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Pharmacokinetics of Midazolam in preterm neonates with an insight in brain Tissue: A PBPK approach

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Abstract: Physiological maturity is a gradual process taking place throughout infancy and childhood. Though for years anatomical growth has been the basis for dose calculation in pediatric population, physiological immaturity can-not be overlooked especially in neonates. The potential difference in physiology can significantly affect the outcomes of treatment and may result in under dosing or over-dosage. For many ethical and logistic constrains, carrying out pharmacokinetic studies of pharmacological agents in neonatal population remains a challenging task and such data is therefore, insufficient. This work presents Physiologically Based Pharmacokinetic modeling approach to predict the disposition of IV Midazolam in preterm neonates of different gestational ages, validated by the experimental studies. Furthermore, midazolam concentration in brain tissue of these neonates- the major site of its action- has been noted. The predicted and observed plasma pharmacokinetic parameters are comparable. This article demonstrates the usefulness of in-silico approach for finding out the PK parameters in neonates which may aid in deciding the frequency of drug administration in this population.

Keywords: Midazolam, gestational age, pre-term neonate, pharmacokinetics, ontogeny, PBPK.

INTRODUCTION

In vitro and *in vivo* research in neonates has revealed that owing to their physiological immaturity they are neither small adults nor little children. Along with the internal growth in size and volume their physiological processes also gain maturity with time. These changes are responsible for different pharmacokinetics of drugs in neonates as compared to children and adults. Conducting clinical studies in this age group for determining the most approximated pharmacokinetics of drugs is, however, hampered by many ethical and logistical concerns causing scarcity of successful clinical trials in this population (Matthew *et al*., 2011). Most drugs are thus often dosed in neonatal patients based on information extrapolated from adults or older children (Kimland and Odlind, 2012) which can however, be erratic.

Owing to physiological changes drugs doses equivalent to safe and efficacious doses in adults can lead to unexpected responses during neonatal period (Kearns *et al*., 2003). Among the term and preterm neonatal subpopulations, their body component of water differ (Loebstein and Koren, 1998) which may affect the volume of distribution of water soluble drugs. For CNS drugs decreased protein binding, a higher relative brain weight, and higher ratio of cerebral to systemic blood flow may culminate in higher cerebral tissue concentrations of drugs in neonates as compared to that in adults (Seyberth and Kauffman, 2011). Similarly, some drug-metabolizing enzymes in children reach the adult values by around one year of age (Anderson and Lynn, 2009). Rates of hepatic metabolism usually correlate with hepatic enzymes' expression which is found to be low at birth and increases steadily over time (Skinner AV 2011; Kearns GL, 2000; Benedetti *et al*., 2007).

Physiologically based pharmacokinetic (PBPK) modeling is an invaluable tool for studying the ADME of various drugs in different stages of life and sub populations. It's a mathematical modeling technique for the prediction of absorption, distribution, metabolism and excretion (ADME) of various natural or synthetic chemical substances in humans or across many other animal species. The research conducted via the PBPK modeling and simulation methodology has significantly increased our understanding regarding pediatric drug disposition. The recently widespread application of PBPK modeling for predicting pharmacokinetics of different drugs in humans estimating doses for different groups of patients can be found in various publications from the Pharmaceutical Industry (Theil *et al*., 2003; Jones *et al*., 2006). It entails scaling of pharmacokinetic parameters taking into account the age-dependent changes in physiological characteristics of the subjects such as their absorption capacity, metabolic rate of different enzymes and elimination capacities for various organs.

PBPK models consist of various compartments which are analogous to different body tissues connected to each other by the circulating blood system. These

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compartments are defined by tissue volumes and blood flow rates, which is specific to the specie used in the study. Different publications have reported these parameters (Brown *et al*., 1997; Chad *et al*., 2009). PBPK models' anatomically and physiologically meaningful compartments integrate biological system specific properties (e.g. organ mass, blood flow) and chemical substance properties (e.g. permeability and binding affinities,) to construct a virtual model. These models use physiologically relevant parameters of the population or specie under study to describe ADME of a drug or toxin yielding in concentration-time profiles in different tissues by which predictions about the overall pharmacokinetic (PK) behavior of the compound can be made.

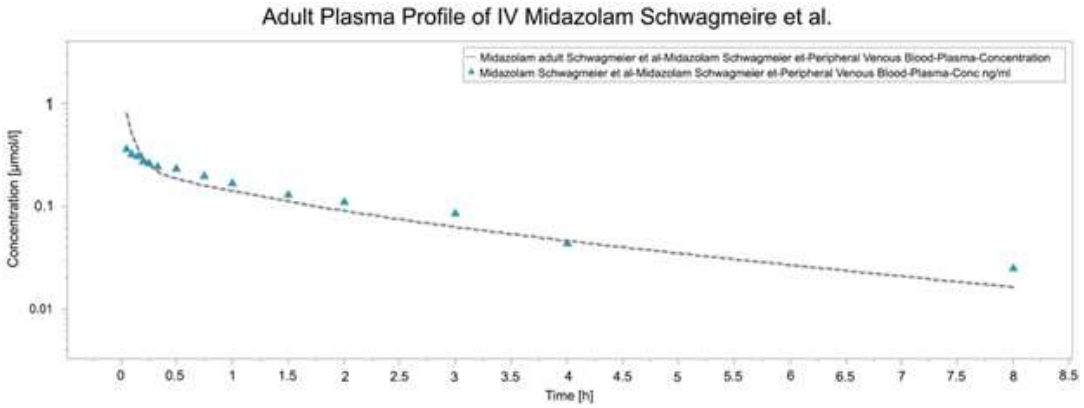
Evidences on the intravenous midazolam infusion for sedation of infants in the "neonatal intensive care unit" can be found in literature (Ng *et al*. 2003; Young and Neofax, 2010). Midazolam reaches the central nervous system and produces its sedative and anxiolytic effects by agonistically binding to the benzodiazepine site on the γ-aminobutyric acid$_A$ ($GABA_A$) receptor complex (De Wildt *et al*., 2003). In neonatal intensive care, midazolam is preferred over other benzodiazepines such as lorazapem and diazepam based on its advantages of shorter half-life, water solubility and rapid clearance (Jacqz-Aigrain *et al*., 1994). In a cohort study, midazolam was given to 25% of neonates who were tracheally ventilated (Ricardo *et al*., 2015) and by far remains one of the most commonly used sedative in neonatal population (Ng *et al*., 2017; Völler *et al*., 2019). It is also used in the treatment of refractory seizures (Favié *et al*., 2019), to induce anesthesia and to produce anterograde amnesia (Slaughter *et al*., 2013). Though active as a parent drug, Midazolam produces an equipotent metabolite, 1-hydroxymidazolam through hepatic hydroxylation which further undergoes glucuronidation before its excretion via renal route. Its elimination has been demonstrated to be delayed in preterm neonates compared with older infants and children. This may result in significant drug and metabolite accumulation during continuous infusion (Anand *et al*., 1999). Clinical studies can be found in literature where adverse neurological effects associated with midazolam in term and preterm neonates have been described (Adams *et al*., 1997; Magny *et al*., 1994; Ng *et al*., 2002; Ng *et al*., 2017). Neonates' have decreased number of $GABA_A$ receptors which increases the risk of myoclonus in neonates by Midazolam use as compared to older populations (Harte *et al*., 1997; van Straaten *et al*., 1992; Brooks-Kayal and Pritchett, 1993; van Alfen-van der Velden *et al*., 2006) Seizure is also encountered as an adverse effect of midazolam, though rarely (Gupta *et al*., 2018). Lliterature also reports the adverse cardio respiratory symptoms and tachycardia in premature neonates during continuous infusion because of low CYP3A activity level (Mio *et al*., 2020). Data in human subjects reporting the long-term neuro-developmental impact of midazolam therapy in newborns is still unavailable. But, preclinical data of benzodiazepine exposure in rodent models has shown significant multifocal neuroapoptosis as well as suppressed neurogenesis (Young *et al*., 2005; Stefovska *et al*., 2008). In addition, prolonged changes in hypothalamic neuron expression and delayed motor development have also been found to be produced if benzodiazepine exposure is given in prenatal period (Kellogg *et al*., 1980; Simmons *et al*., 1984; Kellogg *et al*., 1985). Studies in lower animals exist which have evaluated the extent of uptake of some benzodiazepines into mammalian brain and assessed some physicochemical and molecular factors effecting this uptake (Arendt *et al*., 1987). However, the extent of midazolam diffusion from systemic blood into the human brain or the concentration of midazolam in brain tissue has never been assessed before. This assessment seems to be of utmost importance for gaining the precise knowledge of midazolam effects on a given dose. In the present work we have presented the midazolam concentration and its pharmacokinetics in brain tissue after an iv infusion to preterm neonates of varying gestational ages by using the whole body PBPK simulation and modeling technique.

MATERIALS AND METHODS

Initial model building

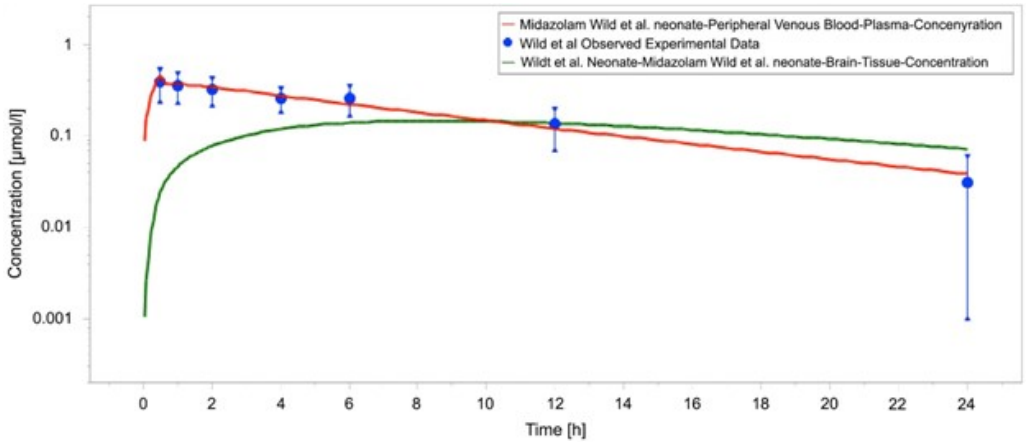
PK-SIM®6.3/ MoBi 6.3 software by Bayer Technology Services GmbH, Leverkusen, Germany was used for carrying out the PBPK modeling. First of all, a virtual whole -body PBPK model for an adult individual was developed and the predicted plasma profile was validated by the experimental study of Schwagmeire *et al.* (1998) (fig. 1). Next, this adult model was scaled down to develop a model of a preterm neonate of 29 weeks gestational age with the specific biometric values of weight 1.63 kg and height 39.02 cm in accordance to the experimental study by Wildt *et al*. (2001). The volumes of different organs of our preterm neonate are shown in table 1 while the blood flow rates to these organs are depicted in table 2.

Required Physicochemical parameters of Midazolam for the model compound generation were collected through an extensive literature survey and were selected as PKa as 6.15, lipophilicity (log MA) as 3.13 log units, solubility as 8.73 microgram/ml at pH 7, fraction unbound as 0.024, molecular weight as 325.8 while the effective molecular weight as 286.8. Hepatic metabolic enzymes were incorporated in biological individual along with hepatic metabolic pathway in the model compound. The mechanistic system of Pk-Sim takes into account all the ontogeny factors of preterm neonates according to its gestational age regarding phase I and phase II enzymes present in liver which were selected for this individual.



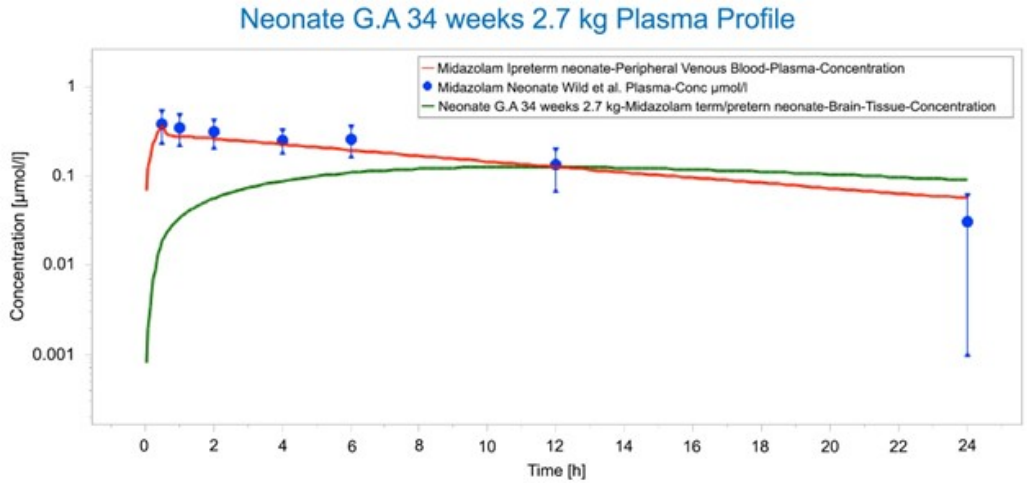
IV Midazolam Simulated Plasma Profile in Adult.
Experimental Observed Data, Schwagmeire *et al.* study.

Fig. 1: Simulated Adult Midazolam Plasma Concentration time profile.



— Simulated Wildt et al Preterm Neonate Midazolam Plasma profile.
● Midazolam Observed Experimental Data Wildt *et al*.
— Midazolam brain tissue Profile of Preterm neonates of G.A 29 weeks.

Fig. 2: Wildt et al Preterm Neonate (29 weeks gestational age) Midazolam 0.1 mg/kg Concentration time profile.



— Midazolam Plasma Profile of Preterm neonates of G.A 35 weeks.
● Midazolam Wildt et al Experimental Plasma levels.
— Midazolam brain tissue Profile of Preterm neonates of G.A 34 weeks.

Fig. 3: Plasma and brain tissue Concentration- time Profile of Midazolam in Preterm neonate of G.A 34 weeks.

The simulation was then created using our individual neonate and midazolam while defining the administration protocol of IV infusion over 30 minutes in compliance to the Wildt *et al*. experimental work.

Table 1: Organ Volumes of Virtual Pre Term Neonate Used in the Simulation

Organs	Volumes (liters)
Venous blood	0.03
Arterial blood	0.03
Bone	0.21
Brain	0.18
Fat	0.52
Gonads	$3.34E^{-4}$
Heart	$8.86E^{-3}$
kidney	0.01
Large intestine	$6.86E^{-3}$
liver	0.05
Liver periportal	0.05
Liver pericentral	0.0
Lung	0.03
Muscle	0.04
Pancreas	$1.236E^{-3}$
Portal vein	0.03
saliva	$1.34E^{-4}$
skin	0.10
Small intestine	0.01
spleen	$3.46E^{-3}$
stomach	$4.47E^{-3}$

Table 2: Blood Flow Rates to Different Organs of Virtual Pre Term Neonate Used in the Simulation

Organs	Blood Flow Rates (liters/min)
Bone	0.01
Brain	0.07
Fat	0.03
Gonads	$1.06E^{-4}$
Heart	$8.43E^{-3}$
kidney	0.04
Large intestine	$8.47E^{-3}$
liver	0.01
Lung	0.24
Muscle	0.01
Pancreas	$2.15E^{-3}$
Portal vein	0.04
skin	0.01
Small intestine	0.02
spleen	$1.64E^{-3}$
stomach	$3.03E^{-3}$

Simulations and models assessment

Afterwards, on running the created simulation of 0.1 mg/kg midazolam we obtained venous plasma concentration-time profile of midazolam in our preterm neonate which was subsequently validated by the clinically observed plasma concentration profile of experimental study by Wildt *et al*. (2003) (fig. 2). Visual predictive checks were used for this validation. Using this profile we determined the pharmacokinetic parameters of midazolam in our virtual individual using PK-Sim soft ware, which were comparable to those obtained by the experimental study (After successful development of this model, we found out the concentration of midazolam and its pharmacokinetics in brain tissue of this individual neonate at this plasma concentration (table 3). In the next step, another model for a preterm neonate of gestational age 34 weeks and biometric values of weight 2.70 kg and height 45.10 cm was developed and the simulation was run for 0.2mg of midazolam infusion to obtain midazolam venous plasma profile in this neonate. (fig. 3). As in previous simulation brain tissue concentration of midazolam was measured for this neonate, too (table 4).

RESULTS

Our Insilico PBPK simulation studies of Midazolam disposition in preterm neonate of gestational age 29 weeks are very well comparable to the experimental data by Wildt *et al*. with predicted AUC 1186.70ng hr/ml, Clearance 1.26 ml/min/kg, MRT10.28 hours and T^{max} 0.50 hours (table 3). Further this study revealed the PK parameters in the brain tissue of this neonate which were AUC 866ng hr/ml, Cmax 47.67ng/ml, Clearance, 1.38 ml/min/kg, MRT 19.2 hr and Tmax, 8.5hr. For the individual neonate of gestational age 34 weeks the plasma PK parameters were AUC 1126.52ng hr/ml, Cmax 123.95 ng/ml, Clearance 0.87ml/min/kg, MRT 14.81 hr and Tmax 0.5 hr. While those in its brain tissue were AUC 810.43ng hr/ml, Cmax 41.49ng/ml, Clearance 0.82 ml/min/kg, MRT 28.86 hr and Tmax 11.25 hr (table 4).

DISCUSSION

Midazolam elimination from the body majorly takes place by hepatic hydroxylation reactions using CYP3A4 and CYP3A5 enzymes yielding 1-hydroxymidazolam. This metabolite of midazolam is pharmacologically active producing sedative effect (Young *et al*., 2005). It further undergoes glucuronidation before its final excretion from the body via renal route. Hepatic CYP3A4 activity involved in this metabolism emerges during the initial few weeks of life and is relatively immature which explains the reduced Midazolam clearance (Cl) in neonates than adults (Ince *et al*., 2013). Only 10% to 30% of neonate liver samples have presented detectable CYP3A5 expression (Thummel *et al*., 2011). Both of these enzymes undergo physiological maturation in neonates with increasing age and approach the adult levels by the ages 3 and 12 months. This physiological immaturity results in higher urinary percent recovery of Midazolam in infants (0.44%) than in adults (0.14). These maturational

Table 3: Plasma and Brain Tissue Pharmacokinetic Parameters of Midazolam in Preterm Neonate (29 Weeks) Obtained by PBPK Simulation and Wildt *et al*. (2003)

Parameter (Units)	Plasma PK Parameters By PBPK	Observed Value Wildt *et al*.	Brain tissue PK parameters By PBPK
AUC(ng hr/ml)	1186.70	804 (153 - 2118)	866.53
CL(ml/min/kg)	1.26	1.8 (0.7-6.7)	1.38
Cmax(ng/ml)	152.05	108 (48.8 – 217)	47.67
t1/2(hours)	7.60	6.3 (2.6-17.7)	10.21
Tmax(hours)	0.50	0.5 (0.5 – 4.0)	8.5
MRT(hours)	10.28	10.3 (4-25.6)	19.20

Table 4: Plasma and Brain Tissue Pharmacokinetic Parameters of Midazolam in Preterm Neonates of 34 Weeks of Gestational Age Obtained by PBPK

Parameters	PK in plasma	PK in brain tissue
AUC (ng hr/ml)	1126.52	810.43
CL (ml/min/kg)	0.87	0.82
Cmax (ng/ml)	123.95	41.49
t1/2 (hours)	10.74	16.56
Tmax (hours)	0.5	11.25
MRT (hours)	14.81	28.86

differences are greatest among the neonates and infants and also observed to be effected by the period a fetus spends in uterine cavity as the clearance of midazolam is found to be decreased in preterm infants.

In our work Physiology-based pharmacokinetic (PBPK) modeling is used to build up models of preterm neonates of different gestational ages to describe the effect of the time spent in utero on the plasma concentration-time profiles and PK parameters of midazolam in preterm neonates by using age specific known physiological parameters (body and organ weights, blood flows, tissue composition etc.) As Midazolam is primarily metabolized by the hepatic metabolic pathway, PBPK models developed by PK-Sim version 6.3, incorporated all the ontogeny differences in these neonatal models of different ages and involved variations in enzyme kinetics considering the fact that along with their anatomical growth, neonates have developmental changes in their physiological processes which are influenced by their gestational ages. Further as midazolam acts in the central nervous system, we also observed its concentration in the brain tissue where the drug must reach to act on its target receptors. It was noteworthy from fig. 2 and 3 that at the time of onset of its action, midazolam concentration is very low in brain tissue as compared to its venous plasma concentration. This concentration in brain tissue keeps rising afterwards and when the drug is in its elimination phase from plasma with plasma concentration being decreasing, the concentration in brain tissue is still high which may explain the reason why the neonates were found more sedated after 18 hours of midazolam administration (Arya and Ramji, 2001). The PK profiles also show that in both of our simulated neonates the MRT in brain tissue is higher than the MRT in plasma, which may result in its prolonged CNS effects.

Based on this study it is recommended that great care should be practiced in planning the dose interval of midazolam in preterm neonates as they may result in drug accumulation in brain tissue. In case subsequent doses are required for any indication, the dose of these following administrations may be reduced to prevent brain from any possible adverse outcome that has been reported in preclinical studies

As there is a paucity of experimental work carried out in neonatal populations in literature, we have validated our preterm neonates' simulated plasma profiles by the available observed data of preterm neonate of 29 weeks of gestational age.

CONCLUSION

Conducting clinical pharmacokinetic studies in preterm neonates is still a challenge owing to multiple reasons. The present work demonstrates the utility of PBPK approach in determining the drugs' pharmacokinetics in preterm neonates. As ontogeny differences have been considered in our simulated preterm neonates and the obtained results in plasma are comparable to the findings observed in clinical studies, this method may be relied upon to predict the specific PK parameters of various drug at their principle site of action. However, further in-silico studies are needed in future to fully validate this use of PBPK approach.

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