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Expression of Genes Encoding for Drug Metabolism in the Small Intestine

BY

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ABSTRACT

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This investigation focused on the mRNA expression of drug metabolising Cytochromes P-450 (CYP) and UDP-glucuronosyltransferases (UGT) and the transport protein P-glycoprotein (Pgp) in the small intestine of humans and rats.

The mRNA expression of the investigated genes in the human small intestine (duodenum) varies between individuals giving each one of us personal profile. In general, the most dominant forms are Pgp, CYPs 2C9, 2D6, 3A4, and UGTs 1A1, 1A10, 2B7. However, which of these is the highest expressed one varies between individuals.

The correlation in expression between some CYP forms and UGT forms respectively is relatively high, which indicates that they have some regulatory mechanisms in common. It was also shown that the mRNA expression of both CYPs and UGTs may be affected by endogenous and exogenous factors. Sex and ethnic background, affected the mRNA expression of CYP2A6 and CYP2E1 respectively. Commonly used drugs such as acetylsalicylicacid (ASA) and omeprazole (omep) affect CYP2A6, CYP2E1 (ASA) and CYP3A4, UGT1A4 (omep). The expression of UGT1A4 is also affected by smoking. All these factors are commonly used and can therefore lead to important drug-drug interactions.

It was also shown that the human small intestinal CYP mRNA expression pattern differs from that found in the rat. The rat CYP expression is rather constant between the different individuals, and the main rat intestinal forms are CYP1A1, CYP2C, CYP2D6 and CYP3A1. The expression is the same for females and males and no difference can be seen between the different segments of the rat small intestine. As metabolic studies have often been done with rat liver we compared the mRNA expression in the two organs. We found that the mRNA expression of CYP1A1 was absent in the liver and that the CYP2B1, CYP2Cs, CYP2D1 and Pgp all had a stronger mRNA expression in the small intestine compared to the liver. It is therefore important to realise that results from metabolic studies on liver may not be directly extrapolated to the small intestine.

Artemisinin is an orally used drug in multidrug treatment of malaria in Southeast Asia. It has been suggested that artemisinin can induce drug metabolism and therefore be involved in drug-drug interactions. This study shows that artemisinin induces mainly the CYP2B via nuclear receptor CAR.

Keywords: CYPs, UGTs, interindividual variation, small intestine, human, rat, artemisinin, CAR-receptor regulation

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To my family

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred to by their Roman numerals (I-IV) in the text.

I M. Lindell, M. Lang, and H. Lennernäs
Expression of Genes Encoding for Drug Metabolising Cytochrome P450 Enzymes and P-glycoprotein in the Rat Small Intestine; Comparison to the Liver.
Eur J Drug Metab Pharmacokin (2003) 28(1) 41-48

II M. Lindell, M.O. Karlsson, H. Lennernäs, L. Påhlman, and M.A. Lang
Variable Expression of *CYP* and *Pgp* Genes in the Human Small Intestine.
Eur J Clin Invest (2003) 33 493-499

III M. Lindell, M.O. Karlsson, L. Påhlman, H. Lennernäs, and M.A. Lang
Expression of UDP-Glucuronosyltransferasese Genes (UGTs) in Human Duodenum.
(Manuscript)

IV M. Lindell, U.S.H. Simonsson, F. Raffalli-Mathieu, A. Lannerbro, P. Honkakoski, and M.A. Lang
Induction of Drug Metabolism by Artemisinin: Evidence for the Involvement of Nuclear Receptor CAR
(Submitted to Biochemical Pharmacology)

ABBREVIATIONS

ABC	ATP-binding cassette
Ah	Aryl hydrocarbon
ASA	Acetylsalicylic acid
CAR	Constitutive androstane receptor
CYP	Cytochrome P450
DR	Direct repeats
DRT-PCR	Duplex reverse transcriptase polycyclic reaction
ER	Everted repeats
GI	Gastro intestinal
hnRNP	Heterogeneous nuclear ribonucleoprotein
IR	Inverted repeats
MDR	Multi drug resistance
NR	Nuclear receptor
Omep	Omeprazole
PAH	Polycyclic aromatic hydrocarbons
PB	Phenobarbital
PBREM	Phenobarbital response element module
Pgp	P-glycoprotein
PPAR	Peroxisome proliferator activated receptor
PXR	Pregnane X receptor
RT-PCR	Reverse transcriptase polycyclic reaction
RXR	Retinoid X receptor
ST	Sulphotransferases
SXR	Steroid X reseptor
UGT	UDP-glucuronosyltransferase
UTR	Untranslated region
XRE	Xenobiotic responseive element
XREM	Xenobiotic-responsive enhancer module

CONTENTS

INTRODUCTION

It is common that drugs and other foreign substances (xenobiotics) are undergoing metabolism before they are eliminated from the body. The routes by which drugs may be metabolised are many and varied. Drug metabolism is normally divided into two phases; phase I and phase II. Phase I enzymes introduce a functional group, such as –OH, into the substrate. In general, they render the compound less lipophilic, but additionally serve to exposes reactive sites, or to add functionally reactive sites to which polar groups may be conjugated. Phase II enzymes then use this functional group as a handle for conjugation with such moieties as glucuronic acid, sulfate, and glutathiol. The end result is a hydrophilic product that can be more easily eliminated from the body [1, 2]. Cytochrome P450 (CYP) probably comprises more than 95% of all phase I enzymes. Phase II enzymes include a group of diverse enzymes, among them the UDP-glucuronosyltransferases (UGT) [2].

The liver is the organ with the highest capacity for both phase I and phase II reactions, but it is not the only organ involved. Drug metabolism has been observed in different organs such as the gastrointestinal tract (GI tract), lung, white blood cells, brain, kidney, and placenta [3]. While the liver has been thought to be the major organ involved in drug metabolism, the small intestine is also capable of performing many of the same metabolic reactions on orally ingested drugs, or potentially toxic compounds ingested with the food [4 - 6]. But the metabolic activities of both phase I and phase II enzymes are considerably much lower in the whole small intestine compared to the liver [7].

The focus of this thesis is on the variation of expression of both *CYP* and *UGT* genes in the small intestine of both humans and rats; in particular variation between the two species, individual variation in expression in human populations, and how the expression is affected by endogenous and/or exogenous factors.

It is important to clarify the differences between the human and the rat expression, and also the differences between the expression of rat small intestine and liver. Rat hepatic tissue has been used extensive by drug metabolism studies. Also in humans the most common used organ in metabolic studies has been the liver, but for orally used drugs the small intestine is the first organ that is exposed for a drug. It is therefore important to elucidate how much the small intestine is contributing in the first-pass drug metabolism. The humans are also exposed to a lot of different exogenous factors, such as other drugs, xenobiotics from the diet and environmental pollutants, factors that may inhibit and/or induce drug metabolism.

The small intestine

The small intestine forms the largest metabolically active external surface of the human digestive system [8]. In humans, the small intestine is about 5 to 6 meter and is divided into three parts: duodenum, jejunum, and ileum [9, 10]. These regions are not anatomically distinct, although there are differences in their absorptive and secretory capabilities. The duodenum is the shortest, widest, and least mobile section. In humans it measures 20 to 30 cm in length and 3 to 5 cm in diameter. The rest of the small intestine is about 5 m long; the proximal two-fifths is referred to as the jejunum and the distal three-fifths is called the ileum [11]. The primary function of the small intestine is to absorb nutrients and water. This is achieved by mixing the passing foodstuff with digestive enzymes to increase its contact with the absorptive cells of the mucosa. Approximately 6 to 12 litres of partially digested foodstuffs, water, and secretions are delivered daily to the small intestine. Of this, only 10 to 20% are passed on to the colon, because most nutrients, electrolytes, and water are absorbed as they are transported through the small intestine [9, 10]. In addition to this fundamental role, a secondary function of the small intestine arises from the fact that it is also a major route of entry into the body for many xenobiotics including pharmaceutical drugs.

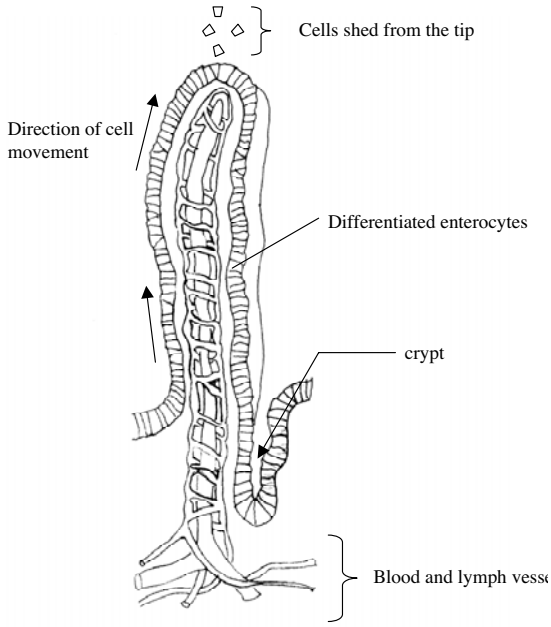

Figure 1. Structure of a single intestinal villius tip. The intestinal mucosa is continuously renewed by new cells that originate from the crypt (modified from Hole and Koos.)

Throughout its length, the inner wall of the small intestine has velvet like appearance. This is due to the presence of innumerable tiny projections of the mucous membrane called intestinal villi. These project into the lumen of the

alimentary canal containing the intestinal contents. The villi increase the surface of the intestinal lining and play an important role in the absorption of digestive products. Each villi consist of a layer of simple columnar epithelium and a core of connective tissue containing blood capillaries, a lymphatic capillary, and nerve fibres (Fig. 1). At their free surface, the epithelial cells possess many fine extensions, called microvilli, that create a brush like border and greatly increase the surface area of the intestinal cells (Fig. 2) [12]. The epithelial cells at the tip of the villi are functionally mature and non-dividing cells, whereas the crypt cells are immature and evolving. The crypt cells continue to mature as they ascend toward the villi and are extruded at its tip (Fig. 1).

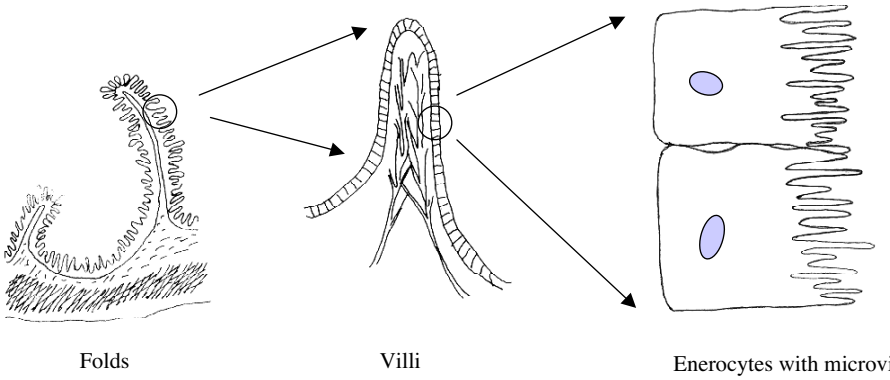

Figure 2. The morphology and surface of the small intestine.

The enterocytes are important for the absorption and metabolism of nutrients and drugs [11]. The enzyme activity is greatest in the villius tips and decreases progressively towards the crypts. The highest concentration of metabolising enzymes in the GI tract is in the upper small intestine: the duodenum and the jejunum followed by the ileum and colon [13]. Epithelial cells of the intestinal mucosa have a programmed life span. They are formed by cell division within the crypts and undergo differentiation of cell function for two to five days as they migrate towards the apical tip. Immunohistochemical analysis of mucosal cross-sections indicates that drug-metabolising enzymes are expressed only after cell transformation, and thus, are found exclusively in mature enterocytes [14].

Although the small intestine is regarded as an absorptive organ in the uptake of orally administered drugs, it also has the ability to metabolise drugs by numerous pathways involving both phase I and phase II reactions [5, 15-17]. It has been shown that the metabolism in the enterocytes are minimal compared to the metabolism in the liver. This is based on the relative rates of metabolism in intestinal microsomes compared to hepatic microsomes. This observation may, however, in part be explained by the localisation of the drug metabolising enzymes (*e.g.* CYPs), in the villius tip, which makes up a relatively small portion of the total intestinal mucosa [18]. Despite significant lower expression of CYP enzymes, the intestine contributes considerably to first-pass metabolism of some substances.

Drug metabolism and Transport

There are essentially four mechanisms (Fig. 3), by which an orally ingested drug may cross the epithelial layer:

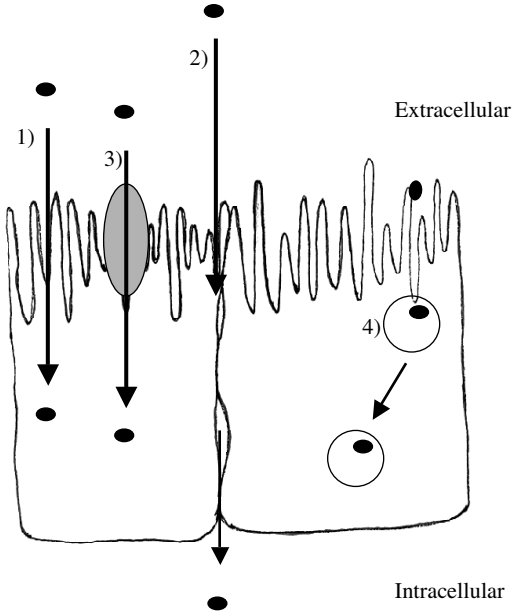

Figure 3. Different transport mechanisms for drugs across the intestinal epithelium; 1) passive transcellular diffusion; 2) paracellular transport; 3) carrier-mediated transport and 4) endocytosis (modified from Barthe et al 1999).

(1) *Passive transcellular diffusion,* a rout most likely used by lipophilic drugs with low molecular weight [19].

(2) *Paracellular transport*. Only small hydrophilic molecules cross the tight-junctions and pass between the cells. Compounds using this paracellular route should not be metabolised by the intracellular enzymes [20].

(3) *Carrier-mediated transport.* This mechanism involves specific interactions between the molecule and the transporter or carrier and is a saturable mechanism, utilised by hydrophilic molecules. Briefly, two types of carrier-mediated transport exist: (I) active transport that requires metabolic energy (ATP) and that can act against a concentration gradient and (II) transport that are driven by a co-transport or a counter transport of various ions. Drug absorption has generally been assumed to occur by passive processes. Transporters in the intestine have been shown to facilitate transport of some drugs possessing structural similarities to natural occurring substances [21].

(4) *Endocytosis.* This is a constitutive process for the uptake of macromolecules that requires metabolic energy and is generally a slow uptake mechanism. [19].

Phase I metabolism

Cytochrom P450 (CYP)

The Cytochrom P450 super family (CYP) is an enzyme group that is critical for oxidation reactions that affect the biological activities of drugs, environmental chemicals and endogenous compounds and that plays a predominant role in the phase I metabolism of xenobiotics.

The CYP enzymes are classified into families and subfamilies based on their amino acid sequence similarity. The root symbol 'CYP', denoting Cytochrome P450 and an Arabic number designating the family (*e.g.* CYP3). Members of the same family are more than 40% identical with respect to their amino acid sequences. If the sequences are more than 55% identical, the enzymes belong to the same subfamily, indicated by an additional letter (*e.g.* CYP3A). Finally, each individual enzyme is represented by an Arabic numeral (*e.g.* CYP3A4) [22]. An updated listing of CYP enzymes can be accessed at http://drnelson.utmem.edu/CytochromeP450.html.

Only three CYP gene families are currently identified to be responsible for drug metabolism in both humans and rats *i.e.* CYP1, CYP2, and CYP3 [23].

CYP1 have two subfamilies CYP1A (*i.e.* CYP1A1 and 1A2) and CYP1B. CYP1A1 is mainly found extrahepatically and CYP1A2 is a hepatic enzyme [24]. Both are induced by polycyclic aromatic hydrocarbons (PAH), found for example in cigarette smoke and charbroiled meat [24-26]. CYP1A2 is responsible for metabolism of several drugs such as caffeine and paracetamol [24].

CYP2 is the largest family of human CYPs identified to date, with up to seven different subfamilies [22]. In the small intestine, mRNA from only three CYP2 subfamilies has been reported: CYP2C, CYP2D6, and CYP2E1 [27-29] whereas the human liver also expresses CYP2A6 and CYP2B6 [30]. All CYP2s are involved in the metabolism of numerous clinically important drugs and endogenous compounds [30]. There are also differences between CYP2 gene family members in terms of response to inducers. For example, CYP2Bs are phenobarbital induced and CYP2E1 is induced by ethanol [31]. Some members of the CYP2C and CYP2D subfamilies appear to be constitutively expressed [32].

CYP3A is the primary CYP subfamily in humans; responsible for metabolism of more than 50% of administered drugs. It is an enzyme family that has shown to be highly conserved, in all mammals examined, including humans [33,34]. To date four members of the CYP3A subfamily are expressed in humans; CYP3A3, CYP3A4, CYP3A5 and CYP3A7 [35]. Studies have shown that CYP3A4 and/or CYP3A3 proteins are the major forms in human liver, jejunum, colon, and pancreas, whereas CYP3A5 is the major protein in the stomach [35]. The subfamily has a broad substrate specificity, where the substrates are structurally diverse including clinically important drugs, covering a wide therapeutic range [23]. The CYP3As has shown to be induced by a multitude of pharmaceutical drugs [32, 36-40] as well as environmental toxins [41, 42]. It therefore appears that CYP3A enzymes may

play a role in some forms of diseases mediated by environmental factors, in addition to playing a role in pharmacokinetics of a vast array of clinically used drugs.

Phase II metabolism

Phase II metabolism is a synthetic or conjugation step that is usually (with the exception of glucuronidation) a nonmicrosomal process occurring in the cytosol [43]. The human intestinal tissues have been reported to contain relatively high activity of glucuronosyltransferase, together with sulfotransferase, and glutathione-S-transferase as the main enzymes in phase II conjugation [44].

UDP-glucuronosyltransferases (UGT)

Glucuronidation is a central process in which many xenobiotics and endobiotics are converted to more hydrophilic compounds, which are readily eliminated from the body. Glucuronidation reactions are catalysed by UDP-glucuronosyltransferases (UGT) that are located on the luminal side of the endoplasmatic reticulum and in the nuclear envelope of hepatocytes and cells in other organs including the intestine [17, 45-47]. The range of target substances is large and spans divergent chemical classes, dietary constituents, environmental pollutants, therapeutic drugs and numerous endogenous substrates [48, 49].

Based on evolutionary divergence of the proteins, at least two UGT subfamilies have been described: UGT1 and UGT2 [48]. In naming the UGTs one uses a similar system as for the CYP enzymes, *i.e.* using UGT as a root symbol, representing UDP-glucuronosyltransferase, followed by an Arabic number denoting the family having ≥45% amino acid identity within a family (*e.g.* UGT2), and a letter designating the subfamily (*e.g.* UGT2B). Mammalian sequences within the same protein subfamily are approximately 60% or more identical. Finally, an Arabic number represents an individual gene within the subfamily (*e.g.* UGT2B15) [2, 48]. An updated listing of the UGT enzymes can be found at: http://som.flinders.edu.au/FUSA/ClinPharm/UGT/

Members of the UGT1 gene complex are encoded by at least 12 unique first exons located at the 5' end of the locus. They are then selectivity spliced with four constant exons (exon 2-5) to form unique RNA transcripts with varied 5' ends and identical 3' ends (Fig. 4) [50]. Members of the UGT1 family conjugate mainly bilirubin, bile acids, phenols and steroid hormones [51, 52]. The gene products of the UGT2 subfamily are, on the other hand, transcribed from unique genes [53, 54]. UGT2B enzymes catalyse the glucuronidation of bile- and fatty acids, and in particular, steroid hormones [2, 51, 52]. Currently, 15 human UGT cDNAs have been identified, eight UGT1A proteins encoded by the *UGT1A* locus (and three pseudogenes) and seven proteins encoded by *UGT2* genes [49].

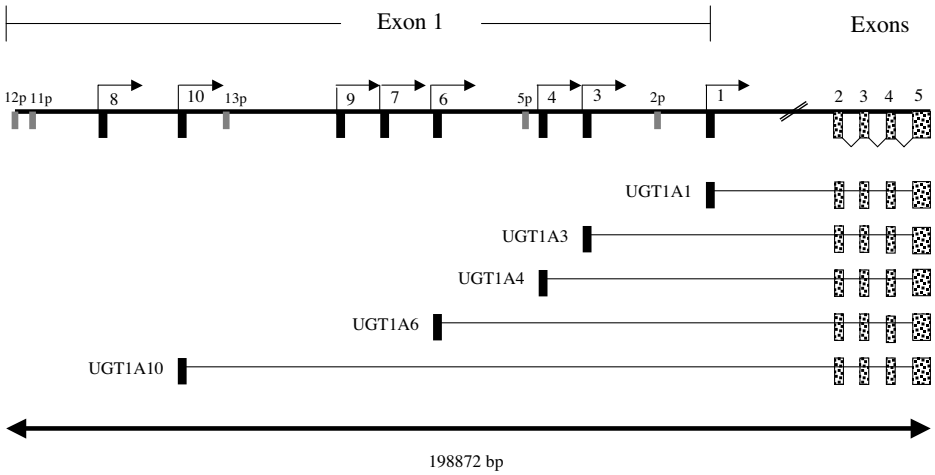

Figure 4 The organisation of the UGT1A1 locus (modified from Tukey and Strassburg 2001 [55]).

In the small intestine the UGT1A proteins have been localised to the epithelial cell layer and the UGT staining of the villi is homogenous. The gene expression of UGT1A is uniform along the length of the human small intestine [8].

Transport through intestinal epithelium

P-glycoprotein (Pgp)

P-glycoprotein (Pgp or ABCB1 [Human Gene Nomenclature Committée: http://www.gene.ucl.ac.uk/nomenclature]) is a member of the ABC-transporter family (ATP binding cassette), a group of proteins that is a participant in our defence against foreign substances. Pgp was first discovered in cancer cells resistant to several chemotheraputic agents. Where the agents were pumped out of the cell [56].

The Pgp:s is encoded by the multidrug resistance genes (MDR). In humans, two *MDR* genes have been found; *MDR1* (*ABCB1*) that encodes a drug transporting Pgp that confers drug resistance, and *MDR2* (*ABCB4*) that encodes an enzyme that is specific for phosphatidylcholine translocation in cells. In rodents, there are three *mdr* genes: *mdr1a* (*mdr1*) and *mdr1b* (*mdr3*) conferring multidrug resistance, whereas *mdr2* is responsible for transport of cell phospholipids [23].

The human MDR1 mRNA encodes 1280 amino acids and the gene product is a 135 to 180 kDa membrane transporter, the variation in molecular mass may be linked to differences in the glycosylation of Pgp [57]. Sequence analysis indicates the presence of 12 transmembrane domains in two homologous halves each containing six regions and a putative ATP-binding site (Fig. 5). The transmembrane segments are thought to form a channel through which the drug is extruded. An ATP-binding site is thought to be involved and ATP hydrolysis required for 'pumping' out drugs against a concentration gradient [58].

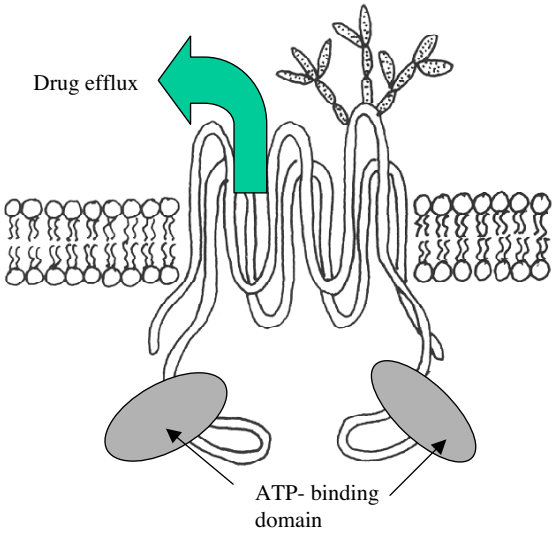

Figure 5 P-glycoprotein structure through the plasma membrane. The transport protein has 12 transmembrane domains in two homologous halves with ATP binding sites on the intracellular side of the molecule (modification from www.nature.com/nre/journl/v2/images/nrc823-f3.gif).

Pgp may play a significant role in drug absorption and disposition in both animals and humans. Pgp is present in normal epithelial cells from liver, kidney, GI-tract among other tissues [59, 60]. The localisation suggests that Pgp functionally can protect the body against toxic xenobiotics by excreting these compounds into bile, urine, and the intestinal lumen [7]. In the GI-tract, the distribution of Pgp is not uniform along the length of the intestine or along the villi within a cross section of the mucosa. The mRNA levels of Pgp increase progressively from the stomach to the colon with low levels in the stomach, intermediate in the jejunum and high levels in colon [61, 62]. However, more studies of the regional distribution of this protein transporter along the gastrointestinal tract are needed. It has been speculated that, since Pgp is located on the epithelium of intestinal cells, it may act as a counter-transport pump that transports drugs back into the intestinal lumen as they begin to be absorbed across the luminal plasma membrane.

CYP3A4 and Pgp

Both CYP3A4 and Pgp function to protect the body from toxic accumulation of hydrophobic xenobiotics via metabolism and excretion. They are both co-localised in the small bowel (being present in villius tip and not in the crypt cells). Moreover, data obtained in rat and man suggest that CYP3A4 are localised primarily at the apex of the enterocytes, just below the brush border containing Pgp [35, 38, 63].

Studies have found that there is an overlap between substrates for CYP3A4 and Pgp [21, 64, 65]. Overlap has also been found among inhibitors [66-68] and inducers of the two proteins [69-72]. A hypothesis has been put forward that CYP3A and Pgp

together form efficient machinery for the elimination of drugs from cells. The drug is entering the enterocyte and is either metabolised by CYP3A or effluxed by Pgp. The drug may then enter the cell again. This recirculation of the drug may allow a greater access to a limited amount of CYP3A, and a greater proportion of the drug will be metabolised [6] (Fig. 6). However, some other evidence suggests that the expression of CYP3A4 and Pgp is independently and non-coordinatedly regulated [64, 73, 74].

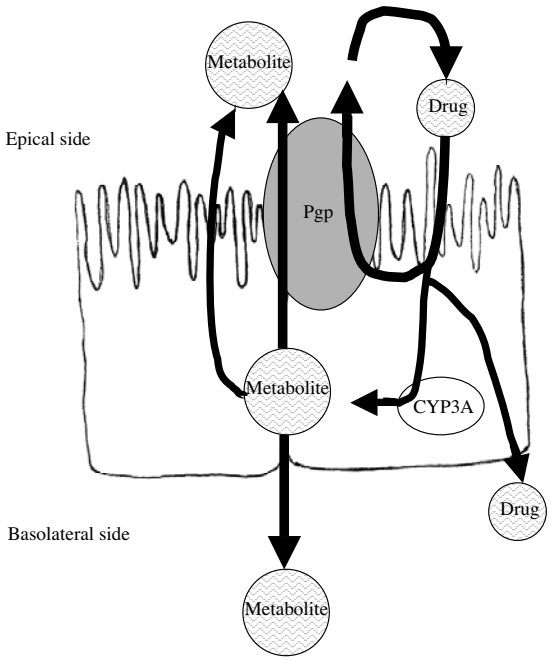

Figure 6. A model of the role of P-glycoprotein and CYP3A4 in the intestinal absorption and gut wall metabolism of drugs (modification from Hunter and Hirst [6]).

Drug metabolising enzymes in the small intestine

The small intestine is the first line of defence against orally ingested xenobiotics including therapeutically used drugs. It has been established that the small intestine has drug metabolising enzymes located in the enterocytes. The duodenum is the intestinal segment that has the highest expression levels of CYP genes and the enzymes are located in the villius tip only [28] The UGT genes are expressed throughout the whole intestine and in both the villius tip and in the crypt [8]. The activity of efflux proteins, Pgp, has been shown to be region dependent [75], whereas the regional aspect of metabolite extrusion has not yet been reported.

Several studies have found high amounts of mainly the CYP3A4, CYP2C, and the CYP2D6 in the human small intestine [28, 29, 38, 76]. And in the rat the prominent forms are CYP1A1, CYP2B1 and CYP3A1 [28, 77, 78].

The major UGT form in the human small intestine is the UGT1A10, but many other forms have also been reported [8, 55]. The role of UGTs in intestinal tissues is not currently known, however, there are several lines of evidence to support a role for UGTs as a part of the detoxification mechanisms; large amounts of intestinal UGT mRNA and protein, as well as the ability to glucuronidate a wide variety of substances that are found in the GI tract [79-81].

Regulation

In accordance with the multiplicity of the *CYP* and *UGT* genes, is the considerable diversity in the mechanisms of their regulation. Not surprisingly, the most common and best understood means of regulation is transcriptional. Post-transcriptional mechanisms include both mRNA and protein stabilisation that may be mediated through transacting regulators or through changes in the phosphorylation status of the enzyme (Fig. 7). Moreover, many CYPs and UGTs are subject to tissue-specific expression, with resulting differences in isoform composition and activities in various tissues [8, 82].

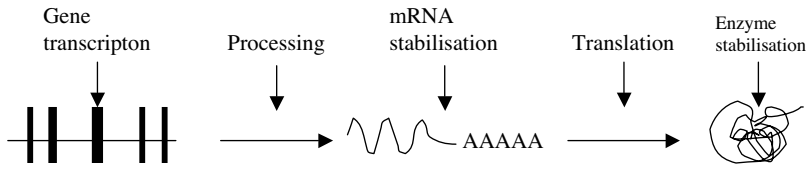

Figure 7. Different mechanisms of regulation of gene expression (modified from Porter and Coon [82]).

Studies on transcriptional regulation have mainly involved the *CYP* gene families and the regulation is now characterised for several isoforms. The regulation of both the *UGT* and *MDR* genes is still poorly understood, but preliminary results indicate that both groups of enzymes use similar receptor-dependent mechanisms as *CYP* genes [83-85].

Transcriptional regulation

Many genes belonging to the *CYP1-3*, *UGT1-2* and *MDR* families can be transcriptionally activated by foreign chemicals that will induce the gene expression through different receptor-dependent mechanisms. The most studied is the Aryl hydrocarbon receptor (Ah-receptor). This is a transcription factor that stimulates the expression of *CYP1* and *UGT1A6* genes [86, 87]. Without a ligand, the Ah-receptor is merged with hsp90, a chaperon protein, and present in the cytosol. Binding an aromatic hydrocarbon ligand in the cytosol activates the Ah-receptor

and the activated receptor, releases the hsp90 then translocates to the nucleus, heterodimerises with the Arnt factor, and binds to DNA enhancer sequences called xenobiotic-response elements (XREs). XREs are found upstream the *CYP1* genes and other Ah-receptor inducible genes such as the *UGT1A6* (Fig. 8) [86, 88-90].

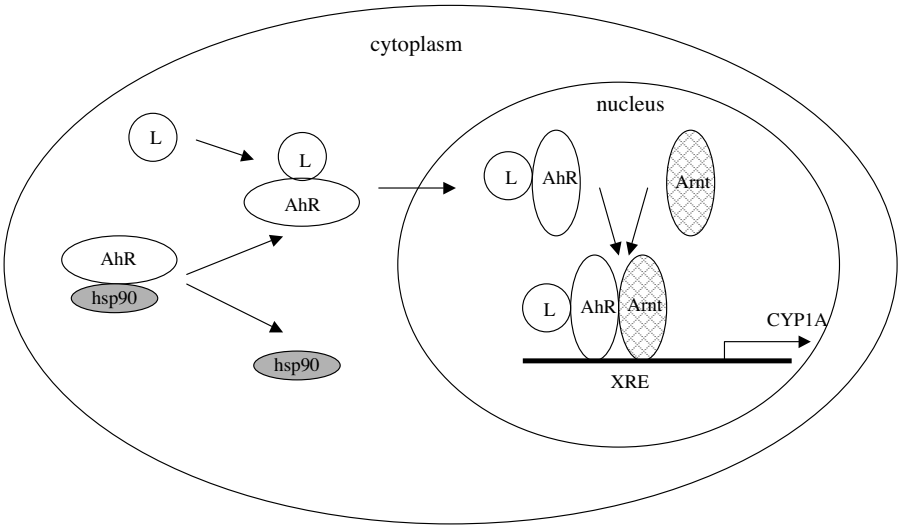

Figure 8. A simplified scheme showing the mechanism of CYP1A gene regulation by the Ah-receptor (AhR): binding of AhR to the hsp90 in the cytoplasm. After binding a ligand (L), drops the hsp90 and enters the nucleus. In the nucleus AhR binds to Arnt before binding to the DNA enhancer sequence XRE.

In addition to the induction of *CYP1* genes at least three other nuclear receptors (NRs) can induce transcription [91, 92] of drug metabolising enzymes. These are the constitutive androstane receptor (CAR or NR1I3 [93] http://www.gene.ucl.ac.uk/nomenclature), the pregnane X receptor (PXR or NR1I2), also named steroid X receptor (SXR) and the peroxisome proliferator-activated receptor (PPAR or NR1C). They all share a common heterodimerisation partner, the retinoid X receptor (RXR or NR2B1) [92].

The CAR has shown to mediate the widely studied induction of *CYP2B* genes by phenobarbital (PB) and many other "PB-like" lipophilic chemicals [94-96]. The CAR/RXR heterodimer interacts with a phenobarbital (PB) response element module (PBREM) located by the promoter of the *CYP2B* gene. The PBREM contains a nuclear factor 1 binding site flanked by two nuclear receptor binding sites composed of imperfect direct repeats of half sides spaced by four nucleotides (DR-4 motif) [97] (Fig. 9). PXR activates *CYP3A* and *CYP2C* genes (*e.g. CYP2C8*) in response to divers chemicals. The ligands include several drugs such as pregnolone derivatives and other steroids [85, 98-100]. The binding site for the PXR/RXR complex of the rodent *CYP3A* promoter/enhancer is a direct repeat spaced by three nucleotides (DR-3), and the human *CYP3A* response elements as everted repeats with six nucleotid space (ER-6) or inverted repeats (IR-6) [98, 100, 101]. Finally

PPARα, which mediates induction of fatty acid hydroxylases of the CYP4 family by many acidic chemicals, classified as "non-genotoxic" carcinogens and peroxisome proliferators [102, 103]. The CYP4 family could be a candidate for metabolism of fatty acid like drugs. The PPARα/RXR complex binds responsive elements (PPRE) in the promoter of target genes. A PPRE is a six nucleotide direct repeat separated by one nucleotide (DR-1) [104, 105].

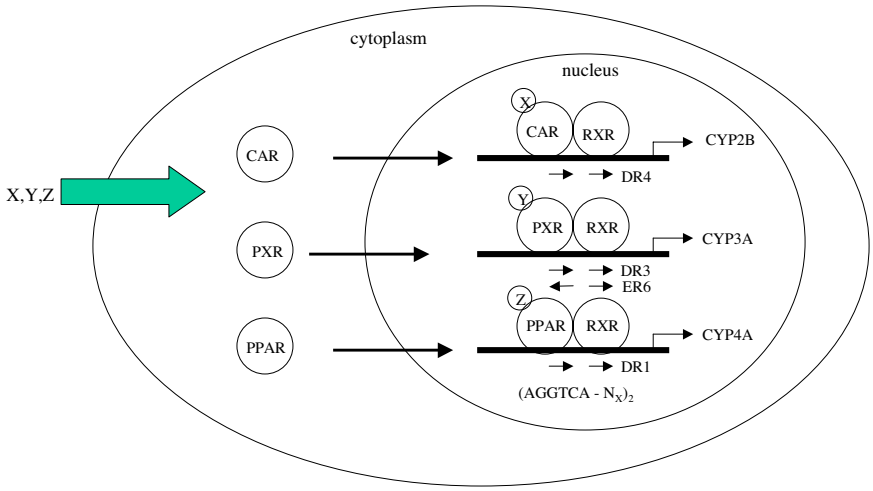

Figure 9. A simplified scheme of the role of nuclear receptors CAR, PXR, and PPAR in drug metabolism. Various drugs (X,Y,Z) are ligands to the receptors. These, drug activated the nuclear receptors form heterodimers with RXR and bind then to their respective enhancer elements on the promoter and activate transcription.

Post-transcriptional regulation

Post-transcriptional regulation mechanisms include both mRNA and protein stabilisation as well as stimulated protein degradation. These processes can result in an increase or decrease of enzyme expression [82].

A good example is CYP2E1 that is regulated by several post-transcriptional mechanisms. Both diabetes and fasting increases the expression by stabilising the mRNA and by an increase in gene transcription, respectively [106-108]. Insulin, on the other hand, decreases the mRNA levels of the CYP2E1 due to RNA-protein interaction mediating the destabilisation of the mRNA [109, 110].

Other CYP enzymes are both transcriptionally and post-transcriptionally regulated. The CYP1A2 mRNA levels are induced by PAH, a ligand to the Ah-receptor, but can also be post-transcriptionally regulated by enhancing the stability and the intracellular processing of the mRNA precursor [111-113].

The murine Cyp2a5 is also both transcriptionally and post-transcriptionally regulated [114-116]; transcriptionally by the transcription factor DBP (DNA binding protein) [117], whereas the post-transcriptional regulation is mediated

by stabilisation of the mRNA by binding of hnRNP1A (heterogeneous nuclear ribonucleo protein) to the 3'UTR (untranslated region) of the CYP2A5 mRNA [118, 119].

Regulation of CYP and UGT genes in the small intestine

As the gene regulation of drug metabolism is mainly studied in the liver, the different mechanisms presented apply basically for this organ only. However, it is established that also several intestinal CYPs are induced by xenobiotics [120-122]. The relevant NRs have also been found in the small intestine, but their role in the small intestinal gene regulation is not clear. [85, 123].

Artemisinin - an antimalarial drug

Malaria is an infectious disease that affects millions of people in the world. The high resistance to commonly used drugs has focused attention on the artemisinin group of compounds, now used in first-line treatment in several regions in Southeast Asia. Artemisinin (Fig. 10) is isolated from *Artemisia annua* L.; a plant with a long history of medical use against malaria in China [124, 125].

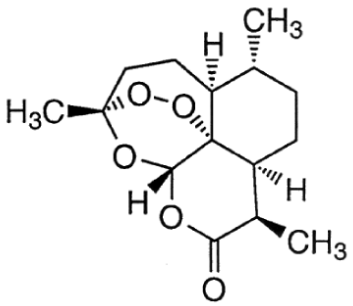

Figure 10. The molecular structure of antimalariae artemisinin (molecular weight 282 Da)

Despite the widespread use of artemisinin, information on its pharmacokinetics and metabolism is limited. Studies using rat liver microsomes indicate that artemisinin metabolism is primarily mediated by CYP2B6 with a minor contribution of CYP2A6 [126]. It has also been demonstrated that after multiple doses of artemisinin, the elimination of omeprazole is enhanced due to an increased capacity of CYP2C19 [127]. Artemisinin also exhibits an autoinduction of its elimination and this phenomenon is possibly due to an induction of CYP2B6 [128].

The evidence so far suggest that artemisinin induction may be similar to that of phenobarbital or other CAR activation compounds, however this has never been demonstrated, and, based on artemisinins structure this is not obvious (Fig.10).

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OBJECTIVES OF THE PRESENT INVESTIGATION

Two overall questions have been addressed in this thesis:

A) How is the expression of drug metabolising CYPs, Pgp and UGTs in the small intestine? For that purpose we performed the following studies:
   1. To investigate the mRNA expression of several major drug metabolising CYPs and Pgp in the small intestine and the liver of rats.
   2. To analyse the expression of eight CYPs and Pgp in human small intestine; interindividual variability and regulation by internal and external factors.
   3. Expression of seven UGTs in the human small intestine; interindividual variability and regulation, by endogenous and exogenous factors.

B) What are the mechanisms of induction of drug metabolising CYP enzymes by artemisinin?

EXPERIMENTAL PROCEDURES

Enterocytes from three segments of the rat small intestines, (*i.e.* duodenum, jejunum, and ileum) were collected and total RNA from both the enterocytes and the liver were purified. The human biopsies, from duodenum, were collected at Uppsala University Hospital, Sweden, in collaboration with Prof. L. Påhlman at the Gastro-intestinal ward. The site were the biopsies were taken showed no visible infection or inflammation. Total RNA was purified within two hours from that the biopsies were taken.

To study gene expression RT-PCR (Reverse transcriptace PCR) technique was chosen allowing us to detect very small amounts of mRNA. This is of importance when using human intestinal biopsies as source of mRNA. Biopsies do not give a large amount of mRNA, and therefore a sensitive method is necessary.

In the study on the human *CYP* genes the mRNA expression levels was compared to the expression of *Pgp* that was set on 100%. Pgp is found in many different tissues in high amounts. In the study on UGT expression the comparison was done to β-actin, an enzyme that is constitutively expressed in several tissues and therefore serves as a good internal standard for a technique called DRT-PCR (Duplex reverse transcriptase PCR). The mRNA expression of β-actin was first standardised so that the band intensity was equal for the different UGT amplifications.

The PCR amplification was visualised on an agarose gel stained with ethidiumbromide which visualise the bands under UV-light. The gel was photographed, and the photo was scanned and the band intensity was determined using the 'NIH Image (1.62)' program; a public domain image-processing and analysis program for Apple Macintosh (http://rsb.info.nih.gov/nih-image/). Details of the used methods are given in the respective papers.

The densitometric data were further statistically analysed to see if any correlation could be found between the expressions of the different CYP forms and UGT forms. We also investigated if any of the endogenous or exogenous factors were affecting the mRNA expression. A detailed description of the statistical analysis is given in papers II and III.

For the artemisinin studies three different techniques were used: *In vivo* experiments where male DBA/2J mice were given artemisinin for three days. After that the animal were killed and the livers removed and microsomes were prepared. The activity of CYP2A5, CYP2B10, and CYP1A2 was determined by measuring the coumarin 7-hydroxylation, pentoxyresorufin O-dealkylase and methoxyresorufin O-dealkylase activity, respectively.

Primary mouse hepatocytes were prepared and treated with three different concentrations of artemisinin and exposed for the drug for 16 – 40 hours. The cells

were then harvested and RNA was isolated and analysed with a Northern blot. The RNA was hybridised with radiolabeled cDNA from CYP2A5, CYP2B10, and CYP1A2.

Based on the result from these experiments we wanted to investigate whether nuclear receptor CAR was responsible for the induction of CYP2B10. HEK293 cells were transfected with a CAR; alternatively PXR, expression vectors and reporter gene, and the cells were exposed to artemisinin. The cells were lysed and the CAR-dependent luciferase and β-galactosidase activities were determined. The PXR vector responding reporter gene was used to see if artemisinin exhibited any receptor specificity and because PXR may cross-regulate CYP2B genes [129]. Further details are given in paper IV.

RESULTS AND DISCUSSION

mRNA expression of some important drug metabolising *CYP* genes and *Pgp* in the rat small intestine, (paper I)

More than 40 forms of CYP enzymes have been described in the rat liver [130]. By using different techniques such as the PCR and immunoblots, it has been established that several of these CYP forms are also expressed in the rat small intestine. Mainly the CYP1A1, CYP2B1, CYP2C, and CYP3A1 have been detected [28, 63, 77, 131]. By using both immuno-, and Northern blot analysis, it has been determined that the total CYP content of the small intestine is much lower than in the liver [131].

In this study we examined the mRNA expression levels of nine known genes encoding for drug metabolising CYP enzymes and Pgp in the three regions (duodenum, jejunum, and ileum) of the rat small intestine and compared the expression with that in the rat liver. We used Sprague-Dawley rats, one of the most used experimental models in studies of drug kinetics.

The intestinal expression of the different *CYP* genes and *Pgp* differed remarkably from the liver. The liver expression was strong for all tested CYP forms except the CYP1A1, which did not show any expression. Four of the tested CYPs, the CYP2B1, CYP2C6, CYP2C11, and CYP2D1 together with Pgp showed higher mRNA expression in the small intestine compared to the liver. On the contrary, CYP2A3, CYP3A1, CYP1A2, and CYP2E1 showed low or no expression in the small intestine. The CYP1A1 that was not expressed in the liver had a very strong expression in the small intestine. Earlier studies have found that the CYP1A1 is a strictly extrahepatic enzyme [111].

Studies on hepatic induction of the CYP1A1 have indicated that glucocorticoid receptors, in addition to the Ah receptor, may play a role in the CYP1A1 induction [132]. However, this receptor does not participate in the intestinal induction despite its presence in the enterocytes [133, 134].

One of the questions addressed was whether or not a gender difference existed in the mRNA levels, however, we could not find any such difference. All tested CYP forms and Pgp were expressed in both males and female at equal levels. There are earlier reports on sex-differences of CYP expression in the rat liver [63, 135, 136]. Yoshioka *et al* [135] found that there was a difference in protein expression between the genders in the rat liver, but when they performed a RNA blot analysis both genders displayed a hybridisation band of the same intensity. These and our results indicate therefore that mRNA could be transcribed in both sexes but that the maturation of the protein could to be an important step in sex-differentiation of some of the CYP enzymes. It is also possible that sex-dependent regulation is tissue specific. This has been seen in studies comparing the liver and the kidney of male and female mice [137]. In liver microsomes the expression of CYP2A4 is higher in

female mice compared to male, but the opposite is the case for the kidney [137]. It is therefore possible that CYP enzymes may show one gender pattern in the liver and a reversed or no gender difference in other tissues. In the case of Pgp one study has shown that the basal protein level was approximately 40% higher in female than in male rat liver [138].

Only CYP2A3 showed variation in mRNA expression along the length of the small intestine, the ileum seemed to lack expression whereas the other two sections had some. Previous studies have shown differential expression of CYPs such as the CYP1A1 and CYP2B1 between the segments of the intestine [28, 63]. When studying the localisation of Pgp, activity was found along the entire length of the small intestine, but with a higher activity in the ileum than in the duodenum [75]. In neither of these studies the mRNA expression was determined and are therefore not directly comparable to our study. However other studies have shown a correlation in mRNA expression and the enzyme activity in human liver for CYP1A1, CYP1A2, CYP3A4, CYP2D6, and CYP2B6 [139]. Whether or not similar correlation exists in the intestine is unclear.

A conclusion from this investigation is that the mRNA expression of both CYPs and Pgp is different in the rat small intestine as compared to the liver. Results from metabolic studies in the liver can therefore not be assumed to be the same in the GI-tract.

Interindividual variation of CYPs and Pgp in the human duodenum (paper II)

To study the CYP and Pgp expression in the human small intestine, biopsies from the duodenum were used. This choice was based on two criteria; (I) earlier studies have shown that the CYP content and activity are generally higher in the duodenum as compared to jejunum and ileum [28, 140]. (II) it is easier to get duodenal sample from patients going through gastroscopic examinations than from the other two sections of the human small intestine, which need more invasive operations.

The mRNA expression was studied using RT-PCR and the analysis was done by comparing expressions of CYP mRNA to the expressions of Pgp mRNA. Pgp is encoded by the *MDR1* gene that is supposed to be expressed in the human small intestine at high levels [60].

An extensive interindividual variation was found in the expression of the eight *CYP* genes and the *Pgp*. Only two CYP forms, the CYP2C9 and CYP3A4, in addition to Pgp were found in all 51 subjects. The other forms were expressed at 82% (CYP1A1), 88% (CYP1A2) and 72% (CYP2A6). The frequency for the remaining three CYP forms was approximately 95%. In addition the level of expression varied extensively between individuals based on densitometric determination. In earlier studies, CYP3A4 was found in high amounts in the human liver and small intestine [28, 35]. Our study shows that the intestinal expression can vary dramatically between individuals which has not been reported before [28, 131, 141, 142].

CYP2C9 has been reported to be expressed in the human small intestine but only at low levels [27, 28]. In our study the CYP2C9 mRNA was expressed at a high average level and with the smallest interindividual variation among the CYP genes indicating that the CYP2Cs could be one of the major forms in the human intestine.

The expression of the different CYP forms and the Pgp were compared to each other with a help of a densitometric analysis. The results show that the correlation was in general low. Only three combinations exhibit a r> 0.5, with a high significance (p< 0,01) CYP2D6/CYP1A2, CYP2E1/CYP1A2, and CYP2E1/ CYP2D6 (Table 1). This was not expected since these three genes are known to be differently regulated, at least in the liver [82, 143, 144]; CYP1A2 is regulated by the Ah-receptor having substances like PAH as substrates. But the enzyme is also posttranscriptionally regulated by RNA stabilisation [88, 111-113].

Table 1. CYPs with the best correlation coefficient (r >5) based on the densitometric analysis.

	CYP1A2	CYP2D6
CYP2D6	0.74 p < 0.001	
CYP2E1	0.55 p < 0.001	0.62 p < 0.01

The CYP2D6 is involved in metabolism of a diversity of drugs [145, 6] and earlier studies indicated that CYP2D6 is not induced by xenobiotics. The co-ordinated transcriptional regulation of the enzyme in both extrahepatic and hepatic tissue should be the same, with no tissue specificity [147].

The CYP2E1 is showing a complex regulation pattern with several post-transcriptional regulatory mechanisms, for example different physiological conditions such as diabetes; that increases the expression by mRNA stabilisation or fasting, where the activation is transcriptionally activated [106-108].

The reason for this high correlation in the expression of the three CYP genes is not known but could be due to some transacting factors working in a tissue specific manner.

It has been speculated that Pgp and CYP3A4 may act synergistically in the small intestine as a major defence against xenobiotics [6]. A suggestion that is based on the fact that they have a broad overlapping substrate specificity [21, 64, 65]. Based on the rather poor correlation of their mRNA expression (r= 0.38) in our study, this does not seem to be the case. Rather, the results indicate that there must be differences in the regulation of these two genes. Both CYP3A4 and Pgp are known to be regulated by the nuclear receptor PXR (NR1I2) [83-85, 99-101, 148], but a differences in the protein-DNA interaction have been discovered: in *CYP3A4* a XREM (xenobiotic-responsive enhancer module) has a DR3 or ER6 motif that mediate the induction whereas in *Pgp* it is a DR4 motif [149]. A second difference is that the PXR dependent induction of CYP3A4 is achieved in co-operation with

a promoter element, which is missing in the MDR1 gene [83]. This could be the reason for their different regulation. Alternatively tissue specific factors may be involved explaining the variation in their expression.

The study does not support the hypothesis of a strict co-regulation of Pgp and CYP3A4 in the small intestine [65].

When comparing the expression of the different CYP forms to the demographic data we could see that CYP2A6 was affected by gender with a lower expression in females compared to male. CYP2E1 was affected by ethnic background with a higher expression among North Europeans compared to individuals from the Middle East. Among the external factors the intake of both acetylsalicylicacid (ASA) and omeprazole (Omep) increased the mRNA of some CYPs: ASA induced both CYP2A6 and CYP2E1, whereas omeprazole upregulated CYP3A4 (Table 2).

Table 2. Correlation between different CYPs and internal and external factors, based on densitometric analysis.

	CYP2A6	CYP2E1	CYP3A4
Sex	P = 0.0087		
Ethnic background		P= 0.0193	
ASA	P= 0.0118	P= 0.0235	
Omep			P= 0.0299

This study showed an extensive interindividual variation in the expression of drug metabolising *CYP* genes in the human small intestine. Accordingly it seems possible that any of the forms included in the study could be a major form in some individuals. The mRNA expression is also affected by the usage of common drugs/xenobiotics such as acetylsalicylic acid, omeprazole and smoking. The possibility that this large interindividual variation may lead to a varied bioavalibility of orally used drugs and complicate optimal drug therapy, should be carefully examined.

Expression of UGTs in the human duodenum (paper III)

The mRNA expression of seven UGT forms was analysed by DRT-PCR and densitometric quantification by comparing with the expression of β-actin set at 100% for each sample.

Interindividual variation in the expression of the different UGT forms was considerable. Only UGT1A1 was expressed in all 45 individuals. The UGT1A10 and UGT2B7 were found in 44/45 of the subjects. These three forms were also expressed at the highest average level based on the densitometric analysis. The lowest frequency was found for UGT2B15; present in 53% of the individuals which also was expressed at the lowest average levels when compared to the other forms (Table 3). Earlier studies have shown activity in most of the studied UGTs found

in the small intestine. [8, 150]. However, in these studies too few individuals were recruited so that reliable conclusions can been drawn from them. In particular on the interindividual variability.

Table 3. A summery of the densitometric analysis on the seven different UDP-glucuronosyltransferases is given. The mean values are based on the densitometric analysis relative units.

	%UGT1A1	%UGT1A3	%UGT1A4	%UGT1A6	%UGT1A10	%UGT2B7	%UGT2B15
mean	265	60	63	41	131	288	46
CV	40%	64%	49%	48%	64%	73%	61%
		No normal distribution			No normal distribution.	No normal distribution	No normal distribution

Table 4. UGTs with the best correlation coefficient (r >5) based on the densitometric analysis.

	UGT1A1	UGT1A3	UGT1A10
UGT1A1			
UGT1A3			
UGT1A10	0.55 P< 0.001	0.52 P< 0.001	
UGT2B7	0.61 P< 0.001		0.58 P< 0.001
UGT2B15		0.64 P< 0.01	

The expression levels of the studied *UGTs* were compared to each other, several of which seemed to correlate well, with a r> 0.5 and a high significance: UGT1A1/ UGT1A10, UGT1A1/UGT2B7, UGT1A3/UGT1A10 UGT1A3/UGT2B15, and UGT1A10/UGT2B7 (Table 4). This rather high correlation could indicate that some common regulation mechanisms are involved. On the other hand the scores (r= 0.5 – 0.6), also suggest some differences in the regulation. The overlapping substrate specificity of some of the UGT forms [8, 55, 89, 151] would speak for a common regulation. Both *UGT1* and *UGT2* genes are known to exhibit tissue specific regulation; for example, *UGT1A10* is only found in extrahepatic tissues such as the small intestine and the biliary tract [55, 152]. Little is known about the regulation of human UGTs, as most of the studies so far have used either hepatocytes or transfected cells It has been established however, that the *UGT1A1* expression is regulated by the CAR receptor [153], and the *UGT2B7* expression is PXR mediated. What factors mediate the *UGT2B15* expression is not yet determined but at least the PXR seems not to be involved [52]. The only studies on regulation that has been done on extrahepatic tissues is on *UGT1A6* showing a CAR dependent expression in Caco 2-cells [86].

None of the endogenous factors included in this study: age, sex, and ethnic background affected the expression of the UGTs. Earlier reports have shown sex differences in drug metabolism by UGTs, however these studies focused on *in vivo* glucuronidation by measuring plasma levels or the urine excretion after an oral dose of the different test drugs [154-157]. Therefore, these studies are not comparable to the present as they measure the systemic activity of *in vivo*.

Only UGT1A4 was significantly increased by the intake of omeprazole and by smoking (Table 5). To our knowledge this is the first report on omeprazole impact on any UGT form.

Table 5. Relationship between UGT1A4 and external factors.

UGT1A4	Omeprazole	p = 0.003
	Smoking	p = 0.029

Which UGT enzyme(s) are involved in nicotine metabolism is not yet known [58]. However, it has been shown that smoking upregulates glucuronidation reactions catalysed by UGT1A6 [87, 159]; but no reports has shown affects on UGT1A4.

This study revealed a large interindividual variation in the mRNA expression of seven UGT forms of the human intestine. The major forms seem to be UGT1A1, UGT1A10 and UGT2B7; they are expressed in most individuals and also at a high average levels.

The expression of these three forms also appeared to correlate with each other. It could therefore be that these three forms together form a major glucuronidation machinery of the intestine. A possibility that should be examined in detail.

The expression of one form; the UGT1A4 is affected by smoking and by usage of omeprazole. This could be of importance for drug-drug interactions at least for some individuals.

Induction of drug metabolism by Artemisinin (paper IV)

Artemisinin is used orally in multidrug treatment of malaria. It has been suggested to induce drug metabolism and therefore be involved in drug-drug interactions.

Earlier experiments, by us and others [128], have indicated that artemisinin can affect CYP1A2, CYP2A, and CYP2B in rodents. Indirect evidence also indicated that artemisinin may act like phenobarbital and other CAR activating compounds in the induction of drug metabolism, but this has never been confirmed. In humans the drug is primarily metabolised by CYP2B6 with some contributions by CYP3A4 and CYP2A6 [127]. In this paper we characterised the inductive properties of artemisinin on CYPs by using three different techniques: *in vivo*, primary hepatocytes in culture and recombinant luc-reproter genes with a CAR or PXR driven promoters

In vivo experiments (Table 6) showed that the drug increased the activity of CYP2B10 and to a lesser extend that of the CYP2A5 of the mouse liver. Hardly any effect was seen on CYP1A2 activity.

Table 6. Effect of artemisinin on the three CYP catalysed enzyme activities of mouse liver microsmes. Activities are given as pmol/min x mg protein. Fold of increased activity is given in parenthesis.

CYP dependant activity	Control	Artemisinin treated
CYP2B10	52 ± 5	390 ± 42 P≥ 0.001
		(7.5)
CYP2A5	135 ± 15	365 ± 30 P≥ 0.05
		(2.7)
CYP1A2	480 ± 60	510 ± 70
		(1.06)

In cultured primary hepatocytes a strong effect was seen on the mRNA expression of CYP2B10 and a weak effect on CYP2A5 upon exposure to artemisinin. The CYP1A2 mRNA expression was not affected. (Fig. 11).

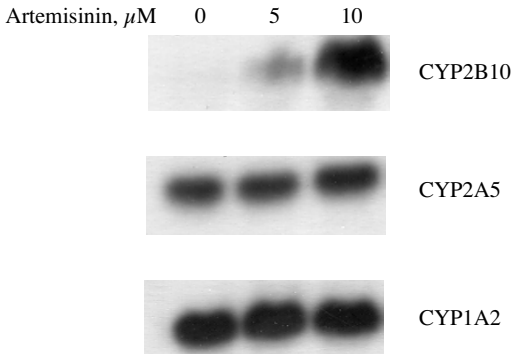

Figure 11. Northern blot analysis of total RNA from mouse primary hepatocytes exposed to artemisinin at three different concentrations (from paper IV).

Based on the results it appeared that artemisinin mainly upregulates the CYP2B enzymes at a pretranslational level.

HEK293 cells were transfected with mouse and human CMX-GAL4-CAR or CMX-GAL4-PXR constructs and treated with artemisinin. The drug showed a dose dependent increase in both mouse and human CAR-mediated expression, with a slightly higher activation with the mouse construct. Neither mouse nor human

PXR driven promoters were affected by artemisinin. (Fig. 12). This results provide evidence that artemisinin acts as an inducer via the CAR receptor, thus confirming the earlier indirect evidence.

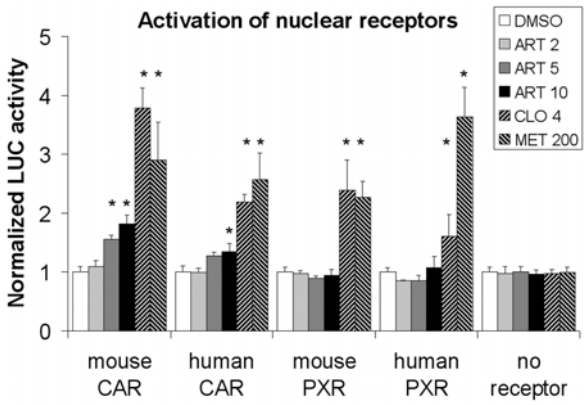

Figure 12. Effects of artemisinin on the expression of CAR or PXR driven luciferase recombinants in HEK293 cells (paper IV).

The study shows that artemisinin induces hepatic CYP2B, at a pretransitional level by a mechanism involving the nuclear receptor CAR. The weaker effect, seen on the CYP2A5 expression, may involve a different regulation mechanism as compared to the CYP2B where artemisinin toxicity may play a role.

GENERAL SUMMARY

This work shows extensive interindividual variations in the intestinal expression of several *CYP* and *UGT* genes encoding for drug metabolising enzymes in humans. Each individual appears to have his or her unique CYP and UGT profile or fingerprint. Due to effects of external factors, the profile may vary over time.

It was shown that the genes expressed at the highest levels in a human population are the *CYP2C*, *CYP2D6*, and *CYP3A4* and the *UGT1A1, UGT1A10,* and *UGT2B7.* Due to the extensive variability, any of the genes included in the study may have a major role in intestinal drug metabolism for a given individual.

Expression levels of the different genes were determined by analysing concentrations of corresponding mRNAs in the human specimen. It was not established how well the mRNA levels reflect the corresponding protein or enzyme activity levels. However, the results should help designing functional studies in search for the key catalysts of drug metabolism in the human intestine.

Some common external factors such as smoking, and clinical drugs omeprazole and acetylsalicylic acid affected the expression of some of the *CYP* and *UGT* genes. This may lead to drug-drug interactions affecting drug absorption and metabolism in the intestine of exposed individuals; a possibility that should be studied in more detail.

The study also established that artemisinin, a widely used antimalarial, affects the expression of drug metabolising enzymes mainly via the CAR receptor with minor effects possibly due to its toxicity. This result should help predicting and avoiding drug-drug interaction in therapies where artemisinin is used.

Monica Lindell

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Monica 15/9

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