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Exposure to acetaminophen and all its metabolites upon 10, 15 and 20 mg/kg intravenous acetaminophen in very preterm infants

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ABSTRACT

Background

Exposure to acetaminophen and its metabolites in very preterm infants is partly unknown. We investigated the exposure to acetaminophen and its metabolites upon 10, 15 or 20 mg/kg intravenous acetaminophen in preterm infants.

Methods

In a randomised trial, 59 preterm infants (24-32 weeks gestational age, postnatal age<1 week), received 10, 15 or 20 mg/kg acetaminophen intravenously. Plasma concentrations of acetaminophen and its metabolites (glucuronide, sulphate, cysteine, mercapturate and glutathione) were determined in 293 blood samples. Area under the plasma concentration-time curves ($AUC_{0\text{-}500\ min}$) were related to dose and gestational age.

Results

Between 10 and 20 mg/kg/dose, median AUCs of acetaminophen, glucuronide, sulphate and cysteine increased significantly resulting in unchanged ratios of AUC of metabolite to acetaminophen. The AUC ratio of glucuronide to acetominophen increased with gestational age, that of sulphate decreased, and the ratio of cysteine and mercapturate remained unchanged.

Conclusion

We found a gestational age-dependent increase in glucuronidation but no evidence for saturation of a specific pathway as there was a proportional increase in exposure of acetaminophen and all metabolites. Compared to adults, very low exposure to glucuronide but higher exposure to sulphate, cysteine and mercapturate metabolites was found, of which the relevance is not yet known.

INTRODUCTION

Preterm infants treated in neonatal intensive care units (NICUs) are often exposed to repetitive or prolonged pain[1, 2]. Opioids and NSAIDs potentially relieve the pain but may result in serious side effects and potential harm to critically ill preterm infants[3]. Intravenous acetaminophen as an opioid-sparing therapy in adults and children has now been introduced in NICUs across the globe[4]. However, only very limited data of its use are available in the most preterm infants[5, 6].

Acetaminophen (N-acetyl-p-amino-phenol; APAP) is extensively metabolized in the liver. The main pathways involved are glucuronidation and sulphation, which in adults account for around 55% and 30% of acetaminophen metabolism, respectively[7-9] (Figure 1). Only 2-5% is excreted unchanged in the urine. Approximately 5-10% of acetaminophen is metabolized by cytochrome P450 (CYP), primarily by the CYP2E1 enzyme[10-12], to the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI)[7, 13-15]. At therapeutic doses, NAPQI is immediately inactivated by conjugation with gluthatione. After formation of acetaminophen-glutathione, acetaminophen-cysteine and acetaminophen-mercapturate are formed consecutively. Without this detoxification route, NAPQI can bind covalently to cellular proteins and form toxic protein adducts, which may cause mitochondrial dysfunction and early oxidant stress[16-18]. This, ultimately, may result in liver cell necrosis[19].

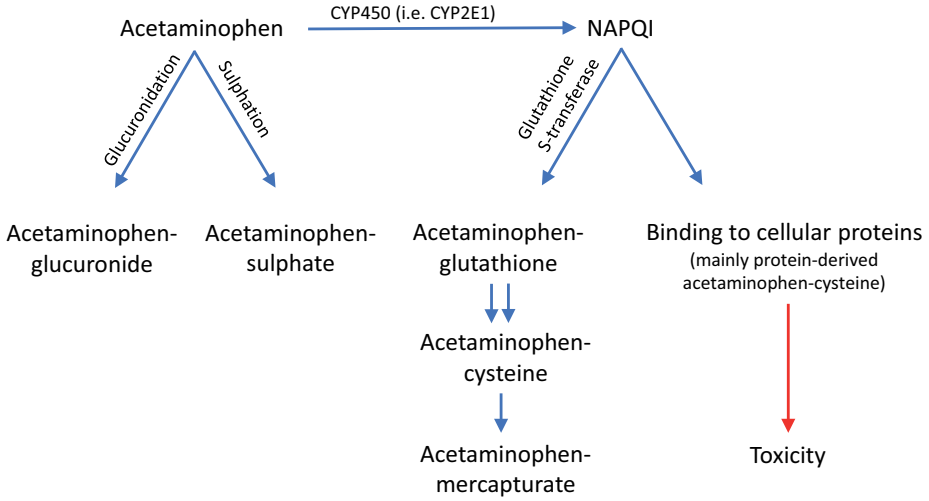

Figure 1. Pathways involved in acetaminophen metabolism.
NAPQI: N-acetyl-p-benzoquinone imine

Neonates have a lower total acetaminophen clearance per kg bodyweight than have adults, with proportionally higher sulphation and lower glucuronidation[20-22]. Acetaminophen sulphation is mainly catalyzed by SULT1A1, 1A3/4, and 1E1[23], of which several sulfotransferase enzymes are already widely expressed in fetal tissue (18-25 weeks)[24]. Glucuronidation of acetaminophen mainly occurs by UGT-1A6, and to a lesser extent by 1A9[25]. Low glucuronidation rates have also been shown for morphine in preterm and term infants[26]. There is, however, very limited data available on the exact contributions of the different pathways in paracetamol metabolism in preterm infants and particularly with respect to the CYP2E1 mediated oxidation pathway in neonates with a gestational age below 28 weeks of age.

To gain more insight in the contributions of the different metabolic pathways upon increasing doses of acetaminophen we studied the exposure to acetaminophen and all of its metabolites in the most premature neonates. To this end, preterm infants were stratified for gestational age (24-28 and 28-32 weeks) and randomised to receive an intravenous dose of 10, 15 or 20 mg/kg acetaminophen.

METHODS

Patients

From October 2010 until October 2013 a randomized, two-center trial was performed at the level 3 Neonatal Intensive Care Units (NICUs) of the Erasmus Medical Center-Sophia Children's Hospital in Rotterdam and Isala Clinics in Zwolle, the Netherlands. Approval of the Ethics Review Committees of both hospitals and written informed consent from parents/legal guardians were obtained prior to study initiation (MEC-2009-250, National Trial Register 2290).

Eligible for inclusion were all preterm neonates with a gestational age < 32 weeks with an indwelling arterial catheter for clinical purposes, undergoing central venous catheter placement in the first 7 days of life. Exclusion criteria were: major congenital anomalies, intraventricular haemorrhage ≥ grade 3, use of neuromuscular blockers, previous acetaminophen, and maintenance dose of analgesics or more than one loading dose of morphine or midazolam any time prior to inclusion in the study.

Study design

Neonates

Sixty neonates were randomly allocated to 10, 15 or 20 mg/kg bodyweight of intravenous acetaminophen (Perfalgan©, Bristol-Meyers Squibb, Utrecht, the Netherlands). The dosages were based on extrapolation of the intravenous dosages of the prodrug propa-

cetamol used in older preterm infants[25]. Patients were stratified for gestational age, i.e. 30 neonates of 24-28 weeks, and 30 of 28-32 weeks gestational age. Acetaminophen was administered via a 15-minute infusion before peripheral central venous catheter placement within the first week of life. Five blood samples (0.2 ml per sample) were collected at different sample schedules, which were based on a previous pharmacokinetic data on acetaminophen in neonates by Allegaert et al.[6]. Samples were randomly taken either at T = 0 (before start of acetaminophen administration), 20, 60, 240 and 540 minutes, or at T = 15 (after the acetaminophen infusion was completed), 30, 120, 360 and 720 minutes. Investigators, nursing and medical staff taking care of the subjects were blinded for the administered dose of acetaminophen.

Drug assay

Acetaminophen, acetaminophen-glucuronide, acetaminophen-sulphate, acetaminophen-glutathione, acetaminophen-cysteine and acetaminophen-mercapturate were measured using high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC–ESI–MS/MS) at the Center for Human Toxicology, University of Utah (Salt Lake City, UT)[27]. The assay was linear over 0.05–50 µg/mL for acetaminophen, acetaminophen-glucuronide and acetaminophen-sulphate, and over 0.025–5.0 µg/mL, 0.01–5.0 µg/mL and 0.01–1.0 µg/mL for acetaminophen-glutathione, acetaminophen-cysteine and acetaminophen-mercapturate, respectively. The lower limits of the ranges represent the lower limits of quantification (LLOQs) of acetaminophen and its metabolites. The lower limit of detection (LOD) for acetaminophen-glutathione was 0.1 ng/ml. Intra and inter-assay accuracies ranged from 80 to 112 %, and intra and inter-assay imprecisions did not exceed 15%.

Data reporting and statistical analysis

Acetaminophen metabolite concentrations were converted to µmol/L molar acetaminophen equivalents using molecular weights for each of the metabolites. Data clearance was performed prior to AUC calculation; the first time a concentration was below LLOQ, this value was set to 0.5*LLOQ for that component. If a level was below LLOQ at two consecutive moments, this value was set to 0 µmol/L. The area under the plasma concentration-time curve for acetaminophen and metabolites over 0-500 minutes ($AUC_{0\text{-}500\ min}$) for each individual patient was calculated by non-compartmental analyses using WinNonLin© software package (version 6.3; Pharsight, Mountain View, CA) and the linear-log trapezoidal rule.

In a per protocol analysis, Kruskal-Wallis tests with post-hoc multiple comparison were applied to test for statistical differences in median $AUC_{0\text{-}500\ min}$ values of acetaminophen and metabolites as well as median $AUC_{0\text{-}500\ min}$ ratios of acetaminophen-metabolite to acetaminophen between dose groups. Linear regression analysis was used to identify

an association between $AUC_{0\text{-}500\ min}$ ratios of each metabolite to acetaminophen and gestational age. A multivariate regression analysis was performed to analyze the significance of small for gestational age (SGA) on the $AUC_{0\text{-}500\ min}$ ratios of the different acetaminophen-metabolites to acetaminophen. Therefore, the three dosage-groups were converted into two dummy variables. Two-sided p-values <0.05 were considered statistically significant. Data were analyzed using SPSS version 22 (IBM, Armonk, NY).

RESULTS

Patients and data

In total, 266 neonates were assessed for eligibility, of whom 60 were randomised to receive acetaminophen in a dosage of 10, 15 or 20 mg/kg intravenously. Data of one patient in the 20 mg/kg/dose group were excluded from analysis because $AUC_{0\text{-}500}$ could not be calculated due to incomplete sampling data. In total 293 samples were available, of 59 preterm neonates with a median gestational age of 27.9 weeks (range 24.0-31.1 weeks) and a median birth weight of 953 grams (range 462-1550 grams) treated with acetaminophen (Table 1). One subject in the 10 mg/kg-group, who was suspected to be an outlier with high AUCs for all components, may have received a higher dose of acetaminophen, although this could not be confirmed.

Table 1. Patient characteristics.

	Acetaminophen dose		
	10 mg/kg (n=20)	**15 mg/kg (n=20)**	**20 mg/kg (n=19)**
Gestational age (weeks)			
Median	27.9	28.0	27.7
(range)	(24.0–31.1)	(24.3–30.6)	(24.3–30.4)
Birth weight (grams)			
Median	970	988	870
(range)	(462–1550)	(475–1440)	(630–1380)
PNA (days)			
Median	5	6	6
(range)	(1–7)	(1–7)	(0–7)
SGA, n (%)	4 (20)	6 (30)	7 (37)
Sex, n (%)			
Boy	10 (50)	10 (50)	8 (42)
Girl	10 (50)	10 (50)	11 (58)
Acetaminophen dose (mg)			
Median	9.7	14.8	17.4
(range)	(4.6–15.5)	(7.1–21.6)	(12.6–27.6)

PNA: postnatal age, SGA: small for gestational age

Exposure of acetaminophen and its metabolites in the three dose groups

The $AUC_{0\text{-}500\ min}$ of acetaminophen and its glucuronide, sulphate and oxidative metabolites in the three dose groups are shown in figure 2. As concentrations for acetaminophen-glutathione were low, $AUC_{0\text{-}500\ min}$ for this metabolite could not be calculated (59 samples above LOD in 27 neonates; 1 above LLOQ) . For the other analytes the percentage of samples below LLOQ was less than 10%, except for acetaminophen-glucuronide with 15% below LLOQ. The median $AUC_{0\text{-}500\ min}$ of acetaminophen and its metabolites increased significantly with dose, except for acetaminophen-mercapturate (Figure 2). To correct the $AUC_{0\text{-}500\ min}$ of the metabolite for the $AUC_{0\text{-}500}$ of acetaminophen in each specific subject, ratios of median $AUC_{0\text{-}500\ min}$ acetaminophen-metabolite to acetaminophen were calculated. No significant difference in AUC ratio for any of the metabolites was found between the dose groups (Figure 3).

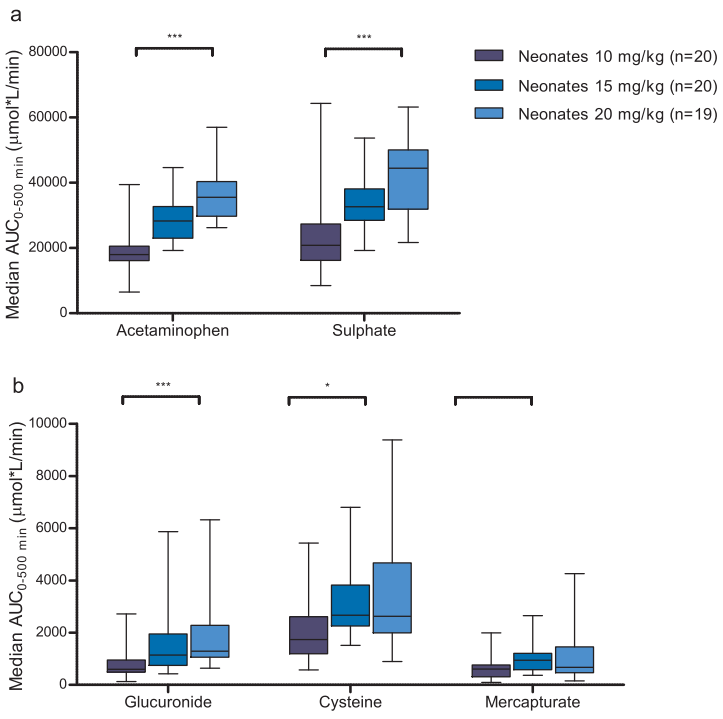

Figure 2. Boxplots of $AUC_{0\text{-}500\ min}$ in preterm neonates (n=59) receiving either 10, 15 or 20 mg/kg of intravenous acetaminophen.

a. Acetaminophen and acetaminophen-sulphate

b. Acetaminophen-glucuronide, acetaminophen-cysteine and acetaminophen-mercapturate

The boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum ranges. Significance of Kruskal-Wallis test with post-hoc multiple comparison was indicated with: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

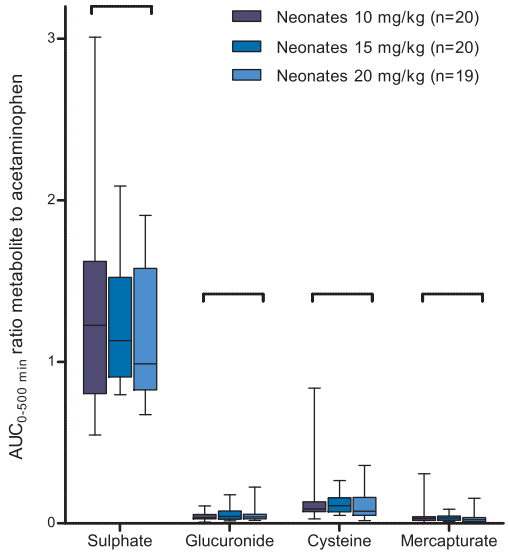

Figure 3. Boxplot of $AUC_{0\text{-}500\ min}$ ratio of sulphate, glucuronide, cysteine and mercaptopurine metabolite to acetaminophen in preterm neonates (n=59) receiving either 10, 15 or 20 mg/kg of intravenous acetaminophen.

The boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum ranges. Significance of Kruskal-Wallis test with post-hoc multiple comparison was indicated with: * p<0.05; ** p<0.01; *** p<0.001.

Metabolism of acetaminophen in relation to gestational age

Figure 4 shows the $AUC_{0\text{-}500\ min}$ ratios of acetaminophen-metabolite to acetaminophen versus gestational age. A positive association was found between acetaminophen-glucuronide and gestational age (slope=0.0054, p=0.020, R=0.089). For acetaminophen-sulphate, a negative association with gestational age was found (slope=-0.093, p=0.001, R=0.174). Paractamol-cysteine and mercapturate were not associated with gestational age: p=0.638 and p=0.124, respectively (Figure 4). Subjects with suspected outlying values were checked, but none could be explained. Therefore, all were kept in the analyses. Multivariate regression analysis showed that SGA was no significant covariate for any of the ratios $AUC_{0\text{-}500\ min}$ acetaminophen-metabolite to acetaminophen. SGA showed a trend towards significance for the ratio $AUC_{0\text{-}500\ min}$ acetaminophen-sulphate to acetaminophen (p=0.075, B=-0.219).

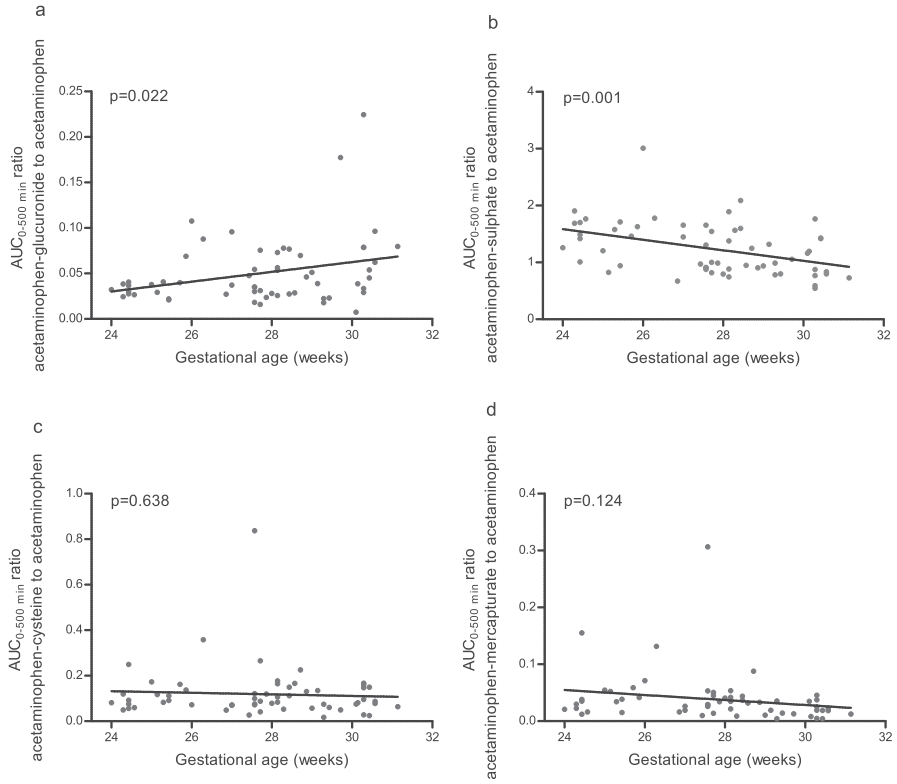

Figure 4. $AUC_{0\text{-}500\ min}$ ratios of acetaminophen-metabolite to acetaminophen versus gestational age for preterm neonates (n=59).

a. Acetaminophen-glucuronide
b. Acetaminophen-sulphate
c. Acetaminophen-cysteine
d. Acetaminophen-mercapturate

DISCUSSION

In this study we have quantified the exposure to acetaminophen and its metabolites in very preterm infants with a gestational age ranging from 24-32 weeks after a single dose of either 10, 15 or 20 mg intravenous acetaminophen per kg bodyweight. Analysis showed that the higher the dose, the higher the exposure, which theoretically can also result in higher efficacy. Importantly, a dose-related increase of exposure was found for none of the acetaminophen-metabolites when corrected for the exposure to acetaminophen in each patient. Furthermore, no saturation was noticed for sulphation even after administration of 20 mg acetaminophen per kg bodyweight. This is particularly of clinical relevance because in preterm neonates the glucuronidation capacity is still

low[28] and the oxidative CYP2E1 pathway is potentially hepatotoxic. The contributions of both the non-toxic sulphation and the glucuronidation pathway in relation to the administered dose are important from a safety point of view because it is the CYP2E1 pathway that is involved in liver toxicity[18].

The finding that with advancing gestational age the contribution of glucuronidation to acetaminophen metabolism increased, while sulphation decreased, might indicate intrauterine maturation of glucuronidation between 24 and 32 weeks, as postnatal age at the time of dose was comparable at different gestational ages. Glucuronidation capacity is low after birth[29] and increases with bodyweight and/or postnatal age as was shown for morphine[21, 26]. Theoretically, it could be postulated that the increase in glucuronidation of acetaminophen with gestational age is related to decreased sulphation. However, given that this increase in glucuronidation capacity was also shown for morphine, the main driver of these changes seems to be the increasing glucuronidation capacity after birth. Also in adults, in whom glucuronidation is at the maximum capacity, sulphation is of less relevance. Another reason for lower exposure to acetaminophen-sulphate with gestational age could be an increased renal elimination clearance of acetaminophen-sulphate[30].

With sulphation being the main pathway for acetaminophen in preterm neonates, and with glucuronidation increasing after birth, an important question is what the relevance is of the exposure to metabolites mediated by CYP2E1 in preterm infants. Plotting the $AUC_{0\text{-}500\ min}$ of acetaminophen and its metabolites from our study (standardized to a dose of 15 mg/kg) versus the $AUC_{0\text{-}500\ min}$ from a previously published study in adults (standardized to a dose of 1000 mg corresponding to 14.4 mg/kg)[31] revealed comparable acetaminophen exposure between neonates and adults ($p=0.296$) (Figure 5). In contrast, not only the AUC of acetaminophen-sulphate, but also those of acetaminophen-cysteine and acetaminophen-mercapturate were higher in neonates (i.e. 2.9, 3.4 and 4.6 fold higher (all $p<0.001$)). As expected, the $AUC_{0\text{-}500\ min}$ of acetaminophen-glucuronide was 18.5 fold lower in neonates compared to adults ($p<0.05$). Immature glucuronidation potentially leads to higher exposure to the potentially toxic CYP2E1-metabolites, NAPQI and protein-derived acetaminophen-cysteine[18, 23]. The level of exposure to acetaminophen-glutathione, -cysteine, and -mercapturate has been claimed to be a measure for exposure to the toxic CYP2E1-metabolites, NAPQI and protein-derived acetaminophen-cysteine[23]. We emphasize, however, that in the present study the metabolite concentrations were obtained after a single dose without reaching steady state, which limits the use of exposure for the calculating the percentage that is being eliminated through a specific pathway. Next to acetaminophen-cysteine and acetaminophen-mercapturate, however, acetaminophen-glutathione was detected in 27 of the 59 preterm neonates. While

these concentrations could not be quantified within the validated range of the assay and need to be interpreted with caution, acetaminophen-glutathione was undetectable in a study in adults[31]. Undetectable acetaminophen-glutathione levels in adults may be the result of faster transformation into acetaminophen-cysteine and subsequently into acetaminophen-mercapturate. Another possible explanation is the fact that in adults a smaller proportion of acetaminophen is metabolized through the oxidative pathway. The detectable concentrations of acetaminophen-glutathione in a substantial number of neonates in the present study add up to the higher exposure of the cysteine and mercapturate metabolites when compared to adults (Figure 5). An important question therefore pertains to the safety of intravenous paracetamol in preterm infants. So far, there is only limited evidence on the safety of acetaminophen in neonates below 32

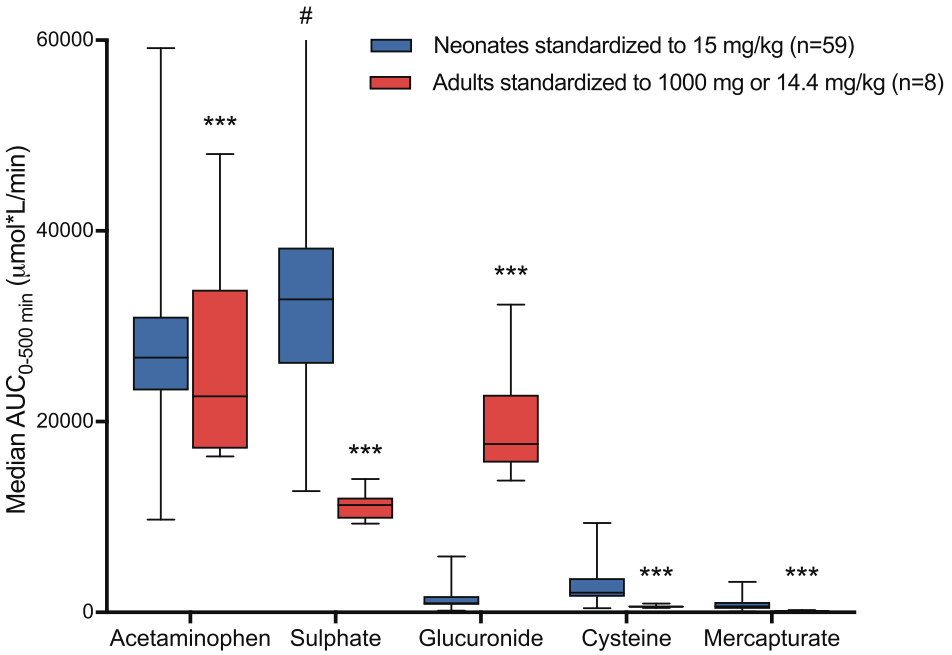

Figure 5. Boxplots of $AUC_{0\text{-}500}$ of acetaminophen and its metabolites in preterm neonates (n=59, 15 mg/kg) compared to data from adults (n=8, 1000 g or 14.4 mg/kg) (31).

The $AUC_{0\text{-}500\ min}$ of acetaminophen and all of its metabolites in preterm neonates is standardized to a dose of 15 mg/kg versus the $AUC_{0\text{-}500\ min}$ in adults standardized to a dose of 1000 mg (corresponding to 14.4 mg/kg). Acetaminophen $AUC_{0\text{-}500\ min}$ is comparable between neonates and adults (Mann Whitney test, p=0.296). In contrast, the $AUC_{0\text{-}500\ min}$ of acetaminophen-sulphate, acetaminophen-cysteine and acetaminophen-mercapturate were 2.9, 3.4 and 4.6 fold higher in neonates (Mann Whitney test, all $p<0.001$). The $AUC_{0\text{-}500\ min}$ of acetaminophen-glucuronide was 18.5 fold lower in neonates compared to adults (Mann Whitney test, $p<0.05$).

The boxes indicate the interquartile ranges, and the whiskers indicate the minimum and maximum ranges. Significance of Mann Whitney test was indicated with: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

# Indicates one AUC value of 96,468 µmol*L/min, which is the maximum value.

weeks. Prior studies on (multiple) acetaminophen dosages from 10 mg/kg to 20 mg/kg in preterm neonates up to 60 mg/kg/day for 9 days have not reported associations with hepatotoxicity[32-35]. Nevertheless, prolonged administration of acetaminophen in these vulnerable neonates may pose a problem. Van Ganzewinkel et al. however, found no depletion of glutathione in very preterm neonates after five six-hourly doses of 7.5 mg/kg acetaminophen[33]. As such, it seems too early to draw conclusions from the concentrations of cysteine and mercaptopurate measured in our study. Future research in preterm neonates should focus on the association between NAPQI protein adducts and hepatotoxicity. Advanced detection of even low grade hepatotoxicity seems important when further considering acetaminophen as analgesic for preterm neonates. Intravenous acetaminophen in a dose as high as 60 mg/kg per day has also been proposed as an off-label treatment of patent ductus arteriosus in preterm neonates[35-38]. Clearly, and particularly in those cases, its safety needs to be further studied.

Despite the strengths of this study such as the relatively large scale, the stratification into two gestational age groups and randomisation to three different dosages, the fact that only a single dose was evaluated may be a limitation. Furthermore, the last sample was drawn at 9 or 12 hours after dosing. Hence, for the AUC calculation we interpolated our data to 500 minutes after dosing. The number of blood samples per newborn was limited for ethical reasons. Sampling at later time-points would have provided more insight in the exposure to the metabolites on a longer term. More data on both PK and safety are needed on prolonged acetaminophen administration, particularly with respect to the CYP2E1 mediated metabolites. In addition, PK studies of consecutive doses above 10 mg/kg have not been performed in very preterm neonates, with even higher dosages gaining more interest with expansion of indications. However, the question is whether doses higher than 10 mg/kg are actually needed when acetaminophen is used for analgesia. To date, in the absence of more evidence, the optimal approach for providing analgesia in preterm neonates seems to aim for similar acetaminophen concentrations as in older children and adults. Using this approach, Wang and colleagues proposed for preterm neonates weighing up to 1500 grams a 12 mg/kg loading dose, with which the target concentration of 9 mg/l is immediately reached, followed by a maintenance dose of around 6 mg/kg administered 4 times daily[22]. Their estimated clearance of acetaminophen of 0.25 L/h/kg versus 0.15 L/h/kg in study by Cook et al[20]. Furthermore, it is important to realize that the concentrations of metabolites are not only determined by the fraction of the dose that is transformed into each metabolite, but also by the metabolites' volume of distribution and rate of elimination. Differences in exposure between preterm infants and adults may therefore not only be caused by an increased formation, but also by differences in volume of distribution or reduced elimimation of the metabolite in question. Another issue is the quantification of acetaminophen-glutathione, and more specifically the instability of acetaminophen-glutathione after

sample collection[27]. Hydrolysis of acetaminophen-glutathione quickly transforms it to acetaminophen-cysteine, presumably by gamma-glutamyl transpeptidase and dipeptidases. This might lead to an underestimation of the concentration glutathione at the time of sample collection, and may lead to an increased acetaminophen-cysteine concentration. For future research, addition of peptidase inhibitors during sample collection could prevent or reduce this degradation.

CONCLUSIONS

In this study we found that acetaminophen glucuronidation is low in very preterm infants and increases with gestational age, already detected from 24 to 32 weeks of gestation. Exposure to acetaminophen sulphate was high, but did not show saturation, not even after administration of 20 mg acetaminophen per kg bodyweight, which is a relevant and comforting finding for clinical practice. Compared to adults, a more than 3-fold increase in exposure to sulphate, cysteine and mercaptopurin metabolites was found, which calls for future investigation on the complete maturation of acetaminophen metabolism during infancy. Furthermore, further research is required on efficacy and safety of acetaminophen in the smallest newborns as well as in older infants, and with respect to prolonged acetaminophen dosing.

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Simulation-based suggestions to improve ibuprofen dosing for patent ductus arteriosus in preterm newborns

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ABSTRACT

Aims

Ibuprofen is the drug of choice for treatment of patent ductus arteriosus (PDA). There is accumulating evidence that current ibuprofen dosing regimens for PDA treatment are inadequate. We aimed to propose an improved dosing regimen, based on all current knowledge.

Methods

We performed a literature search on the clinical pharmacology and effectiveness of ibuprofen. (R)- and (S)-ibuprofen plasma concentration time profiles of different dosing regimens were simulated using a population pharmacokinetic model and evaluated to obtain a safe, yet likely more efficacious ibuprofen exposure.

Results

The most effective intravenous ibuprofen dosing in previous clinical trials included a first dose of 20 mg/kg followed by 10 mg/kg every 24 hours. Simulations of this dosing regimen show an (S)-ibuprofen trough concentration of 43 mg/L is reached at 48 hours, which we assumed the target through concentration. We show that this target can be reached with a first dose of 18 mg/kg, followed by 4 mg/kg every 12 hours. After 96 hours postnatal age, the dose should be increased to 5 mg/kg every 12 hours due to maturation of clearance. This twice daily dosing has the advantage over once daily dosing that an effective trough level may be maintained, while peak concentrations are substantially (22%) lower.

Conclusions

We propose to improve intermittent ibuprofen dosing regimens by starting with a high first dose followed by a twice daily maintenance dosing regimen that requires increase over time and should be continued until sufficient effect has been achieved.

INTRODUCTION

Patent Ductus Arteriosus (PDA) is a potentially very harmful condition in the youngest preterm infants, especially in those born before 28 weeks of gestation[1]. PDA has been associated with a range of adverse outcomes including chronic lung disease, necrotizing enterocolitis, intraventricular haemorrhage, and death[2-4].

Twenty years ago, Varvarigou et al. first reported about the effectiveness of COX-2 inhibition with early ibuprofen treatment in human preterm infants for PDA[5]. Subsequently, ibuprofen has been dosed and licensed as once-daily on 3 consecutive days at 10 mg/kg, 5 mg/kg, and 5 mg/kg/day via intravenous infusion. This dosing regimen leads to closure of the ductus arteriosus in only about 60% of patients[6-9]. Despite the large number of studies on ibuprofen for PDA, an optimal and widely accepted dosing regimen is still lacking. Generally, increased effectiveness has been reported using higher dosages, although the results of reports on the same dosing regimens and comparable cohorts are very divergent[10-18]. Until now, only Desfrere et al.[10] performed an ibuprofen dose-finding study starting in early neonatal life in which they found a clear difference with lower effectiveness at lower gestational age (GA), although this has not yet led to an adapted dosing regimen. Furthermore, three randomized controlled trials have shown more effectiveness for ibuprofen treatment compared with placebo[18], and for high versus low ibuprofen dosage[10, 15]. Recently, a state-of-the-art meta-analyses of randomized controlled trials comparing pharmacotherapeutic interventions by Mitra et al. in the JAMA came to the same conclusions, and besides found higher effectiveness for oral compared to intravenous administration[19]. In current practice, local interpretation of available evidence has led to a large variety of dosing regimens in clinical practice[20]. To avoid further unneccesary blood sampling in this vulnerable population, we aimed to study available data from literature, PK models and simulation to suggest improved ibuprofen doses.

In absence of sufficient evidence, the approach for an optimized ibuprofen therapy should take into account the physiological mechanism that causes active ductal constriction. After term delivery, reduced prostaglandin E2 (PGE2) levels are sensed by the PGE2 receptors (EP4) and promote further constriction of the ductus. Consequently, it is assumed that closure of a patent ductus arteriosus can be enhanced pharmacologically. Inhibition of cyclooxygenase-2 (COX-2) reduces PGE2 generation from arachidonic acid. Intravenous ibuprofen is commercially available as a racemic mixture of R- and S-ibuprofen. The (S)-enantiomer acts through competition for COX-2, followed by a reversible binding and inhibition. (R)-ibuprofen is a relatively weak inhibitor of COX-2[21]. The metabolism of ibuprofen has been shown to mature during early life[12, 22], with CYP2C8 mainly responsible for the metabolism of (S)-ibuprofen, and CYP2C9 for (R)-ibuprofen. The mean half-lives for (S)- and (R)-ibuprofen in preterm infants of about 34 hours and 8

hours at birth, respectively[23], followed by a very rapid increase of (R)-ibuprofen elimination during the first days of life. Furthermore, a spontaneous unidirectional inversion has been described of 63% of (R)- into (S)-ibuprofen in the human body[24]. For durable effect, the (S)-ibuprofen concentration at the COX-2 receptor should remain above a minimal effective concentration[25]. This is confirmed by an increased effectiveness of continuous versus intermittent treatment[13], which has also been reported for oral versus intravenous administration[9, 11, 26, 27]. It seems therefore of relevance to identify a target trough concentration for ductal closure, taking gestational and postnatal age into account. Further, peak concentrations should be minimized regarding safety and toxicity[28, 29]. Namely, the risk of developing side-effects and toxicity of non-steroidal anti-inflammatory drugs seems related to peak concentrations, as continuous administration of indomethacin showed less side-effects than an intermittent regimen[29]. In addition, the meta-analyses by Mitra et al. 2018 found that a continuous infusion of intravenous ibuprofen was associated with the lowest incidence of oliguria compared to all included intermittent dosing regimens[19].

Furthermore, the high remaining proportion of patent open ducts after 3 days of ibuprofen treamtent proves the inhibitory (S)-ibuprofen concentration are too low (or treatment was too short?). after three days depite the. Yet, if ibuprofen treatment is continued, large differences exist between neonatal intensive care units (NICUs) on whether to use the same dosage or to increase ibuprofen dosing with age, so to adjust for the increasing ibuprofen clearance due to maturation.

In this study, we combine evidence on the effect of various intravenous ibuprofen dosing regimens on PDA closure, with a previously developed population PK model. Additionally, we suggest an improved ibuprofen dosing regimen.

METHODS

Effectiveness of ibuprofen

Considering ibuprofen's mechanism of action with competitive, reversible COX-2 binding[25], we aim to maintain a minimal (S)-ibuprofen concentration for optimal effectiveness. In this simulation study, a target trough concentration (S)-ibuprofen was determined from simulations of (R)- and (S)-ibuprofen plasma concentration-time profiles following the most effective reported intravenous dosing regimen. The latter has been recently reported in a state-of-the-art meta-analyses by Mitra et al. in the JAMA incorporating all reported pharmacotherapeutic interventions for PDA closure.

Pharmacokinetic model

Published population pharmacokinetic (PK) models of ibuprofen in preterm infants were investigated using PubMed with MeSH terms: "Ibuprofen"; "pharmacokinetics"; "infant, newborn". The population PK models (see Supplementary File 1) were compared with respect to birth weight, gestational age (GA) and post natal age (PNA) of the cohort, ibuprofen dosing regimen, route of administration, and studied ibuprofen enantiomers[12, 22, 23, 30, 31].

Simulations

In order to illustrate our suggestions for dosage improvements, we simulated plasma concentration-time profiles of (R)- and (S)-ibuprofen after several intravenous dosing regimens for a typical neonate with PDA and a body weight of 840 grams, which we determined from the cohort of all preterm newborns with a significant PDA of the Erasmus Medical Center[32]. First ibuprofen dosage was administered at PNA of 24 hours as an early start has shown to be more effective than a late start.

Simulations were performed using NON-linear Mixed Effects Modeling (NONMEM, version 7.3, Globomax LLC, Ellicott City, Maryland, USA) based on Gregoire et al.[23]. For simulation of intermittent dosing regimens, ibuprofen was administered intravenously in 15 minutes. Higher infusion rates would not be recommended to avoid high peak levels and fluid overload.

We compared the population predicted trough plasma concentrations of (S)-ibuprofen at 48 hours after start of different dosing regimen, which concerned frequently reported regimens as well as new dosing proposals. Furthermore, we compared the peak concentrations as these may be related to safety and toxicity[28, 29]. For the purpose of predicting a peak concentration, a time point at 48.5 hours after start of ibuprofen therapy was chosen, as this was the latest dose that was administered in 15 minutes at 48 hours in all regimens, incorporating 15 minutes after infusion to allow drug distribution.

RESULTS

Effectiveness of ibuprofen

The most effective intravenous ibuprofen dosing regimen determined in meta-analyses by Mitra et al. consisted of a 20 mg/kg first dose, followed by additional 10 mg/kg/doses after 24 and 48 hours, all with 15 minutes infusion rates[19]. Simulation of concentration-time profiles of both ibuprofen enantiomers in a typical neonate with PDA at PNA of 24 hours and a body weight of 840 grams following this dosage regimen, predicted a corresponding (S)-ibuprofen trough concentration at 48 hours after ibuprofen start of 43 mg/L (Figure 1A). Thus, for this neonate, we hypothesized in our study that an

(S)-ibuprofen target concentration of 43 mg/L will achieve optimal effectiveness for a preterm infant with a body weight of 840 grams starting ibuprofen at 24 hours PNA.

Pharmacokinetic model

From the five reported population PK models on ibuprofen in preterm born infants (see Supplementary File 2), the model by Gregoire et al.[23] was selected, as the model was based on the largest, and best matching cohort: 108 premature infants, with a median birth weight of 880 grams (range 300-1700), median GA of 26.9 weeks (range 24.0-30.7), median PNA of 1 day at ibuprofen start (range 0-8), and described the PK of (R)- and (S)-ibuprofen adequately for intravenous ibuprofen administration of 5 to 10 mg/kg/day. Their model consisted of a one-compartment model for both (R)- and (S)-ibuprofen, with unidirectional bioconversion of (R)-ibuprofen into (S)-ibuprofen, and the effect of increasing elimination of the (R)-enantiomer with increasing PNA, being the single covariate in the model. The pharmacokinetic parameter estimates are shown in Supplementary File 3.

Simulations

Population predictions of plasma concentration-time profiles of (R)- and (S)-ibuprofen for a neonate at PNA 24 hours and bodyweight of 840 grams are shown in Figure 1 for several dosages. Table 1 gives an overview of the simulation results following different dosing regimen. This illustration allowed to compare the dosing regimen with respect to the total dose of ibuprofen administered, C_{min}, C_{max}. Comparison of Figure 1B with 1C visualizes that dividing an equal daily dose of 5 mg/kg/day from 1 into 2 administrations, increased the trough concentration from 21 to 25 mg/L at 48 hours after start, and lowered the peak concentration from 34 to 31 mg/L. The determined target

Table 1. Population predicted (S)-ibuprofen concentrations following simulation of various intravenous ibuprofen dosing regimens

	First dose (mg/kg)	MD (mg/kg)	Duration (days)	Cumulative dose (mg/kg)	Pop pred C_{trough} T48 after start (mg/L)	Pop pred C_{peak} T48.5 after start (mg/L)
A	10	5 every 24 hours	3	20	22.2	35.2
B	10	2.5 every 12 hours	3	22.5	26.5	32.9
C	20	10 every 24 hours	3	40	42.5	70.4
D	14	6.5 continuously	3	27	40.9	41.4
E	18	4 every 12 hours	3	38	43.8	54.9

Simulations for a typical neonate with PDA with body weight 840gr, and PNA of 24 hours
On day 1 the loading dose was followed by the first maintenance dose at 12 hours.
MD: maintenance dose – Pop pred: population predictions – T: time after start of ibuprofen therapy

concentration of 43 mg/L for (S)-ibuprofen was reached with a first dose of 18 mg/kg, followed by 4 mg/kg every 12 hours (8 mg/kg/day)(Figure 1D). Table 1 and Figure 1 allow a comparison of our most optimal simulated dosing with the most effective reported regimen of 20-10-10 as is known from previous clinical trials. We show that comparable trough concentrations were reached with a 5% lower cumulative 3-days dosing, and 22 % lower peak concentrations. To reach the same target (S)-ibuprofen concentration with continuous infusion, a first dose of 14 mg/kg followed by a maintenance dose of 6.5 mg/kg/day was required (Figure 1E). This regimen led to the lowest total 3-day dose, and the lowest peak concentration. Simulations upon dosing ibuprofen in neonates with a PNA of 96 hours using the racemic-ibuprofen PK-model of Hirt et al. showed that an increased maintenance dosage was required to 5 mg/kg every 12 hours (10 mg/kg/day) (Figure 1F). These simulations illustrate our proposal to start with a first dose followed by a maintenance dose in twice daily that requires increased dosing over time, and should be continued until sufficient effect has been achieved or treatment needs to be terminated for other reasons.

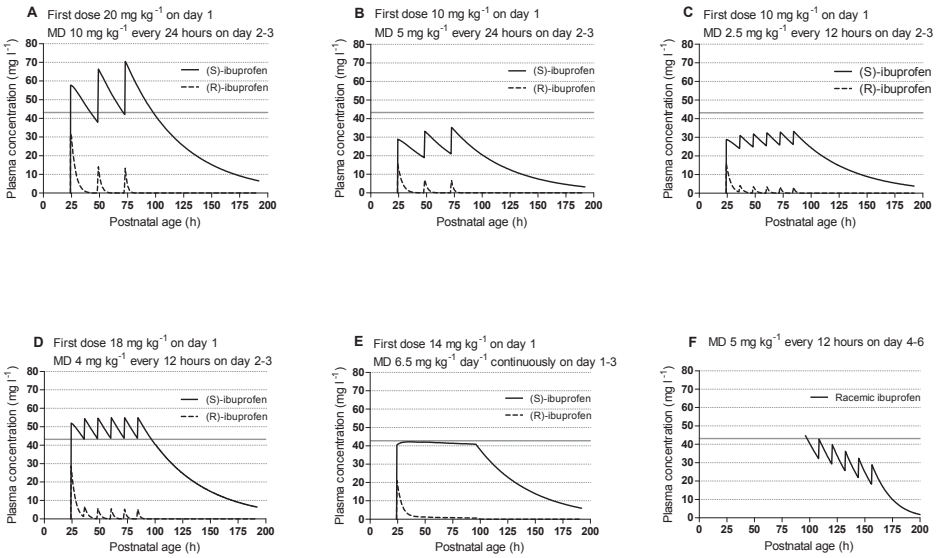

Figure 1 Simulations on population predicted plasma concentration-time profile of (R)- and (S)-ibuprofen.

Population predictions without inter-patient variability were performed for 1 typical neonate with a body-weight of 840 grams.

A grey reference line indicates the S-ibuprofen target concentration.

In figure A – E: Plasma concentrations of the separate (R) and (S)-enantiomers were simulated with the PK-model of Gregoire et al. [23] starting at a postnatal age of 24 hours.

In figure F: Plasma concentrations of racemic-ibuprofen ((R) and (S) not separately) were simulated with the PK-model of Hirt et al. [12] starting at a postnatal age of 96 hours.

Abbreviations: MD: maintenance dose

DISCUSSION

Based on reported effectiveness of ibuprofen and its mechanism of action, we suggest a first loading dose followed by a relatively high intravenous dosage, in twice daily, which is further increased with postnatal age, and continued until ductal closure has been achieved. In our simulation study that needs further validation first, we illustrate a dosage proposal for an intravenous ibuprofen dosing regimen for a typical neonate with PDA with birthweight 840 grams at PNA 24 hours, to start with a first dose of 18 mg/kg, followed by 4 mg/kg every 12 hours. Above 96 hours the dose should be increased to 5 mg/kg every 12 hours until sufficient effect has been achieved or treatment needs to be terminated due to side-effects, contra-indications, or insufficient effect. Thus, COX-2 may be sufficiently inhibited, without exposing preterm infants to unnecessarily high concentrations.

We combined all available evidence on the effectiveness of intravenous ibuprofen dosing regimens on PDA closure and on the mechanism of action, with a previously developed (R)/(S)-ibuprofen population pharmacokinetic model[23]. Herewith, we are the first proposing to maintain a certain (S)-ibuprofen target concentration. Although, the height of the target is most certainly different with gestational and postnatal age, our approach is supported by the reported *in vitro* inhibitory (S)-ibuprofen concentration for COX-2 inhibition leading to 90% reduction of the agonistic effect of PGE2 (IC_{90})[30, 33, 34]. Only the unbound (S)-ibuprofen concentration is able to have an effect on the ductus arteriosus. Aranda et al. found >99% protein binding of (S)-ibuprofen in adult blood compared to 94% in neonates[30]. Neupert et al. found an IC_{90} for unbound (S)-ibuprofen of 2.1 mg/L[34]. Assuming an unbound fraction of 6%, a total (S)-ibuprofen concentration of around 35 mg/L will achieve the IC_{90} for COX-2 inhibition. This finding is in line with our proposed target plasma concentration for (S)-ibuprofen of 43 mg/L.

We propose to divide the daily dose from one into two administrations, either for safety as well as to increase effectiveness. The risk of developing side-effects and toxicity of non-steroidal anti-inflammatory drugs seems related to peak concentrations. The meta-analyses by Mitra et al. reported less side-effects with a continuous administration than an intermittent regimen, although the Odds Ratio for oliguria of 0.07 was not found significant (0.00-1.84)[19]. Gournay et al. reported a placebo controlled trial with major side-effects of ibuprofen concerning gastrointestinal adverse effects, severe intraventricular haemorrhages caused by thrombocytopathy, necrotising enterocolitis, and renal dysfunction[18]. The former has also been illustrated by De Cock et al.[35] reporting a 16% reduced clearance of the renally eliminated amikacin due to combination with ibuprofen. Our proposed maintenance dosage of 4 mg/kg every 12 hours, leads to comparable trough concentrations with 10 mg/kg/day every 24 hours, but with 22 % lower peak concentrations. Secondly, continuous ibuprofen administration has been shown to be

more effective than intermittent by Lago et al.[13] even without a loading dose, leading to 84% vs 64% PDA closure, respectively. The higher responsiveness following a more stable ibuprofen exposure is confirmed by the counterintuitive finding of higher closure rates following oral than intravenous administration, which is thought to be caused by the more graduate absorption following oral intake[36]. The meta-analyses by Mitra et al. also concluded that oral administration is the most effective treatment, followed by high dose intermittend iv dosing regimen[19]. Nevertheless, oral administration is often not tolerated during the first postnatal days of an extremely preterm born infant. Continuous ibuprofen showed low effectiveness in the meta analyses, probably due to the absence of a loading dose and relatively low maintenance. Although, an adequately dosedcontinuous infusion preceded by a loading dose would be higly effective an intermittent regimen is preferred. A continuous infusion has multiple limitations, e.g. requiring a continuously available intravenous catheter, physiochemical incompatibility with intravenous co-medication, and increased risk for infections. All together, for an intermittent regimen we propose to divide the daily dose in two administrations which allows to safely increase the daily dose and trough plasma concentrations of (S)-ibuprofen with limited increase of peak plasma concentrations.

Since Varvarigou et al. first published on ibuprofen for PDA, most reported trials considered ibuprofen a 3-days course, which may be repeated once or twice in clinical practice and seems to improve outcome after initial failure[37-39]. Awaiting the result of echocardiography on day 4, generally, no ibuprofen is administered and often will not be restarted until the next day in case of insufficient ductal closure. This delay in ibuprofen administration leads to an undesirable drop of (S)-ibuprofen plasma concentration on the fourth day of treatment, and an unnecessary high peak concentration following the new loading dose with the start of an additional 3-days course. The success of an additional course has been shown but we considered this regimen with additional 3-days courses suboptimal and potentially unsafe. Instead, we suggest to continue ibuprofen uninterrupted, and therefore maintain the COX-2 inhibition until sufficient closure of the ductus arteriosus is achieved, or until ibuprofen treatment needs to be terminated due to side-effects, contra-indications, or insufficient effect. However, we were not able to propose a reliable dosage above 96 hours PNA using the PK-model by Gregoire, due to the absence of a covariate reflecting maturation of clearance of (S)-ibuprofen. Namely, considering the rapid maturation of (S)-ibuprofen's CYP2C9 metabolism[40], an increased clearance is highly expected and has been described by Hirt et al. in a cohort with median PNA of 69 hours[12]. In addition to the suggested improvements following a higher dose, considering the large inter-individual variability in neonates and mechanism of action, one may also argue that further dose tailoring, e.g. with TDM, may be of added value. Although this off course would first require a validated target concentration in clinical practice. Taking these findings into account, we propose to increase the daily

dosage above a PNA of 96 hours, to 5 mg/kg every 12 hours, as this is the highest, and still safe, investigated daily dose in preterm infants (Figure 1F).

Our approach provides important lessons and allows an unique dose comparison, but is limited by some assumptions we made regarding effectiveness, the target concentration, the performance of the population PK model[23], as well as the sparse knowledge on safety. The large variability in published success rates may partly be caused by maturation; i.e. increased spontaneous closure of the ductus with GA[41], or increased ibuprofen clearance with GA and PNA leading to more subtherapeutic concentrations[10, 12]. In confirmation, Desfrere et al. reported 77% ductal closure following a first dose of 10 mg/kg followed by 5 mg/kg on day 2 and 3 in patients with GA of 27-29 weeks, compared to success in 31% of neonates below 27 weeks of gestation[10]. Further, a target for ibuprofen effectiveness on PDA closure has not yet been determined in clinical trials. If such a target would be available, we would suggest Therapeutic Drug Monitoring in the individual patient. Our simulated dosages would be a good starting point, with further dose adaptations in the individual patient based on bed-side determined plasma levels in the near future. Although we considered the PK model by Gregoire et al. as the best model available for simulations at PNA of 24 hours, the model does not scale (S)-ibuprofen PK on bodyweight nor does it incorporate GA and PNA as a covariate for (S)-ibuprofen. Neither does the model allow simulations following oral administration of ibuprofen, which has been reported with remarkably higher effectiveness compared to intravenous dosing regimens; 83% versus 62%, respectively[14, 27]. Although, oral administration to an extremely preterm born infant is not possible on day 1 after birth due to feeding intolerance, it seems an attractive alternative route of administration. Furthermore, we assumed that the ibuprofen PK is linear over a 5 to 20 mg/kg/dose range, while linearity has only been explored within the 5 to 10 mg/kg range for model development. As such, we could not simulate beyond 96 hours PNA with the PK-model of Gregiore. Finally, safety has not yet been related to ibuprofen dosage and exposure.

Concluding, currently used ibuprofen dosing regimes for PDA seem suboptimal. Based on the best evidence thus far available, we suggest that if decided to treat the PDA intravenously, a high dosage should be used in twice daily, increased with postnatal age, and continued until ductal closure has been achieved. For illustration, a typical neonate with birthweight 840 grams at PNA 24 hours may start with an ibuprofen first dose of 18 mg/kg, followed by a maintenance dose of 4 mg/kg every 12 hours until 96 hours PNA. Above 96 hours a dose increase is suggested to 5 mg/kg per dose every 12 hours until sufficient effect has been achieved or treatment needs to be terminated due to side-effects, contra-indications, or insufficient effect. Thereby, sufficient inhibition of COX-2 may be achieved and maintained, without exposing preterm infants to unnecessarily high (S)-ibuprofen peak concentrations that have not been proven safe. These suggestions should be incorporated in current ibuprofen dosing regimens and require

a prospective evaluation, allowing to bridge the remaining gaps concerning treatment outcome of different administration routes, maturation of spontaneous closure, safety of ibuprofen dosing regimens, and covariates for (S)-ibuprofen PK. Finally, placebo controlled studies are warranted to characterize dynamics of natural PDA closure and to quantify drug related effectiveness.

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SUPPLEMENTARY FILES

Supplementary File 1. Pharmacokinetic models

The second best PK model for our aim was reported by Hirt et al. [1], who found a significant increase of racemic-ibuprofen clearance with increasing PNA. The major limitations were a high median PNA at start of 69 hours which makes the model unsuitable for simulations in the first days of life, and the lack of discrimination between both enantiomers. Therefore, this PK-model can only be used for a suggested dosing regimen after 96 hours. The three other PK-models were judged less suitable: Overmeire et al. [2] included a small cohort of 27 infants with a relatively high median GA of 28.6 weeks and a high median PNA of 69 hours; Aranda et al. [3] studied an even smaller cohort of 21 preterms; the cohort used for the model of Gregoire et al. 2004 [4], was also part of the model that we selected [5].

Supplementary File 2. Overview of reported ibuprofen population PK models in neonates.

	Cohort descriptives N, BW (g), GA (weeks), PNA at start (hours)	Route of administration, Dosage	Analytes, PK parameter estimates, covariates	Limitations
Aranda et al. 1997 [3]	N=21 BW mean = 945 (range 575-1450) GA mean = 26.8 (range 22-31) PNA = range 0-3	Intravenous LD: 10 mg/kg MD: 5 mg/kg/day in 1 dose	Total racemic $CL_{rac\text{-}ibu}$: 2.1 mL/kg/h $Vd_{rac\text{-}ibu}$: 62.1 mL/kg No covariates	Small cohort Total rac-ibuprofen No covariates
Overmeire et al. 2001[2]	N=27 GA mean = 28.6 (SD 1.9) BWmean = 1250 (SD 460) PNA median = 72	Intravenous LD: 10 mg/kg MD: 5 mg/kg/day in 1 dose	Total racemic $CL_{rac\text{-}ibu}$: 10.8 mL/h $Vd_{rac\text{-}ibu}$: 357 mL/kg No covariates	High PNA and GA Total ibuprofen Small cohort Standard 2-stage No covariates
Gregoire et al. 2004[4]	N=62 GA mean = 26.6 (range 24.0-27.9) BW mean = 855 (range 300-1320) PNA range = 0-6	Intravenous LD: 10 mg/kg MD: 5 mg/kg/day in 1 dose	Separate (R)/(S)-ibuprofen $CL_{(S)\text{-}ibu}$: 5.0 mL/h $CL_{(R)\text{-}ibu\ at\ birth}$: 12.7mL/h $Vd_{(S)\text{-}ibu\ \&\ (R)\text{-}ibu}$: 183 mL/kg $CL_{(R)\text{-}\ \&\ (S)\text{-}ibu}$	Smaller cohort than PK model in 2008 by Gregoire et al.
Gregoire et al. 2008 [5]	N=108 GA median = 26.9 (range 24.0-30.7) BW median = 880 (range 300-1700) PNA median = 24 (range 0-192)	Intravenous LD: 10 mg/kg MD: 5 mg/kg/day in 1 dose	Separate (R)/(S)-ibuprofen $CL_{(S)\text{-}ibu}$: 3.5 ml mL/h/kg $CL_{(R)\text{-}ibu\ at\ birth}$: 25.5 mL/h/kg $Vd_{(S)\text{-}ibu}$: 173 mL/kg $Vd_{(R)\text{-}ibu}$: 306 mL/kg Covariates: PNA on $CL_{(R)\text{-}ibu}$	No covariate for PNA on $CL_{(S)\text{-}ibu}$ Low PNA
Hirt et al. 2008 [1]	N=66 GA median = 28 (range 25-34) BW median = 1015 (range 490-1986) PNA median = 69 (range 14-262)	Intravenous LD: 10 mg/kg MD: 5 mg/kg/day in 1 dose	Total racemic $CL_{rac\text{-}ibu}$: 9.49 mL/h $Vd_{rac\text{-}ibu}$: 360 mL/kg Covariate: PNA on $CL_{rac\text{-}ibu}$	Total ibuprofen High PNA at start High GA

Abbreviations: GA: gestational age; PNA: postnatal age; BW: birth weight; SD: Standard Deviation; LD: loading dose; MD: maintenance dose; CL: Clearance; Vd: Volume of distribution; Rac: Racemic

Supplementary File 3. Pharmacokinetic parameter estimates of model Gregoire et al. 2008 [5].

	Parameter	Population mean	Interindividual variability, CV% (95% CI)
S-ibuprofen	Kel1, h^{-1} (95% CI)	0.020 (0.017-0.024)	58 (38-73)
	V1, mL/kg (95% CI)	173 (156-190)	26 (19-32)
	CL_S, mL/h/kg	3.5	
	$T_{1/2\ S}$, h	34.3	
R-ibuprofen	Kel2, h^{-1} (95% CI)	0.069 (0.046-0.093)	26 (0-38)
	Θ_{PNA}, h^{-1} per postnatal day (95% CI)	0.155 (0.133-0.177)	
	K21, h^{-1} (95% CI)	0.014 (-0.006-0.034)	
	V2, mL/kg	306 (240-372)	95 (58-121)
	$CL_{R\ at\ birth}$, mL/h/kg	25.5	
	$T_{1/2\ R\ at\ birth}$, h	8.3	

Kel1 and Kel2 indicated elimination micro-constants of (R)- and (S)-ibuprofen; V1 and V2, volumes of distribution of (R)- and (S)-ibuprofen; K21, bioconversion micro-constant from (R)- to (S)-ibuprofen; CL_R and CL_S, clearance of (R)- and (S)-ibuprofen

a. Kel2 = Kel2 at birth + Θ_{PNA}*postnatal age.

Abbreviations: CI: Confidence interval

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10

Therapeutic Drug Monitoring in Neonates: what makes them unique?

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ABSTRACT

Therapeutic drug monitoring (TDM) refers to the interpretation of quantified drug concentrations in strategically timed samples of bodily fluids, with the aim to maximize therapeutic benefit, while minimizing toxicity. In essence, TDM criteria for neonates are similar to those for adults, but specific issues should be considered. This review focusses on the relevance of these specific issues: Larger variability in pharmacokinetics (PK), and non-PK related factors, sampling opportunities, analytical techniques, therapeutic range.

Larger variability in PK, and non-PK related factors in neonates compared to adults result in a less clear relation between the administered dose and the concentration measured. Sophisticated dosing regimens derived from population PK-models can partly overcome this variability, thereby reducing the need for TDM. Dosing can be further individualized using Bayesian forecasting as a tool for TDM. Besides PK related factors, concentrations of endogenous substances (e.g. immunoglobulin A, plasma protein) in neonates differ from those in adults, which may complicate interpretation of measured drug concentrations. Blood sampling opportunities in neonates are limited by the small blood volume and the need to minimize painful procedures. Dried blood spot sampling may be less invasive. This method has been facilitated by more sensitive analytical techniques, such as chromatography followed by mass spectrometry. For the same reason, saliva is gaining attention as an alternative non-invasive bodily fluid. Lastly, reference values for therapeutic ranges of drugs in neonates are mostly adapted from adult studies, although pharmacodynamics may be quite different in neonates.

This review concludes with recommendations for future research on these specific issues.

INTRODUCTION

Neonates treated in a neonatal intensive care unit (NICU) are exposed to a large number of drugs. Generally, 15 to 20 drugs are administered to a neonate during NICU admission, although this varies by institution and depends on their underlying diseases.[1] Unfortunately, 65% of these drugs are prescribed off-label because their efficacy, dosing and safety have not yet been sufficiently established in neonates.[1-3] As a consequence, the use of these drugs needs to be optimized, for which therapeutic drug monitoring (TDM) may be a useful tool.[4] TDM of aminoglycosides has shown to decrease mortality in adults.[5] Also for neonates, the use of TDM for specific drugs has a large contribution to safely obtaining the desired clinical effects.[4, 6] Hsieh et al. summarized the most commonly administered drugs to infants in NICUs in the United States.[1] The 10 most commonly prescribed drugs are shown in Table 1. TDM is widely used for only two of these drugs, gentamicin and vancomycin.

In this review, we first describe the usefulness of TDM in general, and then focus on the specific issues in neonates that distinguish them from other populations (Figure 1 bold). These issues are: pharmacokinetic (PK) and non-PK related factors, analytical techniques, (blood) sampling or (alternative) bodily fluids, and the concept and reliability of the therapeutic range. This will be translated into recommendations for future research regarding the above described specific issues (Figure 1 *italic*).

Table 1: The most commonly prescribed drugs in neonatal intensive care units in the United States.

1.	Ampicillin
2.	**Gentamicin**
3.	Caffeine citrate
4.	**Vancomycin**
5.	Beractant
6.	Furosemide
7.	Fentanyl
8.	Dopamine
9.	Midazolam
10.	Calfactant

TDM in neonates is primarily used for the drugs presented in bold. The drug exposure ranges from 56 until 681 prescriptions per 1000 infants.
Data obtained from Hsieh et al.[1]

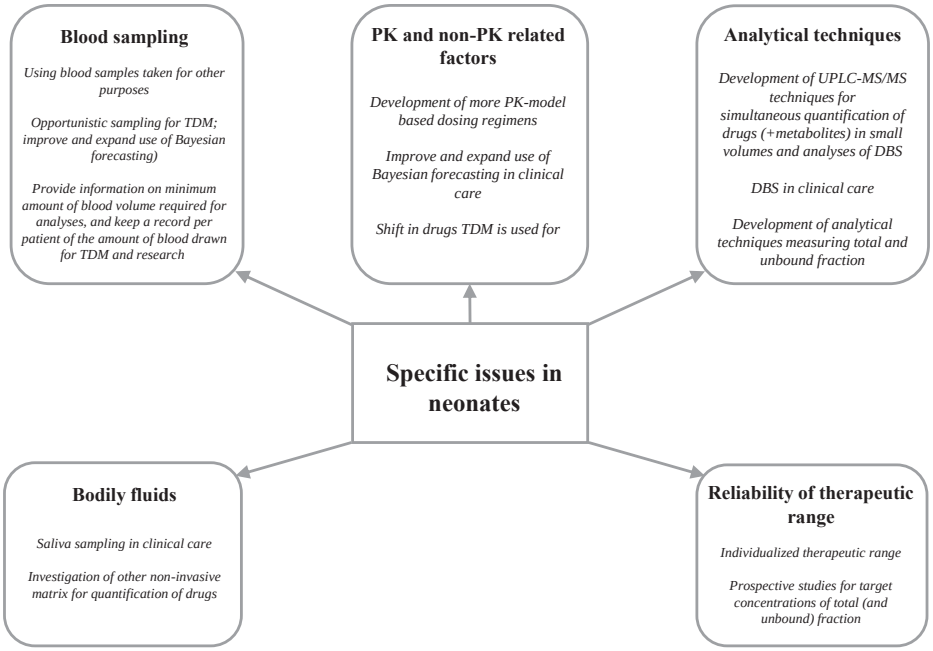


Figure 1: Specific issues in neonates that distinguish them from other populations which could influence TDM are highlighted in bold. In *italics*, recommendations for future research on these specific issues are provided.

DBS= Dried Blood Spot, PK= Pharmacokinetic, TDM = Therapeutic Drug Monitoring, UPLC-MS/MS= Ultra-Performance Liquid Chromatography- tandem Mass Spectrometry

GENERAL RULES FOR ADEQUATE THERAPEUTIC DRUG MONITORING

Different definitions of TDM have been introduced.[7] The International Association for Therapeutic Drug Monitoring and Clinical Toxicology has adopted the following definition:

"TDM is a multi-disciplinary clinical specialty aimed at improving patient care by individually adjusting the dose of drugs for which clinical experience or clinical trials have shown it improved outcome in the general or special populations. It can be based on a a priori pharmacogenetic, demographic and clinical information, and/or on a posteriori measurement of blood concentrations of drugs (pharmacokinetic monitoring) and/or biomarkers (pharmacodynamic monitoring)."

Thus, TDM aims to tailor drug dosages to individual patients, optimizing therapeutic response, while minimizing toxicity or adverse events.[8] Based on this definition, the current review focuses on *a posteriori* TDM, which is based on quantification. Nevertheless, in clinical practice, TDM may be extended to the determination of drug abuse (semi-

quantitative or qualitative process) or newborn screening, which is solely qualitative. The former methods are not included in this review. TDM is indicated primarily for drugs that possess a narrow therapeutic range (i.e. the drug concentration required for therapeutic effect is close to the toxic concentration). Furthermore, drugs need to demonstrate a good correlation between serum concentrations and pharmacologic effect, in those cases where the serum concentration is a better predictor of the desired effect than the dosage.[7] The general rules for a drug to be considered for TDM are presented in Box 1.

Box 1: General rules for a drug to be considered for TDM

General rules for a drug to be considered for TDM (4, 9-12)
1. Drugs with a narrow therapeutic range. *Example: digoxin*
2. Drugs demonstrating a good clinically interpretable correlation between drug concentration and its pharmacological effect, assuming a significant correlation between drug concentration and its concentration in the target tissue. *Example: aminoglycosides, vancomycin*
3. Drugs with extensive inter- and intra-individual variability in pharmacokinetic parameters (e.g. clearance, volume of distribution), for which drug concentrations are generally unpredictable. *Example: vancomycin, phenytoin*
4. The pharmacologic effect of drugs is not easily measurable. *Example: antibiotics*
5. A quick method for quantification of the drug is available, specific, precise and accurate in the bodily fluids of the (neonatal) population, as well as cost effective, and takes account of specific issues of the samples. *Example: inter-assay variability for vancomycin with different immunoassays*

TDM= Therapeutic Drug Monitoring

According to these rules, TDM is not indicated for eight of the top 10 drugs listed in Table 1. For example, fentanyl can be titrated based on individual pain scores, midazolam on scores for sedation, and dopamine on blood pressure. Caffeine dosages used to be guided by TDM, until was found that the majority of preterm infants treated with regular caffeine dosages, attain plasma caffeine concentrations within therapeutic range.[13] On the other hand, TDM can also contribute when a patient does not wake up, despite termination of the midazolam infusion. If concentrations of midazolam and active metabolite are still in the range where sedative effect can be expected, this may help to explain the patient's condition.

Perceptions on the potential benefit of TDM may change over time with increasing knowledge (as has been mentioned for caffeine), or can be divergent. An example of the former is lidocaine used for treatment of neonatal convulsions. TDM has been suggested to be useful because of lidocaine's narrow therapeutic range and large inter-individual variability[14], as well as its value to evaluate the effectiveness (e.g. subtherapeutic concentrations leading to persistent seizures) and safety (e.g. cardiotoxicity).[15, 16] Lidocaine

has both anticonvulsive and anti-arrhythmic properties. Figure 2a illustrates lidocaine metabolism into monoethylglycylxylidide (MEGX), which in turn can be further metabolized into glycylxylidide (GX).[14, 15] Both metabolites are renally eliminated. Fortunately, cardiac side effects only occur at higher lidocaine plasma concentrations (> 9 mg/L) than required to treat seizures (6-7 mg/L) (Figure 2b). (14, 15) In addition, MEGX can also contribute to clinical cardiac toxicity (plasma concentration unknown) and even seizures.[14, 15]

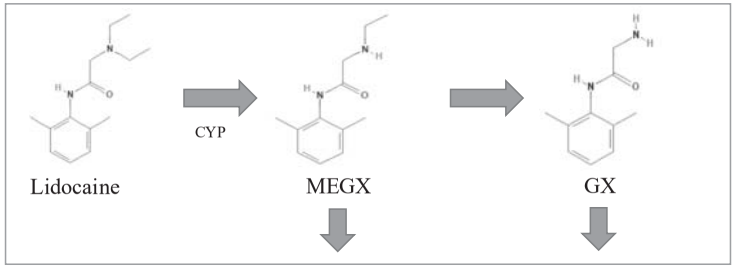
Box 2: Situations when TDM is currently not indicated

Reasons for not using TDM (4, 9-11)
1. Drugs with a large therapeutic range. *Example: penicillin*
2. The value of TDM is limited, as more convenient methods for assessing the effects are present or clinicians can titrate dosage based on available outcome variables. *Example: pain scores, level of sedation or blood pressure*
3. Unknown or incorrect information on dosage, administration, time of sample collection, assay validity.
4. Clinical outcome (therapeutic/toxic effects) is only weakly correlated to either dose or concentration. *Example: penicillin/SSRI*

SSRI= serotonin reuptake inhibitor, TDM= Therapeutic Drug Monitoring

Figure 2c illustrates why TDM, when applying the general rules (Box 1) is not indicated for lidocaine. First, its therapeutic range is unknown, since the plasma concentration range of 6-7 mg/L for convulsive effect and the concentration above 9 mg/L for cardiac toxicity are based on animal data (rule 1 in Box 2). Furthermore, the range of toxicity of the active metabolite MEGX is unknown.[14] Moreover, plasma concentrations of lidocaine and its active metabolite have not yet been correlated with the anti-convulsive effect (rule 2 in Box 2).[17] A dosing regimen based on a population-PK model for both term and preterm neonates (dosage not yet prospectively validated) diminishes inter- and intra-individual differences (rule 3 in Box 2). This PK-model, with bodyweight as a covariate on both clearance and volume of distribution, was developed to provide a plasma concentration between 6-7 mg/L. In 2.4% of all neonates plasma concentrations were above 9 mg/L[15] without observed toxicity. Lidocaine was also studied in neonates undergoing hypothermia, and showed a reduced clearance, which would require an altered dosage. Nevertheless, clinical monitoring is more indicated than TDM.[18] Therefore, in our opinion, TDM is not necessary when lidocaine is used to treat convulsions in neonates.

In clinical practice, TDM may be used for a variety of purposes. Primarily, TDM serves to optimize individual therapy by maximizing drug effectiveness and minimizing its adverse effects.[22] Furthermore, TDM can help to monitor and detect drug interactions,



CYP= cytochrome-P-450, MEGX= monoethylglycylxylidide, GX= glycylxylidide
Structural formulas are obtained from PubChem. (19-21)

Figure 2b: Therapeutic window for lidocaine

Ranges based on adult data or experience.(14-16)

Figure 2c: General rules applied for lidocaine for neonatal convulsions

General rules for a drug to be considered for TDM	General rules for TDM related to lidocaine
1. Drugs with a narrow therapeutic range.	1. Lidocaine and MEGX: therapeutic range unknown in neonates. (15)
2. Drugs demonstrating a good clinical interpretable correlation between drug concentration and its pharmacological effect.	2. No correlation between plasma concentrations of lidocaine and its active metabolites and the anticonvulsant effect. (17)
3. Because of extensive inter- and intra-individual differences in pharmacokinetic parameters, concentrations following a dose administration are often unpredictable	3. Decreased variability due to development of population-PK model (variability explained through combined power estimate of bodyweight on clearance and volume of distribution)
4. The pharmacologic effect of drugs is not easily measurable.	4. Measurable with cEEG, aEEG, cardiac monitoring and clinical symptoms.
5. The method for analysis of the drug is quick, specific, precise and accurate in the (neonatal) population, is cost effective, and takes account of the characteristics of the samples.	5. Easy and fast UPLC-MS/MS method for determination of lidocaine and its active metabolite (MEGX) is developed, especially for neonates (sample volume 10 µl). (14)

Figure 2: Reflections on the use of TDM for lidocaine when used for neonatal convulsions (14-17, 19-21)

to determine the impact of co-medication, to diagnose underexposure (inadequate response), to avoid or confirm toxicity, and to evaluate the effect of changes in clinical condition (e.g. albumin concentration, liver or kidney function).
The above-mentioned criteria for TDM in neonates are the same as those for adults, but several specific issues must be taken into account when it concerns neonates. PK and non-PK related factors may be different (Figure 3), and have been extensively discussed in other reviews[23-29]. Therefore, we will only briefly address this, and mainly focus on the relation between large variability, population PK-modelling, Bayesian forecasting and the (remaining) role of TDM (3.1). This will be followed by a reflection on analytical techniques (3.2), blood sampling (3.3), the use of bodily fluids beyond plasma or serum (3.4), and aspects regarding the therapeutic range (3.5) with specific emphasis on neonates.

SPECIFIC ISSUES IN NEONATES

Pharmacokinetic and non-pharmacokinetic related factors

Pharmacokinetic factors in neonates

PK covariates can be subdivided in maturation-related, and non-maturation-related factors (Figure 3), since both contribute to PK variability in neonates.[28, 29] However, it is not always possible to distinguish between both. For example, genetic polymorphisms are commonly considered to be non-maturation-related factors. Still, the relevance of polymorphisms in the cytochrome-P-450 (CYP) enzyme activity evolves throughout the maturation process.[30] Neonates undergo major and rapid maturational, as well as physiological changes in drug absorption, distribution, metabolism and excretion.[27] These changes result in more extensive inter- and intra-individual variability in PK in this group than in adults, and consequently, in a larger variability in drug disposition.[29]
For example, the changes in PK parameter estimates of vancomycin due to maturation, require dosage adjustments with age.[31] Therefore, the Dutch Children's Formulary suggests a dose of 20 mg/kg/day given in two doses for preterm neonates below 1 week of age and a birthweight below 2.5 kg; 30 mg/kg/day given in three doses for preterm neonates 1-4 weeks of age and a birthweight below 2.5 kg; 32 mg/kg/day given in four doses for term neonates below 1 week of age and birthweight greater than or equal to 2.5 kg; and 48 mg/kg/day given in 4 doses for term neonates 1-4 weeks of age.[32]
Non-developmental factors also contribute to this PK variability, such as genetic polymorphisms (e.g. CYP2D6 and tramadol), environmental factors (e.g. co-medication), treatment modalities (extracorporeal membrane oxygenation (ECMO), cardiopulmonary bypass, hypothermia) and disease characteristics (patent ductus arteriosus, asphyxia).[4]
For example, changes in PK parameters may considerably differ for the situations when

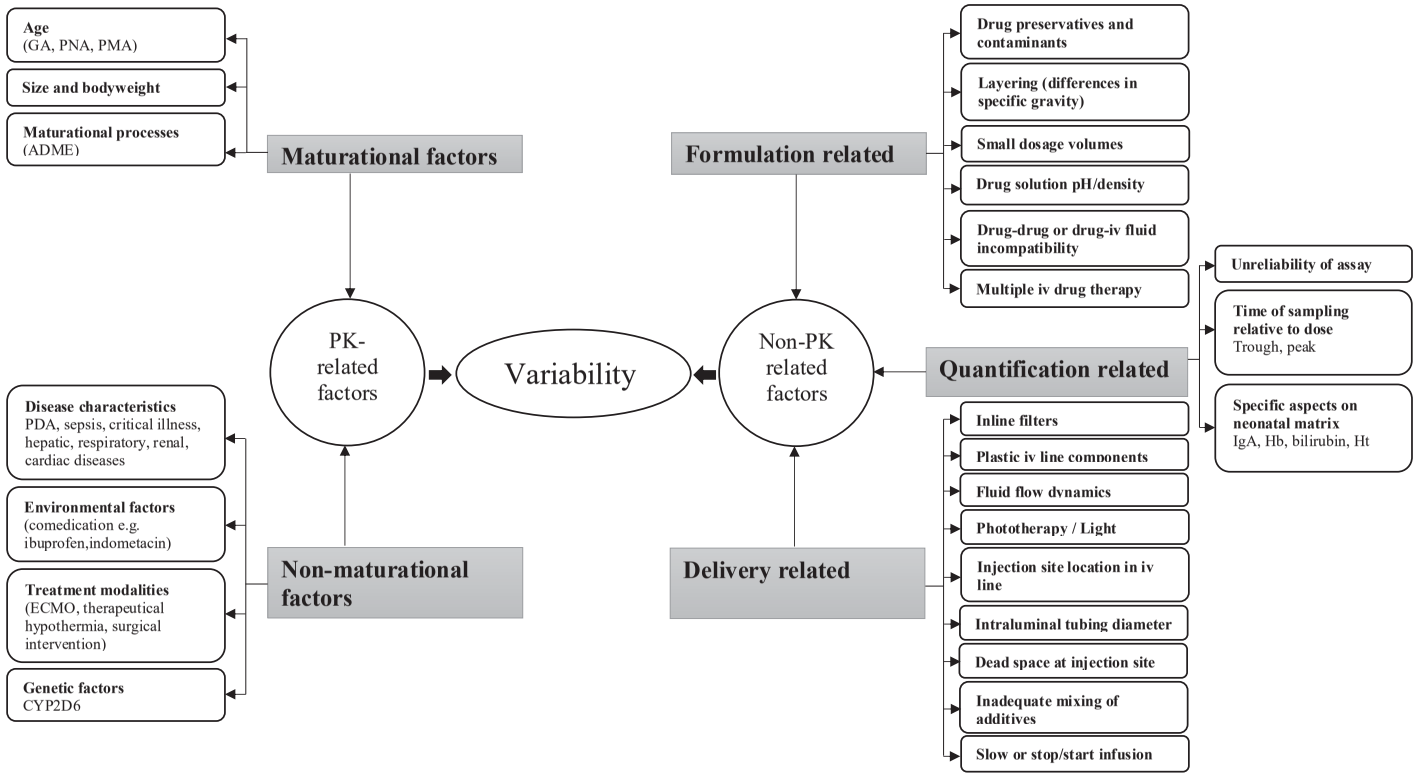


Figure 3: Variability in neonates due to PK (maturational and non-maturational factors) related factors and non-PK (formulation, quantification, delivery) related factors, with specific emphasis on the intravenous route of administration. (4, 24-26, 28)

ADME= Absorption, Distribution, Metabolism, Elimination, CYP= Cytochrome-P-450, ECMO= extracorporeal membrane oxygenation, GA= gestational age, Hb= Hemoglobin, Ht= Hematocrit, IgA = Immunoglobulin A, iv= intravenous, PDA= Patent Ductus Arteriosus, PK= pharmacokinetic, PMA= postmenstrual age, PNA = postnatal age.

the ductus arteriosus is open or closed, as a study has shown that the clearance of ibuprofen was significantly higher after closure of the ductus arteriosus.[33]

Non-PK related factors in neonates

Not only PK related factors make neonates a unique population, but also non-PK related factors contribute to variability (Figure 3). Non-PK factors can be subdivided in formulation, delivery, or quantification related issues. These include errors in drug administration, slow intravenous flow rates, uncertainties on the time of blood sampling related to dose, and unreliability of assays (Figure 3).[26, 34] Evantually, these may all lead to potential problems in the smallest infants[26], and may be of crucial concern for drugs with small therapeutic ranges (e.g. gentamicin).[24] Challenges associated with formulation and delivery are not limited to intravenous formulations, but have also been suggested for other routes of administration.[25] For example, the optimal particle size for inhalation differs between neonates and adults: below 2.4 µm versus 3-4 µm, respectively.[35]

Population-PK modelling and Bayesian forecasting

The large inter- and intra-individual variability in PK and non-PK related factors described earlier, results in a poor relation between the dose administered and the concentration achieved in neonates.[29, 36] Therefore, TDM in neonates is even more relevant. However, this limited predictability and larger variability can be partly overcome by the use of more complex validated dosing regimens derived from population PK models.[37] As illustrated in Figure 4, population PK-models with covariates are based on drug concentrations (obtained from TDM of a drug, randomly or strategically sampled) and patient characteristics (Figure 4a). Covariates may be defined to partly explain the between-subject variability; e.g. size, current weight, birthweight, gestational age, postnatal age, co-administration of drugs, genetic polymorphisms, growth restriction, and disease characteristics (patent ductus arteriosus, critical illness). These covariates can be encorporated in dosing regimens, and may reduce (large) unexplained variability. Consequently, treatment according to a dosing guideline obtained from a population PK-model, will result in a larger proportion of patients with a plasma concentration in the therapeutic range (Figure 4c). Thus, for an individual patient this obtained dosing regimen can reduce required dosage adjustements following TDM (Figure 4d). Finally, success of the suggested dosage based on the model, needs to be evaluated by analysing the achievement of the target (Figure 4e). Best practie is to externally validate this suggested dosage. However, as not all variability of neonates can be explained by covariates in a population PK-model, important and unexplained variability will remain.[4, 11] Therefore, the use of TDM can be reduced once dosing regimens derived from robust models have been developed and validated, but TDM will still be needed to explain part of the remaining unexplained variability or be valuable for specific subgroups (ECMO,

renal or liver impairment, birth asphyxia).[4, 11] Following the steps in Figure 4, dosing regimens for amikacin were developed and validated using population-PK modelling.[38] At steady state, peak concentrations were reached in almost all neonates and trough concentrations were reached in 45-96% of the neonates, depending on their clinical characteristics (age and weight).[38] After this exercise, the question arises if it is still clinically relevant to systematically perform TDM in all neonates.

The next step to further individualize drug therapy is the Bayesian forecasting approach, which is the most advanced TDM application of population-PK.[39] As shown in Figure 4, Bayesian forecasting is based on combining prior PK knowledge of a drug, with individual patient characteristics. Therefore, an a priori developed population PK-model

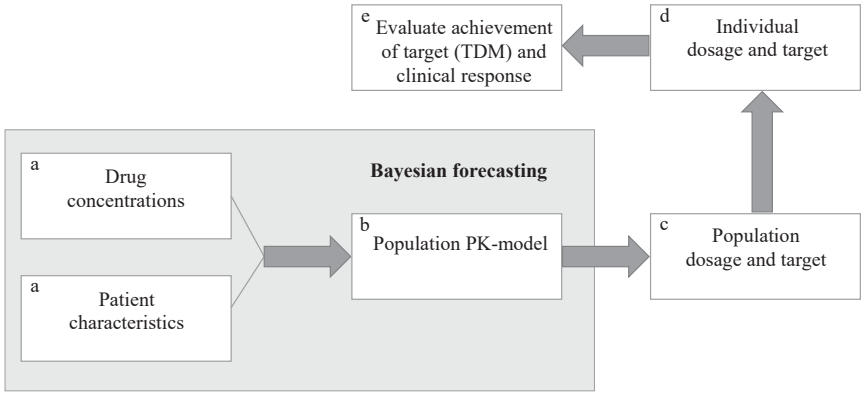


Figure 4: Flow chart of the target-oriented model-based dosing strategy.

with PK parameter estimates and inter-individual variability, is combined with individual patient data; e.g. drug concentrations collected for TDM, gestational age, postnatal age, weight, or renal function.[39] In this way, individual PK parameter estimates can be generated for an individual patient, leading to a tailored dosage adjustment for the individual patient to achieve the target concentration. A major advantage of the Bayesian forecasting approach is that only few samples per individual are needed for these estimations, instead of collecting complete concentration-time profiles.[40] Thus, more accurate dosing can be obtained, while reducing blood sampling. After implementation of a population-PK model and Bayesian forecasting for a drug, TDM can be further optimized, and may eventually be reduced. Still, for some drugs, such as vancomycin, achievement of the target must be closely monitored in the first weeks of life, due to the neonate's fast maturation process. Although Bayesian forecasting looks promising for drugs with an available population PK model, its role needs to be seen in perspective with clinical practice. Moreover, this accounts for the broad spectrum of clinical conditions of neonates, which are not yet sufficiently included in most PK models.[39]

Analytical techniques

The most commonly used techniques for TDM measurements are immunoassays and chromatography combined with mass spectrometry or ultraviolet detection. They both have their advantages, limitations, sensitivities and specificities. Immunoassays are mostly used in clinical practice as this technique is easy to perform, requires a simple sample preparation, run times are short, and various drugs can be measured in random order.[41] On the other hand, the assay may lack specificity when both the parent drug and metabolites need to be measured, as immunoassay antibodies often cross-react with these metabolites.[42] If a metabolite binds to an antibody instead of to the active parent drug, this binding can lead to a falsely increased measured concentration of the active drug, and consequently to over-estimated values compared to chromatography.[42] Furthermore, cross-reaction can arise with drugs resembling the quantified drug[42] or with endogenous substances.[43] A typical cross-reaction in neonates is the interaction between digoxin and endogenous *digoxin*-like substance (EDLS).[10, 42] EDLS can be found in neonatal serum and diminishes with gestational age. EDLS can interfere with the measured digoxin concentration and consequently with the clinical interpretation of digoxin levels. Even neonates not treated with digoxin or not exposed to it in utero, may have measurable concentrations of EDLS, resulting in falsely positive measured concentrations up to the therapeutic range for digoxin. Most assays lack the specificity to completely distinguish EDLS from digoxin.[44] A possible relative resistance to digoxin toxicity has been reported for neonates, but a cross-reaction with EDLS may at least in part explain this 'resistance'.[44, 45] Similar, falsely elevated digoxin concentrations may lead to inadverted digoxin dose adjustments.[44, 45] The label of the kit in general does not provide corrections for cross-reactions as the degree of variability is unknown. Variability depends, for example, on the concentration of the interacting component (in this case EDLS). This problem can be solved by using a more specific immunoassay, such as fluorescence polarization immunoassay, or a chromatographic technique to separate the components prior to detection.[45] Clinicians and pharmacologists should be aware of these cross-reactions and how these affect interpretation of the result for clinical practice.

Ultra-Performance Liquid Chromatography-tandem in combination with Mass Spectrometry (UPLC-MS/MS), ultraviolet (UV) or diode-array detector are newer techniques with chromatographic separation prior to detection.[46] These techniques are more sensitive and specific than immunoassays.[47] The high sensitivity enables measurement of extremely low concentrations in small volumes, which is favorable in neonates.[47] Still, UPLC-MS/MS assays have not yet been developed and standardized for all drugs that require TDM, certainly not for use in small sample volumes. Additionally, the use of UPLC-MS/MS for TDM is less flexible than immunoassays.

Furthermore, interpretation of results could be complicated by the fact that neonatal plasma or serum contains population specific concentrations of endogenous substances which may interact with the drug or the assay (e.g. Immunoglobulin A (IgA), plasma proteins, hemoglobin, hematocrit, bilirubin, lipids).[11] For illustration, IgA concentrations are lower in children[48], while a negative correlation between IgA and the unbound vancomycin plasma concentration has been described.[49] As only the unbound fraction of a drug can exert an effect, this could influence the length of time during which unbound vancomycin is above the minimum inhibitory concentration (MIC).[49] This may apply even more to neonates, considering their relatively low IgA levels.

Similar, neonates have lower plasma protein concentrations, e.g. albumin, and thus a higher unbound fraction of highly protein bound drugs[27, 36]. Oyaert et al. found a higher median unbound vancomycin concentration in children compared to adults, likely explained by lower plasma protein concentrations in children.[49] Theoretically, as neonates have even lower plasma protein concentrations, these findings can likely be extrapolated to neonates. In addition, endogenous substances such as hemoglobin, bilirubin or free fatty acids are also more abundant in neonates, and may thereby influence the interpretation of the results as well.[11] Generally, developed drug assays are not capable of measuring the unbound drug concentration. Although technically possible, the relevance of the unbound concentration-effect relation is mostly unknown at time of assay development. Additionally, extra sample volume is required to distinquish between the unbound and the bound fraction. Finally, in the example of vancomycin, concentrations in neonates may depend on the assay used. Vancomycin is converted to vancomycin crystalline degeneration products, subsequent eliminated by the renal route. Impaired renal function in neonates results in more pronounced accumulation, while the assay displays cross reaction between vancomycin and its degeneration products. To further complicate the setting, a conversion factor for vancomycin has not yet been established because of lack of information on accumulation and formation of vancomycin crystalline degeneration products, and cross-reaction varies among different assays.[12]

Blood sampling

In neonatal care, blood sampling is a balance between risk and benefit for the individual infant. Currently, a heel puncture is a routine, although this is an invasive and painful method for the collection of plasma or serum required for TDM.[34] An ongoing point of discussion is the maximum amount of blood that can be collected from neonates. Their low circulating blood volume (85 ml/kg, which peaks to 105 mL/kg by the end of one month of age) makes them more sensitive to iatrogenic blood loss, and consequently to blood transfusions and anemia.[34, 50, 51] Still, in very low birthweight infants up to 2.3 mL/kg (corresponding with 2.4% of blood volume) could be obtained without influencing basic hemodynamic parameters (hemoglobin, hematocrit), transfusions,

and fluid requirements.[52] Furthermore, as hematocrit is higher in neonates than in older children and adults, more blood must be drawn to obtain a similar volume of plasma or serum. Hematocrit, however, is still not taken into account as a factor influencing blood sampling.[53] Currently, the volume of blood which is drawn depends on the drug assay; therefore a structured approach is necessary in order to protect neonates against unnecessary burden. Guidelines on blood sampling in neonates for clinical care are lacking, but recommendations have been issued for clinical trials.[51, 54] Howie et *al*. reported a wide range in the allowed amount of blood volume drawn from neonates for research purposes, which illustrates the large differences in acceptable burden of blood sampling. This ranged from 1 up to 5% of the total blood volume (TBV) over 24 hours, up to 10% of TBV over 8 weeks.[51] Lower limits of 3 mL/kg within 24 hours (3.8% of TBV) were recommended for sick children. In addition, special caution is needed in cases of anaemia, blood volume depletion, or prematurity.[51] The European Medicines Agency (EMA) suggests not to exceed a blood loss per individual of 3% of TBV during a four weeks' period, and not more than 1% per moment of sampling.[54] The Food and Drug Administration (FDA) states that the TBV which is drawn may not exceed 50 mL or 3 mL/kg in an 8 weeks' period and collection may not take place more than 2 times per week.[55] In the end, it is best to keep blood sampling volume to a minimum, and to draw blood from an indwelling line if present.

In general, recent technical developments have enabled the use of smaller blood volume for quantification of drugs, thereby reducing the risk of anemia and blood transfusions. Micro-analytical methods, developed for e.g. paracetamol and lidocaine, allow measuring drug concentrations in less than 20 µL of blood.[14, 47] Furthermore, new techniques can simultaneously quantify multiple drugs in one run requiring small volumes, for example amikacin, gentamicin and vancomycin in 25 µL of plasma.[56]

Dried blood spot (DBS) sampling is an alternative collection technique in which, for neonatal care, a sample of blood obtained by heel prick is applied onto a special DBS-paper card.[57] In neonates, this was first used for the screening of phenylketonuria. DBS has developed in a quantative manner over the last decade.[58] DBS is a minimally invasive procedure for which at least 50-75µL blood is needed.[58, 59] Therefore, DBS could be a suitable substitute for repeated venous sampling for patients at home, or if no catheter is in situ.[59, 60] The limited amount of blood calls for more sensitive techniques. Consequently, UPLC-UV is in most cases not suitable.[60] Furthermore, proper training is needed to prevent errors like improper placement of the blood drop on the card and variation in blood spot size.[61] A spotting device technique is needed to avoid that the first drop has a large amount of interstitial fluid.[60] Clotting, supersaturating, hemolysis, layering, contamination and insufficient volume may all lead to a sample that is not fit for analysis.[60, 61]

The most important factor that affects DBS is the concentration of hematocrit in blood,[58] which influences the distribution of blood on the paper card[62, 63]. This could have an

impact on the validity of the DBS results, e.g. drying time, homogeneity, spot formation, robustness and reproducibility of assays.[58, 60, 61] Therefore, correction is required for the hematocrit concentration.[64] This is of particular concern in neonates, who have higher hematocrit levels compared to other age groups, and show a large variability in hemocrit.[65] Hematocrit increases by 0.64% with every week of gestational age.[53] As higher hematocrit level disturbs the diffusion of blood, measured concentrations in neonates may be overestimated compared to those in older age groups.[62, 63] As DBS analysis requires a large volume per punch, methods have been developed to minimize the influence of hematocrit.[58, 60, 62, 63] Another disadvantage is the fact that capillary blood in DBS is a mixture of capillary, arterial and venous blood, and intracellular and interstitial fluid.[60] This is essentially different from serum and plasma; therefore venous blood and DBS may show different drug concentrations. As therapeutic ranges are generally defined in serum or plasma, additional clinical validation is required to correlate whole blood to plasma concentrations, followed by a defined conversion factor between plasma and dried blood spot concentrations.[58, 60] At present, DBS analyses for TDM have only been reported in clinical trial settings (e.g. PK studies of metronidazole based on DBS in neonates[66]), but not yet for neonatal clinical care.

Bodily fluids: Saliva as an alternative fluid for TDM

As mentioned above, TDM is a quantitative process, based on the measured concentration of the drug in a specific bodily fluid, usually serum or plasma. As blood sampling has disadvantages such as its painful nature, and risk of anemia and infections, attention has shifted to alternative bodily fluids, such as saliva.[41, 67]

The rationale of measuring concentrations of drugs in saliva is based on the fact that pharmacological effect depends on the drug fraction in plasma that is not bound to proteins.[68] This fraction is capable of binding to the receptor and performing its action.[67] As it is only this unbound fraction that reaches the saliva, the concentration in the oral fluid is thought to directly reflect the unbound drug concentration in plasma.[68] In addition, saliva samples are easy to obtain with a non-invasive procedure, which is advantageous for application in neonates.[69]

Saliva sampling has gained acceptance in PK and PD research, but clinical use is still limited. The main reason for the limited use is the poor correlation between saliva and plasma concentrations,[68] although caffeine is an exception.[70, 71] The Dutch TDM monographs state that, for clinical care, caffeine can be quantified in saliva. It should be noted, however, that the concentration in saliva is approximately 70% of serum concentration.[72] The therapeutic range in blood (10-20 mg/L) cannot be equated to that of saliva, and the latter should always be corrected.[70] In contrast, lithium is detected in much higher concentrations in saliva than in plasma because of ion trapping.[68]

Not all drugs reach the saliva, and for these no correlation can be determined between saliva and plasma. To reach the saliva, drugs must be non-ionized (in the neutral pH range of saliva), lipid soluble and predominantly unbound.[69] Still many other factors determine whether a good correlation can be found between saliva and blood, such as salivary flow rate, pH of saliva and plasma, pKa/pKb, molecular weight, and lipid solubility of the drug.[68] Furthermore, the drug should have little influence on the pH and saliva flow, and should remain stable in fluid as well as its potential metabolites.[68, 69] In neonates, the small volume can be a barrier to use saliva for TDM.[70] The saliva flow is often stimulated with citric acid, but this can influence the properties of saliva and the drug concentration in the collected saliva sample.

In summary, although not all drugs can be quantified in saliva, this may be a good alternative for qualitative analysis, for example for detection whether neonates have been exposed to illicit drugs such as cocaine, cannabinoids, cotinine–metabolite or nicotine.[41, 73, 74]

Therapeutic range

The therapeutic range concept

A drug's therapeutic range is the range of concentrations associated with efficacy and a low risk of dose-related toxicity in the majority of patients. Serum concentrations above the therapeutic range are associated with an increased probability of adverse events, while serum concentrations below the therapeutic range are associated with increased probability of unsatisfactory clinical response.[42] In general, the difference between the therapeutic range and the concentration at which toxic effects occur, may be quite large. Note however, that concentrations at which toxic effects occur are mostly under-documented.

For most drugs the therapeutic range is expressed in steady-state concentration (Css), which is usually only achieved after 4 to 5 elimination half-lives of the drug. In neonates, the elimination half-life may be prolonged because of the combination of lower clearance and higher volume of distribution. Then it takes longer to reach Css, and due to the maturational changes in PK in neonates it is hard to predict when the Css is reached, and if this concentration will be in the therapeutic range. A dose adjustment based on a sample that is collected before Css has been reached, can consequently result in an inadverted adaptation of the dose. Phenobarbital is an example of a drug for which Css in neonates is reached much later than in adults. The elimination half-life of phenobarbital in neonates is between 40-440 hours, in adults between 48-144 hours.[75] However, during the first weeks of life, clearance rapidly increases, and the elimination half-life in neonates shortens due to hepatic enzyme maturation or enzyme induction.[76] In order to

quickly reach Css for phenobarbital, neonates require a loading dose[77]. Similar patterns have been described for vancomycin.[9]

Reliability of the therapeutic range in neonates

TDM of a drug is only useful if reference values of the therapeutic range of that drug are known. Reference values in neonates are mostly derived from adult studies, despite the fact that effects in neonates may be quite different than in adults.[42] For example, the target for effectiveness of vancomycine is validated for adults with *S. Aureus* pneumonia: $AUC_{0\text{-}24h}$/MIC > 400.[78] Despite the protein bound fraction in infants is much lower than the adult fraction of 90%,[49] the target of 400 is currently aimed for in both populations. As only the unbound fraction has pharmacological activity, this could lead to a much higher unbound fraction in infants than required. This may be even more applicable to neonates, considering their plasma protein concentration is lower than in infants.[27]

The validation of a therapeutic range is often limited to a given indication, whereas this could be different for a drug with multiple indications. For example, the target plasma concentration for lidocaine is higher for its anti-arrhythmic than for its anti-convulsive effect.[14, 15] Similar, the target plasma concentration of vancomycin is validated in adults for the treatment of *S. Aureus* pneumonia, an infection that occurs more often in adults than in neonates.[7] *S. epidermis* bacteraemia occurs more often in neonates and is likely to have a different MIC, which could lead to a different target plasma concentration of vancomycin in neonates.

FUTURE RESEARCH RECOMMENDATIONS

This review highlights the possibilities and limitations of TDM in neonates, addressing the specific PK and non-PK related factors of neonates that may lead to large variability, as well as analytical techniques, sampling, bodily fluids, and the concept and reliability of the therapeutic range. In this section, we provide suggestions for future research on these issues (Figure 1 *italic*).

Neonates have a *larger PK variability* compared to adults. This larger variability can in part be overcome by more complex validated dosing regimens derived from population PK models. Once these have been developed, the use of TDM may be altered.[11] Validated dosing regimens are not yet available for all drugs for which TDM is currently used. Not all developed PK-models have been implemented in clinical care[79, 80], although they are essential for Bayesian forecasting. Bayesian forecasting can be used to individualize dosing regimens for each individual patient, taking into account the concentrations obtained and the individual patient characteristics (covariates). To further individualize dosing regimens and thereby optimize the use of TDM, it is necessary to search beyond weight

and age as covariates in population PK-models in neonates. Factors such as small for gestational age, hydrops, infection (sepsis), critical illness, and genetic polymorphisms, should also be investigated as potential covariates.

Another way to optimize, and possibly reduce, the use of TDM is to improve the parameters used to evaluate effectiveness and safety. Therefore, besides PK modelling, the pharmacodynamics (PD) should be better incorporated.

The question arises if there is a need to select different drugs for TDM. For example, caffeine is a substance extensively used as first-line treatment for management of apnea of prematurity.[81] Until a decade ago, TDM was extensively applied for this substance because of fear for cardiac toxicity.[82] However, there has been a shift in the use of TDM of caffeine, because of the absence of a relation between concentration and effect.[13] Furthermore, caffeine has a large therapeutic window (10-20 mg/L). It was even reported that with concentrations > 50mg/L, no adverse events were observed.[83] Thus, the majority (95%), including extreme preterm neonates with decreased clearance, had achieved concentrations within the therapeutic range with the standard dosage regimen.[13] Those findings lead to the conclusion that TDM is no longer indicated for caffeine treatment in neonates (Box 2). In general, the number of drugs requiring TDM is still increasing. These drugs include for example the newer antiepileptic and antifungal drugs. Concerning antiepileptic drugs, TDM has extensively been used for older drugs like phenytoin or phenobarbital. However, for the newer antiepileptic drugs, routinely monitoring plasma concentrations is generally not recommended in adults because of incomplete data on a concentration-effect relationship.[84, 85] So far, target concentrations are neither known for these newer antiepileptic drugs used in neonates. Focussing on adults, TDM of newer antiepileptic drugs with a large inter-individual variability (such as lamotrigine, felbamate, oxcarbazepine) could be beneficial.[86, 87] Due to the additional maturational changes in neonates, PK variability is expected to be larger, and TDM could be an even bigger contribution for the above mentioned drugs. For example, lamotrigine is mostly metabolised through glucuronidation[85, 86], while glucuronidation capacity is lower in neonates and develops with postnatal age.[88] So, extensive PK variability is very likely in neonates and TDM can play an important role to improve pharmacotherapy. For another newer antiepileptic drug like levetiracetam, the anticipated PK variability seems more predictable as it is primarily eliminated through the kidneys, and glomerular filtration rate can be used to predict PK variability.[85, 86]

Similar as for newer antiepileptic drugs, a discussion is ongoing whether TDM should be used for antifungal drugs. Currently, fluconazole is the most commonly used antifungal drug in neonates. However, the need for other antifungal drugs is expected to increase due to the growing risk of resistance patterns. TDM of these drugs is extensively reviewed for adults, while focus on special populations such as neonates is still missing.[89-92] For adults, the guidelines from the British Society for Medical Mycology strongly

recommend TDM of posaconazole (prophylaxis and effectiveness) because of large intra- and interpatient PK variability. The same holds true for voriconazole (effectiveness and toxicity) because of the same PK variability, but also because of non-linear pharmacokinetics.[93] Bruggemann *et al.* also recommends TDM of voriconazole in children.[94] As neonates have an even more extensive PK variability compared to adults and children, it could be worthwhile to investigate the contribution of TDM of these antifungal drugs. In adults, TDM of itraconazole is considered useful because of the variability in absorption following oral administration.[89] This variability will be less pronounced in neonatal treatment, due to the high proportion of intravenous administration. For other antifungals like echinocandins, TDM in adults is not recommended because of lack of sufficient evidence.[95] However, in neonates the clearance of echinocandin micafungin was reported to be proportionally higher than in adults, due to an eight-fold higher unbound fraction.[96] This contributes to a larger PK variability making TDM potentially useful in neonates for micafungin.

TDM can be implemented more efficiently due to the development of more advanced analytical techniques such as UPLC-MS/MS, allowing simultaneous quantification of drugs. Furthermore, increased sensitivity of analytical techniques enables further research in measuring concentrations of drugs and metabolites. These samples can either be obtained from non-invasive bodily fluids (e.g. saliva), as well as from blood through DBS collection. In the future, these non-invasively obtained bodily fluids and newer techniques can be used in clinical care. This is particularly promising as costs and complexity of these techniques decrease, and reliability increases.

The clinical relevance to determine the unbound as well as, or instead of, the total concentration, should be further investigated especially for highly protein bound drugs and in neonates, considering the lower level of plasma proteins compared to adults.[27] Consequently, higher unbound fractions of drugs in neonates are likely, as demonstrated for vancomycin and micafungin.[96] A limitation is that a blood sample volume below 20 µL is not generally sufficient to quantify both unbound as well as the total fraction of a drug. As blood sampling is a major concern in neonates, improvements are needed with respect to the required sample volume and strategically chosen sampling times. Pharmacists should better advice clinicians on the absolute minimal blood volume needed for TDM of each drug. Obviously, clinicians should only collect the minimal volume of blood which is required by the laboratory. In order to avoid excessive blood loss, the neonatology department should keep a daily record of the amount of blood which is drawn from each neonate. Blood sampling for TDM can also be reduced through better communication, which may enable to use left over blood or serum samples, initially collected for other measurements (e.g. blood gasses). The obstacles are mainly logistic; there must be a sufficient amount of blood remaining to quantify the drug, and times of drug administration and blood sampling need to be accurately recorded to enable

reliable interpretation for TDM. Logistic problems can partly be resolved when opportunistic sampling can be performed, instead of samples collected at specific times, such as trough levels. This may be enabled by Bayesian forecasting using opportunistically collected samples for routine blood tests, to estimate the concentration at the necessary time-point for TDM of that specific drug. An additional advantage of Bayesian forecasting is that it probably leads to less blood sampling.[39] Recently, opportunistic sampling instead of trough sampling has been reported for gentamicin in the neonatal population by Germovsek *et al*.[97] They investigated the development and evaluation of a gentamicin population PK-model. This model facilitated opportunistic gentamicin TDM in neonates, which led to the conclusion that opportunistic sampling can reliably predict trough concentrations of gentamicin. This promising concept should be further investigated for other drugs for which TDM is indicated to further reduce the burden of blood sampling for TDM.

Future research should focus on the reliability of the therapeutic range in neonates and rapid quantification of drugs in non-invasive bodily fluids. As described before, saliva can be used for TDM of certain drugs.[70] Consequently, it is necessary to investigate for which drugs, TDM in saliva could be a useful alternative.

Therapeutic ranges for TDM have been extensively discussed, but there is still much to improve. They are mainly population based instead of individualized, and generally not yet evidence-based. Therefore, TDM mostly focusses on dosage adjustments to achieve a drug concentration within the population-based therapeutic range. However, this population-based therapeutic range does not correspond with the optimal concentration for each individual patient. Well-investigated examples are antiepileptic drugs in adults as a lot of patients require a target concentration outside the conventional therapeutic range for that drug.[85, 86] Furthermore, additional individual factors like genetics, type and severity of epilepsy can influence the relation between drug effect and serum concentration.[85, 86] Therefore, further individualization of TDM targets in neonates is essential. Although, this has partly been investigated for adults already, regarding the extensive PK variability, this concept may even be more relevant for neonates to investigate. Nevertheless, this should not be applied too rigidly, as intra-patient variability of pharmacodynamics should be taken into account of the total concept as well, which has been determined for certain antiepileptic drugs in adults.[86] Further, individualization of TDM could also apply for antibiotic drugs, for which treatment, dosing, as well as target concentrations need to be tailored to local resistance patterns per pathogen, hospital, country or area. As mentioned before, therapeutic ranges used in neonates are at present mostly based on adults or animal data.[50] The identification of therapeutic ranges for the neonatal population, with prospective studies is the first step that needs to be taken, and should be used as starting point to further individualize the therapeutic range.

In conclusion, TDM is useful for certain drugs in neonates, but specific issues should be considered when using TDM in this population. We highlighted the relevance of these specific issues, and subsequently provided suggestions on future research to further optimize TDM in this special population.

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PHARMACODYNAMICS IN PRETERM INFANTS

11

Retrospective study shows that doxapram therapy avoided the need for endotracheal intubation in most premature neonates

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ABSTRACT

Aim

Using doxapram to treat neonates with apnoea of prematurity might avoid the need for endotracheal intubation and invasive ventilation. We studied whether doxapram prevented the need for intubation and identified the predictors of the success.

Methods

This was a retrospective study of preterm infants born from January 2006 to August 2014 who received oral or intravenous doxapram. Success was defined as no need for endotracheal intubation, due to apnoea, during doxapram therapy. Univariable and multivariable logistic regression analyses identified predictors of success during the first 48 hours of doxapram therapy.

Results

Data on 203 patients with a median gestational age of 26.1 (interquartile range 25.1-27.4) weeks were analysed. During the first 48 hours of doxapram therapy, 157 (77%) patients did not need endotracheal intubation and 127 (63%) patients were successfully treated over the entire treatment course. The median postnatal age at the start of doxapram therapy was 20 days (interquartile range 12-30). Postnatal age and a lower fraction of inspired oxygen at the start of doxapram therapy were significant predictors of success (odds ratio 0.964, 95% confidence interval 0.938-0.991, p=0.001).

Conclusion

Oral and intravenous doxapram effectively treated most cases of apnoea in preterm infants, avoiding the need for intubation.

INTRODUCTION

A common symptom of neurological and respiratory maldevelopment in preterm born infants is apnoea of prematurity (AOP), which decreases oxygen saturation, resulting in hypoxic episodes that are harmful to the infant's short and long-term neurodevelopmental outcomes[1]. The increasing incidence of AOP over the last decade has created a major clinical problem for which adequate treatment is essential.

The first choice pharmacological agents for the treatment of AOP are methylxanthines: caffeine, theophylline or aminophylline[2,3]. Methylxanthines stimulate the respiratory system, thereby reducing apnoea. Caffeine is the preferred agent, because it has a good efficacy and safety profile[4,5], but in a proportion of patients, AOP persists despite the combination of caffeine treatment and maximal non-invasive ventilation. In these patients, the use of the respiratory stimulant doxapram may be indicated to avoid invasive ventilation.

Doxapram is off-label for use in children and neonates and evidence about efficacy and safety has been limited to a few small studies[6-10] that have reported successful control of AOP cases that were unresponsive to methylxanthines. Other reports on doxapram therapy suggest adverse side effects, such as gastrointestinal disturbances, increased agitation, excessive crying, jitteriness, irritability[11-14], hypokalaemia[15], hypertension[9,16], atrioventricular heart block[12], higher percentages of continuous activity, more electrographic seizure activity and less sleep-wake cycling than in control groups[17]. Because doxapram may affect brain haemodynamics, concerns have been raised about the possible long-term effects on mental development[18,19], although such effects were not confirmed in a cohort study[20].

Consequently, doxapram should be used with caution in preterm born infants and further research into the efficacy and safety of this therapy is needed. This study aimed to evaluate the efficacy and predictors of the success of doxapram treatment on preventing intubation.

PATIENTS AND METHODS

Patients and setting

In this retrospective cohort study, all patients born between January 2006 and August 2014 admitted to the level four Neonatal Intensive Care Unit (NICU) of the Erasmus Medical Centre, Sophia Children's Hospital, who received doxapram during admission were eligible for inclusion. Intubation for any other reason than the failure of doxapram treatment was the only exclusion criterion. The Medical Ethical Committee of the Eras-

mus Medical Centre declared that due to the retrospective design, formal approval was not required (MEC-2015-683).

Data collection

Data on patient characteristics were retrieved from the electronical medical records using Elpado version 2.51.0.1 (Erasmus Medical Centre, Rotterdam, The Netherlands), namely gestational age, gender, date and time of intubation. The following data were obtained from the electronic Patient Data Management System version 8.3.2 (PICIS, Wakefield, Massachusetts, USA): doxapram infusion rates, duration of therapy, route of administration, dosages, haematocrit, fraction of inspired oxygen concentration (FiO_2), positive end expiratory pressure (PEEP), mean airway pressure (MAP) and occurrence of AOP. Data were collected until death or discharge from the NICU. The infants' respiratory condition just before doxapram treatment was assessed from data about respiratory support one hour before the start of treatment. We followed the guidelines in the Reporting of Studies Conducted using Observational Routinely Collected Data (RECORD) statement to report our study[21].

Treatment policy

The local standard of care treatment prescribed caffeine therapy for all infants with gestational ages up to, and including, 28 weeks and in infants born after 28 weeks' gestation with AOP. The treatment comprised a loading dose of 10 mg/kg followed by a daily dose of 5 mg/kg intravenously or gastroenterally. Additional doses of 5 mg/kg caffeine were allowed and the maintenance dose could be increased in the absence of side effects. If the attending physician judged that apnoea persisted despite optimal caffeine therapy and maximal non-invasive ventilatory support, doxapram could be added to avoid endotracheal intubation. This judgement was not defined further in the clinical protocol. To reach a steady state blood concentration immediately, doxapram therapy with 2 mg/mL of Dopram (Eumedica, Manage, Belgium) started with a loading dose of 2.5 mg/kg bodyweight in 15 minutes. The maintenance starting dose was 2.0 mg/kg/h, either by continuous intravenous infusion or continuous gastroenteral administration of the IV solution via a nasogastric tube. Gastroenteral administration was only considered when enteral feeding was well tolerated. The efficacy was evaluated from the absence of apnoea. If it was effective, the doxapram dose could be decreased stepwise, and be switched from intravenous to gastroenteral administration on the judgement of the attending physician. Doxapram treatment was stopped if the patient required endotracheal intubation for mechanical ventilation or if apnoeas were absent with low doses of the drug.

We considered that the first 48 hours of the first doxapram episode would be the most representative period for identifying predictors of successful doxapram therapy.

Outcome

The primary outcome of this study was the success rate of doxapram treatment. Success was defined as no need for endotracheal intubation and mechanical ventilation because of AOP during doxapram therapy. Consequently, treatment failure was defined as the need of intubation due to the persistence of apnoeas despite doxapram treatment.
The secondary outcomes included the potential predictors of success or failure of doxapram treatment during the first 48 hours: gestational age, postnatal age at the start of doxapram, gender, bodyweight, maintenance dosage at start, route of administration, non-invasive respiratory support, haematocrit, FiO2 and PEEP-MAP.

Statistical analysis

Continuous variables are summarised as medians with interquartile ranges (IQR) and categorical variables as frequencies with percentages. The data on successfully treated patients were compared with the data of unsuccessfully treated patients using Fisher's exact tests for categorical data and Mann-Whitney U tests for continuous variables. Univariable and multivariable logistic regression analyses were used to identify the predictors of success of doxapram treatment. The following predictors were considered: gestational age, postnatal age at start doxapram, gender, weight at admittance, maintenance dosage at start, route of administration, non-invasive support, haematocrit within three days from the start of doxapram therapy, FiO2 and PEEP-MAP. The PEEP-MAP variable combines PEEP and MAP and reflects the level of respiratory support with either continuous positive airway pressure or non-invasive positive pressure ventilation, respectively. For the multivariable logistic regression analysis, a stepwise backward method was used to select the predictors in the model, with a cut-off for elimination of $p=0.20$ to avoid the risk of excluding relevant predictors. We had to deal with missing values for some variables. A complete case analysis was used in each step of the stepwise backward method, thereby excluding patients with missing values for predictors that were in the model. Variance inflation factors of the predictor variables were calculated to assess multicollinearity and value of below three was considered acceptable. The calibration of the multivariable logistic regression analysis was assessed using the Hosmer-Lemeshow test and the linearity assumption was tested for each continuous predictor using the Box-Tidwell method.
Two-sided p values of <0.05 were considered statistically significant. Data were analysed using SPSS version 21 (IBM Corp, Armonk, New York, USA).

RESULTS

During the study period, 6,400 infants were admitted to our NICU and 204 (3.2%) received doxapram. These patients represented 7.9% of infants born before 32 weeks and 19.1% born before 28 weeks. One patient was excluded because intubation was needed for surgery rather than the failure of doxapram treatment. In total, 203 patients were included in the statistical analysis and their characteristics are given in Table 1. Their median gestational age was 26.1 (IQR 25.1-27.4) weeks and their median postnatal age at start of the first episode of doxapram therapy was 20 (IQR 12-30) days. Measurement of the primary outcome showed that doxapram was successful in 157 patients (77.3%) as they did not need intubation because of apnoeas in the first 48 hours of the first doxapram episode. During the entire course of doxapram therapy, 127 (62.6%) patients did not need intubation to treat AOP.

Table 1. Patient characteristics

N= 203		Median	IQR	Range	Number	(%)
Gestational age (weeks)		26.1	25.1-27.4	23.9-38.3		
Weight on admission (grams)		840	720-980	370-3,760		
Gender	Male				122	(60.1)
	Female				81	(39.9)
Postnatal age at start of therapy (days)		20	12-30	1-71		
Route of administration at start of therapy	IV				146	(71.9)
	Gastroenteral				57	(28.1)
Doxapram infusion rate at start of therapy (mg/kg/h) (n=201)		2.0	1.9-2.0	0.4-2.9		
Duration of the first episode of doxapram treatment (hours)		70	26-180	0-1,841		
Total duration of doxapram treatment (hours)		120	34-312	0-1,841		
Cumulative dose of doxapram per patient (mg)		123	42-333	2-6,110		
Doxapram dose (mg/kg/h)		1.2	0.9-1.6	0.03-5.8		
Loading dose	Yes				137	(67.5)
	No				66	(32.5)
Number of doxapram episodes	1				148	(72.9)
	2				38	(18.7)
	3				10	(4.9)
	4				6	(3.0)
	5				1	(0.5)

Table 1. Patient characteristics (*continued*)

N= 203		Median	IQR	Range	Number	(%)
Success rate per episode (success / total per episode)	1				135/203	(66.5)
	2				46/56	(82.1)
	3				14/16	(87.5)
	4				6/7	(85.7)
	5				1/1	(100)
Success rate within the first 48 hours of therapy episode 1					157	(77.3)
Overall success rate					127	(62.6)
Respiratory support	CPAP				78	(38.4)
	NIPPV				119	(58.6)
	Nasal				3	(1.5)
	Invasive				3	(1.5)
FiO_2		0.27	0.22-0.35	0.20-0.80		
PEEP (cmH_2O) (n=201)		6.0	6.0-6.0	5.0-7.2		
Pmax (cmH_2O) (n=119)		16.0	14-16.3	10.0-20.0		
Haematocrit (n=194)		0.35	0.31-0.38	0.25-0.54		

CPAP-continuous positive airway pressure; FiO_2-fraction of inspired oxygen; MAP-mean airway pressure; NIPPV-non invasive positive pressure ventilation; PEEP-positive end expiratory pressure; Pmax-peak inspiratory pressure; PNA-postnatal age.

Doxapram treatment was started via intravenous infusion in 146 (71.9%) patients and gastroenterally, via a gastric tube, in 57 (28.1%) patients. A total of 32% of the patients only received doxapram via intravenous infusion, 15% only received it gastroenterally via a gastric tube and 53% received it alternately via both routes of administration. The median doxapram infusion rate at the time of the start of therapy was 2.0 (IQR 1.9-2.0) mg/kg/h. We found that 137 patients (67.5%) received a doxapram loading dose in their first episode of treatment and 55 patients (27.1%) received more than one episode of doxapram. The success rate of doxapram therapy in the respective episodes ranged from 66.5% to 100% (Fig. 1).

Predictors of success

The outcomes of the univariable analyses on patient and clinical characteristics are shown in Table 2. The median postnatal age at the start of doxapram therapy of the successfully treated neonates was significantly higher than that of the unsuccessfully treated neonates, according to the results of the Mann-Whitney U test: 22 (IQR 17) and 14 (IQR 12) days respectively ($p<0.001$). The success rate of doxapram therapy after 48 hours was 69% of cases if started before 20 days of postnatal age versus 87% if started at 20 days of postnatal age or later ($p=0.002$; Fig. 2). Furthermore, the route of administration at the start of therapy was significantly different between both groups, according to Fisher's exact test ($p=0.009$): 33% of the successfully treated patients started with

gastroenteral administration via a nasogastric tube compared to 13% in the unsuccessful treatment group (Table 2).

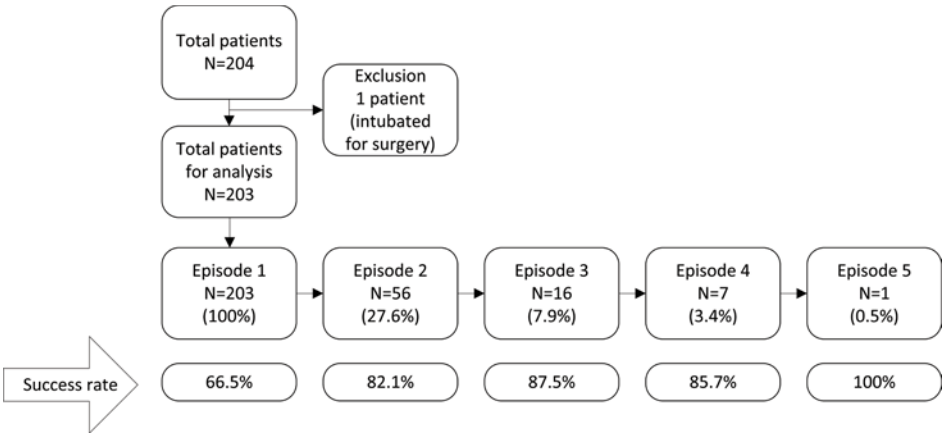

Figure 1. Inclusion of patients.
The flow-chart shows the numbers of patients receiving doxapram therapy during 1 to 5 episodes, and the percentages of all patients. The bottom line gives the success rate of doxapram therapy to avoid intubation per episode.

Table 2. Univariable analyses of intubation within the first 48 hours of the first doxapram therapy episode

		Successfully treated patients n = 157 (77.3%)			**Unsuccessfully treated patients n =46 (22.7%)**			**p value**
		Median	IQR	N (%)	Median	IQR	N (%)	
Gestational age (weeks)		26.3	25.1-27.4		26.0	25.0-27.3		0.39
Weight at admittance (grams)		850	720-1,000		815	705-950		0.45
PNA at start of therapy (days)		22	14-31		14	9-21		<0.001
PNA ≤20 days				72 (45.9)			33 (71.7)	0.002
PNA > 20 days				85 (54.1)			13 (28.3)	
Haematocrit		0.35	0.31-0.38		0.35	0.31-0.38		0.96
FiO_2		0.26	0.22-0.35		0.30	0.24-0.37		0.081
Doxapram infusion rate at start (mg/kg/h)		2.0	1.9-2.0		2.0	1.9-2.0		0.70
Loading dose	Yes			105 (66.9)			32 (69.6)	0.858
	No			52 (33.1)			14 (30.4)	
ROA at start of doxapram	IV			106 (67.5)			40 (87.0)	0.009
	Gastroenteral			51 (32.5)			6 (13.0)	

Mann-Whitney U test
Fisher's exact test
FiO_2-fraction of inspired oxygen; PNA-postnatal age; IQR-interquartile range; IV-intravenous; ROA-route of administration

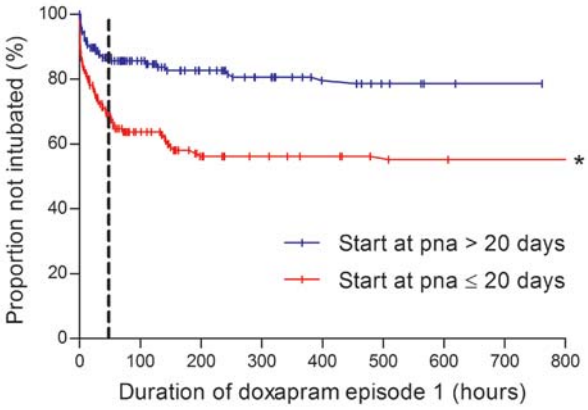

Figure 2. Proportion of successful therapy ('not intubated') during the first doxapram episode starting at postnatal age > 20 and ≤ 21 days.

The red line(–) represents patients who started at postnatal age ≤20 days; the blue line (-) patients starting at postnatal age > 20 days. Each vertical line crossing the graph represents termination of doxapram therapy of one patient at that consecutive time point of episode one.

The proportion of patients not intubated at 48 hours after start of doxapram is indicated by the dashed vertical line (⋮). This corresponds to 69% and 87% if started at postnatal age ≥ 20 days and > 20 days, respectively.

* Episode 1 continued for 2 patients, and stopped without intubation after 1,006 and 1,841 hours, respectively

Variance inflation factors of the predictor variables in the logistic regression analyses were below three, except for postnatal age and squared postnatal age at the start of doxapram, due to a large correlation between these two variables. The univariable logistic regression analysis showed that the route of administration and the postnatal age at the start of treatment were predictors of success (Table 3).

The multivariable logistic regression analysis with stepwise backward elimination of predictor variables revealed that postnatal age, the quadratic term of postnatal age and FiO_2 were significant predictors of success for doxapram therapy (Table 3). In a preliminary analysis with the Hosmer-Lemeshow test, using only linear terms for all continuous predictors, the calibration of the multivariable logistic regression analysis was found to be poor (p=0.016) and a significant violation of the linearity assumption was detected in a preliminary analysis with the Box-Tidwell test, using only a linear term for postnatal age at start of doxapram therapy (p=0.004). Therefore, a quadratic term was included for postnatal age in the multivariable logistic regression analysis, after which no significant violations of the model assumptions were found by the Hosmer-Lemeshow test and the Box-Tidwell test. Due to the quadratic term, postnatal age had a curvilinear relationship with the probability of success of doxapram therapy, with the highest estimated probability of success at a postnatal age of 17 days: the odds ratio (OR) versus the postnatal age of zero days was 2.08. Lower FiO_2 was associated with a higher probability of success

of doxapram therapy (OR per unit increase 0.964, 95% CI 0.938-0.991, p=0.001). We had to deal with two to nine missing values for some variables (Table 1). The final multivariable logistic regression model included data for 201 out of 203 patients and the p value of the Hosmer-Lemeshow test for this model was 0.261.

Table 3. Univariable and multivariable logistic regression analyses with success of doxapram therapy as outcome variable

	Univariable logistic regression analysis			**Multivariable logistic regression analysis**		
Predictor variable	**OR**	**95% CI**	**P-value**	**OR**	**95% CI**	**p value**
Non-invasive support	1.173	0.602-2.284	0.639			
Gestational age	1.017	0.850-1.217	0.854			
PEEP and MAP	0.968	0.755-1.242	0.800			
Weight at admittance	0.941	0.341-2.595	0.906			
Gender	1.174	0.596-2.313	0.643			
Maintenance dosage at start	1.524	0.752-3.090	0.242			
Haematocrit	0.771	0.001-580	0.939			
Route of administration at start	3.047	1.210-7.673	0.018	2.326	0.878-6.165	0.089
FiO_2 [a]	0.984	0.961-1.007	0.173	0.964	0.938-0.991	0.010
PNA at start doxapram	1.046	1.013-1.080	0.005	1.198	1.087-1.319	< 0.001
Quadratic PNA at start doxapram	1.001	1.000-1.001	0.063	0.998	0.996-0.999	0.002

Univariable logistic regression analysis was applied with each of the variables separately.
In multivariable logistic regression analysis with stepwise backward elimination the following variables were subsequently eliminated: non-invasive support (n=183), gestational age (n=183), PEEP and MAP (n=183), weight at admittance (n=192), gender (n=192), maintenance dosage at start (n=192), and haematocrit (n=192). The final multivariable logistic regression model included data of 201 patients and the p value of the Hosmer-Lemeshow test for this model was 0.261.
Parameter just before start of doxapram therapy episode one.
CI-confidence interval; FiO_2-fraction of inspired oxygen; MAP-mean airway pressure; OR-odds ratio; PEEP-positive end expiratory pressure; PNA-postnatal age.

DISCUSSION

We found that 77% of the newborn infants with AOP did not need endotracheal intubation during the first 48 hours of doxapram treatment and almost 63% of patients did not need intubation during the entire treatment course. This might indicate a substantial effect of doxapram on the breathing pattern of the newborn infants who received the therapy. Such an effective treatment is valuable, because prolonged hypoxaemic episodes among extremely preterm infants during the first two to three months after birth have been associated with adverse outcomes at 18 months[1]. Alternatives to doxa-

pram are limited: higher caffeine dosage, with risk of toxicity[22,23], more non-invasive respiratory support and higher FiO_2 or endotracheal intubation followed by invasive ventilation. No treatment at all implies that apnoea will persist, leading to hypoxaemia, whereas intubation and invasive ventilation will increase the risk for bronchopulmonary dysplasia. Nevertheless, doxapram is only licensed for use in children over 12 and the efficacy of doxapram for the treatment of AOP has rarely been studied[6-8,24]. This might be due to the fact that in the UK and USA doxapram is only available in a solvent containing the toxic excipient benzyl alcohol, which raises concerns about toxicity, although these have not been confirmed yet[10,20].

The 63% success rate to prevent intubation during the entire course of doxapram treatment was in line with the 65% reported in 116 patients by Prins et al[25]. Their patient cohort and doxapram treatment were comparable with ours, but they did not study predictors of success.

As they wait for further well-designed trials, clinicians and researchers first need to find the predetermining factors for the success and failure of doxapram therapy. The first step is identifying who might benefit most from doxapram treatment. Secondly, identifying the best dose and further pharmacokinetic and pharmacodynamic studies are needed, to supplement the work by Ogawa et al,[26]. Next, the side effects of doxapram, and the effects on bronchopulmonary dysplasia and neurological outcome, should be studied in a randomised controlled trial. In the present study, the success rate during the first 48 hours was associated with lower FiO_2, the gastroenteral route of administration and the postnatal age at the start of doxapram therapy. All these characteristics seem to reflect the patients' physical condition and wellbeing, although the estimated ORs were relatively small. Lower FiO_2 probably reflects improved cardiopulmonary reserve, with a higher probability of preventing intubation than in the case of higher FiO_2. Gastroenteral administration was only considered when enteral feeding was well tolerated. Furthermore, lower postnatal age was associated with a worse physical condition and more severe apnoea. The poorer response to doxapram may also have reflected the developmental stage of the respiratory system. Doxapram stimulates the peripheral carotid chemoreceptors by inhibiting the potassium channels of type I cells within the carotid body. From animal studies, it is known that carotid chemoreceptors exhibit low sensitivity at birth and become more sensitive with postnatal age[27,28]. Whether this also holds true for human preterm neonates is unknown, but the carotid body will certainly mature with age. Based on these results, we suggest that intubation instead of doxapram treatment is indicated for newborn infants with AOP in a poorer clinical condition. Furthermore, our study suggests good efficacy for the gastroenteral administration of doxapram. The beneficial effect of gastroenteral treatment on AOP has already been suggested[14]. However, little is known about the bio-availability of doxapram after oral administration, as in one study it was estimated to be poor and in another study it was

calculated to be 72%[29,30]. Despite the probability of lower systemic availability after oral administration, dosages were not increased when switching from intravenous to gastroenteral administration in our study. Nevertheless, the effects of gastroenteral and intravenous doxapram dosages were comparable, suggesting that the latter doses were too high. The design of our study does not allow us to draw firm conclusions on this. The gastroenteral route of administration potentially caused harmful side effects, such as gastrointestinal disturbances, nausea and vomiting[13,14,29].

The retrospective design of the current study allowed us to describe and evaluate doxapram therapy as used in our daily clinical setting. An inherent limitation was the lack of data on more efficacy parameters and side effects. For example, more objective data about the effect of doxapram on the respiratory pattern and blood gases would help us to understand more about why doxapram did not work from a physiological perspective. Also, the reason for AOP in our preterm neonates was often unclear, as we were not able to distinguish between apnoea related to lung disease, exhaustion, anaemia or a combination of these. Other limitations that deserve a mention are the single centre setting and the absence of a control group. Although clinicians are always inclined to avoid intubation, as invasive ventilation is related to bronchopulmonary dysplasia, they may feel more reluctant to do this shortly after starting doxapram, as they want to see what effect the doxapram will have.

CONCLUSION

Our results suggest that doxapram may be used effectively for the treatment of AOP in a considerable proportion of patients, administered either intravenously or gastroenterally. As alternatives to doxapram therapy for avoiding intubation are limited, this drug could establish a prominent position in neonatal intensive care, with postnatal age at the start of therapy and FiO_2 being the main predictors of success. Further prospective research is needed to clarify the pharmacokinetics and pharmacodynamics of doxapram and to establish the effective dosages. In addition, the efficacy and safety of doxapram should be studied in placebo-controlled trials.

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Big data analyses for continuous evaluation of pharmacotherapy: A proof of principle with doxapram in preterm infants

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ABSTRACT

Background

Drug effect evaluation is often based on subjective interpretation of a selection of patient data. Continuous analyses of high frequency patient monitor data are a valuable source to measure drug effects. However, these have not yet been fully explored in clinical care. We aim to evaluate the usefulness and applicability of high frequency physiological data for analyses of pharmacotherapy.

Methods

As a proof of principle, the effects of doxapram, a respiratory stimulant, on the oxygenation in preterm infants were studied. Second-to-second physiological data were collected from 12 hours before to 36 hours after start of doxapram loading dose plus continuous maintenance dose in seven preterm infants. Besides physiological data, plasma concentrations of doxapram and keto-doxapram were measured.

Results

Arterial oxygen saturation (SpO_2) increased after the start of doxapram treatment alongside an increase in heart rate. The respiratory rate remained unaffected. The number of saturation dips and the time below a saturation of 80%, as well as the area under the 80%-saturation-time curve (AUC), were significantly lower after the start of doxapram. The AUC under 90% saturation also significantly improved after start of doxapram. Plasma concentrations of doxapram and keto-doxapram were measured.

Conclusions

Using high-frequency monitoring data, we showed the detailed effects over time of pharmacotherapy. We could objectively determine the respiratory condition and the effects of doxapram treatment in preterm infants. This type of analysis might help to develop individualized drug treatments with tailored dose adjustments based on a closed-loop algorithm.

BACKGROUND

Improvements in computer technology have facilitated the analysis of tremendous amounts of data[1]. With the digitization and digitalization of clinical monitoring, most data in intensive care are currently registered continuously. While clinical use of these 'big data' remains mostly limited to the traditional 'snapshot' assessment of a patient's health status, analysis of continuous data brings new opportunities to improve care. The availability of high-resolution vital measurements allows early detection, recognition and treatment follow-up of specific diseases and permits the use of these measurements for therapeutic guidance and evaluation of pharmacotherapy[2].

Current respiratory data in neonatal care

Neonatal intensive care units (NICUs) are pioneers regarding technological innovation in patient care, as is for instance shown by new continuous physiologic data assessment to predict neonatal sepsis[3]. The need for non-invasive patient monitoring has accelerated the development of devices that enable continuous monitoring of breathing, heart action and oxygen levels. Invasive ventilation causes chronic lung disease in very preterm infants. Therefore, respiratory support at the NICU is focused on non-invasive therapy. Meanwhile, frequent episodes of apnea of prematurity (AOP) may be caused by immaturity of the respiratory center of preterm infants, or by a decrease in energy reserves and increased lung disease during admittance. These apneic episodes lead to hypoxemia and are difficult to correct. The underdeveloped central nervous system of the premature infant is prone to hypoxemia[4]. A preventive countermeasure to reduce the effects of apneas is the continuous maintenance of an oxygen buffer by providing a surplus of oxygen. While this surplus is not only often insufficient, it is potentially very harmful to the premature infant. Too high oxygen levels (hyperoxemia) can cause retinopathy of prematurity (ROP) which can lead to decreased vision or even blindness[5]. Furthermore, hyperoxemia is also known to impair brain development[6]. Therefore, oxygen saturation targets need to be kept within specific limits[7].

Apnea definition and saturation targets

The aim of deriving clinical effects from doxapram therapy is subject to the definition of apnea and the clinically applied oxygen targets in neonatology. Evidence and guidelines are lacking to support apnea definitions for preterm and term infants. For example, clinicians and researchers still debate on the definition of the minimum duration of an apneic episode, in which cessation of breathing causes a drop in arterial oxygen saturation. Currently, a large variety of definitions for apnea is used in clinical practice to determine the severity of AOP and to evaluate treatment[8]. Although most alarms on infant breathing monitors are set to alert a respiratory pause of more than 20 seconds, apnea

might be better defined by the associated consequence, such as drops in heart rate or oxygen saturation. However, the degree of change in heart rate or oxygen saturation that defines a respiratory pause as pathological is yet to be defined. Further research is needed to determine the characteristics that differentiate respiratory events with clinical consequence from normal respiratory variability in term and preterm infants. For studies on dips in oxygen saturation it is important to present these apneic moments in the context of the target oxygen saturation that was applied for the patients. Detection of temporary drops in oxygen levels is dependent upon the oxygen target range applied in each individual neonatal intensive care unit. These differences in oxygen saturation targets influence the length and depth of drops in oxygen saturation. Recent large studies in premature neonates have shown that an oxygen target range of 91-95% is beneficial as compared to 85-89% in terms of development of necrotizing enterocolitis (NEC), ROP and mortality[7, 9, 10]. Because of the lack of most basic trend data on oxygen saturation, the indication for a therapeutic intervention for AOP is often based on the frequency of apnea alarms and nurses' observations. Continuous quantitative analysis of periods of hypoxia or apnea could therefore not only allow proper timing of interventions for AOP, but also monitor and evaluate the effects of these interventions.

Treatment of apnea of prematurity

The first-choice pharmacological agents for the treatment of AOP are methylxanthines: caffeine, theophylline or aminophylline[11, 12]. Methylxanthines stimulate the respiratory system, thereby reducing apnea. Caffeine is the preferred agent because it has a good efficacy and safety profile[13, 14]. In a proportion of patients, however, AOP persists despite caffeine treatment together with maximal non-invasive ventilation. In these patients, the use of doxapram, which exhibits another mechanism of the respiratory stimulation, may be indicated to avoid endotracheal intubation and invasive ventilation[15, 16]. Doxapram is frequently used for reducing apnea and hypoxic episodes in preterm infants, although its use in children and neonates is off-label. Evidence about efficacy and safety is limited to a few small studies reporting successful control of AOP unresponsive to methylxanthines[17-21]. Some doxapram solutions contain benzyl alcohol, which forms a contra-indication for use in newborns due to its developmental toxicity. For this reason, doxapram has no indication for newborns in the United States. Nevertheless, the benzyl alcohol free formulation is available in many countries, including most European countries.

Doxapram pharmacology

Available literature suggests that doxapram stimulates respiration through acting on the peripheral chemoreceptors located in the carotid bodies by inhibition of the K channels[22]. Furthermore, doxapram increases minute ventilation and tidal volume via

the respiratory neurons in the central nervous system[23-26]. The onset of effect for both mechanisms is immediate, i.e. within minutes after administration of an intravenously administered dose of doxapram. Despite these promising effects, cardiovascular side-effects like atrioventricular heart block[27] and tachycardia[28] have been reported, probably also caused by the K channel inhibition. Other side-effects concern gastrointestinal disturbances, excessive crying, irritability, increased agitation, jitteriness[26-28], hypertension[20, 24], hypokalemia[29], higher percentages of continuous activity, more electrographic seizure activity and less sleep–wake cycling than in control groups[30]. Because doxapram may affect brain hemodynamics, concerns have been raised about the possible long-term effects on mental development[31, 32], although such effects were not confirmed in a cohort study[33]. Doxapram is primarily metabolized by CYP3A4, and to a lesser extent by CYP3A5 in the liver, which produces the pharmacologically active metabolite keto-doxapram[34, 35]. Keto-doxapram stimulates respiration via the same mechanisms of action as doxapram, although it is less potent[36]. The parameters describing the pharmacokinetics of doxapram and metabolites have been reported to show large interindividual variability, which may be due to certain covariates[35, 37, 38]. Gender and postmenstrual age should be taken into account, as these were found to influence variability of doxapram pharmacokinetics[39]. Furthermore, enzyme maturation might play a role in pharmacokinetics, and thereby also in the pharmacodynamics of the drug. This enzyme maturation might be non-linear and ultimately lead to dosage recommendations which will be based on factors other than body weight alone. For this reason, it's even more important to develop tools to improve personalization of therapy, i.e. a potential benefit of quantification of doxapram and keto-doxapram concentration in plasma, and/or define an objective relevant effect parameter for AOP.

New developments

Recently developed closed-loop systems for automatically adjusting the inspired fraction of oxygen based on the measured arterial oxygen saturation[40, 41] offer a partial solution, since apneas are not prevented. Consequently, pharmaceutical intervention remains the main treatment modality for AOP to prevent endotracheal intubation and invasive ventilation. In the future, closed-loop systems using clinical monitor data might be envisioned in which dosing of respiratory stimulants is based on accurate and current clinical effects. In this study, we aim to show that pharmacological therapy can be closely monitored with the use of high-frequency physiological data, and can eventually be individualized. Doxapram for AOP treatment serves as the example for this proof of principle.

METHODS

Patients and setting

In this proof of principle study, patients were selected from an ongoing trial on the pharmacokinetics (PK) and pharmacodynamics (PD) of off-label drugs (i.e. doxapram) in preterm infants with a gestational age below 32 weeks, the DINO-study (Drug dosage Improvement in NeOnates). This trial started in September 2014 at the level 3 NICUs of the Erasmus Medical Center-Sophia Children's Hospital in Rotterdam (the Netherlands) and is due to close in June 2017. The Erasmus MC ethics review board approved the protocol and written informed consent from parents/legal guardians was obtained prior to study initiation (MEC-2014-067, ClinicalTrials.gov by NCT02421068).

Eligible for inclusion for the current study were patients who received a doxapram loading dose followed by continuous administration for at least 36 hours, and for whom, efficacy data were successfully stored and at least one blood sample was collected from 12 hours before until 36 hours after start of doxapram therapy. Excluded were patients with aberrant saturation targets, such as in specific congenital cardiac anomalies.

Data collection

The following data were prospectively collected from the electronic Patient Data Management System (version 8.3.2, PICIS, Wakefield, MA): doxapram infusion rates, route of administration, dosages, hematocrit, applied fraction of inspired oxygen (FiO_2) and ventilation mode. Data were obtained from 12 hours before start of doxapram until 36 hours after start. Infants' respiratory condition just before doxapram treatment was recorded in the form of FiO_2 and non-invasive positive pressure ventilation (NIPPV) frequencies set on the ventilator one hour before the start of treatment. Physiological data on arterial oxygen saturation (SpO_2), respiratory rate and heart rate were collected from bedside monitors (Inifinity M540, Drägerwerk AG & Co. KGaA, Lübeck, Germany) on a second-to-second basis from 12 hours before until 36 hours after the start of doxapram therapy. The per-second data acquired from this system had a 12 second averaging time for SpO_2 and 10 seconds for electrocardiogram(ECG)-derived heart rate. Doxapram and keto-doxapram plasma concentrations measured in randomly collected samples during the first 36 hours of therapy were incorporated in the analysis. A software tool was developed in LabVIEW (version 2015 SP1, National Instruments, Austin, TX, USA) for sorting per-second data based on specified time frames (e.g. hours or minutes) and providing data from these time frames on the number of oxygen saturation dips and the total area under the SpO_2 curves for a specified oxygen saturation. Data points where no SpO2 value was registered were excluded from the analysis. In these cases no value was registered due to e.g. a low signal strength or too much environmental light. All values

from 0% up to and including 100% SpO2 were included and checked for artefactual data by quantifying the contribution of values under 30% to the results.

AOP treatment policy

The local standard of care treatment proposed caffeine therapy in all infants of gestational age ≥28 weeks, and in infants >28 weeks with AOP. Caffeine is largely studied and currently registered as a safe drug to treat AOP. Treatment consisted of a loading dose of 10 mg/kg followed by a daily dose of 5 mg/kg intravenously or gastro-enterally. Additional doses of 5 mg/kg caffeine were allowed and the maintenance dose could be increased in the absence of side effects. If the attending physician judged that apnea persisted despite optimal caffeine therapy and maximal non-invasive ventilatory support, doxapram (Dopram®, Manage, Belgium) could be added to the therapy regimen. This was not defined further in the clinical protocol. To reach steady state blood concentration immediately, doxapram therapy started with a loading dose of 2.5 mg/kg bodyweight in 15 minutes. The suggested maintenance starting dose was 2.0 mg/kg/h, either by continuous intravenous infusion or continuous gastro-enteral administration of the IV solution via a nasogastric tube. Gastro-enteral administration was only considered when enteral feeding was well tolerated.

The effect of breathing and existence of AOP was evaluated by current international standards. The nurses and attending physicians gave their interpretations of the presence or absence of apnea. In addition, alarm-signals for oxygen saturation, heart rate and respiratory rate supported the effect evaluation. These alarms were set to indicate saturation below 90% or over 95%, heart rate below 100 or over 200 bpm, and AOP if the respiratory pause exceeded 20 seconds. If treatment was effective, doxapram dose could be decreased in a not standardized stepwise manner, and route of administration could be switched from intravenous to gastro-enteral, both on the initiative of the attending physician. Doxapram treatment was stopped if the patient required endotracheal intubation for mechanical ventilation or if apneas were absent with low dosage below 0.5 mg/kg/h.

Drug assay

Doxapram and keto-doxapram plasma concentrations were measured using ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) at the Pharmacy Department of the Erasmus Medical Center, Rotterdam, the Netherlands. The validated assay (according to FDA guidelines) was linear over 0.05–4.50 mg/L for doxapram, and over 0.05–5.00 mg/L for keto-doxapram. The lower limits of the ranges represent the lower limits of quantification (LLOQs). Intra-assay accuracies ranged from 1.2–4.8%, and inter-assay accuracies ranged from 1.4–2.4%.

Statistical analysis

Collected physiological data (1 Hz) were grouped in time-slots per hour and per minute. After setting a saturation target, the number of times an oxygen saturation dip occurred below this target, the time of each dip below the target, and the area under the curve (AUC) of each dip below the target were calculated from specifically selected oxygen saturation levels. The effect of a doxapram loading dose was determined comparing the average per hour of each parameter during four hours before start of doxapram in comparison with the first four hours after start, as well as one hour before with one hour after start, using a paired T-test. Analysis of a four-hour time interval allows to evaluate the effect of intravenously and orally administered doxapram, and to average the physiological respiratory changes. The effect of the doxapram maintenance dose was determined by comparing the average per hour of each parameter during four hours before start of doxapram therapy, a four-hour time window 24 hours after the start of doxapram (24–28 hours). Two specific oxygen levels were selected for analysis due to their relevance. An arbitrary oxygen saturation level of 80% was used to indicate the clinically relevant dips, where a level of oxygen below 90% was analyzed, based on the internationally accepted lower limit of oxygen saturation. Two-sided p-values <0.05 were considered statistically significant. Data were analyzed using SPSS version 21 (IBM, Armonk, NY, USA).

RESULTS

Table 1. Patient characteristics of seven preterm infants.

	Median	Interquartile Range
Birthweight (g)	910	630-1040
Gestational age (weeks)	25.4	24.3-26.9
Gender male	43%	
Postnatal age start doxapram (days)	24.0	13-28
Bodyweight at start doxapram (g)	1050	840-1200
Loading dose (mg/kg in 15 min)	2.14	2.1-2.6
Maintenance dose at start (mg/kg/hr)	2.00	1.9-2.0

Data of seven preterm infants with median gestational age of 25.4 weeks (IQR 24.3–26.9) and median postnatal age at start of doxapram therapy of 24.0 days (IQR 13–28 days) were incorporated in the analyses (Table 1). A total of 1174900 seconds of monitoring were registered, out of which 6613 seconds (0.56%) were excluded because no SpO2 value was registered. Out of the remaining 1,168,287 registered SpO2 values a value of less than 30% was registered during 115 seconds (0.0098% of all data). This was considered to be a negligible influence on the results. All infants were receiving NIPPV at the start of doxapram therapy. The SpO_2 increased immediately after start of doxapram

treatment, mainly affecting the lower percentiles. The four-hour average SpO_2 before start of doxapram therapy was significantly lower than in the first four hours after start (p=0.010, paired T-test, Table 2), and showed higher variability (Figure 1a). Levels of FiO_2 had briefly increased before the start of doxapram, most likely as an effort to compensate for a deteriorating respiratory condition. During the four-hour time window 24 hours after the start of doxapram, FiO_2 levels were lower when compared to the four

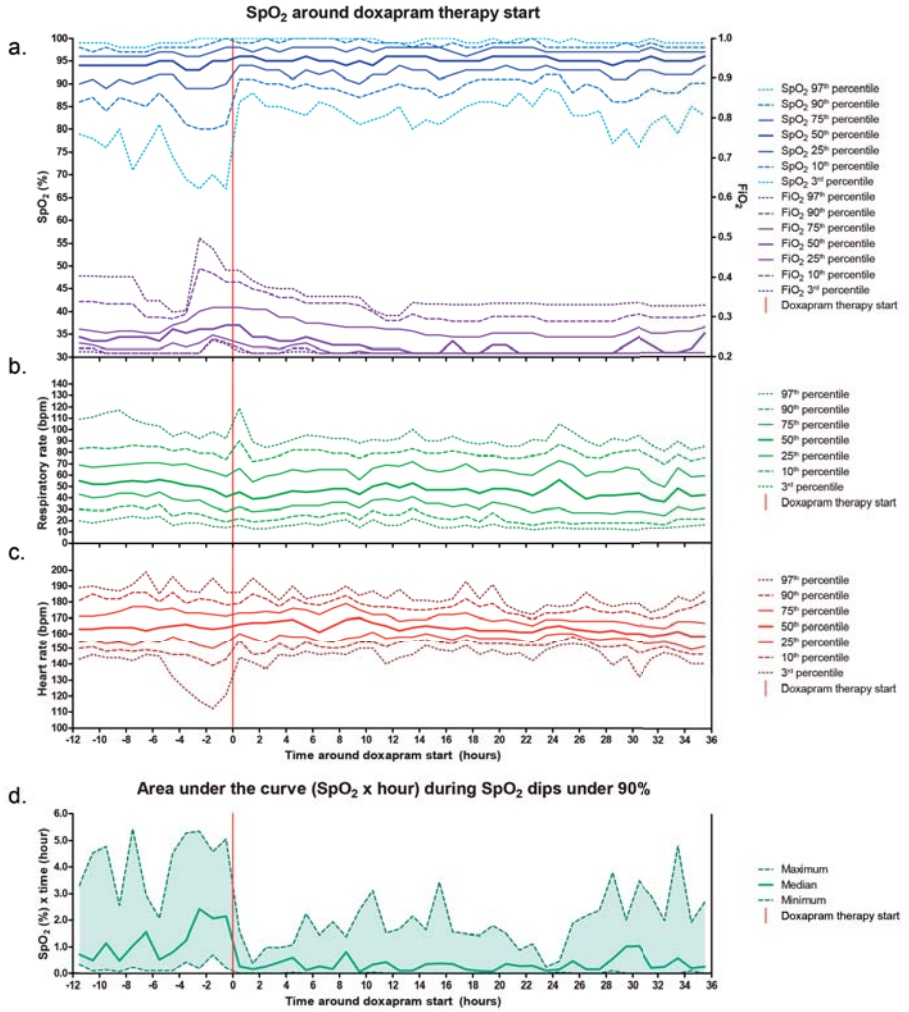

Figure 1. Averages per hour of measurements from 12 hours before until 36 hours after the start of doxapram treatment for 7 patients, therapy start indicated by the vertical bar.

a) Arterial oxygen saturation (SpO_2) and fraction of inspired oxygen (FiO_2)

b) Electrocardiogram impedance-derived respiratory rate

c) Heart rate. Values are percentiles of per second measurements averaged over one-hour time windows

d) Area under the curve below a saturation target of 90% per hour

hours before start (table 2). ECG impedance-derived respiratory rate had temporarily increased during the first hour of doxapram therapy. Heart rates increased by the start of doxapram, which is a familiar side-effect of doxapram (Figure 1b and c, Table 2). With regard to a saturation level of 80%, the average per hour of the number of oxygen saturation dips, time below a saturation, as well as the AUC under 80%-saturation in the four hours before start of doxapram were significantly higher than in the first four

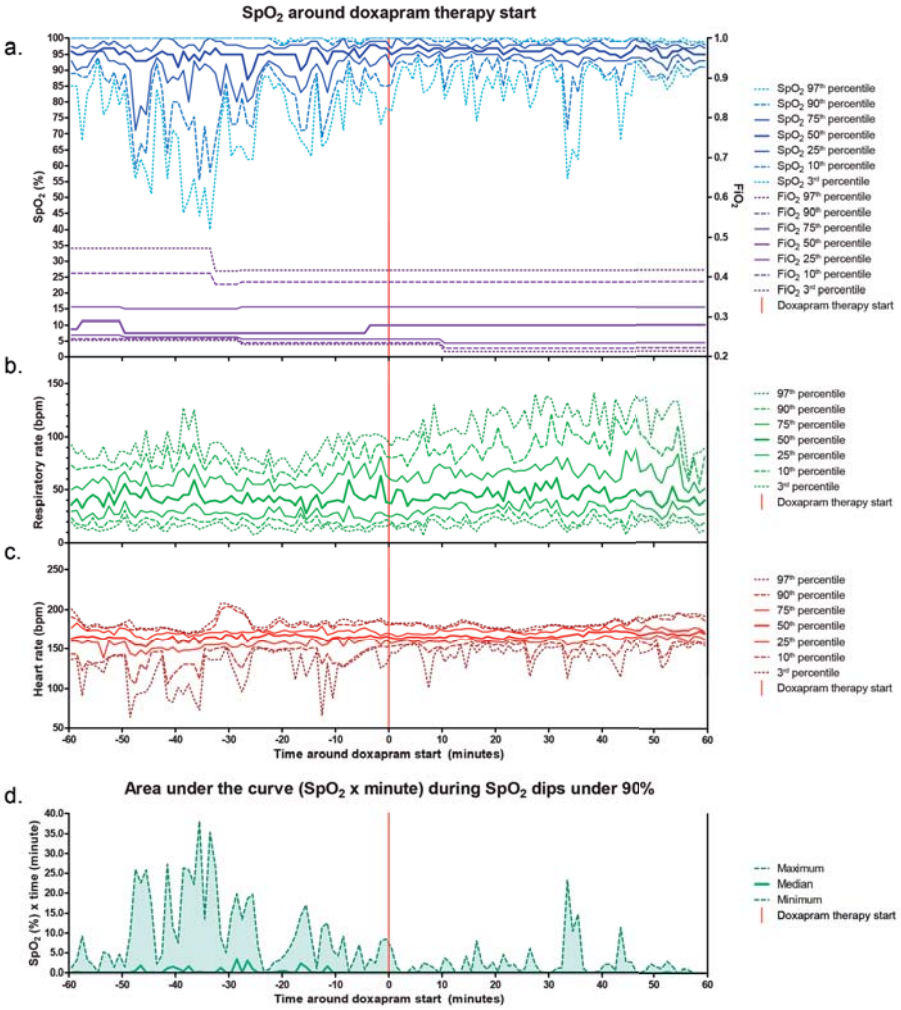

Figure 2. Averages per minute of measurements with a one-hour radius around the start of doxapram treatment for 7 patients, therapy start indicated by the vertical bar.

a) Arterial oxygen saturation (SpO_2) and fraction of inspired oxygen (FiO_2)

b) Electrocardiogram impedance-derived respiratory rate

c) Heart rate values are percentiles of per second measurements averaged over one-hour time windows

d) Area under the curve below a saturation target of 90% per minute

hours after start of doxapram, p=0.016, p=0.014, p=0.011, respectively (Figure 2, Table 2). These differences were also significant comparing one hour before with one hour after start of doxapram (p<0.05, Table 2). Subsequently, the improvement of respiratory parameters was sustained with maintenance dosage, comparing four hours before, with 24-28 hours after start of doxapram (p<0.05, Table 2). Using the alarm setting with lower oxygen saturation limit of 90% as the clinically accepted reference, the average AUC per hour under 90% saturation also significantly improved in the four hours after start of doxapram (p=0.006, Table 2).

Table 2. a. Comparison of respiratory parameters before and after start of doxapram therapy.

	Average per hour around start of doxapram			Average per minute around start of doxapram			Average per hour around start of doxapram		
	4 hours before	4 hours after	P-value #	1 hour before	1 hour after	P-value #	4 hours before	24-28 hours after	P-value #
SpO_2 (%)	91.5	95.0	0.010	92.4	95.4	<0.001	91.5	94.4	0.007
FiO_2	29.2	27.8	0.43	29.7	29.1	0.356	29.2	24.1	0.019
Respiratory rate (bpm)	50.1	46.3	0.194	44.7	51.7	<0.001	50.1	48.2	0.289
Heart rate (bpm)	161.6	165.7	<0.001	161.4	166.6	<0.001	161.6	163.6	0.038

Paired T-test

b. Comparison of converted respiratory parameters before and after start of doxapram therapy.

	Average per hour around start of doxapram			Per hour around start of doxapram			Average per hour around start of doxapram		
	4 hours before	4 hours after	P-value #	1 hour before	1 hour after	P-value #	4 hours before	24-28 hours after	P-value #
Number of dips < 80% SpO_2	14	3	0.016	13	3	0.004	14	3	0.029
AUC < 80% SpO_2 (%·hour)	0.92	0.06	0.011	0.94	0.10	0.037	0.92	0.085	0.010
Time < 80% SpO_2 (seconds)	350	33	0.014	296	37	0.006	350	43	0.015
AUC < 90% SpO_2 (%·hour)	2.49	0.36	0.006	2.38	0.41	0.016	2.49	0.48	0.009

Paired T-test

Figures 1c and 2c show a reduction of bradycardia after start of doxapram, indicated by decreased variation of heart rate at the lower percentiles. Consequently, the average heart rate did increase significantly comparing four hours before, with four hours after start of doxapram therapy, and even with a four-hour time window 24 hours after the start of doxapram ($p<0.001$ and $p=0.038$, see Table 2). Respiratory rate was temporarily increased during the first hour after the start of doxapram therapy.
For each patient, one blood sample was collected during the first 36 hours of doxapram therapy. The measured median plasma concentrations of doxapram was 2.5 mg/L (range 1.8-4.3 mg/), and median 0.6 mg/L (range 0.3-0.8 mg/L) of keto-doxapram.

DISCUSSION

We showed a new principle of translated high-frequency monitoring data that can be used to evaluate pharmacotherapy. Using second-to-second physiological data, we could objectively determine the respiratory condition and therewith the detailed effects of doxapram treatment in preterm infants. Combining such 'big' monitor data with patient characteristics, drug dosages and drug concentrations, will help to quantify drug therapeutic effects in preterm infants in future clinical trials and practice. This strategy might also be used to individualize treatment by giving detailed dosing advise tailored to the individual patient.
Previous studies showed that doxapram treatment–by preventing oxygen desaturation–is successful in preventing endotracheal intubation[15, 16]. Our study confirmed an immediate increase of SpO_2-level upon start of treatment, without a substantial effect on respiratory rate over four hours, but with a short increased respiratory rate comparing the first hour. This may indicate that the mechanism of action is associated with an increased inhalation or depth of breathing.
The major advantage of the presented approach lies in the continuous evaluation of the effectiveness and safety of treatment. The doxapram loading dose was effective in all patients, but for some patients the effectiveness had decreased 24 hours later, as illustrated by the enlarged SpO_2 variability in figure 1a. The loss of effectiveness may be due to a decrease of doxapram and keto-doxapram concentrations over time because of insufficient continuous maintenance dosages, but may also be ascribed to a declining respiratory condition before doxapram initiation. Two patients received doxapram by oral administration, which may have led to a delayed onset of the effect and a lower bioavailability of doxapram, both due to the absorption-phase. The bioavailability has hardly been studied[28, 42]. Consequently, the overall effect of doxapram may be even more evident if all patients would have received doxapram intravenously. The current proof of concept study is too small to further analyze important covariates on the effects of

doxapram such as the effect of route of administration, dose, genotype, etcetera. Future larger studies are needed.

On the other hand, it is also possible that a variable response to doxapram in neonates might reflect the development of the respiratory system. Doxapram stimulates the peripheral carotid chemoreceptors by inhibiting the potassium channels of type I cells within the carotid body. From animal studies, it is known that carotid chemoreceptors exhibit low sensitivity at birth and become more sensitive with age[43, 44]. Whether this also holds true for human preterm neonates is unknown, but the carotid body will certainly mature with age. Therefore, age should be considered in further investigation. Doxapram and keto-doxapram plasma concentrations were measured. Although the number of samples is yet too small to perform a firm analysis and draw conclusions, more samples will be collected during the ongoing trial, and non-compartmental mixed effects modeling will serve to investigate interpatient variability of PK parameters. Recently, gender and postmenstrual age were found to influence variability of doxapram pharmacokinetics, and should be investigated in population pharmacokinetic model development[39].

Limitations of the study concern the small cohort of seven patients and the heterogeneity in their respiratory conditions. Furthermore, physiological parameters were compared as an average per time frame of one and four hours. The use of shorter time frames might lead to more sensitivity/accuracy. For this concept, an arbitrary saturation level of 80% was chosen as an indicator for oxygen saturation dips with a substantial clinical impact. The recorded high-frequency data also allowed analysis of dips in relation to the clinical saturation targets, in our patients included in this study the lower limit was 90%. With this proof of principle, we demonstrate the possibilities of evaluating pharmacotherapy using high-frequency monitor data. Showing saturation dips under specific saturation levels allows quantification of pharmacotherapeutic effects with regard to the applied clinical saturation targets as applied in each specific hospital[7, 9, 10]. Further studies in larger cohorts of patients over longer periods of time might be needed to verify the findings. By doing so, new outcome variables can be defined that can be used in randomized controlled trials in the near future.

A strength of our study is that this type of analysis can be easily used in larger numbers of patients to serve as a guidance in future trials. As respiratory parameters are generally measured with high frequency at NICUs, our approach may be easily implemented in more NICUs to support clinical care.

Clinical implementation of continuous feedback of a patient's condition and treatment effectiveness of the administration of a drug, ultimately in a closed loop design (Figure 3), can be especially beneficial for the most vulnerable preterm infants. Our principle can potentially be expanded to other high frequency patient parameters during NICU clinical care, such as neonatal convulsions, which may be diagnosed earlier from amplitude-

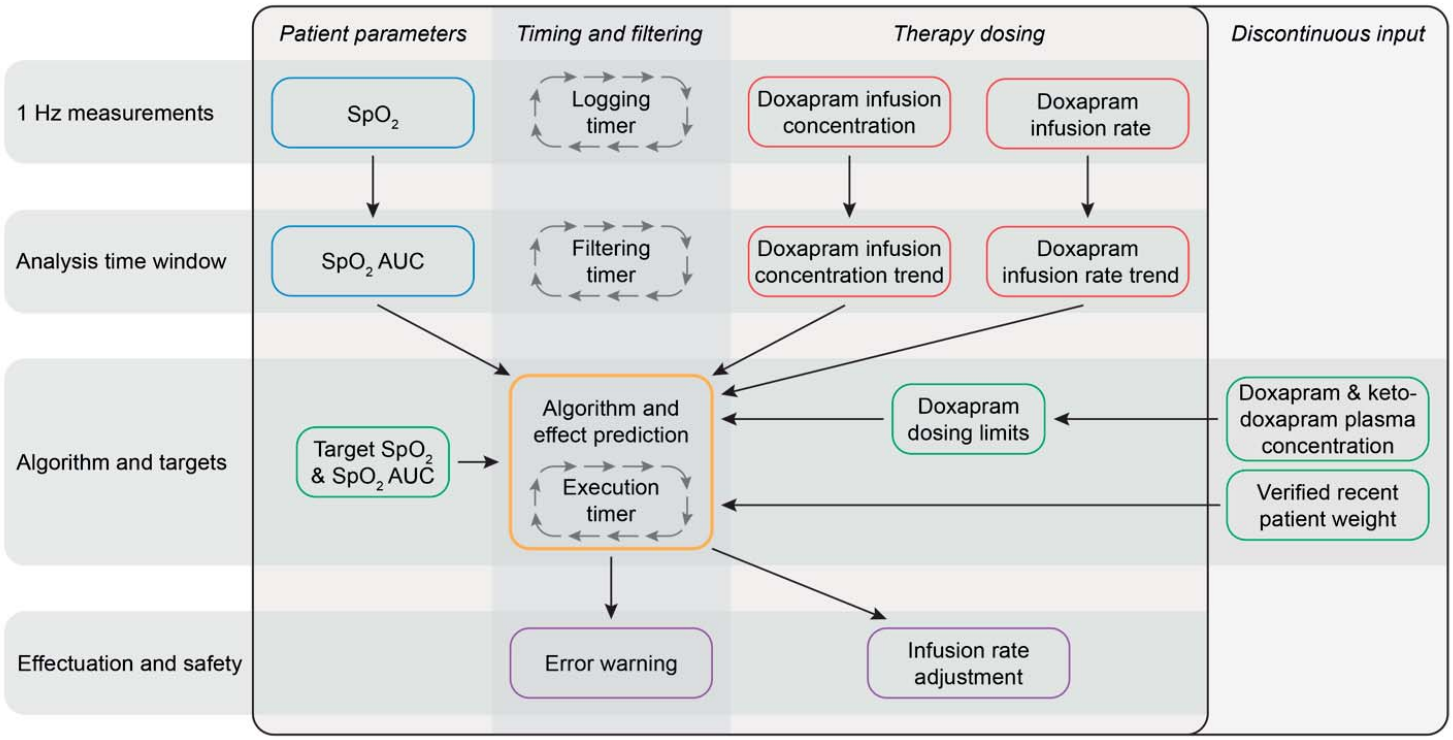

Figure 3. Closed loop design of doxapram treatment for AOP

All relevant parameters are incorporated in this closed loop design of a continuous feedback of a patient's condition and doxapram treatment effectiveness.
Blue: High frequency monitor data, Red: Dosage doxapram, Green: Settings, Yellow: Algorithm, Purple: Output
Description of the loop: *SpO_2* monitor data is collected and compared to *Target SpO_2 AUC* and may lead to *Infusion rate adjustment* of the *Current doxapram dosage* (infusion rate * infusion concentration), if within targets. Together, these are considered in the algorithm, which leads to output. A discontinuous input with Doxapram *plasma concentrations* may lead to an individualized adjustment of the *Dosing limits*.

integrated electroencephalography data[45]. Also, its applicability has been suggested for the early diagnosis and treatment of infections in preterm infants, measured by heart rate characteristics[46]. In addition, pain may be assessed using heart rate variability[47], skin-temperature, skin conductance, video-data of movements or facial expression, eye-movement, and high frequency cortisol measurement in saliva. Thus, a solely reply on subjective scores by nurses will be avoided. Apart from the effectiveness of drugs, certain side effects may be identified more easily, like agitation and respiratory depression caused by opioids.

Our approach may finally help to objectively treat apnea of prematurity, by-passing the ongoing discussions on the definition of apnea and interpretation of subjective parameters. Furthermore, the detailed information gathered by our monitoring device can facilitate circadian rhythm research, and inter- and intra-patient variability of respiratory parameters in patients with different respiratory conditions or causes for respiratory failure. This may help to determine characteristics that differentiate respiratory events of clinical consequence from normal respiratory variability in term and preterm infants. Moreover, different treatment strategies can be evaluated in real-time. This may also be interesting from a safety perspective, if these respiratory parameter data could be paralleled with amplitude integrated electroencephalography (aEEG)[30, 32]. Currently, contradictory neurodevelopmental effects have been reported of doxapram therapy in preterm infants[31, 33].

CONCLUSION

An algorithm based on profound analyses of defined patterns in oxygen saturation profiles may be able to propose dose adjustments tailored to the individual intensively monitored child. By using big data in this study, we confirmed that doxapram therapy on the one hand may be effective to treat AOP in preterm infants, but also is associated with side effects. Ultimately, dosing algorithms based on real-time monitor data need to be compared to the current golden standards in clinical practice with respect to outcome parameters such as duration of illness, side effects, and a patient's clinical condition. For some drugs and indications, implementing such algorithms may replace the need for future PK/PD studies as effectiveness and safety are continuously monitored.

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SUMMARY, CONCLUSIONS AND PERSPECTIVES

13

General discussion

AIM AND MAIN FINDINGS

The overall aim of this thesis was to optimize pharmacological treatment for preterm born infants using pharmacokinetic (PK) and pharmacodynamic (PD) principles. The investigator-initiated DINO-study (Drug dosage Improvements in NeOnates) in four different centers was funded by ZonMw and observationally investigated nine drugs with a negligible burden to subjects; i.e. acetaminophen (also known as paracetamol), fentanyl, midazolam, phenobarbital, doxapram, ibuprofen, fluconazole, sildenafil and levetiracetam.

Main findings on pharmacokinetics

Population PK-models in preterm infants have been developed, and dosage recommendations have been made for phenobarbital, fentanyl and doxapram (Table 1). Birth weight, postnatal age (PNA) and actual bodyweight were required to guide dosing of phenobarbital in term and preterm neonates (**Chapter 5**). Consequently, to immediately reach the therapeutic window the loading dose should be adjusted from 20 mg/kg to 30 mg/kg and because clearance matures with increasing PNA, at a PNA of 15 days a maintenance dose of 5 mg/kg/day should be increased by 1 mg/kg/day. For fentanyl, gestational age (GA) and PNA were found the primary covariates for clearance and actual bodyweight for volume of distribution (**Chapter 6**). The developed model showed that the youngest preterm infants, those with the lowest GA and PNA have a much higher exposure to fentanyl than older newborns when fentanyl is dosed according to bodyweight. Compared to PNA above 8 days, only 50% of the dosage would achieve a comparable exposure in preterm infants at PNA 0-4 days, and 75% of the dosage should be administered at PNA 5-8 days. Doxapram clearance was also found to be importantly influenced by GA and PNA in preterm infants (**Chapter 6**). Bioavailability of doxapram following oral administration was estimated to be 75%. The relatively low clearance associated with the lowest GA and PNA results in a higher exposure to doxapram in preterm compared to older newborns when doxapram is dosed per kg bodyweight. Doses should be increased with higher GA and increasing PNA, and when i.v. administration is switched to oral therapy, a 33% dose increase is required to reach the same plasma concentrations. An earlier randomized controlled trial, stratifying for a single dose of 10, 15 or 20 mg acetaminophen, showed that none of the acetaminophen-metabolites (glucuronide, sulphate, cysteine, mercapturate and glutathione) showed a dose-related increase of exposure (**Chapter 8**). Acetaminophen glucuronidation was found low in very preterm infants and increased with gestational age. Exposure to acetaminophen sulphate was high, but did not show saturation, which is a relevant and comfortable finding for clinical practice. For ibuprofen, simulation-based suggestions on treatment optimization showed that intermittent intravenous ibuprofen dosing regimens should

Table 1. Current neonatal dosages in Dutch Pediatric Formulary and suggestions from DINO-study for term and preterm born infants.

Drug	Current dosage Pediatric Formulary	Optimized dosage from DINO-study
Phenobarbital	Under and above 36 weeks GA IV LD: 20 mg/kg/dose, add 10 mg/kg if necessary. MD: 5 mg/kg/day in once daily	Term and preterm born infants IV LD: 30 mg/kg MD: PNA < 15 days, 5 mg/kg/day in once daily PNA > 15 days, 6 mg/kg/day in once daily
Fentanyl	Term neonate (no preterm dose) IV LD: 0.5 – 3 µg/kg/dose MD: 0.5 – 3 µg/kg/hour	Preterm born infant < 32 weeks IV LD: 2 µg/kg MD: Compared to neonates at PNA of 8 days: PNA 0-4 days 50% PNA 5-8 days 75% PNA > 8 days 100%
Doxapram	Under 37 weeks GA IV LD: 2-2.5 mg/kg MD: 0.5-2 mg/kg/hour Oral MD: 24-48 mg/kg/day in 4-24 hours per day or continuously oral	Under 30 weeks GA IV LD: 2,5 mg/kg MD: Dosage at PNA > 15 days: GA < 26 weeks: 1.2 mg/kg/h GA 26-27 weeks: 1.6 mg/kg/h GA 28-29 weeks: 2.0 mg/kg/h PNA < 15 days reduce dosage: PNA day 5-9: 50% PNA day 10-15: 75% PNA dag > 15: 100% Oral MD: 33% higher dose than IV
Ibuprofen	GA < 34 weeks IV LD: 10 mg/kg MD: 5 mg/kg/day in once daily on day 2 & 3	For illustration: a typical preterm neonate with PDA, bodyweight 840 g at PNA 1 day Suggestions for improvement: 1. Maintain above target concentration (i.e. 43 mg/L) 2. Loading dose 3. Maintenance dose divided into 2 doses 4. Increase dosage with PNA 5. Continue therapy until sufficient effect For illustration: Dosage for neonate with bodyweight 840 g at PNA 1 day IV LD: 18 mg/kg MD: < PNA 96 h: 8 mg/kg/day in twice daily > PNA 96 h: 10 mg/kg/day in twice daily

Abbreviations. IV: intravenous – LD: loading dose – MD: maintenance dose – GA: gestational age – PNA: postnatal age – PDA: patent ductus arteriosus

start with a loading dose followed by a twice daily maintenance dose that should be increased over time (**Chapter 9**). Treatment should be continued until sufficient effect has been achieved or may be terminated due to side-effects, contra-indications, or insufficient effect.

To describe the PK of drugs from the DINO-study (and other studies as well), two high performance assays have been developed for simultaneous quantification of multiple analytes in a minimal plasma volume; either for acetaminophen and six metabolites (**Chapter 3**), and for doxapram, keto-doxapram, fentanyl, cefazolin and sufentanil (**Chapter 4**).

Main findings on pharmacodynamics

The PD of doxapram in neonates was first investigated retrospectively and next with an innovative prospective approach. First, doxapram administration could prevent apnea of prematurity (AOP) and from consequent endotracheal intubation in 77% of cases during the first 48 hours treatment. Sixty-three percent of the patients did not need intubation during the entire treatment course (**Chapter 11**). Second, the effectiveness of doxapram was also shown from analyses of high frequency physiological data (**Chapter 12**). Arterial oxygen saturation (SpO_2) increased after the start of doxapram treatment alongside an increase in heart rate. This approach may help to objectively diagnose and treat AOP, by-passing the ongoing discussions on the definition of apnea and clinical interpretation of subjective parameters.

PHARMACOKINETICS

Neonatal life is characterized by fast maturational changes, mainly driven by increasing age (GA, PNA, PMA) and weight (birthweight, actual bodyweight). PK and subsequent PD display extensive between- and within-subject variability[1, 2]. PK processes concern absorption, distribution, metabolism, and excretion (ADME). Specific absorption issues for neonates are gastric acid production, skin permeability[3], and gut permeability, including first-pass effects and the ontogeny of intestinal transporters[4]. The physiology-related maturation in distribution processes is reflected in changes in body composition, protein binding and subsequent compartment size changes. Cerebral bioavailability of drugs changes during (embryonic) development, which will affect cerebral drug exposure[5]. All phase I (e.g. oxidation with cytochromes) and phase II (e.g. glucuronidation with UGTs) metabolic processes of medicines mature in an enzyme-specific pattern, while renal function (glomerular filtration rate, tubular absorption/excretion) also displays age-dependent clearance.

Changes in PK characteristics have been described through allometric approaches for interspecies scaling. In drug development, the outcomes may serve to predict PK parameters and to predict PK parameters translated from adults to children[1]. The present population PK/PD modeling approaches improved the prediction of clearance from preterm neonates to infants and may have practical use for first-in-pediatric dose selection[6]. The use of physiologically based pharmacokinetic (PBPK) modeling combining physiological knowledge of the specific subject and collected PK data may be helpful for this purpose. The ultimate aim is to find 'the tool' for the design of First-in-Human and First-in-Neonate trials, and the individualization of dosing in these therapeutic orphans[7]. Such tools could be able to incorporate environmental covariates, that is, either disease characteristics or the medical interventions related to these diseases–e.g., whole-body cooling following perinatal asphyxia[8], extracorporeal membrane oxygenation (ECMO) to treat severe circulatory or respiratory failure[9], critical illness, or nutritional strategies. The impact of these environmental covariates on compound-specific disposition or effects is highly relevant and can be better quantified when integrated in a covariate analysis[10].

Discussion on PK findings

From the PK results described in this thesis it appears that maturation is the most important covariate for clearance of phenobarbital (**Chapter 5**), fentanyl (**Chapter 6**) and doxapram (**Chapter 7**). Interestingly, for each of the drugs the separate influences of intra-uterine and extra-uterine maturation could be distinguished, reflected by gestational age or birthweight, and postnatal age or actual bodyweight, respectively. For all three drugs the most of clearance maturation takes place during the first 15 days of life and stabilizes afterwards. This means that for these three drugs neonatal dosing guidelines for at least three different periods are needed: (1) at birth (based on GA or birth weight); (2) PNA days 1-15; (3) PNA beyond day 15 (based on postnatal age or actual bodyweight). For acetaminophen, intra-uterine maturation of glucuronidation could be detected from 24 to 32 weeks GA (**Chapter 8**).

Fentanyl, phenobarbital and doxapram are mainly cleared hepatically by biotransformation; doxapram into keto-doxapram by CYP3A4 and 3A5[11]; fentanyl mostly into norfentanyl by CYP3A4[12-14]; and phenobarbital by CYP2C9, with minor metabolism by CYP2C19 and CYP2E1[15]. Although in young infants the lower CYP3A4 enzyme activity is overtaken by CYP3A7 during the first months of life, this does not hold for the metabolism of all CYP3A4 metabolized drugs, e.g. doxapram[11]. For doxapram and fentanyl comparable non-linear scaling exponents on clearance were estimated for GA and PNA. For phenobarbital and fentanyl, but not doxapram, bodyweight proved a covariate for volume of distribution. A possible effect of bodyweight on the volume of distribution of doxapram may have been missed because the small bodyweight range, and the availability of only continuous administration data prevented making an accurate estimation of the

volume of distribution. Despite the low glucuronidation capacity of acetaminophen we found in preterm neonates, sulfation did not show saturation, not even after administration of 20 mg acetaminophen per kg bodyweight. This is a relevant and comfortable finding for clinical practice, because this implicates that even high dosages can still be cleared via non-toxic sulfation instead of forcing more acetaminophen clearance via the potentially hepatotoxic oxidative CYP2E1-route. Compared to adults, very low exposure to glucuronide but higher exposures to sulfate, cysteine and mercapturate metabolites were found, which indicates different exposures to potentially hepatotoxic metabolites formed by the oxidative CYP2E1 pathway.

One of the additional values of a population PK model is the prediction of concentration-time profile for future study designs or TDM for individualized therapy. This was illustrated in this thesis with the simulation approach of ibuprofen dosages (**Chapter 9**). Although the suggestions cannot clarify the correct dosage for PDA closure at different GA and PNA, the proposed framework may help further dosage investigation.

Future steps in neonatal PK

This thesis reports three newly developed population PK-models for drugs administered to preterm born infants. Although these add to the sparse evidence, the ball needs to keep on rolling. The next step is to use the suggested dosages in clinical practice in a small cohort and to validate them by measuring blood concentrations. While the latter step is essential for a reliable translation of the PK model into optimized dosages, it is often neglected, which may even be considered unethical.

Both retrospectively and prospectively collected data could be more efficiently used. Previously collected and analysed data should be pooled and re-analysed with modern sophisticated statistical methods. The population PK model for phenobarbital (**Chapter 5**) is a clear example of the importance of data-pooling, as a PK model could not have been created based on the data of the DINO-study alone[16]. The use of data from larger cohorts may allow describing more diverse covariates with a lower incidence, such as the diversity of co-medication that may have an interaction or a co-morbidity, such as PDA or infections. Prospective data collection may be improved by anonymously collecting treatment data of all currently admitted patients to the NICU, which enables to learn from every treated patient. The use of scavenged samples collected from surplus blood or plasma from routine laboratory tests represents another opportunistic approach. These left-over samples can be used to measure drug concentrations within the context of a research protocol. Scavenged PK sampling is an attractive method to use in infant clinical trials because it increases the feasibility of biological sample collection by reducing the minimum required volume of blood as well as the number of vascular punctures (Table 2, improvement 12). The same accounts for collection of cerebrospinal fluid samples from diagnostic lumbar punctures, which is even more invasive to collect.

This approach can be combined with traditional PK samples to characterize the drug concentration-time profile and is acknowledged by the FDA as a method for minimizing risk in pediatric PK trials.

New methods should be further explored. First, a combined approach of physiology-based PK (PBPK) modeling may serve to identify gaps in our knowledge on maturational physiology (e.g. drug receptor activity, receptor expression, drug absorption, passage of blood brain barrier) and in this way may also guide fundamental research. Second, the maturational patterns and the impact of different covariates can subsequently be applied to predict in vivo time–concentration profiles for compounds that undergo similar routes of elimination. Through improved predictability, mechanism-based models can help to improve clinical care and feasibility of clinical studies in neonates[17]. De Cock et al. showed that an amikacine population PK model could adequately describe the PK of other renally eliminated drugs[18]. Third, pharmaco-metabolomics may have additional value on top of the conventional covariates in predicting drug PK[19]. Including metabolomics biomarkers can help to increase our understanding of the processes involved in drug action. Based on that, evidence-based drug dosing regimen can be developed, which will eventually reduce negative outcomes.

Such methods may enable to describe the pathophysiology of neonates over the gestational- and postnatal age spans and may even give insight in intra-uterine and pre-conceptional determinants. Due to the increasing long-term survival of preterm newborns, we are facing more chronic diseases due to morbidities at neonatal age that require chronic treatment or prophylaxis. On the other end of the spectrum, antenatal interventions enable postponing the date of delivery and/or improve the child's condition and that of the mother[20, 21].

PHARMACODYNAMICS

Age-dependent PD differences are much less explored than PK, but also relate to age and population-specific effects[22]. Consequently, dosing of medication in (preterm) neonates should be based on integrated knowledge concerning the underlying diseases, the physiological characteristics of the newborn receiving the medication, and the PK-PD parameters. Neonatal PD has specific challenges to deal with. First, specific sensitivities of receptors, organs and tissue for exposure to drugs lead to specific patterns of efficacy, toxicity and adverse drug reactions. Second, knowledge on morbidities related to birth and prematurity cannot be learned from other populations, e.g. patent ductus arteriosus, pulmonary hypertension of the newborn, AOP, perinatal asphyxia, neonatal convulsions. Third, not all morbidities may yet have been recognized. For it was only 30 years ago that one came to realize that neonates can feel pain[23]. Fourth, adequate objec-

tive effect parameters have been missing. And if an effect parameter has been defined, the challenge is to determine the target value for optimal treatment. As such, a target blood pressure for preterm born infants with hypotension has not yet been agreed on and may differ per individual newborn[24]. For most preterm neonatal drug indications, important considerations have only recently been recognized, including the maturing of receptors, genetic polymorphisms, and other differences in the pharmacokinetics and pharmacodynamics.

Discussion on PD findings

By using big data, we confirmed that doxapram therapy on the one hand may be effective to treat AOP in preterm infants, but also is associated with side effects (**Chapter 12**). Furthermore, the high frequency physiological data can facilitate circadian rhythm research, and between- and within-individual variability of respiratory parameters in patients with different respiratory conditions or causes for respiratory failure. Next steps are awaiting for a combined analyses of the PD of doxapram with either the population PK model to explore a possible target concentration, and with the administered dosage (and route of administration) to define subgroups with different targets. An algorithm based on profound analyses of defined patterns in oxygen saturation profiles may be able to propose dose adjustments tailored to the individual, intensively monitored child. This would be a very innovative approach to provide personalized medicine.

Future big (data) steps in neonatal PD

For doxapram, complete pharmacology could be reported: Quantification of drug and metabolite, population PK model, retrospective and prospective PD analyses, and implementation in daily neonatal care. Although PK-PD relationships for five drugs were major objectives of the research grant, it appeared not possible to deliver the PD for all five drugs and find clear target concentrations or exposure. This failure should not discourage investigation of PD objectives; it clearly points out the difficulty to study such endpoints, which is also experienced with treatment evaluation in daily clinical practice. Clinical implementation of continuous feedback of a patient's condition and drug effectiveness, ultimately in a closed loop design, can be especially beneficial for the most vulnerable preterm infants. Our principle can potentially be expanded to other high frequency patient parameters during NICU clinical care, such as neonatal convulsions, which may be diagnosed earlier from amplitude-integrated electroencephalography data[25]. Also, its applicability has been suggested for the early diagnosis and treatment of infections in preterm infants, measured by heart rate characteristics[26]. In addition, pain may be assessed in a multimodal way integrating heart rate variability[27], skin-temperature, skin conductance, video-data of movement- or facial expression and eye-movement tracking analyses[28]. Furthermore, determination of relevant biomarkers for

the effect of treatment using a targeted metabolomics approach may help to understand the underlying mechanisms of disease and drug effect[19, 29]. Altogether, a reply only on subjective scores by nurses and physicians will be avoided. Apart from the effectiveness of drugs, certain side effects may be identified more easily, like agitation and respiratory depression caused by opioids. Figure 1 shows a schematic representation of the implementation of PK, PD, and metabolomics into individual neonatal pharmacotherapy.
This approach may open doors to discover the role of artificial intelligence and computer learning in neonatology and detect the basic rhythm and physiology of a 'normal' preterm infant without treatment but comparable physiology. Furthermore, the detailed information gathered by our monitoring device can facilitate circadian rhythm research, and inter- and intra-patient variability of respiratory parameters in patients with different respiratory conditions or causes for respiratory failure (Table 2, Improvement 11). Ultimately, this may enable personalized pharmacotherapy by objective titration of AOP therapy and help to differentiate respiratory events of clinical consequence from normal respiratory variability. Moreover, different treatment strategies can be evaluated in real-time. This may also be interesting from a safety perspective, provided these respiratory parameter data can be paralleled with amplitude integrated electroencephalography (aEEG)[30, 31]. Ultimately, dosing algorithms based on real-time monitor data need to be compared to the current golden standards in clinical practice with respect to outcome parameters such as length of stay, duration of illness, side effects, and a patient's clinical condition.

CURRENT NEONATAL PHARMACOTHERAPY

Current neonatal pharmacotherapy differs largely between neonatal intensive care units (NICUs) and patients (**Chapter 2**). The main reason is the sparse evidence on neonatal pharmacology and the absence of consensus on the optimal treatment of most indications.

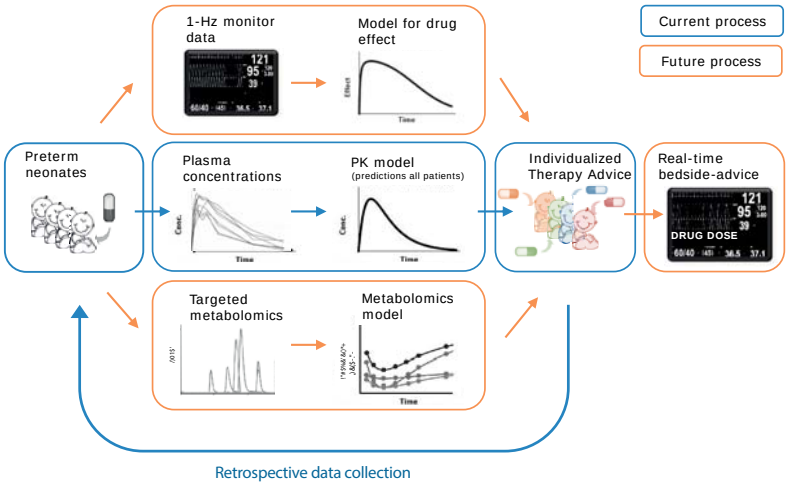

Figure 1. Schematic representation of currently implemented PK and PD in neonatal care and future perspectives.

Future process includes: automated multi center data pooling; high frequency (side-)effect registration; follow up of each patient, short and long term.

Future process stakeholders: pharmaceutical companies (finance and support); government institution (host data and anonymization); hospitals (privacy policy with patients consent).

Based on a figure designed by Swantje Völler.

'Off-label' and 'off with the label'

The differences between NICUs in prescribed drugs are the largest for drug classes with the highest proportion of off-label drugs in relation to neonatal age and are larger with decreasing PMA, although the proportion off-label prescriptions became smaller (**Chapter 2**). The high proportion of off-label drug use in neonatology is mainly due to limitations of the Summary of Product Characteristics (SmPC). These limitations concern, first, the large physiological differences between neonates. Second, premarketing studies in neonates without a known benefit and harm are generally unethical. Third, as outcome measures in neonates are hard to define, the risk of failing to find a contributive effect of a new treatment is high. Fourth, if a new drug therapy for adults may also be promising for a neonatal indication it will at some point be used in neonates, even if neonatal treatment is not mentioned in the label. Therefore, from a commercial point of view there is too little financial benefit for the manufacturer from a neonatal registration. Fifth, the process of updating the SmPC with new findings is a slow and inefficient process. Sixth, SmPCs are often unclear on their definition of a neonate, which is by the book 'an infant during the first 28 days of life'[32]. Seldom, preterm infants are addressed or specified in SmPCs, let alone a recommendation covering the whole gestational and postnatal age range.

Taking these limitations into account, the SmPC cannot be expected to describe up to date evidence for the use of each particular drug in neonates. Therefore, the main relevance of the SmPC for neonatal care could be to make new, high quality drugs available on the

market of every country. Adequate neonatal drug use should thus be determined from the current level of evidence, instead of the SmPC (Table 2, Improvement 1). Consequently, the call for pharmacological trials in clinical practice remains urgent, although trials involving neonates deal with multiple challenges. Appropriate dosing is hampered by the rapid physiological changes occurring at this stage of development. Although only few dose-finding studies in neonates have been performed, many randomized controlled trials have compared drug treatments of which the adequate dose has not been established yet. The selection of proper end-points and biomarkers is complicated by the limited knowledge of the pathophysiology of the specific diseases of infancy. Copinni et al. have provided some recommendations to stimulate drug research in neonates and infants[33]. They suggest that clinical research should be evaluated by ad hoc ethical committees with specific expertise, and that a maturational GFR model for a renally eliminated drug like amikacin should be used for dosing suggestions of multiple drugs following renal clearance.

Table 2. Suggestions to improve neonatal pharmacotherapy.

No	IMPROVEMENTS
	CLINICAL IMPLEMENTATION
1	Adequate neonatal drug use should be determined from the current level of evidence, instead of the SmPC.
2	Consensus meetings on the interpretation of available evidence about the treatment of common diseases, and development of (inter)national guidelines should receive priority.
3	Translation of research findings by PK-PD modeling into clinical practice should be discussed by clinical experts, researchers and the PK-PD modelers.
	GOVERNMENT–PHARMACEUTICAL INDUSTRY
4	Proper drug formulation should be made available worldwide, instead of being limited to certain countries due to legislation issues.
5	A global neonatal pharmacovigilance database should enable automated collection of treatment information for short- and long-term outcome, patient characteristics, (side-) effects.
6	Pharmaceutical companies should financially support a continuous international collection of data from NICUs in which their drugs are being prescribed
7	Healthcare and the pharmaceutical industry should overcome the barriers to pool data.
8	An international platform should be mandated and supervised by the government to bring consensus in treatment protocols and optimal therapy per drug–similar to the Dutch Pediatric Formulary
	FUTURE STUDY DESIGN
9	Bayesian simulations should have a more prominent role in trial design, power analyses and strategic sampling.
10	Parents should consent to the use of data generated during treatment of their infants.
11	Detailed information gathered by monitoring devices can detect the basic rhythm and physiology of a 'normal' preterm infant without treatment and facilitate circadian rhythm research and inter- and intra-patient variability of physiological parameters.
12	Scavenged samples should be used for PK analyses as an attractive method for infant clinical trials.

Availability

Despite the above-mentioned limitations, the SmPC is a requirement in each country to ensure drug availability on the market. The content of the SmPC for a certain drug often differs per country, as well as the drugs that have been made available on the market. For both issues the manufacturer of the drug is responsible. Although these differences have been reduced within the European Union, the variable availability of drugs and their formulations enlarges the between-country differences in drug use. For example, doxapram preparations in the United States contain excipients that are contra-indicated for use in neonates, while in Europe a formulation is available that is suitable for neonates. Furthermore, therapies that have proved their effectiveness in preterm newborns, such as indomethacin and vitamin A injections, are unavailable because no manufacturer is willing to market these. Strikingly, in such case a proper formulation for a drug in neonates may be developed, but legislation and too little profit are more important than optimal treatment of patients. It is unacceptable that these issues should inhibit optimal treatment of infants in certain countries (Table 2, Improvement 4).

Consensus

Chapter 1 describes drugs and indications on which little consensus has been reached in the Netherlands[16]. Consensus meetings on the interpretation of available evidence about the treatment of common diseases, and development of (inter)national guidelines should receive priority (Table 2, Improvement 2). This should be even more realistic to achieve as neonatal intensive care consists of around 10 main neonatal indications compared to the much larger variety of indications presenting at the pediatric intensive care unit. Meetings on the optimal treatment of common diseases and development of guidelines should be attended by pediatricians, neonatologists, and others.

New legislation

Despite the new legislations introduced more than a decade ago in the United States with the Pediatric Research Equity Act in 2003[34], the Food and Drug Administration Reauthorization Act of 2017[35], and in the European Union with the Pediatric Regulation in 2006[36], many drugs are still used off-label and the variability in drug prescriptions reflects the lack of evidence on drug use especially in the smallest newborns. Although these legislations have not yet led to increased licensing[37, 38]. To improve pediatric drug therapy, pediatric drug research in the pre- and post-marketing phases should be encouraged. To also encourage the generation of sufficient pediatric evidence on existing drugs, the EMA issued the *'Priority list for studies on off-patent paediatric medicinal products'*[16].

The USA and European Union have adopted pediatric clinical trial regulations to address historic deficiencies in this research. These reflect the recent paradigm shift in attitudes,

recognizing that 'the time has come to protect children and young people through research not from research'.

For that goal, an incentive for the pharmaceutical industry is required to deliver relevant evidence for safe and effective treatment. So far, trials by the pharmaceutical industry performed for this purpose generally concern a highly selected cohort. This selection is based upon the biggest chance to find an expected effect, and therefore to increase the possibility of extending the indications in the label which enables a prolonged patent. Herewith the drug remains expensive as competition is not yet allowed.

For this reason, expanding drug registrations hardly serves the aim to reach improvement of pediatric pharmacological treatment. Facilitating more investigator-initiated research would be more important as pharmaceutical companies have little benefit of incorporating new findings in pediatrics, which has led to only few drug labeling changes made under pediatric legislation.

Choosing the right dose

As stated before, adequate neonatal drug use should be determined from the current level of evidence, instead of the SmPC, although the latter is generally suggested[39]. Expert interpretation should be described in future (inter)national guidelines. Hopefully this may bring a halt to the endless number of reports on the proportion of off-label drug use in infancy and a move forward to descriptions of evidence-based treatment. It should be realized that off-label therapy does not necessarily mean off-knowledge and therefore inadequate treatment.

Recently, a continuously updated online pediatric formulary has been released in the Netherlands–the Dutch Pediatric Formulary[40]–as a next step from the periodically updated sources such as the British National Formulary, Pediatric Dosage Handbook, etc. Despite the valuable interpretation regarding dosages and safe drug use, this formulary does not suggest which drug to choose for certain indications and therefore does not help to reduce the differences in prescriptions between physicians and hospitals. For that reason, an international platform such as the International Neonatal Consortium (INC)(41), should be mandated to bring consensus in pharmacological treatments (Table 2, Improvement 8). Contradictory statements on efficacy and safety can then be placed in a good perspective, for example studies with midazolam indicating increased risk for apoptosis and worse neurological development[42], and acetaminophen with an increased asthma risk at older ages[43].

Narrowing or closing knowledge gaps through collaboration and changes

The knowledge gaps in neonatal pharmacology are tremendous and may only be narrowed or closed through collaboration of all stakeholders involved in pharmacotherapy in newborns. Ideally, all neonatal wards on the globe should be considered one obser-

vational research cohort. Ethically we are obliged to learn from exposing neonates to drugs that have not yet been properly studied. We should do better than the current situation in which thousands of (often too) small observational pediatric trials have been reported, which generally describe findings following underpowered analyses leading to a large variety in suggested adjustments. Therefore, a global database would be required for automated collection of treatment information and short- and long-term outcomes, patient characteristics and adverse drug reactions (ADRs) (Table 2, Improvement 5). Current ADR registration through governmental institution is hardly used for neonates, although prescribers are obliged by law to report ADRs (Figure 2). Over the past 5 years only 452 ADRs in neonates have been reported following drug administration during pregnancy or to neonates to the Netherlands Pharmacovigilance Centre Lareb. One hundred forty-four (32%) of these reported ADRs were related to neonatal drug administration, and 308 upon fetal drug exposure. Clearly, a very small proportion of suspected ADRs in neonates is reported here. High-quality ADR data can only be obtained if all stakeholders, including clinicians and pharmacists, are aware of the importance. Next to that, they need to be facilitated, either financially, as well as with tools for ADR recognition, and with a minimal-time-consuming infrastructure to collect and report ADRs[44, 45]. The administrative burden of health care is increasing and inefficient, for which more electronic data collection is needed. A suggestion may be to oblige pharmaceutical companies to financially support a continuous international collection of data from NICUs in which their drugs are being prescribed (Table 2, Improvement 6). Pharmaceutical companies then will contribute to learning from the exposure of their drugs to a population they have not yet sufficiently investigated pre-marketing. The cash flow for this purpose may optimally pass a governmental agency, like the EMA. The collected data may also be owned by the government, and shared with the appropriate institutions, which will optimally benefit future treatment of neonates.

Number of reported ADRs observed in neonates collected by Netherlands Pharmacovigilance Centre Lareb. The left graph concerns reported suspected ADRs upon drug administration to a neonate. The right graph reflects reported ADRs noticed either antenatal or postnatal due to fetal exposure following drug administration during pregnancy.

Therapeutic drug monitoring for interpretation

The clinical interpretation of pharmacokinetic knowledge with therapeutic drug monitoring (TDM) in neonates (reviewed in **Chapter 10**) focusses on the relevance of specific issues in neonates: Larger variability in PK, and non-PK related factors, sampling opportunities, analytical techniques, therapeutic range. Sophisticated dosing regimens derived from population PK-models can partly overcome this variability, thereby reducing the need for TDM. Dosing can be further individualized using Bayesian forecasting

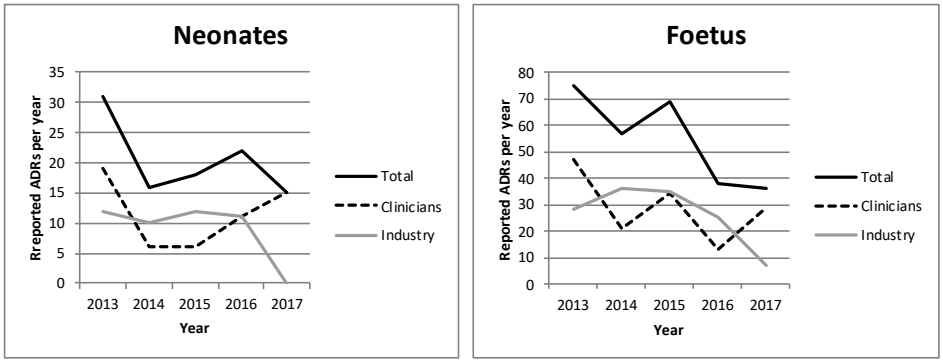

Figure 2. Reported Adverse Drug Reactions at Lareb from 2013-2017.

based on population PK models as a tool for TDM but requires an easier software-shell and a better translation towards clinicians. Also, Bayesian simulations should have a more prominent position in trial design, power analyses and strategic sampling (Table 2, Improvement 9).

Besides PK related factors, concentrations of endogenous substances (e.g. immunoglobulin A, plasma protein) in neonates differ from those in adults, which may complicate interpretation of measured drug concentrations. Blood sampling opportunities in neonates are limited by the small circulating blood volume and the need to minimize painful procedures. Consequently, most PK studies are limited to opportunistic sampling schemes. TDM as well as PK-studies need assays for simultaneous quantification of drugs and their relevant metabolites in a minimal volume of bodily fluids. Two assays have been developed for simultaneous quantification of multiple analytes in a minimal plasma volume; either for acetaminophen and six metabolites (**Chapter 3**), and for doxapram, keto-doxapram, fentanyl, cefazolin and sufentanil (**Chapter 4**). Lastly, reference values for therapeutic ranges of drugs in neonates are mostly adapted from adult studies, although PD may be quite different in neonates. Eventually, TDM will remain valuable for determination of individual exposure, but may also be unnecessary with continued individualization of treatment leading to better dose-effect relationships taking newly discovered patient characteristics into account.

Neonatal pharmacological research

Neonatal pharmacological research deals with many challenges that need to be overcome. The DINO-study was designed to observationally investigate nine drugs with additional blood collection in preterm infants born before 32 weeks GA. Despite this little burden of additional blood collection with negligible risk, only around 60% of parents of eligible children provided informed consent for participation. This relatively low rate is common in studies with preterm infants and remains a big challenge[46]. Pharmacological

treatment must be started immediately after birth, so that parents need to be asked for consent while being in an emotional roller-coaster.
If important research findings and concrete suggestions are finally not implemented, this would undermine the value of the research and moreover be unethical with regard to the participants. Responsibility for implementation lies with the clinicians, who can often not oversee the reliability and limitations of the analyses on which the suggestions were based. These limitations may concern the GA or PNA range of the studied cohort, incorporated outcome measures, and co-morbidities. Therefore, translation of research findings into clinical practice should be discussed by clinical experts and the researchers (Table 2, Improvement 3). For example, simulations using the developed phenobarbital population PK model clearly state that a higher loading dose of 30 mg/kg is required instead of 20 mg/kg (Chapter 5). Although this suggestion is in line with on-ward experience that for sufficient effect often an additional 10 mg/kg is needed on top of the 20 mg/kg loading dose, clinicians appear too reluctant to adapt this recommendation.

FUTURE BIG STEPS FOR SMALL PATIENTS

Ethical obligations on data generation

In the present time, it seems unethical to continue making only small steps towards better treatment of neonates. A change in clinical practice and research infrastructure requires the mandate of governments to decide that treatment of every individual child needs to generate evidence for collaborated optimization of future treated infants. Without an exception, every healthcare professional agrees on this perfect picture but is limited in the availability of tools for a uniform collection of patient data, scientific collaborations, and above all the required parental consent.
The available evidence cannot be changed tomorrow. Nevertheless, healthcare professionals are obliged to provide optimal treatment to their patients. This means the most we can do today would be to find consensus and use available knowledge and evidence on the optimal therapy for each indication. Just as important is the ethical obligation to constantly learn from the treatment of patients, even more when this concerns the most vulnerable preterm born infants. Every day, patients are exposed to experimental treatments in neonatal care. Strikingly, data of these practice based experimental treatments are hardly used to improve the care for newborns in the future. In modern times of high performance technological health care and electronic registration of practically all healthcare patient data, we are obliged to organize a uniform anonymous data collection that can benefit future treated patients. National, but preferably international neonatal care should be considered one big cohort of study patients. Parents should automatically allow the use of generated anonymized data during treatment of their

infants (Table 2, Improvement 10). It seems more unethical to continue treating infants with drugs that have hardly been investigated. Large studies that take the large inter- and intra-patient variability into account, are needed. The possibility for deferred consent could be considered as well[47]. This approach allows the researcher to start collecting data early in an observational study with minimal additional burden. With respect to therapeutic and intervention studies, this may only be allowed if an expert in the field of the intervention and population of the cohort approves the study protocol. Therefore, a medical ethical review board should consult a neonatologist when judging a protocol for research in (preterm) neonates to determine the burden, judge the feasibility, reliability and usability of the selected outcome parameters.

Currently available data should be used efficiently by pooling, which may overcome the often too small sample sizes in reported trials leading to contradictory reported results and doubtful interpretation in each neonatal ward. In addition, pre- and post-registration data collected by pharmaceutical companies should be made available for pooling as well. Healthcare and the pharmaceutical industry should break the borders to pool data, and to find international consensus on the interpretation of reported efficacy and safety of treatments (Table 2, Improvement 7).

The final gap, implementation

The next important gap to overcome concerns knowledge translation: the translation and implementation of relevant new evidence into clinical practice. This gap would benefit from improved communication and collaboration between the PK-PD modelers who analyzed the data and the clinicians who should implement the suggested new dosage regimens. Top Institute Pharma (TI Pharma) has been founded for this goal, as an independent research enabler of drug discovery and development. TI Pharma sets up and runs multidisciplinary partnerships that advance the development of socially valuable medicines. More comparable initiatives are yet required. A publication of a new dosage suggestion generally does not lead to a change in clinical practice. For the clinicians it may not be possible to oversee the reliability and limitations of the model-based analyses, whereas the PK-PD modelers on the other hand may suggest very complicated simulation-based dosages (Table 2, improvement 3)[48, 49]. Study design and implementation may also benefit from the close involvement of parents as ambassadors of the importance of performing trials, and the relevance of translating study results into practice.

In addition to a collaborative interpretation afterwards, the study design and success would benefit from close collaboration before start, where researchers and clinicians should discuss the aim of the population PK and PD model, the desired route of administration, co-medication, possible covariates, factors influencing between and within-subject variability.

Contribution by pharmacists

As we gain more knowledge on individualized dosages (based on GA, PNA, gender, interacting co-medication, etc.), dosing regimen become complicated and require more guidance. The dosage suggestion for paracetamol for preterm infants consists of more than ten different doses per kg for neonates depending upon their body weight group[48], which also holds for vancomycin dosage suggestions for neonates[49]. A pharmacist should be involved to translate these schemes into a workable dosage regimen for implementation in clinical practice, as well as for validation of the population PK model. Even if these schemes could be automated within drug prescribing systems, every adjustment in healthcare introduces a risk for mistakes and should be minimized to essential adjustments. Consequently, it is beyond doubt that the need is increasing for a closer participation of a pharmacist to guide drug prescribing, formulating drugs, evaluating effects, adverse drug reactions and interactions[50]. A large added value may also lie in education of clinical staff implementation-schemes, discussions on treatment consensus, pharmaco-economic choices, and prioritizing the gaps yet to be filled. Let's bring the pharmacist to the ward!

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Summary

INTRODUCTION

A prematurely born infant has not yet fully matured at the time of birth. Organs such as the lungs, central nervous system and eyes, kidneys, gastro-intestinal tract, and liver, are still developing, as well as physiological processes such as enzyme activity, coagulation and inflammatory response. This is why it is vital to start invasive, non-invasive and pharmacological support immediately after birth. There is still a general lack of evidence on the optimal treatment, and these children therefore are sometimes referred to as 'therapeutic orphans'.

CURRENT NEONATAL PHARMACOTHERAPY

The thesis opens with a short Introduction **(Chapter 1)** followed by the report of a study in which we evaluated and compared drug prescriptions in four neonatal intensive care units (NICUs) in the Netherlands during one year (**Chapter 2).** This revealed that a considerable part of the drugs administered in these NICUs is still used off-label and that drug prescriptions widely vary between NICUs. The largest variability was found for drug classes with the highest proportion of off-label drugs, i.e. cardiovascular and nervous system drugs. The lower the infants' postmenstrual age, the higher the difference in prescribed drugs between NICUs, and the lower the proportion of off-label prescriptions. This study showed for what drugs and indications little consensus has been reached, and which therefore should be prioritized for expert-interpretation of current evidence and for future research. Consensus meetings attended by pediatricians, neonatologists and clinical pharmacologists on the treatment of common diseases and development of (inter)national guidelines should have the highest priority. New investigator-initiated research is urgently required as pharmaceutical companies have little benefit of drug development for therapeutic orphans. In spite of the new pediatric legislation, only few drug labelling changes have been made.

QUANTIFICATION OF DRUGS AND METABOLITES

Chapter 3 described an assay for quantification of acetaminophen (APAP, also known as paracetamol) and its metabolites in plasma using ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-MS/MS). This assay is suitable for research purposes as well as therapeutic drug monitoring (TDM) in specific patient populations. TDM of metabolites may be indicated in case of toxicity or as part of standard clinical care in certain populations where metabolites may be used as a marker

for suspected liver injury. The assay allowed quantification of APAP and six metabolites, APAP-glucuronide, APAP-sulfate, APAP-cysteine, APAP-glutathione, APAP-mercapturate, and protein-derived APAP-cysteine in human plasma. Its strength is the combination of minimal injection volume of only 10 µL, a short runtime of 4.5 minutes, an easy sample preparation method, and the ability to quantify acetaminophen and all six metabolites. This assay was successfully validated for clinical practice and greatly facilitates further research into acetaminophen and metabolites, as well as for TDM purposes, even in the smallest plasma volumes obtained from preterm infants.

Chapter 4 describes a method for the simultaneous quantification of fentanyl, sufentanil, doxapram, its active metabolite keto-doxapram and cefazolin in human plasma by UPLC-MS/MS. This method greatly facilitates further research into these frequently used drugs from different therapeutic classes as well as possible TDM purposes, even in the smallest plasma volumes. The strength of this method is the combination of a small sample volume, a short run-time, a deuterated internal standard, an easy sample preparation method and the ability to simultaneously quantify all analytes in one run.

PHARMACOKINETICS IN PRETERM INFANTS

In the study reported in **Chapter 5,** a population pharmacokinetic model for phenobarbital was developed based on previously collected TDM data of term and preterm newborns. This model was subsequently externally validated using prospective phenobarbital data from the pharmacokinetic DINO-study (Drug dosage Improvements in NeOnates) in preterm neonates. Clearance (CL) and volume of distribution for a child with a birthweight of 2.6 kg at postnatal age (PNA) day 4.5 were 0.0091 L/h (9%) and 2.38 L (5%), respectively. Birthweight and PNA were the best predictors for clearance maturation. Clearance increased by 36.7% per kg birthweight and 5.3% per PNA day of living. The best predictor for the increase in volume of distribution was actual bodyweight (0.31 L/kg). External validation showed that the model can adequately predict the neonatal pharmacokinetics in a prospective study. From the results it seems that for phenobarbital, both PNA and bodyweight should guide dosing, both in term and preterm neonates. To immediately reach the therapeutic window, the loading dose should be adjusted from 20 mg/kg to 30 mg/kg, while the maintenance dose should be increased from 5 to 6 mg/kg/day after a PNA of 15 days. This study illustrates that data sharing and external validation can lead to model-based clinical dose optimization when ethical and practical constraints limit the possibilities to perform large studies, such as in the neonatal age group.

Chapter 6 describes a two-compartment population pharmacokinetic model of fentanyl in preterm infants. Clearance and central volume of distribution for a neonate with a

gestational age (GA) of 26.9 weeks and a bodyweight of 1kg at PNA day 3 were 0.409 L/h (7%) and 6.49 L (12%), respectively. GA and PNA were the best predictors for clearance maturation. Clearance increased with an exponent of 0.545 (9 %) for PNA per day and an exponent of 5.35 (13 %) per week of gestation, respectively. The best predictor for the increase in volume of distribution was actual bodyweight with an exponent factor of 2.41 (12 %). Simulations show that when fentanyl dosing is based on actual bodyweight (same dose in microgram/kg for all subjects), a lower PNA and GA are associated with higher initial concentrations and a slower decrease in plasma concentrations. Nevertheless, currently worldwide one equal dosage per kg actual bodyweight is used for all preterm infants, on account of which the exposure to fentanyl is highest in the youngest preterm infants. Consequently, we suggest lower dosages per kg bodyweight in the youngest preterm infants, especially in the first days of life. Compared to PNA above 8 days, only 50% of the dosage would achieve a comparable exposure in preterm infants at PNA 0-4 days, and 75% of the dosage should be administered at PNA 5-8 days.

Chapter 7 reports a population pharmacokinetic model of doxapram and its active metabolite keto-doxapram in preterm infants. Such a model was not available in the literature. Based on a relatively low clearance compared to newborns at older ages when dosed per kg bodyweight, the lowest GA and PNA were found associated with a higher exposure to doxapram. At PNA day 1 clearance of doxapram for a neonate born after 25 weeks GA was estimated to be 15% of clearance on day 30, and 82% on day 15. Therefore, doses should be increased with higher GA and increasing PNA. Oral bioavailability was estimated to be 75%. When intravenous administration is switched to oral therapy, a 33% increase in dose is required to reach similar plasma concentrations.

Chapter 8 addresses the exposure to acetaminophen and its metabolites (glucuronide, sulphate, cysteine, mercapturate and glutathione) in very preterm infants with a GA ranging from 24-32 weeks after a single dose of 10, 15 or 20 mg intravenous acetaminophen per kg bodyweight. Analysis showed that the higher the dose, the higher the exposure, which theoretically can also result in higher efficacy. Importantly, in none of the patients a dose-related increase of exposure was found for any of the acetaminophen-metabolites when corrected for the exposure to acetaminophen. Already detected from 24 to 32 weeks GA, acetaminophen glucuronidation was low in extremely preterm infants and increased with GA. Exposure to acetaminophen sulfate was high, but did not show saturation, not even after administration of 20 mg acetaminophen per kg bodyweight. This is a relevant and comforting finding for clinical practice because in preterm neonates the glucuronidation capacity is still low and metabolites formed through the oxidative CYP2E1 pathway are potentially hepatotoxic. Compared to adults, very low exposure to glucuronide but higher exposure to sulfate, cysteine and mercapturate metabolites was found, of which the relevance is not yet known.

Chapter 9 combined evidence on the effectiveness of intravenous ibuprofen dosage regimens on closure of the patent ductus arteriosus (PDA) and on the mechanism of action, with a previously developed (R)/(S)-ibuprofen population pharmacokinetic model. Based on reported effectiveness of ibuprofen and its mechanism of action, we suggest that intravenous treatment should start with a high loading dose followed by a maintenance dosage given twice daily. The latter should be increased with PNA, and continued until ductal closure has been achieved. For a typical neonate with PDA with birthweight 840 grams at PNA 24 hours, we recommend a loading dose of 18 mg/kg, followed by 8 mg/kg/day given in two doses. After 96 hours the dose should be increased to 10 mg/kg/day given in two doses until sufficient effect has been achieved or treatment needs to be terminated due to adverse drug reactions, contra-indications, or insufficient effect. Thus, COX-2 may be sufficiently inhibited, without exposing preterm infants to unnecessarily high concentrations ibuprofen.

Chapter 10 reviews the implementation in neonatal care of pharmacokinetic knowledge via TDM, with a focus on specific issues concerning TDM in neonates: larger variability in pharmacokinetics (PK), and non-PK related factors, sampling opportunities, analytical techniques, therapeutic range. Sophisticated dosing regimens derived from population PK-models can partly overcome the variability in exposure, thereby reducing the need for TDM. Dosing can be further individualized using Bayesian forecasting. Besides PK related factors, concentrations of endogenous substances (e.g. immunoglobulin A, plasma protein) in neonates differ from those in adults, which may complicate interpretation of measured drug concentrations. Blood sampling opportunities in neonates are limited by the small blood volume and the need to minimize the number of painful procedures. Lastly, reference values for therapeutic ranges of drugs in neonates are mostly adapted from adult studies, although pharmacodynamics may be quite different in neonates.

PHARMACODYNAMICS IN PRETERM INFANTS

Chapter 11 evaluates whether doxapram could prevent the need for endotracheal intubation and identifies the predictors of success. We found that 77% of the newborn infants with apnea of prematurity (AOP) did not need endotracheal intubation during the first 48 hours of doxapram treatment and that 63% of patients did not need intubation during the entire treatment course. Such an effective treatment is valuable, because prolonged hypoxemic episodes among extremely preterm infants during the first two to three months after birth have been associated with adverse outcomes at 18 months (increased risk of late death or disability). As alternatives to doxapram therapy to avoid endotracheal intubation are limited, this drug could obtain a prominent position in

neonatal intensive care, with high PNA at the start of therapy and a lower fraction of inspired oxygen (FiO_2) being the main predictors of success.

Chapter 12 reports a pilot study on the usefulness and applicability of high frequency physiological data for analyses of pharmacotherapy. Arterial oxygen saturation (SpO_2) increased after the start of doxapram treatment alongside an increase in heart rate. The respiratory rate remained unaffected. The number of saturation dips and the time below a saturation of 80%, as well as the area under the 80%-saturation-time curve (AUC), were significantly lower after the start of doxapram. The AUC under 90% saturation also significantly improved after start of doxapram. This new principle of translated high-frequency monitoring data can be used to evaluate pharmacotherapy. Using second-to-second physiological data, the respiratory condition could objectively be determined and therewith the detailed effects of doxapram treatment in preterm infants. This confirmed that while doxapram therapy may be effective to treat AOP in preterm infants, it also is associated with side effects. Combining such 'big' monitor data with patient characteristics, drug dosages and drug concentrations will help to quantify drug therapeutic effects in preterm infants in future clinical trials and practice. It will then be possible to improve treatment by giving detailed dosing advice tailored to the individual patient. An algorithm based on profound analyses of defined patterns in oxygen saturation profiles may be able to propose individual dose adjustments. For some drugs and indications, implementing such algorithms may replace the need for future PK/PD studies as effectiveness and safety are continuously monitored.

In **Chapter 13**, we discuss our main findings, compare them with the current literature and make recommendations for implementation and future research to improve drug therapy in neonates.

The developed population PK models for fentanyl, phenobarbital and doxapram all show maturation of clearance leading to adjusted dosages with respect to gestational and postnatal age, instead of the currently used equal dosage per kg bodyweight for all ages. Despite the difficulty of evaluating therapy in neonates, our progress with regard to doxapram PD may serve as a basis for other drug therapies. We made a relevant contribution to neonatal care, nevertheless we emphasize the need for more knowledge on drug therapy specifically in preterm born infants, which concerns a combination of pharmacokinetics, pharmacodynamics, metabolomics, and physiology.

Several improvements have been suggested for neonatal care as well as for future research. For neonatal care; appropriate formulations of drugs for neonates should be made available in all countries, consensus on the interpretation of currently available evidence should be published in (inter)national guidelines, and more research findings should be translated to neonatal practice. Future neonatal research would benefit from; a more efficient organisation of neonatal clinical trials, the use of scavenged samples for PK analyses, a global neonatal pharmacovigilance database, more collaboration

and data sharing in special populations. The discussion ends with the statement that a pharmacist has an important contribution to the improvement of neonatal pharmacotherapy.

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Samenvatting

INTRODUCTIE

Een prematuur geboren baby is nog niet volledig ontwikkeld bij geboorte. Organen zoals de longen, hersenen, ogen, nieren, maag-darmkanaal en lever zijn zich nog aan het ontwikkelen, alsmede de fysiologische processen zoals enzymactiviteit, stolling en ontstekingsrespons. Daartoe is het van levensbelang om direct na geboorte invasieve, non-invasieve en farmacologische ondersteuning te starten. Er bestaat nog steeds een groot gebrek aan bewijsvoering voor de optimale therapie, waardoor deze kinderen ook wel '*therapeutic orphans*' worden genoemd.

HUIDIGE NEONATALE FARMACOTHERAPIE

Het proefschrift start met een korte introductie **(Hoofdstuk 1)** gevolgd door een onderzoek waarin we de voorgeschreven geneesmiddelen gedurende 1 jaar evalueren en vergelijken tussen de 4 neonatale intensive care units (NICUs) in Nederland **(Hoofdstuk 2)**. Hieruit bleek dat een aanzienlijk deel van de toegediende medicatie op deze NICUs nog steeds off-label is en dat er grote verschillen bestaan in de voorgeschreven geneesmiddelen. De grootste verschillen werden vastgesteld bij de geneesmiddelgroepen met het grootste aandeel off-label, te weten cardiovasculaire en centraal werkende medicatie. Verder bleek dat bij lagere postmenstruele leeftijd, de verschillen in voorgeschreven medicatie tussen de NICUs groter werd, en het aandeel off-label voorschriften juist kleiner. Middels dit onderzoek konden we de geneesmiddelen en indicaties benoemen waarover nog weinig consensus is bereikt en daartoe prioriteit verdienen voor beoordeling van bewijsvoering door experts en voor toekomstig onderzoek. Consensus bijeenkomsten met kinderartsen, neonatologen en klinisch apothekers over de behandeling van veelvoorkomende aandoeningen en de ontwikkeling van (inter)nationale richtlijnen, dienen prioriteit te krijgen. Nieuw *investigator-initiated* onderzoek is noodzakelijk aangezien ontwikkeling van geneesmiddelen voor '*therapeutic orphans*' de farmaceutische industrie weinig oplevert. Ondanks de nieuwe wetgeving voor onderzoek bij kinderen heeft dit nog maar in enkele aanpassingen van het label geresulteerd.

BEPALEN VAN GENEESMIDDELEN EN METABOLIETEN

Hoofdstuk 3 beschrijft een bepalingsmethode voor paracetamol (APAP) en zijn metabolieten in plasma met *ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-MS/MS)*. Deze methode is zowel geschikt voor onderzoeksdoeleinden als voor *therapeutic drug monitoring (TDM)* in specifieke

patiënt populaties. TDM van de metabolieten kan toegevoegde waarde hebben in geval van toxiciteit of tijdens standaardtherapie van specifieke patiënt populaties waar metabolieten een marker zouden kunnen zijn bij verdenking op levertoxiciteit. Middels deze bepalingsmethode kunnen APAP en 6 metabolieten gemeten worden, APAP-glucuronide, APAP-sulfaat, APAP-cysteine, APAP-glutathion, APAP-mercapturaat, en uit eiwit verkregen APAP-cysteine in plasma. De sterkte van de methode is de combinatie van een minimaal injectievolume van 10 µL, een korte looptijd van 4,5 minuten, een eenvoudige sample voorbewerking en de mogelijkheid om paracetamol en 6 metabolieten te bepalen. De methode was eveneens gevalideerd op patiëntensamples en is van grote toegevoegde waarde voor toekomstig onderzoek naar paracetamol en metabolieten, alsook voor TDM-doeleinden, zelfs in de kleinste volumina verkregen van prematuur geboren kinderen.

Hoofdstuk 4 beschrijft een bepalingsmethode voor gelijktijdige meting van fentanyl, sufentanil, doxapram, zijn actieve metaboliet keto-doxapram en cefazoline in humaan plasma met UPLC-MS/MS. Deze methode maakt toekomstig onderzoek mogelijk van deze frequent toegepaste geneesmiddelen uit diverse geneesmiddelgroepen alsmede de mogelijkheid voor TDM, zelfs in de kleinste plasma volumina. Deze methode onderscheidt zich door de combinatie van een kleine sample volume, een korte looptijd, het gebruik van een gedeutereerde interne standaard, een eenvoudige sample voorbewerking en het gelijktijdig kunnen meten van meerdere componenten in 1 run.

FARMACOKINETIEK IN PREMATUUR GEBOREN KINDEREN

Het beschreven onderzoek in hoofdstuk 5 betreft de ontwikkeling van een populatie farmacokinetiek model van fenobarbital op basis van eerder verzamelde TDM-data bij aterm en prematuur geboren kinderen. Dit model is vervolgens gevalideerd met prospectief verzamelde fenobarbital data van de farmacokinetiek DINO-studie (Drug dosage Improvements in NeOnates) in prematuur geboren kinderen. Klaring (CL) en verdelingsvolume van een kind met een geboortegewicht van 2,6 kg bij een postnatale leeftijd (PNA) van 4,5 dagen waren 0,0091 L/u (9%) en 2,38 L (5%), respectievelijk. Geboortegewicht en PNA waren de beste voorspellers voor de maturatie van klaring. Klaring nam met 36,7% toe per kg geboortegewicht en met 5,3% per PNA dag, respectievelijk. De beste voorspeller voor de toename van verdelingsvolume was actueel lichaamsgewicht (0,31 L/kg). De externe validatie liet zien dat het model een adequate voorspelling kan geven van de prospectief verzamelde neonatale farmacokinetiek data. Uit de resultaten is af te leiden dat de fenobarbital dosering rekening moet houden met zowel PNA als lichaamsgewicht, in atermen en in prematuren. Om onmiddellijk in therapeutisch gebied te bereiken, dient de oplaaddosering verhoogd te worden van 20

mg/kg naar 30 mg/kg, en de onderhoudsdosering van 5 mg/kg naar 6 mg/kg bij een PNA boven 15 dagen. Dit onderzoek illustreert dat het delen van data en het uitvoeren van een externe validatie kan leiden tot geoptimaliseerde doseringen gebaseerd op het model, daar waar ethische en praktische bezwaren de mogelijkheden beperken voor het uitvoeren van een groot onderzoek, zoals bij neonaten.

Hoofdstuk 6 beschrijft een 2-compartiment farmacokinetiek model van fentanyl in prematuur geboren kinderen. Klaring en verdelingsvolume van een neonaat met een zwangerschapsduur (GA) van 26,9 weken en een lichaamsgewicht van 1 kg bij PNA dag 3 waren 0,409 L/u (7%) en 6,49 L (12%), respectievelijk. GA en PNA waren de beste voorspellers voor maturatie van klaring. Klaring nam toe met een exponent van 0,545 (9%) voor PNA per dag en een exponent van 5,35 (13%) per week zwangerschapsduur. De beste voorspeller voor de toename van verdelingsvolume was actueel lichaamsgewicht met een exponent van 2,41 (12%). Simulaties laten zien dat wanneer fentanyl enkel wordt gedoseerd op actueel lichaamsgewicht (een gelijke dosering in microgram/kg voor alle patiënten), bij een lagere PNA en GA hogere aanvang concentraties worden bereikt en een langzamere afname van de plasmaconcentraties. Desalniettemin wordt er momenteel wereldwijd één gelijke dosering per kg lichaamsgewicht gebruikt voor alle prematuur geboren kinderen, waardoor de blootstelling aan fentanyl het grootst is in de jongste prematuren. Daartoe stellen we voor een lagere dosering per kg lichaamsgewicht te geven aan de jongste prematuren, met name in de eerste dagen na geboorte. In vergelijking met een prematuur bij PNA boven 8 dagen, zou een prematuur bij PNA dag 0-4 slechts 50% van de dosering moeten krijgen, en bij PNA 5-8 dagen slechts 75%.

Hoofdstuk 7 beschrijft een populatie farmacokinetiek model van doxapram en de actieve metaboliet keto-doxapram in prematuur geboren kinderen. Een dergelijk model is nog niet eerder gepubliceerd. Vanwege de relatief lage klaring bij prematuren was bij doseren per kg lichaamsgewicht een hogere blootstelling gevonden bij lagere zwangerschapsduur en postnatale leeftijd. Op dag 1 na geboorte bij 25 weken zwangerschapsduur werd de klaring geschat op 15% in vergelijking met dag na geboorte 30, en 82% op dag 15. Daartoe dient de dosering toe te nemen bij hogere zwangerschapsduur en postnatale leeftijd. Orale biologische beschikbaarheid werd geschat op 75%. Dit betekent dat wanneer intraveneuze toediening wordt veranderd naar orale toediening, de dosering 33% verhoogd moet worden om vergelijkbare plasmaconcentraties te bereiken.

Hoofdstuk 8 beschrijft de blootstelling aan paracetamol en metabolieten (glucuronide, sulfaat, cysteine, mercapturaat en glutathion) in zeer prematuur geboren kinderen met een zwangerschapsduur van 24 tot 32 weken na een enkele intraveneuze dosis van 10, 15 of 20 mg per kg lichaamsgewicht. Uit de analyse blijkt dat een hogere dosering tot een hogere blootstelling leidt, wat theoretisch in meer effectiviteit zal resulteren. Belangrijk is de bevinding dat bij geen van de patiënten een verhoogde blootstelling aan

een van de paracetamol-metabolieten werd gevonden als deze gecorrigeerd werd voor de blootstelling aan paracetamol. Bij de patiënten met een zwangerschapsduur van 24 tot 32 weken werd een lage paracetamol glucuronidering vastgesteld, waarbij al wel een toename zichtbaar was met toenemende zwangerschapsduur. De blootstelling aan paracetamolsulfaat was hoog, maar liet geen teken van saturatie zien, zelfs niet bij hoge doseringen van 20 mg paracetamol per kg. Dit is een belangrijke en geruststellende bevinding voor de klinische praktijk, met name omdat de glucuronideringscapaciteit in prematuren nog laag is en de alternatieve oxidatieve route via CYP2E1 tot de vorming van potentieel hepatotoxische metabolieten leidt. In vergelijking met volwassenen zien we in prematuren een erg lage blootstelling aan glucuronide maar een hoge blootstelling aan sulfaat, cysteine en mercapturaat metabolieten, waarvan de betekenis nog niet bekend is.

In **Hoofdstuk 9** wordt de bewijsvoering voor de effectiviteit van intraveneuze ibuprofen doseerregimes voor de behandeling van patent ductus arteriosus (PDA) en het werkingsmechanisme gecombineerd met een eerder ontwikkeld populatie farmacokinetiek model voor (R)/(S)-ibuprofen. Gebaseerd op de gepubliceerde effectiviteit van ibuprofen en he werkingsmechanisme, stellen we voor de intraveneuze behandeling te starten met een hoge oplaaddosering gevolgd door een onderhoudsdosering verdeeld over 2 giften per dag. De onderhoudsdosering dient te worden verhoogd met de postnatale leeftijd, en gecontinueerd te worden tot de ductus gesloten is. Voor een typische neonaat met PDA met een geboortegewicht van 840 gram en een postnatale leeftijd van 24 uur stellen we een oplaaddosis van 18 mg/kg voor gevolgd door een onderhoudsdosering van 8 mg/kg/dag in 2 doses. Na 96 uur dient de dosering verhoogd te worden naar 10 mg/kg/dag in 2 doses tot voldoende effect is bereikt of de behandeling gestaakt dient te worden vanwege bijwerkingen, contra-indicaties of onvoldoende effect. Hierdoor wordt voldoende COX-2 remming bereikt, zonder prematuur geboren kinderen bloot te stellen aan onnodig hoge concentraties ibuprofen.

Hoofdstuk 10 betreft een review over de implementatie van farmacokinetische kennis in neonatale zorg door gebruik van TDM, met een focus op specifieke aspecten voor TDM bij neonaten: grote variabiliteit in farmacokinetiek, en niet farmacokinetiek-gerelateerde zaken, sample mogelijkheden, analysetechnieken, en de therapeutische range. Verfijnde doseerregimes afgeleid van populatie farmacokinetiek modellen kunnen deels de variabiliteit in blootstelling verkleinen, en daarmee de noodzaak voor TDM verminderen. Verdere individualisatie van de dosering kan bereikt worden dor gebruik te maken van Bayesiaanse voorspellingen. Naast de farmacokinetiek gerelateerde aspecten, kunnen concentraties van endogene componenten (bijv. immunoglobuline A plasma eiwit) sterk verschillen in neonaten ten opzichte va volwassenen, waardoor de interpretatie van gemeten geneesmiddel concentraties bemoeilijkt kan worden. Bloedafname mogelijkheden in neonaten zijn erg beperkt door het klein circulerend

bloedvolume en de noodzaak tot het minimaliseren van het aantal pijnlijke handelingen. Tot slot, de referentiewaarden van therapeutische ranges van geneesmiddelen in neonaten zijn over het algemeen overgenomen van die van studies met volwassenen, hoewel de farmacodynamiek aanzienlijk kan verschillen in neonaten.

FARMACODYNAMIEK IN PREMATUUR GEBOREN KINDEREN

Hoofdstuk 11 evalueert in hoeverre doxapram in staat is om endotracheale intubatie te voorkomen en benoemt de voorspellers voor succes. Daarbij bleek dat 77% van de neonaten met apneus door prematuriteit geen endotracheale intubatie nodig hadden gedurende de eerste 48 uur van de doxapram behandeling en dat 63% überhaupt niet hoefde te worden geïntubeerd gezien ver de totale behandelduur. Een behandeling met dermate veel effectiviteit is zeer waardevol omdat aanhoudende hypoxische episodes bij extreem prematuur geboren kinderen in de eerste 2-3 maanden na geboorte in verband staat met slechte uitkomsten op een leeftijd van 18 maanden (verhoogde kans op overlijden en gehandicapt). Aangezien de alternatieven voor doxapram behandeling om intubatie te voorkomen beperkt zijn, zou dit geneesmiddel een belangrijke plek kunnen krijgen binnen de intensieve neonatale zorg, waarbij een hoge postnatale leeftijd bij start van doxapram en een lage zuurstof fractie in de toegediende lucht (FiO_2) de belangrijkste voorspellers voor succes bleken.

Hoofdstuk 12 beschrijft een pilot-studie naar de meerwaarde en de toepasbaarheid van hoog-frequente fysiologische data voor beoordeling van farmacotherapie. Arteriële zuurstofspanning (SpO_2) nam toe na start van doxapram behandeling, tezamen met een toename van de hartfrequentie. De ademhalingssnelheid bleef onveranderd. Het aantal dalingen van de zuurstof saturatie, de tijd onder een saturatie van 80%, en het oppervlak onder de 80%-saturatie-tijd-curve (AUC), waren allen significant lager na het starten van doxapram. De AUC onder de 90%-saturatie was ook significant verbeterd na start van doxapram. Deze nieuwe benadering van vertaling van hoog-frequente patiëntmonitordata kan gebruikt worden voor de evaluatie van farmacotherapie. Met gebruik van perseconde fysiologische data kan de respiratoire conditie objectief worden beoordeeld, en daarmee het effect van de doxapram behandeling bij prematuur geboren kinderen. Hiermee werd bevestigd dat doxapram naast het effect, ook bijwerkingen tot gevolg heeft. Het combineren van 'big' monitor patiënten data , geneesmiddeldoseringen en de geneesmiddelconcentraties zijn veelbelovend voor het kwantificeren van geneesmiddeleffecten in prematuur geboren kinderen in toekomstig onderzoek en in de praktijk. Daarbij kan behandeling worden verbeterd door een exact doseeradvies toegespitst op de individuele patiënt. Een algoritme gebaseerd op grondige analyses van patronen in zuurstofsaturatie zou ik staat kunnen zijn individuele doseeradviezen voor te stellen.

Voor enkele geneesmiddelen en indicaties zou de implementatie van dergelijke algoritmes de noodzaak tot farmacokinetiek-farmacodynamiek studies kunnen vervangen, aangezien effectiviteit en veiligheid continu gemonitord worden.

In **hoofdstuk 13** bediscussiëren we de belangrijkste bevindingen, vergelijken deze met de huidige literatuur en formuleren aanbevelingen voor implementatie en toekomstig onderzoek om de farmacotherapie bij neonaten te verbeteren. De ontwikkelde populatie farmacokinetiek modellen voor fentanyl, fenobarbital en doxapram laten alle drie maturatie van klaring zien, wat aangepaste doseringen vraagt op basis van zwangerschapsduur en postnatale leeftijd in plaats van de huidige gelijke dosering per kg lichaamsgewicht voor alle leeftijden. Ondanks het feit dat evalueren van therapie bij neonaten erg moeilijk is, kan onze progressie op het gebied van doxapram farmacodynamiek als basis dienen voor andere geneesmiddelen. We hebben een relevante bijdrage geleverd aan de neonatale zorg, desondanks benadrukken we de behoefte aan meer kennis over farmacotherapie met name bij prematuur geboren kinderen. Deze kennis betreft een combinatie van farmacokinetiek, farmacodynamiek, metabolomics, en fysiologie.

Diverse verbeteringen worden voorgesteld voor zowel neonatale zorg als voor toekomstig onderzoek. Ten aanzien van neonatale zorg betreft dit onder andere; geschikte geneesmiddelformuleringen voor neonaten dien beschikbaar te zijn in alle landen, consensus dient te worden bereikt over de interpretatie van beschikbare bewijsvoering en zou opgenomen moeten worden in (inter)nationale richtlijnen, en meer onderzoeksresultaten zouden vertaald moeten worden naar de neonatale praktijk. Toekomstig onderzoek zou erg gebaat zijn bij; een efficiëntere organisatie van neonataal onderzoek, het gebruik van restmateriaal van samples voor farmacokinetiek analyses, een wereldwijde farmacovigilantie database, meer samenwerking en het delen van data van bijzondere patiëntgroepen. De discussie sluit af met de stelling dat een apotheker een belangrijke bijdrage levert aan de verbetering van neonatale farmacotherapie.

APPENDICES

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PHD PORTFOLIO

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PhD Period	June 2013 - May 2018
Promotors	Prof. dr. D. Tibboel
	Prof. dr. D.M. Burger
Co-promotor	Dr. S.H.P. Simons

PHD TRAINING	**YEAR**	**ECTS**
General courses		
Scientific Integrity [Erasmus MC]	2016	0,3
Biomedical English Writing [Erasmus MC]	2016	1,0
Biostatistical Methods I: Basic Principles [NIHES]	2015	2,0
BROK-course [NFU BROK Academy]	2013	1,0
Endnote and Pubmed course [Erasmus MC]	2013	1,0
Specific Courses & Workshops		
European Medicines Agency workshop, London, United Kingdom	2018	0,3
ZonMw GGG meeting, Amsterdam, the Netherlands	2016	0,2
NONMEM PK modeling course, Slotervaart, Amsterdam	2015	0,5
Interpretation of population pharmacokinetics, Leiden	2015	0,2
Open Clinica course [Erasmus MC]	2015	0,2
PhD Day [Radboudumc]	2014	0,3
ZonMw GGG meeting, Amsterdam, the Netherlands	2014	0,2
WinNonLin course, Nijmegen [Radboudumc]	2013	1,0
Presentations at Conferences		
Dutch Society for Pediatrics meeting, Arnhem, the Netherlands [oral]	2018	0,3
Joint European Neonatal Societies, Venice, Italy [oral]	2017	1,0
Pediatric Academic Societies, San Francisco, USA [poster presentation]	2017	1,0
Dutch Hospital Pharmacy Congress, Utrecht, the Netherlands [oral]	2017	0,3
Dutch Hospital Pharmacy Congress, Den Bosch, the Netherlands [oral]	2015	0,3

Joint European Neonatal Societies, Barcelona, Spain [oral]	2015	1,0
Dutch Society for Pediatrics meeting, Vedhoven, the Netherlands [workshop]	2015	0,3
Dutch Hospital Pharmacy Congress, Rotterdam, the Netherlands [oral]	2014	0,3
European Acacemy for Paediatric Society, Barcelona, Spain [oral]	2014	1,0
Dutch Society for Pediatrics congress, Veldhoven, the Netherlands [oral]	2014	0,3
Dutch Hospital Pharmacy Congress, Den Bosch, the Netherlands [poster]	2013	0,3

Conferences

Farewell symposium prof. Tibboel, Rotterdam, the Netherlands	2018	0,3
FIGON Dutch Medicines Days, Ede, the Netherlands	2017	0,3
Young Researchers Day, Tulips, Maarssen, the Netherlands	2016	0,3
Child Health symposium, Tulips, Noordwijk, the Netherlands	2016	0,3
Pharmacometrics meeting, Nijmegen, the Netherlands	2015	0,3
Young Researchers Day, Tulips, Maarssen, the Netherlands	2015	0,3
Farewell symposium prof. Lie-A-Huen, Amsterdam, the Netherlands	2014	0,1
Pediatric anesthesiology 'Jackson Rees' symposium, Roterdam, the Netherlands	2013	0,3

Local research meetings

Research meetings neonatology (weekly)	2015-2018	2,0
Pediatric pharmacology meetings (weekly)	2015-2018	2,0
Clinical pharmacology meetings, Erasmus MC (weekly)	2015-2018	2,0
Clinical pharmacology meetings, Radboudumc (weekly)	2013, 2014	1,0

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Pediaric and obstetric nurses, perinatal pharmacology [Radboud Academy]	2013-2018	1,0
Pediatric course organisation for resident hospital pharmacists	2014-2018	2,0
Master medical students, drug during pregnancy [Erasmus MC]	2017-2018	0,5
Supervision master thesis Nienke Halbmeijer	2014-2015	2,0

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ABOUT THE AUTHOR

Robert Flint was born on December 17 1978 in Winschoten. He received his Atheneum degree at Laar & Berg in Laren in 1998 and subsequently enrolled in the Pharmaceutical Sciences program at Utrecht University. During his study years, he completed a research project at the University of Santiago in Chile in 2002 on the characterization of dopaminergic cell lines, assessing which cells may be beneficial for treatment of Parkinson's disease (supervisors dr. E. Ronken and dr. P. Caviedes). Furthermore, he performed a project on the implementation of a pharmaceutical management system at the Nsambya Hospital in Kampala, Uganda (supervisor dr. W. Rutten). In Utrecht, he had joined the Utrecht student sailing union 'Histos' and for a full year acted as a board member. He was supported by the Utrecht University to participate in international sailing regattas.

After having obtained the master's degree in 2006, Robert started his professional career as a pharmacist at the Department of Hospital Pharmacy of the Academic Medical Center in Amsterdam and completed a residency in hospital pharmacy (supervisor prof.dr. L. Lie-A-Huen) in 2012, where he performed a study on the pharmacokinetics of S-ketamine in children at the pediatric intensive care unit. He then did an internship as a pediatric clinical pharmacist in the Evelina Children's Hospital in London. In June 2012, he started working as a hospital pharmacist at the Vlietland hospital in Schiedam.

In June 2013 he started this PhD research project on dosage optimization of five drugs used in preterm born infants, which had been awarded a ZonMw grant to prof.dr. R. de Groot and colleagues. This was a multi-center research project at the Radboudumc in Nijmegen under supervision of prof.dr. D.M. Burger, and at the Erasmus MC in Rotterdam under supervision of prof.dr. D. Tibboel and dr. S.H.P. Simons. In 2015, Robert left the Vlietland hospital for a position as a laboratory pharmacist at the Erasmus MC, and in 2017 was appointed as pediatric pharmacist at the Erasmus MC-Sophia Children's Hospital and the Department of Neonatology.

Robert aims to further develop as a pediatric clinical pharmacist and help improve the infrastructure for pediatric research and implementation of new findings through close collaboration. As of June 2018, Robert is a clinical pharmacist at the Erasmus MC with a focus on pediatrics in the Sophia Children's Hospital.

Robert is married to Marie-Rose. They are proud parents of two daughters, Liselotte (2015) and Marilou (2017).

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