Drug metabolism in human and rat intestine
van de Kerkhof, Esther Gesina

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2007

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
**Introduction to the gastrointestinal tract**

The gastrointestinal tract forms the first line of defense against xenobiotics including drugs that are taken orally. As oral administration is the most convenient and therefore most frequently used route of drug administration, this gastrointestinal barrier determines to a large extent the bioavailability of drugs.

The barrier function of the intestine is ensured by the epithelial cells that line the luminal surface of the intestine and the specialized tight-junctions between them [1]. The intestine controls the uptake of water, electrolytes, nutrients and xenobiotics, and it secretes ions, enzymes, mucus and excretes endogenous and exogenous compounds from the blood towards the lumen. The absorptive area of the intestine is enormous due to folding, villi and microvilli, increasing the surface area approximately 600-fold [1]. Intestinal motility causes mixing of the components and ensures absorption and transport along the tract [1,2].

![Histology of the intestinal wall](image)

**Figure 1: Histology of the intestinal wall [3]**

Every region of the intestinal tract consists of the same cell layers (figure 1 [3]): serosa, muscularis, submucosa and mucosa. The mucosa is covered with a continuous layer of cells, consisting of enterocytes and goblet cells [2]. Anatomically, the intestine is divided into duodenum, jejunum, ileum and colon. In each of these regions, the enterocytes have a different composition of enzymes and transporters that as part of the homeostatic function of the intestine, are able to metabolize and transport endogenous and exogenous compounds [4-7].

**Drug metabolism and transporters in the intestinal tract**

Although the liver has long been thought to play the major role in drug metabolism, the metabolic capacity of the intestine in this respect is increasingly recognized [8,9]. In the seventies of the 20th century, intestinal drug metabolism was already reported [10]. Nonetheless, until recently the clinical significance of intestinal drug metabolism remained under debate [11,12]. Basic knowledge concerning appreciation of drug metabolizing enzymes (DMEs) and drug transporters (DTs) in the human intestine has been collected in the last decade [13,14]. An important indication for the clinical significance of intestinal metabolism was inferred from *in vivo* studies using cyclosporine A, verapamil and midazolam as substrates [8,9,15]. DMEs and DTs are broadly expressed along the intestinal tract and can contribute together to all three phases of drug metabolism as depicted in figure
Introduction

2: introduction of functional groups in the drug molecule (phase I), conjugation of drug molecules or phase I products (phase II), excretion by efflux transporters (commonly referred to as phase III) and deconjugation in the lumen (phase III of drug metabolism).

Figure 2: Phases of drug metabolism in the intestine

The intestine is not a homogeneous organ. The expressions of drug metabolizing enzymes and drug transporters in the enterocytes follow different patterns both along the crypt-villus axis and along the length of the intestine. For DMEs, the activity has been reported to be the highest in the differentiated epithelial cells of the villus region and to decline progressively towards the crypt region [16,17]. This heterogeneity is introduced by cell migration from crypt to villus, which appears to be a concerted program of differentiation leading to apoptosis and cell extrusion into the lumen [18]. The migration from crypt to villus axis takes place in approximately 3 days in human and 2 days in rat intestine [5]. Data on the presence of the most prominent drug metabolizing enzymes and drug transporters on mRNA level or protein level and on activity measurements are summarized in table I (p. 14-15).

Phase I drug metabolism
The members of the cytochrome P450 (CYP) superfamily are the principle enzymes involved in the biotransformation of drugs [11]. The amounts of CYPs in the intestine show a large inter-individual variation as has been described for CYP1A1 activity [19] and CYP3A protein [20] differing over 15-fold between individuals. This variation may originate from several interindividual differences (polymorphism, age, gender, drugs, diet, etc)
In the human intestine, at protein level, CYP3A is the most abundant (59-94% of which 33-87% is CYP3A4), followed by CYP2C9 (4-38%), CYP2C19 (0.5-7%), CYP2J2 (0.2-4%) and CYP2D6 (0.2-4%), which clearly differs from liver in which CYP3A4 and CYP3A5 are the most abundant CYPs, but representing only 40% of the total CYP. In the liver, this is followed by CYP2C (25%), CYP1A2 (18%), CYP2E1 (9%), CYP2A6 (6%), CYP2D6 (2%) and CYP2B6 (<1%) [21]. CYP2A6 activity [22], has not been detected in the human
Another remarkable difference between human intestine and liver is the distribution of CYP1A isoforms. CYP1A1 is predominantly expressed in the intestine, whereas CYP1A2 is the major CYP1A isoform in the liver \[23\]. CYP3A4 is a major player in the conversion of drugs, being involved in the metabolism of approximately 50-60% of the drugs, followed by CYP2C and CYP2B6 that are involved in the conversion of another 20 to 25% \[12,24,25\]. This suggests that the intestine is in principle able to convert over 60% of all drugs, as is illustrated by the finding that CYP3A4 activity plays a significant role in the first-pass metabolism of, for example, midazolam \[26\]. Therefore, drug metabolism by the intestinal wall should not be neglected and requires thorough evaluation.

In the proximal part of the intestinal lumen, the concentrations of orally taken drugs, but also of dietary components are highest. To form an optimal barrier preventing xenobiotics to enter the body, the highest density of DMEs should also be present in the proximal part of the intestine. This is indeed the case for CYP3A4, CYP2C8\textendash10 and CYP2D6 \[14,27,28\]. The total enterocyte microsomal protein content decreases markedly in distal direction, whereas for the total P450 content a slight increase from duodenum to jejunum is followed by a sharp decrease towards ileum \[5\]. The distribution pattern of NADPH-dependent P450 reductase activity, which is required for the P450 reactions \[29\], closely parallels that of CYP3A \[20\]. In contrast, mucosal microsomal cytochrome b5 protein content and b5 reductase activity (enhancers of the mono-oxygenase reaction) tend to increase slightly in distal direction \[20\]. The gradient along the intestinal tract is not similar for all members of the CYP superfamily. The CYP2S1 protein is equally expressed along the tract \[30\], whereas the expression of CYP2J2 increases \[31\]. In the proximal part of the human small intestine CYP3A4 is the predominant enzyme, whereas in the large intestine CYP3A5 is more abundant \[32\]. Like in the human intestine, in the rat intestine CYP3A is the most abundantly present CYP. However, which isoforms are expressed is still under debate. Up to now, clear evidence exists for the presence of 5 different isoforms in rat small intestine: CYP3A1 \[33\], CYP3A2 \[33,34\], CYP3A9 \[35\], CYP3A18 (predominantly in males \[35\]) and CYP3A62 \[36\]. As for human intestine, also in rat the metabolic activity of phase I metabolism in general is higher in the duodenum and jejunum than in the ileum and colon \[34,37\] as described in chapter 3 of this thesis. 7EC O-deethylation (among others mediated by CYP1A) decreases in distal direction \[37\], as well as 17β-HSD activity (androstenedione formation) \[38\], but CYP3A9 and CYP3A18 mRNA expression first increases and then remains constant along the small intestine \[35\].

### Phase II drug metabolism

The significance of intestinal phase II metabolism to total drug metabolism has not been extensively described. For sulphation, Lin et al. hypothesized that the intestine could be the major site for first-pass metabolism of compounds such as terbutaline and isoproterenol \[11\]. Furthermore, Pelkonen et al. reviewed the significant contribution of phase II intestinal metabolism to pre-systemic clearance for several β2-adrenoceptor agonists \[32\]. The significance of phase II metabolism in rat has been proven in vivo showing that more than 50% of orally dosed morphine is glucuronidated in the intestinal wall during absorption \[39\]. Many conjugation enzymes and isoforms are expressed in the human intestinal tract, as is summarized in table I. As for phase I metabolism, conjugating enzyme gradients are generally decreasing in distal direction as has been proven for UGT protein \[40\] and for
sulphotransferase activity [41,42]. For GST activity, a decreasing gradient was also found from proximal to distal colon [43].

Conjugating enzymes are not equally distributed along the rat intestinal tract either. Sulphation rates decrease in distal direction in small intestine, but the highest activity has been found in colon [37]. With regard to glucuronidation, UGT2B1, UGT2B3 and UGT2B6 expression decreases in distal direction [44], whereas UGT1A1 and UGT1A6 activities are homogeneously distributed along the small intestine [34]. In colon, UGT1A1, 1A3, 1A6 and 1A8 expressions are higher than in small intestine [44], which is in line with activity data (as described in chapter 3) showing that 7-hydroxycoumarine glucuronidation (mainly UGT1A6) is higher in colon than in small intestine [37]. For GST activity, a decreasing gradient in distal direction has been reported [45] and glutathione conjugation has also been shown in mucus of rat intestine [46].

Knowledge about the gradients of drug metabolizing enzymes in the intestine is very valuable for the interpretation of local absorption data. Low bioavailability of drugs can be the result of low water solubility or high polarity leading to a low rate of mucosal uptake, but it can also be due to a high rate of drug metabolism. With the distribution patterns of drug metabolizing enzymes in mind, the localization of high first-pass metabolism in the intestine can be estimated. When necessary, the drug formulation can be adapted such that the drug is released at the most optimal region in the gut. Since most drug metabolizing enzymes have the highest expression in the proximal part, a programmed release preparation could decrease the intestinal first-pass effect, provided that mucosal uptake can also take place efficiently in the distal part of the small intestine.

**Phase III transporters**

Apart from drug metabolizing enzymes, drug uptake and efflux transporters, such as PEPT1 [47] or MRP2 and Pgp [48], form another obstacle to drug absorption. The major membrane transporters have been classified into the solute carrier (SLC) transporter family and the ATP-binding cassette (ABC) transporter family by the Human Genome Organization (HUGO) Gene Nomenclature (SLC1-45: http://www.gene.ucl.ac.uk/nomenclature/genefamily/slc.php or ABCA to ABCG: http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html) [47]. The most important transporters detected in human and rat intestine are listed in table I. Furthermore, the localization of these transporters is depicted in figure 3A.

For influx transporters, such as PEPT1, clinical significance of determining the bioavailability of several β-lactam antibiotics such as cefadroxil, ampicillin and cefixime has been proven. Concomitant administration of cefadroxil and cephalaxin in humans decreased the AUC values of cefadroxil after oral administration presumably due to the competitive inhibition of intestinal PEPT1 mediated transport of cefadroxil by cephalaxin [47,49].

Since efflux transporters excrete drugs back from the enterocyte into the lumen, they may prevent drug entrance into the circulation [4,50,51]. In addition, the intestinal efflux pumps may contribute to the extrahepatic excretion of drugs present in the blood. Talinolol, for example, is actively secreted into the human small intestine after intravenous administration.
Table I: Summary of drug metabolizing enzymes and drug transporters currently known to be present (detected at mRNA (M), protein (P) or activity (A) level or not detectable (ND) in both rat and human small intestine (SI) and colon tissue.

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Figure 3: Localization of transporters in the intestine (A) and the hypothesized role of Pgp, as formulated by Benet to increase the possibility for drug metabolism along the intestinal tract [4,54] (B)
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</table>
Chapter 1

This secretion was reduced by concomitant intraluminal administration of R-verapamil (MDR1 substrate) [49]. The importance of intestinal secretion of drugs or metabolites for drug absorption [48,52] has been further confirmed with the in situ perfusion technique, where inhibition of P-glycoprotein (Pgp) increased the apparent permeability of tacrolimus in rat ileum and colon [53]. Benet has launched the hypothesis that Pgp by itself increases the opportunity for the intestine to metabolize a drug. In this hypothesis (depicted in figure 3B) it is assumed that a part of the drug after entering the cells is effluxed back into the lumen by Pgp, thereafter it is re-absorbed further down the intestine and ‘re-exposed’ to the drug metabolizing enzymes in these cells. The residence time of these drugs within the intestinal cells is thereby prolonged and the opportunity for drug metabolism is increased [4,54]. According to Lin et al., the Km values of Pgps are relatively low, indicating that they are readily saturated when drugs are administered at high doses. In contrast to Benet, these investigators question the significance of Pgp in the in vivo situation, since drug concentrations normally exceed the Pgp Km values [11]. On the other hand, oral administration of digoxin to volunteers with silent polymorphism in exon 26 (G 3435T) of MDR1 results in significantly higher digoxin plasma levels and AUC in comparison with healthy (non-polymorphistic) volunteers which provides direct evidence for a role of intestinal MDR1 in determining the oral bioavailability [49]. The exact contribution of Pgp to the inhibition of intestinal permeability, however, remains difficult to assess [55]. Nevertheless, new drug candidates are routinely screened for their potential to interact with Pgp and these results can influence the future development of the compounds [54].

Also for transporters, different gradient patterns along the tract exist as has been summarized by Pang [56]. In human tissue, for example, in distal direction, the density of some transporters increases (Pgp [57-59], MRP1 [59], OCTN2 [60]), whereas BCRP (ABCG2 [61]), MRP2 [59,62], CNT1-2 [60], SERT [60], PEPT1 [60,62] and OCTN2 [60,62] decrease or remain constant as was also reported for ATPB [60,62] OCTN1 [60]. Furthermore, Mrp3 is highest in colon [59,62] and ASBT is higher in ileum than in both duodenum and colon [60,63]. In rat intestinal tissue, several transporters have been detected as are listed in table I. As for human intestine, Mrp2 decreases [64,65] in distal direction along the tract. In contrast, Bcrp, Mdr1a, Mdr1b, Mrp3 and Ostα-Ostβ expression increases along the length of the tract [35,64,66-68].

Drug-drug/diet interactions

The significance of the intestine in determining the fate of drugs in the body is not only due to the high intrinsic capacity to metabolize drugs, but is further complicated by its sensitivity to induction and inhibition of the DMEs involved [32,69-72]. This can result in a marked variation in the bioavailability of drugs and may cause an imbalance between toxification and detoxification [73]. It was suggested by Lin et al. that intestinal enzymes respond to a greater extent than hepatic enzymes to orally administered inducers like drugs and food components because of exposure to their relatively high concentrations in the intestine [11]. A well-known example is the induction of CYP3A4 and Pgp by St Johns Wort in transplantation patients, causing a serious decrease in cyclosporine A plasma concentration, which in several cases has lead to organ rejection after transplantation.
Furthermore, significant inhibition of CYP3A4 by grapefruit juice has been shown to increase the felodipine AUC 3-fold in hypertensive patients [70].

**Mechanisms of drug induced induction**

Regulation of gene expression at the transcriptional level, mediated by orphan nuclear receptors (such as PXR, CAR, GR) and the aryl hydrocarbon receptor (AhR), plays a crucial role in drug metabolism and clearance of drugs and xenobiotics as has recently been reviewed [24,73,76,77]. Nowadays it is commonly known that CAR, PXR and RXR, termed as orphan receptors [24], function as sensors of toxic by-products derived from endogenous metabolism and of exogenous compounds [78,79].

PXR (pregnane X receptor) is one of the nuclear receptors involved in the regulation of drug metabolizing enzymes and drug transporters (figure 4A). The human receptor of PXR has also been referred to as steroid and xenobiotic receptor (SXR) or pregnane activated receptor (PAR) [24] and according to the new nomenclature PXR has been classified as NR1I2. PXR is predominantly expressed in liver and intestine and to a lower level in kidney and lung (human, mouse, rat). Binding of a ligand to PXR (figure 4A) gives rise to its nuclear translocation. In the nucleus, it binds to RXR and then to the xenobiotic responsive enhancer module (XREM [80]) in the promoter regions of responsive genes, inducing, amongst others, CYP3A, UGT1A1 and MRP2 transcription [24,78]. For PXR, important species differences have been described with respect to ligand specificity. Rifampicin, for example is a potent inducer of CYP3A in rabbit and human liver, but not in rat and mouse liver where PCN is a potent inducer [24].

CAR is another member of the orphan nuclear receptor family (figure 4B) and originally defined as constitutively activated receptor, but according to the new nomenclature is now called NR1I3. It is mainly expressed in liver and to a lesser extent in intestine. CAR is located in the cytoplasm of cells partially in the phosphorylated form. In this form, CAR can activate responsive genes in the absence of ligands. Ligands such as phenobarbital (PB) enhance the phosphorylation of CAR, which causes nuclear translocation, where CAR is re-phosphorylated by CaM Kinase, which is also activated by PB, and subsequently forms a heterodimer with RXR (figure 4B). This complex can bind to the PB responsive enhancer module (PBREM), inducing the transcription of, for instance, CYP2B [76].

Cross-talk among the orphan nuclear receptors PXR and CAR has been extensively discussed in the literature [76]. There is significant overlap in the response of PXR ligands and CAR ligands. This cross-talk may take place, because both CAR and PXR recognize each others response elements and trigger gene expression upon activation by either common or selective ligands [24].

Activation of the GR (glucocorticoid receptor, figure 4C) by ligands such as dexamethasone, can introduce nuclear translocation inducing the transcription of for example CYP2C9. Since GREMs (glucocorticoid receptor enhancer module) are also present on the genes coding for PXR, CAR and RXR, it indirectly also leads to induction of PXR and CAR related genes [81] and thereby it may enhance the transcription of PXR and CAR regulated genes provided that an endogenous ligand is present.

A fourth mechanism of transcriptional activation and induction of metabolism is represented by AhR (aryl hydrocarbon receptor, figure 4D). AhR is known to recognize a range of
chemical structures, including (non-) aromatic and (non-) halogenated compounds [76], such as β-naphthoflavone. The unliganded AhR is found in the cytoplasm as a complex with a heat shock protein 90 (HSP90) dimer, which prevents nuclear translocation. Ligand binding causes a conformational change, which leads to the release of HSP90 and an increased affinity of AhR for DNA and nuclear translocation. In the nucleus, binding to ARNT (Ah receptor nuclear translocator), results in AhR-ARNT complex formation that has a high affinity to the XREM on DNA, thereby inducing CYP1A transcription [76].

**Figure 4:** Induction mechanism via PXR (A), CAR (B), GR (C), AhR (D), Nrf2 (E)

Activation of Nrf2 (NF-E2-related factor) by oxidative/electrophilic stress or MAP (mitogen-activated protein) kinases represents another mechanism of transcriptional activation [82,83]. Phytochemicals, such as phenethyl isothiocyanate (PEITC) and sulforaphane, have been reported to activate Nrf2 (figure 4E). In the cytosol, Nrf2 is present in a latent complex with the cytoskeleton-associated protein Keap1 (kelch-like ECH-associated protein 1) [84].
Oxidative/electrophile stress oxidizes Keap1 that uncouples Nrf2 and Keap1 and causes nuclear translocation of Nrf2. In the nucleus, Nrf2 binds to small Maf proteins and these heterodimers can associate with ARE/EpREs on the DNA (antioxidant response element/electrophile response element) [85], thereby inducing the transcription of GSTA2 and UGT1A6 among other genes [82,83]. The AhR and Nrf2 pathways are coupled [82,83], as has been reported for the PXR, CAR and GR mediated induction pathways [81]. Linking of the induction via AhR and Nrf2 occurs via several mechanisms. For example, Nrf2 itself is up-regulated by AhR mediated induction. In addition, AhR ligand binding up-regulates CYP1A1, which generates reactive oxygen species, which in return activates Nrf2. Furthermore, as a third mechanism direct cross-interaction of AhR/XRE and Nrf2/ARE signaling has been described [83].

**Induction pathways in the intestine**

In the human and rat intestine, all of the above-mentioned pathways were shown to be active and DME induction was found both in vivo and in intestinal cell lines after administration of rifampicin [86], dexamethasone [87-89], PB [87-89], β-naphthoflavone [88-90] and quercetin [91-96] prototypical inducers for PXR (human), GR/PXR, CAR, AhR and Nrf2 respectively [24].

The important notion thus is that the drug metabolizing capacity of the intestine shows large intrinsic interindividual differences. These differences are further enlarged by the presence of inducing and inhibitory substances available from the diet and ingestion of xenobiotics. These interindividual differences potentially lead to marked variability in drug bioavailability as well as interactions at the transport and metabolic levels.

**Aim of the thesis**

To study drug metabolism in intestinal tissue in detail, *in vitro* methods are required, since *in vivo* methods are often technically complex or give rise to ethical problems, when experiments in humans are involved. These *in vitro* methods cannot only increase our fundamental knowledge on drug metabolism in the human intestine. Information can also be gathered that is of great benefit for research in the pharmaceutical industry. Among others, it may allow a better prediction of drug metabolism and drug interactions in the intestine of animals and humans.

Such *in vitro* test systems should meet at least three criteria. First, the model should consist of intact cells with DMEs and DTs remaining functional for approximately 24 h of incubation. This is important to allow investigation of slowly metabolized drugs as well as to characterize induction mechanisms. Secondly, the technique should be applicable to both animal and human tissue. Animal models are commonly used, but drug metabolism and especially drug-drug interactions, are highly species specific. Thirdly, the *in vitro* test system should require only small amounts of tissue in view of the limited access to human small intestinal tissue [97].

Cell lines, such as Caco-2 and LS180, can be cultured for a long term, do not require human tissue and have been used for induction studies [98-100]. These cells, however, differ in many aspects from intact human intestinal tissue and cannot be used for quantitative or qualitative metabolism studies, since they lack several important drug metabolizing
enzymes. An alternative is the use of intact tissue systems, such as intestinal tissue mounted in Ussing chambers. This technique is applicable to animal and human tissue, but the limited viability only allows incubations up to 4 h. Tissue biopsies can also be used for human and animal gut material, and can be cultured for longer time periods to study induction. Yet their viability decreases readily after 3 h of incubation [101]. Recently, the intestinal precision-cut slice model has been developed in our laboratory by De Kanter et al. [101] showing high metabolic rates in the slices up to 3 h of incubation [101]. Longer incubation periods were not tested at the start of this PhD project. This technique enables very efficient use of intestinal tissue, can potentially be applied to both tissue from all animal species and that of humans. Furthermore, the incubation period could possibly be extended up to 24 h. In addition, it has some highly appreciated features in comparison with cell lines. In tissue slices, all cell types are present; location dependent metabolism and regulation can be studied and the enzyme systems, co-factors and transporters, in principle, remain present in their physiological context.

The aim of this project, therefore, was to evaluate the application of rat and human intestinal precision-cut slices for drug metabolism studies.

Therefore, the intestinal tissue slice model was first characterized up to 3 h of incubation with respect to viability and metabolic functionality. The slice technique was then further applied to measure the metabolic rates of both phase I and phase II drug metabolizing enzymes in different segments of the intestinal tract. In the studies described in chapter 3, the sensitivity of slices to induction of drug metabolizing enzymes by β-naphthoflavone after 24 h of incubation was assessed. Subsequently, the ability of precision-cut slices to serve as a model for the detection of drug mediated induction of drug metabolizing enzymes in the rat intestinal tissue was investigated by incubating them during various incubation periods with several prototypical inducers (chapter 4). In chapter 5, we investigated the induction along the rat intestinal tract.

Furthermore, it would be of great benefit to show that this in vitro test system is applicable not only for rat intestine, but also for human tissue. Therefore, we aimed to validate the slice system for the use of human tissue. In addition, we had the opportunity to directly compare the precision-cut slice method with the Ussing chamber set-up (validated and present at AstraZeneca, Mölndal, Sweden) (chapter 6). In chapter 7, we describe the validation of the precision-cut slice system to study DME and DT induction in human intestine up to 24 h of incubation. Subsequently, we investigated the response of a broad spectrum of drug metabolizing enzymes, drug transporters and nuclear receptors to induction pathways via GR, PXR, CAR, AhR and Nrf2 (chapter 8).

In the discussion section, we compare the intestinal induction data obtained in human intestine with published responses in liver. Finally, we compare the induction data obtained in rat intestine and human intestine to form an opinion on the predictability of rat induction studies for the situation in man (chapter 9).
**Introduction**


http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/basics/gi_microanatomy.html

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[20] van de Kerkhof EG, Ungeil AL, Sjoberg AK, de Jager MH, Hilgendorf C, de Graaf IA,


Introduction


2007) Regional distribution of solute carrier (SLC) mRNA expression along the human intestinal tract. Drug Metab Dispos in press


[65] Cherrington NJ, Hartley DP, Li N, Johnson DR, Klaassen CD. (2002) Organ distribution of multidrug resistance proteins 1, 2, and 3 (Mrp1, 2, and 3) mRNA and hepatic induction of Mrp3 by constitutive androstane receptor activators in rats. J Pharmacol Exp Ther 300: 97-104


Introduction

Nuclear receptor-mediated transcriptional regulation in Phase I, II, and III xenobiotic metabolizing systems. Drug Metab Pharmacokinet 21: 437-57


(multidrug resistance protein 2) in Caco-2 cells. *Biochem Pharmacol* **59**: 467-70


[102] van de kerkhof E, de Graaf IA, Ungell AL, Groothuis G. unpublished observation.  


Introduction

cytochrome P-450 2J4. Drug Metab Dispos 27: 1123-7


