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Prediction of human pharmacokinetics—biliary and intestinal clearance and enterohepatic circulation

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Abstract

The main objective was to evaluate and propose methods for predicting biliary clearance (CL_{bile}) and enterohepatic circulation (EHC) of intact drugs in man. Another aim was to evaluate to role of intestinal drug secretion and propose a method for prediction of intestinal secretion CL (CL_i). Animal data poorly predict the CL and CL_{bile} of biliary excreted drugs, and the suggested molecular weight threshold for bile excretion as the dominant elimination route does not seem to hold. Active transport, low metabolic intrinsic CL (CL_{int}) and, as an approximation, permeability (P_e) less than that of metoprolol is required for substantial CL_{bile} to occur. The typical EHC plasma concentration vs time profile (multiple peaks) is demonstrated for many low metabolic CL_{int}-compounds with efflux and moderate to high intestinal P_e and fraction absorbed. Physiologically-based in-vitro to in-vivo (PB-IVIV) methodology with in-vitro intrinsic CL_{bile}-data obtained with sandwich-cultured human hepatocytes has generated 2- and 5-fold underpredictions for two compounds with intermediate to high CL_{bile}. This is despite not considering the unbound fraction. Possible explanations include low transporter activity and diffusion limitations in the in-vitro experiments. Intestinal reabsorption and EHC were also neglected in these predictions and in-vivo CL_{bile} estimations. The sandwich model and these reference data are still very useful. Consideration of an empirical scaling factor and a newly developed approach that accounts for intestinal reabsorption and EHC could potentially lead to improved PB-IVIV predictions of CL_{bile}. Apparently, no attempts have been made to predict CL_i. Elimination via the intestinal route does not appear to be of great importance for the few compounds with available data, but could be equally as important as bile excretion. Net secretion in-vitro P_e and newly estimated in-vivo intrinsic CL_i data for digoxin and rosuvastatin could be useful for approximation of CL_i of other compounds.

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Introduction

Excretion via the biliary and intestinal routes could be important for the elimination of drugs and metabolites from the body. The ability to predict the biliary and intestinal clearance (CL_{bile} and CL_i) of intact drugs in man is therefore valuable in the design and selection processes of candidate drugs (CDs), and for prediction and understanding of the pharmacokinetic (PK) profiles and drug–drug interaction (DDI) potential. An understanding of each of the processes involved in bile and intestinal excretion and intestinal uptake (intestinal reabsorption is an important determinant that should be considered) is necessary to fully comprehend the processes of CL_{bile} and CL_i, and these determinants must be accounted for, either individually or by grouping and approximation, if predictive models are to be developed.

It is common that drugs excreted via the biliary pathway have been metabolized by phase II enzymes within hepatocytes and then transported into bile, and for those which have been directly degraded by phase II enzymes (without intermediate phase I metabolism), intestinal deconjugation and reabsorption as intact substance might occur (Shou et al 2005). A compound that is excreted via bile into the small intestine and then reabsorbed and excreted into bile again (wholly or partly) is said to undergo enterohepatic circulation (EHC) (Roberts et al 2002).

Active transport is a characteristic of bile and intestinal excretion, and transport-related DDIs occur for many excreted drugs (Ho & Kim 2005). Known clinically important bile excretion DDIs include those found for digoxin, pitavastatin, pravastatin

and rosuvastatin (Ho & Kim 2005). Other statins, such as atorvastatin, fluvastatin, lovastatin and simvastatin, have instead been shown to be subject to metabolism-related DDIs (Shitara & Sugiyama 2006). This can be explained by their higher hepatic clearance (CL_H) and passive permeability (P_e), and (thereby) lower degree of excretion.

Predictions of PK in man are commonly done using allometric and physiologically based in-vitro to in-vivo (PB-IVIV) methods, or from molecular properties (Fagerholm 2007a–h). Simple allometry is based on a weak rationale and also performs poorly for prediction of renal, hepatic and total CL (Ward & Smith 2004; Fagerholm 2007a,f). PB-IVIV methods are generally scientifically more sound, and with such approaches it is possible to reach quite good PK predictions for many compounds (Fagerholm 2007a, c, f, g). There are also limitations with PB-IVIV methodology, such as the requirement to include empirical scaling factors to correct for underpredictions of CL_H, uncertainty regarding dispersion and organ extraction models, difficulty in predicting the intestinal uptake of poorly soluble compounds, and uncertainty regarding the actual number of cells and amount of enzymes/transporters involved in drug absorption and disposition (Fagerholm 2007a, e, f). Due to the underprediction potential of CL_H (when excluding empirical scaling factors) there is also a potential to overestimate the impact of renal, bile and intestinal excretion.

The main objective was to evaluate and propose methods for prediction of CL_{bile} and EHC of intact drugs in man. Another objective was to evaluate the role of intestinal secretion and propose a method for prediction of CL_i.

Methods

The literature was searched for studies where bile and intestinal excretion data in man and animals have been estimated or predicted. To eludicate the requirements and difficulties, and to propose potential ways towards improved CL_{bile} predictions, factors determining bile excretion and CL_{bile} were evaluated.

Results and Discussion

Evaluation of factors determining biliary and intestinal clearance

Physiological, PK and clinical implications of bile excretion and EHC have been excellently presented by Roberts et al (2002).

Determinants of CL_H, such as unbound fraction (f_u) in blood ($f_{u,bl}$), metabolic intrinsic CL (CL_{int}), flow rate (Q_H) and convection/mixing of liver blood, hepatocyte P_e and surface area (S), and drug concentration are also important for the CL_{bile}. CL_{bile} is also determined by the stability of drugs and phase II metabolites in bile and intestinal fluids, P_e across the bile duct epithelium and intestinal wall, and flow rate of bile and intestinal contents.

For significant bile excretion to occur a compound must have a sufficiently high P_e to be absorbed into the liver and then into bile, and a comparably low metabolic CL_{int}. If the intestinal P_e is too high then the bile-excreted compound will eventually be completely reabsorbed by the intestine (bile excretion will then only be a part of distribution). Highly permeable compounds excreted into the intestine via bile are expected to be more rapidly and extensively absorbed than low P_e compounds, and (consequently) to have more distinct additional (EHC) peaks in the systemic circulation.

A high passive P_e also increases the potential for a compound to be redistributed from hepatocytes back to blood and, thereby, escape potential transport into bile. Passive transport of a compound from the inside of hepatocytes back to blood is favoured by the higher S and P_e of the sinusoidal membrane (blood side), and sink conditions (provided by a high Q_H and binding to blood components), whereas passive transport in the bile direction is limited by the smaller S and lower passive P_e of the canalicular membrane (bile side), slow bile flow rate (less than a per cent of the Q_H) and potentially high drug concentration in bile (compounds may concentrate up to more than 1000-fold in bile (Rowland & Tozer 1995)). The S of the sinusoidal and canalicular membranes of hepatocytes have been estimated to be 15–37% and 13% of the total S, respectively (Weibel et al 1969), and the canalicular membrane has a 2-fold higher cholesterol content than the sinusoidal membrane (Meier et al 1984). Cholesterol has a condensing effect on the acyl region of lipid bilayers and makes membranes more rigid and less permeable (for passive transport) (Meier et al 1984). Antipyrine is a highly permeable (mainly passive P_e) compound with complete gastrointestinal uptake, low metabolic CL_{int} and high f_u (0.97) (Fagerholm et al 1996; Shibata et al 2002). Its oral bioavailability is near complete (0.96) and appearance in the circulation rapid (Shibata et al 2002), which demonstrates that transport into bile (and EHC) probably is negligible in comparison with transport (diffusion) back to blood. This is despite the low binding capacity in blood.

Sinusoidal and canalicular membranes also have their own specific transporters and functions and many of these are involved in the hepatic uptake and bile excretion of drugs (Roberts et al 2002; Chandra & Brouwer 2004). Bile excretion seems to require active transport across at least the canalicular membrane. The rate-limiting step in bile excretion could be either the sinusoidal absorption or the canalicular secretion. As for metabolic CL_{int}, interindividual differences and regulation/interactions of active hepatic/bile transport occur and this makes predictions more complicated.

Compounds transported across the canalicular hepatocyte membrane enter the bile. Bile is formed primarily by hepatocytes, released into the canalicular space between two hepatocytes, and then sporadically secreted into the upper small intestine at an average rate of 0.5–0.8 mL min^{-1} (Rowland & Tozer 1995). The pH of bile averages about 7.4 (Rowland & Tozer 1995). The bile ducts occupy <1–2% of the liver volume (Brauer 1963). The epithelium of the bile duct is a potential route for drug and metabolite transport (Boyer 1996). Transport of bicarbonate and ions from blood via bile-duct epithelial cells to bile has been shown (Boyer 1996). Little is known, however, about transport of drugs directly from bile to the blood stream (and vice versa). High P_e of a compound would facilitate such transport.

There are anatomical, physiological and biochemical differences between species that make interspecies extrapolation difficult. These include differences in expression and activity of hepatic/bile and intestinal transporters, and metabolizing enzymes (Ishizuka et al 1999; Suzuki & Sugiyama 2000; Roberts et al 2002; Shitara et al 2005; Hilgendorf et al 2007; TP-search transport database, 2007). Animals and man often differ with regards to metabolic CL_{int}, hepatic, renal and total CL, and intestinal and hepatic uptake of compounds with pronounced active transport (Sandker et al 1994; Bogaards et al 2000; Clarke & Jeffrey 2001; Ward & Smith 2004; Fagerholm 2007a, b, e, f). On this basis it is doubtful whether animal data are useful for prediction of CL_{bile} and CL_i in man. Biliary excretion data (% of dose excreted in bile) for 7 compounds (including 3 glucuronides) with molecular weight (MW) between 288 and 794 g mol^{-1} indicated that rats and dogs are good excreters and rabbits, guinea-pigs and monkeys are poor excreters (Lin 1995). Human data were obtained for two high-MW substances, and both had a high % excreted. This parameter is dependent on other elimination routes and CL data appear to be lacking. Therefore, little is known about the CL_{bile} values of these compounds and overall bile excretion potential in the different species.

CL_i is determined by the intestinal secretion CL_{int} ($CL_{int,i}$), $f_{u,bl}$, intestinal metabolic CL_{int}, intestinal mucosal Q (Q_i; ~250 mL min^{-1}; Fagerholm 2007e) convection/mixing of intestinal blood and drug concentration. The overall $CL_{int,i}$ is influenced by the amount/fraction of mature enterocytes involved (functionally mature cells are found at the villi tips), and the villous counter-current exchange system (which enables transport of molecules from arterioles to venules without accessing the enterocytes) (Fagerholm 2007e). Important intestinal efflux proteins, such as MDR1 (P-glycoprotein), MRP2 and BCRP, are not localized on the blood side of enterocytes (TP-search transport database, 2007), which indicates a potential for limited active intestinal active secretion capacity. CL_{bile} is also determined by the intestinal reabsorption capacity. Due to a continuous intestinal secretion/reabsorption process distinct multiple peaks in plasma are not anticipated following reabsorption.

Methods for prediction of bile excretion and clearance

Due to the difficulty in obtaining human in-vivo CL_{bile} data, and that human and animal CL_{bile} data of intact compounds are not obtained routinely (and for scaling purposes) within the pharmaceutical industry, there is a limited amount of data on bile excretion and CL_{bile} of drugs and CDs.

Approaches used to predict bile excretion and CL include MW threshold, allometry and PB-IVIV. MW-threshold and allometry approaches seem to be based on poor rationales and do not perform well. PB-IVIV methodology has recently been applied with reasonably good results. A drawback with these PB-IVIV predictions is that factors such as intestinal reabsorption, EHC and binding to blood components were not considered. On this basis, suggestions how to improve PB-IVIV predictions of bile excretion potential, CL_{bile} and EHC in man are presented.

Molecular weight threshold for bile excretion potential

It has been suggested that bile excretion has a MW threshold (biliary excretion in man predominant pathway if the MW > 500–600 g mol^{-1}), and that different species have different thresholds (MW > 200–325, > 400 and > 475 g mol^{-1} in rats, guinea-pigs and rabbits, respectively) (Lin 1995, 1998; Roberts et al 2002). If this is actually true it means that MW is an important determinant for CL_H and intestinal fraction absorbed ($f_{a,i}$), compounds with MW above the thresholds have minor hepatic and renal CL, and different species have different uptake and efflux capacities. Based on the current knowledge about metabolism, renal excretion, intestinal absorption and species similarities/differences, such a generalization appears doubtful. Determinants such as active transport, CL_{int} and f_u do generally not appear to be strongly related to MW (at least not for compounds of normal size for drugs) (Bogaards et al 2000; Shibata et al 2000, 2002; Clarke & Jeffrey 2001; Fagerholm 2007a, c). Other molecular descriptors have been shown to be important for bile excretion (many drugs excreted in bile contain both polar and non-polar groups) and intestinal reabsorption (Lin 1998; Roberts et al 2002). High-MW compounds generally have low P_e and low renal tubular reabsorption potential and, therefore, renal excretion (≥ glomerular filtration rate • plasma f_u ($f_{u,pl}$)) could potentially be greater than bile excretion for such substances. Compounds shown to be excreted in human bile, such as digoxin (MW 781 g mol^{-1}) and indometacin (MW 358 g mol^{-1}) (Roberts et al 2002), are also excreted unchanged in urine. Renal excretion is the major route of elimination of digoxin (~60% of an intravenous dose is excreted unchanged in urine) (Goodman Gilman 2001; Drescher et al 2003). Bile excretion was not clearly the main route of elimination in the pig either (44% of an intravenous dose is excreted unchanged in bile) (Tannergren et al 2006). Less than a percent of orally dosed erythromycin (MW 1056 g mol^{-1}) and intramuscularly administered novobiocin (MW 612 g mol^{-1}) was recovered (as drug + metabolites) in bile within 12 h (Rollins 1984). Considering the intestinal reabsorption potential, there are strong relationships for passive intestinal P_e and f_a between man and rats, and many compounds with MW > 200–325 g mol^{-1} are well absorbed in several species, including the rat (Fagerholm et al 1996; Chiou & Barve 1998; Zhao et al 2003). A MW of > 500–600 g mol^{-1} generally appears to be associated with a low extent of intestinal uptake and reabsorption in man (Lipinski et al 2001), but it is not a definite cut-off level. Some compounds with considerably greater MW, such as ciclosporin (MW 1202 g mol^{-1}), are well absorbed following oral administration. The intestinal uptake is more strongly related to P_e than to MW. Furthermore, there are many examples of compounds with MW above the proposed thresholds that are extensively metabolized by the liver and eliminated mainly via metabolism (including ciclosporin). Thus, the suggested MW threshold does not have a strong rationale or empirical support.

Predictions from animal data

Mahmood & Sahawalla (2002) and Mahmood (2005) used various allometric approaches to extrapolate the total CL of biliary excreted compounds from animals to man. In the

report by Mahmood & Sahawalla (2002), the eight selected compounds were known to be excreted in bile in at least one species, and available % of dose excreted (including metabolites) data varied between 19 and >98%. Data on % excretion of unchanged drug in man was only available for two compounds – napsagatran and susalimod. When simple allometry was used, the total CL in man was overestimated (+46% to 17-fold) for all compounds. Maximum life-span- and brain-weight-based allometric approaches also overpredicted the CL. Mahmood (2005) used seven other allometric methods (including corrections for species differences in bile flow and UDGPT) for prediction of human total CL of these compounds. No considerable improvement of the alternative allometric scaling methods was observed compared with simple allometry. Results such as these are anticipated based on the known species differences in metabolic CL_{int}, $f_{u,bl}$ and active transport, and the poor rationale and predictability of allometry. Considerable differences in the excretion pattern of digoxin have been observed between man and pigs. CL, CL_{bile} and the fraction excreted in bile in man and pigs have been estimated to be 3.1 vs 8.3 $mL\,min^{-1}\,kg^{-1}$, ≤0.25 vs 3.7 $mL\,min^{-1}\,kg^{-1}$ and ≤8 vs 44%, respectively (Rollins 1984; Drescher et al 2003; Tannergren et al 2006). These results for digoxin demonstrate that the pig is not a reliable model species for predicting drug excretion CL in man.

Physiologically based in-vitro to in-vivo *prediction* PB-IVIV prediction of CL_{bile} requires appropriate methodologies for prediction of in-vitro intrinsic CL_{bile} ($CL_{int,bile}$) and $f_{a,i}$, consideration of $f_{u,bl}$, the use of an appropriate liver extraction model (the well-stirred, parallel-tube and dispersion models are commonly used) and estimates of hepatocellularity and liver weight.

In-vitro *systems.* Suspended hepatocytes (in-vitro) have 3- to 7-fold larger S compared with the in-vivo situation (and sandwich-cultured hepatocytes) (Weibel et al 1969). There is also an unphysiological direct contact between compounds and canalicular efflux proteins when such cells are used. This indicates a potential to mispredict the sinusoidal uptake CL (P_eS), canalicular P_eS and metabolic CL_{int} from data obtained with isolated hepatocytes.

Hoffmaster et al (2004) found that the sandwich-cultured human and rat hepatocytes repolarize and traffic functional canalicular MDR1 and MRP2 to the appropriate cellular domain. The sandwich-cultured hepatocyte method has other apparent advantages over suspended hepatocytes (for studies on bile transport and CL): intact canalicular networks are developed; protein expression and function are retained; and metabolizing capacity decreases more slowly (Liu et al 1999; Chandra & Brouwer 2004; Griffin & Houston 2005). A proposed drawback with this method is that drug diffusion in the collagen layers may affect the metabolic and transport rates (Treijtel et al 2004).

Efflux proteins in the canalicular membrane, such as MDR1, MRP2 and BCRP, also exist as efflux proteins in the human intestine (TP-search transport database, 2007), and therefore it is desirable that the absorption model is able to account for both diffusion and efflux. The Caco-2 and Ussing chamber models/techniques are available for such studies (Lennernäs et al 1997; Ungell et al 1998; Obradovic 2005; Fagerholm 2007b). The Ussing approach has been used to study intestinal absorption, transport and metabolism in the human intestinal mucosa (Söderholm et al 1998; Ungell et al 1998; Sjöberg et al 2000; Berggren et al 2003). Similar P_e values and regional characteristics to those in rat Ussing studies were shown, and both D-glucose and L-dopa had high active and low passive P_e (Sjöberg et al 2000). Furthermore, it was possible to demonstrate that the human intestine in-vitro was viable (monitored using electrical measurements), had functional MDR1-efflux and was able to metabolize testosterone (a CYP3A4-substrate) (Sjöberg et al 2000). Obradovic (2005) showed that human in-vitro (Ussing chamber) small intestinal P_e values for a large set of structurally diverse passively absorbed compounds were well correlated with the in-vivo $f_{a,i}$. Due to comparably low drug amounts in excreted bile (vs those in the intestines following oral dosing) there is also a lower potential for solubility/dissolution problems for excreted compounds.

Predictions in rats. A PB-IVIV method with in-vitro $CL_{int,bile}$ data obtained with sandwich-cultured rat hepatocytes has been used to examine the relationship between in-vitro and in-vivo rat CL_{bile} (quantitated in bile-cannulated rats) of 5 substances (inulin, salicylate, methotrexate, [D-pen^{2,5}]enkephalin and taurocholate) (Liu et al 1999). In-vitro and in-vivo CL_{bile} ranged between 0 and 56, and 0.04 and 117 mL min^{-1} kg^{-1}, respectively. It was assumed that biliary excretion was the predominant elimination pathway, and intestinal reabsorption was neglected. A strong and linear relationship (r^2=0.99) was established between in-vitro and in-vivo CL_{bile}. A slope of ~2 demonstrates an underprediction potential and that an empirical scaling factor is required for obtaining good predictions.

Sasaki et al (2004) estimated the $CL_{int,bile}$ for seven substances in double-transfected Madin–Darby canine kidney II (MDCK II) cell layers that express sinusoidal and canalicular transporters from rats. They used a PB-IVIV approach with the well-stirred model (including $f_{u,bl}$ data) to predict the rat in-vivo blood CL_{bile}, and compared these estimates with in-vivo blood CL_{bile} data. Intestinal reabsorption was not considered. The in-vivo CL_{bile} was not well predicted, but after an empirical scaling factor of 18 had been added the predictions were reasonable.

Rather complex PB models for illustration/description of metabolic and biliary CL in rats have been built by Ploeger et al (2000a) and Liu & Pang (2005).

Predictions in man. Ploeger et al (2000b; see above) used the rat data and model for glycyrrhizic acid (which is metabolized presystemically and undergoes EHC), and built a whole-body PB model (including biliary excretion) that forecasted the PK in man quite well. A variety of PK parameters were included in their model, including transit times, volume, binding, hydrolysis, solubility, dissolution and uptake rate in the gastrointestinal tract, hepatic efflux and hydrolysis rate constants, and f_u. CL_{bile} data were, however, not generated/predicted with this approach.

A human hepatocyte sandwich culture able to produce in-vitro $CL_{int,bile}$ data is available (Bi et al 2006; Ghibbelini et al 2006, 2007). With this cell culture Ghibellini et al (2007)

estimated the human in-vitro $CL_{int,bile}$ and predicted the in-vivo CL_{bile} (not considering intestinal reabsorption) for three biliary excreted substances: sestamibi (low bile excretion ratio (E_{bile}); 0.3), mebrofenin (E_{bile} 0.8) and piperacillin ($E_{bile} < 0.01$). The biliary and urine recoveries, and total, biliary and renal CL for sestamibi were estimated to be 15 and 19%, and 19, 5.5 and 4.6 $mL\,min^{-1}kg^{-1}$, respectively. Corresponding CL_{bile} estimates for mebrofenin and piperacillin were 16 and 0.03 $mL\,min^{-1}kg^{-1}$, respectively. More than 70% of the piperacillin dose was excreted unchanged in urine, demonstrating negligible bile excretion also of this compound. The well-stirred liver extraction method (including hepatocellularity and liver weight estimates) was applied for predictions, but binding to blood components was not considered. Intestinal reabsorption and EHC were not fully considered in these predictions either. In-vivo CL_{bile} estimates were calculated based on the appearance of the compounds in duodenal aspirates (collected with an oro-enteric tube with an inflated occlusive balloon placed in the duodenum). Their PB-IVIV methodology correctly predicted negligible in-vivo CL_{bile}-for piperacillin. The in-vivo CL_{bile} -for the two other compounds were underpredicted by 2.2-(mebrofenin) and 4.6-fold (sesamibi) (despite the neglect of blood component binding). This suggests that the in-vitro transporter activity in these experiments was quite low (down-regulated) and/or that there had been diffusion limitations (previously shown for the sandwich-cultured hepatocyte model; Treijtel et al 2004).

Additional in-vivo *references values.* Proost et al (2000) collected bile from anaesthetized patients via a T-drain (inserted during surgery) and estimated the bile recovery (mean recovery 7%) and bile concentration–time profile (mean half-life 16 h) of intravenously administered rocuronium ($MW = 546\,g\,mol^{-1}$). The plasma CL and $f_{u,pl}$ of this compound is 260 $mL\,min^{-1}$ and 0.54, respectively (Proost et al 2000; Roy & Varin 2004). Using these data, the in-vivo $CL_{int,bile}$, CL_{bile} and E_{bile} were estimated to be 18 and 10 mL min^{-1} and 0.02, respectively. Napsagatran ($MW = 559\,g\ mol^{-1}$) is extensively excreted unchanged in the bile in rats, rabbits, dogs and man (Lavé et al 1999; Mahmood & Sahawalla 2002). Following intravenous administration, 61, 60, 97 and 60% of the dose was excreted unchanged in bile in these species, respectively. Total CL values (17, 245, 405 and 459 mL min^{-1}, respectively) are low to moderate in relation to the Q_H (Lavé et al 1999; Lindstedt & Schaeffer 2002). Based on these data, the CL_{bile} and E_{bile} (calculated based on plasma Q_H; blood cell binding data missing) in man were estimated to be 275 $mL\,min^{-1}$ and 0.30, respectively. It has not been possible to find its human $f_{u,pl}$ in the literature. By using an approximated $f_{u,pl}$ value of 0.5 ($f_{u,pl}$ is 0.33 in rats and 0.52 in dogs) the in-vivo $CL_{int,bile}$ was estimated to be 800 mL min^{-1}. Two other compounds with known bile excretion as unchanged drug in human bile are ethinylestradiol (42% of dose) and susalimod (60% of dose) (Maggs et al 1983; Påhlman et al 1998).

Suggested improvements It is recommended that $f_{u,bl}$, intestinal reabsorption and EHC are considered in CL_{bile} predictions, and an empirical scaling factor for correction for potential underpredictions (as indicated by available data) is probably also required.

The newly developed PCS, a P_e-based classification system that demonstrate the relationships between in-vitro P_e and in-vivo f_a (or fraction reabsorbed) in the human intestines, liver, brain and kidneys, is believed to be useful for prediction of both metabolic (liver and gut-wall) and non-metabolic (renal and biliary) CL and DDIs, and to understand the interplay between drug metabolism and passive/active P_e (Fagerholm 2007h). With the use of predicted CL_{bile} and $f_{a,i}$ and the PCS it is possible to make predictions/approximations of the true CL_{bile} (CL_{bile*}; where intestinal reabsorption is considered; Equation 1) and EHC potential. Fagerholm (2007h) used Equation 1 to simulate the maximum bile excretion potential of hypothetical compounds with various passive and active P_e in the human liver/bile and intestine.

$$CL_{bile*} = CL_{bile} \bullet [(1 - f_{a,i}) + (E_{bile} \bullet f_{a,i} \bullet (1 - f_{a,i})) + (E_{bile}{}^{2} \bullet f_{a,i}{}^{2} \bullet (1 - f_{a,i})) + \ldots\ldots\ldots + (E_{bile}{}^{\infty} \bullet f_{a,i}{}^{\infty} \bullet (1 - f_{a,i}))] \quad (1)$$

$$E_{bile} = CL_{bile}/Q_H \quad (2)$$

P_e, metabolic CL_{int} and predicted or measured substrate potential for the transporters could be used as an initial screen for bile excretion potential. Metoprolol (log D = 0.0) is a compound with high intestinal P_e and f_a (0.98) (Kasim et al 2004; Willmann et al 2004), and this makes it suitable as a reference substance with an upper P_e limit for CL_{bile}. Compounds with a higher intestinal P_e than that are expected to be completely reabsorbed following bile excretion and have zero/negligible CL_{bile}. As expected, many compounds with intermediate to high passive P_e, low metabolic CL_{int} and efflux have the typical EHC plasma concentration vs time profile (multiple-peaks) in man. Examples include amlodipine (Raušl et al 2006), warfarin and cardiac glycosides (digoxin; MDR1- and OATP-substrate) (Roberts et al 2002; TP-search transport database, 2007). Morphine (moderate P_e; MDR1-substrate; significantly conjugated; deconjugation potential in the intestine) and indometacin (high P_e; MDR1-substrate; low CL_{int}) have also been shown to undergo some degree of EHC in man (Roberts et al 2002; TP-search transport database, 2007).

By aiming for the development/selection of CDs with high(er) passive P_e and moderate metabolic CL_{int} it would be possible to avoid excretion CL and related DDIs, and to improve CL predictions (it appears that CL_H and CL can be well/better predicted for such compounds; Fagerholm 2007a, f, h).

Prediction and impact of intestinal excretion and clearance

Available data show that intestinal secretion is not a major route of drug elimination. CL_i values are low, and approximately 10, 20 and 0.2% of the MDR1 substrates digoxin and talinolol and BCRP substrate rosuvastatin (MW 482 g mol^{-1}), respectively, is secreted by the human small intestine (colonic secretion and reabsorption not considered) (Gramatté & Oertel 1999; Greiner et al 1999; Drescher et al 2003; Lin & Yamazaki 2003; Bergman et al 2006; Lennernäs 2007). These findings are in agreement with the absence of MDR1, MRP2 and BCRP in basolateral enterocyte membranes, low Q in the

intestinal mucosa compared with the liver and kidneys (~1/6 to ~1/5) and (anticipated) limited amount/fraction of enterocytes involved. For digoxin, CL_i and CL_{bile} are similar. Approximately 8% of an intravenous digoxin dose is excreted (primarily unchanged) in bile (Rollins 1984) whereas approximately 60% unchanged in urine (Goodman Gilman 2001; Drescher et al 2003). For rosuvastatin the CL_{bile} is approximately 160-fold higher than CL_i (Bergman et al 2006; Lennernäs 2007). A minor role of intestinal secretion (CL_i approximately 2% of total CL and 8% of CL_{bile}) has also been demonstrated for the extensively excreted fexofenadine in the porcine model (Petri et al 2006).

Apparently, no attempts have been made to predict CL_i. Net secretion in-vitro P_e (serosal-to-mucosal P_e – mucosal-to-serosal P_e; obtained with a cell model with relevant efflux proteins) and available in-vivo CL_i and f_u data for digoxin and rosuvastatin could be useful (references) for approximation of CL_i of other effluxed compounds (Equation 3). Based on the fraction excreted by the small intestine (~10%), total CL (230 mL min^{-1}) and $f_{u,pl}$ (0.75) of digoxin (Greiner et al 1999; Goodman Gilman 2001; Drescher et al 2003; Lin & Yamazaki 2003), Q_i (250 mL min^{-1}) and the well-stirred model, its $CL_{int,i}$ was estimated to be ~30 mL min^{-1}. Corresponding data for rosuvastatin are 0.2%, 830 mL min^{-1}, 0.1 and ~15 mL min^{-1}, respectively. Regional differences in uptake and efflux capacity, colonic reabsorption and blood-cell binding have not been considered in these estimations.

$$CL_{int,i} \approx \text{in-vivo } CL_{int,i,ref} \text{ (30 or 15 mL min}^{-1}\text{)} \bullet \text{ net in-vitro } P_e / P_{e,ref} \quad (3)$$

The CL_i of the test substance(s) could then be approximated by using an extraction model including $CL_{int,i}$, $f_{u,bl}$ and Q_i. The CL_i could also be neglected when predicting CL.

Conclusion

This evaluation demonstrates that animal data poorly predict the CL and CL_{bile} of biliary excreted drugs; the suggested MW threshold for bile excretion as the dominant elimination route does not seem to hold; it is possible to obtain reasonably good PB-IVIV predictions of CL_{bile}; the typical multiple peak plasma concentration vs time profile is demonstrated for low metabolic CL_{int} compounds with efflux and moderate to high intestinal P_e and $f_{a,i}$; CL_i could be similar to CL_{bile} (as for digoxin), but is generally of minor importance vs other excretion routes (for the few compounds with available intestinal secretion data). Consideration of intestinal reabsorption (negligible CL_{bile} is expected for compounds with an intestinal P_e and $f_{a,i} \geq$ metoprolol), EHC, $f_{u,bl}$ and an empirical scaling factor could possibly lead to improved CL_{bile} predictions. The newly developed P_e-based prediction approach, PCS, could be valuable in these improvements. It appears that no attempts have been made to predict CL_i. The use of newly estimated in-vivo $CL_{int,i}$ values for digoxin (~30 mL min^{-1}; ≤8% of an intravenous dose excreted by the small intestine; MDR1 substrate) and rosuvastatin (~15 mL min^{-1}; ~0.2% excreted by the small intestine; BCRP substrate), and net secretion in-vitro P_e data of these two drugs and test substance(s) could be useful for predictions of CL_i. CL_i could also be neglected.

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