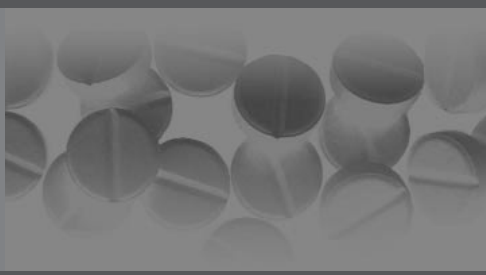
Glucuronidation in humans: Pharmacogenetic and developmental aspects

Chapter 3



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Summary

During human development impressive changes in drug disposition occur. An important determinant of drug clearance is metabolism, something that is not only determined by ontogenic regulation but also by genetic processes which add to the variability of drug metabolism during different stages of childhood. Therefore, and understanding of the developmental regulation of different metabolic pathways, together with information on the genetic determinants of drug metabolism, will increase the knowledge of inter- and intraindividual variability in drug disposition during childhood.

Conjugation has historically received less attention than cytochrome P450 metabolism. An important group of conjugation reactions are catalysed by the uridine 5'-diphosphate(UDP)-glucuronosyltransferases (UGTs); to date at least 10 different UGT isoforms have been identified. The UGTs are not only involved in the metabolism of many drugs [e.g. morphine, paracetamol (acetaminophen)] but also capable of the biotransformation of important endogenous substrates (e.g. bilirubin, ethinlyestradiol) and several xenobiotics. Isoform specificity for these substrates has, however, not been fully characterised.

Serious adverse effects associated with chloramphenicol toxicity in the neonate have highlighted the importance of developmental changes in UGT activity. However, isoform-specific differences preclude the generalisation of a simple developmental pattern for UGT activity. UGT 2B7 is the only UGT isoform for which ontogeny has been characterized both in vitro and in vivo, using morphine as the probe drug. However, no general developmental pattern for the individual UGT isoforms which might be of value for the clinician is currently available.

Genetic polymorphisms have been identified for the UGT family. Not only for the UGT1A gene, which is associated with impaired bilirubin conjugation, leading to genetic hyperbilirubinemia (the Crigler Najjar and Gilbert's syndromes), but also for 3 other UGT isoforms. However, the impact of these genetic differences on drug metabolism remains to be established because overlapping isoform specificity of the drugs studied, as well as a lack of specific probe substrates to test the activity of individual UGT isoforms in relation to these gene mutations.

Clearly, an information gap exists regarding the developmental and genetic aspects of UGT regulation and its potential impact on therapy. More research is needed on the pharmacogenetics and ontogeny of the UGTs for effective translation of scientific information into clinically applicable knowledge.

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 the activity of drug-metabolising enzymes 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 metabolism and drug excretion (1).

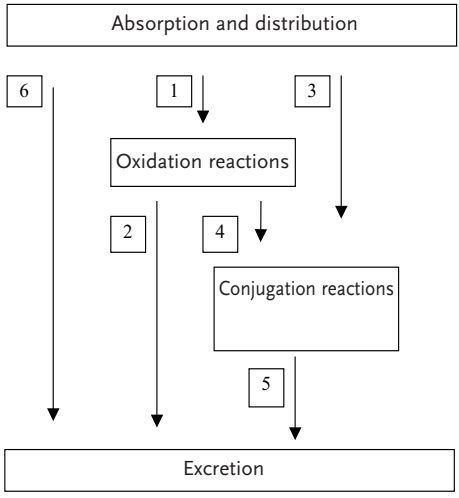
Drug biotransformation reactions by the cytochrome P450 (CYP) family have been subject to extensive research. Historically, less attention has been given to the discussion of conjugation reactions [e.g. by sulfotransferases or uridine 5'-diphosphate (UDP)-glucuronosyltransferases (UGTs)]. Nevertheless, enzyme conjugation reactions play an important role in drug metabolism and detoxification. In humans, a large group of conjugation enzymes is represented by the UGTs (fig. 1).

Failure to recognise the impact of ontogeny on UGT activity has produced therapeutic tragedies in paediatrics such as the 'grey-baby' syndrome associated with administration of chloramphenicol to neonates (2).

Individual differences in drug metabolism during childhood are only partially explained by the developmental changes involving drug-metabolising enzyme(s). They may also be because of differences in diet, exposure to xenobiotic compounds and concomitant administration of other drugs. It is now recognised that genetic variation directs the expression of many drug-metabolising enzymes and therefore, also contributes to interindividual differences in drug metabolism (3).

Consequently, physicians who prescribe drugs to neonates, infants and children should be aware of the interindividual and intraindividual differences that might result from the interaction between developmental and genetic factors. The goal of this review is to provide the practitioner with insight concerning pharmacogenetic and developmental aspects of the UGTs.

Figure 1 *Biotransformation pathways*



The concept of biotransformation via oxidation and conjugation pathways (see bracketed numbers in text)

Hydrophilic compounds as drugs and other xenobiotics are transformed by adding a functional group (oxygen, sulfur, carbon) to form more water-soluble compounds [1]. They can either directly be excreted in bile or urine [2], or serve as substrates for conjugation [4]. The cytochrome P450 system constitutes the largest group of these oxidative enzymes. Subsequently a substrate [3] [4] is conjugated to an endogenous compound, such as glucuronic acid, sulfate acetate, glutathione, thereby further enhancing water solubility with subsequent excretion [5]. In addition, some drugs are not subject to any drug metabolism and are excreted unchanged in urine and/or bile [6].

Uridine 5'-diphosphate (UDP)-glucuronosyltransferases (UGTs)

Structure and function

The glucuronosyltransferases have been previously characterized with respect to their activity and also their physiologic and pharmacological importance. (4) (5) (6) (7) (8) (9)

Mammalian UGTs are part of a gene superfamily consisting of enzymes that catalyze the addition of the glycosyl group from a nucleotide sugar to a small hydrophobic molecule (aglycone). (10) Homologous enzymes are found in plants, animals and bacteria.

Hydrophobic compounds that have been metabolised by phase I enzymes, providing them with an essential functional group (e.g. carbon, sulfur, and oxygen) are acceptor substrates for glucuronidation. Glucuronic acid is associated with the substrate molecule through the catalytic activity of an UGT (Figure 2).

The mammalian UGTs are responsible for the glucuronidation of hundreds of hydrophobic endogenous and xenobiotic compounds. Endogenous substrates are bilirubin, bile acids, thyroxin and steroids. (11) Numerous therapeutic agents [e.g. morphine, paracetamol (acetaminophen), Non-steroidal anti-inflammatory drugs (NSAIDs)] are also glucuronidated. Finally, the UGTs are responsible for detoxifying an extensive group of potentially carcinogenic or teratogenic xenobiotics that enter the body as components of the diet or as airborne pollutants (6).

Although UGTs do enable drug detoxification by enhancing renal excretion of hydrophilic intermediates, glucuronide metabolites are not necessarily inactive and in some instances may be pharmacologically active or toxicologically reactive. For example, morphine-6-glucuronide is approximately 100 times more potent as an analgesic than is morphine. Reduced clearance of this metabolite may therefore lead to a prolonged analgesic effect, with an increased risk of side effects (12). Drug bioactivation may be involved in the pathogenesis of drug hypersensitivity reactions. For example, acylglucuronides of NSAIDs can interact with mammalian host proteins, leading to immune-mediated anaphylaxis and other adverse effects (13) (14).

The nomenclature for the UGT superfamily is based on divergent evolution of the genes that control their expression. The root symbol UGT is commonly used. UGTs that share at least 50% homology are grouped in a family which is annotated by an Arabic number, followed by a letter, indicating a subfamily and an Arabic numeral representing the individual gene (e.g. UGT1A1 or UGT2B4). (15) To date, at least 18 different human UGT isoforms have been identified by gene sequencing and cDNA cloning (15). These advances have enabled the evaluation of a broad variety of possible substrates.

The determination of substrate specificity for the different UGTs is complicated by overlapping substrate activities (i.e. one substrate is metabolised by more than one isoform) and broad substrate specificity (i.e. one isoform glucuronidates a wide range of substrates). Representative substrates for different UGT isoforms are contained in table I.

UGT Gene Structure and Genetic Polymorphisms

The human UGT1 gene is located on chromosome 2 at locus 2q.37. (16) The UGT1 gene consists of at least 13 unique varieties of exon 1 and four common exons 2 to 5. A role for alternative splicing was suggested when cDNA sequencing showed significant homology between the different UGT cDNAs for the C-terminal region, but a lack of overlap for the N-terminal coding region (17). Each exon 1 is preceded by its own promoter region

Table I *Glucuronosyltransferase isoform specificity: representative substrates*

	Endogenous substrates	Exogenous substrates	References
UGT isoform			
UGT1A1	Bilirubin	SN-38 (irinotecan metabolite) Ethinylestradiol	(47) (91) (79) (44)
UGT1A3	Estrone	Norbuprenorphine	(61)
UGT1A4	Androstanediol	Imipramine Amitriptyline	(95)
UGT1A6	?	Acetaminophen Naftazone Naproxen 1-Napthol 2-Napthol	(91) (90) (92) (76) (85)
UGT1A9	Estrone	Propofol Acetaminophen*	(80) (90) (76) (47)
UGT1A10	?	Mycophenolic acid	(96)
UGT2B4	Hyodeoxylicholic acid	?	(97)(98)
UGT2B7	Androsterone Epitestosterone	Morphine Codeine Buprenorphine Naloxone (and other morphinan derivatives) Lorazepam NSAIDs: Naproxen Salicylate Ketoprofen Ibuprofen Valproic acid Menthol Propanolol	(85) (99) (40) (100) (62)
UGT2B15	Androgen steroids #	Eugenol 4-Hydroxy-biphenyl	(21)(101)
UGT2B17	Androsterone Testosterone Dihydrotestosterone		(63)

Vmax = maximum enzyme activity; ? = unknown

Table II *Known polymorphisms of human UGT genes*

Gene	Mutation	Distribution	Expression	In vitro activity and substrate specificity	In vivo activity	Reference
UGT1*	In exons 1 to 5	Different	Absent (CNI) Reduced (CNII)	Absent or Reduced	Leading to CN I and II	Cf. ref (31)
UGT1A1	Promoter region TA replicate of TATA-box	Homo- (up to 13%) and heterozygous (45 %)	Reduced	Reduced	Leading to Gilbert's syndrome	Cf. ref (31)
UGT1A6	Double mutation in exon 1	Heterozygous in 33% of population	Protein expression similar	Lower for salicylic acids	?	(102)
UGT2B15	?	Heterozygous and homozygous	25% of mRNA and protein expression	Substrate specificity is similar	?	(103)
UGT2B7	Single amino acid substitution	?	Similar?	Different substrate specificity	?	(62)

mRNA= messenger RNA; ? = unknown

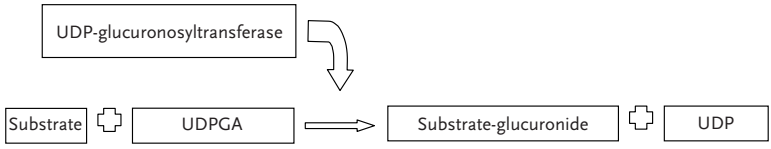
and encodes a unique UGT isoform. The messenger RNA encoding each UGT isoform is formed by the fusion of one of the exons 1 to the four exons 5 (17) (18, 19)(fig 2).

Consequently, gene mutations in the common exon 2 to 5 region can lead to changes in activity and/or expression of additional isoforms, while gene mutations in the unique exon 1 or promoter region may only affect the unique isoform involved. In contrast to the UGT1 family, molecular comparison of the different UGT2B family members suggests that they are encoded by independent genes, probably all located on the human chromosome 4 (20-22). The consequences of a genetic polymorphism for a drug-metabolising enzyme are generally most pronounced when a given pharmacological substrate has a narrow therapeutic range or when the genetic defect leads to a complete lack of enzyme activity. To date, a genetic polymorphism for the UGTs (by the definition of Meyer (3)) has only been observed in association with bilirubin glucuronidation (23, 24).

Genetic Hyperbilirubinaemia

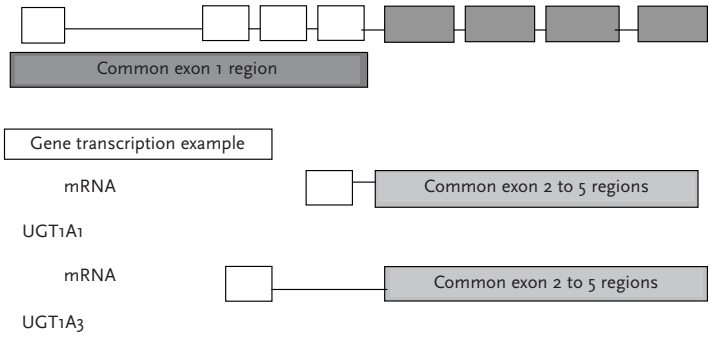
Unconjugated hyperbilirubinaemia may result from a defect in the UGT1 gene responsible for the glucuronidation of bilirubin (25). The clinical manifestation of this genetic hyperbilirubinaemias is very heterogeneous, which can partially be explained by the complicated regulation of UGT1 gene expression. Some individuals have a defect in the bilirubin specific exon 1 region, which results in reduced activity of the UGT1A1 isoform. (26) (27) (28) (29) Other individuals with defects in the common exon 2 to 5

Figure 2 *Mechanism of glucuronidation.*



Abbreviations: UDP = uridine 5'- diphosphate; UDPGA = uridine 5'diphosphoglucuronic acid

Figure 3 *UGT1 gene structure*



An example of alternative splicing leading to different mRNAs with subsequent different UGT proteins
Adapted from (31).

region (26) (30) may also have changes in activity of the other UGT1 isoforms sufficient to alter the biotransformation of endogenous or exogenous substrates (e.g. drugs and environmental chemicals).

Defects in the UGT1A gene (which occur in less than 1% of the population) lead to serious and often lethal hyperbilirubinaemia, a condition known as the Crigler Najjar syndrome type 1 (CNI) or type 2 (CNII). CN1 and CNII can be differentiated by absent and reduced enzyme (UGT1A1) activity, respectively (26). To date, over 30 mutations leading to Crigler Najjar syndrome have been reported (31).

Gilbert's syndrome constitutes a milder form of congenital unconjugated hyperbilirubinaemia, which may be considered a genetic polymorphism in the UGT1 gene. Recent studies (32) (33) have shown that a mutation in the promoter region of the UGT1 gene is associated with Gilbert's syndrome. This genotype could be correlated with the Gilbert's syndrome (phenotype) in healthy volunteers who fasted and abstained from alcohol and drug use. Genotyping performed in three different populations (i.e. Japanese, Eastern Scottish and Inuit Indians from Canada) revealed that about 3, 12 and 17%, of these populations, respectively, were homozygous for the promoter region mutation while approximately 20% of the Japanese and 50% of the other populations were heterozygous. (34) (32) (35) Three additional mutations have been identified in causal association with Gilbert's syndrome, one of them producing a clinically more serious phenotype. (31) (29) (36)

Other UGT Genetic Polymorphisms

Since the availability of heterologously expressed UGT isoforms, mutations in both the UGT1 and UGT2 gene have been found (Table II). With exception of the genetic hyperbilirubinaemias, to date a genetic polymorphism caused by these mutations has not been identified, in large part due to the absence of isoform-specific probe substrates. Consequently, polymorphic drug glucuronidation has not been extensively studied in humans. Earlier investigation of fenofibrate, paracetamol, dextromethorphan and clofibrate were difficult to interpret since these drugs are known to be metabolised by more than one UGT isoform. However, for none of these drugs could a genetic polymorphism be found. (37) (38) (39)

Extrinsic factors such as diet, exposure to pollutants and the concomitant use of other drugs may also cause a bimodal distribution of pharmacokinetic data suggestive of a genetic polymorphism. For example, dietary differences of Chinese compared to Caucasians were suggested to explain a 50% higher urinary excretion of codeine glucuronides in the Chinese group. The opposite was found for morphine glucuronidation. Given the fact that the most important UGT isoform for the metabolism of both morphine and codeine is UGT2B7, the assertion that factors other than UGT2B7 activity may explain these interethnic differences in pharmacokinetics appears to be supported. (40-42)

Impact of Genetic Defects in UGT Activity on Drug Metabolism

Reduced or absent UGT1A1 activity may not only impair the metabolism of bilirubin, but also that of exogenous compounds that are metabolised by UGT1A1. Genetic defects in the common exon 2 to 5 region of the UGT1 gene, in the case of Crigler Najjar or Gilbert's syndrome, may also lead to absent or decreased UGT activity towards

substrates of the other UGT1 gene products. Accordingly, these patients may be at an increased risk for drug toxicity from treatment (or exposure) to substrates of UGT1.

Little research has been performed on the impact of the genetic hyperbilirubinaemias on drug glucuronidation. Recently, Wasserman et al. (43) reported that the administration of the cytostatic drug irinotecan (with the active metabolite SN-38, which is metabolised by UGT1A1) led to severe toxicity in oncology patients with Gilbert's syndrome. This toxicity appeared to be related to accumulation of SN-38, as may be expected when UGT1A1 activity is reduced (44).

Conflicting results were found when paracetamol disposition was examined in patients with Gilbert's syndrome. Some researchers found a decrease in paracetamol clearance and an increase in plasma concentrations of bioactivated paracetamol metabolites, the latter being attributed to a deficient glucuronidation pathway. Ullrich et al. (45) could not confirm this change in paracetamol metabolite formation or clearance when using urinary metabolite/paracetamol ratios after oral administration. Different routes of administration as well as different observations (plasma *vs* urinary data) used to determine glucuronidation activity may explain these conflicting results. Interestingly, Esteban and Perez-Mateo (46) studied 6 patients with Gilbert's syndrome, 4 of whom did not show a difference in paracetamol glucuronidation when compared with controls. Nonetheless, 2 patients in this study had a significant lower formation of paracetamol glucuronide. This is a potentially interesting finding given the fact that paracetamol is mainly metabolised by UGT1A6 and to a lesser extent by UGT1A9, as opposed to UGT1A1, which has reduced activity in Gilbert's syndrome. A possible explanation may be that other UGT1 gene defects located in the common exon 2 to 5 region may also lead to the clinical manifestation of Gilbert's syndrome, as was recently suggested by Maruo et al. (36)

Finally, ethinylestradiol is also a substrate for UGT1A1 (47). Therefore, in women using ethinylestradiol as component of an oral contraceptive and who have Gilbert's or Crigler Najjar's syndrome, an increase in the severity of their hyperbilirubinaemia and/or estrogen-related adverse effects may occur.

In conclusion, the consequences of genetic hyperbilirubinaemias on drug glucuronidation are not clearly defined. This can be partially be attributed to the diversity in gene defects leading to Crigler Najjar and Gilbert's syndrome, and issues concerning specificity and/or overlap in the UGT isoforms responsible for the biotransformation of widely used drugs.

Developmental Changes in UGT Activity

The clinical importance of developmental reductions in UGT activity has been acknowledged since the occurrence of serious adverse events associated with chloramphenicol toxicity in neonates (2) (48). Other, less dramatic, changes in the pharmacokinetics of widely used drugs which are also UGT substrates (e.g. morphine, paracetamol and lorazepam (49) (50) (51)) highlight the dependence of the activity of this enzyme on development. Finally, developmentally regulated changes in UGT activity have also been demonstrated *in vitro*.

Glucuronidation During Embryogenesis

Immunoreactivity studies (52) (53) performed with an antibody shown to cross-react with multiple human UGT isoforms found in human and fetal livers, document the

presence of this enzyme during development. UGT immunoreactivity was found early in gestation in liver, spleen and kidney tissue. As early as 32 days post-ovulation, UGT immunoreactivity could be shown in red blood cells, mainly in the fetal precursor cells. After 12 weeks of gestation, the number of immunopositive red blood cells decreases to nearly undetectable levels. Early in gestation, UGT immunoreactivity in liver and kidney tissue is considerably lower when compared with the fetal red blood cells. Therefore, it may be possible that UGTs in circulating red blood cells prevent the fetus from possible detrimental effects of xenobiotics at a time when the liver and kidney are not fully developed to perform this task.

Endogenous Substrates and Ontogeny of UGT Activity

Fetal and neonatal UGT activity has been studied *in vitro* during the fetal and neonatal period. For example, bilirubin has been proposed as probe substrate for UGT1A1 (25) (11). Immunoreactivity and catalytic UGT activity towards bilirubin are nearly undetectable in fetal liver and the activity increases directly after birth, reaching adult levels around 3 to 6 months of age. (54) In addition, enzyme activity develops in parallel with immunodetectable protein levels. This finding suggests that the decreased activity of UGT1A1 in the fetus and neonate is related to regulation at the level of transcription or translation and not to the existence of a 'fetal' or otherwise inactive UGT1A1 isoform. Finally, the increase in catalytic activity directly after birth is not dependent upon gestational age, suggesting that birth-related events play a role in the expression and activation of the UGT1A1 gene. (55) (56) (57) (54) (58).

The impact of reduced UGT1A1 activity in the development of neonatal hyperbilirubinaemia has not, as yet, been clarified. Only recently have investigators studied the impact of the promoter region defect associated with Gilbert's syndrome on the development of icterus during the neonatal period. Gilbert's syndrome appears to accelerate the development of neonatal jaundice, although other researchers could not find a relationship between Gilbert's syndrome and neonatal hyperbilirubinaemia with plasma levels above 275 μmol/l. (2) (59) (60).

Estrone represents an endogenous substrate for UGT1A3 (61). This hormone is glucuronidated by fetal and neonatal human liver microsomes at a level of approximately 30% of adult activity. (55) While the ontogeny of UGT1A3 remains to be characterised, these data suggest that a developmental pattern for activity does exist.

UGT2B17 plays an important role in the metabolism of androgen steroids. The catalytic activity of fetal and neonatal liver microsomes towards testosterone to form glucuronides is 3% and 13% of adult levels, respectively (57). Androsterone, which is, among other biotransformation pathways, glucuronidated by both UGT2B7 (62) and UGT2B17 (63) is similarly metabolised in fetal and neonatal livers (i.e. 8 and 11% of adult UGT activity). (57)

In contrast, serotonin (5-hydroxytryptamine) is glucuronidated by fetal and liver microsomes at the same level as in the adult (57). Although the UGT isoform responsible for the glucuronidation of serotonin has not been determined to date, its identification could have important clinical implications regarding prenatal and postnatal drug exposure and possibly, the neurobehavioral effects modulated by this neurotransmitter.

Exogenous Substrates and Ontogeny of UGT Activity

The most extensive data on developmental changes of UGT activity for a single drug that can be linked with one specific UGT isoform are available from *in vitro* and *in vivo* data from morphine. Recently it was shown that morphine is largely metabolised by UGT2B7 to morphine-6-glucuronide and morphine-3-glucuronide. (40) Therefore, morphine has been proposed as a probe substrate for this UGT isoform. (19) In contrast to most other drugs, morphine undergoes considerable glucuronidation by the fetus.

In vitro studies using liver microsomes from fetuses aged 15-27 weeks indicated that the rate of morphine glucuronidation was approximately 10 to 20 % of that seen with adult microsomes. (64) (65) No correlation was found between gestational age (i.e. 15 to 27 weeks) and the rate of glucuronidation. The mean rate of morphine glucuronidation was 2-fold higher in fetal livers obtained after hysterectomy as compared to those after induced abortion (65) which suggests a regulatory mechanism for UGT activity related to labor and/or the birth process.

Morphine metabolism in premature neonates and older infants has also been studied *in vivo* (66). Morphine glucuronidation has been demonstrated in premature infants as young as 24 weeks of gestation. The mean plasma clearance of morphine was 5 fold lower in neonates (gestational age 24 to 37 weeks, postnatal age 2 to 12 days) when compared to children aged 1 to 16 years. Plasma morphine clearance from studies of continuous intravenous infusion increases slowly after birth, reaching adult values between 6 months and 30 months of age, when using the per-kg size model. Anderson et al. (67) corrected available clearance data from different age groups, using the $^3/_4$ power model. This correction showed that adult levels are reached at an earlier age between 2 and 6 months of age (67). Given the predominance of glucuronidation in the biotransformation of morphine, the maturation of morphine clearance appears to be related to developmental differences in UGT activity. (51)

The ratios of morphine-3-glucuronide : morphine and morphine-6-glucuronide : morphine in urine were both found to be significantly lower in the premature infants as compared to older infants. However, the ratio morphine-3-glucuronide : morphine-6-glucuronide was not different between the neonates and the older children, suggesting that both metabolic pathways develop in parallel (68). This knowledge has potentially important clinical implications given that morphine-6-glucuronide has more potent analgesic effects than morphine. (12) Accordingly, the clinical effect of morphine is thus not only dependent upon the clearance of morphine but also on the subsequent formation of its glucuronide metabolite. (12)

Naloxone, an opiate antagonist, was recently shown to be mainly metabolised by UGT2B7 (other pathways are N-dealkylation and 6-ketone reduction). (51, 69) The elimination half-life of naloxone is approximately 3-4 times longer in neonates than in adults, a finding which may be suggestive of reduced UGT2B7 activity in the neonate. (69)

The benzodiazepines and NSAIDs are, at least partially, glucuronidated by UGT2B7. The rate of elimination of lorazepam in the human neonate, when prenatally administered to the mother is slow when compared with that of the adult. (70) The reduced clearance of lorazepam was similar in term and preterm infants as young as 32 weeks gestational age. In a 33-week gestational age neonate, severe hypotonia necessitating prolonged assisted ventilation was associated with a toxic lorazepam concentration after administration of 1.5 mg/kg lorazepam over 27 hours, suggesting

reduced glucuronidation of the drug early in life. (50) In children aged 2.3 to 17.8 years, lorazepam plasma clearance normalised to bodyweight is similar to values reported in adults. (71) However, when lorazepam clearance was normalised for body surface area, it was lower in children. (71) When compared to healthy adults, the $t^{1}/_{2}ß$ of oxazepam is only slightly reduced in the neonate after administration to the mothers before delivery. (72) However, the isoform specificity of oxazepam remains controversial, as some authors suggest a role for UGT2B7 (73) and others could not confirm this finding. (62) Finally, the elimination of salicylate is also reduced in neonates, and, as suggested by previous investigators, appears to be partially attributable to reduced UGT activity. (74) (75)

Summarising, data pertaining to the disposition of UGT2B7 substrates suggest that activity is present in the fetus from week 15 of gestational age, with an increase in activity to approximately 10% of adult activity around birth. Adult levels appear to be attained between 2 months and 3 years of age.

Paracetamol (acetaminophen) is mainly metabolised by UGT1A6, but also to a much lesser extent by UGT1A9 (76). The rate of paracetamol glucuronide formation is negligible in the fetus (77) and rather low after birth, not reaching adult values before 10 years of age (49, 78). This apparent lack of UGT activity is, however, compensated for by higher sulfotransferase activity in infants and young children. (49) Formation of salicylamide glucuronide is similarly reduced in older children when compared to adults. (78)

Propofol is mainly glucuronidated by UGT1A9 (79, 80) and has been proposed as probe for UGT1A9 activity. (19) The clearance of this anesthetic agent, corrected for body weight, is 20 to 55% higher in children (aged 1 to 11 years) than in adults. (81, 82) However other investigators failed to detect a correlation between pharmacokinetic parameters of propofol and age. (83) Given that propofol is a high-extraction drug, which makes its clearance primarily dependent upon liver blood flow and to a lesser extent on enzyme activity (81, 84), it may not represent a suitable *in vivo* probe for UGT1A9. The pharmacokinetic data of propofol can thus not be used to predict UGT activity.

Although the reduced glucuronidation of chloramphenicol in neonates, and especially the resulting toxicity, have been well known for decades, the UGT isoforms responsible for chloramphenicol glucuronidation have not been clarified. (2) UGT2B7 glucuronidates chloramphenicol, but at negligible low levels when compared to the UGT2B7 probe morphine (85). Hence, the polyfunctionality of chloramphenicol glucuronidation does not lead to its use as probe for UGT2B7 activity.

Zidovudine, the first drug to be approved for the treatment of HIV disease, is mainly metabolised via glucuronidation, although the isoform specificity for zidovudine conjugation has not been characterised to date (86). In the first 2 weeks of life zidovudine clearance is 10.9 ml/kg/min, considerably lower than that (19.0 ml/kg/min) in children 14 days and older (range 14 to 99 days). (87) In older children, 5.4 ± 3.23 years old, the pharmacokinetics of zidovudine did not appear to be altered by age, using weight or body surface area. (88)

Ritodrine is a ß2-agonist used for management of preterm labor. Catalytic activity towards ritodrine is barely detectable in fetal liver samples. Ritodrine glucuronides account for 38% and 23% of total urinary ritodrine conjugates excreted in mothers and neonates, respectively. (89) In a manner similar to that observed for paracetamol, (49) reduced glucuronidation of ritodrine is compensated for by increased sulfation, thereby protecting the neonate from potentially toxic ritodrine plasma concentrations. (89)

More specific information on the ontogeny of the activity for the individual UGT isoforms can be deducted from *in vitro* studies with xenobiotic compounds of which the substrate specificity has been elicited. 1-Naphthol has been proposed as probe substrate for UGT1A6. (90) (91) (92) (11) In fetal, premature and neonatal liver microsomes glucuronidation of 1-naphthol occurs at 5 to 10% of adult activity, which is considerably higher than the rate of bilirubin conjugation in the fetus and neonate. (56) UGT activity towards 1-naphthol increases slowly after birth, with approximately 50 % of adult activity being reached by 6 months of age. The developmental pattern for 1-naphtol glucuronidation was also not found to be different for premature and term infants. (55)

Finally, the adult : fetal ratio of the glucuronidation activity towards 2-naphthol, which is also metabolised by UGT1A6, is more than 100 (7.89 *vs* 0.07 nmol/min/mg protein). (64) The lower enzymatic activity when compared to 1-naphthol may be explained by different analytical methods used or the overlapping substrate specificity of UGT1A6.

Implications of UGT Ontogeny in Drug Development

Isoform specific differences preclude the generalisation of a simple developmental pattern for UGT activity. Given the heterogeneity of the different UGT isoforms, developmental differences in activity for the individual UGTs may be expected. In table III we summarised *in vitro* and *in vivo* (pharmacokinetic) developmental data for various UGT substrates in order to illustrate possible developmental patterns.

Table III *Postulated ontogeny of UGT activity*

	Activity	
	In vitro[a]	**In vivo[b]**
UGT isoform		
1A1	Nearly undetectable during fetal life, increasing directly after birth, reaching adult levels 3 to 6 months after births	NA
1A3	During fetal and neonatal period around 30% of adult activity	NA
1A6	Glucuronidation during fetal life at 1 to 10% of adult levels, increasing slowly after birth, at 6 months 50% of adult activity. Independent of gestational age	Not reaching adult levels before 10 years of age
1A9	NA	NA
1A10	NA	NA
2B4	NA	NA
2B7	15 to 27 weeks of fetal life: 10 to 20% of adult levels. Change in activity regulated by birth related events?	First two weeks of life 20% of levels of older children (1 to 16 years). No apparent change during adolescence
2B15	NA	NA
2B17	Reduced activity during fetal (<10%) and neonatal (around 10%) period when compared to adults	NA

A *data from immunoreactivity or catalytic studies with fetal, neonatal or adult human liver microsomes using UGT substrates that are mainly metabolized by 1 specific UGT isoform*

B *data from pharmacokinetic studies using UGT substrates that are mainly metabolized by 1 specific UGT isoform*

NA *no information available*

These developmental patterns should however be interpreted with care, as the complete ontogeny for those UGT isoforms important in human drug metabolism is not known. A lack of complete knowledge on substrate specificity and isoform specificity complicates the interpretation of both *in vitro* and *in vivo* data with respect to the individual UGT isoforms. Only recently, 6 probe substrates for different isoforms were proposed (e.g. bilirubin for UGT1A1) by Burchell and Coughtrie. (19) Most *in vitro* data are limited to the fetal and neonatal period, resulting in a lack of data during the first year of life and thereafter.

The use of clearance expressed in L/kg is a weak size model when used to address developmental issues. The use of the per-kg model will lead to an underestimation of clearance by up to 200% in children with body weight under 3.4kg. As discussed by Holford and colleagues, (67, 93) the application of the $^3/_4$ power model, instead of the per-kg model, will generate the following equation for clearance:

$CLi = CLstd\ (Wi/Wstd)^{3/4}$

where CLi is clearance in the individual, CLstd is clearance in a standardised individual, Wi is the individual's bodyweight and Wstd is the bodyweight of the standardised individual. The use of this model will be more accurately correct for size, and will thus be more useful to detect the influence of age on differences in pharmacokinetics. Since the pharmacokinetic data used, are expressed in L/kg and not using the 3/4 power model, the interpretation of these data with respect to UGT activity should be undertaken with care and is merely speculative.

Moreover, considering that other organs are also capable of glucuronidation (53, 84, 94) with organ-specific activity and possibly an organ-specific developmental patterns, further complicates the accurate assessment of UGT ontogeny derived from data generally obtained from experiments using human liver microsomes.

Overlapping isoform specificity, the availability of alternative metabolic pathways or differences in the other factors which may change pharmacokinetics (e.g. renal function) should be considered before translating plasma clearance or drug : metabolite ratios as surrogate marker for UGT activity. At present, morphine is the only drug primarily metabolised by glucuronidation with known single-isoform specificity (i.e.UG2B7). The pharmacokinetic data for morphine may, therefore, be reliably used to reflect the ontogeny of UGT2B7.

Future directions

In contrast to the cytochrome P450, knowledge concerning the impact of development on UGT activity is lagging. Due to the importance of UGTs in human drug metabolism, more complete characterisation of these critical enzymes is important and deserves increased attention.

In vitro studies using human UGT isoforms should be performed with probe substrates in order to characterise specificity and determine the impact of ontogeny. Moreover, for drugs that are widely used in paediatric practice, and are extensively glucuronidated, the metabolic profile should be determined at different stages of development in order to search for 'breakpoints' in UGT activity of a magnitude that might warrant age-specific modifications in drug therapy.

Pharmacokinetic studies of glucuronidated drugs are also needed to supplement the *in vitro* information on the impact of ontogeny on the activity of the different UGT

isoforms. When combining these *in vitro* and *in vivo* data, a developmental 'road map' could be constructed for the individual UGT isoforms that would allow prediction of the impact of age on enzyme activity.

Patients with genetic hyperbilirubinaemia are at increased risk for drug toxicity or drug failure caused by reduced UGT activity. This group may also benefit from combined *in vitro* and *in vivo* studies, combining their specific gene defect and isoform specific *in vitro* UGT activity with pharmacokinetic data. Moreover, the impact of ontogeny on development of UGT activity in this patient population should also be studied.

Conclusion

Clearly, an information gap exists regarding the developmental and genetic aspects of UGT regulation and its potential impact on therapy. Case reports illustrate the danger of drug accumulation due to developmentally reduced UGT activity. However, no general developmental pattern for the individual UGT isoforms that might be of value for the clinician is currently available. More research on the pharmacogenetics and ontogeny of the UGTs is necessary for effective translation of scientific information into clinically applicable knowledge. As has been shown with the cytochrome P450 enzymes, coupling of basic and clinical science will be necessary to continually improve our understanding of the UGTs.

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