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Determinants of Drug Metabolism in Early Neonatal Life

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Abstract: Clinical pharmacology intends to predict drug-specific effects and side effects based on pharmacokinetics (i.e. absorption, distribution, metabolism and elimination) and pharmacodynamics (i.e. dose/effect relationship). Developmental pharmacology focuses on the maturational aspects of these phenomena during perinatal life and later stages of infancy.

In general, phenotypic variation in drug metabolism is based on constitutional, environmental and genetic factors but in early neonatal life, it mainly reflects ontogeny. Although the major site of drug metabolism is the liver, the gastro-intestinal tract, blood cells and other organs like kidneys or lungs might also be involved in drug metabolism.

Important alterations in hepatic drug metabolism occur in early neonatal life. These alterations are of relevance when age-dependent aspects of pharmacokinetics, -dynamics or toxicology are considered.

Age dependent maturation of various phase I and II processes will be illustrated based on recently reported observations on the *in vivo* disposition of various analgesics (paracetamol, tramadol) in human neonates and young infants. However, age only in part explains the interindividual variability observed.

Therefore, concerted efforts should be developed to simultaneously assess the impact of age, environmental factors, co-morbidity and polymorphisms in this specific population. The implementation of multivariable models like non-linear mixed effects (NONMEM) models hereby provide us with the tools to disentangle the impact of various co-variables in this specific population.

Key Words: Metabolism, ontogeny, cytochrome P450 iso-enzymes, uridine diphosphate glucuronosyltransferase iso-enzymes, tramadol, paracetamol.

1. INTRODUCTION

Clinical pharmacology intends to predict drug-specific effects and side effects based on pharmacokinetics (i.e. absorption, distribution, metabolism and elimination) and pharmacodynamics (i.e. dose/effect relationship). Developmental pharmacology focuses on the maturational aspects of these phenomena during perinatal life and later stages of infancy. Important alterations in renal clearance and hepatic metabolism occur in perinatal life, resulting in maturational trends in drug metabolism and elimination in preterm and term infants [1-3].

We recently reported on the determinants of amikacin clearance variability in preterm neonates to illustrate the impact of renal maturation on drug clearance [4]. In the present paper, we would like to focus on the determinants of drug metabolism in early neonatal life and would like to illustrate the relevance of these maturational processes based on some recently reported *in vivo* observations on disposition of various analgesics in early neonatal life.

In general, phenotypic variation in drug metabolism is based on constitutional, environmental and genetic factors but in early neonatal life, it mainly reflects ontogeny. Although the major site of drug metabolism is the liver, the gastro-intestinal tract, blood cells and other organs like kidneys or lungs might also be involved in drug metabolism [1-3,5-8].

Drug-metabolizing enzymes are generally divided into phase I and phase II reactions. Phase I reactions are non-synthetic reactions like oxidation, reduction and hydrolysis. The most important group of enzymes involved in phase I processes are the cytochrome p 450 (CYP) iso-enzymes located on the endoplasmic reticulum. Other phase I enzymes like dehydrogenases or esterases may also be of relevance in the disposition of a given drug or compound [5].

CYP enzymes are a superfamily of haem-containing enzymes, classified into several families based upon sequence similarity, subsequently further classified in subfamilies. All CYP enzymes have rather broad compound specificities, with both exogenous and endogenous compounds. The most relevant families involved in drug metabolism in humans are families 1,2,3 and 4. CYP3A (29 %), CYP1A2 (13 %), CYP2C (18 %) and CYP2E1 (7 %) are the most abundantly expressed CYP iso-enzymes in hepatic tissue, but CYP3A (52 %), CYP2D6 (31 %) and CYP2C9 (10 %) iso-enzymes contribute most to the metabolism of drugs currently marketed for human use [3,5].

Phase II reactions are synthetic reactions like glucuronidation, sulfation, methylation or acetylation. The largest group

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of enzymes involved in phase II reactions are the uridine diphosphate glucuronosyltransferase (UGT) iso-enzymes. These UGT iso-enzymes are not only involved in the metabolism of many drugs (e.g. morphine, paracetamol, chloramphenicol, zidovudine) but are also of relevance in the biotransformation of endogenous compounds such as bilirubin or steroids [3,6,7].

Although the metabolism of a given drug most frequently results in more aqua-soluble inactive compounds (e.g. chloramphenicol glucuronidation), this metabolism might also be involved in the transformation of the mother compound or pro-drug to a more potent drug (e.g. codeine to morphine by CYP2D6, propacetamol to paracetamol by esterase, morphine to morphine-3 and 6 glucuronides by UGT 2B7) or into a toxic compound: paracetamol hepatotoxicity is caused by N-acetyl-p-benzoquinone-imine (NAPQI), produced by the oxidative enzyme CYP2E1 and normally mopped up by glutathione reserves. The assessment of iso-enzyme specific phenotypic activity or ontogeny is therefore of relevance when age-dependent aspects of pharmacokinetics (e.g. chloramphenicol glucuronidation), pharmacodynamics (e.g. codeine to morphine, CYP2D6) or toxicology (NAPQI production, CYP2E1 ontogeny) are considered [1,8].

In the assessment of phenotypic hepatic drug metabolism in humans, two experimental approaches can be considered. First, based on *in vitro* observations in hepatic samples, data on iso-enzyme specific phenotypic expression of protein, mRNA and protein activity can be documented. Alternatively, *in vivo* assessment involves the assessment of phenotypic iso-enzyme activity based on metabolic ratio's following the administration of a probe drug [1,3-8].

2. ONTOGENY OF DRUG METABOLISM

Distinct patterns of isoform-specific developmental changes in drug biotransformation are apparent for many of the above mentioned Phase I and Phase II drug metabolizing enzymes but seem to be most prominent for the CYP iso-enzymes.

Although the total CYP content in the foetal liver equals about 30 to 60 % of adult values, iso-enzyme specific ontogeny precludes the generalisation of a simple single developmental pattern for overall CYP activity, necessitating iso-enzyme specific assessment [9,10]. In contrast, ontogeny of the conjugation pathways (glucuronidation, sulfation, glutathione conjugation) in early neonatal life is not that well characterised yet but seems to display much less isoform specificity compared to CYP iso-enzymes [1,3,6,7,11].

To assess age-related changes in drug metabolism, the above mentioned experimental approaches can be considered. Based on *in vitro* observations in hepatic tissues mostly collected shortly post mortem, data on iso-enzyme specific ontogeny of protein, mRNA and protein activity can be documented. In contrast, *in vivo* assessment involves the description of age-related changes in iso-enzyme specific phenotypic activity based on the use of probe drugs [1,8].

The advantage of an *in vitro* approach is the possibility to simultaneously assess various ontogenic processes or interactions between different drugs in an *ex vivo*, safe setting. However, *in vitro* assessment does not take the age-dependent hepatic weight/body weight ratio nor the effect of hepatic blood flow or hepatic microstructure on the phenotypic activity into account. In contrast, the disadvantage of *in vivo* phenotypic assessment is that extrahepatic metabolism (e.g. renal, gastro-intestinal, lungs) might also contribute to the phenotypic variability observed. It is a 'whole body assessment' [1,3,4,5,7,8].

Finally - for both the *in vitro* and *in vivo* approach - we have to be aware that the phenotypic activity observed likely only in part can be explained by age-dependent maturation, i.e. ontogeny. Altered phenotypic activity is not only explained by age, since polymorphisms, co-morbidity, environmental factors or co-medication likely also contribute to the interindividual variability observed in line with observations in adults (Fig. **1**) [12-15]. We would like to illustrate the age dependent maturation of various phase I and II processes based on recently reported observations on the *in vivo* disposition of various analgesics (paracetamol, tramadol) in human neonates and young infants.

2.1. Illustrations of Ontogeny of Phase I Enzymes

Although the total CYP content in the foetal liver is about 30 to 60 % of adult values, iso-enzyme specific ontogeny precludes the generalisation of a simple single developmental pattern for CYP activity [9,10]. CYP3A7 is the most abundant CYP iso-enzyme at birth with a subsequent decrease in CYP3A7 activity most prominent during the first year of life, whereas CYP3A4 is the major CYP3A isoform present in adulthood involved in drug metabolism [9,16]. CYP3A4 activity increases during growth and development, but the number of *in vivo* observations to support the *in vitro* observations of these changes are limited [1,3,4,8,10,18].

CYP2D6 only represents 2 % of total hepatic CYP content in adults, but mediates metabolism of multiple therapeutics and xenobiotics including β-receptor antagonists, anti-arrhythmics, selective serotonin re-uptake inhibitors, antipsychotics and opioids like codeine and tramadol, potentially administered to either neonate or mother before (foetal) or after (breastfeeding) birth [1,3,5,8]. Observations on CYP2D6 ontogeny in neonatal life are still very limited and mainly based on *in vitro* studies. Key observations on *in vitro* ontogeny of both CYP3A4 and CYP2D6 are summarized in Table **1** [10,16-18].

Tramadol (M) hydrochloride is a 4-phenyl-piperidine analogue of codeine. It acts as a central analgesic partially as μ-receptor agonist, partially by its effects on re-uptake mechanisms of mono-amines. *O*-demethyl tramadol (M1) is produced by *O*-demethylation of M by CYP2D6. M1 has a much higher affinity for the μ-receptor and therefore is of pharmacodynamic relevance. The other metabolite (*N*-demethyl tramadol, M2) is produced after *N*-demethylation, mainly by CYP3A4. Tramadol disposition in the first months of life can therefore be used as a probe drug to simultaneously assess CYP2D6 and CYP3A4 ontogeny in the first months of life [19].

As part of a broader project on maturational aspects of tramadol disposition in early neonatal life, the contribution of tramadol (M), *O*-demethyl tramadol (M1, CYP2D6 mediated) and *N*-demethyl tramadol (M2, CYP3A4 mediated) to

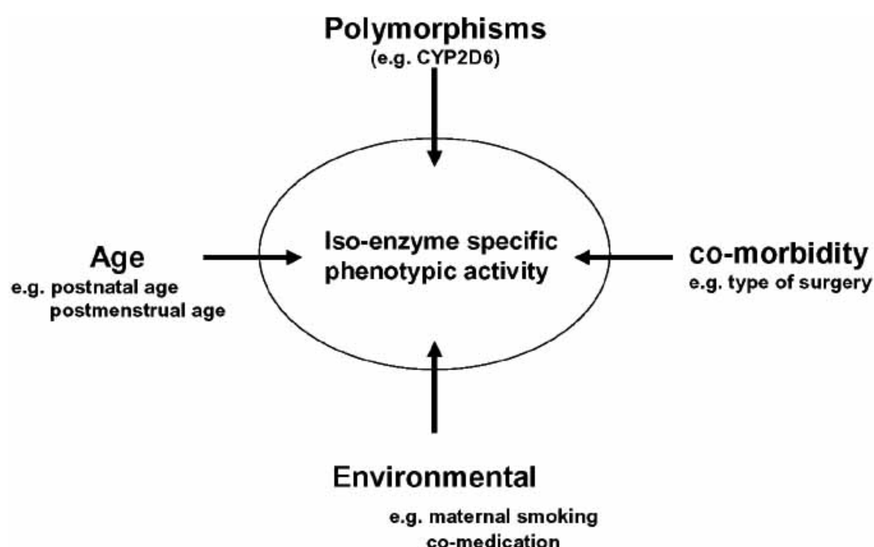

Fig. (1). Schematic presentation of various determinants of potential relevance on the iso-enzyme specific phenotypic activity observed. Co-variables mentioned are discussed in the paper.

overall tramadol elimination and the log M/M2 was assessed in 24 hours urine collections during continuous intravenous tramadol administration. Correlations with perinatal characteristics [postnatal age (PNA), postmenstrual age (PMA)] were studied.

Of the total amount of tramadol administered in a 24 hours interval, 34.5 % (SD 6.1) was retrieved in the urine as parent compound or metabolite in the first 24 hours in 25 neonates and young infants (PMA 25-53 weeks).

This retrieval primarily consisted of tramadol 79 % (SD 18), M1 contributed 10 % (SD 17) and M2 3 % (SD 3.4). Correlations of the contribution of M (r = - 0.73), M1 (r = 0.68) and M2 (r = 0.4) to overall M elimination with increasing PMA were observed [20,21].

Table 1. Data on *In Vitro* Assessment of Maturational CYP3A4 (Testosterone 6β-hydroxylase) and CYP2D6 Activity (Dextrorphan Production) Compared to Adult Values [10,16-18]

	Population	Activity
CYP3A4 activity		
Lacroix *et al*. [16]	foetal, < and > 30 weeks	< 5 %
	neonatal, < 24 hour	5 %
	neonatal, day 1-7	8 %
	neonatal, day 8-28	20 %
	day 28 - 90	25-30 %
	day 90 – 365	30-40 %
	> day 365	120 %
CYP2D6 activity		
Ladona *et al*. [10]	foetal, 14-24 weeks	0 %
Treluyer *et al*. [17]	foetal, 17-40 weeks	< 5 %
	neonatal, < 24 hour	< 5 %
	neonatal, day 1-7	5 %
	neonatal, day 8-28	5-10 %
	> day 28	25-30 %
Jacqz-Aigrain *et al*. [18]	foetal, 17-40 weeks	< 5 %
	neonatal, < 24 hour	1-3 %
	neonatal, day 1-7	10 %
	neonatal, day 8-28	30 %
	> day 28	30 %

In line with earlier reported observations on tramadol disposition in adults, the urinary log M/M2 ratio reflects CYP3A4 activity while the urinary log M/M1 ratio reflects CYP2D6 activity [22,23].

In a cohort of neonates and young infants (25-52 weeks PMA), the mean log M/M2 was 1.44 (SD 0.46) compared to an adult phenotypic ratio of 0.47. An inverse correlation between log M/M2 ratio and PMA (r = - 0.66, p = 0.0006) and PNA (r = - 0.5, p = 0.008) was observed with a maturational half life of the log M/M2 ratio of 16 to 20 weeks (Fig. **2**). In a multiple regression model, PMA remained the only significant variable to explain interindividual log M/M2 variability [20].

In the same cohort, mean urine log M/M1 was 0.94 (SD 0.7) compared to an adult phenotypic ratio of – 0.1. Significant correlations of the urine log M/M1 ratio with PMA (r = - 0.73, p < 0.0001) and PNA (r = -0.56, p = 0.0035) were observed. In a multiple regression model with the urine log M/M1 ratio as dependent variable, only PMA remained an independent variable. The maturational half-life of the log M/M1 ratio in early neonatal life is about 12 to 16 weeks without plateau in the age range evaluated (Fig. **2**) [21].

Compared to the developmental changes in CYP2D6 activity, CYP3A4 activity is relatively delayed in the first months of life (Fig. **2**) [20,21]. This is in line with the *in vivo* observations on the ontogeny of CYP2D6 and CYP3A4 summarized in Table **1** [10,16,17,18]. Finally, it is important to stress that the overall weak correlations reflect that PMA only in part explains the interindividual variability observed.

Besides CYP iso-enzyme specific ontogeny, other phase I enzymes like esterases might also display ontogeny. We are unaware of any *in vitro* assessment of esterase ontogeny but propacetamol might be used as an *in vivo* test probe to assess age-dependent esterase activity since propacetamol is a prodrug of paracetamol and is hydrolysed by esterases after intravenous administration [24,25].

The individual standardized predicted hydrolysis half-life in a pooled population pharmacokinetic study in neonates, toddlers and children of propacetamol was used to assess potential age-dependent maturation of esterase function during childhood. Anderson *et al*. documented that there was no age-dependent effect on the hydrolysis half-life. These data suggest that the esterase function is already mature at birth and confirm earlier reports on cholinesterase activity during pregnancy and in newborns (Fig. **3**) [26].

2.2. Illustrations of Ontogeny of Phase II Enzymes

Phase II reactions are synthetic reactions including glucuronidation, sulfation, methylation or acetylation [1,3,6,7]. Paracetamol, N-acetyl-p-aminophenol (acetaminophen), is a readily available antipyretic and analgesic agent. It is the most often prescribed drug for treatment of mild to moderate pain in infants, including neonates and can be administered by either oral, rectal or intravenous route. Paracetamol is either sulfated or glucuronidated, and thus provides us with a drug substrate to simultaneously assess ontogeny of sulfation and glucuronidation in neonates and young infants [25,27].

As part of a broader project on the assessment of maturational aspects of intravenous paracetamol disposition in neonates and young infants, paracetamol-glucuronide (APAP-G), paracetamol-sulfate (APAP-S) and free paracetamol were determined in urine samples of neonates during repeated administration of propacetamol. Spearman rank and linear multiple regression were used to study the effect of postnatal age, postmenstrual age and of repeated administra-

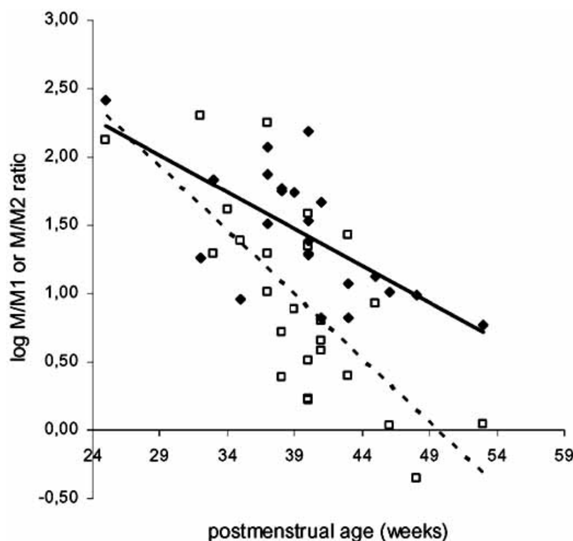

Fig. (2). Ratio (individual observations and trend) of log M/M2 (full line, closed squares, reflecting CYP3A4 phenotypic activity) and of log M/M1 (dashed line, open squares, reflecting CYP2D6 phenotypic activity) in 24 hour urine collections in neonates and young infants during continuous administration of intravenous tramadol [20,21].

M = tramadol, M1 = *O*-demethyl tramadol, M2 = *N*-demethyl tramadol

X-axis = postmenstrual age in week, Y-axis = log metabolic ratio (either log M/M1 or log M/M2)

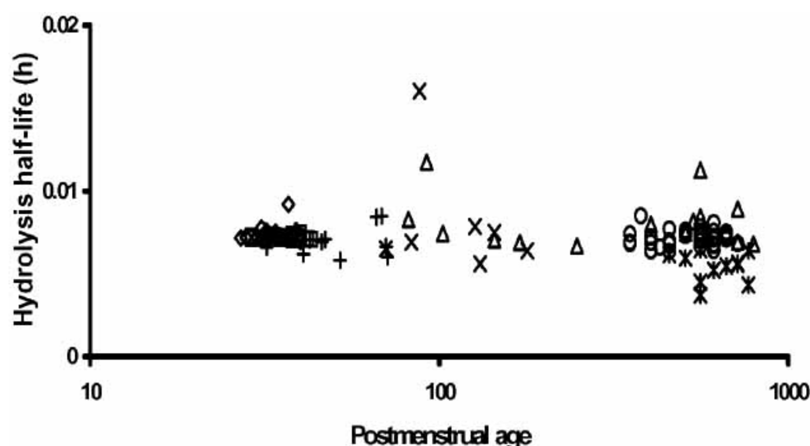

Fig. (3). Hydrolysis half-life (h) of propacetamol as reported by Anderson *et al.* based on pooled population pharmacokinetics in neonates, toddlers and children. This hydrolysis depends on esterase activity and displays no age-dependent maturation (figure adapted from ref. [25]).

tion on the relative contribution of APAP-G to overall urine paracetamol (APAP-G + APAP-S + free paracetamol) elimination (G/T ratio) since this ratio mainly reflects UGT-1A6 ontogeny [28].

Based on 147 urine samples collected in 23 neonates, the molar median G/T ratio was 14 % (range 1-53). Besides increasing G/T ratio with increasing postnatal ($p < 0.0001$) and postmenstrual age ($p < 0.01$), repeated administration ($p < 0.01$) also correlated with an increasing G/T ratio and repeated administration remained significant ($p < 0.01$) after correction of postnatal and postmenstrual age in a multiple regression model [28].

We therefore initially concluded that the observed increase in the contribution of glucuronidation to overall paracetamol urinary elimination during repeated administration reflected induction of phenotypic UGT-activity. However, Ghanem *et al.* more recently found – at least during repeated administration of paracetamol in rodents – strong arguments in favour of a shift from biliary to urinary elimination of paracetamol glucuronide (APAP-G) during repeated administration [29].

This shift from biliary to urinary elimination was associated with increased expression of basolateral multidrug resistance-associated protein 3 (Mrp3) relative to canalicular Mrp2, and decreased entero-hepatic recirculation in the absence of alterations in UGT-activity. Such a switch is biologically sound since this results in the reduction of entero-hepatic recirculation during repeated administration of paracetamol. It is therefore more likely that the progressive increase in the contribution of glucuronidated paracetamol to overall paracetamol elimination observed in the first months of life at least in part reflects such a switch from biliary to urinary elimination in humans in line with observations in rodents [30].

3. DISCUSSION

In the present paper, we were able to illustrate the relevance of age on drug metabolism (phase I and phase II processes) based on some recently reported *in vivo* observations on drug metabolism of analgesics in humans in early life. However, visual inspection of Figs. **2** and **3** already strongly suggests that age is only one of the determinants of drug metabolism in early life since still important interindividual variability independent of the age was observed.

Although the number of observations in neonates are still limited, it has to be anticipated that the birth process itself (switch from foeto-maternal to individual metabolism), disease characteristics, co-morbidity, environmental factors or polymorphisms all contribute to the interindividual phenotypic activity observed in the first months of life [12-15].

Birth itself, either preterm or at term age, seems to be of relevance when drug metabolism is considered. Using a ^{15}N methacetin urine test, Krumbiegel *et al.* documented the postnatal maturation of both CYP and glucuronidation capacity in term and preterm infants illustrating the relevance of both postnatal and postmenstrual age [31]. This is well known for the endogenous bilirubin metabolism (UGT1A1) but has also been documented by Bouwmeester *et al.* for morphine glucuronidation (UGT2B7) [7,32]. Our observations on paracetamol disposition confirm the relevance of both postmenstrual and postnatal age on UGT-activity [28,30].

In vitro observations on fetal and neonatal hepatic CYP ontogeny further illustrated the impact of birth itself on CYP activity. The observations on the impact of age on tramadol disposition serve as *in vivo* illustrations of this maturational process [9,10,33,34].

Disease severity also has an effect on drug disposition as these effects are not limited to adult populations [13-15]. However, it might be more difficult to document a modest additional decrease in an iso-enzyme specific phenotypic activity when the a priori ‘healthy’ phenotypic activity is still low. In contrast, this additional limited decrease in phenotypic activity might even be of more clinical relevance. Lynn *et al.* documented that morphine clearance in postcardiac surgery children is slower compared to non-postcardiac children while Carcillo *et al.* reported on the negative effect of

sepsis-mediated multiple organ failure on overall phenotypic CYP activity in children [14,15].

At present, there is still limited information on the impact of various environmental factors. Maternal tobacco consumption during pregnancy is associated with an enhanced UGT-activity [7]. More recently, Blake *et al*. documented the effect of either breastfeeding or artificial feeding on drug metabolism in the first 6 months of postnatal life: caffeine (3-demehylated metabolites) disposition was enhanced while no differences in dextromethorphan (3-hydroxy morphinan) metabolism were observed in formula-fed infants in the first year of life [35]. It should also be taken into account that the type of feeding itself has an impact on the gastro-intestinal transit time in neonates. It is therefore still unclear whether the observations reflect differences in transit time or in phenotypic activity gastro-intestinal and/or hepatic CYP activity [36].

There is only limited and conflicting information on the impact of prenatal betamethasone administration on drug metabolism in early neonatal life. Elimination half-life of metronidazole was inversely related to gestational age, and ranged from 22.5 to 109 hours. Hepatic hydroxylation of metronidazole was not evident in infants less than 35 weeks gestation, unless they had been exposed prenatally to betamethasone [37].

In contrast, Baird-Lambert *et al*. evaluated the effects of prenatal steroids on theophylline metabolism in infants of 27-32 weeks gestation. Although in utero exposure to betamethasone was associated with a more mature theophylline metabolite pattern in the first days of life, initial elimination half-life (t1/2 beta) did not differ significantly between groups. By the second or third week of life the metabolite pattern was similar in all infants. The decline in terminal half-life seen during theophylline treatment was not directly related to increased metabolite formation. These data suggest that other factors, such as renal clearance, are more important in determining the pharmacokinetics of theophylline in neonates than hepatic metabolism is [38]. The conflicting evidence on the potential impact of betamethasone administration on renal drug clearance has been discussed in an recent population pharmacokinetic analysis on amikacin clearance in preterm neonates at birth [4].

Finally, hepatic and extra-hepatic polymorphisms likely contribute to the phenotypic activity observed in an iso-enzyme specific way in early neonatal life [12,39-42]. Extreme preterm neonates all are phenotypic CYP2D6 slow metabolizers, but it is very likely that with increasing age, the individual CYP2D6 activity will progressively more reflect the various polymorphisms (wild type, slow metabolizer or ultrarapid metabolizer) of this iso-enzyme in line with observations in children and adults [39-41]. Since phenotypic CYP3A4 develops at a relatively slower pace compared to CYP2D6, it has to be anticipated that polymorphisms will only marginally contribute to overall phenotypic activity in the first months of life. In contrast, CYP3A7 is expressed most abundantly before and at birth with subsequent decrease in the first year of life. If CYP3A7 polymorphism ever were of clinical relevance in drug disposition, associations with perinatal drug disposition variability should be investigated during perinatal life.

Population modelling using mixed effects models (NONMEM) hereby provide us with the tools to study the impact of various co-variables on the variability in drug disposition in early neonatal life. Covariate investigation in children can improve the understanding of the developmental aspects of drug disposition - including drug metabolism – in children ultimately leading to more effective use of drugs. In neonates, besides allometric scaling, ontogeny is of relevance. Since the discussion of these models is beyond the scope of the present paper, we refer the interested reader to some recent reviews on this issue [43-46].

CONCLUSIONS

Important alterations in hepatic drug metabolism occur during early life. These alterations are of relevance when age-dependent aspects of pharmacokinetics, -dynamics or toxicology are considered. Since age only in part explains the interindividual variability observed, concerted efforts should be developed to simultaneously assess the impact of age, environmental factors, co-morbidity and polymorphisms in this specific population.

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