majority of the ~800 mL of bile secreted per day is concentrated and stored in the gallbladder (Figure 1). Bile freely flows into the gallbladder, because this organ has a much lower internal pressure than the intra-hepatic ducts, common bile duct and the Sphincter of Oddi (Figure 1). The gallbladder fills to its maximum capacity (40–70 mL) in approximately 6 hours, while re-absorbing water and electrolytes from hepatic bile.[3,38–40] After ingestion of a meal, fats, proteins and acid reach the duodenum and endogenous neurohormones such as cholecystokinin (CCK) and secretin are released by endocrine cells in the small intestine.[41] CCK, acting on the CCK-A type receptors of smooth muscle fibers, contracts the gallbladder and relaxes the Sphincter of Oddi. A similar effect can be obtained by intravenous infusion of physiological amounts of CCK analogs (e.g., CCK-8).[42,43] CCK blood concentrations are decreased to minimal values during fasting, which promotes contraction of the Sphincter of Oddi and relaxation of the gallbladder walls, facilitating storage of bile in the gallbladder. CCK also delays gastric emptying in order to coordinate the digestive postprandial process. In the blood of humans and animals, CCK-33, 8 and 58 are the predominant active forms of this polypeptide [44] generated by differential post-translational modifications of the same gene; functional activity is dependent upon the last seven amino acids at the carboxyl terminus. In addition to the role of CCK in gallbladder contraction, CCK also functions as a neurotransmitter in the cerebral cortex to regulate satiety, and on the peripheral nerves of the GI tract to regulate bowel movement.[45,46]

Methods Used to Determine *In Vivo* Biliary Clearance

Several preclinical tools involving *in vivo* or *ex vivo* rodent models can be utilized to evaluate the extent of biliary excretion of drugs and the interaction of xenobiotics with bile salt transport proteins. These tools employ animal species with well characterized differences from humans, making the prediction/extrapolation to humans less accurate.[47] Determination of the extent of biliary excretion can be attained in human subjects with different methodologies as described in detail below. However, only a few studies have been performed *in vivo* in humans to evaluate biliary clearance of drugs, and little is known about the contribution of biliary clearance to the overall systemic clearance for most drugs. Table I summarizes those studies published in the literature along with the techniques used for the determination of the amount of drug and metabolites excreted into bile.

I. Patients as Study Subjects—Historically, there has been little success in the development of valid and reliable techniques to quantify biliary excretion of drugs or endogenous compounds in healthy human volunteers. Such data is obtained more easily from patients diagnosed with diseases of the gallbladder and biliary tract who require medical procedures that allow measurements of drug concentrations in bile. Usually these patients suffer from cholestasis and/or occlusion of the biliary or cystic ducts, and some may have undergone liver transplantation.

i. Cholecystectomy: Several groups have investigated gallbladder bile concentrations and disposition of drugs into bile by administering the compound of interest to patients prior to cholecystectomy. Drug concentrations in the gallbladder bile and tissue after removal are quantified and compared to the plasma concentrations. Although this approach only allows the determination of drug content in bile and tissue at a single time point, it is still very useful and has been employed to establish whether an antibiotic reaches the necessary therapeutic concentrations to treat bacterial infections of the gallbladder and biliary tract.[48,49]

ii. T-tube: Patients that require a temporary bile shunt (T-tube) that diverts part of the bile flowing from the liver to a transcutaneous port for external collection have been employed commonly to study biliary excretion of drugs. T-tubes are used primarily in patients with cholangitis, distal bile duct obstruction or edema, and often are inserted after cholecystectomy and choledochotomy. T-tubes allow for partial collection of liver bile, and provide an estimate of the biliary excretion of administered drugs during the time that the shunt is in place.[50–55] A limitation of this technique is that bile collection in these patients is not complete. Furthermore, the composition of bile may be very different in patients due to underlying hepatobiliary disease.[56]

iii. Nasobiliary Drainage: Patients with bile duct stenosis can be treated with the temporary insertion of a nasobiliary tube. This patient population has been recruited less frequently for drug disposition studies, probably due to the fact that this procedure is relatively new. This intervention theoretically allows for complete collection of bile while the tube is in place. The nasobiliary drainage interrupts delivery of bile into the duodenum and can be used to evaluate the influence of enterohepatic recirculation on the plasma concentration-time profile for the drug of interest. In these studies, the same patients have been used as their own control when the stricture has resolved and the tube has been removed thus restoring the enterohepatic recycling process.[26,50]

Less commonly, bile has been collected from patients equipped with other types of drainage devices such as biliary-enteric diversions or percutaneous transhepatic cholangiodrainage.[51] Although the details of the surgical procedures are beyond the scope of this review, all these techniques allow free access to partial or complete collection of hepatic bile from patients.

In recent years laparoscopic cholecystectomy with duct exploration and endoscopic retrograde cholangiopancreatography (ERCP) have largely replaced more invasive methods for removing common duct stones. As a result, many fewer patients with T-tubes have been available to enroll in studies examining the biliary excretion of drugs. The relatively few patients who are treated with nasobiliary drainage are seldom well enough or maintain the tube long enough to be suitable study candidates.

II. Healthy Volunteers as Study Subjects—One of the major limitations of the techniques described above is the use of subjects with significant hepatobiliary disease. Disease status may significantly influence transport protein expression, function, localization and/or bile flow, thereby altering drug excretion and reabsorption patterns.[57,58] It is well known that biliary obstruction decreases the expression of BSEP and MRP2 (ABCC2) in hepatocytes, and

increases P-gp, MDR3 (ABCB4) and MRP3 (ABCC3) expression.[59,60] Moreover, cholestatic syndromes may be associated with genetic polymorphisms in transport proteins.[61,62] More relevant information may be obtained by enrolling healthy volunteers as study subjects. In this case, the physiology of the hepatobiliary tract is intact and healthy. However, the collection of bile from the gallbladder or upon immediate secretion into the intestine is more challenging, due to the difficulty of accessing the gallbladder and the anatomy of the human hepatobiliary tract.

i. Fecal Recovery: The approach most commonly used in the determination of biliary excretion as a route for drug elimination is the quantification of the drug of interest in feces. Often these studies are performed with a radiolabeled compound in order to increase the selectivity and the recovery of drug and unknown metabolites, resulting in good mass balance.[63–69] More recently, sensitive analytical techniques such as HPLC coupled with mass spectrometry have been applied widely to the analysis of biological matrices to identify the excretion routes of parent drugs and metabolites. Several drawbacks are associated with the quantification of drugs in feces. Most importantly, this method does not distinguish between biliary excretion and intestinal secretion processes. For orally administered drugs, this method does not distinguish between unabsorbed drug and that secreted by the liver and/or gut back into the intestinal lumen, thus, the percentage of the dose in feces does not necessarily reflect the dose excreted into bile. Moreover, relatively unstable drugs, or compounds that are transformed into unstable metabolites, may not be recovered in feces due to the long exposure to the intestinal contents and colonic flora. Finally, drugs subjected to entero-hepatic or entero-enteric recycling, such as glucuronide conjugates, can be reabsorbed in the colon upon intestinal degradation by β-glucuronidases of the microbial flora, and will not be recovered in the feces.

ii. Aspiration of Duodenal Fluids: An ideal methodology would provide information on the amount of parent drug and metabolites excreted into bile, and would be flexible enough to allow for studies examining potential drug-drug interactions. This type of information can be obtained by sampling duodenal fluids to collect bile upon discharge from the biliary tract into the small intestine.[11,12,70,71] Duodenal bile is representative of gallbladder bile in terms of bile composition, although it is more dilute.[72] The use of oroenteric tubes to withdraw pancreatico-biliary secretions from the duodenum is a common practice in the medical field, and has been used to study the biliary excretion of endogenous compounds such as radiolabeled bile acids.[35,73] Some of these investigations have utilized occlusive balloons to facilitate more complete bile collection, along with the perfusion of a marker to evaluate leakage of bile or duodenal fluids distal to the balloon seal.[74] Other groups have corrected for any incomplete bile collection by perfusing non-absorbable markers, such as polyethylene glycol or sulphobromophthalein, into the duodenum.[71,75,76] Modified versions of these methodologies have been applied successfully to the determination of intestinal absorption and secretion of drugs, thus enhancing the knowledge regarding intestinal processes, which complements information acquired regarding biliary excretion of drugs.[77–82] All the methods cited are particularly sophisticated, and the execution of these studies is not trivial. These clinical studies often require custom-made catheters, specialized personnel and equipment, and they need to be approved by local Institutional Review Boards and Research Ethics Committees. For these reasons, these methods are not used widely. Although a direct comparison of methods has not been published, it is reasonable to believe that the information obtained is, in many cases, an estimate of the amount of drug excreted via the biliary route. Nevertheless, many of the studies conducted in healthy human volunteers have resulted in incomplete and highly variable recovery of compounds excreted in bile. This is primarily due to the difficulty in obtaining and assessing the completeness of bile collection, and an inherent lack of control over gallbladder contraction. A novel method to increase the recovery of biliary secretions and correct for the degree of gallbladder contraction, with the intention of reducing the level of complexity of the procedure, has been developed recently by our group.[83] This method assures the quantitative

collection of bile secreted into the duodenum by temporary occlusion of the intestine with an inflatable balloon. At the same time, the administration of a hepatobiliary imaging agent (e.g. Tc-99m mebrofenin) is used to evaluate the degree of gallbladder contraction in response to pharmacological stimulation, and to detect any leakage of bile due to partial occlusion of the intestine. One of the major factors affecting the variability in the determination of biliary excretion in humans is related to differences in the amount of bile and drug delivered into the duodenum during the collection interval. Partial spontaneous contractions of the gallbladder during fasting are not uncommon, and can deliver over 25% of total gallbladder bile into the intestine. To obtain more complete gallbladder contraction, CCK-8 can be administered intravenously to pharmacologically stimulate gallbladder emptying. CCK-8 administered at a low dose (0.02 μg/Kg) infused slowly over 30–60 min has been reported to provide the most complete gallbladder contraction[43]; however, inter-individual response is variable, and possibly influenced by the collection procedure. Therefore, it is important to assess the degree of gallbladder contraction in each subject. The administration of trace amounts of a gamma-emitting probe that accumulates quickly in bile, such as Tc-99m mebrofenin, can assist in the determination of the overall gallbladder response, and indicate when intestinal occlusion is not complete. Alternatively, ultrasound may be used to determine the extent of gallbladder contraction together with perfusion of the occluded tract with a non-absorbable marker to identify leakages. A gallbladder ejection fraction (EF) can be calculated from the abdominal gamma images of the volunteers during gallbladder contraction, and this number can be incorporated as a correction factor when determining the amount of bile (containing drug) excreted into the duodenum and collected by aspiration.[83] This method has been shown to correct reasonably well for the high variability in the gallbladder contraction which influences the amount of drug excreted into the duodenum.[84] Quantification of parent drug and metabolites in bile aspirated directly from the duodenum rather than in feces can be extremely advantageous in the identification of unknown metabolites, uniquely formed in humans, that are unstable and would not be found after prolonged contact with intestinal enzymes (such as unstable glucuronides).

Finally, the possibility of repeating a pharmacokinetic study in the same volunteers with and without the balloon inflated enables an evaluation of the importance of biliary excretion in systemic drug exposure, and in studying the enterohepatic recirculation process.[85] Biliary excretion and intestinal reabsorption may be one explanation for multiple peaks in the plasma concentration-time profiles of some drugs.[86,87] Often, pharmacokinetic modeling of these data with a model that incorporates enterohepatic recycling has been used to explain these experimental observations.[88] Unfortunately, this approach does not provide direct evidence for biliary excretion, and does not allow for precise quantification of the biliary clearance parameter. Experimentally, the pharmacokinetic disposition of drugs and glucuronide conjugates have been compared in patients before and, upon removal of nasobiliary drainage, after the restoration of enterohepatic recirculation.[26] Other studies have interrupted enterohepatic recirculation with antibiotics, to eliminate the colonic flora, and/or cholestyramine, a resin that prevents the availability of lipophilic compounds for absorption in the gut lumen.[4,89] Improved methods for studying drug disposition in healthy volunteers in the presence or absence of enterohepatic recirculation should enable the investigation of hepatobiliary disposition mechanisms in much more depth than what has been attempted to date.

Methods Used to Determine *In vitro* Biliary Excretion

Several *in vitro* models are available to investigate the processes governing biliary excretion and potential interactions of drugs with the hepatic uptake and excretion of bile salts and other endogenous and exogenous substances. These tools span from the whole hepatocyte to membrane vesicles prepared from cell lines transfected with specific transport proteins. Such

techniques complement human and animal *in vivo* studies, and allow characterization of transport processes at the molecular level. Unfortunately, the results obtained with *in vitro* models are difficult to compare to human *in vivo* data. This is primarily due to the fact that the data generated *in vitro* are used to determine values for the intrinsic biliary clearance of a drug. In contrast, *in vivo* studies often are designed to obtain more crude estimates such as the percentage of the dose excreted into bile rather than pharmacokinetic parameters. The *in vivo* biliary clearance values in humans usually are not reported (Table I). Whole liver perfusion, a common technique in rodent models to obtain biliary clearance values, is rarely possible in humans due to limited availability of viable whole human livers.

i. Sandwich-Cultured Hepatocytes—Primary hepatocytes isolated from humans and animals are useful tools for studying hepatic metabolism and transport due to the expression of the full array of proteins involved in these processes. In conventional culture, a rapid decline in expression of both metabolizing enzymes and transport proteins occurs over time after cell isolation.[90] In addition, collagenase digestion of liver, required for hepatocyte isolation results in the disruption of the canalicular membrane and consequently, the cellular internalization of transport proteins expressed at this membrane.[91] A novel *in vitro* model system using primary hepatocytes from various species, including rat and human, cultured in a collagen-sandwich configuration (SC) has several distinct advantages over conventional hepatocyte culture. For example, SC hepatocytes develop functional and extensive bile canalicular networks, acquire and maintain normal cell polarity, and allow direct access to the hepatocyte and adjacent biliary compartment.[92–96] Thus, hepatocytes maintained under these culture conditions offer a unique opportunity to study basolateral uptake and canalicular efflux transport processes. Using simple alterations in buffer components, accumulation of substrates of interest can be measured in hepatocytes alone, or in hepatocytes plus canalicular networks. This allows determination of the *in vitro* intrinsic biliary clearance. To date, most SC experiments have been conducted using rat hepatocytes. [93,96–100] Recently, SC human hepatocytes also have been characterized; uptake and efflux transport proteins are expressed, properly localized on the apical and basolateral membranes and functional.[91,101] Since SC human hepatocytes also retain metabolic capabilities, this model allows for investigation of the interplay between many of the processes that take place *in vivo*. Additionally, these cells can be maintained in culture for several days allowing for the study of drug interactions involving induction mechanisms. Human hepatocytes are not readily available and are associated with high costs. Furthermore, each culture is performed with cells from a single donor reflecting the inherent variability among donors in protein expression, function, and canalicular network formation. SC human hepatocytes serve as a unique, comprehensive tool to investigate hepatobiliary transport mechanisms.

ii. Suspended Hepatocytes—Viability of primary hepatocytes can be maintained for several hours in suspension when supplemented with appropriate culture media and oxygen. Fresh or cryopreserved rat and human hepatocytes have been used extensively to determine the hepatic metabolic clearance of drugs. [102,103] Suspended hepatocytes are a useful tool to characterize hepatic uptake and metabolism processes and inhibition studies can be perfomed with this system. However, due to the limited viability of these cells, it is not possible to study induction processes.[104–106] Additionally, internalization of canalicular transport proteins during collagenase perfusion limits the use of suspended hepatocytes to predict an overall value for biliary clearance when transport into bile is important.

iii. Vectorial Transport Using Polarized Cell Lines—The Madin Darby Canine Kidney, strain II (MDCKII) cells are polarized cells that have been used extensively to express various combinations of recombinant uptake and efflux transport proteins. Initially, MDCKII cells transfected with different combinations of a single basolateral uptake and a single canalicular

efflux pump were established.[107–109] More recently, MDCKII cells transfected with a single efflux pump (MRP2/*ABCC2*) and three basolateral uptake transport proteins (OATP1B1/*SLCO1B1*, OATP1B3/*SLCO1B3* and OATP2B1/*SLCO2B1*) have been established in an attempt to mimic vectorial transport processes in human liver.[110] The combination of quadruple, double and single transfected cells is very useful for determining the contribution of individual transport proteins to vectorial transport processes. These cell lines can be transfected with transport proteins of interest, grown on a permeable membrane, and used to study the transcellular passage of selected substrates. Many model substrates have been studied using MDCK cells, and correlated with biliary clearance values obtained from rats *in vivo.*[108] Although the polarized transfected cell systems are powerful tools for studying vectorial transport, they lack a full complement of transport proteins, metabolic enzymes and co-factors present in hepatocytes, and expression levels of transport proteins may not be comparable to hepatocytes *in vivo*. These issues must be considered in data interpretation and when extrapolating to *in vivo* hepatic or biliary clearance values. Nevertheless, transfected systems are simple to use and more readily available than primary human hepatocytes and are useful as a high-throughput screening tool.

iv. Whole Cells and Membrane Vesicles—Whole cell expression systems such as *Xenopus laevis* oocytes or immortalized cell lines transfected with the transporter of interest are ideal tools when functional studies of the kinetics of transport for individual transport proteins are needed.[111] Such expression systems have been used extensively in the literature to demonstrate substrate specificity and for functional characterization of mutant proteins, such as genetic polymorphisms.[112,113] Although oocytes are a transient expression system and need to be injected individually with cRNA of the protein of interest, oocytes often are used to study uptake processes. The commercial availability of this model facilitates their use for transport studies.[114] These expression systems are more applicable to studying the effect of genetic diseases and the molecular mechanisms of transport than for the determination of hepatobiliary clearance.[115] Because of technical difficulties in loading whole cells with a substrate of interest prior to measuring efflux, these models are more suitable for the study of uptake transport proteins. To circumvent this issue, the double transfected MDCKII cells have been used, or more commonly, efflux transport processes have been studied with inside-out membrane vesicles. Such vesicles can be prepared from primary hepatocytes or from cell lines transfected with a transport protein of interest. Basolateral and apical membrane vesicles prepared from polarized cells such as primary hepatocytes can be isolated through various centrifugation steps and membrane fractions enriched in basolateral or canalicular transporters can be used to investigate drug transport and transport inhibition.[116,117] Canalicular vesicles are more commonly prepared from rat than human hepatocytes due to limited human tissue availability. However, several recent studies have used canalicular membrane vesicles isolated from human hepatocytes.[117–119] Membrane vesicles prepared from primary hepatocytes have distinct advantages over transfected systems: the native membrane environment is in place during isolation from the other cellular components, and all the hepatic transport systems are present. Membrane vesicles can be prepared, stored frozen, and used as needed; however, isolation of basolateral and canalicular fractions is labor intensive and complete purity is never achieved.

Membrane vesicles prepared from cell lines transiently and stably expressing membrane proteins of interest have been used extensively to characterize polymorphisms, substrate specificity and structure-function relationships of efflux transport proteins.[120–122] The expression systems used are many (insect cells: e.g., Sf9 cells [123,124]; mammalian cells: e.g. HEK293 cell line [125]) and expression of endogenous transporters may vary. The background activity of membrane vesicles must be accounted for by performing control experiments in untransfected cells; moreover, post-translational protein modifications may be different

depending on the host cells and may affect the affinity of the transport protein for the substrate of interest.

CONCLUSIONS

Novel and important technologies for evaluation of biliary excretion and the related toxicological/pharmacological issues derived from drug-drug interactions at the hepatobiliary transport level are under development. Currently, several *in vitro* models are used as screening tools in drug discovery and early development to eliminate molecules that present challenges in this particular aspect of disposition.[126–128] Comparison of results obtained using *in vitro* systems to data generated *in vivo* in humans would be extremely beneficial to substantiate *in vitro* observations. Unfortunately, information on *in vivo* biliary excretion of drugs is not readily available, and studies in volunteers will continue to be challenging and limited to few subjects. However, the findings of these studies can be extremely valuable, not only to explain results obtained using *in vitro* systems, but also to determine the importance of biliary excretion and enterohepatic recirculation in the overall disposition of drugs.

In the future, it is possible that a more systematic comparison between *in vivo* and *in vitro* hepatobiliary clearance will confirm the accuracy of *in vitro* scaled predictions, and allow the development of accurate models to predict drug disposition in humans with a higher degree of confidence. We believe that this combination of tools, together with extensive use of mathematical modeling and simulations, will not only improve our understanding of drug disposition, but will also help predict drug-drug interactions and/or the impact of genetic polymorphisms on drug exposure and toxicity.

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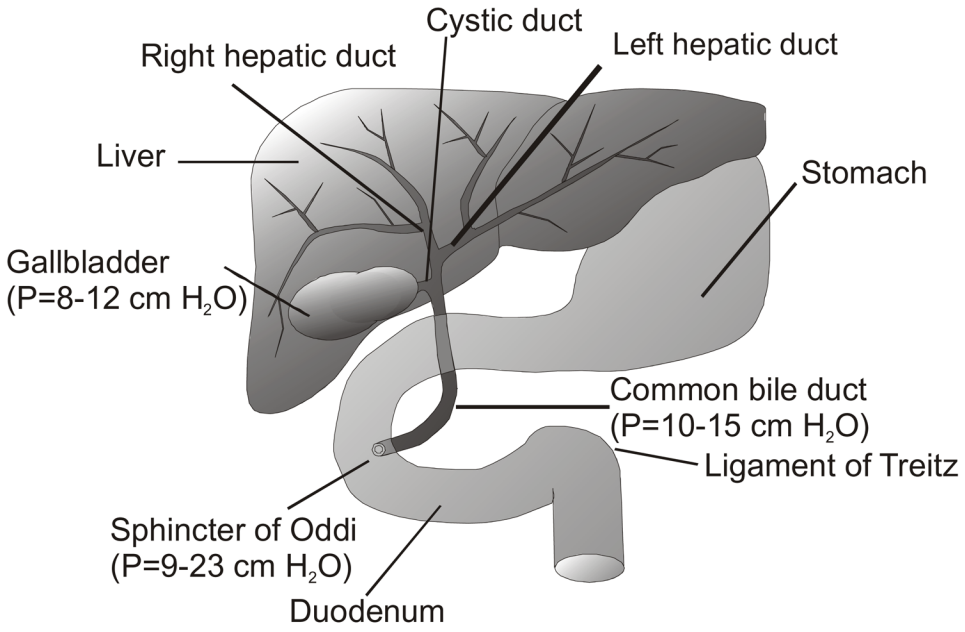

Figure 1.
Macro-anatomy of the hepatobiliary system: bile flow into the gallbladder is facilitated by lower internal pressure when compared to the rest of the biliary tract (pressure values are in parenthesis[40]). Water and solutes are reabsorbed within the gallbladder.

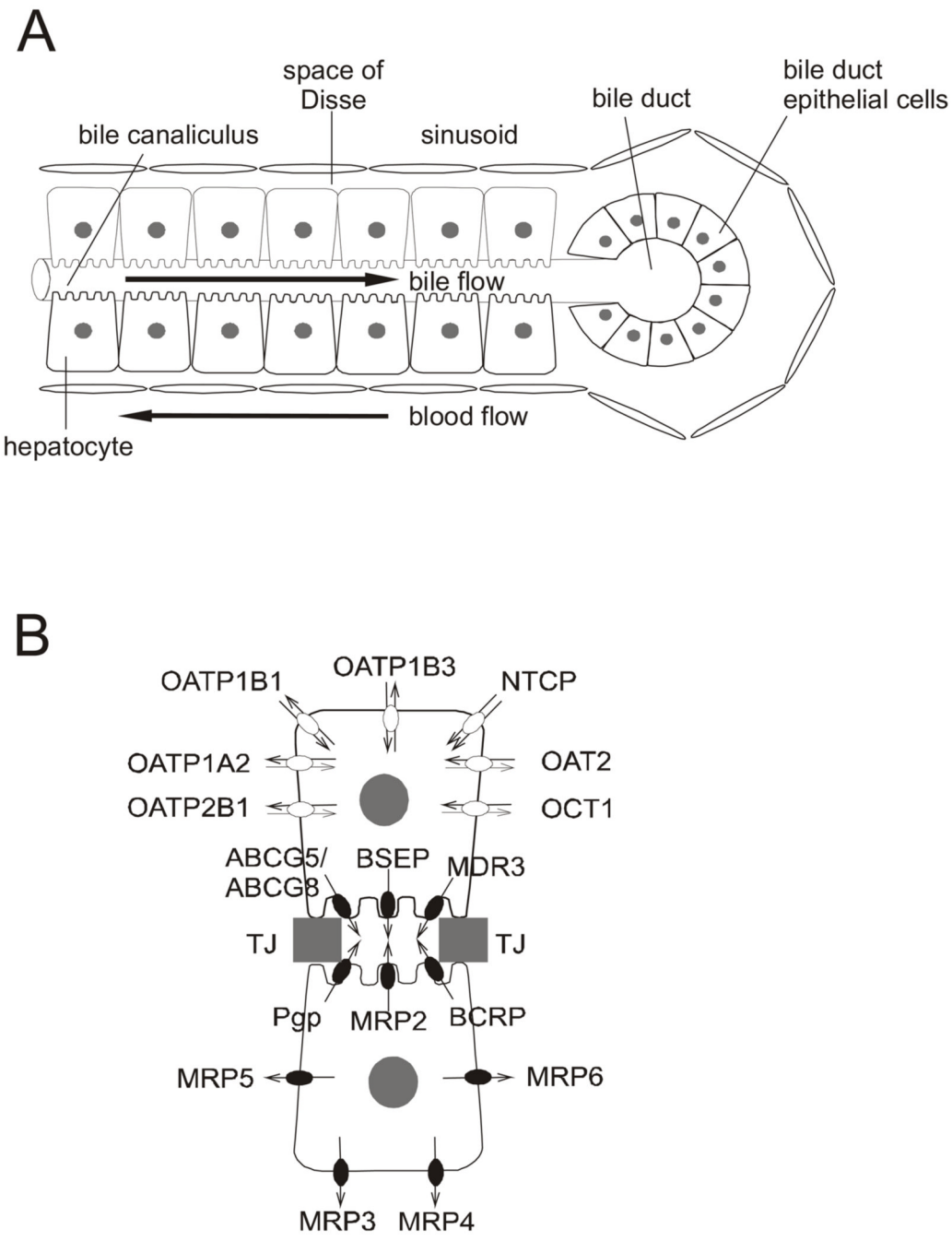

Figure 2. Micro-anatomy of the hepatobiliary tract

(A) At the cellular level, hepatocytes are organized in cords and bathed by sinusoidal blood from the basolateral side; the canalicular membranes form the bile canaliculi. Bile flows in the opposite direction to blood and drains into bile ducts

(B) An enlarged view of two hepatocytes from Figure A. Transport proteins are involved in the uptake and excretion of endogenous substances and xenobiotics into blood and bile. Filled symbols represent unidirectional primary active transport by ATP-binding cassette (ABC) transport proteins (Pgp, *ABCB1*; BSEP, *ABCB11*; BCRP, *ABCG2*; MRP2, *ABCC2*; MRP3, *ABCC3*; MRP4, *ABCC4*; MRP5, *ABCC5*; MRP6, *ABCC6*; ABCG5, *ABCG5*; ABCG8, *ABCG8*). Open symbols represent transport by members of the solute carrier protein families

including the SLC family (OAT2, *SLC22A7;* OCT1, *SLC22A1*; NTCP, *SLC10A1*) and the SLCO family (OATP1B1, *SLCO1B1*; OATP1B3, *SLCO1B3*; OATP1A2, *SLC01A2*; OATP2B1, *SLCO2B1*). Each functional hepatocyte contains all the transport proteins depicted in this figure. Adjacent hepatocytes form tight junctions (TJ) to seal the canalicular domain from the basolateral domain.

Table I

Summary of human studies that report the amount of parent drug and/or metabolites excreted via the biliary route. The percentage of the dose excreted into bile and/or feces as parent drug or total radioactivity is/are summarized where possible. The administration route and methods used to obtain this information, the particular population of subjects studied, and systemic and biliary clearance values, if published, also are listed. Antibiotics are a therapeutic class that has been studied in particular detail in terms of biliary excretion and distribution within the gallbladder tissue. In addition to the studies reported here, the reader is referred to comprehensive reviews on this topic.[71,129–131]

Therapeutic Class	Compound	Administration route	Population studied	Bile collection methodology (number of subjects)	% of dose excreted in bile or feces (collection interval)	Clearance parameters	Reference
Antineoplastic Agent	Elsamitrucin	IV	Cancer patients	Indwelling biliary catheter (n=1)	~22 unchanged (51 hr)	Cl_{total}=10–19 L/h/m^2	132
	Irinotecan	IV (^{14}C)	Cancer patients	Feces (n=7); T-tube bile and feces (n=1)	54–75 total in feces, 30 total in bile; 32.3 unchanged, 14 total in feces	Cl_{total}=13.5 L/h/m^2	67 133
	Taxol	IV	Cancer patients	Feces (n=5)	5 and 71.1 unchanged and total (4–5 days)	N.D.	134
	Vincristine	[a]IV (^{3}H)	Cancer patients	T-Tube bile and feces (n=1)	[a]49.6 total in bile and4.2 in feces (72 hr), 46.5 of bile radioactivity at 2 hr as unchanged	[b]Cl_{total}=189 mL/min/m^2	[a] 55 [b],135
Hepatobiliary Imaging Agent	Tc-99m Mebrofenin	IV	Healthy volunteers	Duodenal aspiration (n=4)	67.1/84.2 unchanged/corrected by EF (3 hr)	Cl_{total}=17.3 $Cl_{biliary}$=12.5 *in vivo* $Cl_{biliary}$ =16.1 mL/min/Kg	83
Cardiac Imaging Agent	Tc-99m Sestamibi	IV	Healthy volunteers	Duodenal aspiration (n=7)	14.7/21.5 unchanged/corrected by EF (3 hr)	Cl_{total}=25.8 $Cl_{biliary}$=3.9 *in vivo* $Cl_{biliary}$=6.0 mL/min/Kg	136
NSAID	Aspirin	PO and IV	Patients	T-tube bile (n=3); nasobiliary tube bile (n=2)	0.02–1.89 primarily conjugated (24 hr)	N.D.	50
	Diclofenac	PO	Patients	Percutaneous transhepatic cholangiodrainage (n=2)	4.6 total (24 hr), 1.1 unchanged and active phase II metabolites	N.D.	51
	Indomethacin	PO	Patients	Percutaneous transhepatic cholangiodrainage (n=3)	0.23–15 (24 hr)	N.D.	51
	Ibuprofen	PO	Patients	Percutaneous transhepatic cholangiodrainage (n=3)	0.82 (24 hr), 0.15 unchanged and active phase II metabolites	N.D.	51
Analgesic, Antipyretic	Paracetamol/Acetaminophen	PO	[c]Patients; [d]healthy volunteers	[c]T-tube bile (n=6); [d]duodenal aspiration for 15 min post CCK administration(n=10)	[c]2.6 total and [c]0.57 unchanged; [d]7.3 total	N.D.	[c] 52 [d],137
Antihyperlipidemic	Ezetimibe	PO (^{14}C)	Healthy males	Feces (n=8)	78 mostly unchanged (10 days)	N.D.	138
	Fluvastatin	IV/PO (^{3}H)	Healthy volunteers	Feces (n=6)	86–92 total (120 hr), 1.8 of total radioactivity as unchanged	Cl_{total}=0.97 L/hr/Kg	64
	Pravastatin	IV	Healthy volunteers	Feces	34 total and 23 unchanged	Cl_{total}=13.5 mL/min/Kg	139
	Rosuvastatin	PO (^{14}C)	Healthy volunteers	Feces (n=6)	90 total at 10 days (~70 at 72hr); 78.8 as unchanged (10 days)	N.D.	68
	Simvastatin	PO (^{14}C)	Patients	T-tube bile and feces (n=4)	25 total in bile and 20 in feces	N.D.	53
Antihistamine	Desloratadine	PO (^{14}C)	Healthy volunteers	Feces	47 primarily metabolites	N.D.	140
	Ebastine	PO	Healthy volunteers	Feces	6 total (24hr), 28 total (312 hr)	N.D.	141
	Fexofenadine	PO (^{14}C)	Healthy volunteers	Feces	80 total (~95 of radioactivity as unchanged)	Cl/F=1.10 L/hr/Kg	140,141
	Levocetrizine	PO (^{14}C)	Healthy volunteers	Feces	13 total and 86 unchanged	N.D.	140
	Mizolastine	PO (^{14}C)	Healthy volunteers	Feces	84–95 primarily glucuronidated	Cl/F=0.69 L/hr/Kg	140,141
Antihypertensive	Eplerenone	PO (^{14}C)	Healthy volunteers	Feces (n=8)	32.0 primarily metabolites (96 hr)	N.D.	63
	Ramipril	PO	Patients	T-tube bile (n=8)	~17 total and 0.1 unchanged (24 hr)	N.D.	54

Therapeutic Class	Compound	Administration route	Population studied	Bile collection methodology (number of subjects)	% of dose excreted in bile or feces (collection interval)	Clearance parameters	Reference
	Spironolactone	PO (^{3}H)	Healthy males	Feces (n=5)	22.7 total (5 days)	N.D.	66
	Valsartan	IV	Patients	Biliary-enteric diversion (n=1)	89 mostly unchanged	N.D.	142
Antidiabetic	Rosiglitazone	PO/IV (^{14}C)	Healthy volunteers	Feces (n=4)	21.6 from PO, 25.2from IV (21 days)mostly metabolites	N.D.	143
Immuno Suppressant/Modulator	Leflunomide	PO (^{14}C)	Healthy volunteers	Feces (n=4)	48 (28 days) as methylhydroxy-metabolite (M1)	Cl_{total} for M1=0.051 L/h	144
Immunosuppressant	Mycophenolic acid	PO	Healthy volunteers; patients	Feces (n=4); T-tube bile (n=17)	5.5 in feces; 16.5–26 in bile as glucuronide conjugate, <0.1 unchanged in bile	Cl_{total}=8.5–11.6 L/min	4
	Tacrolimus	[e]IV/PO (^{14}C) [f]IV	[e]Healthy males; [f]patients	[e]Feces (n=6)	[e]0.29 (IV) and 0.12 (PO) unchanged, [e]5.4 (IV) and 92.6 (PO) total (11 days)	[f]Cl_{total}= 0.3–5.4 L/hr/Kg	[e] 69 [f], 145
Gastrointestinal Stimulant	Metoclopramide	PO	Patients	Nasobiliary tube (n=10), repeated with restored enterohepatic recirculation	<1 as unchanged and conjugated metabolites	Cl/F= 0.5 L/hr/Kg	146
Antiarrhythmic	Digoxin	PO	[g]Healthy Males; [h]patients	Intestinal perfusion(n=6)		[g]Cl_{total}=255 Cl_{bile}= [g]72–163 or [h]79–343 or [I]58–142 mL/min	[g], 147 [h], 12 [I], 11
Antipsychotic	Quetiapine	PO		Feces	21 total, < 1 of total radioactivity as unchanged (168 hr)	Cl/F=86–105 L/hr	148
Skeletal Muscle Relaxant	Cyclobenzaprine	IV and PO (^{14}C)	Healthy volunteers	Feces (n=5, PO) (n=3, IV)	13.5 (PO) and 15.1 (IV) primarily as glucuronide (120 hr)	N.D.	149
Antifungal	Ketoconazole	PO (^{3}H)	Healthy males	Feces (n=3)	57 total, 20–65 of total radioactivity as unchanged (4 days)	N.D.	150
Benzodiazepine	Lormetazepam	PO	Patients	Nasobiliary drainage bile (n=5)	0.3–2.8 total (24 hr)	Cl_{total}=1.9–6.2 mL/min/Kg	26
Analgesic Opioid	Methadone	PO (acute, intermediate, chronic administration)	Post-addict volunteers	Feces (n=12)	3.0, 7.4, 20.1 total and 0.14, 0.47, 1.2 unchanged (acute, intermediate, chronic, respectively)	N.D.	151
Anti-asthma	Montelukast	PO (^{14}C)	Healthy volunteers	Duodenal secretions suction at 2–8 and 8–12 hr post dose (n=6)	3–20 total	N.D.	152
Antiretroviral	Lopinavir	PO (^{14}C in combination with ritonavir)	Healthy volunteers	Feces (n=5)	82.6 total, 19.8 unchanged (0–8 days)	N.D.	153
	Ritonavir	PO (^{14}C)	Healthy males	Feces (n=5)	86.3 total, 33.8 unchanged (6 days)	N.D.	154
Antibiotic	Azlocillin	IV	Healthy volunteers	Duodenal perfusion (n=5)	3.4 as unchanged	Cl_{total}=173–375	71
	Ceftriaxone	IV	Healthy males	Duodenal perfusion for 6–8 hr (n=5)	11–65 total	Cl_{bile}=1.4–25.9 mL/min Cl_{total}=9–20	155
	Mezlocillin	IV	Healthy volunteers	Duodenal perfusion(n=5)	29.9 unchanged	Cl_{bile}=1–13 mL/min Cl_{total}=187–477 Cl_{bile}=45.8–122.8 mL/min	71
	Piperacillin	IV	[j]Cholecystectomy patients; [k]healthy volunteers	[j]T-tube (n=5); [k]duodenal aspiration (n=3)	[j]0.7 as total (12 hr); [k]0.41/1.1 unchanged/corrected by EF (6 hr)	[j]Cl_{total}=276 [j]Cl_{bile}=1.74 mL/min [k]Cl_{total}=2.9 [k]$Cl_{biliary}$=0.012 [k] *in vivo* $Cl_{biliary}$=0.032 mL/min/Kg	[j], 48 [k], 84

Therapeutic Class	Compound	Administration route	Population studied	Bile collection methodology (number of subjects)	% of dose excreted in bile or feces (collection interval)	Clearance parameters	Reference
	Quinupristin/Dalfopristin	IV	Healthy volunteers	Feces (n=24)	74.4–77.5 total, <15 unchanged	Cl_{total}=0.83–0.87 Cl_{total}=0.85–0.82 L/hr/Kg	156
	Temocillin	IV	Healthy volunteers	Duodenal perfusion(n=6)	2.2 unchanged (6 hr)	N.D.	70
	Trovafloxacin	PO (^{14}C)	Healthy volunteers	Feces (n=4)	63.3 total, 43.2 of total radioactivity as unchanged	N.D.	157

IV= intravenous administration

PO= oral administration

EF= gallbladder ejection fraction

in vivo $Cl_{biliary}$= biliary clearance corrected for EF