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Review

Cytochrome P450-mediated metabolism in the human gut wall

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Abstract

Objective Although the human small intestine serves primarily as an absorptive organ for nutrients and water, it also has the ability to metabolise drugs. Interest in the small intestine as a drug-metabolising organ has been increasing since the realisation that it is probably the most important extrahepatic site of drug biotransformation.

Key findings Among the metabolising enzymes present in the small intestinal mucosa, the cytochromes P450 (CYPs) are of particular importance, being responsible for the majority of phase I drug metabolism reactions. Many drug interactions involving induction or inhibition of CYP enzymes, in particular CYP3A, have been proposed to occur substantially at the level of the intestine rather than exclusively within the liver, as originally thought. CYP3A and CYP2C represent the major intestinal CYPs, accounting for approximately 80% and 18%, respectively, of total immunoquantified CYPs. CYP2J2 is also consistently expressed in the human gut wall. In the case of CYP1A1, large interindividual variation in the expression levels has been reported. Data for the intestinal expression of the polymorphic CYP2D6 are conflicting. Several other CYPs, including the common hepatic isoform CYP2E1, are expressed in the human small intestine to only a very low extent, if at all. The distribution of most CYP enzymes is not uniform along the human gastrointestinal tract, being generally higher in the proximal regions of the small intestine.

Summary This article reviews the current state of knowledge of CYP enzyme expression in human small intestine, the role of the gut wall in CYP-mediated metabolism, and how this metabolism limits the bioavailability of orally administered drugs. Possible interactions between drugs and CYP activity in the small intestine are also discussed.

Keywords cytochrome P450; CYP; first-pass metabolism; gut wall metabolism; intestinal metabolism

Introduction

Although a role for the intestine in the metabolism of drugs has been recognised for many years, it is often overlooked or its importance is understated. Instead, the major site of first-pass metabolism of the majority of orally administered drugs is often assumed to be the liver. However, the possibly greater capacity of the liver compared with the small intestine with respect to first-pass metabolism does not detract from the potential of the small intestine to directly metabolise orally ingested xenobiotics prior to systemic uptake, and thus, to reduce their bioavailability. This is because an orally ingested drug must pass sequentially from the gastrointestinal lumen, through the gut wall and then through the liver in order to enter the systemic circulation and become bioavailable (Figure 1).[1]

Oral bioavailability (F_{oral}) is thus the product of the fraction of dose absorbed (F_a), the fraction of the absorbed dose which passes through the gut into the hepatic portal blood unmetabolised (F_g) and the fraction of drug not metabolised in the liver (F_h), as shown in the following equation:[2] $F_{oral} = F_a \times F_g \times F_h$.

Many of the enzymes involved in phase I and II reactions in the human liver have also been detected within intestinal epithelial cells. These include cytochromes P450 (CYPs),[3–5] uridine diphosphate glucuronosyltransferases,[6–8] sulfotransferases,[9,10] acetyl transferases,[10,11] glutathione S-transferases,[3,10,12] esterases,[13] epoxide hydrolase[3,10] and alcohol dehydrogenase.[14]

The CYP enzymes are of particular relevance because they are responsible for the majority of phase-I-dependent drug metabolism and for the metabolism of a huge variety of dietary constituents and endogenous chemicals.[15,16] Intestinal first-pass metabolism has

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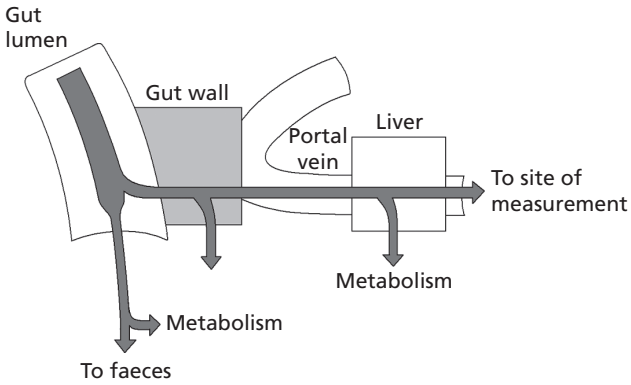

Figure 1 Steps involved in the bioavailability of an orally administered compound (redrawn from Rowland and Tozer[1]).

been shown to be clinically relevant for several drugs, particularly for those that are substrates for CYP3A, such as ciclosporin,[17–19] midazolam,[20–22] tacrolimus,[23] nifedipine,[24,25] felodipine[26–28] and verapamil.[29,30]

The hypothesis that the small intestine plays an important role in first-pass metabolism of orally ingested xenobiotics is supported by the position of the small intestine as the first site of exposure of xenobiotics to metabolic systems and by the large surface area available in the small intestine for absorption and subsequent metabolism.

Physiological and biochemical aspects

Basic physiology and morphology of the small intestine

Anatomically, the small intestine is a thin-walled tube with a physiological length of about 3 m and a gradually decreasing diameter. It is divided into three regions: the duodenum, jejunum and ileum.[31] Although these regions are not anatomically distinct, their functional and histological characteristics differ.[32] The small intestinal wall is made up four basic layers of tissues, as is the majority of the gastrointestinal tract, with certain modifications to support the processes of digestion and absorption. From the lumen towards the interior tissues, these layers are the mucosa, submucosa, muscularis, and either a serosa or adventitia, depending on location (Figure 2).[33]

The small intestinal mucosa itself consists of three distinct layers, namely the epithelium, the lamina propria and the muscularis mucosae. The muscularis mucosae, the deepest layer, is a continuous thin sheet of smooth muscle that separates the mucosa from the submucosa. The lamina propria is a structural support for the epithelial cells, containing blood capillaries, lymph vessels and nerve fibres. The third layer, the epithelium, faces the gut lumen and consists of a continuous monolayer of epithelial cells.[34–36] Several macro- and microscopic features greatly increase the total surface area available for absorption: the circular folds, the villi and the microvilli. The circular folds (also called valves of Kerckring/plicae circulares), 10 mm permanent ridges in the mucosa,[35] increase the surface area of the small intestine relative to the geometrically derived area of the wall by a factor of 3.[37] The villi, which are mucosal projections that extend into the lumen,

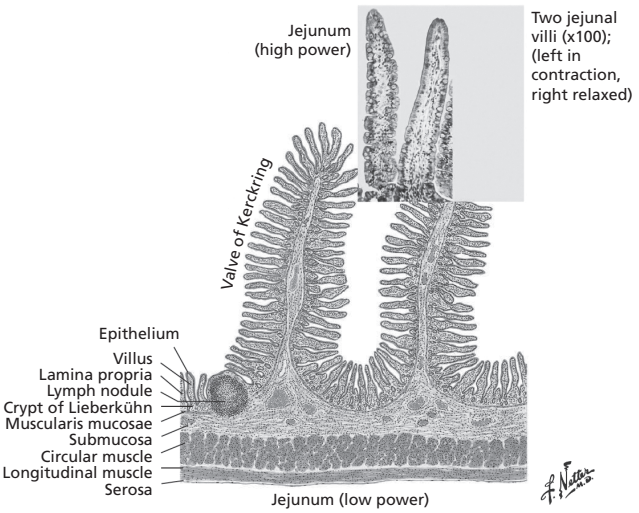

Figure 2 Small intestine microscopic structure. (Selected from the original artist's image.[33] Netter illustration used with permission of Elsevier Inc. All rights reserved.)

vary in both height (~500–1000 μm)[32,35,38,39] and form in different regions of the small intestine. The villi in the duodenum are short and leaf-like; the villi in the jejunum are rounded, finger-like projections whereas those in the ileum tend to have a club-like form. They are typically taller and more numerous in the jejunum than in the ileum.[31,32,38,40–42] It has been estimated that the presence of villi (20–40 per mm^2) increases the surface area tenfold compared with a simple cylinder.[35–37,39,43] As shown in Figure 2, the villi have a single columnar epithelial cover and a core of highly cellular reticular connective tissue, the lamina propria. The villus, which serves as an absorptive unit, is supplied by a single eccentrically located arteriole that passes to the villus tip. There, it breaks up in a fountain-like pattern, from which small capillaries are formed, which then drain into a villus venule.[44–46] At the base of the villi reside simple tubular invaginations, called crypts of Lieberkühn, from which the epithelial cells originate and differentiate. The surface epithelium consists of a sheet of heterogeneous cells which includes some mucus-secreting goblet cells, endocrine cells, Paneth cells and M cells. The dominant cell type on the villi is the enterocyte or absorptive cell, which is responsible for the majority of the digestion and absorption of drugs and nutrients in the human small intestine and contains the metabolic enzymes, including CYPs.[47,48] At their luminal side, the enterocytes possess densely packed fine extensions, the microvilli, which create a brush-like border and greatly increase the surface area by another factor of 20.[31,36,37,43] There are an estimated 200 million microvilli per mm^2 of small intestine.[35]

Unlike hepatocytes, which are intended to restore the liver mass and regenerate only when untimely cell death occurs,[32,49] the epithelial cells of the intestinal mucosa have a programmed limited life span. In general, the cells in the crypt region are specialised as production zones where cell division occurs, whereas the epithelial cells at the tip of the villi are non-dividing functionally mature cells. Enterocytes move from the crypt region upwards to the villus tip, where they are sloughed

off and excreted into the gut lumen at the end of their life span. Approximately 10^{10} cells are shed per day in the human small intestine, corresponding to a turnover time of human intestinal epithelial cells of 2–6 days.[50–55]

Factors that influence gut wall extraction

Apart from the intracellular content of the relevant enzymes, the rate of an enzyme-catalysed reaction is determined in particular by the available substrate concentration. Factors that affect the intracellular residence time and thus the concentration of the drug substance in the cytosol can therefore greatly influence the extent of drug metabolism in the small-intestine enterocytes.[32]

Absorption

Absorption of orally ingested drugs from the human intestine can occur by two mechanisms: either through the cell (transcellular) or between the cells (paracellular), shown in Figure 3.[56] Transcellular absorption of a molecule can take place by a passive mechanism, can be mediated by a specific carrier or can occur via endocytosis.

The most frequent route of absorption, especially in the case of lipophilic drugs, is passive diffusion through the cell membrane.[57–59] Transcellular absorption from the gut lumen to the blood requires uptake across the apical membrane, followed by transport across the cytosol, then exit across the basolateral membrane into the blood. According to Fick's first law of diffusion, the driving force for drug transfer is the concentration of the diffusing species in the compartments on either side of the membrane; the net rate of penetration (NP) is: $NP = P \times SA \times (C_{side1} - C_{side2})$, where P is the permeability coefficient, SA is the surface area of the membrane and $(C_{side1} - C_{side2})$ is the difference between the concentrations of drug.[1] Since membranes are not inert barriers, active mechanisms such as carrier-mediated transport can be involved in the transport of drugs across the membrane, and either facilitate or slow down the transport. This carrier-mediated transcellular pathway involves specific interactions between the compound and the carrier and is important for the absorption of some hydrophilic molecules such as L-dopa[60] and some cephalosporins.[61] Endocytosis of compounds is supposed to be minimal in the small intestine and is not a quantitatively significant mechanism for drug absorption in the intestine.[57,62]

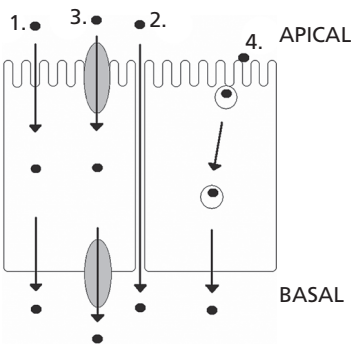

Figure 3 Pathways by which drugs cross the intestinal epithelium: **1** passive transcellular diffusion; **2** paracellular pathway between adjacent cells; **3** carrier-mediated transport; **4** endocytosis (modified from Hillgren *et al.*[56]).

Some substances cannot be absorbed across the cell membranes (i.e. they are excluded from the transcellular pathway). Of these, only small hydrophilic molecules (molecular mass < 100–200 Daltons) are able to diffuse across the tight junctions between the cells,[1,63–65] although absorption by this route is quite limited because the paracellular pathway comprises a relatively small percentage of the total epithelial surface area.[58,64]

The route by which the substance is absorbed is of particular importance, since compounds using the paracellular route will not be metabolised by the intracellular CYP enzymes.[4,57,66,67]

Mucosal blood flow

Several authors have suggested that mucosal blood flow rather than total intestinal blood flow or portal vein blood flow should be used when estimating intestinal metabolism.[67–70] Indeed, from an anatomical point of view, the one-cell-thick epithelial layer that contains the oxidative enzymes is supplied exclusively by the mucosal blood flow. Since the drug enters the enterocytes from the luminal side, the mucosal blood flow is not involved in the delivery of drug to the site of intestinal metabolism after oral administration, but it highly influences the intracellular residence time and hence the time during which the drug is exposed to the intracellular enzymes.[67] Thus, an increase in blood flow relative to a fixed intrinsic clearance should reduce intestinal first-pass extraction, resulting in increased bioavailability.[32,67,71,72]

The blood supply to the small intestine, proximal portions of the colon and the pancreas is provided by the the superior mesenteric artery, the largest single branch of the abdominal aorta.[73] The blood that courses through the small intestine, constituting about 10% of the cardiac output or 4% of total blood volume,[31,44,74] flows thereafter into the portal vein and subsequently enters the liver. Within the small intestine, the blood flow is further distributed in the various layers of the gut wall. In fasted animals at rest, approximately three-quarters of the blood perfusing the gut wall is distributed to the mucosal layer, and approximately 60% of mucosal blood flow perfuses the vessels that terminate as end loops supplying the epithelial cells in the intestinal villi. The remaining 40% of mucosal blood flow supplies the crypts and goblet cells.[44,73]

In each region of the small intestinal wall, as well as in each layer, the blood flow is related to the metabolic demands and the functional activity of the cells. A number of factors, such as neural, humoral and metabolic mediators, serve to regulate the blood flow to meet the tissue's need for delivery of oxygen and nutrients and removal of waste. The presence of food in the gut lumen initiates and stimulates intestinal absorption, secretion and motility via local nerves and chemicals. Of particular interest is the observation that after a meal, blood flow increases by as much as 30–130% of basal flow, depending on the composition of the chyme. In dogs, this increase in blood flow during nutrient absorption has been demonstrated to be diverted to the mucosal layer in particular – the site of nutrient absorption and drug metabolism.[32,44,73,75]

Protein binding

Another factor that may influence the extent of gut wall metabolism is the fraction of unbound drug presented to the metabolising enzymes. However, the evidence on the impact of plasma protein binding on the extent of intestinal first-pass metabolism is conflicting.[32,67,72,76–80] Recently, Fisher & Labissiere[77] reported that in one of their early studies plasma protein binding corrections were applied to systemic clearance, but were not required for predictions of intestinal midazolam extraction in cynomolgous monkeys, since the drug has not yet contacted plasma binding components during absorption through the outer mucosal enterocyte layer containing the active CYPs. Hall and colleagues hypothesised that a high degree of protein binding would modulate the clearance within the enterocytes when the drug is delivered to the site of metabolism via the systemic circulation, since protein binding limits diffusion out of the capillary; however, it would not influence enterocyte metabolic extraction when the drug is presented to the enzyme via direct absorption from the intestinal lumen.[78] They concluded that the rate of CYP3A-dependent metabolism of midazolam by enterocytes is a function of total intracellular drug concentration rather than a concentration reduced by the plasma-free fraction. Likewise, in recent work by Yang and co-workers, the prediction of gut wall metabolism of several CYP3A substrates by their so-called 'Q_{Gut} Model' was clearly improved when the free fraction was assumed to be unity.[72] On the other hand, studies with CYP3A4-expressing Caco-2 cell monolayers supported a role for serum protein binding in determining the extent of intestinal first-pass extraction of saquinavir, an extensively metabolised and actively secreted HIV protease inhibitor.[79] In summary, whether or not plasma protein binding influences the rate at which a drug passes through the cell, and thus has an impact on gut extraction, is unclear and requires further examination.

The cytochrome P450 system

A spectrum of biotransformation reactions occurs in the mammalian body, the most common of which are oxidations catalysed by the CYP system, a superfamily of haem proteins located mainly on the membrane of the endoplasmic reticulum and the inner mitochondrial membrane of cells.[67,81–86] Structurally, the CYP isoenzymes are classified into families and subfamilies based on their amino acid sequence similarity.[82,87] In this system, CYP proteins that have more than 40% amino acid identity are placed in the same family, designated by an Arabic numeral. If the sequences are more than 55% identical, the enzymes belong to the same subfamily, indicated by a capital letter. Finally, each individual enzyme is represented by an Arabic numeral after the letter. The gene associated with the enzyme is denoted in italics.[87,88] Humans have 57 putatively functional genes and at least 58 pseudogenes, divided among 18 families of *CYP* genes and 43 subfamilies; only a relatively small number of the encoded proteins significantly contribute to the metabolism of drugs.[83,84,89,90] It appears that in humans, 15 CYP enzymes are primarily involved in xenobiotic metabolism, virtually all of them being from three main P450 families: CYP1, CYP2 and CYP3.[85–87] Metabolism by the CYP system is of particular importance because the vast majority of drugs, drug candidates and preclinical candidates are substrates of CYPs. Collectively, approximately 80% of oxidative metabolism of commonly used drugs can be attributed to the CYP enzymes.[16,86,89,91] Usually, biotransformation results in more hydrophilic and less biologically active compounds, which are easier to excrete. However, this strategy fails in a number of cases, for example when biotransformation yields a more lipophilic and/or more reactive metabolite.[92,93]

In addition to drug metabolism, CYP enzymes are important in the oxidative, peroxidative and reductive metabolism of endogenous compounds such as steroids, bile acids, fatty acids, prostaglandins, biogenic amines and retinoids.[86,87,94]

Intestinal cytochrome P450 enzymes

The considerable absorptive function of the human small intestine provides the framework for an enhanced metabolic role of this organ, constituting an important barrier to the systemic uptake of xenobiotics. Although the liver is generally the organ with the highest drug-metabolising activity,[3,95–97] the enterocytes in the epithelium of the small intestine are also a potentially important site of drug metabolism and may considerably influence the general metabolism, activity and carcinogenicity of xenobiotics. Advances in protein purification and immunochemical methods in recent decades have facilitated the characterisation and quantification of individual CYP isoenzymes even in tissues with lower abundance of individual enzymes.

To date, several attempts have been made to quantify the CYP content in human small intestine, revealing a mean protein content ranging from 20 to 210 pmol/mg, which indicates significant variability in intestinal CYP expression.[4,5,80,98] The distribution of CYPs along the length of the small intestine is not uniform.[3,96,98] Total CYP content, as a function of microsomal protein, slightly increases from the duodenum to the jejunum and then decreases toward the ileum. Furthermore, the expression of CYP enzymes varies within the small intestinal villus, with the highest concentration found in mature enterocytes lining the villus tip, the main site of absorption for orally administered compounds. The goblet cells and the epithelial cells of the crypts between the villi contain considerably lower levels of drug metabolising enzymes.[99–103]

The procedure of correcting for the cytoskeletal protein villin, which serves as an internal standard for enterocytes, is frequently used to normalise the content of other proteins with respect to factors such as depth of biopsy and proteolysis.[104] Nevertheless, caution should be exercised when comparisons of enzyme protein and catalytic activity are made, since the studies often vary in the source of the intestinal tissue (e.g. individual vs pooled), the segment of the small intestine used, and the method used to isolate the enterocytes.[105–108] As a result, metabolic rates obtained with different methods are usually expressed in different units: data from studies with precision-cut slices are typically expressed per mg intestinal tissue protein; data from microsomes prepared after scraping are usually expressed

per mg of mucosal microsomal protein; data obtained with microsomes prepared after elution are expressed per mg of enterocyte microsomal protein.[109] To adequately compare results obtained in different studies, however, the data must be expressed in the same units.

CYP3A

It has become apparent over the past 20 years, and most notably during the past 10 years, that CYP3A (i.e. CYP3A3/CYP3A4, CYP3A5, CYP3A7 and CYP3A43[84,110,111]) is the predominant CYP subfamily in the small intestine, accounting for 70–80% of total intestinal CYP content.[3–5,80,96,98–101,104,112] Total CYP3A protein content in microsomes prepared from mucosal scrapings obtained from proximal regions of 31 human donor small intestines averaged 50 pmol/mg microsomal protein and ranged from 18 to 151 pmol/mg, representing the most abundant CYP subfamily in each of the donors.[4] The CYP3A protein content obtained in jejunal homogenate protein by Lin and colleagues averaged 19 pmol/mg homogenate protein in 31 samples.[113] Along with total CYP content, the distribution of the CYP3A subfamily is not uniform along the small intestine, being generally higher in proximal regions of the small intestine.[3,78,80] CYP3A content in intestinal microsomes prepared from mucosal scrapings from 20 full-length human small intestines was found to be 30.6 pmol/mg in duodenum, 22.6 pmol/mg in jejunum and 16.6 pmol/mg in ileum and exhibited large interindividual variability, ranging from less than 3 to 91 pmol/mg in the duodenum, from 2 to 98 pmol/mg in the jejunum and from less than 2 to 60 pmol/mg in the ileum. Accounting for the median microsomal protein mass within each region of the small intestine, total CYP3A amount was estimated to be 9.7, 38.4 and 22.4 nmoles for duodenum, jejunum and ileum, respectively.[80] The results of Paine and co-workers were in good agreement with the values reported by Schmiedlin-Ren and colleagues, who analysed CYP3A content and midazolam hydroxylation activity in microsomes prepared from mucosal scrapings of paired duodenum and jejunum samples from eight human donors. Microsomal CYP3A content was 41.3 ± 30.5 pmol/mg in the duodenum and 39.7 ± 31.2 pmol/mg in the jejunum.[114] Generally speaking, microsomal CYP3A protein content was strongly correlated with the mucosal intrinsic clearance of the known CYP3A substrate midazolam,[22,78,80,104,114,115] revealing a generally higher metabolic clearance in liver preparations than in the small intestine; within the small intestine, metabolic capacity was considerable lower for the distal portion compared with the proximal small gut. Likewise, the median duodenal, jejunal and ileal microsomal CYP3A content reported by Paine and colleagues represented 44%, 32% and 24% of the median total hepatic CYP3A content, respectively. However, accounting for microsomal protein content and organ wet weight, total intestinal amount of CYP3A protein was quite low compared with the calculated amount of CYP3A protein in the liver (70 nmoles in the small intestine vs 5490 nmoles in the liver).[78,80,116] As a result, the total unbound intrinsic clearance of midazolam for the entire small intestine, estimated on the basis of microsomal intrinsic clearance, microsomal CYP3A content and total regional CYP3A amount, was very low at approximately 1.4% of that of an average human liver (0.21 vs 15.8 l/min).[80] Nevertheless, several studies indicated that the intestinal extraction ratio was similar to that of the liver (e.g 0.43 ± 0.24 vs 0.44 ± 0.14 for midazolam[22]), suggesting that intestinal metabolism can contribute significantly to the first-pass metabolism of orally administered CYP3A substrates.[17,18,20–22,29] The hepatic and intestinal availability of several CYP3A substrates, calculated from in-vivo data, has been summarised recently by Galetin and co-workers, revealing that for certain substrates (e.g. triazolam and tacrolimus), intestinal extraction rivals or even exceeds that of the liver.[106]

CYP3A4

As the major congener among the CYP3A subfamily, CYP3A4 represents the most consistently expressed and most abundant isoenzyme in adult small intestine and liver,[3–5,80,96,98–101,104,108,112,117–120] even though it is generally agreed that *CYP3A4* is not coordinately regulated in these tissues.[22,80,104,113,121] Conflicting results have been published concerning the extent of expression of CYP3A4 in human small intestine at the mRNA, protein and activity levels. As indicated earlier, the conditions of organ procurement and the preparation procedure of intestinal microsomes affect the results concerning the amount of CYP and the catalytic activity. Furthermore, it should be noted that the reported gradient of CYP3A4 expression along the small intestine, as well as up to 30-fold interindividual variability in intestinal CYP3A4 expression, contribute to the inconsistent results reported by different authors.[3–5,21,22,26,80,99–101,104,108,118,119,122,123]

To give some examples, the median CYP3A4 content (17 pmol/mg homogenate protein) among 31 samples of jejunal mucosa obtained by Lin and colleagues was considerable lower than the values reported by Watkins and colleagues (70 pmol/mg microsomal protein) and by de Waziers and colleagues (approximately 160 pmol/mg in the duodenum, 120 pmol/mg in the jejunum and 70 pmol/mg microsomal protein in the ileum).[3,5,32,113] Paine and colleagues reported large interindividual variability, with a CYP3A4 protein content ranging from 8.8 to 150 pmol/mg (mean 43 pmol/mg) in microsomes prepared from mucosal scrapings obtained from the proximal portion of 31 human donor small intestines.[4] The average CYP3A4 content in enterocytes isolated from duodenal or jejunal mucosa found by von Richter and colleagues (76 pmol/mg homogenate protein, corresponding to 210 pmol/mg microsomal protein) was as high as or even higher than the values reported for the intestinal content of the entire CYP3A subfamily.[4,80,113,121] The authors concluded that the high content of CYP3A4 protein obtained in their study could be attributed to the use of isolated enterocytes as opposed to mucosal scrapings; they proposed a factor (2.8 for intestinal CYP3A4 content) to facilitate the comparison of results derived from tissue homogenates with studies that have relied on use of microsomal protein. With this correction, the results were in reasonable agreement with the values obtained in shed intestinal cells with a multilumen perfusion catheter (36 pmol/mg homogenate protein).[107] Nevertheless, the observation of von Richter and co-workers that the

CYP3A4 content in small intestinal specimens was about three times higher than in the liver homogenates obtained from the same subjects[121] conflicts with the current opinion that the specific intestinal CYP3A4 content is in general lower than that of the liver,[3,34,113] or at most shows a range in values that overlaps with those in the liver.[4,5,21,22,80] However, it should be remembered that even though the specific CYP3A4 content in the proximal small intestine is comparable to, or somewhat lower, than that determined in the liver, the estimated total CYP3A4 content in the whole intestine is considerable smaller than that in the whole liver[3,78,80] because of the lower yield of microsomal protein in the small intestine[10,124] and the higher organ weight of the human liver (ca. 1800 g) compared with the small intestine (ca. 650 g).[31] Consistent with the protein levels of CYP3A4, the enzyme activity has also been reported to be higher in the liver than the small intestine even if expressed per mg of microsomal protein.[3,98,104,125,126] For example, erythromycin demethylase activity was closely correlated with the amounts of CYP3A4 in the liver and in the small intestine, being clearly higher in the liver than in duodenum and jejunum and, in turn, higher than in ileum.[3,98] By contrast, the values reported for enterocyte testosterone 6β-hydroxylase activity (3.2 nmol/min per mg) were only slightly lower than the published values for hepatic microsomes (4.5–4.9 nmol/min per mg),[127] indicating that the relative contributions of hepatic and intestinal CYP3A4 to the overall first-pass metabolism vary between medications. However, as discussed earlier, large interindividual variability in both catalytic activity and CYP3A4 expression makes it difficult to determine or even predict the extent to which a given CYP3A4 substrate undergoes intestinal metabolism.[80,104,118,121,126] The reasons for interindividual differences in CYP3A enzyme expression and the current understanding and implications of genetic variation in the CYP3A enzymes have been reviewed by Lamba and coworkers.[111]

CYP3A5

The expression of intestinal CYP3A5 follows a polymorphic pattern, similar to that found in the liver,[104,111,113,118,128–130] resulting in some disagreement concerning the extent of CYP3A5 expression at the mRNA as well as at the protein level. Several authors reported that CYP3A5 mRNA was detectable in all small intestinal probes tested,[100,112,118,119,123] but an absence of CYP3A5 mRNA has also been reported.[99] Later, some of the same authors[100] detected CYP3A5 mRNA in microsomes from all regions of the gastrointestinal tract from a single female human donor. However, the detection of mRNA does not necessarily imply the expression of the corresponding protein, since the mRNA may or may not be translated into protein.[98] Gibbs and colleagues reported that CYP3A5 protein was found in five of 13 liver and three of eight intestinal microsomal preparations, suggesting that CYP3A5 is variably expressed in both tissues.[131] In an earlier study, CYP3A5 protein was readily detectable in five of 20 duodenums, and could be detected with additional experiments in another nine samples, indicating that CYP3A5 is commonly expressed in human small intestine;[104] however, no data were presented. Recently, CYP3A5 protein was readily detected in 11 of 31 human donor small intestines (35%) and represented 3–53% (average 27%) of total CYP3A (CYP3A4 + CYP3A5) content.[4] The mean protein content obtained in the 11 samples with readily detectable CYP3A5 was 16 pmol/mg microsomal protein (range 4.9–25). Similarly, Lin and colleagues reported that of the 31 jejunal samples from Caucasian donors, 87% had a faint or quantifiable CYP3A5 band, ranging from 0.5 (detectable but non-quantifiable) to 19.8 pmol/mg of homogenate protein.[113] Von Richter and colleagues were able to detect CYP3A5 protein in all intestinal samples investigated, but the levels were below the limit of quantification (< 0.25 pmol/mg homogenate protein) in most samples,[121] whereas Zhang and colleagues and Kaminsky and Zhang were unable to detect CYP3A5 protein in any of the samples tested.[98,132] In another study, Western blot analysis of 20 enterocyte preparations showed the presence of CYP3A5 protein along the entire small intestine in four preparations.[80] Interestingly, for the two intestines in which the CYP3A5 band was quantifiable, the CYP3A5-to-CYP3A4 ratio decreased from duodenum to jejunum and then increased in the ileum to values comparable to or greater than those observed for the duodenum. This is consistent with previous reports of CYP3A5 representing the major CYP3A enzyme present in the colon.[26,100,101,118,133]

Because CYP3A5 and CYP3A4 have overlapping substrate specificity, it is difficult to segregate the relative contributions of the two enzymes to CYP3A-mediated metabolism.[134] Lown and colleagues suggested that the presence of CYP3A5 detected in human proximal small intestine did not influence the total midazolam metabolism measured,[104] whereas the characterisation of CYP3A5 genotype and phenotype in a large number of livers and small intestines revealed a better correlation between total midazolam hydroxylation activity and CYP3A content when the contribution of CYP3A5 was included.[113] In addition, intestinal CYP3A5, together with hepatic CYP3A5, has been shown to play an important role in the first-pass metabolism of orally administered tacrolimus.[135]

Nevertheless, several studies indicate that CYP3A4 expression greatly predominates over that of CYP3A5 in human small intestinal enterocytes, suggesting that CYP3A5 plays only a minor role in the intestinal metabolism of CYP3A substrates in most subjects. However, CYP3A5 may still account for inter-individual differences in the clearance of some CYP3A substrates.[80,100,104,111,118,121,129]

CYP3A7

The third major congener of the CYP3A subfamily is CYP3A7, the predominant isoform detected in human fetal liver.[112,136–139] CYP3A7 mRNA has been detected in 64% of 59 duodenal biopsies from white patients aged 1 month to 17 years, but at a much lower level than CYP3A4 and CYP3A5.[140] Likewise, Canaparo and colleagues and Burk and colleagues reported measurable, albeit very low, expression of CYP3A7 mRNA in most of the small intestine samples investigated.[118,141] By contrast, most authors were unable to detect and/or quantify CYP3A7 in human small intestine,[98,100,112,119] suggesting that the contribution of

CYP3A7 to intestinal CYP3A-dependent drug clearance is negligible.[120]

CYP3A43

The least important CYP3A appears to be CYP3A43, being undetectable in human small intestine.[112,120,142] It has been suggested that CYP3A43 is likely to be a pseudoprotein without any function in mammalian cells.[143]

Summary of CYP3A

In summary, the CYP3A subfamily as a whole, and specifically CYP3A4, represents the predominant phase I drug-metabolising species found in humans and thus greatly influences oral drug bioavailability. In addition, differences in the expression and activity of the minor CYP3As in the liver and in the small intestine could account for inter-individual variability in CYP3A-mediated metabolism.

CYP2C

Apart from CYP3A, only a very limited number of CYPs are expressed to a notable extent in human small intestine compared with in the liver.[4,97] The second most abundantly expressed CYP subfamily in human liver and along the small intestine is CYP2C.[3,4,97,98,127] It has been estimated that the CYP2C subfamily accounts for the metabolism of approximately 20% of clinically prescribed drugs.[16,144,145] All common human CYP2Cs (i.e. CYP2C8, CYP2C9, CYP2C18 and CYP2C19) seem to be expressed in human small intestine, at least at the mRNA level.[146] Recently, Paine and colleagues characterised the contributions of individual CYP enzymes to total proximal small intestinal CYP content in 31 donor small intestines. As described above, CYP3A accounted for the majority (~80%) of the 11 P450s examined. CYP2C, the second most predominant isoenzyme, represented approximately 18% of total intestinal CYP content.[4] As for CYP3A4, the content of CYP2C decreased dramatically in the distal small intestine, although the levels of each form varied differently along the length of the intestine.[98] Within the CYP2C subfamily, the most abundant and most important member was found to be CYP2C9, followed by CYP2C19.[4,127,145–149] However, the average microsomal protein contents were considerably lower than those reported for the liver (~8 pmol/mg for intestinal CYP2C9 vs 73 pmol/mg for hepatic CYP2C9 and 1 pmol/mg for intestinal CYP2C19 vs 14 pmol/mg for hepatic CYP2C19).[4,147,150] Both enzymes were readily detected in all 31 donor intestines but showed large interindividual variation. By contrast, CYP2C8 protein was not detected in any of the donors.[4] The results were in good agreement with the observations of Läpple and colleagues in an earlier study, in which the rank order of CYP2C protein expression in samples of proximal small intestine obtained from 15 patients was CYP2C9 (2 pmol/mg protein) > CYP2C19 (1.5 pmol/mg protein) > CYP2C8 (content below the limit of quantification in all intestinal samples) > CYP2C18 (not detected).[148] In accordance with the data of Paine and colleagues, the intestinal CYP2C9 protein content was estimated to be approximately 10-fold lower in the intestine (~2 pmol/mg protein) than in the liver (~20 pmol/mg protein).[148]

Contrary to the above-mentioned observations, Glaeser and colleagues reported CYP2C8 and CYP2C9 protein expression in almost all samples, whereas they were unable to detect CYP2C19 in shed intestinal cells.[107] This discrepancy may have been due to the use of shed enterocytes preparations as opposed to microsomes prepared from intestinal biopsies. It appears that CYP2C18 mRNA is not translated into protein to a significant extent, since CYP2C18 protein has not been found in detectable amounts in human tissues,[146,148] even though CYP2C18 mRNA contributes substantially to the overall CYP2C mRNA expression. In any case, CYP2C18 seems to be less clinically important, since comparatively few substrates of CYP2C18 are known.[144,151] In terms of CYP2C activities, Galetin and Houston suggested comparable intestinal and hepatic catalytic activity (per pmol of CYP enzyme) for CYP2C9 and CYP2C19 after correction for the activity loss in intestinal microsomes due to the enterocyte isolation method.[105] However, most observations suggest that the intestine would make a minimal contribution to the overall first-pass metabolism of CYP2C drug substrates. For example, the activity of CYP2C9 towards the marker substrate diclofenac is clearly higher in the liver than in the small intestine.[127,148] Likewise, the mean V_{max} value for enterocyte *S*-mephenytoin 4′-hydroxylase activity (CYP2C19) was found to be markedly lower than published values for human liver microsomes,[127] and paclitaxel 6α-hydroxylation via CYP2C8 in intestinal microsomes was reported to be negligible.[105,150] But, because of the considerable interindividual variability in CYP2C content and activity, enteric CYP2C might be important in some individuals for substrates with a low oral bioavailability (e.g. fluvastatin).[4,127,144,152,153]

CYP2J

Another isoform readily detectable in human small intestine and several other tissues, including the heart, kidney, placenta and skeletal muscle, is CYP2J2.[4,112,154–158] It has been suggested that CYP2J products are involved in the release of intestinal neuropeptides, the control of intestinal motility and/or the modulation of intestinal fluid/electrolyte transport.[158] Moreover, CYP2J2 generates cardioprotective epoxyeicosatrienoic acids (EETs), the major CYP2J2-mediated epoxidation products of arachidonic acid.[159,160] In-vitro studies have suggested that, in addition to these potential physiological roles, CYP2J2 may contribute to the first-pass metabolism of therapeutic drugs such as astemizole,[161–163] ebastine[164,165] and terfenadine.[163] In contrast to CYP3A and CYP2C content and associated catalytic activity, which tend to be highest in the proximal region and decline progressively toward the distal region, CYP2J2 protein expression is relatively constant throughout the entire gastrointestinal tract from oesophagus to colon.[158] Furthermore, interindividual differences in the intestinal expression of CYP2J2 proteins have been reported to be relatively low. The different pattern of CYP2J2 expression was suggested to be due in part to its lack of response to inducing agents, coupled with its documented expression in other cell types, including the autonomic ganglion cells of nerves and smooth muscle cells.[158] The mean CYP2J2 protein content in proximal human small intestine was 0.9 pmol/mg, being detectable in all 31 samples analysed. However, the low specific content (only 1.4% of total intestinal immunoquantified CYP protein)[4] raises the question as to whether it

really contributes significantly to the first-pass metabolism of drugs.

CYP2D6

Several other CYPs are reported to be expressed in the human small intestine, even though often to a very low extent and often only in some of the individuals tested. CYP2D6, a highly polymorphic CYP isoenzyme,[48,94] is one of the most dominant cytochromes in terms of the number of drugs that are substrates.[16,143,166,167] Conflicting reports can be found in the literature on the role of intestinal CYP2D6-mediated metabolism. Although CYP2D6 was detectable in 29 of the 31 individuals tested, its contribution to total intestinal CYP was minimal (<1%; mean protein content 0.5 pmol/mg microsomal protein). Thus, the authors concluded that the intestinal form is unlikely to play a significant role.[4] Likewise, Madani and colleagues[168] reported that median CYP2D6 content in human jejunal microsomal preparations was less than 8% of median hepatic content (0.9 vs 13 pmol/mg microsomal protein). Furthermore, total in-vitro intrinsic clearance (per mg microsomal protein) of the CYP2D6 substrate metoprolol was about 30-fold lower in jejunal compared with hepatic microsomes (0.7 vs 19.7 μl/min per mg). Similarly to most other CYP enzymes, oxidative activity as well as CYP2D6 protein content tended to peak in the proximate jejunal sections and decreased toward the distal segment of the ileum. Based on the assumption of a well-stirred model for liver and intestinal clearance, the predicted average in-vivo intestinal extraction ratio for metoprolol was negligible compared with the predicted average hepatic extraction ratio (0.0085 vs 0.48). The authors supposed that, unless a CYP2D6 substrate has an exceptionally high microsomal intrinsic clearance and/or long residence time in the mucosa, intestinal CYP2D6 would not be expected to contribute significantly to overall first-pass metabolism.[168] In a study by de Waziers and co-workers, the estimated intestinal CYP2D6 content represented approximately 20% of the concentration found in the liver, whereas the total amount in whole intestine was about 40 times lower than in whole liver.[3] Zhang and co-workers were unable to detect CYP2D6 protein in 10 human donor small intestines when probed by immunoblots, although RT-PCR of enterocytes revealed the expression of CYP2D6 mRNA.[98] In another study, Lindell and colleagues analysed the expression of eight different *CYP* genes in 51 human duodenum biopsies, also using RT-PCR. They observed a relatively high level of CYP2D6 mRNA expression in the duodenum and concluded that, considering the large number of drugs metabolised by CYP2D6, this could mean a significant role of CYP2D6 in intestinal drug metabolism.[149] Furthermore, based on a recent systematic comparison of intestinal and hepatic metabolism using bufuralol as a probe substrate for CYP2D6, Galetin and Houston suggested comparable intestinal and hepatic microsomal intrinsic clearance (2.8 vs 3.2 μl/min per pmol CYP enzyme in human intestinal and human liver microsomes, respectively), albeit only after normalisation for the CYP relative abundance and after correcting the clearance estimates obtained in intestinal microsomes prepared by mucosal scraping for activity loss.[105] In summary, most studies have shown that CYP2D6 is expressed in the intestine but do not suggest that it plays a significant role in drug metabolism of orally administered CYP2D6 substrates.[4,34,168,169] The extensive variety of human CYP2D6 genotypes and the considerable variability in the expression of CYP2D6 could contribute to apparently conflicting results.[107]

CYP1A

CYP1A1, referred to historically as aryl hydrocarbon hydroxylase, is expressed predominantly in extrahepatic tissues and has been reported to be the most prominent inducible form in rat small intestine.[13,85,112,167,170] Although, in contrast to most *CYP* genes amongst the families 1–3, the *CYP1A1* gene is relatively well conserved,[143,152] large interindividual variation in the intestinal expression levels of CYP1A1 have been reported.[98,171,172] Although CYP1A1 protein was reported in one study to be undetectable along the human gastrointestinal tract,[173] most authors were able to detect CYP1A1 mRNA and/or protein in some human intestinal samples, at least at low levels.[4,13,26,98,112,149,171,172] Zhang and colleagues reported only weakly detectable CYP1A1 protein in two of eight human samples examined,[98] suggesting that, in accordance with the results of earlier studies, CYP1A1 is likely to be induced rather than constitutive.[171,174] Similarly, Paine and co-workers reported that CYP1A1 was detectable in only three of 18 human small intestine preparations.[172] Using 7-ethoxyresorufin *O*-deethylation (EROD) as an indicator of CYP1A1 catalytic activity, resorufin formation was measurable in one-third of the intestinal microsomal samples tested, including those with detectable CYP1A1 expression. The activities displayed by some of these samples were comparable with, or even exceeded, that for two human liver microsome preparations. In contrast to the results of Buchthal and colleagues,[171] the variation in intestinal CYP1A1 was not accounted for by either smoking habits or concomitant medications. Similarly, in a recent study CYP1A1 protein was readily detected in three of 31 donor small intestines, with a range of 3.6–7.7 pmol/mg (mean 5.6 pmol/mg), irrespective of smoking status.[4] However, dietary histories were not available for any of the donors, suggesting that environmental chemicals could have accounted for the variation in CYP1A1 expression.

The second member of the CYP1A subfamily, CYP1A2, which is responsible for the metabolism of several drugs,[16,67,84] is an almost exclusively hepatic CYP enzyme, being virtually undetectable in human small intestine.[3,4,97,98,112] Accordingly, McDonnell and co-workers reported that CYP1A2 mRNA was not present constitutively in human duodenum. By contrast, CYP1A2 mRNA was detectable at low levels in two of six individuals following treatment with omeprazole, an inducer of CYP1A.[175]

CYP2E1

The common hepatic CYP2E1 isoenzyme[97,143] shows only low or no expression in the small intestine.[3,4,112,149] Zhang and colleagues reported only weak signals for CYP2E1 mRNA, whereas CYP2E1 protein was not detected.[98] Only Thörn and colleagues found a high expression of CYP2E1 mRNA in almost all of the samples at the different sites

along the human gastrointestinal tract – even higher than CYP3A4.[123] However, they suggested that there is no clear association between CYP2E1 mRNA expression and metabolic activity.

Other isoenzymes

A relatively newly discovered member of the cytochrome P450 superfamily, CYP2S1, exhibited relatively strong expression in epithelial cells throughout the gastrointestinal tract.[112,176–178] Likewise, the intestinal expression of CYP4F12, which catalyses metabolism of the antihistamine ebastine, has been shown by RT-PCR analysis.[164,179] Nevertheless, Paine and colleagues suggested that the contribution of CYP2S1 and CYP4F12 or unknown P450s to the whole intestinal P450 content would be relatively low.[4]

Another minor enzyme in the human gut wall is CYP1B1, a key enzyme in the metabolism of 17β-estradiol and in the activation of a variety of environmental carcinogens and mutagens.[180,181] As for CYP2D6, CYP2E1 and CYP3A5, Zhang and colleagues reported CYP1B1 mRNA expression in some human small intestine samples (two of four), but were unable to detect the corresponding protein in enterocyte microsomes from 10 human small intestines.[98]

Most authors agree that CYP2B6 is not expressed, or only weakly detectable, in human small intestine samples.[4,98,182] Only Lindell and co-workers reported a relatively high level of CYP2B6 mRNA expression in the intestine.[149]

Further CYPs such as 2A6, 2A7, 2A13, 2F1 and 4B1 were either not detected in human small intestine or were detected only faintly.[4,98,112,183] Similarly, it is unlikely that the relatively new CYP enzymes 2R1, 2U1 and 2W1 contribute substantially to total intestinal CYP content, although Bièche and colleagues reported moderate expression of the mRNAs in jejunal and ileal samples.[4,112,176]

Inhibition and induction of gut wall metabolism

Selective inhibition or induction of gastrointestinal enzymes either by dietary or environmental xenobiotics or by co-administered drugs has been identified as an important source of drug interactions and a major contributor to variability in oral drug bioavailability.[19,26,99,184]

Inhibition of CYP enzymes

Inhibition of CYP3A by grapefruit juice

The most prominent example is the effect of grapefruit juice on the oral availability of many drugs such as felodipine (Figure 4),[26,185] nifedipine,[25,185] verapamil,[186] terfenadine,[187] ethinylestradiol,[188] midazolam,[189] saquinavir[190,191] and ciclosporin.[18,192,193] Grapefruit juice has been shown to contain substances that inhibit intestinal CYP3A and increase bioavailability of co-administered CYP3A substrates (for reviews see[194–198]). For example, in 1997 Lown and co-workers demonstrated the inhibition of first-pass metabolism of felodipine, a well established substrate for CYP3A4, by grapefruit juice.[26,199] In this study, 10 healthy volunteers received 8 fl oz of grapefruit juice three times a day for 6 days. Although the magnitude of the interaction was highly variable between individuals, the overall effect of the first glass of grapefruit juice was an increase in the mean maximum plasma concentration C_{max} of felodipine of 225% and an increase of 116% in the mean area under the plasma concentration–time curve (AUC). Interestingly, administration of grapefruit juice for 6 days resulted in an unequivocal decrease in enterocyte CYP3A4 protein concentration, whereas the mean CYP3A4 mRNA concentration was unchanged. This decrease in enterocyte CYP3A4 protein concentration was accompanied by a similar decrease in enterocyte levels of CYP3A5 protein in the subjects with measurable CYP3A5 protein. By contrast, there was no consistent change in enterocyte levels of CYP2D6 or CYP1A1 protein with recurrent grapefruit juice intake. Furthermore, the effect of grapefruit juice appeared to be selective for intestinal CYP3A, as liver CYP3A4 activity, measured by the [^{14}C-*N*-methyl] erythromycin breath test, was not affected.

Given the relatively narrow therapeutic index of some of the drugs affected by the interaction with grapefruit juice (e.g. ciclosporin, terfenadine), some authors have expressed the need to highlight grapefruit juice as a source of risk for some patients.

Drug–drug interactions affecting CYP3A

Another inhibitor of intestinal (and hepatic) CYP3A-mediated metabolism is the antifungal agent ketoconazole. It was noted that treatment with ketoconazole increased the oral bioavailability of the immunosuppressive agents ciclosporin, tacrolimus and sirolimus, exerting greater inhibitory effects on intestinal than on hepatic extraction.[23,106,184,200,201] A similar differential effect has been noted for inhibitory interactions between erythromycin and ciclosporin,[202] and between erythromycin and midazolam.[203] Likewise, the CYP3A-mediated first-pass extraction of midazolam has been shown to be inhibited by lopinavir/ritonavir in human liver and small intestine.[204] Similar results were observed by Gorski and colleagues in their drug–drug interaction study of midazolam and clarithromycin, which is also a CYP3A inhibitor.[20] Intestinal and hepatic midazolam 1′-hydroxylation activity is also inhibited by antifungal agents *in vitro*.[131] Interestingly, liver and intestinal microsomes containing equal or greater amounts of CYP3A5 in addition to CYP3A4 were less susceptible to inhibition by ketoconazole and fluconazole, compared with those containing only CYP3A4. The authors suggested that this may contribute significantly to the interindividual variability associated with ketoconazole– and fluconazole–midazolam interactions. Besides CYP3A, intestinal CYP1A1 has also been shown to be highly sensitive to inhibition by ketoconazole *in vitro*.[172]

Effect of excipients

There is some evidence to suggest that, in addition to co-administered drugs and dietary constituents, several commonly used excipients inhibit intestinal cytochrome P450 activity, specifically CYP3A.[205–208] Using rat jejunal tissue mounted in diffusion chambers, Johnson and co-workers demonstrated significant inhibition of the CYP3A-mediated metabolism of verapamil by the block copolymer Pluronic P85 as well as polyethylene glycol 400 in a concentration-dependent manner. By contrast, 0.01% vitamin E D-alpha-tocopheryl polyethylene glycol 1000 succinate had little effect on the formation of norverapamil from verapamil.[208] Similarly, Ren and

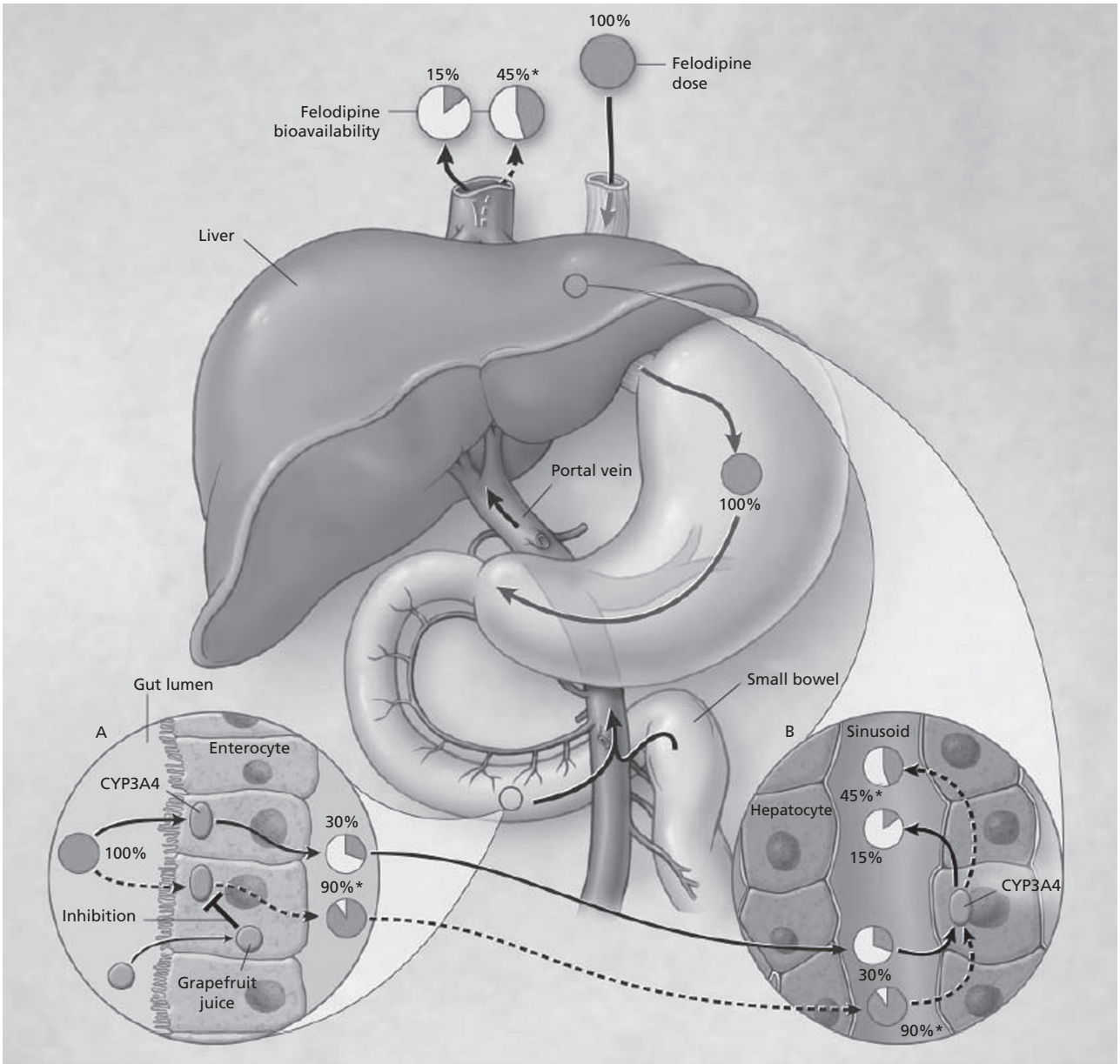

Figure 4 First-pass metabolism after oral administration of a drug, as exemplified by felodipine and its interaction with grapefruit juice. CYP3A enzymes (e.g. CYP3A4) present in enterocytes of the intestinal epithelium extensively metabolise felodipine during its absorption, and on average only 30% of the administered dose enters the portal vein (solid line). CYP3A enzymes in the liver further metabolise the drug so that only 15% of the dose is bioavailable and finally reaches the systemic circulation and is able to exert its effects. Grapefruit juice selectively inhibits CYP3A in the enterocyte, the net result being an increase in the oral bioavailability of felodipine by a factor of three, denoted by the asterisks and dashed lines. (Reproduced from Wilkinson[89] Copyright © 2005 Massachusetts Medical Society. All rights reserved.)

co-workers recently reported that four different non-ionic surfactants inhibited the 1′-hydroxylation of midazolam by intestinal microsomes obtained from male Sprague-Dawley rats.[207] However, another study suggests only minor effects of excipients on intestinal CYP3A activity. Although some of the examined excipients (e.g. Tween 20, Tween 80, taurocholic acid/lecithin mixed micelles) exhibited inhibition of enzyme activity in cDNA-expressed human CYP3A4 and human liver microsomes, in many instances this was in the millimolar range and was therefore considered insignificant.[206] Furthermore, one should generally be careful before extrapolating from in-vitro and animal data to predict human drug interactions *in vivo*.

Induction of CYP enzymes

Effect of rifampicin

Although not as common as drug–drug interactions caused by inhibition, those resulting from induction of CYP3A are thought to be just as profound and clinically important.[209]

Results from in-vivo interaction studies suggest that both hepatic and intestinal metabolic extraction are sensitive to the effect of the most potent inducer of CYP3A, the antituberculosis drug rifampicin.[19,29,94,99,210,211] Rifampicin treatment (300 mg twice daily for 7 days) resulted in a 5–8-fold increase in the concentration of CYP3A4 mRNA in human duodenal enterocytes obtained from five healthy volunteers. This was accompanied by an increase in CYP3A4 enzyme protein levels, as well as catalytic activity measured by erythromycin *N*-demethylation in one study subject.[99] Likewise, rifampicin has clearly been shown to induce the expression of intestinal CYP3A4 mRNA and protein, and CYP2C8 and CYP2C9 protein content in shed human enterocytes.[212] By contrast, no significant difference before and during rifampicin intake was observed for expression of CYP2D6 in enterocyte, an isoenzyme supposed not to be inducible by pharmacological agents.[94]

Induction of intestinal CYP3A4 has also been reported for other compounds, for example the glucocorticoid dexamethasone and the herbal medicine St John's wort (*Hypericum perforatum*).[5,213,214]

The effect of an orally co-administered modulator of CYP3A function was expected to be more pronounced at the level of the intestine compared with the liver, based on presumed local concentration differences during the period of modulator absorption. Thus, relatively selective induction of intestinal CYP3A-dependent first-pass by rifampicin is thought to explain the differential effects of rifampicin on the pharmacokinetics of intravenous and oral nifedipine.[24] A similar differential effect has also been noted following CYP3A induction produced by rifampicin with regard to ciclosporin,[19] midazolam[215] and verapamil.[29] By contrast, Lin and colleagues hypothesised that the assumption of a pronounced response of intestinal enzymes compared with hepatic enzymes to the inducer is valid only when a small dose of the inducer is given orally. Based on animal data, they argued that, at a low dose, the inducer may be metabolised significantly by the small intestine, and only a very small fraction of the inducer would reach the liver intact. In accordance with this hypothesis, the extent of hepatic induction is much higher than intestinal induction when the inducer is given at high doses.[32]

Induction of CYP1A1

Another enzyme reported to be inducible in human small intestine is CYP1A1.[171,174,175] CYP1A1 activity in human duodenal mucosa was determined by measuring EROD in biopsies from 20 smokers (3–30 cigarettes/day), 10 non-smokers receiving omeprazole treatment (20–60 mg/day for at least 1 week), and 21 non-smokers. Median intestinal EROD activity was significantly higher in smokers and in omeprazole-treated patients than in non-smoking controls; immunoblot analysis revealed that EROD activity correlated well with CYP1A protein levels.[171] Likewise, McDonnell and colleagues found that omeprazole (20 mg/day for 1 week) induced CYP1A1 mRNA and enzymatic activity in the duodenum of five of six volunteers. The extent of increases in both mRNA and enzymatic activity of CYP1A was quite variable, ranging from 2- to 16-fold induction in CYP1A1 mRNA.[175] In a further study, healthy adults were fed a diet enriched with chargrilled meat for 7 days, which resulted in a marked induction in the enterocyte content of CYP1A1 protein and mRNA of each subject, whereas CYP3A4 and CYP3A5 mRNA and protein levels were not significantly changed.[216] Since Paine and colleagues observed that CYP1A1 was not detectable in any of the four small intestines of donors known to be chronic smokers, they also concluded that other CYP1A-inducible environmental chemicals, such as polycyclic aromatic hydrocarbons and heterocyclic aromatic amines contained in the diet, could have accounted for the CYP1A1 expression observed in some of the other individuals tested.[4,172]

Summary

In summary, it has been shown that, particularly for the most abundant and most important intestinal cytochrome P450 enzyme subfamily, CYP3A, inhibition of these enzymes increases and induction of them diminishes oral availability of CYP3A substrates. Intestinal interactions between oral CYP3A substrates may add to interindividual variability in intestinal presystemic metabolism. In addition, there is the possibility of severe drug–drug interactions when an inhibitor or inducer is co-administered orally, because both hepatic and intestinal CYP3A is inhibited or induced (see also Flockhart[217]).

Interaction between CYP3A4 and P-glycoprotein

One factor that is likely to affect the first-pass metabolism of CYP3A4 substrates is the interaction between CYP3A4 and the membrane transporter P-glycoprotein (P-gp) in enterocytes.[2,78,103,218–224] P-gp is a member of the adenosine triphosphate-binding cassette superfamily of proteins, located in the apical brush border of the enterocytes, where it functions to shunt xenobiotics from the enterocytes back into the intestinal lumen.[122,225,226] A substrate for both P-gp and CYP3A4, upon entering the enterocyte, may be absorbed directly into the systemic circulation, metabolised by CYP3A4 in the enterocyte or secreted back into the intestinal lumen by P-gp. P-gp-mediated efflux and CYP3A4 metabolism may be functionally linked and act in concert to limit the passage of drugs across the enterocyte. There are several lines of evidence to support this hypothesis. First, CYP3A4 and P-gp are both localised to mature enterocytes on the villus tip;[99,103,226] Second, there is extensive overlap among the substrates for P-gp and CYP3A4, with the two proteins also sharing common inhibitors and inducers.[227–235] Finally, some evidence suggests that P-gp and CYP3A4 could be coordinately regulated.[234,236] This information has led to the hypothesis that CYP3A4 and P-gp form a coordinately regulated alliance to maximise the effectiveness of xenobiotic excretion. Since a portion of the extruded drug can be reabsorbed into the enterocytes and thus circulates between the gut lumen and the epithelial cells, it is possible that P-gp increases metabolism as a result of prolonged exposure to the intracellular drug-metabolising enzymes and by keeping the intracellular drug concentration within the linear range of the CYP3A4 enzyme (Figure 5).[2,168,237,238]

According to this hypothesis, subjects with relatively high enterocyte P-gp levels could have increased intestinal metabolism of CYP3A4 substrates simply as a result of a prolonged absorption phase. Hence, even low levels of intestinal CYP3A4

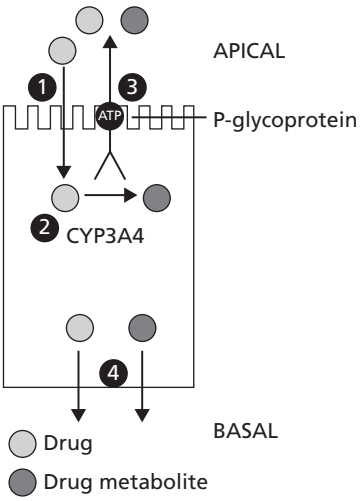

Figure 5 Functional interaction between drug transport (P-glycoprotein) and drug metabolism (CYP3A4) in enterocytes. **1** Absorption of an orally administered drug from the lumen of the gastrointestinal tract into the enterocyte. **2** Intestinal metabolism via CYP3A4. **3** Transport of the parent compound and/or its metabolite from the enterocyte into the gut lumen via P-glycoprotein. **4** Translocation of drug and/or metabolite across the basal membrane of enterocytes. (Reproduced from Fromm[238] with permission of Blackwell Publishing.)

may be significant in subjects with active intestinal P-gp. For example, co-administration of ciclosporin with rifampicin, an inducer of CYP3A and P-gp in humans, resulted in a 2.7-fold decrease in ciclosporin bioavailability. By contrast, concomitant administration of ketoconazole (an inhibitor of CYP3A and P-gp in humans) resulted in a 2.5-fold increase in ciclosporin bioavailability.[78,184,201] Using a CYP3A4-transfected Caco-2 cellular system, Cummins and co-workers demonstrated that, for flux in the apical to basolateral direction, inhibition of P-gp caused a decrease in the extraction ratio of sirolimus, a substrate for both CYP3A4 and P-gp, whereas under the same conditions, there was no significant change in the extraction ratio for midazolam, which is a substrate only for CYP3A4 in the cellular system.[239] Although some in-vitro results suggest that metabolic changes following addition of an interacting substance will not necessarily yield a quantitative prediction,[240] at least one study performed in humans indirectly supports the interplay between P-gp and CYP3A4 in the gut wall.[241] In this study, the influence of intestinal P-gp and CYP3A4 on plasma concentrations of quinidine, a substrate of both P-gp and CYP3A4, was demonstrated in eight healthy male volunteers. The dose-corrected plasma AUC from time 0 to 3 h of quinidine was negatively correlated with both intestinal P-gp content and intestinal CYP3A4 expression in shed enterocytes obtained from six of the subjects. That is, higher amounts of CYP3A4 and P-gp due to rifampicin treatment were associated with lower plasma quinidine concentrations.

By contrast, some evidence suggests that the expression of CYP3A4 and P-gp in the intestine are regulated separately and that the overlap in substrate specificity of CYP3A4 and P-gp is fortuitous rather than indicative of a more fundamental relationship.[209,242] Some (e.g. ciclosporin, quinidine) but not all (e.g. nifedipine) CYP3A4 substrates were found to be P-gp substrates. And, vice versa, certain P-gp substrates (e.g. digoxin) appear not to be metabolised by CYP3A4.[228,242] Furthermore, there is experimental evidence that the expression regions of CYP3A4 and P-gp are somewhat different, as the expression of P-gp increases longitudinally along the gastrointestinal tract, whereas CYP3A4 protein, and therefore metabolic activity, decrease.[80,98,123,226,243,244] Accordingly, Lown and colleagues were unable to find a significant intrasubject correlation between enterocyte concentration of P-gp and CYP3A4 in healthy volunteers.[26] In another study, some of the same authors also found no correlation between intestinal P-gp and CYP3A4 content in 25 kidney transplant patients who underwent small-bowel biopsy for measurement of CYP3A4 and P-gp.[245] However, because of the concerted nature of CYP3A4 and P-gp, it is difficult to discriminate between the relative roles of intestinal P-gp and CYP3A4 in determining the oral bioavailability of CYP3A4/P-gp bisubstrates.

Conclusions

Knowledge about gut wall metabolism has increased enormously over the last two decades and it is well known that the small intestine epithelium of humans expresses an array of phase I and phase II metabolic enzymes, with the CYP enzymes representing the most important class in phase-I-dependent drug metabolism. The strategic localisation of CYP enzymes in the tips of the villi of the intestinal mucosa provides the first site for metabolism of orally ingested drugs, lending further support to the view that metabolism in the gut wall can substantially contribute to the overall first-pass metabolism.

The growing number of in-vitro models, as well as the availability of several animal models and in-silico approaches, has enormously increased our ability to understand the influence of first-pass metabolism in the gut wall on oral bioavailability. Translating these data into a quantitative prediction of the situation in humans *in vivo* represents the next big challenge. Many methods that are available to study intestinal drug metabolism *in situ* or *in vivo* are technically and ethically difficult in man. Furthermore, it still remains difficult to discriminate between the contribution of the liver and the small intestine and/or other extrahepatic sites to the overall metabolism of drug substances. Likewise, if there is an overlap among the substrates for various metabolising enzymes and/or intestinal transporters, it is difficult to distinguish their individual contributions to the bioavailability of drugs. Another unsolved question centers on the reasons for variability in intestinal drug metabolism. Variations and changes in the activity of CYP enzymes can result from genetic polymorphisms, enzyme inhibition, enzyme induction and physiological and environmental factors. These alterations may have clinical implications, as the resulting changes in the pharmacokinetics of drugs can lead to reduced efficacy on the one hand and increased toxicity on the other hand. Clinically, this is of particular importance when the fraction of the dose administered that escapes metabolism is small and variable. In these cases, investigators, and especially clinicians, must consider the impact of intestinal metabolism of orally administered drugs when interpreting pharmacokinetic data and to optimise therapy in patients.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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