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Prediction of human pharmacokinetics—gut-wall metabolism

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Abstract

Intestinal mucosal cells operate with different metabolic and transport activity, and not all of them are involved in drug absorption and metabolism. The fraction of these cells involved is dependent on the absorption characteristics of compounds and is difficult to predict (it is probably small). The cells also appear comparably impermeable. This shows a limited applicability of microsome intrinsic clearance (CL_{int})-data for prediction of gut-wall metabolism, and the difficulty to predict the gut-wall CL (CL_{GW}) and extraction ratio (E_{GW}). The objectives of this review were to evaluate determinants and methods for prediction of first-pass and systemic E_{GW} and CL_{GW} in man, and if required and possible, develop new simple prediction methodology. Animal gut-wall metabolism data do not appear reliable for scaling to man. In general, the systemic CL_{GW} is low compared with the hepatic CL. For a moderately extracted CYP3A4-substrate with high permeability, midazolam, the gut-wall/hepatic CL-ratio is only 1/35. This suggests (as a general rule) that systemic CL_{GW} can be neglected when predicting the total CL. First-pass E_{GW} could be of importance, especially for substrates of CYP3A4 and conjugating enzymes. For several reasons, including those presented above and that blood flow based models are not applicable in the absorptive direction, it seems poorly predicted with available methodology. Prediction errors are large (several-fold on average; maximum ~15-fold). A new simple first-pass E_{GW}-prediction method that compensates for regional and local differences in absorption and metabolic activity has been developed. It has been based on human cell in-vitro CL_{int} and fractional absorption from the small intestine for reference (including verapamil) and test substances, and in-vivo first-pass E_{GW}-data for reference substances. First-pass E_{GW}-values for CYP3A4-substrates with various degrees of gastrointestinal uptake and CL_{int} and a CYP2D6-substrate were well-predicted (negligible errors). More high quality in-vitro CL_{int}- and in-vivo E_{GW}-data are required for further validation of the method.

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Introduction

Gut-wall metabolism/extraction can contribute significantly to the elimination of drugs from the human body. Methods for prediction of first-pass (or pre-systemic) and systemic gut-wall extraction ratio (E_{GW}) and clearance (CL_{GW}) in man, and an understanding of mechanisms determining these processes are required/desired for predicting plasma concentration–time profiles and interaction potentials, and for selecting appropriate candidate drugs. To be able to predict E_{GW} and CL_{GW} well (with a physiologically accurate model), data on gut-wall mucosal blood flow rate (Q_{GW}), gut-wall in-vitro intrinsic CL ($CL_{int,GW}$) and unbound fraction (f_u) in blood ($f_{u,bl}$) are needed. It is also required that high quality pre-systemic and systemic in-vivo CL_{GW} and E_{GW} data are available. In addition, local and regional differences in enzyme contents, $CL_{int,GW}$ and permeability (P_e), efflux and reabsorption potentials, absorption region, characteristic of gut-wall mucosal blood flow (mixing/convection), intestinal and mucosal drug concentrations, intestinal distribution and time for absorption, potential for saturation of metabolism, and the fraction and amount of intestinal mucosal cells involved in drug absorption and metabolism need to be known/understood/predictable and considered. These determinants are generally difficult to estimate and/or poorly understood, and this makes predictions of E_{GW} and CL_{GW} problematic. To understand the contribution of the gut-wall metabolism to the overall elimination it is also required that the differences between and similarities of gut-wall and hepatic metabolism are known or predictable.

The objectives of this review were to evaluate determinants and methods for prediction of the first-pass and systemic CL_{GW} and E_{GW} in man, and if required and possible, develop and propose new simple prediction methodology.

Methods

The first step in the process of analysing available prediction methods and developing new methodology was to evaluate/review the factors that determine gut-wall drug metabolism. Suitable reference compounds were required for validation of prediction methods. The literature was investigated to find compounds with available intestinal extraction, metabolism and absorption data in man.

Evaluation of factors determining gut-wall metabolism

Expression of metabolizing enzymes along the intestine

Intestinal mucosal cells (enterocytes in the small intestine; colonocytes in the large intestine) have the ability to metabolize drugs by numerous pathways involving both phase I and II reactions (Lin et al 1999; Wacher et al 2001; Doherty & Charman 2002; Kaminsky & Zhang 2003). Colonocytes have a different expression and generally lower activity of drug metabolizing enzymes than enterocytes, and therefore, these cells are expected to contribute less to drug metabolism (De Waziers et al 1990; Cao et al 2006; van de Kerkhof et al 2006). CYP3A4, the dominant CYP450 in human enterocytes (~80% of total CYP450), is expressed at very low levels in colonocytes (1/40 of intestinal levels) (De Waziers et al 1990; Lin et al 1999; Kaminsky & Zhang 2003; Cao et al 2006; Galetin & Houston 2006; Paine et al 2006), and the expression of this enzyme also varies along the length of the small intestine (Galetin & Houston 2006). Median levels of CYP3A4 in the human duodenum, distal jejunum and distal ileum are reported to be 31, 23 and 17 pmol (mg microsomal protein)$^{-1}$ (Paine et al 1997). A similar trend has been demonstrated for the CYP2C family (including CYP2C9 and CYP2C19), which is the second most abundant CYP450 in the human small intestine (CYP2C9 ~15% of total CYP450) (Lin et al 1999; Galetin & Houston 2006; Paine et al 2006). Many other CYP450s (including CYP1A1, CYP2D6, CYP3A5, CYP2E1 and CYP2J2), and a number of phase II metabolizing transferases (UDP-glycosyltransferase (UDPG), sulfotransferase and glutathione S-transferase) are also present in the enterocytes (Lin et al 1999; Wacher et al 2001; Doherty & Charman 2002; Kaminsky & Zhang 2003; Thörn et al 2005; Cao et al 2006; Galetin & Houston 2006; Paine et al 2006). Thörn et al (2005) showed that CYP2E1 had the highest mRNA expression along the human gastrointestinal (GI) tract (higher than CYP3A4 and CYP3A5). In general, however, there is no clear association between mRNA expression and metabolic activity (Thörn et al 2005). The expression level of UDPG appears to be greater than for CYP3A4, and this enzyme is also present at high levels in the colonic mucosa (Cao et al 2006). van de Kerkhof et al (2006) demonstrated that conjugation (sulfation and glucuronidation) rates in human colonic slices were equal to those in the human small intestine.

Local intestinal expression of metabolizing enzymes

The levels and activity of metabolizing enzymes demonstrate local differences. It has been demonstrated that enterocytes in the crypts between villi contain considerably lower levels of drug metabolizing enzymes (Lin et al 1999). Immature enterocytes in the crypts migrate to the tip of the villi over a period of two to six days, and during this migration, they become functionally mature (Lin et al 1999). In a biopsy sample, these mature enterocytes only represented a fraction of the obtained cells (von Richter et al 2004). Artursson et al (2001) hypothesized that the villi localization of absorption was dependent on the P_e of a compound. They suggested a possibility that highly permeable substances were absorbed mainly from the villi tips, whereas substances with lower P_e had greater potential to diffuse into and be absorbed from a region further down the length of the villi. If such a hypothesis holds, we could expect low P_e compounds (which are to a greater extent absorbed from regions with lower enzymatic activity—small intestinal villi crypts and colon) to have comparably lower first-pass E_{GW}. Despite low/moderate GI P_e and fraction absorbed (f_a) (Borgström et al 1989), the highly hydrophilic terbutaline has a high first-pass E_{GW} (approximated to 0.74; see below). The high E_{GW} suggests that this compound is not absorbed from the villi crypts to any significant extent (opposite of the suggested hypothesis), or that it is absorbed to some extent by crypts by cells with high sulfation capacity. It cannot be ruled out that the P_e of terbutaline (moderate) is too high for significant crypt absorption to occur. The high E_{GW}-value of terbutaline also suggests a minor role of the paracellular pathway for GI absorption of hydrophilic compounds of similar and greater size.

Species differences in expression of intestinal mucosal metabolizing enzymes

Cao et al (2006) demonstrated that man (males and females) and rats (gender not specified) exhibited distinct expression levels and patterns of metabolizing enzymes in the duodenal and colonic mucosa. The expression of CYP3A4/CYP3A9 (rat CYP3A9 corresponds to the human CYP3A4) was greater in the rat (11-fold in duodenum and 193-fold in colon), and the opposite was found for UDPG (12- and 36-fold higher expression in the human duodenum and colon, respectively). The considerable differences in oral bioavailability (F) that have been observed between animals and man could have been due partly to these differences (Sietsema 1989; Cao et al 2006). On this basis, it appears that animal E_{GW}-data are not suitable to use for prediction of E_{GW} and F in man.

Comparison between the intestines and the liver

Compared with the liver, the small intestine has lower weight (0.7 vs 1.8 kg), blood flow rate (0.25 L min^{-1} (Q_{GW}) vs 1.50 L min^{-1}) and total CYP450 enzyme level and activity, and cells with considerably lower P_e and a programmed limited life span (1–2% renewal of cells h^{-1}) (Hultén et al 1977; Alpini et al 1986; Leggett & Williams 1995; Fagerholm et al 1999; Lin et al 1999; Lindstedt & Schaeffer 2002; Lundquist et al 2002; Shin et al 2003; Galetin & Houston 2006). The intestinal

and hepatic forms of CYP3A4 seem to behave similarly (Pelkonen et al 2001), and the intestinal level of the enzyme is estimated to be ~10–50% lower than in the liver (Doherty & Charman 2002). The total amount of CYP3A4 expressed only represents 1% of that in the liver (Paine et al 1997). The small intestine of man has been reported to contain ~1/26 of the hepatic amount of microsomal protein (Paine et al 1997), ~1/10 of the hepatic CYP2C9 content (Läpple et al 2003), ~1/40 of the hepatic CYP2D6 content (Doherty & Charman 2002), and very low amounts of CYP1A1 (Doherty & Charman 2002). Galetin & Houston (2006) found that the in-vitro CL_{int} for CYP2C8-, CYP2C9-, CYP2C19-, CYP2D6- and CYP3A4-substrates with human intestinal microsomes were 4.5- to 50-times lower compared with estimates obtained with human liver microsomes. On this basis, it is expected that the contribution of the small intestine to systemic drug metabolism of most common CYP450-substrates is low/negligible in comparison with that of the liver. This has been confirmed in studies by Paine et al (1996) and Soars et al (2002). Paine et al (1996) estimated the systemic E_{GW} (calculated based on plasma concentration data and plasma flow rate) of intravenously administered midazolam (CYP3A4 substrate with moderate hepatic extraction ratio; E_H) in man to be 0.08. This corresponds to a plasma CL_{GW} of 11 mL min^{-1}, which is only 1/35 of the hepatic CL (CL_H), 385 mL min^{-1} (estimated from an E_H of 0.55 (Paine et al 1996)). In-vitro glucuronidation activity is also lower in intestinal cells than in hepatocytes (van de Kerkhof et al 2006). In contrast, sulfation rates in human proximal jejunal (approximately 250–300% higher) and colonic cells are higher than in hepatocytes. Due to the comparably low intestinal weight, Q and cell P_e, and anticipated small fraction of intestinal cells involved in drug absorption and metabolism, the systemic plasma CL_{GW}-values for sulfated compounds are probably also low in comparison with the corresponding CL_H.

Intestinal mucosal blood flow characteristics

The characteristic of mucosal blood flow (mixing/convection) is not well known, and therefore, the most appropriate extraction model for systemic gut-wall metabolism is unknown. The architecture of the mucosal blood vessel system indicates, however, that it is not as well-stirred as in the liver. This would suggest that the well-stirred model is not appropriate to use when/if the E_{GW} is high. As indicated above, a high systemic E_{GW} is not expected. Therefore, the choice of extraction model (e.g. well-stirred or parallel-tube) would probably not be of any significant importance for the predictions. The microcirculation of the villi (counter-current exchange) enables permeable molecules to diffuse from arterioles to venules without accessing the enterocytes (Lin et al 1999). Such diffusion can prohibit systemic gut-wall metabolism, and it complicates CL_{GW}-predictions.

Other factors that make predictions difficult

Other factors that make prediction of CL_{GW} and E_{GW} difficult include inter- and intra-individual variability in enterocyte metabolism, potential contribution by enterocytes that have been sloughed off from the mucosa, efflux, and different expression of drug transporters in mucosal (for example, P-gp, BCRP and MRP2 are efflux proteins in this membrane) and serosal enterocyte membranes (Lin et al 1999; Wacher et al 2001; Doherty & Charman 2002; Glaeser et al 2002). The expression of P-gp along the human intestine has been shown to increase (opposite of CYP3A4 and CYP2C) (Thörn et al 2005; Galetin & Houston 2006). Gut-wall metabolism could be facilitated by a synergy between drug transporters and metabolic enzymes (for example, between P-gp and CYP3A4) (Lin et al 1999; Wacher et al 2001; Doherty & Charman 2002). A compound with a relatively low enterocyte $CL_{int,GW}$ and comparably low/moderate passive P_e, trapped in an enterocyte circulation process, could therefore (at least theoretically) have a high first-pass E_{GW}.

First-pass gut-wall mucosal extraction

In contrast to systemic gut-wall metabolism, the first-pass extraction is not hindered by binding to blood components, and molecules pass or have to pass through the mucosal cells (paracellular uptake is generally negligible according to data by Fagerholm et al (1999)) before reaching the blood circulation. Substantial first-pass E_{GW}, often similar to or greater than E_H, is demonstrated for some substances, especially substrates for CYP3A4 and conjugating (especially sulfating) enzymes (Thummel et al 1996; Lin et al 1999; Kharasch et al 2004; Mizuma et al 2005). The first-pass E_{GW} and E_H of midazolam, which has a f_u in plasma of ~0.03–0.04 and systemic E_{GW} of 0.08, are estimated to be 0.43 and 0.55, respectively (Paine et al 1996). The first-pass E_{GW} for other CYP3A4-substates, ciclosporin, methadone and verapamil, are estimated to be 0.4–0.6 (depending on estimate used for CL_H), 0.22 and 0.49, respectively (von Richter et al 2001; Wacher et al 2001; Kharasch et al 2004). Salbutamol and terbutaline, which are primarily metabolized to sulfate conjugates, also have high first-pass $E_{GW,}$ and this is also the case for the UGT1A1 probe ethynylestradiol (Borgström et al 1989; Mizuma et al 2005). Based on a f_a value from the GI tract of 0.60, oral F of 0.14, hepatic plasma flow rate of 700 mL min^{-1} and metabolic plasma CL of 82 mL min^{-1} (Borgström et al 1989), the first-pass E_{GW} and E_H of terbutaline are estimated to be 0.74 and 0.12, respectively. As shown above, gut-wall contents of other CYP450s are generally lower than for CYP3A4. Thus, it is anticipated that the first-pass E_{GW} and CL_{GW} of such substrates are low. This has been exemplified by Madani et al (1999), who estimated the first-pass E_{GW} and E_H for the highly permeable CYP2D6 substrate metoprolol to be 0.0085 and 0.48, respectively.

The role of permeability

As demonstrated above, the P_e is or could be an important determinant for gut-wall metabolism. Substances with low/moderate GI P_e are or are expected to be absorbed to some extent from regions with lower enzymatic activity (the colon and possibly from small intestinal crypts). Highly permeable substances are or are anticipated to be absorbed from the small intestine (from upper small intestine if the P_e and dissolution rate are very high) where the enzymatic activity is highest (at least for CYP3A4 and CYP2C), and these also have higher potential to escape systemic E_{GW} due diffusion from arterioles to venules in the villi microcirculation.

Entrapment in an enterocyte circulation could be of importance, especially for compounds with low and moderate passive P_e (assumed to be more sensitive to efflux) and during the first-pass. Permeation, rather than the Q_{GW}, could be the rate-limiting step for systemic enterocyte metabolism. This is because GI mucosal cells have relatively low P_e (e.g. much lower than of hepatocytes). Despite a considerably longer residence time in the intestines compared with the liver (hours vs seconds), the uptake capacity of the intestines appear to be somewhat lower than that of the liver. For example, enalaprilat and atenolol have low and moderate GI f_a (0.1 and 0.5, respectively) (Fagerholm et al 1996; Chiou & Barve 1998), and their hepatic uptake CL (in rats; human data are not available) is estimated to be 20% of hepatic blood flow rate (Q_H) and $> Q_H$, respectively (Schwab et al 1990; Hung et al 1997). Available human in-vivo small intestinal P_e data obtained during Loc-I-Gut perfusion experiments (lumen-to-blood direction; 10-cm intestinal segment length), and small intestinal transit time (3 h) and length (7 m) enable an estimation of the small intestinal uptake CL of various compounds (Davies & Morris 1993; Fagerholm et al 1996). P_e-values of D-glucose (10×10^{-4} cm s^{-1}; measured), metoprolol (1.1×10^{-4} cm s^{-1}; estimated from f_a-data because of uncertainty of measured value), atenolol (0.2×10^{-4} cm s^{-1}; estimated) and enalaprilat (0.03×10^{-4} cm s^{-1}; estimated) predict the small intestinal f_a to be 1.00, 0.75, 0.22 and 0.04, respectively. These values correspond to uptake CL of 135, 22, 4 and 0.6 mL min^{-1} (or 54, 9, 2 and $< 1\%$ of Q_{GW}), respectively. D-Glucose has a very high P_e, much higher than drugs in general (Fagerholm et al 1996). These estimations indicate a high probability that the systemic metabolism is not mainly limited by Q_{GW}. This is supported by a potentially greater mucosal (GI lumen side) than serosal (blood side) surface area and accessibility (due to the villi structure). On the other hand, serosal membranes of enterocytes are reported to be more permeable than mucosal membranes (Fagerholm 1997), which indicates that the systemic enterocyte uptake could be less dependent on P_e than predicted from mucosal uptake data. These data also suggest that the intestinal mucosa, in general, is not expected to contribute significantly to the systemic metabolic CL, at least not for substrates of CYPs 2C9, 2D6, 1A1, and 3A4 with low and moderate CL_{int} (consistent with in-vivo data for midazolam; see above). The systemic CL_{GW} for CYP3A4-substrates with very high CL_{int} and P_e could reach maximally 1/6 (Q_{GW}/Q_H) of CL_H. Substrates for other CYPs are not expected to have high CL_{GW} and E_{GW}. Substrates for metabolizing enzymes specific for the GI mucosa or with comparably high GI mucosal levels and activity could, however, have systemic CL_{GW} of greater relative importance. The P_e and uptake data also suggest that GI mucosal excretion is not anticipated to be of great importance for passive drug elimination. That is because compounds with low/moderate P_e are expected to have low enterocyte uptake and excretion CL, and substances with high P_e are expected to have high re-absorption and counter-current exchange potentials.

Limitations with microsomes

In-vitro $CL_{int,GW}$-data obtained with gut-wall microsomes are probably of limited value for estimation and prediction of gut-wall metabolism. This is because microsomes lack complete sets of membranes to permeate through (P_e could be of great importance for the gut-wall uptake and metabolism), lack complete sets of metabolizing and transporting enzymes and cell components to bind to, and often have reduced metabolic activity (Lavé et al 1999; Obach 2001; Masimirembwa et al 2003; Fagerholm 2007a). As a result, liver microsome in-vitro CL_{int}-data (together with $f_{u,bl}$- and Q_H-data, and a liver extraction model) generally cause considerable underpredictions of the in-vivo CL_H (on average 5- to 9-fold underprediction) and CL (Iwatsubo et al 1997; Ito et al 1998; Obach 1999; Naritomi et al 2001; Ito & Houston 2005; Fagerholm 2007a). Thus, there is a potential to greatly underestimate the metabolic capacity and overestimate the uptake capacity of enterocytes from enterocyte microsome CL_{int}-data. Gut-wall interaction data obtained with microsomes could therefore also be misleading. For example, this could potentially happen for two interacting compounds with different P_e and binding capacities to various cell components. The P_e and binding potential are important determinants for the intracellular concentrations at the metabolic site (and consequently the K_m and CL_{int}). It is, therefore, recommended that microsome in-vitro $CL_{int,GW}$-data are not used for prediction of E_{GW} and CL_{GW}.

Saturation

Saturation of gut-wall metabolism could occur, especially during the first-pass when local drug concentrations in the GI lumen are high (up to mM range), and if the P_e and dissolution rate are very high. Available human data for the highly-permeable verapamil showed that the duodenal wall extraction was not impaired at luminal concentrations above the K_m-values for two metabolic pathways (Drescher et al 2003). This finding could possibly be explained by lower concentrations at the metabolic sites caused by the permeation and cellular binding processes, and a concentration gradient between GI lumen and blood.

Prediction of systemic gut-wall metabolic clearance

According to the evaluation above, it seems reasonable to assume that the systemic metabolic CL_{GW} for most compounds is low. For the moderately extracted and highly permeable CYP3A4-substrate midazolam the gut-wall/hepatic CL-ratio is only 1/35. For most CYP450-substrates (including CYPs 3A4, 2D6, 2C and 1A1-substrates) it can also be assumed that the systemic metabolic CL_{GW} is low/negligible in comparison with the CL_H. The systemic metabolic blood CL_{GW} for CYP3A4-substrates with very high CL_{int} and P_e can reach maximally 250 mL min^{-1} (Q_{GW}), which is 1/6 of Q_H. A high relative CL_{GW} is reachable for permeable substrates for metabolizing enzymes specific for the GI mucosa or with comparably high GI mucosal levels and activity (such as for substrates of sulfating enzymes). As a suggestion and a general rule, CL_{GW} can be neglected when predicting the total CL. Consequently, systemic gut-wall metabolism interactions could also generally be assumed to be non-significant.

Low systemic drug CL_{GW} was also predicted by Soars et al (2002), who used a physiologically-based in-vitro-to-in-vivo (PB-IVIV) approach to predict the human systemic CL for

eight glucuronidated compounds (substances with low/moderate P_e and incomplete GI f_a inclusively). The well-stirred model was applied, human intestinal microsome in-vitro CL_{int}-data were used, and the in-vivo CL_{int} was estimated using the intestinal weight (approximately 2 kg in an adult) and mg microsomal protein per g intestine ($3\,mg\,g^{-1}$). The small intestinal weight has been estimated to be 620 g (Thummel et al 1997). Due to the expected P_e-dependency for intestinal cell metabolism (overprediction potential), use of microsomes (underprediction potential (Fagerholm 2007a)), and assumptions that all small and large intestinal cells are involved in the glucuronidation (overprediction potential), the results could be misleading. The highest predicted systemic CL_{GW} in the study was small, only $3\,mL\,min^{-1}$, which suggested a minor role of systemic gut-wall metabolism. Hepatic CL_{int} and CL-values were 0.5- to 3.3 (median 1.5)-fold and 5- to 37 (median 14)-fold higher than corresponding small intestinal estimates. Despite the uncertainties, the data demonstrated that the contribution by the intestine was low in comparison with that by the liver.

Although predicted CL_{GW}-values might be low/negligible and the gut-wall extraction process is complicated, a PB-IVIV approach could still be applicable. The recommendation is that the parallel-tube model is applied (less stirring of blood than in the liver is anticipated), that $f_{u,bl}$-data are used, and that in-vitro $CL_{int,GW}$-data are obtained with human enterocytes or small intestinal mucosa. A considerable portion of the large amount of enterocytes that are shed from the mucosa into the lumen has retained functionality (Glaeser et al 2002), and such cells could be useful for estimation of the in-vitro $CL_{int,GW}$. It has also been possible to demonstrate that the human intestine in-vitro (Ussing chamber technique) is able to metabolize testosterone (a CYP3A4-substrate) and to have functional P-gp efflux (Sjöberg et al 2000). Both this method and precision-cut intestinal slices could be useful for estimating in-vitro $CL_{int,GW}$ (van de Kerkhof et al 2006). Phase I and II metabolic activity with these two techniques were similar and were retained for up to 3–4 h (van de Kerkhof et al 2006). Advantages with the slice technique are that a lower amount of tissue is required (4 vs 160 mg) and that it is easier to use. An advantage with the Ussing method is that vectorial transport can be studied.

In-vitro data have been used to extrapolate $CL_{int,GW}$ to a whole human small intestine by using a total small intestinal weight of 620 g (Thummel et al 1997). Such an upscaling is problematic, because the small intestinal mucosa does not only consist of mature enterocytes with high and similar activity. Although it is difficult, if not impossible, to approximate the actual weight of intestinal mucosal cells involved in metabolism, the "effective" small intestinal weight for highly permeable CYP3A4-substrates can be approximated by comparing measured ($11\,mL\,min^{-1}$ and 0.08, respectively; see above) and predicted systemic in-vivo plasma CL_{GW} and E_{GW}-estimates of midazolam.

Prediction of first-pass gut-wall extraction ratio

As demonstrated above, prediction of first-pass E_{GW} is of particular importance for compounds degraded by CYP3A4 or that are directly conjugated (sulfated in particular), and Q_{GW}-based models (such as the well-stirred and parallel-tube models) are not applicable for prediction and estimation of this parameter.

Thummel et al (1997) used a PB-IVIV approach to predict the first-pass E_{GW} of 12 CYP3A4-substrates. Intestinal $CL_{int,GW}$-data were obtained using microsome CL_{int}-data and the estimated amount of CYP3A4 in the small intestine. The well-stirred extraction model was applied. The predictions were poor (see Table 1). Average and maximum prediction errors were several-fold and maximum ~15-fold (ciclosporin; underprediction), respectively. Other examples were ~5-fold (0.09 vs 0.49) underprediction for verapamil and a >3-fold (0.575 vs 0–0.2) overprediction for quinidine. This could be explained, at least partly, by the use of microsome data (underprediction potential of metabolic capacity and overprediction potential of enterocyte uptake capacity), the assumption

Table 1 The performances of two methods for prediction of first-pass E_{GW}

Drug	Measured first-pass E_{GW}	Estimated[a] first-pass E_{GW}	Predicted first-pass E_{GW}	Estimated total first-pass E	Hepatocyte in-vitro CL_{int}[b] (units)	GI f_a
Method in Thummel et al (1997)						
Verapamil	0.49	–	0.09	0.78	18	1.00
Midazolam	0.43	–	0.38	0.56	14	1.00
Ciclosporin	–	0.4–0.6	0.033	–	3.5	0.35
Diltiazem	–	~0[c]	0.025	0.59	9	0.92
Quinidine	–	–	0.58	0–0.2	–	0.80
Method presented in this paper						
Verapamil	0.49	–	0.49 (reference)	0.78	18	1.00
Midazolam	0.43	–	0.38	0.56	14	1.00
Bromocriptine	–	0.36	0.40	0.84	37	0.28
Nifedipine	–	0.13	0.15	0.50	5.6	1.00
Diltiazem	–	~0[c]	0.16	0.59	9	0.92
Metoprolol[d]	0.0085	–	negligible	0.61	7	0.98

[a]Estimated based on f_a, F, CL_H and Q_H-data. It was assumed that blood and plasma CL_H were similar, which might not be correct. This adds some uncertainty to the estimated in-vivo E_{GW}. [b]Taken from McGinnity et al (2004). [c]E_H~total first-pass E. [d]CYP2D6-substrate (all other compounds are CYP3A4-substrates).

that f_a was complete (which it was not for all compounds), and that the well-stirred model was not applicable for prediction of gut-wall metabolism in the absorptive direction.

Rostami-Hodjegan & Tucker (2002) proposed to use a hybrid parameter reflecting the absorption CL (estimated to be 20 L h^{-1}, or 330 mL min^{-1}), instead of the Q_{GW}, for prediction of first-pass E_{GW} with the well-stirred model. The suggested uptake CL is, however, a magnitude higher than for compounds in general and more than 2-fold greater than the highly permeable D-glucose (see above). Such an assumption underestimates the impact of P_e on gut-wall extraction.

One possible approach would be to develop a model which would take all the important factors for gut-wall extraction into account, such as the intestinal distribution and absorptive area, the impact of efflux/reabsorption processes, and the number, distribution (along the intestine and villi), and overall metabolic activity of intestinal cells. It could be virtually impossible to know/predict the amount and fraction of intestinal cells involved in absorption and metabolism, and to estimate the average metabolic activity of these cells, and therefore it was decided to take another approach based on data for reference substances with known absorption characteristics and gut-wall metabolism/extraction. A PB_IVIV scaling approach was developed, taking the fraction of the GI f_a that is estimated to be absorbed by the small intestinal mucosa ($f_{a,si}$; fraction of absorbed drug that may undergo small intestinal extraction) and metabolism/extraction data for reference substances into account (equations 1–3):

$$E_{GW} = E_{GW,ref} \times (CL_{int}/CL_{int,ref}) \times f_{a,si} \quad (1)$$

where $E_{GW,ref} \times (CL_{int}/CL_{int,ref}) > 1$ is set to unity

$$f_{a,si} = \frac{1 - e^{-2 \times Pe \times 3\,h/r}}{1 - e^{-2 \times Pe \times 8.4\,h/r}} \quad (2)$$

$$P_e = \frac{-\ln(1 - f_a) \times r}{2 \times 8.4\,h} \quad (3)$$

$E_{GW,ref}$ and $CL_{int,ref}$ are the first-pass in-vivo E_{GW} and in-vitro CL_{int} for a reference compound with high P_e and complete small intestinal uptake. CL_{int} and $f_{a,si}$ are the in-vitro CL_{int} and predicted $f_{a,si}$ for a test compound, respectively. The P_e is the human small intestinal in-vivo P_e, r is the radius of the small intestine (1.75 cm), 3 h is the small intestinal transit time, and 8.4 h is the effective total GI transit time (small intestinal transit time × correction factor f; 3 h × 2.8) (Fagerholm et al 1996; Fagerholm 2007b). The transit time estimates should be converted to seconds if the P_e unit is cm s^{-1}. The effective total GI transit time is shorter than the actual time (estimated to average 36 h (Davis 1986)), and an explanation is a lower absorptive capacity of the colon for substances with low and intermediate P_e (Fagerholm et al 1997). From equation 2, $1 - e^{-2 \times Pe \times 3\,h/r}$ denotes the f_a from the small intestine, whereas $1 - e^{-2 \times Pe \times 8.4\,h/r}$ denotes the f_a from the whole intestine (Fagerholm et al 1996). The P_e can be estimated from relationships between in-silico, in-vitro or animal in-vivo P_e and human P_e, or alternatively, from predicted f_a in an absorption model and the relationship between this f_a and P_e in man (equation 3) (Fagerholm et al 1996; Lennernäs et al 1996). The $f_{a,si}$ for compounds with complete f_a must be set to 1 (equation 3 does not allow insertion of a $f_a = 1$).

This approach assumes that no gut-wall extraction occurs in the colon, and that the impact of efflux and enterocyte recirculation is negligible. The f_a of compounds with high passive P_e is or is expected to be unaffected by efflux (such as shown for verapamil (Sandström et al (1998)). The method could be rearranged so that the small intestine is divided into two compartments with 1.5-h transit times, and where the upper part (where the largest portion of the dose generally is absorbed) has twice as high enzymatic activity. The use of several reference substances with different CL_{int}- and E_{GW}-levels (low/moderate/high) and absorption characteristics (rapid/slow/passive/efflux/influx) could also improve the applicability.

The in-vitro CL_{int} is preferably obtained with GI mucosal cells (enterocytes or small intestinal mucosa; see above). Hepatocyte in-vitro CL_{int}-data could also be used, especially for CYP3A4-substrates (because intestinal and hepatic forms seem to behave similarly (Pelkonen et al 2001)). Differences in P_e between enterocytes and hepatocytes and potential dependency of P_e on the metabolism (especially in the small intestinal mucosa) are compensated for by using data for a highly permeable reference substance and by incorporating the $f_{a,si}$-term.

It is recommended that the in-vitro CL_{int} is estimated from the area-under-the curve and amount of substance at the start of the experiments, and not (as is common) from terminal half-life and the physical volume of the incubation medium. This is because the latter approach does not account for cell binding accurately.

Due to the saturation potential at high concentrations during absorption (especially for highly permeable and rapidly dissolving compounds given at high doses) it is important that experiments are performed (if possible) at concentrations that are physiologically relevant (maximal oral dose/GI fluid volume (usually set to 250 mL)).

The CYP3A4- and P-gp substrate verapamil appears to be a suitable reference substance. It is highly permeable (average P_e~4–6 × 10^{-4} cm s^{-1} in the small intestine of man; ~3–4-times higher than for metoprolol), completely and rapidly absorbed despite efflux, moderately extracted by the gut-wall (first-pass $E_{GW} = 0.49$) and has a relatively high in-vitro hepatocyte CL_{int}-value (von Richter et al 2001; Kasim et al 2004; McGinnity et al 2004). Its $f_{a,si}$ is estimated to be > 99% (~80% within 1 h, which indicates that its E_{GW} is mainly determined by the upper small intestinal enzymatic capacity). Compounds with a greater P_e than verapamil are therefore expected to have a $f_{a,si}$ of 1. The $f_{a,si}$ of compounds with a P_e comparable with those of metoprolol ($f_a = 0.98$) and atenolol ($f_a = 0.52$) are approximated to 0.8 and 0.5, respectively. The impact of influx and efflux could be considered (at least to some extent) by estimating the P_e with a model with transport activity.

To validate the method, verapamil data were used and compared with those for other CYP3A4-substrates with high enterocyte or hepatocyte CL_{int} and that have been studied together with verapamil. Four suitable compounds were found in the literature: midazolam, bromocriptine, nifedipine and diltiazem (see Table 1) (Heizmann et al 1983; Chiou & Barve 1998; Goodman & Gilman 2001; Shibata et al 2002; Tolle-Sander et al 2003; McGinnity et al 2004). Hepatocyte

CL_{int}-data for these drugs were found in a report by McGinnity (2004). The first-pass in-vivo E_{GW} for midazolam was measured by Paine et al (1996). Estimates for bromocriptine, nifedipine and diltiazem were based on available GI f_a, F, CL_H and Q_H-data. For these three compounds it was assumed that blood and plasma CL_H were similar (which might not be correct). This adds some uncertainty to the estimated in-vivo E_{GW}, and could explain parts of the prediction errors. The first-pass in-vivo E_{GW} for midazolam (high CL_{int}, high GI f_a), bromocriptine (high CL_{int}, low GI f_a) and nifedipine (low/moderate CL_{int}, high GI f_a) were well predicted (Table 1). Prediction errors were negligible: 0.38 (predicted) vs 0.43 (measured), 0.40 vs 0.36, and 0.15 vs 0.13, respectively. The first-pass in-vivo E_{GW} for diltiazem (low/moderate CL_{int}, high GI f_a) was poor (0.16 vs ~0 (the estimated in-vivo value was actually < 0)), which could have been due to a poor estimation of its in-vivo E_H and E_{GW} (by assuming that plasma CL and blood CL were similar). Nevertheless, the new method correctly predicted low/negligible E_{GW} for this substance.

The proposed prediction method suggests that substrates of other CYP450s have negligible first-pass E_{GW}. The in-vivo first-pass E_{GW} for the highly permeable CYP2D6 substrate metoprolol was estimated to be 0.0085 (Madani et al 1999). Substrates of CYPs 2D6, 2C9 and 2C19 may show genetic polymorphism. Due to the negligible role of these enzymes in gut-wall metabolism, this was most likely without clinical importance for the first-pass E_{GW}.

The results show that the proposed simple methodology has the potential to work well for CYP3A4-substrates with various degrees of GI uptake and CL_{int}, and for substrates of other CYP450s. CYP3A4-substrates with a $CL_{int} < 1/5$ of that of midazolam or verapamil are expected to have a first-pass $E_{GW} < 0.1$. For readily absorbed CYP3A4-substrates with higher CL_{int} than for verapamil and midazolam extensive first-pass E_{GW} (> 0.5) is anticipated.

Terbutaline (first-pass $E_{GW} = 0.74$) could be a useful reference substance for sulfated compounds with moderate P_e. If this compound is used as a reference substance it must be taken into account that it is incompletely absorbed from the GI tract and that gut-wall metabolism could occur along the whole intestine.

Conclusion

It has been suggested (as a general rule) that systemic CL_{GW} can be neglected when predicting the total CL, and that gut-wall extraction data in animals should not be used for prediction of CL_{GW} and E_{GW} in man. Based on rationales and performances of available first-pass E_{GW}-prediction methodology it was assumed that new methodology was required. A new simple first-pass E_{GW}-prediction method, based on human cell in-vitro CL_{int}- and in-vivo first-pass E_{GW}-data for reference substances and CL_{int} for test compounds, and the fractional absorption from the small intestine, was developed. Cell CL_{int}-data (preferably of enterocytes, but hepatocyte-data could also be used) are required for full consideration of the potential impact of permeation and cell component binding. This simple approach compensates for differences in absorption and metabolic activity along the intestine and villi. Other advantages are that it does not require knowledge about the amount and fraction of cells involved in absorption and metabolism, and that it performs well (negligible prediction errors) for a limited number of CYP3A4-substrates with various degrees of GI uptake and CL_{int}, and a CYP2D6-substrate. The highly permeable and moderately extracted verapamil appears to be a suitable reference compound for CYP3A4-substrates. More high quality in-vitro CL_{int}- and in-vivo E_{GW}-data are required for further validation of the method.

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