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REVIEW ARTICLE

Developmental Pharmacokinetics in Neonates: Maturational Changes and Beyond

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Abstract: ***Background:*** Effective and safe pharmacotherapy in an individual neonate necessitates understanding the pharmacokinetic (PK) and pharmacodynamic (PD) properties of a specific drug together with the characteristics of this neonate.

Methods: Developmental PK hereby provides estimates of the concentration-time profile. Multiple maturational, disease and treatment related differences can result in differences in PK and probably also in PD in neonates compared to other populations. All these PK processes (absorption, distribution, metabolism and elimination, ADME) display maturation but are also affected by non-maturational covariates. Maturational covariates relate to age or weight dependent changes, while non-maturational covariates relate to variables in disease, environment, treatment – including co-medications - or genetic background.

Results: We will describe general PK related aspects of ADME in neonates with emphasis on both maturational and non-maturational covariates of the variability observed, followed by compound specific illustrations (tramadol, amikacin) to further underscore the impact and interaction of these maturational and non-maturational changes.

Conclusions: Future efforts should focus on integration of the already available knowledge and the collection of data on the impact of non-maturational covariates. These kinds of PK efforts will become clinically important when subsequently linked to PD, ultimately covering both wanted effects and undesired side-effects.

Keywords: ??????????.

1. INTRODUCTION: AIMING AT A MOVING TARGET

The goal of administering a given compound is to provide an effective treatment for a specific disease while minimizing side effects. Clinical pharmacology aims to predict drug-specific (side)-effects based on pharmacokinetics (PK) and pharmacodynamics (PD). PK (***a***bsorption, ***d***istribution and elimination, through either ***m***etabolism or primary renal ***e***limination, ADME) hereby describes the relationship between a drug concentration at a specific site (e.g. plasma, cerebrospinal fluid) and the time after its administration ('*what the body does to the drug'*).

Neonates are a particularly vulnerable subgroup of pediatrics covering the period from birth up to 28 days of postnatal age or - more accurate - the equivalent maturational age (44 weeks post-menstrual age), and include both preterm (<37 weeks gestational age at birth) and term neonates. Safe and effective pharmacotherapy for all neonates necessitates the understanding of PK and PD properties of a specific drug, combined with the individual characteristics of the neonate [1,2]. Developmental PK hereby provides estimates of changes in the concentration-time profile during growth and development, while PD describes the relationship between a given concentration and response. PK processes (ADME) are subject to maturational changes but are also affected by non-maturational covariates. Maturational covariates relate to age or weight dependent changes while non-maturational covariates relate to variables in disease, environment, treatment – including co-medications - or genetic background (pharmacogenetics). Human growth and development consist of a sequence of physiologic events that link somatic growth with maturation. Weight gain hereby displays co-linearity, but is not similar to maturation. Across the pediatric age, both organ size and function change as well as body composition and (patho)physiology. We should be aware that these changes are most prominent in early infancy [3]. These maturational changes in physiology are further affected by co-morbidity characteristics [e.g. renal impairment, perinatal asphyxia, cardiac failure, sepsis, patent ductus arteriosus] or treatment modalities [e.g. whole body cooling, extracorporeal membrane oxygenation (ECMO), pharmacotherapy]. The combined effects of maturational (e.g. age, weight) and non-maturational covariates result in extensive variability. As a consequence, neonatal pharmacotherapy is as diverse as the neonates who are treated in our units [1-3].

In this review, we will describe PK related aspects of absorption, distribution, metabolism and elimination in neonates with emphasis on both maturational and non-maturational covariates responsible for the observed variability. In addition, compound specific illustrations will be presented to further stress the impact of maturational and non-maturational changes. The overall goal of this paper is to provide a framework of neonatal PK in this special issue on neonatal pharmacology.

2. ABSORPTION

Absorption describes the concentration-time profile of a given compound following non-intravenous administration, commonly captured by the bioavailability and rate of absorption. Drugs administered intravenously are completely available to the systemic circulation, while compounds administered by other routes (e.g. sublin-

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gual, oral, enteral, rectal, buccal) may not enter the systemic circulation completely or intact. Compared to intravenous administration, a reduced percentage of the drug will enter the central circulation. This is because there are barriers (e.g. skin, intestinal mucosa) that can obstruct, limit, delay but also alter (first pass metabolism) the compound during passage. The proportion of the dose that enters intact the systemic circulation is defined as the drug's bioavailability. To assess this bioavailability, the area under the drug concentration-time curve (AUC) is needed as a measure of the total amount of drugs that reaches the systemic circulation. When compared to the intravenous route, this is called the absolute bioavailability (absolute availability = $[AUC]_{oral/dose\text{-}\boldsymbol{oral}}/[AUC]_{intravenous/dose\text{-}\boldsymbol{intravenous}}$, whereas when compared to another non-intravenous one, this is called the relative bioavailability. As well as the extent (bioavailability), the rate of absorption also matters. The rate is most commonly reflected by the time to reach the peak concentration (t_{max}) after administration of the compound.

The above mentioned barriers relate to chemical, mechanical and physical ones. It is obvious that both maturational and non-maturational covariates can affect the extent and the rate of absorption in neonates. To illustrate this, we like to refer to the maturational changes in midazolam bioavailability following oral administration. The bioavailability of midazolam displays a progressive *decrease* with increasing age, probably reflecting a progressive *increase* in first pass metabolism (cytochrome P450 3A) [4]. This phenomenon can be bypassed by using the sublingual, the intranasal or buccal route and is further affected by drug transporter ontogeny and activity.

2.1. Enteral Routes

Gastrointestinal absorption is affected by different maturational covariates, including gastric pH, the rate of gastric emptying, the ontogeny of intestinal motility and the development of intestinal enzymes and transporters related to drug disposition. Table **1** provides examples on the impact of maturational changes on oral absorption of specific compounds in neonates. The main driver of absorption but also metabolism for most drugs is the intestinal mucosa and the related first pass effect. Besides the intestinal surface area, developmental maturation in the activity of drug metabolizing enzymes (e.g. cytochrome p450 3A) and efflux transporters (e.g. efflux transporter P-glycoprotein (P-gp) are important covariates of the bioavailability and – related to this - the first pass process [5]. In addition to maturational covariates non-maturational ones also matter.

Gastric emptying in neonates is slower than in children or adults, but is also affected by the type of feeding (faster for human milk > hydrolysate > regular formula) in neonates [6]. Interestingly, naso-gastric feeding tubes are very commonly used in preterm neonates, while the impact of these tubes on the bioavailability of specific compounds is very rarely considered. Extrapolating from observations on losses of nutrition, it seems that lipophilic compounds are more likely to adhere to syringes and tubes [7]. The same holds true for the use of naso-duodenal tube feeding in neonates. Besides similarities related to tube adherence, this practice does bypass gastric emptying. We are unaware of any structured evaluation on the impact of this specific route, but it is fair to anticipate a faster absorption profile compared to oral administration. In neonates intra-gastric pH is elevated (>4). This may increase the bioavailability of acid-labile compounds (penicillin G) and decrease the bioavailability of weak acids (phenobarbital) when given orally [5,8].

The intestinal permeability is higher in neonates and decreases with the initiation of enteral feeding. This decrease evolves faster in breast-fed compared to formula-fed neonates [9]. The knowledge on the patterns related to e.g. intestinal length, intestinal motor activity, exocrine pancreatic functions, the ontogeny of intestinal enzymes and – be it much more limited – of transporters has increased. The lower bile acid synthesis and pool may affect both micelle formation as well as enterohepatic recirculation in infants. Pancreatic lipase is low at birth and will reach adult levels from the age of 9-12 months of life onwards [5,10]. However, fresh human milk contains considerable amounts of lipase activity and can be considered as an exogenous source of lipase activity [11].

In a recent paper, Somani et al. evaluated the changes in oral drug absorption in (pre)term neonates for the Biopharmaceutics Classification System (BCS), using two paradigm products for both class I (paracetamol, theophylline) and class II (indomethacin, ibuprofen) compounds with available PK data after both the intravenous and oral route. The authors hereby concluded that these changes in oral absorption occur within the first few days after birth, and are a system-specific property [10]. Consequently, these system-specific properties can be used to guide oral dosing estimates for other BCS class I and BCS class II compounds in preterm neonates.

Besides maturational changes, these patterns are also affected by non-maturational covariates. Feeding tolerance differs between appropriate for gestational age preterm infants as compared to those infants who are small for gestational age [12]. The administration of prenatal steroids (for 'lung maturation') improves the clinical outcome of necrotizing enterocolitis while the combined use of postnatal steroids and non-steroidal anti-inflammatory drugs is associated with an increased risk of spontaneous intestinal perforation [13,14]. Bosentan absorption in critically ill neonates with pulmonary hypertension is delayed and therefore steady state concentrations were only achieved from day 5 onwards [15]. There is only limited knowledge on the ontogeny of intestinal transporters and its covari-

Table 1. Examples on the impact of maturational changes on oral absorption of specific compounds in neonates.

Oral	
Swallowing	liquids, but also mini-tablets can be considered in neonates.
Gastric emptying	the peak concentration of a given compound, e.g. paracetamol is delayed and lower in neonates.
Gastric pH	increased bioavailability following oral penicillin administration in neonates.
Intestinal enzymatic activity	increased bioavailability following oral midazolam administration in (pre)term neonates because of lower intestinal drug metabolism.
Pancreas activity and bile	reduced uptake of lipophilic drugs, fat-soluble vitamins or enterohepatic recirculation.
Co-morbidity	bioavailability after oral administration may be different in the setting of critical illness. One may consider another, more reliable route, if available (e.g. intravenous).

ates, but some data suggest that this is at least in part driven by the intestinal microflora [16].

Other enteral routes of administration are rectal or sublingual. Rectal administration can bypass the enteral first-pass metabolism and is associated with higher and earlier peak concentrations of lipophilic compounds like thiopentone or methohexitone as compared to the oral route [5]. In contrast, the available observations on paracetamol illustrate that rectal administration results in extensive variability and more limited bioavailability [17,18]. Compared to oral administration, the relative bioavailability is 0.67 (30 %) and 0.61 (21 %) for triglyceride base elixir and capsule suppositories, respectively [17]. This necessitates higher doses, but remains associated with limited predictability. This makes the rectal route less suitable for repeated administration of paracetamol. This is very likely also a system-specific property, and should be considered when developing and interpreting studies using the rectal route (paracetamol, ibuprofen) of administration to e.g. induce closure of a patent ductus arteriosus [17,18].

The sublingual or buccal route is only rarely used in neonates, but there are case reports on e.g. midazolam administration for seizures or sildenafil to treat pulmonary hypertension [19,20]. These routes resulted in faster and more extensive absorption as compared to the oral route. A similar pattern has recently been observed in a PK study on sublingual buprenorphine to treat neonatal abstinence syndrome [21].

2.2. Non-Enteral Routes

The absorption of drugs by non-enteral routes (skin, inhalational, conjunctiva, intravitreal, intramuscular) also changes in early infancy. For example, changes in skin thickness during development can affect the extent of absorption after topical application. Based on trans-epidermal water loss and percutaneous absorption studies, term neonates likely have stratum corneum barrier properties similar to adults. It is thought that postnatal life accelerates stratum corneum maturation, so that even preterm infants have barrier function similar to term infants from 2-3 weeks of postnatal age onwards [22]. This suggests that the neonatal skin mainly adjusts to the extra-uterine environment, irrespective of the gestational age at birth. There are several case reports on the systemic exposure following topical application of iodine antiseptics (secondary hypothyroidism), lidocaine-prilocaine cream (methemoglobinemia), steroids (adrenal suppression) or excipient related toxicity (e.g. anilin, hexachlorophene, propylene glycol) in ointments or other skin care products. To illustrate this, the absorption of chlorhexidine gluconate (aqueous formulation, 2%) used for skin antisepsis prior to catheter insertion in preterm neonates resulted in detectable concentrations of chlorhexidine gluconate with the highest concentrations 2-3 days after exposure [23]. Besides maturational changes, disease characteristics may also affect absorption, as illustrated for topical timolol to treat infantile hemangioma [24]. Besides the skin permeability itself, the higher body surface area (BSA) to weight ratio in (pre)term neonates is also of relevance. Using the Mosteller formula, the BSA/kg ratio in average neonates of 26, 30, 34 and 38 weeks is 0.090, 0.087, 0.075 and 0.064 respectively, as compared to an infant (0.046) or adult (0.025). Again, this reflects the important changes that already happen in infants within the neonatal age range.

Inhalational absorption is mainly of relevance for inhalational anesthetics. Absorption of inhalational agents (e.g. sevoflurane) relates to the alveolar surface area and functional residual capacity (FRC). Since neonates have a proportional higher alveolar capacity and lower FRC, absorption is more rapid in neonates. This absorption pattern is further affected by e.g. right-to-left shunting of blood, while left-to-right shunting usually has much more limited impact on uptake. The Neurosis trial evaluated the impact of early inhaled budesonide for the prevention of bronchopulmonary dysplasia in preterm neonates in a randomized, placebo controlled setting. The authors hereby concluded that the incidence of bronchopulmonary dysplasia was lower among those who received early inhaled budesonide than among those who received placebo, but the advantage may have been gained at the expense of increased mortality in exposed cases [25]. It is worth to explore the potential relevance of systemic absorption on these outcome variables and the impact of cytochrome P450 3A polymorphisms [26].

Absorption following conjunctival application is commonly non-intentional, but the use of topical mydriatic agents to enable screening for retinopathy of prematurity has been associated with feeding intolerance, paralytic ileus and cardiorespiratory events in these preterm neonates [27]. Similarly, propranolol 0.1 % eye micro-drops in preterm neonates included in an open-label pilot trial to prevent retinopathy of prematurity, resulted in systemic exposure to propranolol, be it less as compared to the oral propranolol studies [28].

Intravitreal injection of anti-vascular epithelial growth factor (VEGF) products has been reported repeatedly as pharmacological treatment to avoid laser therapy in preterm neonates with threshold retinopathy of prematurity. However, following intravitreal injection, these drugs (e.g., bevacizumab) can be detected in the serum of these preterm neonates as early as 2 days after the injection, showing a peak concentration at 14 days of life, and persist for up to 60 days. This indicates a deep compartment behavior with subsequent slow release. As a consequence, serum free VEGF levels decreased [29]. The clinical impact may be relevant, since preterm infants initially treated with bevacizumab compared to laser treatment had higher odds of severe neurodevelopmental disabilities [30].

Reduced skeletal muscle blood flow and inefficient muscular contractions may prevent or alter absorption from the site of intramuscular injection in neonates, but this can be counterbalanced by the relatively higher density of capillaries in skeletal muscles. Besides maturational changes, this can also be affected by e.g. sepsis, or co-administration of muscle relaxants or analgosedatives. Despite the known factors of variability in absorption, the intramuscular administration of benzylpenicillin and gentamicin has been evaluated as part of neonatal sepsis treatment (AFRINEST studies) due to the ease of administration in resource limited settings [31]. Nevertheless, this route to treat infectious diseases in neonates has to be avoided as much as possible because it is painful. The use of the muscular route is more common when the aim is to attain a deep compartment pattern, with subsequent slow release. This pattern has extensively been documented for palivizumab, but has also been suggested for a specific long-acting nevirapine formulation to provide Human Immune Deficiency Virus (HIV) prophylaxis in breastfeeding infants. The aim is hereby to facilitate adherence [32]. The same concept holds true for vitamin K. In a recent position paper by the ESPHAN committee on nutrition on the prevention of vitamin K deficiency bleeding in newborns, it was suggested that intramuscular application is the preferred route for efficiency and reliability of administration, since the success of an oral policy depends on compliance with the protocol and this may vary between populations and healthcare settings [33].

3. DISTRIBUTION

The apparent volume of distribution (L, and L/kg) is a theoretical measure of the extent to which a drug will distribute in the intravascular compartment and migrate into extravascular tissues. These shifts to and from extravascular compartments can depend on passive processes (protein binding, free concentrations, permeability) or active processes (both influx and efflux, transporters). Besides chemical properties of a given compound (e.g. lipophilicity, protein binding, molecular weight), the volume of distribution will be affected by population specific characteristics. Similar to absorption, metabolism or elimination, distribution can be affected by both maturational as well as non-maturational covariates.

Maturational changes are related to changes in weight, body composition or plasma protein concentrations. The changes in weight in early life is rather complex, with an initial weight loss in the first days of life with a return to the birth weight at day 8-14 of postnatal life and a subsequent 50 % increase compared to the initial birth weight at 6 weeks of postnatal life. This initial weight loss is more pronounced and the regain somewhat delayed in neonates on breastfeeding compared to formula feeding [34].

Besides the weight and weight changes, the age dependent changes in body composition are also quite impressive. In neonates, the extracellular and total body water is higher compared to infants or adults (60 to 40 to 35 %). Within the neonatal age range, there is a progressive decrease in free fat mass from 94 to 92 and 88 % in neonates born at 30, 34 and 38 weeks of gestational age [35,36]. The reverse (increase in proportional body fat) is illustrated in Fig. **1**. This figure provides estimated (10-50-90th centiles) trends for body fat (fat/total body weight, %) in appropriate for gestational age (AGA) grown neonates at birth between 30 and 40 weeks of gestational age. This figure combines data from two studies in preterm (30-36) and term (36-40) neonates (all <72h postnatal life) that generated this kind of data using the same air-displacement plethysmography technique [35,36].

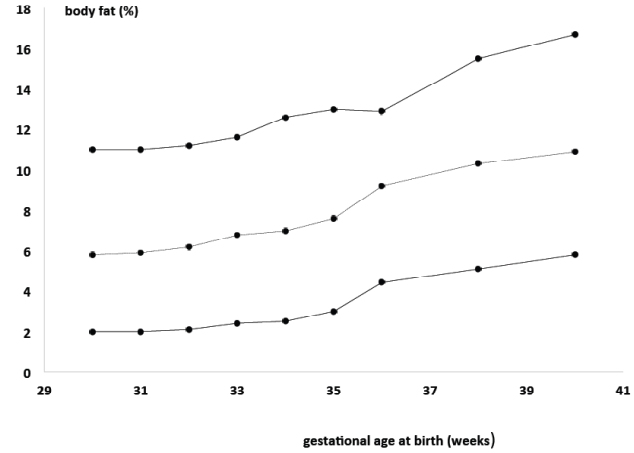


Fig. (1). Trends (10, 50 and 90th centile) in body fat composition (% of total body weight) at birth in neonates with a gestational age between 30 and 40 weeks [35,36].

These differences in body composition result in higher volumes of distribution and lower (peak) concentrations of water soluble antibiotics (e.g. aminoglycosides, vancomycin, beta-lactam antibiotics) or water soluble compounds (e.g. paracetamol) when administered on a mg/kg basis. Similar, this means that – in the absence of the use of a loading dose (20 mg/kg) – the higher volume of distribution will result in delayed attainment of a steady state when only the recommended 10 mg/kg q6h is used (Fig. **2**). When a loading dose is not used, the median analgesic paracetamol plasma concentration (10 mg/l) is only reached from the 3th dose onward, equal to 12 h after initiation of treatment [37]. In contrast, a highly lipophilic compound like propofol will have a lower distribution volume and this may result in higher concentrations in the effect compartment when a similar dose (mg/kg) is administered in a preterm compared to a term neonate (Fig. **1**). Finally, also the plasma protein composition displays maturation. The most relevant binding proteins in the plasma are albumin and alfa-1-acid glycoprotein. The total plasma protein and albumin concentration display a gestational age-dependent increase from 44-46 and 24-27 g/l between 24-28 weeks, to 50-52 and 30-32 g/l at 36 weeks of gestational age

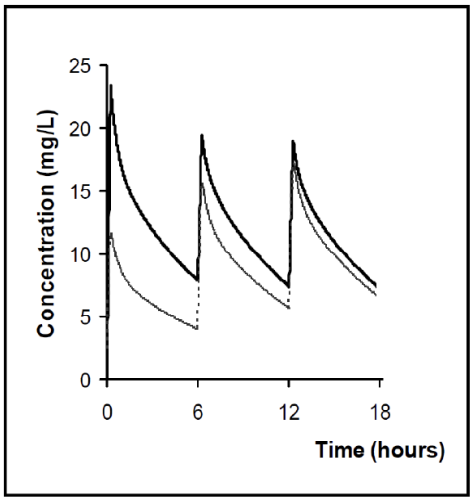


Fig. (2). Concentration time profiles with or without the use of an intravenous loading dose (20 mg/kg) of paracetamol, followed by 10 mg/kg q6h in a term neonate. Without the loading dose, it takes up to the 3rd administration before a similar profile will be reached [37].

[38]. Alfa-1-acid glycoprotein in newborn is about 50 % of the adult values, but there is already a broad range (3 fold, 0.32-0.92 g/L) within the neonatal population. This is likely due to the fact the alfa-1-acid glycoprotein is an acute phase protein, that increases after surgical stress. In this circumstance it is of importance to know that alfa-1-acid glycoprotein binds local anesthetics.

Increased capillary permeability, increased hydrostatic pressure, or decreased tissue oncotic pressure due to hypoproteinemia is commonly encountered in critically ill neonates and may increase the volume of distribution. These increases in volume of distribution may necessitate the use of a higher dose (mg/kg) to reach a given concentration. To illustrate this, Lingvall et al. documented that the gentamicin volume of distribution was significantly higher in blood culture confirmed septic neonates compared to non-septic cases [39]. Competitive binding of co-administered drugs or endogenous substances may also have an impact on the degree of drug-protein binding. In neonates, competitive binding of antibiotics (e.g. ceftriaxone, cefazolin) and bilirubin to albumin has been described [40,41]. Specifically in neonates, unconjugated bilirubin and free fatty acid concentrations will also display relevant competition for albumin binding places [40,41].

As a clinical consequence, the highly albumin-bound antibiotic ceftriaxone is currently contraindicated because of displacement of unconjugated bilirubin, which could potentially result in kernicterus [41]. We should be aware that the amount and type of circulating plasma proteins does not only influence drug distribution, but also drug action and elimination, since only the unbound drug can be distributed throughout the body, is available for elimination and can have a pharmacological effect. To illustrate this, differences in plasma protein binding explain the higher clearance of micafungin in neonates [42].

During ECMO, the volume of distribution for water soluble compounds like morphine and midazolam is increased (+200 %) [43]. Similarly, the presence of a patent ductus arteriosus, cardiogenic shock, or the use of whole body hypothermia also affects the volume of distribution because of their impact on cardiac output, regional blood flow characteristics, and tissue permeability [44].

4. METABOLISM AND ELIMINATION

Drug clearance represents the capacity to excrete a given drug or its metabolites from the body and hereby reflects the average

steady state or median concentration achieved with a maintenance dose. Clearance is defined as the volume of fluid that - for a given time interval - is completely cleared of a specific compound through either metabolism or primary elimination. The main routes of clearance of drugs and its metabolites are through the hepatobiliary system, the kidney and the lungs. While primary elimination is mainly through renal elimination (glomerular filtration, renal tubular excretion), metabolism occurs mainly – but is not limited – to the liver since also the kidney, intestinal mucosa, lung or the central nervous system contribute to drug metabolism [5,45].

4.1. Metabolic Clearance

Metabolic clearance relates to the regional blood flow, liver size, the compound specific extraction rate and the intrinsic iso-enzyme specific capacity. The pathways involved in drug metabolism are commonly classified in Phase I or Phase II reactions. Phase I mainly covers intramolecular, 'destructive' processes, while Phase II reactions are 'synthetic', conjugative processes. Phase I involves processes like oxidation, reduction, hydration or hydrolysis. Phase I covers both non-CYP and CYP mediated reactions. The most relevant group of iso-enzymes are the cytochrome P450 (CYP) enzymes, with a major contribution of CYP3A4/5 iso-enzymes since these are involved in the metabolism of about 50-60 % of all therapeutic drugs currently on the market. CYP3A4, CYP2D6 and CYP2C9 together account for about 85 % of all human drug oxidation activity. Other relevant iso-enzymes are CYP1A2, CYP2B6, CYP2C8-10, CYP2C19, and CYP2E1. Non-CYP mediated iso-enzymes relate to esterases, flavin-containing mono-oxygenase (FMOs), alcohol or aldehyde dehydrogenases (ADHs). Phase II involves glucuronidation, sulphation, methylation, acetylation or glutathione conjugation. The most relevant groups of iso-enzymes involved are UDP-glucuronosyltransferases (UGT).

Liver microsomal protein content (20-25 mg/g liver proteins) is low in neonates, and subsequently increases with age to reach a maximum level of microsomal protein content (40 mg/g) at about 30 years of age. However, a single maturational trend is too simple [5,45]. Hines et al. suggested almost a decade ago three different developmental patterns for drug metabolizing enzymes: high in fetal life to low or absent after birth (Class 1), stable throughout development (Class 2) or low in fetal life to increasing and high after birth (Class 3). Consequently, for a given iso-enzyme there is a specific window of hypervariability [46]. Both CYP1A2 hepatic protein concentrations and in vitro activity are very low with a slow developmental pattern after birth, starting with 5% at birth, 25% at the end of infancy, up to only 50 % of the adult level of activity at the age of 6 years. This is in line with the available in vivo observations on e.g. caffeine. CYP2B6 expression and activity increases significantly (2 fold) in the first month of life. The CYP2C subfamily ontogeny is faster and earlier (from birth onwards) when compared to CYP2C19 ontogeny (only slowly rising in the first 6 month of life). CYP2D6 is already present in fetal liver tissue, with a subsequent increase in neonatal life. CYP3A7 has a high activity during fetal life and early infancy, with a subsequent decrease. In contrast, CYP3A4/5 matures slowly, and only reaches an adult level of activity at the end of infancy [46].

Esterase function already matures from 28 weeks onwards, as reflected in e.g. remifentanil degradation. It seems that the FMO-1 activity is high at birth with a subsequent decrease, while FMO-3 ontogeny is the opposite, being low at birth with an age-dependent increase. ADH capacity in the newborn liver is about 10 % of the adult capacity, but there are different patterns of ontogeny for different iso-enzymes.

Sulphation activity is already higher in early infancy but has a low capacity. Glutathione conjugation is already at a relevant level of activity at birth (65-70 % of the adult level) and is of relevance for e.g. paracetamol detoxification. The UGTs are responsible for the glucuronidation of hundreds of hydrophobic endogenous compounds and drugs. Genetics, co-morbidity or environmental issues further interact with these developmental changes and ontogeny is only one of the covariates involved [45].

4.2. Renal Function

The final destiny of the majority of drugs and their metabolites is elimination by the renal route. Consequently, it is important to understand its maturation. Maturation of renal elimination capacity is a continuous process, already started during fetal organogenesis and only completed at the end of childhood. In neonates, glomerular filtration rate (GFR) is mainly based on weight at birth, and the postnatal age with a 2-4 fold increase in GFR in the first 4 weeks of postnatal age. However, renal elimination covers both GFR as well as renal tubular transport activity (both excretion and absorption). Intriguingly, these processes do not mature simultaneously. To further put this into some perspective, the GFR is 20-45 ml/min/1.73m^2 in the term neonate, with a subsequent progressive increase of 5–10 ml/min/1.73 m^2 for each consecutive week. Median GFR reference values in infants aged 27 to 31 weeks gestation ranged from 7.9 to 30.3 on day 7, 10.7 to 33.1 on day 14, 12.5 to 34.9 on day 21, and 15.5 to 37.9 ml/min/1.73 m^2 on day 28 [47]. Besides maturational changes, GFR estimates can also be affected by disease characteristics, like perinatal asphyxia or respiratory distress or co-medication (ibuprofen, indomethacin, methylxanthines) [48]. Vieux et al. also quantified the impact of ibuprofen on creatinine clearance in 148 preterm neonates either or not exposed to ibuprofen (median estimate 12.8 vs 18.1 ml/min/1.73m^2, - 29 %) [49].

4.3. Drug Transporters in Liver and Kidney

The liver and the kidney display drug transporter activities. Drug transporters are present on the hepatocyte (blood-hepatocyte, and hepatocyte-biliary surface) and the renal tubular cell. These transporters can either exert an uptake or efflux. Renal tubular functions (secretion, absorption) also display maturation, but with a somewhat later onset and rate, to reach adult capacity at the end of the first year of life. Tubular secretion of organic anions at birth is about 20-30 % of adult values, but the ontogeny of individual transporters in the renal tubular cells is largely unknown. Digoxin clearance hereby serves as an excellent example of renal ontogeny of both GFR and renal P-gp transporter activity. The available data on the ontogeny of drug transporters has recently been summarized. It seems that different developmental patterns – similar to the Hines classification for the maturation of hepatic drug metabolism - for individual transporters emerge, but more data are needed to describe these patterns [50].

5. ILLUSTRATIONS ON THE IMPACT OF DIFFERENT COVARIATES ON PHARMACOKINETICS IN NEONATES

We aim to illustrate the complex pattern of ontogeny (i.e. age-related maturation), genetic polymorphisms (metabolism, transporters) and changes in renal elimination capacity during infancy on the phenotypic concentration-time profiles of tramadol and its metabolites [51]. This will be followed by an illustration on maturational (age, weight) and non-maturational covariates (co-medication, perinatal asphyxia) relevant for amikacin disposition in neonates [52].

5.1. Tramadol

Tramadol is a 4-phenyl piperidine analogue of codeine. Its analgesic effect is mediated through noradrenaline re-uptake inhibition, increased release of serotonin and decreased serotonin re-uptake in the spinal cord. Tramadol also has a weak μ-opioid receptor effect with a receptor affinity that is 6000 times weaker than morphine. Tramadol is metabolized by O-demethylation (CYP 2D6) to the pharmacodynamic active, μ-opioid receptor related metabolite O-demethyl tramadol (M1). This specific metabolite is subsequently eliminated by renal elimination or undergoes hepatic re-uptake (Organic Cation Transporter-1, OCT-1). M1 formation in

part depends on ontogeny, i.e. age dependent activity and CYP2D6 polymorphisms [51]. Similar, hepatic M1 re-uptake also depends on age-dependent activity and OCT-1 allele frequency [53]. However, none of these pathways matures simultaneously, resulting in extensive variability in tramadol disposition and subsequent effects in early infancy. Maturational absorption further adds to this variability. Tramadol can be administered by oral, rectal, caudal or intravenous route [Fig. **3**].

5.1.1. Absorption

PK following oral administration has been reported in children, but not in neonates. In these children, absorption of oral drops (1.5 mg/kg) was fast with tramadol peak concentration at 30 minutes, and peak M1 concentrations at about 4-5 h. Compared to observations in adults, this tramadol peak is somewhat earlier and likely reflects gastric emptying while the first pass metabolism results in extensive metabolic clearance to the pharmacodynamic more relevant M1 [54]. Extrapolating these findings to newborns (delayed gastric emptying, ontogeny of metabolic and elimination clearance) we assume that tramadol absorption and subsequent M1 elimination will be delayed. This may explain the prolonged sedation and respiratory depression observed in former preterm neonates exposed to a single oral tramadol dose (2 mg/kg) [55]. PK following rectal administration (single dose, 25 mg) has been documented in 12 infants and young children (12.5-25 kg) [56]. Tramadol is absorbed at a reasonable rate and variability. Compared to oral administration, the tramadol peak (2.4 h instead of 0.5 h), but not the M1 peak (3.9 h instead of 4-5 h) was somewhat delayed, but the variability in absorption was not quantified. Extrapolating from these available data in neonates and infants, it is reasonable to postulate that rectal administration results in additional variability due to variability in absorption, and depends on age and formulation [17].

5.1.2. Metabolism

Tramadol enters the hepatocyte by passive diffusion from the blood compartment to the liver. In the hepatocyte, tramadol is subsequently metabolized by O-demethylation (CYP2D6) to the pharmacodynamic active metabolite O-demethyl tramadol (M1), or by N-demethylation to the inactive N-demethyl tramadol (M2) [Figure 3]. Subsequently, both metabolites diffuse back to the blood compartment by passive diffusion, in part after additional metabolism (e.g. glucuronidation). Moreover, there is an additional active re-uptake of a M1 mechanism from the blood compartment back to the hepatocyte through the OCT-1 transporter. All these processes display age-dependent maturation, while CYP2D6 and OCT-1 also display genetic polymorphisms [53,57]. In vivo observations on CYP2D6 ontogeny in infancy have been described by Blake et al. using dextromethorphan O- and N-demethylation throughout the first year of life. The authors hereby documented that N-demethylation ontogeny is slower compared to O-demethylation (CYP2D6) [58]. Similar observations were reported for tramadol disposition throughout human life in 295 human subjects. Differences in clearances were largely accounted for by age driven maturation once size was considered. Both tramadol clearance as well as formation clearance to M1 displayed fast maturation, while M1 elimination clearance was somewhat delayed and similar to the maturation of the glomerular filtration rate pattern.

In an attempt to focus on early infancy, we used a dataset of tramadol and M1 concentrations collected in 50 infants (median postmenstrual age 39.5, interquartile range 36.8 to 41.3 weeks) that were treated with intravenous tramadol (loading dose, 2 mg/kg, followed by a continuous infusion, 5-8 mg/kg/24 h). Because of continuous administration, the M/M1 plasma value to a large extent reflects the phenotypic variability in drug disposition. We hereby

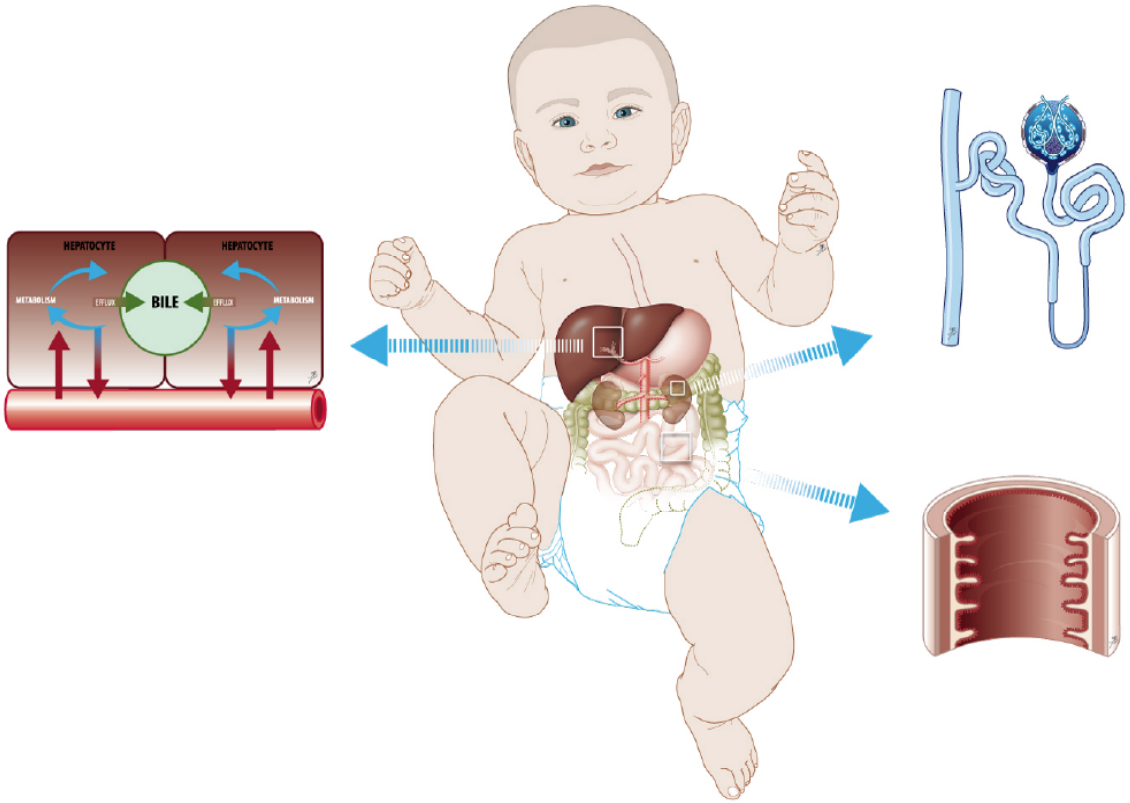


Fig. (3). Schematic overview on aspects of pharmacokinetics of tramadol in neonates. The routes of administration are oral, rectal or intravenous. Tramadol metabolism relates to hepatic metabolism with subsequent diffusion of these metabolites back to the blood compartment. However, the most relevant metabolite (O-desmethyltramadol) undergoes active hepatic re-uptake, while elimination clearance is by renal route [51].

illustrated the age-driven trend to lower log M/M1 plasma values during continuous iv tramadol administration, reflecting the progressive increase in M1 formation (Fig. **4a**) [51,57]. Besides age, genetic variants further contribute to the interindividual variability in metabolic clearance. The CYP2D6 iso-enzyme is highly polymorphic, and these variants can result in increased (e.g. duplication/multiplication), decreased or even absence of CYP2D6 activity. One approach to link polymorphism with phenotypic CYP2D6 activity is the CYP2D6 activity score (range 0-3), as described by Gaedigk et al [59]. However, we should realize that such a CYP2D6 'activity' score has been validated in adults and once the phenotypic CYP2D6 activity is fully expressed. Using the same dataset of log M/M1 plasma observations, we illustrate the impact of CYP2D6 polymorphisms on tramadol disposition, using this CYP2D6 activity score (unknown or 0, 0.5, 1, 1.5, 2 or 3) (Fig. **4b**). Similar, hepatic re-uptake of M1 is driven by the OCT-1 transporter, and also this transporter displays polymorphisms. Linear mixed-model analysis illustrated that the OCT1 genotype (allele frequency) was another independent covariate in the same dataset of plasma log M/M1 observations in newborns and young infants [53]. As illustrated in Fig. **4c**, the plasma log M/M1 values are significantly higher in neonates with 2 active alleles compared to 0 or 1 active allele, reflecting a higher phenotypic re-uptake capacity in the presence of 2 active alleles. Besides confirming the impact of PG on tramadol disposition in neonates similar to earlier observations in adults these pharmacogenetic observations also show us that these enzymes or transporters in young infants are indeed already yet showing their phenotypic activity.

5.1.3. Elimination

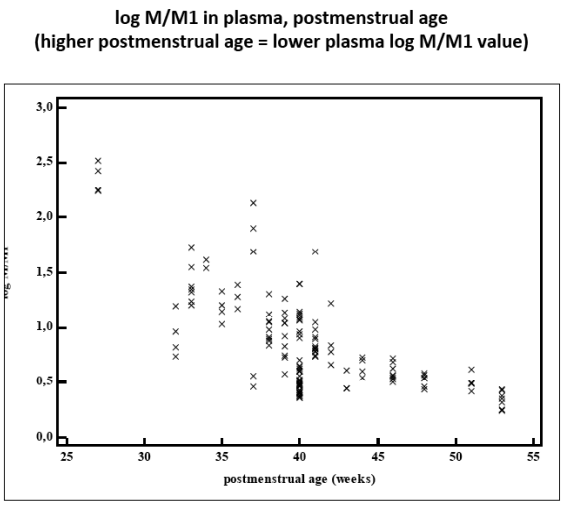
Tramadol, and even more its metabolites, undergo renal elimination (Figure 3). This means that the plasma M1 concentration will not only depend on its formation, but also on its subsequent renal elimination. As mentioned earlier, tramadol clearance as well as formation clearance to M1 displayed fast maturation, while M1 elimination clearance was somewhat delayed and similar to the maturation of the glomerular filtration rate pattern. This means that M1 accumulation is more likely to occur in early infancy, since the M1 formation is already quite advanced while its subsequent elimination capacity [51].

5.2. Amikacin

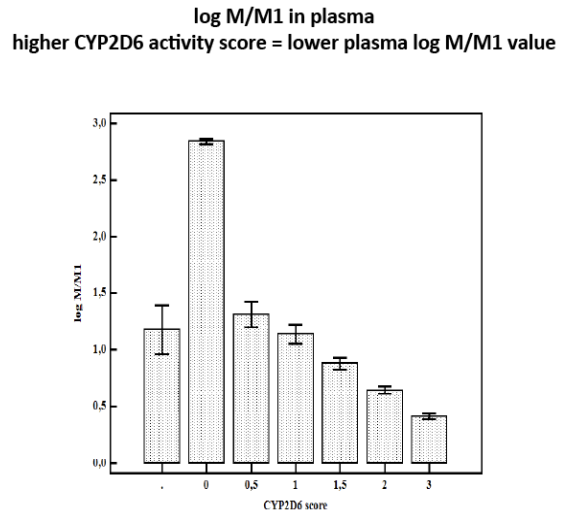
Amikacin is a commonly administered aminoglycoside in neonates. The bactericidal effect of aminoglycosides relates to the inhibition of bacterial protein synthesis. This effect goes by binding to the bacterial 30S ribosomal subunit and interfering with transfer RNA binding. This also explains the 'post-antibiotic' effect. For effective therapy a peak/minimal inhibitory concentration (MIC) ratio >8 should be targeted. The concentration-dependent response supports the use of high doses to attain peak concentrations for aminoglycosides. Because toxicity relates to the Area Under the time vs concentration Curve (AUC), and to avoid adaptive resistance, these higher doses should be combined with extended dosing intervals. Since aminoglycosides are hydrophilic, these compounds distribute to the extracellular water, and subsequent undergo glomerular filtration. Since neonates have a proportionally high extracellular water content and a proportionally low glomerular filtration, this means that even higher doses should be combined with extended dosing intervals. In adults, this is commonly once daily, but in neonates these dosing intervals can be further extended. Both phenomena display maturational and non-maturational covariates [52].

5.2.1. Volume of distribution

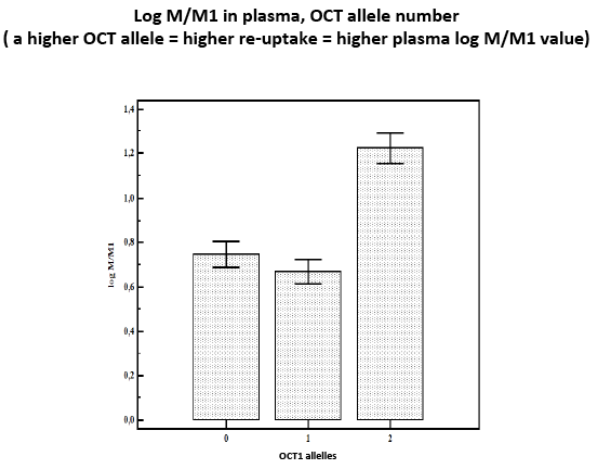
The extracellular water content (l/kg) displays maturational changes and these changes are most prominent in early infancy. When we focus on neonates at birth, the extracellular water content is higher in the most immature preterm neonates. This is translated



(a)



(b)



(c)

Fig. (4). The impact of postmenstrual age (**a**), CYP2D6 activity score (**b**, . = unknown, otherwise 0.5-3 activity score) and OCT1 allele frequency (**c**) on the plasma log M/M1 observations as collected during continuous intravenous tramadol administration in preterm and term neonates. The lower the

log M/M1, the higher the phenotypic CYP2D6 activity or the lower the OCT1 re-uptake activity [51,53,57,59].

in recent suggestions to consider higher doses of gentamicin (6 mg/kg) in the most preterm infants [60], while dosing regimens of 5 mg/kg every 48 h, 5 mg/kg every 36 h, and 5 mg/kg every 24 h for patients with gestational ages of <37 weeks, 37 to 40 weeks, and ≥40 weeks, respectively, were recently recommended [61]. Besides these age-driven maturational changes, disease characteristics can also affect these estimates. Based on a dataset of 576 gentamicin concentrations in 277 neonates, of whom 15 had a confirmed sepsis, Lingvall et al. quantified a 14 % increase in volume of distribution in these neonates [39]. A similar increase in the distribution volume of distribution (0.58 vs 0.45 l/kg, + 28 %) has been observed in term neonates either or not on ECMO [62].

5.2.2. Elimination

Since elimination of aminoglycosides is exclusively by glomerular filtration, covariates of GFR will affect clearance [63]. In neonates, this means that gestational age or birth weight - reflecting the renal maturation until birth - and postnatal age - reflecting renal maturation after birth - are the most relevant covariates. Besides these maturational differences, there are again also relevant non-maturational covariates. Co-administration of ibuprofen or indomethacin results in a transient reduction of aminoglycoside clearance of about 20 % [64]. Similarly, whole body cooling and perinatal asphyxia result in a reduction of aminoglycoside clearance of 40 % with a progressive increase (+29 %) after rewarming [52,65].

DISCUSSION

The maturation of the individual ADME processes in early infancy is driven by age (postnatal, gestational or postmenstrual age) or weight as main covariates. However, all these processes have their specific maturational pattern. This time-dependent physiology is further affected by additional non-maturational covariates like genetic variation or environmental (drug-drug, drug-nutrition, drug-treatment modalities, disease) characteristics as discussed and illustrated in this paper. We would like to stress the need to integrate these different maturational and non-maturational processes in order to predict the concentration time profiles in individual neonates. The maturational pattern of the individual renal or hepatic elimination processes may differ. This can only be solved by integration of available knowledge on the ontogeny of the different elimination routes (metabolic *vs* elimination clearance) to predict compound specific, concentration-time profiles in neonates, as has been suggested in the tramadol illustration. This can be achieved through modelling, a potential very powerful tool to turn integrated knowledge into PK prediction [1,45].

Although the topic of modeling and simulation in pediatric drug therapy is discussed elsewhere in this special issue, we still would like to draw attention to an interesting attempt to integrate this fast evolving time-dependent physiology of early infancy as compared to later pediatric life into physiologically based (PBPK) model building approaches. In general, covariates are fixed throughout a PK study. However in the setting of fast maturation as in neonates, redefinition of these covariates during the study is warranted. Based on a dataset on sildenafil disposition, Abduljalil *et al.* illustrated that the resampling time in the first day of life is hourly, to increase to every 6 hours in the second part of the first week of postnatal life to every 48 hours after the first month of life [66].

PK efforts are most likely to be clinically impactful when linked to pharmacodynamics (PD), as this impact on both desired effects and unwanted side-effects. Newborns differ from other populations, potentially also in their drug response. Maturational changes in early infancy can significantly affect PK processes, but also PD. Developmental pharmacodynamics is the study of age-related maturation of the structure and function of biologic systems and how this affects response to pharmacotherapy. This may manifest as a change in the potency, efficacy, or therapeutic range of a drug [67]. The next steps to be taken in neonatal clinical pharmacology are the development and validation of pharmacodynamic measurements ('biomarkers'), and validation of longterm neonatal outcome markers to assess safety. Improved data collection and analyses of continuous physiological parameters, including heart rate variability, near infrared spectroscopy or amplified electroencephalography (aEEG) may provide better pharmacodynamic effect registration [68]. Such an approach is described in a paper on doxapram use and effects in neonates, integrating dosing, concentrations and outcomes as part of the routine clinical care. Long term neonatal outcome markers are not limited to neurodevelopment outcome, but also cover aspects of cardiovascular and renal health in former preterm neonates. This is because in preterm neonates, the growth of the vascular tree and microcirculation, including glomerulogenesis becomes a postnatal instead of an intrauterine event that can be affected by environmental factors, including drugs and nutrition [69].

In conclusion, we have provided an overview of the available evidence on the impact of maturational and non-maturational covariates on PK in neonates. Future efforts should focus on integration of the already available knowledge, the collection of data on the impact of non-maturational covariates, and the development and validation of PD tools in neonates with the ultimate aim to understand the (patho-)physiological processes involved as a pivotal tool towards improved pharmacotherapy in these vulnerable patients.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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