Cytochrome P450 3A.
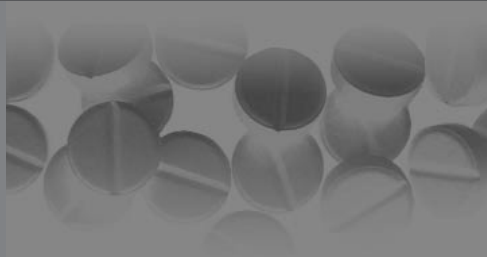# Ontogeny and drug disposition

Chapter 2

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Summary

Maturation of organ systems during fetal life and childhood exerts a profound effect on drug disposition. The maturation of drug-metabolizing enzymes is probably the predominant factor accounting for age-associated changes in non-renal drug clearance. The group of drug-metabolizing enzymes most studied is the cytochrome P450 superfamily. The CYP3A subfamily is the most abundant group of cytochrome P450 enzymes in the liver and consists of at least three isoforms, CYP3A4 , CYP3A5 and CYP3A7. Many, frequently described drugs are mainly metabolized by the CYP3A subfamily. Therefore, maturational changes in CYP3A ontogeny may impact on the pharmacokinetics of many of these drugs.

CYP3A4 is the most abundantly expressed CYP and accounts for approximately 30-40 % of the total CYP content in human adult liver and small intestine. CYP3A5 is 83% homologous to CYP3A4, it is expressed at a much lower level than CYP3A4 in the liver, but is the main CYP3A isoform in the kidney. CYP3A7 is the major CYP isoform detected in human embryonic, fetal and newborn liver, but is also detected in adult liver, although at a much lower level than CYP3A4. Many drugs have been identified as important substrates for the CYP3A family. However, substrate specificity for the individual isoforms has not been fully elucidated. Because of large interindividual differences in CYP3A4 and CYP3A5 expression and activity genetic polymorphisms have been suggested. However, although some gene mutations have been identified, the impact of these mutations on pharmacokinetics of CYP3A substrates has to be established.

Ontogeny of CYP3A activity has been studied in vitro and in vivo. CYP3A7 activity is high during embryonic and fetal life and decreases rapidly during the first week of life. On the contrary, CYP3A4 is very low before birth, but increases rapidly thereafter reaching 50% of adult levels between 6 and 12 months of age. During infancy CYP3A4 activity appears to be slightly higher than in adults. Large interindividual variation in CYP3A5 expression and activity was observed during all stages of development, but no apparent developmental pattern of CYP3A5 activity has been identified to date.

Concluding, profound developmental changes occur in the activity of CYP3A isoforms during all stages of development. These changes have, in many instances, proven to be of clinical significance with respect to treatment, using substrates, inhibitors or inducers of CYP3A. Investigators and clinicians should consider the impact of ontogeny on CYP3A in both pharmacokinetic study design and data interpretation, as well as when prescribing drugs to pediatric patients

Introduction

Maturation of organ systems during fetal life and in the neonatal and paediatric period exerts a profound effect on drug disposition. From fetal life through adolescence, dramatic changes in pharmacokinetics occur. Accordingly, effective and safe drug therapy in newborns, infants and children requires a thorough understanding of human developmental biology and of the dynamic ontogeny of drug absorption, distribution, metabolism and drug excretion (1).

Drug metabolism is one of the major determinants of drug clearance in a given individual. Although the relative size of the liver and of hepatic blood flow may affect the rate of drug metabolism during development, the maturation of the drug-metabolising enzymes is probably the predominant factor accounting for age-associated changes in nonrenal drug clearance (2).

The group of drug-metabolizing enzymes most studied includes the cytochrome P450 (CYP) superfamily. The CYP3A subfamily, the most abundant subfamily of cytochrome P450 isoforms in the liver, consists of at least three isoforms: CYP3A4, 3A5 and 3A7 (3, 4). Recently, developmental changes in CYP3A expression and catalytic activity have been studied (5-9) demonstrating a different ontogenic pattern for the individual CYP3A isoforms. Accordingly, the pharmacokinetics of CYP3A substrates may change as a function of developmental changes in CYP3A activity (10).

In view of the profound age-related differences in CYP3A activity, physicians who prescribe drugs for neonates, infants and children should be aware of the interindividual differences that often result from developmental patterns of enzyme expression and activity. The goal of this review is to provide the practitioners with insight into the developmental aspects of CYP3A activity and their possible impact on drug therapy.

Cytochrome 3A, structure and function

Cytochrome P450 superfamily

The cytochrome P450 superfamily represent a superfamily of heamcontaining proteins that catalyze the metabolism of many lipophilic endogenous substances and exogenous substrates. The biological and pharmacological relevance of this gene family has been the subject of several recent publications (11-13).

The nomenclature for the P450 superfamily is based on divergent evolution of the genes. CYPs that share at least 40% homology, are grouped in a family, which is annotated by an Arabic number (e.g. CYP3). A subfamily, indicated by a letter represents highly related genes (e.g. CYP3A). Another Arabic numeral (e.g. CYP3A4) sequentially numbers the individual genes. In humans, 17 CYP gene families have been described to date; the first three families are mostly involved in the biotransformation of pharmaceuticals and xenobiotics, wheras the other gene families represent genes responsible for the biotransformation of endogenous compounds (4). The most abundant and most involved in drug metabolism are the isoforms of the CYP3A subfamily (11).

The CYP3A subfamily

The CYP isoforms belonging to the 3A subfamily account for the majority of drug metabolising enzymes present in adult human liver and intestine (14-17). The CYP3A

subfamily consists of at least three functional genes: CYP3A4, CYP3A5 and CYP3A7 (4) which are located on chromosome 7 (18). The enzymes compromising the CYP3A subfamily share at least 85% amino acid sequence homology. However, they have been shown to differ substantially in substrate specificity and expression (11). The existence of many additional CYP3A genes is not likely, since a single CYP3A gene is approximately 30 kbp in length and only 90 kbp of human genomic DNA hybridize with CYP3A complementary DNA under low-stringency conditions (19).

CYP3A4

CYP3A4 is the most abundantly expressed CYP in both the human liver and in the small intestine and accounts for approximately 30%-40% of total CYP content in both liver and intestine (14, 20). In adult human liver, CYP3A immunoreactivity was detected in midzonal and centrilobular regions (21), wheras intestinal CYP3A occurs in the enterocytes lining the lumen of the small intestine (14, 20). Inoue et al. (18) assigned the CYP3A4 gene to chromosome 7 at band q22.1. The gene is divided in 13 exons and 12 introns with a length of approximately 27 kbp (22).

The catalytic properties of CYP3A4 have been studied extensively using expressed enzyme, immunoinhibition experiments and the correlation of metabolic activity and immmunoreactive CYP3A4 content in microsomal samples (11). The list of exogenous substrates of CYP3A4 is growing fast (table I) and includes a large number of therapeutically important drugs such as erythromycin, midazolam, cyclosporin, lidocaine (lignocaine) and nifedipine (10). Endogenous substrates include steroids as testosterone, cortisol, progesterone, androstanediol, dehydroepiandrosterone 3-sulfate (DHEA-S) and estradiol (11). CYP3A4 also metabolizes procarcinogens as sterigmatocystin and aflatoxin B1 (23).

CYP3A5

CYP3A5 is 83% homologous to CYP3A4 and is also found in hepatic tissue, although at a lower level than CYP3A4. Large interindividual differences for CYP3A5 expression are found. Initially, CYP3A5 mRNA and protein were detected in 10 to 30% of adult human liver samples (15, 24-27). More recently, however, Jouanaidi et al. (28) detected CYP3A5 mRNA in all livers and CYP3A5 protein in 74% (14 out of 19) of all livers examined . This apparent difference in expression of CYP3A5 may be explained by the use of more sensitive analytical methods (28). It should also be noted that expression of CYP3A5 mRNA differed widely between individuals, ranging from little CYP3A5 mRNA in some individuals, to CYP3A5 being the only CYP3A expressed in others (28).

The substrate specificity of CYP3A5 appears to be similar to that of CYP3A4. However, some differences in catalytic properties have been found. In a reconstituted system, the formation rate of 1-OH-midazolam is considerably higher with CYP3A5 than with CYP3A4. In contrast, the rate of formation of 4-hydroxy-midazolam with CYP3A4 and CYP3A5 is similar (29). No CYP3A5 catalytic activity was found towards quinidine, 17α-ethinylestradiol and aflatoxins (30), all substrates for CYP3A4. However, Gillam et al. (31) did find considerable catalytic activity of CYP3A5 towards both erythromycin (about six times higher when compared to CYP3A4) and ethylmorphine. Interestingly, Gorski et al. (29) found a much better correlation between midazolam hydroxylation and

Table 1 *List of important CYP3A substrates, adapted from (67, 116).*

Drugs			Xenobiotics	Endogenous substrates
Antihistamines	**Anti-fungals**	**Anesthesia/analgetics**	Aflatoxin B1	DHEA-S
Astemizole	Ketoconazole	Alfentanyl	Sterigmatocystin	DHEA
Mizostaline	Miconazole	Fentanyl	Benzphetamine	Testosterone (6-β-OH)
Terfenadine	**Immunosuppressants**	Lidocain	Heterocyclic amines	Testosterone (2-β-OH)
Anti-reflux	Cyclosporin (M17 formation)	Ethylmorphine	Benzopyrene activation	Testosterone (15-β-OH)
Cisapride	Cyclosporin (M1 formation)	**Antihypertensives**		Cortisol (6-β-OH)
Anti-emetic	Tacrolimus (FK506)	Amlodipine		Progesterone (6-β-OH)
Ondansetron	**Chemotherapeutics**	Felodipine		Androstanedione (6-β-OH)
Anti-epileptic	Busulfan	Isradipine		Estradiol
Carbamazepine	Doxorubicin	Nicardipine		17-α-ethinylestradiol
Clonazepam	Etoposide	Nifedipine		
Ethoxisumide	Tamoxifen (also 2D6)	**Anti-arrhythmics**		
Zonisamide	Vinblastine	Verapamil		
Anti-HIV	Vincristine	Quinidine		
Indinavir	**Benzodiazepines**	**Antidepressants**		
Ritonavir	Alprazolam	Imipramine		
Saquinavir	Diazepam (minor)	Nafzadone		
Antibiotics	Midazolam (1-OH formation)	Sertraline		
Clindamycin	Midazolam (4-OH formation)	**Miscellaneous**		
Erythromycin	Temazepam	Dextromethorphan		

erythromycin *N*-demethylation when livers containing both CYP3A4 and CYP3A5 were excluded from analysis; a finding which supports the different isoform specificity for these drugs. Wrighton et al. also reported that erythromycin is not metabolized by CYP3A5 (30).

The formation of carbamazepine 10,11-epoxide from carbamazepine and 2-sulphamoylacetylphenol (SMAP) from zonisamide catalyzed by CYP3A5 was approximately 33% and 10% , respectively, as compared with CYP3A4 activity (32).

CYP3A5 actively metabolizes estradiol, DHEA-S and cortisol (30). However, Ohmori et al. (32) did not find any activity towards DHEA-S by heterologously expressed CYP3A5. The 6β-hydroxylation of testosterone by CYP3A5 occurred at about 10% of CYP3A4 activity in one study (32), but was similar in another (31).

In summary, the specificity of CYP3A4 and CYP3A5 for biotransformation of many substrates appears to be similar although the extent and rate of metabolic conversion by the individual isoforms may be quite different for a given substrate. The discrepant results of *in vitro* studies probably reflect the sensitivity of CYP3A metabolic activities to incubation conditions.

CYP3A7

CYP3A7 is the major CYP isoform detected in embryonic, fetal and newborn liver, but is also detected in adult liver, although at much lower levels than CYP3A4 (7, 9, 33). The CYP3A7 gene is also located on chromosome 7 and its nucleotide sequence is nearly 90% homologous to that of CYP3A4 (18, 19).

Only a few substrates have been studied with regard to a role for CYP3A7 in their biotransformation. Formation of 1-hydroxy-midazolam and carbamazepine-10,11-epoxide are only marginally supported by CYP3A7 as compared to CYP3A4 (5). The metabolism of zonisamide by CYP3A7 was approximately 70% of that by CYP3A4 activity (32). In contrast, the biotransformation of cisapride to either nor-cisapride or its two primary ring-hydroxylated metabolites by CYP3A7 is at least 10-fold less than that observed with CYP3A4 under the same experimental conditions (34).

CYP3A7 also plays an important role in the biotransformation of endogenous compounds. CYP3A7 catalyzes the 16α-hydroxylation of DHEA-S, a physiologically important reaction for the formation of estriol in pregnancy with a higher affinity and maximal velocity than CYP3A4 (32, 35). CYP3A7 is minimally involved in the 6β-hydroxylation of testosterone, although this conversion is essentially supported by CYP3A4 (5). Finally CYP3A7 is capable of metabolising potential environmental pollutants (e.g. aflatoxin B1) (5, 36). Data derived from fetal liver microsomes may provide additional information on the role of CYP3A7 in the biotransformation of several substrates, since it is the main CYP isoform present in the fetal liver (33).

Interindividual and intraindividual variation of CYP3A activity

Interindividual variation in CYP3A expression (3, 21, 37) is reflected by large interindividual differences (e.g. 4- to 13-fold) in plasma clearance of CYP3A substrates (38-43). Using a human liver bank (24 adult Caucasian men and women), Transon et al. (27) found a 3.8 fold (1.9 to 7.2 µl/L) difference in the Michaelis-Menton constant (Km) and a 13 fold (9.5 to 123.4 nmol/mg protein/h) difference in the maximum rate of metabolism (V_{max}) for the 1'-hydroxylation of midazolam. Thummel et al. (44), using a human liver bank assembled from organ donors (n = 21) found a 29 fold variation of V_{max} for midazolam 1-OH hydroxylation, whereas Kronbach et al. (45) only found a 5 fold variation. Thummel et al. (46) determined CYP3A content in intestinal microsomes from 15 donors and found an interindividual variation exceeding 18-fold for each region of the gut.

Intraindividual variation in midazolam plasma clearance [CV% as a measure of CYP3A activity *in vivo*] ranged from approximately 5 to 20%, which is considerably smaller than interindividual variation (47). Ten adult patients, not receiving known CYP3A inducers, underwent two erythromycin breath tests within two weeks which showed a <27 % change in test results (43). As reviewed by Fahr (38), the pharmacokinetics cyclosporin exhibit up to two-fold intraindividual variability, which may partially be attributed to variability in CYP3A activity.

In contrast, the interindividual variation in CYP3A7 mRNA in fetal liver was only 2.5-fold, which is considerably lower when compared with the 10-fold variation in CYP3A4 expression levels found in adult liver (37).

Genetic variation in CYP3A expression

Racial and gender influences, which may explain part of the variability in CYP3A activity; however, study results remain inconclusive. In one study, no differences in hepatic CYP3A mRNA and protein were found between Caucasians and Japanese, but CYP3A dependent activities appear to be higher in Caucasians when compared to Japanese (3). Others could not detect differences in catalytic activity towards nifedipine, a well-known CYP3A substrate (48). Recently, Chavez-Teyes et al. (49) have suggested that Mexican (Mestizo) males may have reduced CYP3A4 activity as compared with Caucasian males based upon higher midazolam bioavailability and reduced plasma clearance of midazolam in a small population (n=11) of adults.

CYP3A activity measured *in vivo* by means of different probe drugs (50) was slightly higher in women when compared to men in two studies (39, 43), but other investigators could not confirm this gender difference (47, 51). Christians et al. (52) did not find a

gender difference in the area under the concentration-time curve (AUC) after oral cyclosporin administration, but when co-administered with the CYP3A inhibitor diltiazem, the AUC of cyclosporine was significantly more increased in women than in men. *In vitro* studies, using human liver microsomes, failed to support a gender difference in CYP3A activity(3, 37), but when using human intestinal microsomes, cyclosporine was metabolized significantly faster by microsomes from female than from male patients (51). Finally, the menstrual cycle phase does not appear to influence CYP3A4 activity evaluated using midazolam in adults (47). No correlation has been found between age or gender and heterogeneous CYP3A5 expression (26, 30).

To date, the large interindividual variation in CYP3A4 activity and expression could not be attributed to a genetic polymorphism of the CYP3A4 gene (39). Recently, however, Rebbeck et al. (53, 54) and Felix et al (54) identified a mutation in the CYP3A4 promoter region which was associated with an increased severity of prostate cancer at diagnosis compared to patients without the mutation. The authors speculated that this may be because of CYP3A4 mediated differences in testosterone metabolism. They also reported a strong correlation between the incidence of secondary tumors after acute lymphocytic leukemia in children and this CYP3A4 promoter region mutation. However the consequence of allelic variation in the CYP3A4 promoter region for CYP3A4 activity *in vivo* has not been addressed.

CYP3A5 cDNA sequencing revealed a point mutation, which was detected in two out of five individuals with absent CYP3A5 protein (28). The authors suggest that one or more mutations may explain the heterogeneous expression of CYP3A5, but no additional studies have pursued this possibility.

Extrahepatic sites of CYP3A expression

The presence of CYP3A in other organs may also contribute to interindividual variability in the disposition of CYP3A4 substrates. CYP3A4 is expressed in esophagus, duodenum, small intestine and colon, but not the stomach, with the upper small intestine serving as the major drug metabolising site of the gastrointestinal tract (15-17, 55). In adults, approximately 40% of total CYP3A4 content is thought to reside in the small intestine (46). Significant biotransformation of selected CYP3A4 substrates has been observed in the intestinal wall, considerably affecting the oral bioavailability of these drugs (14, 42, 56-58). For example, when midazolam was given to healthy volunteers, the extraction ratio for intestine and liver were comparable (approximately 40%) resulting in an overall oral availability of 30% (46, 58). Consequently, disease states that affect the intestinal epithelium may reduce CYP3A4 activity, resulting in an increased bioavailability of CYP3A4 substrates (59). In contrast, when midazolam was administered parenterally and delivered by arterial blood to the intestinal mucosa, only less than 10% of the delivered dose was extracted in the intestine, compared with almost 60% of the dose when delivered, after oral administration, to the luminal side of the intestine (46).

Although intestinal CYP3A4 activity plays an important role in the first pass-extraction of many CYP3A4 substrates, interindividual variability in the activity of the intestinal transporter P-glycoprotein (P-gp) may be an equally significant determinant of oral bioavailability, at least for cyclosporin (46). Moreover, overlapping substrate specificity exists for intestinal CYP3A and P- glycoprotein, which should also be

accounted for when comparing disposition of CYP3A substrates after intravenous and oral administration.

CYP3A5 appears to be the main CYP3A isoform in the stomach and esophagus, although data are conflicting regarding the expression pattern of CYP3A5 in the remainder of the gastrointestinal tract. In general, depending on the analytical method used, CYP3A5 appears to be polymorphically expressed in the human intestinal tract, with considerably lower activities, when compared to CYP3A4. (15, 16, 55, 60). CYP3A5 is the major CYP3A isoform detected in human kidney, lungs, blood and pituitary gland. Haehner et al. (61) detected CYP3A5 protein and mRNA in 100% of kidney samples. Interestingly, the investigators found a bimodal distribution in CYP3A5 content and activity. Renal CYP3A5 content and activity were 100- to 1000-fold lower than that found for hepatic CYP3A4. (61). Renal CYP3A4 mRNA was detected in 40% of kidney samples and in 70% of these samples, catalytically active CYP3A4 protein could be measured (61).

CYP3A is expressed in neutrophils and B-lymphocytes, but not T-lymphocytes, although the analytical method was unable to distinguish between CYP3A4 and CYP3A5 (62). Since CYP3A content could not be induced by rifampicin (rifampin), it was suggested that the CYP3A isoform detected in human blood cells was more likely to be CYP3A5 than CYP3A4 (63) (64).

Interestingly, CYP3A5 mRNA and protein were detected in the human pituitary gland and were located in growth hormone containing cells. This finding suggests that CYP3A may be involved in the regulation of growth hormone production (65). Finally, low or negligible CYP3A7 and CYP3A5 expression was found in extrahepatic embryonic and fetal tissue (7, 61, 66).

CYP3A drug interactions

The vast majority of drug interactions mediated via CYP3A are the result of either induction or inhibition of this enzyme (67).

The effect of induction is to increase CYP3A4 content and to enhance drug clearance by this route (68). CYP3A4 activity can be induced *in vitro* and *in vivo* by corticosteroids (e.g. dexamethasone), anticonvulsants (e.g. phenobarbital, phenytoin, carbamazepine) and several antimicrobials (e.g. rifampin, rifapentine) (69-75). The molecular mechanism of CYP3A induction has not been fully elucidated. Only recently, Lehmann et al. (76) identified a specific nuclear receptor in the CYP3A promoter region, which may explain the induction mechanism of CYP3A activity at least for some compounds. Inhibition of CYP3A4 activity *in vitro* and *in vivo* has been reported for several compounds such as imidazole derivatives, erythromycin, clarithromycin, troleandomycin, gestodene, ritonavir, fluvoxamine and grapefruit juice (71, 77-81). Known inducers of CYP3A4, such as dexamethasone, rifampicin or phenobarbital, do not appear to induce CYP3A5 activity *in vitro* (24, 25). Nevertheless, Schuetz et al. (82) showed that the CYP3A5 gene contains the sequence information (encoding a dexamethasone responsive element in the promoter region) needed for corticosteroid regulation of transcription. *In vitro* inhibition of CYP3A5 activity has been demonstrated for triazolam (32) and gestodene (30). Ketoconazole and fluconazole are only weak inhibitors for CYP3A5 activity in human hepatocytes relative to their inhibiting effect on CYP3A4 activity(83). Pretreatment of HepG2 cells expressing human CYP3A7, with dexamethasone,

rifampicin, troleandomycin, erytromycin, phenobarbital, phenobarbital-like inducers or lovastatin produced dose-dependent increases of CYP3A7 mRNA and immunoreactive protein (24). Finally, rifampicin has been shown to induce CYP3A7 expression in adult human hepatocytes (84), while gestodene inhibits its activity (36).

Development of CYP3A activity

Although several exogenous factors are important determinants of interindividual variation in CYP3A activity, the impact of ontogeny on the activity of CYP3A isoforms contributes significantly from birth through adolescence. *In vitro* techniques have been used to assess the impact of development on CYP3A activity, but the majority of information currently available is derived from either pharmacokinetic studies of CYP3A substrates or the *in vivo* examination of biotransformation of CYP3A pharmacological 'probe' compounds.

Ontogeny of CYP3A activity assessed in vitro

The benefits and problems of the different methods used to determine CYP3A expression and activity *in vitro* have been discussed in recent reviews (8, 85). Briefly, problems with sensitivity or quantitation may arise, depending on the methods used to detect CYP expression. Specifically, studies which have determined total amounts of immunoquantifiable CYP3A protein from liver may not have used assays capable of differentiating the respective CYP3A isoforms (48). Such limitations may explain conflicting results concerning the assessment of ontogeny of CYP3A activity *in vitro*. The limitations may now be overcome with the use of antibodies raised against isoform-specific peptide sequences or individual isoforms expressed in cells (5).

Total CYP content

Total hepatic CYP content appears to be fairly stable from early fetal life to the first year after birth with reported levels between 30% and 60% of adult levels (86-88). Other authors, (21, 89) however, reported levels in neonates and infants similar to those in adults.

CYP3A content

Fetal CYP3A content (immunoquantitated from liver microsomes) ranges from 30% to 100% of adult CYP3A content (88, 90). An early report showed that total CYP3A content represents 85% of total CYP content in fetal liver, increasing in parallel with the total amount of CYP during development (86). However, another study specifically measuring CYP3A7 showed that its content represents only around 30% of total fetal CYP content (88). During the transition from fetal to neonatal life total CYP3A content appears to be relatively stable (5).

CYP3A4

In embryonic hepatic tissue (6-12 weeks gestational age) CYP3A4 mRNA could not be detected (37). In contrast, CYP3A4 mRNA was detected in fetal liver microsomes (at between 11 and 30 weeks gestational age) at 10% of adult levels, increasing immediately after birth and reaching approximately 50% of adult levels between 6 and 12 months

of age (5, 84, 91). As was shown by Lacroix et al. (5) testosterone β-hydroxylase activity in human liver microsomes, mainly an activity of CYP3A4, was extremely low in the fetus with an increase after birth reaching 30-40% of adult levels from 3 months to one year of age and 120% of adult levels after the age of one year. Gestational age at birth (25 to 40 weeks) did not have an influence on this ontogenic pattern of CYP3A4 activity (5).

CYP3A5
CYP3A5 is consistently demonstrated in embryonic liver (37). However, CYP3A5 protein could be detected in only 10% of fetal livers (30). Gender does not appear to affect fetal expression of CYP3A5 (37). Wrighton et al.(30) showed that CYP3A5 was expressed in nearly 50% of all infant livers studied, while in the same study expression of CYP3A5 was only found in 29% of adult livers. CYP3A5 protein was detected in the livers of a 2-month and a 14-year-old patient in another study (25). Given the dependence of CYP3A5 quantification on various (i.e. different) analytical methods, the aforementioned results provide a useful, albeit limited, picture of CYP3A5 ontogeny.
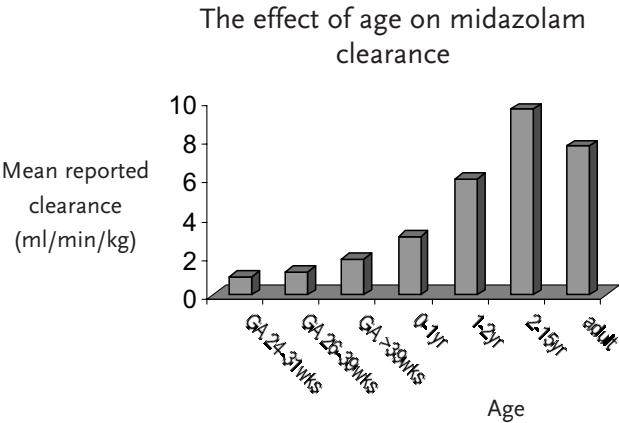
CYP3A7
CYP3A7 constitutes about 32% of total CYP content in the human fetal liver (88) and is not detected in other organs during embryogenesis (days 50 to 60) (7). The hydroxylation of DHEA-S, which is mainly catalyzed by CYP3A7 and to a much lesser extent by CYP3A4, was considerably higher in liver samples from 10- to 20 weeks fetuses, when compared with a liver sample from a term infant (5, 35). Immediately after birth, DHEA-S hydroxylation more than doubled, with the highest activity being reached between postnatal day 1 and 7. The activity decreased dramatically after the first week of life to only 10% of newborn levels between 3 and 12 months of age (5). Reported CYP3A7 mRNA expression in human adult livers ranges from 54% to almost 90% of samples (37, 91). However, the amount of CYP3A7 mRNA detected was only 1.7% to 10% in adults when compared to the fetus (37, 84).

CYP3A7 mRNA and protein are also detected in adult endometrium and placenta and in higher amounts in the endometrium of pregnant women. The placental and endometrial CYP3A7 content appears to increase significantly from the first to the second trimester of pregnancy (92). CYP3A7 content in placenta and endometrium per gram of tissue is between 0.6 and 5.5 % of CYP3A7 content in fetal liver. During gestation, the contribution of CYP3A7 in endometrium and placenta to the metabolism of substrates for this isoform should not be underestimated since placenta weight is approximately five times more than total fetal liver weight.

In summary, before birth CYP3A7 activity is high whereas CYP3A4 activity is very low. Directly after birth, a transition from predominantly CYP3A7 activity to mainly CYP3A4 activity occurs (5).

Finally, Table II summarises *in vitro* studies on CYP3A-mediated metabolism during development.

Figure 1 *Mean midazolam clearance (corrected for body size using kilogram bodyweight) after intravenous midazolam administration using data from: (100, 102, 104-106, 163-167).*

GA = gestational age, postnatal age <15 days

Figure 2 *The ontogeny of CYP3A4 and CYP3A7 activity expressed as activity measured using isoform specific probes in human liver microsomes. Due to a lack of information on CYP3A5 activity during development we did not include an ontogenic pattern for CYP3A5 in the figure.*
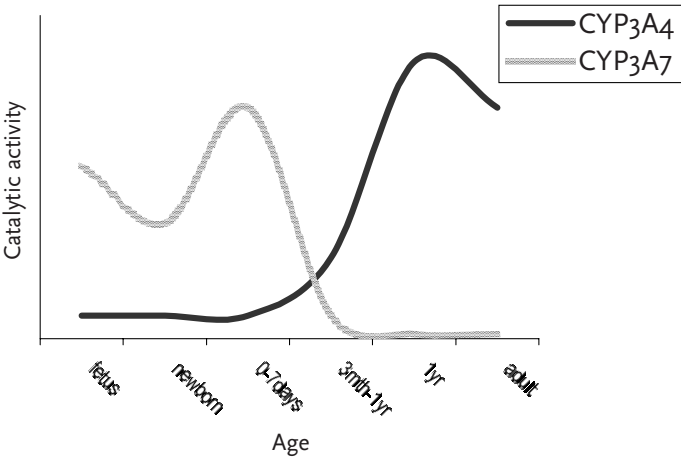*For references see text.*

Table II CYP3A Substrates

Substrates	Isoforms expressed in cells			Catalytic activity towards CYP3A substrates in human liver microsomes7				References (67, 116)	Comments
	CYP3A4	CYP3A5	CYP3A7	Adult (%)	Child (%)	Newborn (%)	Fetus (%)		
Drugs									
Carbamazepine	+++	++	+	Present			CBZ-E formation (?)	(32)(155)	age-dependent change in metabolites formed / CBZ-E present in stillborn fetus of mother receiving CBZ
Zonisamide	+++	+	++					(32)	
Indinavir	+++						32	(90)	
Erythromycin	+++	ND/++++	ND/++++	100	67 (6-11yrs)			(30)(31)	
Cyclosporin (M17 formation)	+++	ND	ND					(26)	3A5, less metabolites formed
Cyclosporin (M1 formation)	+++	+++						(26)	
Diazepam (minor)	+	?		100	140 (3-12 months)	15 (<24h pp), 40-50 (1-7days pp)	<5	(142)	Temazepam formation rate (CYP3A mediated)
Midazolam (1-OH formation)	+	+++	+/-				5(29)	(5)(29)	
Midazolam (4-OH formation)	++	+++						(29)(156)	
Lidocaine	++	+++	?					(117)	
Ethylmorphine	+	++		100			100	(31)(66)	
Dextromethorphan				100			30	(157)(158)	3 MM formation (CYP3A)
Nifedipine	+++	+++	+++	100	44		18	(26)(30)(90)	
Quinidine	+++		ND					(30)	
Endogenous substrates									
DHEA-S	++	++	+++					(30)(32)	
DHEA (16α-OH)	+	+++	+++	+			+++	(5)(30)(35)(86)	
DHEA (16β-OH)			+++	+++			+	(86)	
Testosterone (6-β-OH)	++	+	+	100	30-40%	30-40	2-10	(88)(159)(160)	
Testosterone (2-β-OH)	++	++	++ / ++++	<1% or 100	12-38		12	(5)(25)(32)(90)	
Testosterone (15-β-OH)	+/-	+/-						(26)	
Cortisol	+++	++						(30)(112)	
Progesterone (6-β-OH)	+++	++						(26)(37)	
Androstanedione (6-β-OH)	+++	++	++					(26)(159)	
Xenobiotics									
Aflatoxin B1	+++	ND		100			200	(23)(36)(88)(161)	activation of afb1 in vitro
Sterigmatocystin	+++	+++	+++	100			50	(36)(88)	
Benzphetamine	+++	+++						(31)(33)	
Heterocyclic amines	+	++	+++					(162)	(activation of IQ and MeIQ from cooked meat and fish and tobacco)
Benzopyrene activation	Activity?	+++		100			600	(88)	

Isoforms expressed in cells: Catalytic activity measured using CYP3A isoforms expressed in cells, + (little) ++++ (extensive); catalytic activity in relation to the other CYP3A substrates, ND: Not detected

Catalytic activity towards CYP3A substrates: percentage catalytic activity measured in pediatric liver microsomes/hepatocytes when compared to activity in human adult liver microsomes/hepatocytes

Ontogeny of CYP3A activity in vivo
Methods to measure CYP3A activity in vivo

As reviewed by Watkins (50), CYP3A activity can be assessed *in vivo* with the use of different pharmacologic probes. According to the validation criteria for non-invasive probes for CYP3A4 activity as defined by Watkins (50), the erythromycin breath test (ERMBT) is currently one of the best validated method to assess CYP3A activity *in vivo* (93). The ERMBT results correlate significantly with the plasma clearance of orally given cyclosporin (94) and intravenously administered midazolam (93), the latter also correlates with *in vitro* CYP3A4 content and catalytic activity (44, 58). Furthermore, the plasma clearance of midazolam significantly correlates with that of nifedipine (95) and nifedipine oxidation activity *in vitro* is significantly correlated with CYP3A4 protein levels (48). Another important CYP3A probe is the urinary 6β-hydroxy-cortisol:cortisol (6βOHF:C) ratio which, however, does not correlate with either ERBMT or midazolam clearance (96, 97). As discussed by Watkins (50) a possible explanation for this lack of correlation may be that renal CYP3A5 also catalyzes the metabolism of cortisol and therefore, urine based assays, such as the 6βOHF:C ratio, may not correctly reflect hepatic CYP3A activity. Nevertheless, cortisol is frequently used as a probe to assess CYP3A induction (72).

Because of the intravenous use of [N-methyl¹⁴-C]-erythromycin, the ERBMT has formidable ethical obstacles to overcome before it can be used in children. An attractive alternative is the use of oral ¹³C-labeled (stable isotope) erythromycin which, because of technical difficulties has not yet been used to study CYP3A activity in this population. In contrast, the plasma clearance of erythromycin does not provide information on CYP3A activity since the drug is mainly cleared unchanged into bile and only a small part proceeds by hepatic N-demethylation (98).

The plasma clearance of midazolam has also been used to assess CYP3A activity. Jacqz-Aigrain et al. (99-101) studied the pharmacokinetics of midazolam in premature and term neonates. The plasma clearance of midazolam was significantly reduced in newborn infants (1.8 - 2.2 ml/kg/min) when compared with that of adults (6.3-11 ml/kg/min) (100, 102) and was even lower in preterm infants younger than 39 weeks gestational age (1.2 – 1.6 ml/kg/min) (5, 100, 103). Since midazolam is only slightly metabolized by CYP3A7 (29), the reduced clearance of midazolam in the newborn may be explained by developmentally low CYP3A4 activity following birth [5]. A marked increase in midazolam clearance (per kg bodyweight) in the first weeks of life, as may be expected to occur in parallel with a developmentally regulated surge in CYP3A4 activity, is not, however, observed. Conflicting data exist on midazolam clearance after the newborn period. In one study, clearance was similar in older infants, children (i.e., 8.0 ml/kg/min) (104) and adults (i.e., 6.3 –11.1 ml/kg/min) (102, 105). While in another, Hughes et al. (106) found lower clearances in children (2.3 and 3 ml/kg/min) until two years of age. In older children, however, (3 to 13 years old) midazolam clearances were higher (13ml/kg/min) than those observed in adults (6.3-11 ml/kg/min). These changes are summarised in figure 1.

The oral clearance of midazolam has been proposed as a measure of combined hepatic and intestinal CYP3A activity in adults (46, 50) Although the cDNA of hepatic and intestinal CYP3A4 is similar (56), they appear not to be regulated in concert (107). Therefore, it is unclear if intestinal CYP3A activity changes in parallel with hepatic

CYP3A activity during ontogeny. Oral midazolam bioavailability is approximately 36% in adults (108), somewhat higher than that found in children where bioavailability ranged from 15% to 27% with doses ranging from 0.15 to 1.0 mg/kg (109). No data are currently available on the oral clearance of midazolam during the first weeks of life.

Cortisol is hydroxylated by CYP3A4 and CYP3A5 (110, 111) and to a much lesser extent by CYP3A7 (112). The 6β-OHF : C ratio has been used to study CYP3A activity in neonates up to one year of age. Nakamura et al. (113) measured the 6β-OHF : C ratio at different time points during the first two weeks of life. Within 24 hours of after delivery, the ratio was significantly larger compared to adults and declined to adult levels within 3 days after birth. A similar pattern of urinary 6βOHF : C ratios was found by Vauzelle-Kervoedan et al. (114) in term infants at a single occasion between 1 and 15 days after birth. In infants from one month to one year of age, the mean 6β-OHF : C ratio was lower compared with that of neinates and adults. No correlation could be found between the high 6βOHF : C ratio of term neonates on the day of birth and the 6βOHF : C ratio of their mothers, measured two hours after delivery (111). The authors speculate that, given their observation that cortisol is also metabolised by CYP3A7 and that CYP3A7 activity is high directly after birth (5), the observed elevated 6βOHF : C ratio seen on the first day of life may be caused by age-related changes in CYP3A7 activity. This is, however, in marked contrast to the low testosterone 6β-hydroxylase activity observed *in vitro* in fetal livers, CYP3A7 has a higher catalytic activity towards testosterone than cortisol *in vitro* (5).

The 6βOHF : C ratio in premature infants was significantly lower directly after birth, than in term infants, with no change during the first 2 weeks of life (113). It is unclear if these differences in 6βOHF: C ratio between preterm and term infants accurately reflect the differences in CYP3A4 activity during the first two weeks of life. Lacroix et al. (5) did not find a gestational age related (25 to 40 weeks) difference in postnatal development of CYP3A7 and CYP3A4 activity in human liver microsomes. Since the reduced renal function seen in preterm infants may reduce the renal clearance of cortisol and its metabolite, gestational age and postnatal age related changes in CYP3A activity may be obscured.

Nifedipine is a well-defined CYP3A substrate and its plasma clearance may therefore be associated with CYP3A activity (50). Pharmacokinetics of oral nifedipine were studied in children with bronchopulmonary disease aged 5 to 68 months. The elimination half-life ($t^{1}/_{2}\beta$) of nifedipine was lower in children (1.8 h.) when compared with the previous data in adults (2.4 to 3.4 h) (115). As was pointed out by Kearns (116), the plasma clearances varied considerably, which obscured age-dependent changes (from 5 to 68 months of age) in clearance.

Lidocaine is also metabolized by CYP3A4 (117) to its main metabolite monoethyl-glycinexylodide (MEGX). The MEGX formation rate has been proposed as a general test for hepatic dysfunction (as was recently reviewed by Tanaka (118)). However, the use of MEGX formation rate as a measure of CYP3A4 activity *in vivo* seems less appropriate, since only few of the criteria for a CYP3A probe, as discussed by Watkins et al. [48] have been met. Moreover, lidocaine is a high extraction drug, and variability in its clearance may therefore be more importantly explained by hepatic blood flow differences than by variation in CYP3A activity (50).

Pharmacokinetics of important CYP3A substrates in children
Cyclosporin

Cyclosporin is a widely used immunomodifier in peadiatric transplant recipients. In their comprehensive review, Cooney et al. (119) discussed the age-related differences in cyclosporin pharmacokinetics in transplant recipients. Akthough the apparent volume of distribution does not appear to change as a function of age, cyclosporin plasma clearance (on a per kg or body surface area basis) is considerably higher in paediatric patients younger than 8 years when compared to adults. As a result age-specific cyclosporin dosage requirements are necessary to prevent allograft rejection (120, 121).

This increased dose requirement for cyclosporin in children is suggestive of increased hepatic CYP3A activity in this age-group. Furthermore, as cyclosporin is also metabolized by intestinal CYP3A (122), developmental changes in intestinal CYP3A activity may also contribute to the observed lower oral bioavailability in paediatric patients as opposed to in adults (119). Differences in surgical approach to liver transplantation between children and adults may also contribute to higher dose requirements in children (119). Moreover, it is possible that age-dependent expression and activity of transporter protein P-glycoprotein which is also located in the intestinal wall may contribute significantly to the presystemic clearance of cyclosporin (123). As illustrated by a recent case-report where CYP3A activity was induced following rifampin administration (75), the altered activity of CYP3A, which occurs consequent to development, may be of significant magnitude to impact upon the toxicity profile of this drug.

Tacrolimus

Tacrolimus (FK 506) is also an immunosuppressant used in paediatric patients to prevent post-transplant allograft rejection. As with cyclosporin, a correlation appears to exist between whole blood concentration of tacrolimus and both efficacy (i.e. no rejection) and toxicity in adults and children (124). Yasuhara et al. (125) found marked interindividual variability in the pharmacokinetics of tacrolimus in children aged between 3 months to 15 years after liver transplantations. Part of this variability was explained by a decrease in clearance (per kg bodyweight) of tacrolimus with increasing bodyweight and an increase in clearance with postoperative days. This finding supports the observation, that children younger than 3 years old need 2 to 3 times higher oral doses per kg bodyweight of tacrolimus to maintain the same plasma trough concentrations as older children and adults (125).

Filler et al.(126), who studied the disposition of tacrolimus in renal transplant patients, also found considerable interindividual variation in AUC values after oral administration of tacrolimus. Therefore, they recommend therapeutic drug monitoring of tacrolimus in paediatric patients. Interestingly, they did not find substantial pharmacokinetic differences in tacrolimus disposition in this group of patients when compared with adult data, which may be explained by the older age of the children studied and the inclusion of young adults (age range was to 23 years).

Etoposide

After oral administration of the antineoplastic agent etoposide (VP-16) in paediatric patients between 1.1 and 17 years of age, the AUC and half-life values were only slighter lower than those reported for adults (127). However, the systemic clearance of etoposide

was not significantly altered by the age in contrast with disease-related changes in serum albumin, which accounted for 46% of the variability of etoposide clearance(127). Likewise, Boos et al. (128) did not observe any age-related changes in the clearance of etoposide, in children and young adults between 3 months and 28 years of age when corrected for bodyweight or body surface area(128).

Carbamazepine

CYP3A4, and to a lesser extent CYP2C8, catalyse the biotransformation of carbamazepine to its main metabolite carbamazepine-10,11-epoxide (73). The observation of this metabolite at very low concentrations when compared to carbamazepine concentrations in the circulation of stillborn fetuses of mothers receiving carbamazepine may be interpreted as evidence that carbamazepine is metabolised by CYP3A7. (129). A significant correlation was found between age and carbamazepine dose ratio (reciprocal of apparent oral clearance) in patients with epilepsy, ranging between 3 months and 29 years of age (130). In addition, Korinthenberg et al.(131) found an inverse relationship between the ratio of the epoxide metabolite to carbamazepine and age in children ranging in age from 2 weeks to 15 years who were at steady-state, either on carbamazepine monotherapy or with concomitant administration of other aromatic anticonvulsants capable of inducing CYP.

Astemizole

Shortly after its introduction, the histamine H_1- receptor antagonist astemizole, which is a CYP3A4 substrate (67), was associated with cardiotoxic effects in children following accidental overdosing of this drug. Several investigators reported a prolonged QT interval, ventricular dysrrhythmia, atroventricular block and brief episodes of ventricular tachycardia and fibrillation (132-134). However, no data are available on the pharmacokinetics of astemizole in children, so the effect of coadministration of a CYP3A inhibitor or predisposition for a prolonged QT-interval cannot be determined at this moment.

Cisapride

The prokinetic drug cisapride is widely used in young children for the treatment of gastro-oesofageal reflux. The biotransformation of cisapride is mediated by CYP3A4 with little contribution of the other CYP3A isoforms (34). Reports of prolonged QTc interval and in some cases serious ventricular arrhythmias, when cisapride was ingested with known CYP3A4 inhibitors (135-137) suggest that reduced CYP3A4 activity present in young infants (5) may predispose them to concentration-related adverse effects. Despite reduced CYP3A4 activity in the first month of life, an increased clearance of cisapride (normalized to bodyweight) was seen in premature infants with postnatal ages from 15 to 199 days when compared to the dose requirements with adults. There was no evidence of significant increases in the plasma cisapride concentrations (137). However, further clinical studies will be needed to clarify the association between the pharmacokinetics of cisapride and the consequences of a prolonged QT-interval in preterm infants.

Caffeine

In the neonate, CYP1A2 activity is severely reduced, while CYP3A isoforms are present. Cazeneuve et al. (138) demonstrated that the biotransformation of caffeine in the newborn

is primarily dependent on CYP3A and not on CYP1A2, as is the case in older infants and adults. Moreover, in contrast with the adult, 85% of a caffeine dose is excreted unchanged by the kidney in neonates (139). Consequently, reduction in the activity of CYP1A2 and CYP3A activity early in life is less important than renal function with respect to caffeine pharmacokinetics in the neonate (138-140).

Inhibition and induction of CYP3A activity during development

In vitro inhibition studies with known CYP3A substrates (midazolam, DHEA en progesterone) have revealed significant inhibition of ethylmorphine metabolism (66). Significant inhibition of CYP3A7 expressed in COS cells was seen for triazolam, but not for troleandomycin (32).

Tateishi et al.(9) detected CYP3A7 protein in two out of 10 livers of patients aged 2 years and older (8 and 10 year of age, respectively). The authors speculated that the long-term treatment of these children with drugs known to induce CYP3A activity may have induced their CYP3A7 expression. However, CYP3A7 mRNA but not functional protein was induced by rifampicin in human adult hepatocytes.

No controlled studies have been performed studying the effect of inhibitors or inducers on CYP3A activity in children. Some limited information can, however, be gained from examination of relevant case-reports. Sereni et al. (141) reported that in neonates who received phenobarbital during fetal life or early after birth, the urinary excretion of diazepam metabolites was significantly higher than in neonates who did not. Treluyer et al. (142), showed that in fetal liver microsomes from the offspring of mothers who received a CYP3A inducer (e.g. phenobarbital, prednisone), concomitedly with diazepam, the formation of both temazepam (CYP3A-catalyzed) and N-desmethyldiazepam (CYP2C-catalyzed) were dramatically increased when compared with controls.

Hiller et al. (143) described the case of an 8 year old boy who received an oral doze of midazolam (0.5mg/kg orally) as premedication, followed one hour later by an erythromycin infusion. Shortly thereafter he lost consciousness which was subsequently regained after discontinuation of the erythromycin, a known CYP3A4 inhibitor. As expected, the plasma concentrations of midazolam were significantly elevated when erythromycin was coadministered. Hughes et al. (106) reported a similar inhibitory effect of erythromycin on the kinetics of midazolam, resulting in reduced consciousness in a child.

Cyclosporin produced a two-fold increase in the AUC of the CYP3A substrate etoposide when given as combination chemotherapy for solid tumors in children (144, 145). This inhibitory effect of cyclosporin on clearance of etoposide is similar in children when compared with adults (67) and suggestive of competitive inhibition of hepatic CYP3A by cyclosporin. The authors advised reducing etoposide dose by 50% when given to paediatric patients in combination with cyclosporin (145).

The $t^1/_2\beta$ of carbamazepine in neonates exposed to the drug *in utero* was comparable with that seen in adults (146). Given the fact that CYP3A4 activity is considerably lower in neonates (5) and that all mothers in this study also received phenytoin, it would appear that CYP3A isoforms in the fetus and neonate are inducible (146). Detection of carbamazepine-10,11-epoxide in fetuses of mothers who received carbamazepine before delivery not only suggest a role for CYP3A7 in the epoxidation of carbamazepine, but

also the inducibility of this process. However, it should be noted, however, that -10,11-epoxide concentrations are also determined by the activity of the drug-metabolising enzyme epoxide hydrolase which further metabolises epoxide, and that the activity of this enzyme may also be altered by drugs such as phenytoin (147).

Finally, carbamazepine concentration : dose ratios were decreased, and formation of the 10,11-epoxide metabolite was significantly increased, in paediatric patients who received phenytoin in addition to phenobarbital (148). Despite clear evidence which demonstrates that CYP3A isoforms are inducible during development, the impact of ontogeny on the rate and/or extent of such interactions is not generally known.

Regulation of CYP3A activity during ontogeny

Little is known about the factors that govern CYP3A7 expression in the fetus and the transition from CYP3A7 to CYP3A4 activity after birth. Hashimoto et al. (22) showed that both CYP3A7 and CYP3A4 have characteristic sequences in the 5'-flanking region with an identity of 91%. The 5' flanking region of CYP3A5 is only 74% related to both CYP3A4 and CYP3A7 (149). Several consensus sequences for transcription factors (e.g. estradiol response element, CAAT box) were found in all three 5'flanking regions. Moreover, the 5'flanking region of CYP3A has specific deletions and/or sequences which seem characteristic for each gene. However, whether these sequences and/or deletions are involved in the regulation and differential expression of the individual CYP3A genes during development remains, however, to be established.

Given the ability of human growth hormone to modulate the effect of many general transcription factors, and the effect of human growth hormone to enhance CYP3A mediated drug metabolism, growth hormone may be an important factor in the transcription of CYP3A, as discussed by Leeder et Kearns (10). Interestingly, Liddle et al. (150) showed recently that human growth hormone and also dexamethasone regulate CYP3A4 gene expression, possibly at the pretranslational level, in cultured human hepatocytes. Moreover, they demonstrated an inhibitory effect of tri-iodothyronine on the transcription of the CYP3A4 gene. The role of these hormonal factors in the regulation of CYP3A4 expression during development needs, however, to established.

Ontogeny of CYP3A activity and the impact on drug development

The CYP3A family is involved in the biotransformation of numerous therapeutically important drugs. Clearly, distinct ontogenic patterns exist for the three isoforms of this subfamily.

Because of the difficulty in obtaining liver and other tissue material from children, scarce data are available on the ontogeny of the individual CYP3A isoforms in the neonatal period, infancy and childhood. Moreover, data on the disposition of individual substrates and their specific metabolic profile at different phases of ontogeny are lacking for many CYP3A substrates.

Accordingly, application of existing data to modify the design of pharmacokinetic studies of CYP3A substrates, inducers or inhibitors is limited in most instances to inferential information gleaned from individual published studies. Given the apparent

lack of correlation between liver size and hepatic microsomal enzyme activity (151), and between hepatic CYP activity and the amount of hepatic microsomal protein (152) caution must be exercised when plasma drug clearance is used to assess the impact of development on CYP3A activity. More specifically, when extrapolating pharmacokinetic data for CYP3A substrates in relation to CYP3A activity, it is important to note that corrections made for bodyweight or body surface area may not accurately reflect age-related differences in drug metabolism (153). *In vitro* (5) and *in vivo* (116) data clearly support a marked reduction in the activity of CYP3A4/5 during the first one to two months of postnatal life. However, during infancy, and for the first two to three years of life, CYP3A4 activity appears to exceed adult values as reflected by clearance of midazolam (106), cyclosporin (119) and tacrolimus (125). When these data are converted using the allometric model (2), the age-dependent changes in clearance persist, which is suggestive of elevated CYP3A activity in this age group. Accordingly, pharmacokinetic data would appear to support at least two developmental 'breakpoints' for CYP3A activity during the first three years of life. It must be noted, however, that these apparent developmental 'patterns' of CYP3A activity, may be significantly influenced by both exogenous (e.g. drugs capable of altering CYP3A expression, activity and /or the access of a substrate to the enzyme) and endogenous (e.g. intrinsic hepatic disease, or the effect of cytokines associated with systemic infection on CYP3A activity (118, 154)). Such factors, depending upon the direction and magnitude of effect, could well obscure the apparent impact of ontogeny (10). Nonetheless, the design of pharmacokinetic studies and ultimately of dosing regimens for CYP3A substrates during the first two to three years of life must capture the developmental pattern of enzyme function so as to ensure adequate characterisation of the disposition characteristics of a drug and the translation of this information into the provision of safe drug therapy.

Future directions

Clearly, several critical information gaps exist with regard to the overall impact of ontogeny on CYP3A activity. First, the pharmacokinetically important CYP3A substrates should be completely characterized over the first 3 to 5 years of life. Also, other important drugs used in the newborn period should be studied in order to clarify a possible 'compensatory' role for CYP3A when the CYP isoforms may be markedly reduced.

Second, the factors governing the transition from CYP3A7 to CYP3A4 around birth should be elucidated since this may provide us insight into the genetic and possibly, neurohumoral factors, that upregulate the activity of this and possibly other CYPs.

Thirdly, the role of both hepatic and renal CYP3A5 on xenobiotic disposition must be better characterised. Several authors suggest that the lack of correlation between different probe drugs to assess CYP3A activity may be because of the differential contribution of hepatic and renal CYP3A5 to their metabolism. This is a potentially important consideration when CYP3A4 activity is low.

Finally, the role of intestinal CYP3A and the impact of ontogeny on its activity must be studied because of its potential importance in determining presystemic drug clearance. It is quite possible that age-dependent differences in the rate and/or extent of oral bioavailability for CYP3A substrates may be more a function of changes in the activity of intestinal CYP3A than age-associated differences in gastrointestinal motility.

Conclusion

Profound developmental differences in the activity of CYP3A isoforms occur *in utero*, in the neonatal period, through infancy and early childhood and to adolescence. In many instances these changes have proven to be of clinical significance with respect to treatment using substrates, inhibitors or inducers of CYP3A. Investigators and clinicians must consider the impact of ontogeny on CYP3A in both the design of studies and the interpretation of pharmacokinetic data, as well as in the provision of therapy to paediatric patients.

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