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Physiologically based pharmacokinetic modelling of Aminophylline and Caffeine

Surulivelrajan Mallayasamy Dr
Manipal College of Pharmaceutical Sciences, msv.rajan@manipal.edu

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELLING OF AMINOPHYLLINE AND CAFFEINE

A REPORT SUBMITTED TO

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In partial fulfillment for the degree of Doctor of Pharmacy (PharmD)

SUBMITTED BY

Tummala Hari Prabhath
(Reg No: 150614020)

Thotakura Sahithi
(Reg No: 150614006)

Balusu Rachana
(Reg No: 150614038)

Pasnoor Achyuth Kumar
(Reg No: 180615004)

PharmD 5th Year / PharmD P.B. 2nd Year,
Department of Pharmacy Practice,
Manipal College of Pharmaceutical Sciences,
MANIPAL ACADEMY OF HIGHER EDUCATION, MANIPAL

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UNDER THE GUIDANCE OF

Dr Surulivelrajan Mallaysamy
Associate professor,
Department of Pharmacy Practice,
Manipal College of Pharmaceutical Sciences, MAHE.

Dr. Leslie Edward S Lewis
Professor and Unit Head
Division of Neonatology, Department of Pediatrics
Kasturba Medical College,
MAHE, Manipal

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CERTIFICATE

This is to certify that this project report entitled, "PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELLING OF AMINOPHYLLINE AND CAFFEINE" by Mr. Tummala Hari Prabhath, Ms. Thotakura Sahithi, Mr. Pasanoor Achyuth Kumar and Ms. Balusu Rachana for the completion of 5th year PharmD / 2nd year PharmD P.B. comprises of the bonafide work done by them in the Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences and Kasturba Hospital, Manipal, under the guidance of Dr Surulivelrajan Mallaysamy, Associate professor, Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences, Manipal and co-guide Dr Leslie Edward S Lewis, Professor and Unit Head, Division of Neonatology, Department of Pediatrics, Kasturba Medical College, MAHE, Manipal

I recommend this piece of work for acceptance for the partial fulfilment of the completion of the 5th year PharmD / 2nd year PharmD P.B. program of the Manipal Academy of Higher Education, Manipal for the Academic year 2019-2020.

Date:

Place: Manipal

Dr Surulivelrajan Mallaysamy
Associate Professor,
Department of Pharmacy Practice,
Manipal College of Pharmaceutical Sciences,
MAHE, Manipal - 576104

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Date:

Place: Manipal

Dr C Mallikarjuna Rao

Principal,

Manipal College of Pharmaceutical Sciences,

Manipal Academy of Higher Education,

Manipal - 576104

MANIPAL COLLEGE
OF PHARMACEUTICAL SCIENCES
MANIPAL
(A constituent unit of MAHE, Manipal)

DECLARATION

We hereby declare that the project entitled "PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELLING OF AMINOPHYLLINE AND CAFFEINE" was carried out under the guidance of Dr Surulivelrajan Mallaysamy, Associate Professor, Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal. The extent and source of information derived from the existing literature have been indicated throughout the project work at appropriate places. The work is original and has not been submitted in part or full for any diploma or degree purpose for this or any other university.

Date:

Place: Manipal

Tummala Hari Prabhath
(Reg No. 150614020)

Thotakura Sahithi
(Reg No. 150614006)

Pasnoor Achyuth Kumar
(Reg No. 180615004)

Balusu Rachana
(Reg No. 150614038)

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List of abbreviations

µg / mcg	Microgram
min	Minute
ml	Milliliter
µmol	Micromole
mg	Milligram
kg	Kilogram
cAMP	cyclic Adenosine Monophosphate
cGMP	cyclic Guanosine Monophosphate
CPAP	Continuous Positive Airway Pressure
ADME	Absorption, Distribution, Metabolism, Excretion
I.V.	Intravenous
P.O.	Per os (Oral Administration)
t1/2	Half-life
hr / hrs	Hour / Hours
Vd	Volume of distribution
CL	Clearance
AUC	Area Under Curve
Km	Michaelis constant
Cmax	Maximum Concentration
MRT	Mean Residence Time
NICU	Neonatal Intensive Care Unit

List of tables

Table No.	Table Title	Page No.
1	Aminophylline: Patient demographics data	13
2	Aminophylline: Compound parameters	13
3	Aminophylline: Michaelis-Menten Constants for CYP enzymes	14
4	Aminophylline: Postnatal age-based subgroup demographic data	14
5	Aminophylline: weight-based subgroup demographic data	15
6	Aminophylline: Clearance in various groups	22
7	Aminophylline: Pharmacokinetic parameter output representing mean individual from each population	23
8	Caffeine: Organism parameters used for the population model	26
9	Caffeine: Drug parameters used for the population model	27
10	Caffeine: Vmax and Km values for the hepatic clearance used for the population model	28
11	Caffeine: Classification of subgroups based on bodyweight	29
12	Caffeine: Mean and range values of bodyweight sub-population	29
13	Caffeine: Classification of subgroups based on PNA	30
14	Caffeine: Mean and range values of PNA subgroups	30
15	Caffeine: Parameter output of the population simulation of caffeine	32
16	Caffeine: Pk-parameter output for the bodyweight subgroup analysis of caffeine	36
17	Caffeine: Pk-parameter output for the PNA subgroup analysis of caffeine	39

List of figures

Fig No.	Figure Title	Page No.
1	Metabolism of aminophylline	03
2	Building blocks of the PBPK modelling	10
3	Aminophylline: Time vs Concentration for the unabridged population	16
4	Aminophylline: Time vs Concentration profile for Preterms weighing less than 1 kg	17
5	Aminophylline: Time vs Concentration profile for Preterms weighing 1-1.5 Kg	18
6	Aminophylline: Time vs Concentration profile for Preterms weighing greater than 1.5 kg	19
7	Aminophylline: Time vs Concentration profile for preterms with postnatal age ranging from 1 to 6 days	20
8	Aminophylline: Time vs Concentration profile for preterm with postnatal age greater than 6 days	21
9	Caffeine: Time vs Concentration profile whole preterm population	31
10	Caffeine: Time vs Concentration profile for preterm population with bodyweight range 0.5 to 1.0 Kg	33
11	Caffeine: Time vs Concentration profile for preterm population with bodyweight range 1.0 to 1.5 Kg	34
12	Caffeine: Time vs Concentration profile for preterm population with bodyweight above 1.5Kg	35
13	Caffeine: Time vs Concentration profile for preterm population with PNA range of 0 to 10 days	37
14	Caffeine: Time vs Concentration profile for preterm population with PNA range of greater than 10 days	38

Abstract

Background: Irregularity in temperature regulation and control of breathing during the first few days are the reasons for the abrupt deaths in preterm neonates, of which apnea in premature neonates constitutes the major reason for these deaths. Methylxanthine's (aminophylline and caffeine) are used in its treatment. Majority of the hospitals follow a standard treatment regimen for both aminophylline and caffeine irrespective of preterm's age and body weight which leads to toxic or sub-therapeutic concentrations of the drug. Using Physiologically based pharmacokinetic modelling (PBPK), various drug characteristics and its deportment can be studied. This helps in predicting the Pharmacokinetic parameters of the drug which in turn aids in the individualization of dosing regimen with respect to body weight and postnatal age.

Objectives: To develop a PBPK model for aminophylline and caffeine to aid in optimizing and individualizing the dosage regimen with respect to body weight and postnatal age for the treatment of apnea in preterm neonates.

Methodology: Anonymized data of 108 and 61 preterm neonates with apnea from a previously reported study were obtained for building an aminophylline and caffeine model respectively. The data was obtained from a previous study conducted at NICU of Kasturba Hospital, Manipal University. Preterm neonates with less than or equal to 34 weeks of gestational age and greater than 6 apneic episodes in 24 hrs were included in the study. A standard treatment protocol of 5mg/kg loading dose and 2mg/ kg maintenance dose for every 8 hours and a standard dosing regimen of 10mg/kg loading dose followed by 2.5mg/kg maintenance dose for every 24 hours were used in this PBPK model development for aminophylline and caffeine respectively. Pk-Sim software package was employed to build a predictive model. The predictions were compared to the reported data through visual inspection and also by pharmacokinetic parameters comparison.

Results: Subgroup simulations provided evidence for the maturation of enzymes with the progression of time, which in turn increases the clearance for both aminophylline and caffeine, which can be interpreted from the visual predictive curve. The study also provides an evidence of decreased half-life of the drug in the body, where t1/2 of 32.83 hrs and 29.87 hrs was reported in the Sub-groups PNA 1-6 and PNA > 6 respectevely in the aminophylline group. Similarly, t1/2 of 67.04 hrs and 57.5 hrs was reported in the subgroups PNA 0-10 and PNA > 10 respectively in the caffeine group. These results also provide an evidence for an improved renal function with age in both the case groups (aminophylline and caffeine).

Conclusions: The study provides an evidence for the maturation of enzymes with the time and alteration of drug characteristics like the volume of distribution and clearance concerning covariates body weight and postnatal age. Hence it provides evidence in delivering optimized concentrations of the drug when dosing regimen is individualized with respect to body weight and postnatal age.

CONTENTS

1. Introduction 01

1.1 Apnea of Prematurity 01

1.2 Treatment of AOP 01

1.3 Methylxanthines 02

1.3.1 Aminophylline 02

1.3.2 Caffeine 04

2. Physiology Based Pharmacokinetic Models. 08

2.1 PBPK Models and building blocks 08

2.2 Significance of Pediatric PBPK Modeling 10

2.3 Software's Available for PBPK Modeling 11

2.4 PK-SIM for PBPK Modeling 11

3. Aminophylline

3.1 Methodology 12

3.1.1 Materials and Methods 12

3.1.2 Model Building 12

3.1.3 Results 21

3.1.4 Discussion 24

4. Caffeine

4.1 Methodology 26

4.1.1 Material and Methods 26

4.1.2 Model Building 26

4.1.3 Results 31

4.1.4 Discussion 40

5. Conclusion. 41

6. Bibliography 42

7. Appendix 45

INTRODUCTION

1.1 APNEA IN PRETERM NEONATES

Major neonatal deaths occur during the first week of life. Nearly one million newborns die within 24 hours of their birth. Irregularity in temperature regulation and control of breathing during the first few days are reasons for their abrupt deaths. Birth asphyxia (lack of breathing at birth), apnea of prematurity, infections and birth defects are the major complications. Apnea of prematurity (AOP) occurs secondary to a physiological immaturity of respiratory control (Barrington K et al, 1991; Zing Zhao et al, 2011). It is a developmental disorder described as respiration cessation for more than 20 seconds or respiration cessation < 20 seconds followed by bradycardia and cyanosis (Eric C Eichenwald, 2016). AOP involves diverse clinicopathological events, Depression of cerebral hemodynamics which makes the ventral surface of medulla and the adjoining areas of brainstem vulnerable to the inhibitory mechanisms like hypoxia, adenosine secretion, hypercapnia and hyperthermia, leading to the apneic episodes in preterm infants. Apnea during infancy is frequent during active or REM sleep compared to being infrequent during quiet sleep. There exists a complex relationship between the central respiratory control and the central chemosensitive areas (Eldridge L F et al, 1983).

The decreased chemosensitive factors lead to an inadequate respiratory response (Darnall et al, 2006). Reduced lung volumes leading to hypoventilation may also be an initial trigger. Gastroesophageal reflux being difficult to diagnose in this age group due to the non-acidic nature could also be a suspected cause for apnea (Varsha Bhatt et al, 2012). Although establishing the consequences of apnea of prematurity on neurodevelopment in preterm remains a challenge, Regulation of the cerebral hemodynamics and respiratory rate are few of the pivotal objectives to prevent premature death in preterm neonates with apnea.

1.2 MANAGEMENT OF AOP:

Apnea resolution and respiration rate control is the primary objective of avoiding preterm death. Continuous positive airway pressure and nasal intermittent positive pressure ventilation, methylxanthine therapy and doxapram therapy are the treatment strategies available.

Methylxanthine therapy:

The most commonly used agents for the management of AOP are Caffeine (1-3-7-trimethylxanthine) and theophylline (1-3-dimethylxanthine) (Varsha Bhatt et al, 2012). The exact mechanism of action by which these agents act in AOP remains poorly defined. Theophylline, an inhibitor of phosphodiesterase, and an adenosine antagonist, operates by different mechanisms. Theophylline improves central respiratory enhancement by antagonizing the role of adenosine, a core respiratory depressant and respiratory stimulant throughout the periphery (Church M K et al, 1986). Phosphodiesterase inhibition of isoenzyme activity results in a breakdown of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). This leads to a

rise in cAMP and cGMP in the blood, which results in the relaxation of the airways (Trophy T J et al,1991; Barnes P J et al, 1994). Theophylline also controls the release of catecholamines and modulates the calcium ion flux (Ramirez G et al,1995; Kolbeck R C et al, 1988). Increased alveolar ventilation and carbon-dioxide sensitivity are the physiological changes obtained following the administration of theophylline which provides evidence of the respiratory centre's stimulation as a mechanism for AOP relief (Varsha Bhatt et al, 2012). Caffeine being a non-adenosine antagonist modulates through neurotransmitters such as nor-adrenaline, acetylcholine, dopamine, gamma-aminobutyric acid, and glutamine, increasing cAMP and cGMP leading to bronchodilation. Caffeine stops apnea and initiates regular breathing by increasing the activation of the peripheral chemoreceptors. In the immature lung, caffeine exhibits an anti-inflammatory action. Following caffeine administration, the higher success rate for early nasal continuous positive airway pressure therapy (nasal-CPAP) was recorded. Caffeine therapy also decreases ventilator-induced lung injury, which is critical in the early neonatal phase. Early nasal CPAP therapy may not be effective in all infants during the prevalent AOP. (Varsha Bhatt et al, Aug 2012).

1.3 METHYXANTHINES

1.3.1 Aminophylline

Aminophylline, a theophylline derivative, is a mixture of drugs containing 2:1 proportion of theophylline and ethylenediamine. Theophylline once in the body acts as a blocker of adenosine receptor & histone deacetylase activator which increases cAMP & cGMP causing bronchial smooth muscle dilation. The standard treatment regimen of 5mg/kg of loading dose and 2mg/ kg of maintenance dose of aminophylline is used for treating AOP (Amir MA et al, Aug 2014).

1.3.1.1 Absorption

When given orally aminophylline is immediately released and peak concentration is reached within 1 to 2 hrs. Its absorption is least affected in the presence of food. (Product Information: aminophylline IV injection Hospira, 2004) (Product Information: aminophylline oral tablets, West- ward Pharmaceutical, Corp, Eatontown, NJ, 2002).

1.3.1.2 Distribution

Once theophylline is released into systemic circulation 40% is protein-bound (primarily albumin). The volume of distribution (Vd) ranges from 0.3L/kg to 0.7L/kg. Vd increases in preterms due to a decrease in plasma protein binding which leads to an increase in the serum concentration of aminophylline leading to toxic effects.

1.3.1.3 Metabolism

Metabolism by the liver in adults is a saturable process which primarily occurs through CYP1A2 mediated N-Demethylation. Biotransformation of the aminophylline takes place through demethylation (1-methylxanthine and 3-methylxanthine) and hydroxylation (1,3-dimethyluric acid). About 6% is metabolized to caffeine through N-Methylation (Cheng et al, 1990; Denaro et al, 1990; Tang-Liu et al, 1982; Dahlqvist et al,). Metabolic clearance is significantly higher compared to renal clearance (approximately 10% of the dose) in adults, whereas in neonates due to the immaturity of metabolizing enzymes (CYP1A2, CYP2E1, CYP1A1) 50% of the drug is cleared unchanged through renal route (Ha et al., 1995; Bonati et al., 1981; Ogilvie). The N-demethylation pathway is absent in neonates while the hydroxylation pathway role is significantly lacking. The activity of these pathways increases gradually with enzyme maturation and reaches maximum levels by the age of one. 3-Methylxanthine and Caffeine are the only pharmacologically active metabolites. 3-Methylxanthine produces about one-tenth of theophylline's pharmacological activity. The half-life (t1/2) of aminophylline in adults is around 8-9 hrs which substantially increases to 17-43 hrs in preterms with 3-15 days of postnatal age. Due to these differences in ADME of the drug in adults compared to preterms, dose adjustment is needed. The therapeutic range of 5-20 mcg/ml serum concentration is proven to be safe and effective in the treatment of AOP. Serum concentration exceeding greater than 20 mcg/ml produces toxic effects. (Product Information: aminophylline IV injection Hospira, 2004) (Product Information: aminophylline oral tablets, West-ward Pharmaceutical, Corp, Eatontown, NJ, 2002).

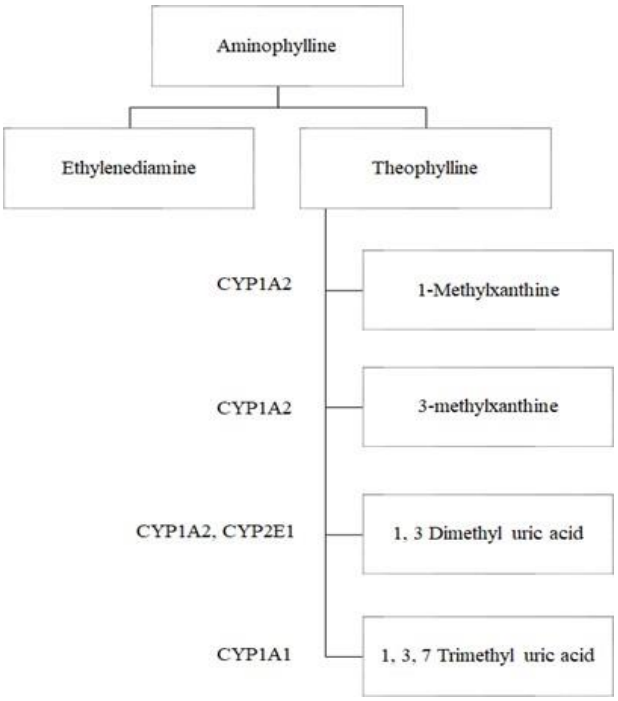


Fig 1. Metabolism of Aminophylline

1.3.1.4 Excretion

In neonates, 50 percent of the aminophylline dose is excreted unchanged by urine, but only 10 percent is excreted unchanged in the urine after about three months of age and the rest is metabolized into 1-methylxanthine, 3-methylxanthine, 1,3-dimethyluric acid, 1,3,7-trimethyluric acid and caffeine. Since there is minimal evidence regarding the accumulation at clinically significant levels of active metabolites (i.e. caffeine, 3-methylxanthine) even in the end-stage renal disease. Dosage adjustment is not required in adults and children older than 3 years for renal insufficiency. In comparison, close consideration is required to reduce the dose and track serum theophylline concentrations in neonates under 3 years of age with decreased renal function. This is because a large fraction of the theophylline dose is excreted in the urine as unchanged theophylline and caffeine (Product Information: aminophylline IV injection Hospira, 2004) (Product Information: aminophylline oral tablets, West-ward Pharmaceutical, Corp, Eatontown, NJ, 2002).

1.3.2 Caffeine:

Caffeine Citrate is the salt form of caffeine base belonging to the class of methylxanthines. This is a known competitive inhibitor of the phosphodiesterase enzyme which is responsible for the inactivation of the cyclic adenosine monophosphate (cAMP).

Mechanism of action involved in the treatment of apnea of prematurity:

According to Anari et.al, 2017 the mechanism is not well established because the molecular and cellular targets involved in the CNS are not clearly known but it is hypothesized to be involved in the

1. Simulation of the respiratory core by adenosine receptor's competitive antagonism (Fred- holm,1995). Increases in minute ventilation by increasing central CO2 sensitivity and improving function of the respiratory muscles (Abhu shaweesh et al,2011).
2. Decreased diaphragmatic failure
3. Increased metabolic rate and increased oxygen consumption.

1.3.2.1 Pediatric Dosing in The Treatment of Apnea of Prematurity:

1. FDA Labeled Dosage for Apnea of Prematurity:

Loading Dose: 20mg/kg/IV over 30min given as a single dose with a syringe infusion pump. Maintenance Dose: 5mg/kg/IV over 10min given with a single infusion pump every 24 hours (start after 24hrs of the loading dose)

2. Off-label Dosage for Apnea of Prematurity:

Loading Dose: 20mg/kg-25mg/kg IV/PO over 30minutes every 24hours.

Maintenance Dose: 5mg/kg – 10mg/kg IV/PO over 30minutes every 24 hours (start 24hrs after the loading dose).

High Dose Maintenance: 10mg/kg – 20mg/kg/day IV/PO every 24 hours (start 24hrs after the loading dose).

According to the product information IV/PO, Sun pharma Ltd. "The dose of caffeine citrate is twice the dose of the caffeine base." For example, 10mg of caffeine citrate is equal to the 5mg of caffeine base.

1.3.2.2 Pharmacokinetics:

The pharmacokinetic profile of the caffeine is very well described in the product information IV/ PO, Sun pharma Ltd. According to this product information label the absorption, distribution, metabolism and excretion of the caffeine citrate is as follows

Absorption:

Caffeine is well absorbed via oral, rectal and percutaneous routes of administration. IV route of administration is preferred in the treatment of apnea of prematurity in infants and the bioavailability of caffeine base is half the concentration of caffeine citrate.

Distribution:

The unbound plasma concentration of caffeine in neonates is 70%. Caffeine being a hydrophilic drug it distributed widely across the tissues. The volume of distribution of caffeine in preterm neonates is found to be 0.8-0.9L/Kg. Scott NR et.al,1989 described a decrease in volume of distribution in chronic liver disease patients.

Metabolism:

In adult's caffeine is extensively metabolized by the liver. The enzymes involved in the metabolism of caffeine are CYP1A1, CYP1A2, CYP3A4, CYPD6 and CYP2E1. In neonates the hepatic enzymes are not well developed, and the metabolites are paraxanthine, theobromine, theophylline and trimethyl uric acid.

Excretion:

The average t1/2 of caffeine is expected to be between 52 to 96 hours in neonates as per De Caraolis et.al, 1991. The adult half-life 5-6 hrs is achieved by the age of 9 months. The 86% of the parent compound is excreted unchanged in urine in 6 days.

1.3.2.3 Adverse Drug Reactions:

Common

1. Psychiatric: Irritability
2. Other: Feeding problem symptom

Serious

1. Endocrine metabolic: Acidosis (rare), Hyperglycemia, Hypoglycemia, Impaired wound healing (rare)
2. Gastrointestinal: Gastritis (rare), Gastrointestinal hemorrhage (rare)
3. Hematologic: Disseminated intravascular coagulation (rare), Hemorrhage.
4. Neurologic: Central nervous system stimulation, Cerebral hemorrhage (rare)
5. Ophthalmic: Retinopathy of prematurity (rare)
6. Renal: Renal failure (rare)
7. Respiratory: Dyspnea (rare), Pulmonary edema (rare)
8. Other: Sepsis (rare)

1.3.2.4 ADRs of Caffeine Citrate associated with the AOP Treatment:

1. Vascular perfusion of the organs or tissues decreased: palpitations, flushing, arrhythmias, tachycardia, prolonged QRS interval, myocardial infarction and reduced blood flow in neonates with caffeine use. (Somani & Gupta, 1988; Palmer et al, 1995; Donner- stein et al, 1998)

2. Dermatologic Effects: Rash, dry skin, and skin breakdown were recorded in 8.7 percent, 2.2 percent, and 2..2percent, respectively, of infants in a randomized trial (n=85) (Prod Info Cafcit (R), 2000).

3. Endocrine/Metabolic Effects: Acidosis and Abnormal Healing were each reported in 2.2% of infants during the treatment. (Prod Info Cafcit (R), 2000).

4. Gastrointestinal Effects: In a randomized trial (n=85), there were records of feeding aversion, necrotizing enterocolitis, gastritis, and gastrointestinal hemorrhage in 8.7%, 4.3%, 2.2%, and 2.2%,

respectively. (Prod Info Cafcit (R), 2000).

5. Hematologic and neurological Effects: Hemorrhage and Cerebral Hemorrhage was reported in 2.2% of infants (Prod Info Cafcit (R), 2000).

6. Ophthalmic Effects: Retinopathy of prematurity was reported in 2.2% of (Prod Info Cafcit (R), 2000).

7: Renal Effects: Kidney Failure was reported in 2.2% of infants (Prod Info Cafcit (R), 2000).

8. Respiratory Effects: Dyspnea and Lung Edema were each reported in 2.2% of infants (Prod Info Cafcit (R), 2000). Hyperventilation and Tachypnea have been associated with caffeine use (usually in doses greater than 250 mg/day).

9. Oher: Sepsis and Accidental Injury were reported in 4.3% and 2.2% of infants (Prod Info Cafcit (R), 2000).

10. Withdrawal symptoms in neonates: Chronic ingestion of excessive amounts (2-18 cups ap- proximately 200-1800mg) of caffeine throughout pregnancy has been associated with the withdrawal symptoms in newborn infants. The primary withdrawal symptoms observed were tremulousness, irritability, and non-bilious vomiting. Maternal consumption of as low as 200 to 360 mg caffeine daily (2 to 3 cups of coffee per day) produced vomiting and irritability in 1 neonate. All these symptoms resolved within 7 days with no treatment.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELLING

2.1 PBPK Models and building blocks:

Definition:

It is a mathematical modeling technique for human and other animal species to predict the absorption, distribution, metabolism and excretion of synthetic or natural chemical substances.

Types of PBPK Model:

1. Whole Body PBPK Model:

Peter S A et.al defined the whole body PBPK model as a schematic representation of the organs that are associated with the absorption, distribution, metabolism and excretion of the drug due to their physiological/ pharmacological function/their function. Considers every organ as a different compartment with organ specific drug input and output rates.

2. Partial PBPK Model:

In this type of PBPK Model the highly perfused tissues or organs are grouped as one compartment and the blood flow to all these organs grouped is assumed to be same. According to L Kuepfer et.al the PBPK model is typically made up of four building blocks. These building blocks serve as the inputs to the model and the model predictions are made based on these input parameters.

Organism Parameters:

These properties depend on the species and population specific. Each organ is represented based on the prior knowledge of the anatomy and physiology of the organism of interest. This section includes organ volumes, organ composition, blood flows, surface areas, and expression levels. For an individual simulation, the parameters should be based on the parameters of the subject/ participant from the clinical study. To build a population model first you have to build an individual representing the mean of the population and create the required number of the virtual individuals based on the range of the clinical population.

Drug Properties:

Includes both physiochemical properties and the biological properties of the specific drug. The physiochemical properties such as lipophilicity, solubility, PKa of the drug are also called as drug specific

parameters as they solely depend on the specific compound. Whereas the drug biologic properties such as fraction of unbound drug and tissue plasma partition coefficient depends on the organism's parameters as well along with the drug parameters. Along with both physiochemical and biological properties a detailed input on the metabolizing enzymes, transport proteins and the clearance processes such as renal, biliary and hepatic concentrations is also necessary.

Formulation:

This has a very big role in the design of the dosage form for a new chemical entity in the invitro bioavailability studies. During invitro in vivo correlation (IVIVC) which is one of the applications of the PBPK modelling the input of the type of formulation and the rate at which the drug is absorbed from the oral formulation has a role in the invitro bioavailability testing.

In the PK-SIM there are four different options available under the oral formation based on the release of the drug from the formulation they are dissolved, Weibull, lint80, particle dissolution, table, first order and zero order. This input is very important in the simulation of absorption models.

Administration Protocol:

This section of the building blocks mainly comprises of three main parts

1. Route of administration.
2. Amount of the dose administered.
3. In case of the multiple dosing the dosing schedule.

The model administration protocol given as input will be similar to that of the clinical study from which the observed concentration will be used for the model validation. In the PK-SIM there are two different types of the protocol that is the simple and advanced protocol. The simple protocol is usually used when giving an input for the single dose administration whereas the advanced section is used when the multiple doses are administered.

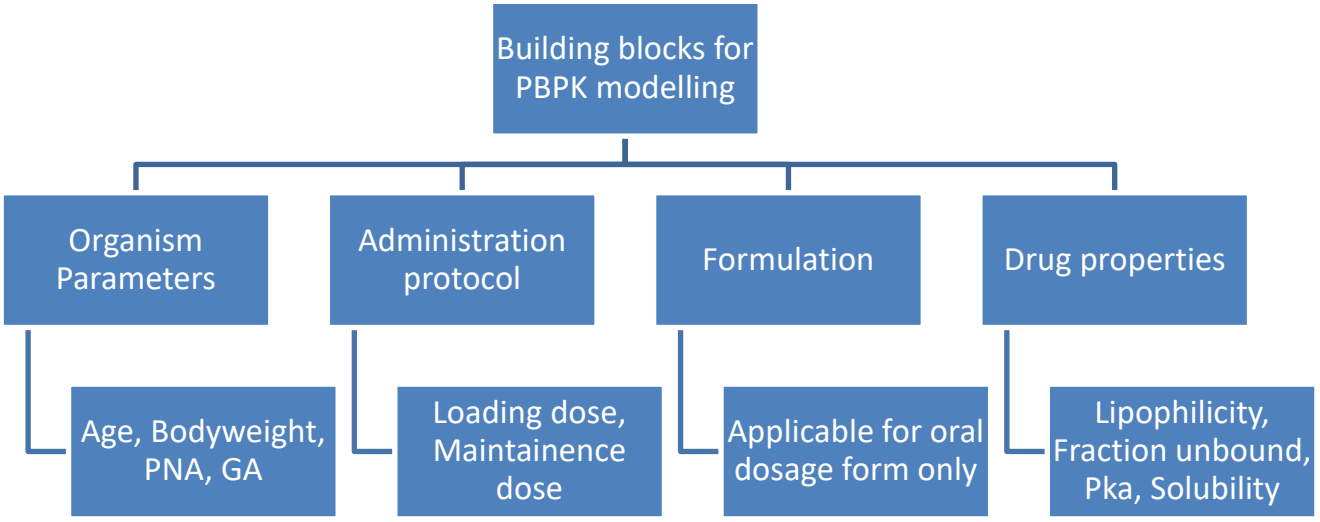


Fig 2. Building Blocks of the PBPK Modelling

Applications of PBPK models:

1. Useful in the prediction of concentration profiles in the special populations (Pediatric extrapolations, Special Populations, Disease Populations.)
2. Evaluation of Drug-Drug interactions.
3. Cross Species extrapolation.
4. In-vitro- In-vivo Correlation (IVIVC).

2.2 Significance of The Pediatric PBPK Model:

In pediatrics a traditional pharmacokinetic study is very tough to perform due to various factors such as the number of samples to be collected, the blood volume (Howie SR et.al), requirement of the same protocol , dosing schedule and sample collection at the regular intervals and various ethical concerns (Roth-Cline M et.al; Barker CIS, et.al). Hence Population Pharmacokinetic approach is employed in order overcome the major limitations of the traditional PK studies which uses sparse sampling technique. The Pop PK studies do not include the developmental differences in the pediatric group which is one of the major challenges determining the dosage adjustments in the pediatrics. The PBPK models which include the anatomy and physiology of the subject in order to determine the concentration in the neonates have gained a significant importance in the pediatric dosage adjustments (Penkov D et.al). Apart from this they can also be used to determine the first dose in pediatrics as they facilitate cross species extrapolation. The regulatory authorities incorporated the guidelines for the model development and qualification (Committee for Medicinal Products for Human Use (CHMP) et.al)

2.3 Software's Available for The PBPK Modeling:

The development of commercial platforms integrating physiological databases and the implementation of PBPK modeling approaches facilitates the construction of PBPK models. These software's differ from in various approaches made to facilitate using friendly but at the core they all include physiological databases that are combined with the compound. Various software's include GastroPlus (Simulations Plus, Lancaster, PA), SimCyp (SimCyp, Sheffield, UK), and PK- Sim and MoBi (Bayer Technology Services, Leverkusen, Germany). PK-SIM is an open source from open systems pharmacology. These commercial PBPK modeling systems have the physiology of predefined organisms and populations with a standardized model framework.

2.4 PK-Sim for Physiologically Based Pharmacokinetic Modelling:

Pk-Sim and MoBi are part of Open Systems Pharmacology, an open-source tool designed to perform efficient multi-scale modelling and simulations based on modular concepts. These software tools make use of building blocks where Pk-Sim works on the whole-body concept, the focus of its counterpart MoBi lies at the molecular level. PK-Sim provides access to all the relevant anatomical and physiological parameters from the integrated database for humans and common laboratory animals like mouse, rat, pig and monkey. It also provides different Physiologically Based Pharmacokinetic (PBPK) calculation and parameterization methods. Relevant processes, such as the distribution of the drugs through blood flow as well as specific active processes are automatically taken into consideration by PK-Sim. It provides various model structures to opt for such as, to explore differences for small molecular compounds and large molecular compounds etc. These building blocks include Individuals, Populations, Compounds, Formulations, Administration Protocols, Events, and Observed Data. Pk-Sim further can be used to compare two individual simulations and derive results out of it (Stephanie Laer, 3rd March,2011).

AMINOPHYLLINE

3.1 Methodology:

3.1.1 Material and Methods

Anonymized data of 108 preterm neonates with apnea were obtained from the previous study conducted at NICU of Kasturba Hospital, Manipal University. Preterm neonates with less than or equal to 34 weeks of gestational age and greater than 6 apneic episodes in 24 hrs were included in the study (Shivakumar M et al.). Dosing began as early as from day 2 with follow up dosing every 8 hrs. All the subjects were prescribed doses ranging from 1.2 - 3mg/kg every 8hrs following a loading dose ranging from 4 - 10mg/kg. A standard treatment protocol of 5mg/kg loading dose and 2mg/ kg maintenance dose for every 8 hours is used in this PBPK model development. The plasma and urine amount of theophylline were calculated from the model using Area under curve and individualized dose.

Clearance (Cl) = Dose / AUC

Need for study:

Aminophylline is recommended as a prophylactic treatment for AOP. The major concern is the higher chance of toxicity or achieving subtherapeutic levels of the drug due to the immaturity of metabolizing enzymes and clearance of the drug and its metabolites from the body. By predicting the ADME of a drug in preterms dose can be adjusted accordingly.

3.1.2 Model Building

Individual and Population building blocks.

PBPK models were built using the building blocks from PK-Sim. A mean individual representing the unabridged population (whole population) was created using the Individual block. This mean individual is used to produce a set of 100 and 50 virtual individuals for building a population representing the unabridged population and subgroup populations which were divided based on covariates weight and PNA, respectively. The Population were built around the range of demographics represented in Table 1. All these functions were performed using the population building block from PK-Sim. Table 1 represents the demographic inputs used in building an individual and unabridged population around the mean individual.

Table 1. Patient Demographics Data	
Number of patients	108
Body weight (Kgs)	1.123 (0.56 - 2.34)*
Postnatal age (Days)	6.85 (2 - 23)*
Height (cm)	35.84 (28.31 - 43.41)*
Gestational age (weeks)	30 (26 - 34)*
*Mean (Range)	

Compound Parameters:

Aminophylline is a derivative of theophylline which is combined with ethylenediamine to enhance the solubility. Upon conversion to theophylline, 40% of the agent is primarily bound to albumin. Aminophylline is primarily metabolized in the liver and follows mixed order kinetics. A fraction of parent compounds and metabolites are thought to be eliminated through urine. CYP450 iso-enzymes (CYP1A2, CYP2E1, CYP1A1) play an important role in its metabolism. CYP1A2 catalyzes the metabolism of theophylline to demethylated products. Hydroxylation occurs at a rapid rate with the substantial activity of both CYP1A2 and CYP2E1. CYP1A2 having higher affinity is predominant at lower doses and is readily saturable compared to CYP2E1. CYP1A1 manifests some activity in the formation of 1-Methylxanthine (Ginsberg et al, Aug 2010). Expression of CYP genes in the recombinant systems of human B-lymphoblastoid cell lines was used to study the various activities of the CYP enzymes towards these xanthine substrates. Biotransformation of these CYP enzymes was evaluated by Ha et al (1995). Michaelis-Menten Constants for these CYP enzymes which demonstrate their activity on the metabolism of theophylline are shown in Table 3.

Table 2. Compound Parameters		
Parameter:	Input values:	Reference:
LogP	-0.02	Drugbank
Fraction Unbound	0.6 Log Units	Drugbank
Molecular Weight	180.1 g/mol	Drugbank
Solubility at pH 7	7360 mg/L	Drugbank

Table 3. Theophylline metabolism to 1-MX, 3-MX and 1,3-U (Ginsberg et al, Aug 2010)						
	1-Methylxanthine (1-MX)		3-Methylxanthine (3-MX)		1,3-Dimethyluric acid (1,3U)	
CYP isoform	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m
CYP 1A1	0.22	0.31	NIL	NIL	NIL	NIL
CYP 1A2	6	0.38	2.44	1.09	7.32	0.23
CYP 2E1	NIL	NIL	NIL	NIL	68.67	15.3
Vmax in µmol metabolite formed/min/µmol CYP, Km in mmol/L						

Administration protocol:

A standard protocol of 5mg/kg loading dose of theophylline infused over 30 min and 2mg/kg maintenance dose of theophylline infused over 10 minutes was used for constructing the model. Dose adjustments from aminophylline to theophylline were made using the salt factor of 0.8 to have an effective measure of serum theophylline levels (Product Information: aminophylline IV injection, aminophylline IV injection. Hospira, Inc, Lake Forest, IL, 2004.) (Product Information: aminophylline oral tablets, aminophylline oral tablets. West-ward Pharmaceutical, Corp, Eaton- town, NJ, 2002).

Subgroup Population:

As stated in the section previously, subgroup populations were built around the mean individual. 50 virtual individuals in each population were generated around the range of demographic data represented in Table 4 and 5 respectively. Subgroups were created for the covariates PNA and weight. Table 4 and 5 represent the demographic inputs used in constructing the subgroup population.

Table 4. Postnatal age-based Subgroup Demographic Data		
	Group 1	Group 2
Number of patients	57	51
Weight (Kgs)	1.22 (0.65 - 2.34)*	1.02 (0.56 - 1.76)*
Postnatal age (Days)	4.89 (2 - 6)*	9.04 (7 - 23)*
Height (cms)	36,.24 (28.75 - 41.15)*	35. 28 (28.31 - 43.41)*
Gestational age (Weeks)	30.22 (26 - 34)*	29.82 (26 - 34)*
*Mean (Range)		

Table 5. Weight based Subgroup Demographic Data [Mean (Range)]			
	Group 1	Group 2	Group 3
Number of patients	44	50	14
Weight (Kgs)	0.854 (0.56 - 0.99)*	1.17 (1 - 1.49)*	1.8 (1.59 - 2.34)*
Postnatal age (Days)	7.36 (3-23)*	6.76 (2 - 22)*	5.57 (4 -10)*
Height (cms)	35.41 (29.96 - 40.24)*	36.03 (28.31 - 43.41)*	36.45 (33.53 - 39.8)*
Gestational age (Weeks)	28.84 (26 - 34)*	30.54 (26 - 34)*	32 (26 - 34)*
*Mean(Range)			

Simulation:

The Model was simulated for the unabridged population of 100 virtual individuals with the predetermined standard protocol of 5mg/kg loading dose and 2mg/kg maintenance dose with a dosing interval of 8 hrs for 424 hrs. Clearance post-loading dose was compared to the clearance post final dose. The clearance was calculated using the obtained PK parameters from the simulation. The predicted time-concentration profile showed an increase in clearance with time due to the maturation of liver enzymes with age.

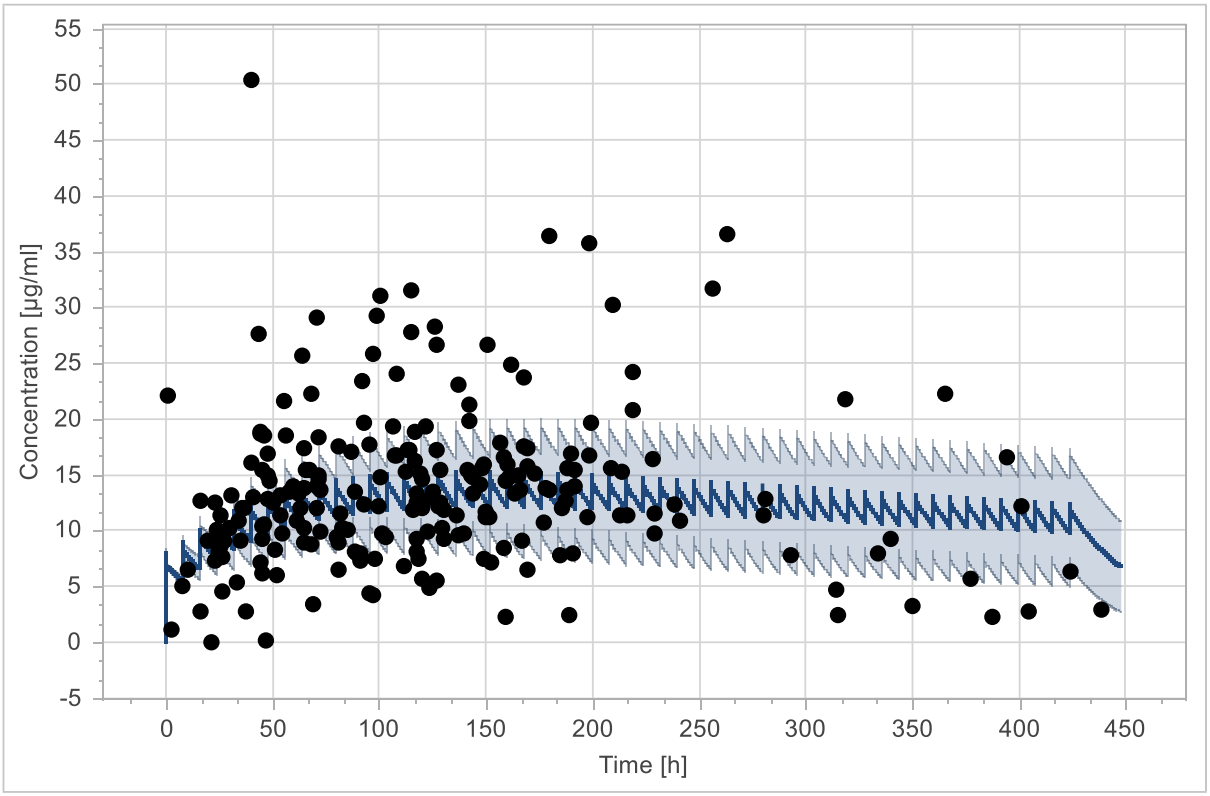


Fig 3. Time vs Concentration for the unabridged population

Further simulations were performed for the subgroups with 50 virtual individuals in each sub-group. The preterms were sub-grouped into 3 categories based on weight (less than 1 kg, 1-1.5 kg, greater 1.5 kg) and the models were simulated. Results are represented in the figures below (Fig 3, Fig 4, Fig 5).

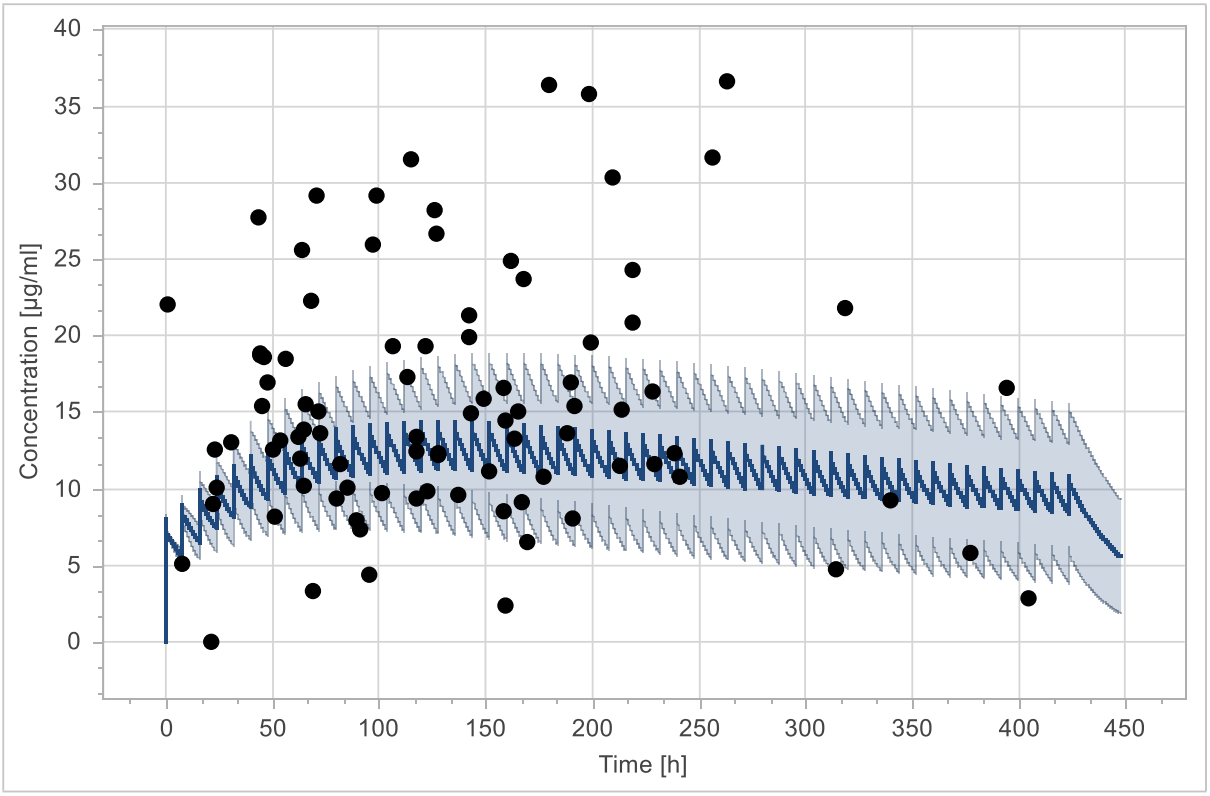
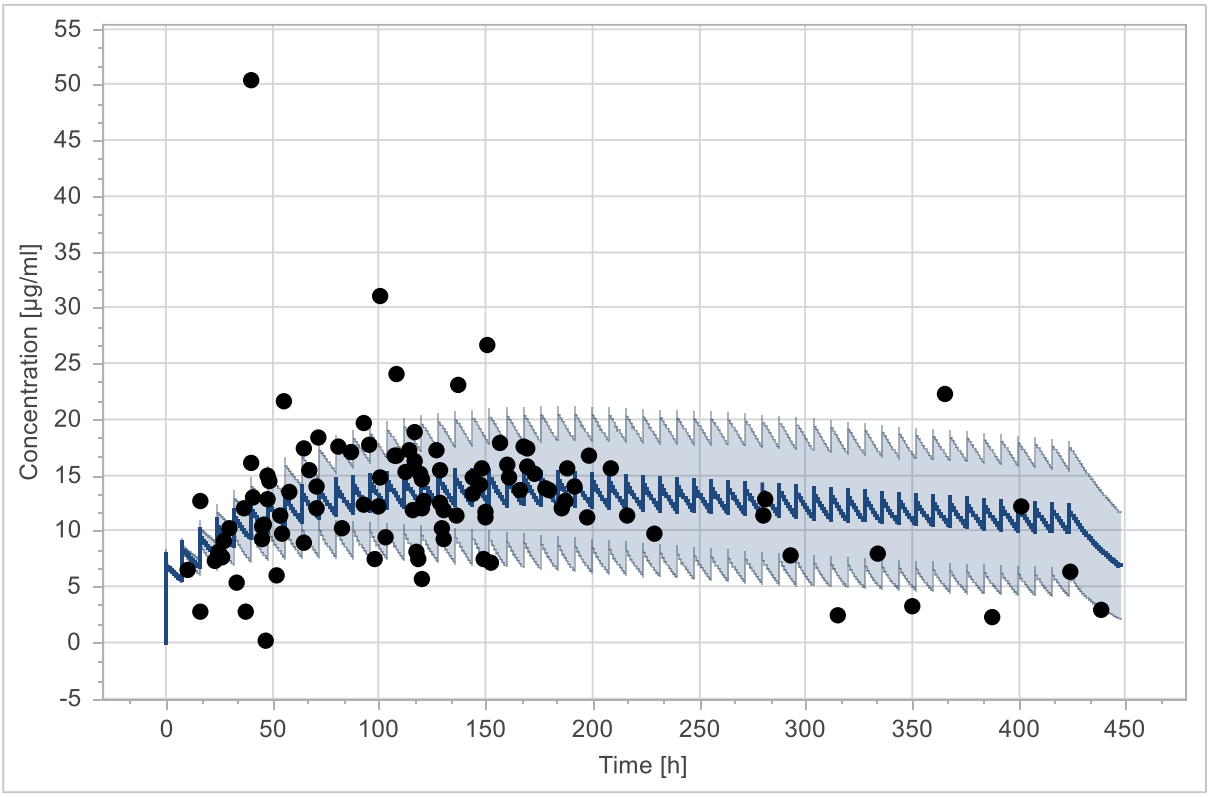


Fig 4. Time vs Concentration profile for Preterms weighing less than 1 kg



Predicted peripheral venous blood-plasma concentrations of theophylline (Arithmetic mean)

Predicted peripheral venous blood-plasma concentrations of theophylline (Arithmetic standard deviation)

• Observed concentrations

Fig 5. Time vs Concentration profile for Preterms weighing 1-1.5 Kg

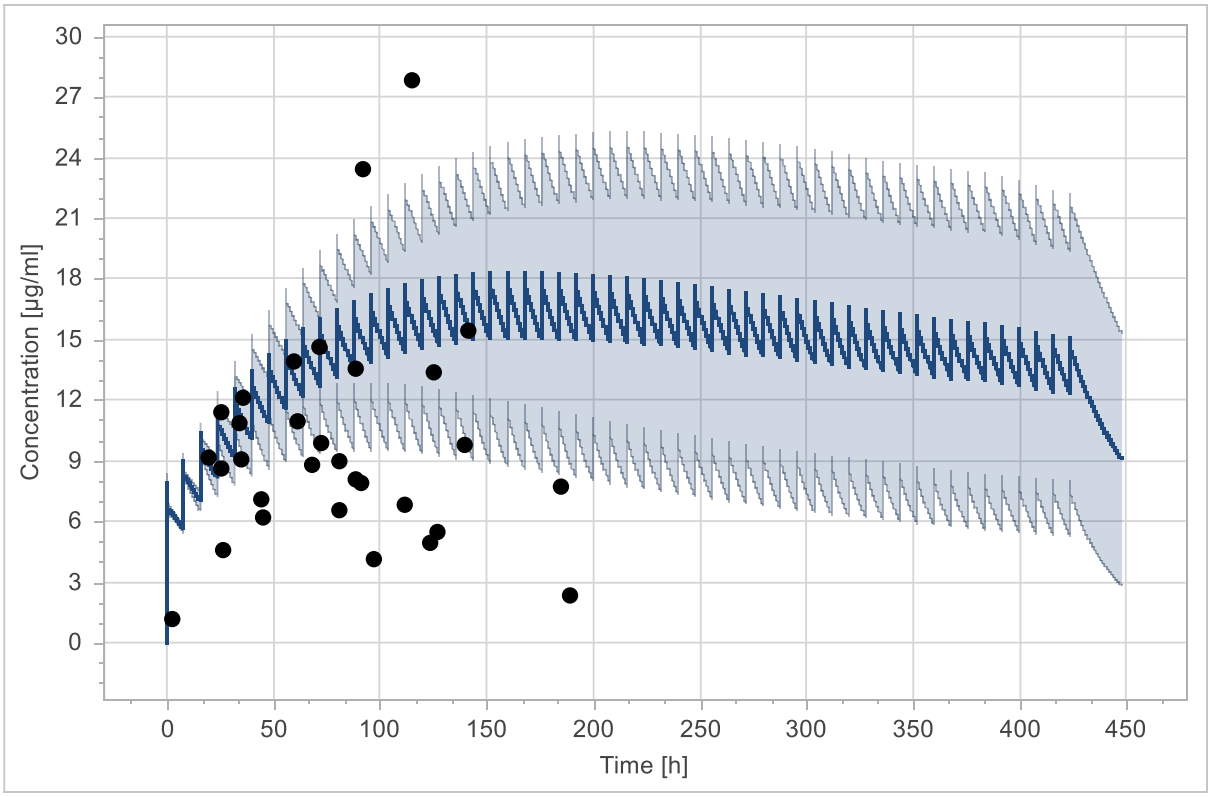
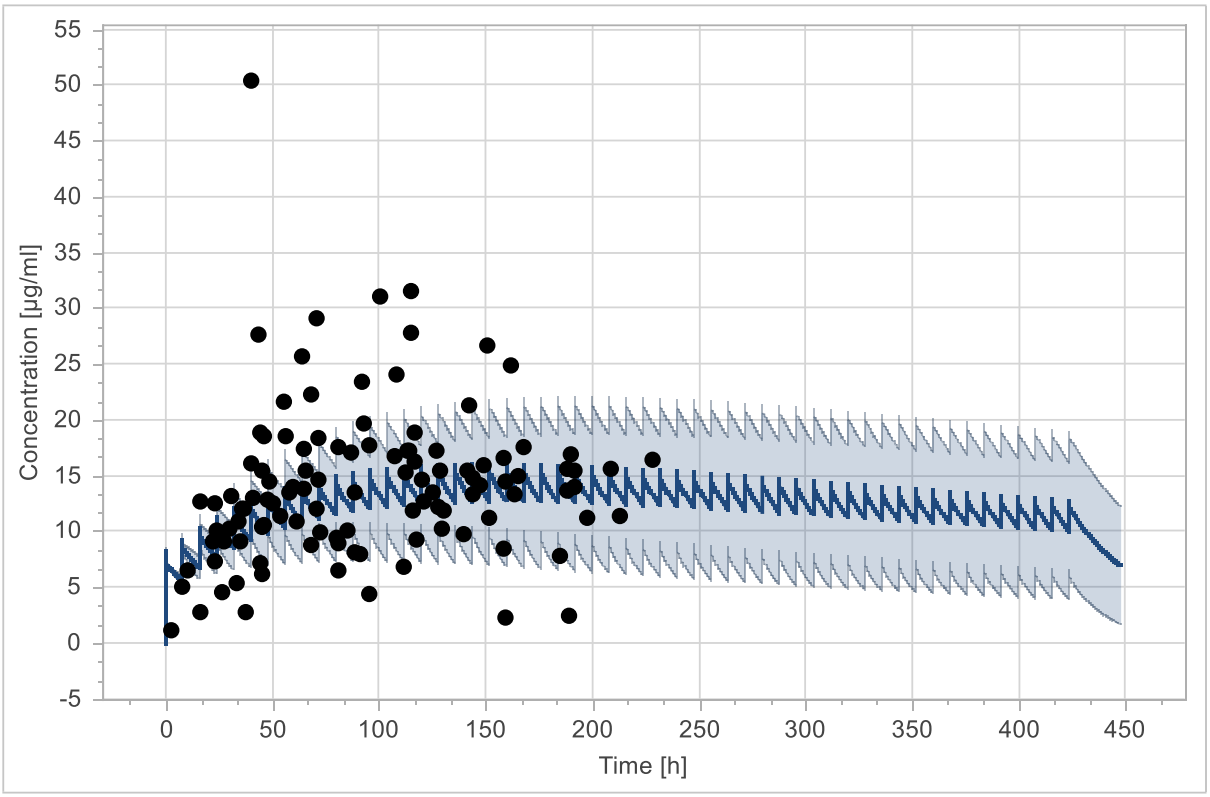


Fig 6. Time vs Concentration profile for Preterms weighing greater than 1.5 kg

The preterms were sub-grouped into 2 PNA categories (ranging from 1 to 6 days and PNA greater than 6 days). The models were simulated, and the results are represented in the figures below (Fig 6, Fig 7)



Predicted peripheral venous blood-plasma concentrations of theophylline (Arithmetic mean)

Predicted peripheral venous blood-plasma concentrations of theophylline (Arithmetic standard deviation)

Observed concentrations

Fig 7. Time vs Concentration profile for preterms with postnatal age ranging from 1 to 6 days

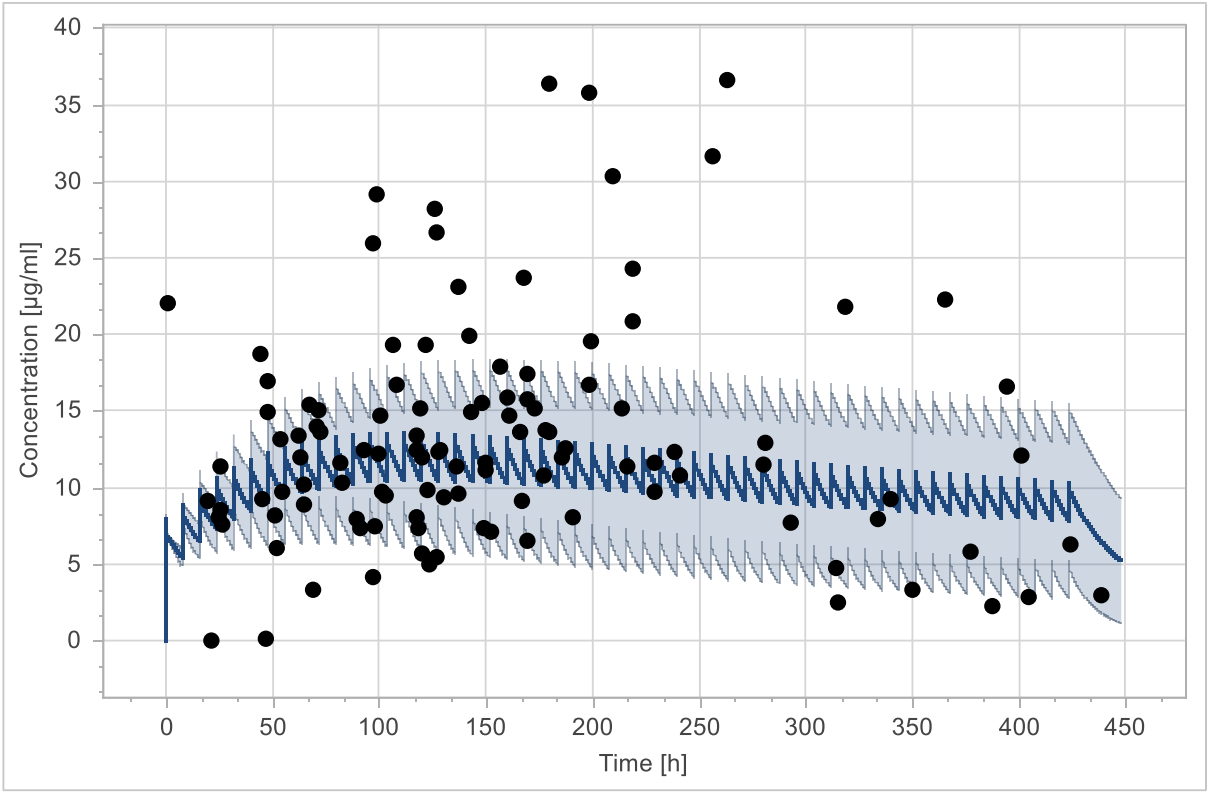


Fig 8. Time vs Concentration profile for preterm with postnatal age greater than 6 days

Clearance calculation:

Clearance (CL) post-loading dose and clearance post last maintenance dose was calculated using dose, individualized to each preterm of the virtual population and the AUCinf_t1 and AUCinf_tLast. The obtained CL was multiplied by 60 to obtain CL in L/hr.

Clearance post LD = Dose (mg)/ AUCinf_t1 (µg*min/ml) Clearance post last MD = Dose (mg)/ AUCinf_tLast (µg*min/ml)

3.1.3 Results

The study was conducted from the previously obtained data from 108 preterms. Demographics of these patients (Table 1, 4 and 5) were used to build a virtual population representing an unabridged population and subgroup populations. The primary measure of outcome was clearance. Clearance post LD in unabridged population was 0.026 L/hr which substantially increased to 0.055 L/hr post last maintenance dose. PNA

based Subgroup simulations provide a shred of evidence for the maturation of enzymes with the progression of time which in turn increases the clearance. Weight-based subgroup simulations also provide a similar trend of increase in clearance with time. There is also increase of clearance with the increase in bodyweight post-loading dose (CL post LD of BWT 0-1 < BWT 1-1.5 < BWT greater than 1.5) but this reverts with the progression of time (CL post last MD of BWT 0-1 > BWT 1-1.5 > BWT greater than 1.5). This provides evidence for the alteration in distribution characteristics with regards to body weight and progressing time and this results in altered standard dosing patterns which leads to increased plasma concentration in preterms with BWT 0-1 and sub-therapeutic levels of plasma concentration in preterms with BWT greater than 1.5 post LD. CL in various populations is represented in Table 6.

The Pharmacokinetic parameters representing the mean individual from each population are represented in Table 7.

Table 6. Clearance (CL)

	CL Post LD	CL Post Last MD
Unabridged population	0.026388188	0.054788956
BWT 0-1 (Kg)	0.016237354	0.090393858
BWT 1-1.5 (Kg)	0.019849332	0.058491951
BWT greater than 1.5 (Kg)	0.023381645	0.033613176
PNA 1-6 (Days)	0.024577978	0.0597144
PNA greater than 6 (days)	0.027330422	0.092659935
Clearance in L/hr		

Table 7. Pharmacokinetic Parameter output representing mean individual from each population					
	AUC_inf_t1 (µg*min/ml)	AUC_inf_tLast (µg*min/ml)	Cmax (µg/ml)	MRT (hr)	Half-Life
Unabridged Population	14492.29581	31585.54898	15.276082	35.1	31.71
BWT 0-1 (Kg)	13249.78591	25272.95393	14.35397	31.11	29.44
BWT 1-1.5 (Kg)	15581.72071	33089.07601	15.396749	37.61	33.74
BWT greater than 1.5 (Kg)	18059.42664	46795.66396	18.323374	44.69	38.37
PNA 1-6 (Days)	14915.55782	33073.24522	16.016293	35.36	32.83
PNA greater than 6 (Days)	13655.69168	23777.47738	13.541719	32.97	29.87

- AUC_inf_t1 - AUC from the first data point extrapolated to infinity
- AUC_inf_tLast - AUC from the time of last dose extrapolated to infinity
- Cmax - Maximum concentration
- MRT – Mean Residence Time of the drug molecule
- Half-Life – Half-life time associated with terminal slope

3.1.4 Discussion

Preterm births lead to various complications, one of it includes apnea of prematurity. Methylxanthine therapy is the most used way of management and this hurdles in achieving an absolute therapeutic concentration necessary with the standard dosing regimen. The benefits of methylxanthine treatment for premature apnea outweigh potential short-term risks. Postnatal methylxanthine therapy may lead to altered behavioral and respiratory control in offspring's (Tracey H Reilly, Pediatric Theophylline Toxicity, Medscape). Aminophylline / Theophylline, due to narrow therapeutic range, when prescribed, due to immaturity in the development of the physiological system in the preterms the prescribed drugs may cause toxicity or end up with subtherapeutic concentrations. Hence individualized dosing adjustment is necessary. In the current study, a standard dosing protocol of 5 mg/kg loading dose and 2 mg/kg maintenance dose with a dosing interval of 8 hrs was compared in preterms with different body weights and its effect with the progression of age. Serum concentration ranging from 15-20 mcg/ ml is considered effective and anything greater than 20mcg/ml leads to toxicity. The predicted mean concentration for the unabridged population was found to be 15.27 mcg/ml. Which when compared to the subgroups based on weight, predicted concentrations were in stoop with the actual concentrations only in the averaged weighted preterms (BWT 1-1.5) were predicted mean concentration was found to be 15.4 mcg/ml (Fig 4, Table 7). The predicted mean theophylline concentration in preterms weighing less than 1 kg was found to be 14.4 mcg/ml (Table 7) which when compared with the concentrations from the actual patient data, it was found that the patients were having serum concentrations greater than the predicted range (Fig 3). This reverts with the preterms weighing greater than 1.5 kg where the actual concentrations were below the predicted concentrations (Fig 5). This can provide an evidence for the relationship between alteration of distribution and bodyweight. With maturation the relative amount of body water and fat levels change. When measured as a percentage of body weight, the total body water is decreased from 80 to 60% from birth to 1 year. (Hong Lu and Sara R, 2014). Similarly, when compared the subgroups based on PNA, clearance post-loading dose was greater in subgroup PNA > 6 days when compared to PNA 1-6 days (Table 6). With the progression of time, after the last maintenance dose i.e. after 482 hrs the clearance was found to be in the same fashion with the mean clearance of 0.06 L/hr in subgroup PNA 1-6 days and 0.09 L/hr in subgroup PNA > 6 days. These results suggest lower maturation of hepatic enzymes and renal function which lead to lower clearance and increased bioavailability of the compound. The maturation of CYP1A2 begins from day 8, which reaches the adult levels by the age of 12 to 18 months (Hong Lu and Sara R, 2014). This provides evidence for the greater clearance in subgroup PNA > 6 days compared to subgroup PNA 1-6 days. Similarly, the maturation of CYP2E1 begins from the less than 24 hrs which contributes to the majority of metabolism of theophylline till the third month (Hong Lu and Sara R, 2014). The significant findings of the present developed model were that body weight and PNA has a notable influence on clearance (Fukuda M.S, et all November 2005). In a study, the effect of age on preterm metabolism of theophylline (Grygiel J.J and Brikett D.J, 1980) it was stated that adults and children have a dual pathway to metabolize theophylline which is N-demethylation and 8 - hydroxylation which is not developed in preterm neonates up to 40 weeks of gestation. In the current study, there is an increase in

clearance with post-natal age (Table 6) due to maturation of liver enzymes (Aranda et al, 1976). Theophylline is 60 % bound to albumin in adults and about 53 to 65 % theophylline is bound reversibly bound to circulating plasma proteins in adults but due to lack of this plasma protein in neonates the free drug availability is elevated in the blood leading to increased bioavailability which in turn increases the half-life by almost 8 fold and reversible binding to circulating plasma proteins is reduced to an average of 36% in neonates which leads to toxicity and adverse effects (Jacob V et al,1976).

CAFFEINE

4.1 Methodology

4.1.1 Materials and Methods:

Anonymized data of 61 preterm neonates with apnea were obtained from the previous study conducted at NICU of Kasturba Hospital, Manipal University. Though the study was done based on the standard dosage regimen that is 10mg/kg of Caffeine base as a loading dose followed by 2.5mg/kg of caffeine base as maintenance dose. But the clinically unresponsive subjects were titrated to a dose of 7.5mg/kg. To maintain dosage uniformity the subjects deviated from the exact standard dose were excluded.

4.1.2 Model Building

Organism Parameters:

The modeling was done using PK-SIM Software. We created an individual representing the whole population and 100 virtual individuals were simulated around the mean individual using the range of the population parameters. The mean and the range values used for creating the mean individual and Population are listed below.

S.No	Parameter	Mean	Range
1.	Body weight	1.22 Kg	0.56 – 2.01 Kg
2.	Height	36.33 Cm	27 - 43 Cm
3.	Post Natal Age	3.29 days	1 - 18 Days
4.	Body Mass Index	9.24	------
5.	Gestational Age	30.14	26-35

Table 8. Organism Parameters Used for The Population Model of Caffeine.

Compound Parameters:

In the compound parameter section, all the processes involved in the absorption, distribution, metabolism and excretion of the drug. The base line compound parameters for caffeine are as follows:

S.No	Parameter	Value With Units	Reference
1.	Lipophilicity	-0.07 Log units	PubChem
2.	Fraction Unbound	70%	PubChem
3.	Solubility	21600mg/L	PubChem
4.	Pka (Base)	0.80	PubChem

Table 9. Drug Parameters Used for The Population Model of Caffeine.

The metabolism of caffeine is a saturable process and it follows mixed order kinetics with an initial zero order followed by a first order process. Caffeine is mainly metabolized via four hepatic enzymes namely CYP1A2, CYP2E1, CYP3A4, CYP1A1. The major metabolites of caffeine are Paraxanthine, Theobromine, Theophylline and Trimethyl uric acid. The Vmax and Km values were taken from the literature of the invitro recombinant CYP enzyme system (Data of Ha et al., 1995, 1996).

S.No	Enzyme	Metabolite	Vmax (mol/min/ mol rem.enzyme)	Km(mmol/ l)
1.	CYP1A1	Paraxanthine	0.05	0.59
		Theobromine	0.01	0.41
		Theophylline	0.06	0.26
2.	CYP1A2	Paraxanthine	0.51	0.19
		Theobromine	0.05	0.16
		Theophylline	0.02	0.25
		Trimethyl Uric Acid	0.03	0.27
3.	CYP3A4	Trimethyl Uric Acid	0.46	46.0
4.	CYP2E1	Theobromine	0.0008	1.44
		Theophylline	0.0006	0.84
		Trimethyl Uric Acid	0.05	1.04
5.	CYP2D6	Paraxanthine	0.56	11.0
		Theobromine	0.28	15.90
		Theophylline	0.63	12.50
		Trimethyl Uric Acid	0.21	9.13

Table 10. Vmax And Km Values for The Hepatic Clearance Used for The Population Model of Caffeine.

Administration Protocol:

The Administration protocol for the caffeine is as followed as per the clinical study first a loading dose of 10mg/kg is given followed by a maintenance dose of 2.5mg/kg .If there was no response the dose was increased to 7mg/kg. The subjects which required higher doses any alteration in the dosing regimen were excluded. The clinically observed concentration versus time was added to the observed data tab and pulled over the predicted concentration with standard deviation versus time.

Subgroup Analysis:

The population was divided into three subgroups based on the weight. To build a model based on the subgroups the compound parameters and the administration protocol were cloned as per the population model and the group specific mean and range of the organism parameters are given as an input.

The subgroups based on the weight are as follows.

S.No	Name	Weight Range (Kg)
1	Group 1	0.5-1.0
2	Group 2	1.0-1.5
3	Group 3	>1.5

Table 11. Classification of Subgroups Based on Body Weight

Input values of range and mean of the sub populations used to create the subgroup organism parameters are as follows:

Parameter	Group 1		Group 2		Group 3	
	Mean	Range	Mean	Range	Mean	Range
Body Weight (Kg)	0.825	0.56-0.99	1.24	1.01-1.5	1.7	1.6-2.01
Gestational Age (Months)	29	26-32	30.66	27-35	32.14	31-33
Post Natal Age (Days)	4.09	1-14	2.96	1-18	3.285	2-6
Height (Cm)	33.18	27-38	36.88	30-41	39.14	34-43

Table 12. Mean and range values of Body weight sub population

The subgroups based on PNA are as follows:

S.No	Name	PNA range (days)
1	Group 1	0-10 days
2	Group 2	>10 days

Table 13. Classification of Subgroups Based On PNA

Parameter	Group 1		Group 2	
	Mean	Range	Mean	Range
Body Weight (Kg)	1.2675	0.615-2.005	1.214	0.56-1.8
Gestational Age (Months)	30.5375	27-33	30.86	27-35
Post Natal Age (Days)	6.6375	2-10	14.74	11-37
Height (Cm)	36.325	28-43	36.71	27-43

Table 14. Mean and range values of PNA subgroups

4.1.3 Results

From the available dataset, the PBPK model for caffeine population was generated and the results are presented below as figures and tables. The figure below shows the time vs conc profile for the whole caffeine population

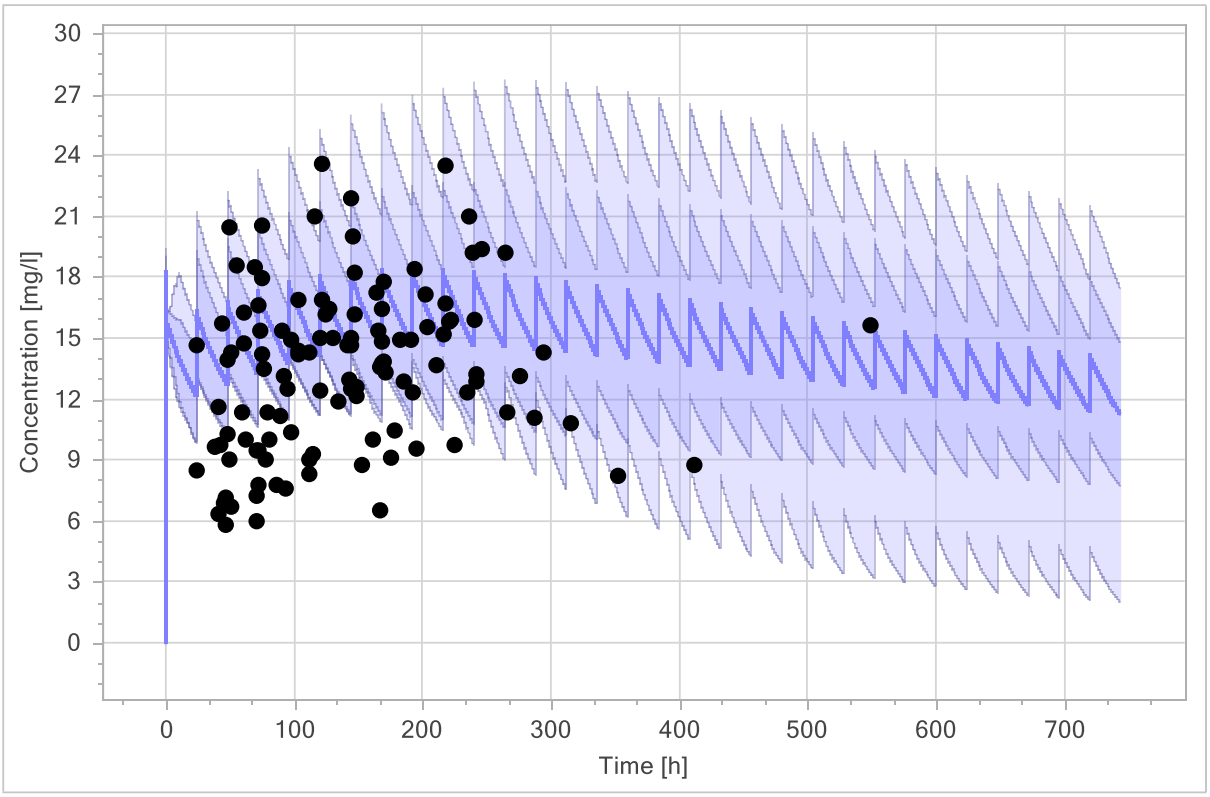


Fig 9. Time Vs Concentration profile- whole preterm population

PARAMTER	VALUE
AUC_inf_tD1 [µmol*min/l]	465013.44
AUC_inf_tD1_norm [µg*min/l]	9.03E+12
AUC_inf_tDLast [µmol*min/l]	482007.66
AUC_inf_tDLast_norm [µg*min/l]	3.744E+13
AUC_tD1-tD2 [µmol*min/l]	101948.83
AUC_tD1-tD2_norm [µg*min/l]	1.98E+12
AUC_tDlast-1_tDlast [µmol*min/l]	94410.422
AUC_tDlast-1_tDlast_norm [µg*min/l]	7.333E+12
C_max [µmol/l]	94.501282
C_max_tD1-tD2 [µmol/l]	93.913841
C_max_tD1-tD2_norm [mg/l]	1823712.8
C_max_tDlast-tEnd [µmol/l]	72.840813
C_max_tDLast-tEnd_norm [mg/l]	5657982.8
C_trough_tD2 [µmol/l]	62.880585
C_trough_tDlast [µmol/l]	57.953384
MRT [h]	96.416032
t_max [h]	192.75
t_max_tD1-tD2 [h]	0.75
t_max_tDlast-tEnd [h]	720.75
Half-Life [h]	66.702417
Half-Life_tDlast-tEnd [h]	77.49541

Table 15. Parameter output of the population simulation of caffeine

Sub-group analysis:

The preterm population was divided into subgroups based on neonatal body weight and Post Natal Age (PNA). Three subgroups based on body weight (0.5Kg-1.0Kg, 1.0Kg-1.5Kg, >1.5Kg) and two subgroups based on PNA (0-10 days and >10 days). The tables represent the output PK parameters of the sub-populations.

I. Based on Body Weight

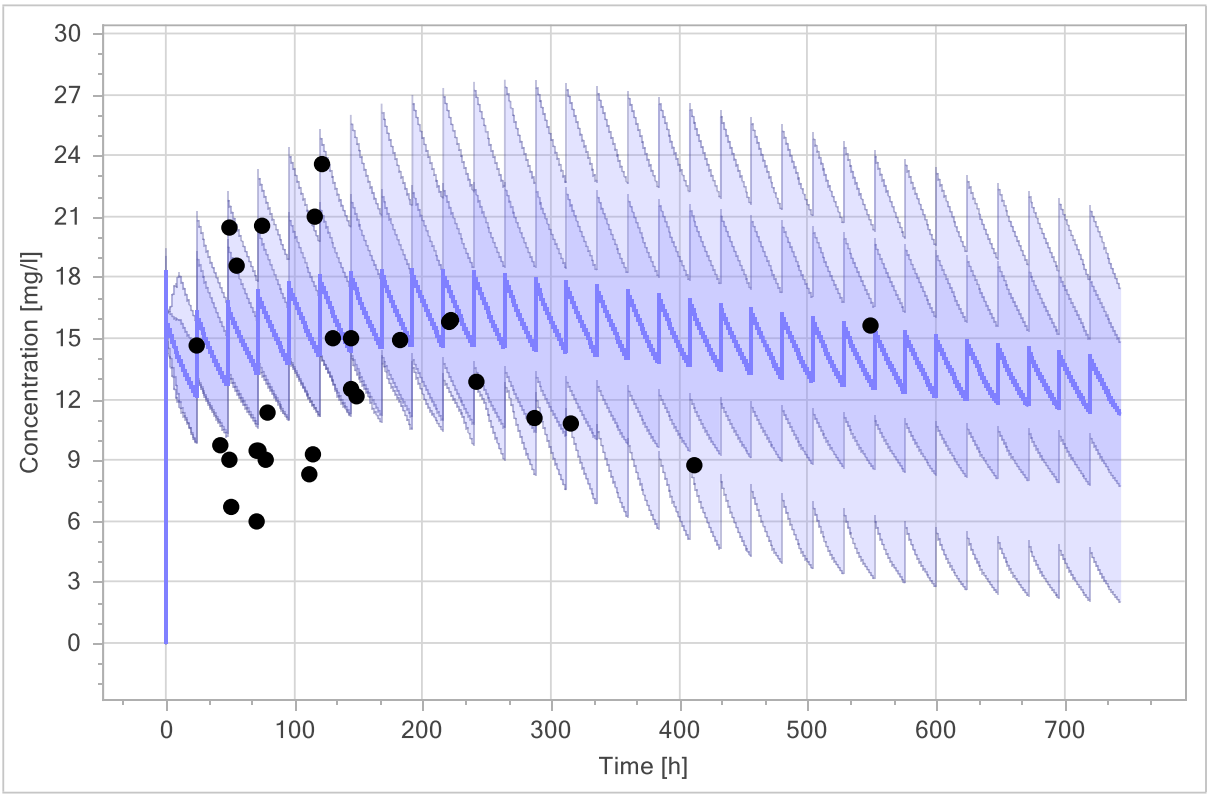


Fig 10. Time Vs Concentration profile for preterm population with body weight range 0.5 to 1.0 Kg

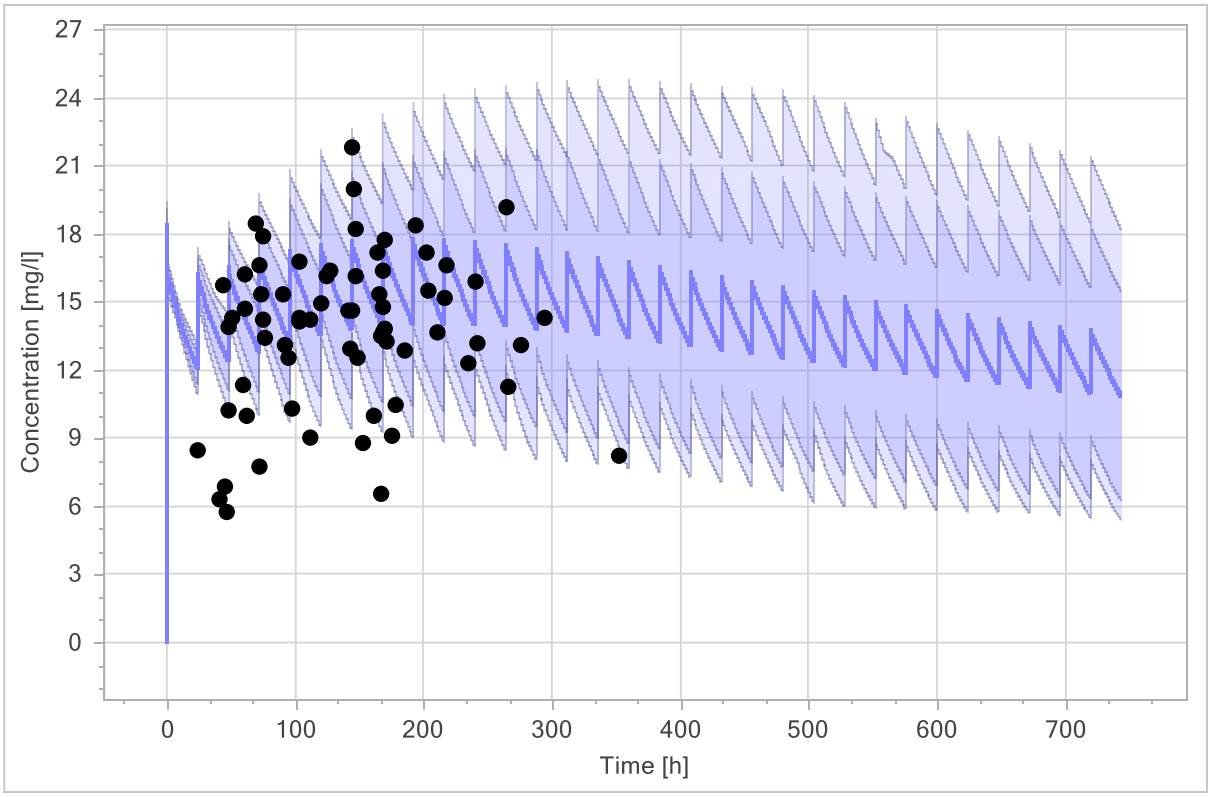


Fig 11. Time Vs Concentration profile for preterm population with body weight range 1.0 to 1.5 Kg

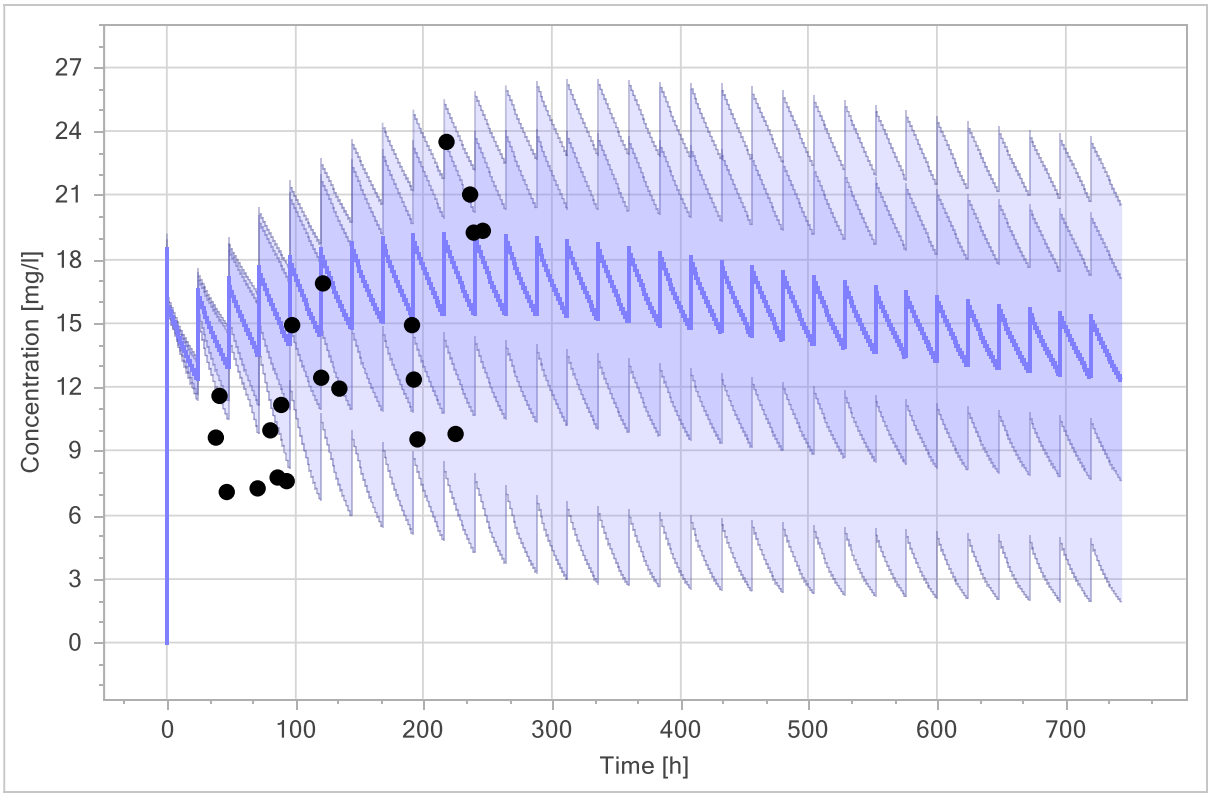
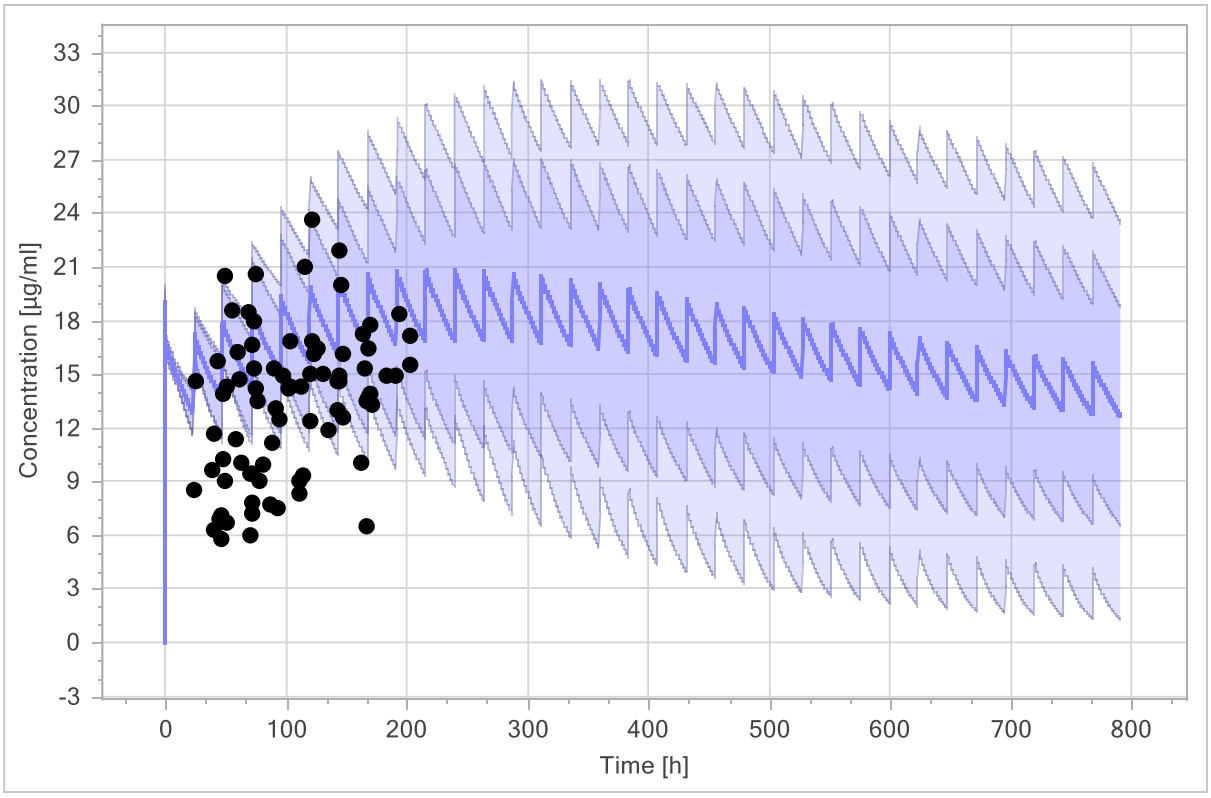


Fig 12. Time Vs Concentration profile for preterm population with body weight above 1.5Kg

PARAMETER	GROUP 1	GROUP 2	GROUP 3
AUC_inf_tD1 [µmol*min/l]	465013.44	431324.2	481387.4
AUC_inf_tD1_norm [µg*min/l]	9.03E+12	8.38E+12	9.35E+12
AUC_inf_tDLast [µmol*min/l]	482007.66	457738.9	557130.7
AUC_inf_tDLast_norm [µg*min/l]	3.744E+13	3.56E+13	4.33E+13
AUC_tD1-tD2 [µmol*min/l]	101948.83	103127	103568.8
AUC_tD1-tD2_norm [µg*min/l]	1.98E+12	2E+12	2.01E+12
AUC_tDlast-1_tDlast [µmol*min/l]	94410.422	91561.48	102695.6
AUC_tDlast-1_tDlast_norm [µg*min/l]	7.333E+12	7.11E+12	7.98E+12
C_max [µmol/l]	94.501282	94.79954	98.69127
C_max_tD1-tD2 [µmol/l]	93.913841	94.79954	95.08429
C_max_tD1-tD2_norm [mg/l]	1823712.8	1840912	1846442
C_max_tDlast-tEnd [µmol/l]	72.840813	70.85256	78.92624
C_max_tDLast-tEnd_norm [mg/l]	5657982.8	5503543	6130674
C_trough_tD2 [µmol/l]	62.880585	62.3846	63.89842
C_trough_tDlast [µmol/l]	57.953384	56.0673	63.60205
MRT [h]	96.416032	87.73719	98.67993
t_max [h]	192.75	0.75	216.75
t_max_tD1-tD2 [h]	0.75	0.75	0.75
t_max_tDlast-tEnd [h]	720.75	720.75	720.75
Half-Life [h]	66.702417	60.77594	68.30734
Half-Life_tDlast-tEnd [h]	77.49541	75.67403	82.73179

Table 16. PK-parameter output for the Body Weight subgroup analysis of caffeine

II. Based on PNA



Predicted peripheral venous blood-plasma concentrations of caffeine (Arithmetic mean)

Predicted peripheral venous blood-plasma concentrations of caffeine (Arithmetic standard deviation)

- Observed concentrations

Fig 13. Time vs Conc profile for preterm population with postnatal age (PNA) range of 0 to 10 days

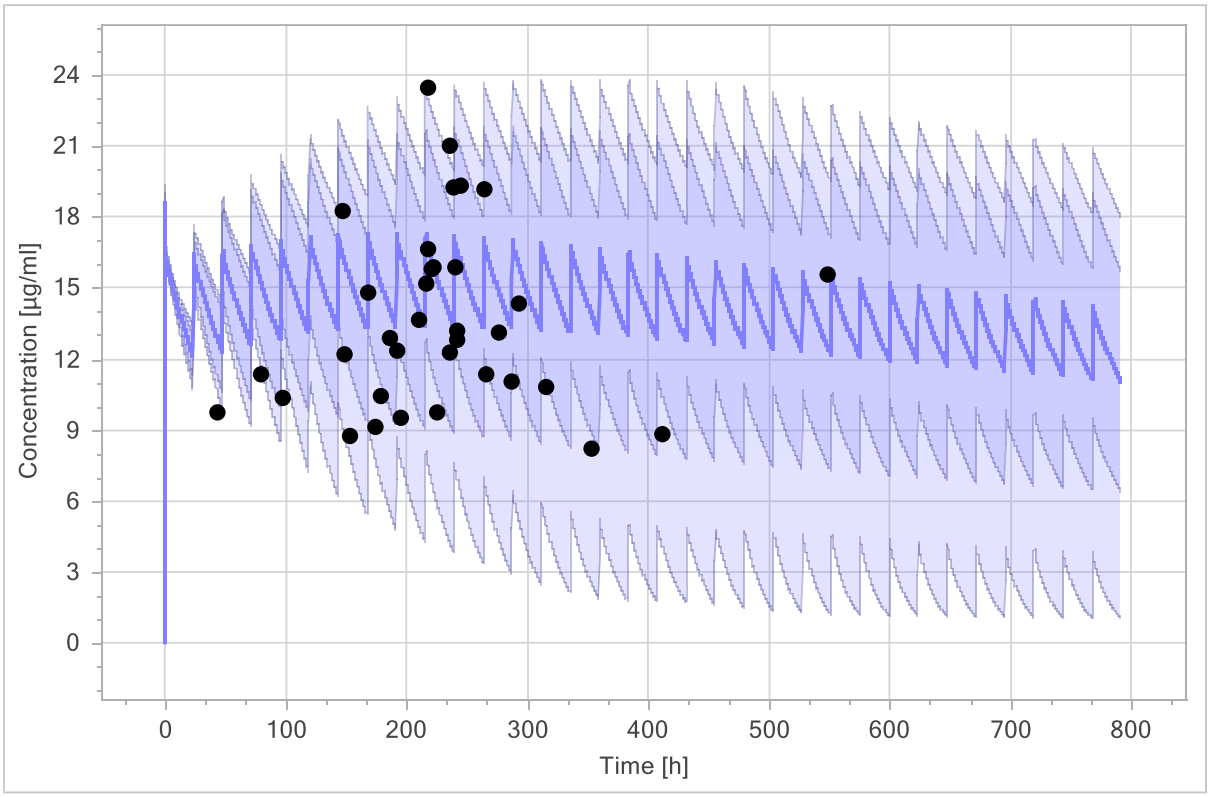


Fig 14. Time vs Conc profile for preterm population with postnatal age (PNA) range of greater than 10 days

PARAMETER	GROUP 1	GROUP 2
AUC_inf_tD1 [µmol*min/l]	491035.0313	415170.6563
AUC_inf_tD1_norm [µg*min/l]	9.16866E+12	7.75211E+12
AUC_inf_tDLast [µmol*min/l]	574660.5	446638.8125
AUC_inf_tDLast_norm [µg*min/l]	4.16393E+13	3.2363E+13
AUC_tD1-tD2 [µmol*min/l]	107675.5859	103911.1484
AUC_tD1-tD2_norm [µg*min/l]	2.01053E+12	1.94024E+12
AUC_tDlast-1_tDlast [µmol*min/l]	105139.6875	93482.32813
AUC_tDlast-1_tDlast_norm [µg*min/l]	7.61831E+12	6.77363E+12
C_max [µmol/l]	107.3054962	95.71002197
C_max_tD1-tD2 [µmol/l]	98.02106476	95.71002197
C_max_tD1-tD2_norm [mg/l]	1830260.634	1787108.541
C_max_tDlast-tEnd [µmol/l]	80.44139862	73.33685303
C_max_tDLast-tEnd_norm [mg/l]	5828699.589	5313911.915
C_trough_tD2 [µmol/l]	66.05621338	62.54024506
C_trough_tDlast [µmol/l]	64.98568726	56.94951248
MRT [h]	96.79428711	83.0654541
t_max [h]	240.75	0.75
t_max_tD1-tD2 [h]	0.75	0.75
t_max_tDlast-tEnd [h]	768.75	768.75
Half-Life [h]	67.04505208	57.49594727
Half-Life_tDlast-tEnd [h]	83.71397298	71.82002767

Table 17. PK-parameter output for the PNA subgroup analysis of caffeine

4.1.4 Discussion

Bodyweight, postnatal age, postmenstrual age and gestational age are the major parameters concerning the dosing in the neonatal population (Lawrence C. Ku et.al.). Pharmacokinetics of the drug are not only different between neonates, older children and adults, but they also vary among neonates with different ranges of maturity. As the age increases the renal and hepatic metabolism is very well developed leading to the changes in the potency, efficacy and toxicity of the drug (Hong Lu, PhD et.al, Warner A.et.al.). Of these wide differences, body weight and postnatal age are considered to be having a potential role in determining the safe dose in neonates as they are directly related to the growth and development in the neonates (Loebstein R et.al).

Caffeine citrate which is used in the treatment of the apnea of prematurity is among the most frequently used drug in the neonatal group (Hsieh E M et.al). The therapeutic range of caffeine in neonates is 5-25 mg/L and the drug should measure >40mg/L to show the toxic levels (Johnson P J et.al). According to the current treatment guidelines, the dose of caffeine is titrated according to the body weight of an infant to attain the therapeutic range (Hey E, Ed et.al). The dosage regimen according to the guideline is 20mg/kg of the caffeine citrate as a loading dose followed by 5-10 mg/kg of the maintenance dose (Caffeine Citrate label.).

The population model simulated is validated by the visual predictive curve comparing the predicted and observed outcomes. The half-life and Cmax obtained are similar to the PBPK model of caffeine by Gary Ginsberg et.al and is within the range of 42-103 hrs in neonates (*Abdel-Hady H et.al*). In the first few weeks of birth, caffeine is mainly excreted by the kidneys due to the lack of hepatic enzymes which leads to decreased drug metabolism which picks up as the age increases and the drug is cleared more rapidly (Abdel-Hady H et.al). This could probably explain the drop in the Cmax as the treatment duration increases (Multi-dose study). In the simulation, the AUC and Cmax in all the three groups based on the bodyweight are very close with minimal variance. This can be inferred as the dose increased with respect to the bodyweight could effectively manage the increased clearance due to the hepatic enzyme maturation.

The decrease in the Cmax with an increase in the age should be explained in the output. In the covariate analysis of the population pharmacokinetic studies, the concentration of the caffeine is found to be affected only by the bodyweight but there is evidence of the effect of the postnatal age.

CONCLUSION

From the aminophylline study, it can be concluded that dose adjustments are needed for aminophylline / theophylline in the treatment of apnea of prematurity in neonates. With several instances of toxicity being reported with aminophylline / theophylline (Tracey H Reilly, 2019) it is necessary to individualize the dose to each individual. As stated earlier, PBPK modelling can be used to estimate pharmacokinetic (PK) parameters of each individual which in turn helps in individualizing the dosage regimen.it was also found out that covariates bodyweight and postnatal age are the two factors that influence the dosing regimens in these patients due to increase in the maturation of enzymes with time. By developing a PBPK model from the data available, the obtained pharmacokinetic parameters can be used to effectively calculate, adjust and administer the optimal dose of aminophylline / theophylline in patients with apnea of prematurity. This helps in preventing toxic or sub-therapeutic concentrations of the drug.

Caffeine study results suggest that the dosage regimen of caffeine citrate in the treatment of apnea in preterm neonates is efficacious and results in appropriate plasma concentrations. Previous studies have suggested that conventional plasma concentrations of caffeine can be obtained when dosed based on body weight and postnatal age of neonates. Our study which was based on PBPK modelling of caffeine, using PK-Sim supports the above study. It also provides an optimal dosing strategy to individualize the dose to each patient.

BIBILOGRAPHY

1. Shivakumar, M, Jayashree P, Najih, M, Lewis L, Kamath A, Shashikala. Erratum To: Comparative Efficacy And Safety Of Caffeine And Aminophylline For Apnea Of Prematurity In Preterm (≤34 Weeks) Neonates: A Randomized Controlled Trial, 2020
2. Muhe, L., McClure, E., Nigussie, A., Mekasha, A., Worku, B., Worku, A., Demtse, A., Eshetu, B., Tigabu, Z., Gizaw, M., Workneh, N., Girma, A., Asefa, M., Portales, R., Bekele, T., Bezabih, M., Metaferia, G., Gashaw, M., Abebe, B., Berta, H., Alemu, A., Desta, T., Hailu, R., Gebreyesus, G., Aynalem, S., Abdissa, A., Pfister, R., Bonger, Z., Gizaw, S., Abebe, T., Berhane, M., Bekuretsion, Y., Dhaded, S., Patterson, J, Goldenberg, R.. Major causes of death in preterm infants in selected hospitals in Ethiopia (SIP): a prospective, cross-sectional, observational study. The Lancet Global Health, 2019, 7(8), pp.e1130-e1138.
3. Zhao, J., Gonzalez, F. & Mu, D. Apnea of prematurity: from cause to treatment. Eur J Pediatr 170, 1097–1105 (2011).
4. Bhatt-Mehta, V. and Schumacher, R.. Treatment of Apnea of Prematurity. Pediatric Drugs, 2003, 5(3), pp.195-210.
5. Armanian A M, Badiee Z, Afghari R, Salehimehr N, Hassanzade A, et al. Prophylactic Aminophylline for Prevention of Apnea at Higher-Risk Preterm Neonates, Iran Red Crescent Med J. Online ahead of Print; 16(8):12559
6. Product Information: aminophylline IV injection, aminophylline IV injection. Hospira,Inc, Lake Forest, IL, 2004.
7. Product Information: aminophylline oral tablets, aminophylline oral tablets. West-ward Pharmaceutical,Corp, Eatontown, NJ, 2002.
8. Ginsberg, G., Hattis, D., Russ, A. and Sonawane, B.. Physiologically Based Pharmacokinetic (PBPK) Modeling of Caffeine and Theophylline in Neonates and Adults: Implications for Assessing Children's Risks from Environmental Agents. Journal of Toxicology and Environmental Health, Part A, 2004, 67(4), pp.297-329.
9. Maharaj, A.R., Barrett, J.S. & Edginton, A.N. A Workflow Example of PBPK Modeling to Support Pediatric Research and Development: Case Study with Lorazepam. AAPS J 15, 455–464 (2013).
10. HR Ha J Chen, AU Freiburghaus, F Follath; Metabolism of theophylline by cDNA-expressed human cytochromes P-450, British Journal of Pharmacology, 1995
11. Khalil, F. and Läer, S. Physiologically Based Pharmacokinetic Modeling: Methodology, Applications, and Limitations with a Focus on Its Role in Pediatric Drug Development. Journal of Biomedicine and Biotechnology, 2011, pp.1-13.
12. Fukuda, T., Yukawa, E., Kondo, G., Maeda, T., Shin-o, T., Kondo, Y., Imamura, T., Irikura, M. and Irie, T. Population pharmacokinetics of theophylline in very premature Japanese infants with apnoea. Journal of Clinical Pharmacy and Therapeutics, 2005. 30(6), pp.591-596.
13. Grygiel, J. and Birkett, D. Effect of age on patterns of theophylline metabolism. Clinical Pharmacology and Therapeutics, 1980, 28(4), pp.456-462.

14. Aranda, J., Sitar, D., Parsons, W., Loughnan, P. and Neims, A. Pharmacokinetic Aspects of Theophylline in Premature Newborns. New England Journal of Medicine, 1976, 295(8), pp.413-416.
15. Ogilvie, R. Clinical Pharmacokinetics of Theophylline. Clinical Pharmacokinetics, 1978, 3(4), pp.267-293.
16. Tracey H Reilly, Pediatric Theophylline Toxicity, Drugs & Diseases; Pediatrics: Cardiac Disease and Critical Care Medicine, 2019 (Medscape)
17. Eric C. Eichenwald et al; Apnea of Prematurity, Journal of the American academy of pediatrics, 2016
18. Barrington, K. and Finer, N. The Natural History of the Appearance of Apnea of Prematurity. Pediatric Research, 1991, 29(4), pp.372-375.
19. Eldridge, F., Millhorn, D., Waldrop, T. and Kiley, J. Mechanism of respiratory effects of methylxanthines. Respiration Physiology, 1983, 53(2), pp.239-261.
20. Church M., Featherstone R., Cushley M., Mann J. and Holgate S, Relationships between adenosine, cyclic nucleotides, and xanthines in asthma. Journal of Allergy and Clinical Immunology, 1986, 78(4), pp.670-675.
21. Torphy, T. and Undem, B. Phosphodiesterase inhibitors: new opportunities for the treatment of asthma. Thorax, 1991, 46(7), pp.512-523.
22. Barnes, P. and Pauwels, R.. Theophylline in the management of asthma: time for reappraisal?. European Respiratory Journal, 1994, 7(3), pp.579-591.
23. Gayan-Ramirez, G., Janssens, S., Himpens, B. and Decramer, M.. Mechanism of theophylline-induced inotropic effects on foreshortened canine diaphragm. European Respiratory Journal, 1995, 8(11), pp.1915-1921.
24. Kolbeck, R. and Speir, W. Diltiazem, Verapamil, and Nifedipine Inhibit Theophylline-enhanced Diaphragmatic Contractility. American Review of Respiratory Disease, 1989, 139(1), pp.139-145.
25. Peters, S.A. Physiologically Based Pharmacokinetic (PBPK) Modeling and Simulations: Principles, Methods, and Applications in the Pharmaceutical Industry (John Wiley & Sons, Hoboken, NJ, 2012).
26. Maharaj, A.R. & Edginton, A.N. Physiologically based pharmacokinetic modeling and simulation in pediatric drug development. CPT Pharmacometrics Syst. Pharmacol. 3, e150 (2014).
27. Jones, H. & Rowland-Yeo, K. Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. CPT Pharmacometrics Syst. Pharmacol. 2, e63 (2013).
28. Zhao, P. et al. Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. Clin. Pharmacol. Ther. 89, 259–267 (2011).
29. Huang, S.M., Abernethy, D.R., Wang,Y., Zhao, P. & Zineh, I. The utility of modeling and simulation in drug development and regulatory review. J. Pharm. Sci. 102, 2912–2923 (2013)
30. Stephen RC Howiea, Blood sample volumes in child health research: review of safe limits Bull World Health Organ 2011;89:46–53 | Bull World Health Organ 2011;89:46–53.

31. Charlotte I S Barker, Joseph F Standing, Lauren E Kelly, Lauren Hanly Faught, Allison C Needham, Michael J Rieder,Saskia N de Wildt,Martin Offringa, Pharmacokinetic studies in children: recommendations for practice and research,Arch Dis Child 2018;103:695–702.
32. Roth-Cline, Michelle & Nelson, Robert. (2015). Ethical Considerations in Conducting Pediatric and Neonatal Research in Clinical Pharmacology. Current pharmaceutical design. 21. 10.2174/1381612821666150901105146.
33. Caffeine. In: In Depth Answers [database on the Internet]. Available from: www.micromedexsolutions.com.
34. Committee for Medicinal Products for Human Use (CHMP), Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation, 13 December 2018, EMA/CHMP/458101/201613.
35. L Kuepfer, C Niederalt , T Wendl1, J-F Schlender , S Willmann , J Lippert, M Block , T Eissing and D Teutonico Applied Concepts in PBPK Modeling: How to Build a PBPK/PD Model, CPT Pharmacometrics Syst. Pharmacol. (2016) 5, 516–531.
36. Warner A. Drug use in the neonate: interrelationships of pharmacokinetics, toxicity, and biochemical maturity. Clin Chem.1986 May;32(5):721-7.
37. Lawrence C. Ku ,P. Brian Smith. Dosing in neonates: Special considerations in physiology and trial design, Pediatr Res. 2015 Jan; 77(0): 2–9.
38. Loebstein R1, Koren G. Clinical pharmacology and therapeutic drug monitoring in neonates and children. Pediatr Rev. 1998 Dec;19(12):423-8.
39. Hong Lu, PhD and Sara Rosenbaum, Developmental Pharmacokinetics in Pediatric Populations, Developmental Pharmacokinetics in Pediatric Populations.
40. Hsieh EM, Hornik CP, Clark RH, et al. Medication use in the neonatal intensive care unit. Am J Perinatol 2014; 31: 811–821.
41. Johnson PJ. Caffeine citrate therapy for apnea of prematurity. Neonatal Netw 2011; 30: 408–412. doi:10.1891/0730-0832.30.6.408.
42. Caffeine Citrate www.drugs.com/pro/caffeine-citrate.html Date last accessed: November 11, 2019. Date last updated: March 1, 2019.
43. Hey E, Ed. Neonatal Formulary, 6th edition, Chichester, John Wiley & Sons, 2011; p. 64.
44. Abdel-Hady H, Nasef N, Shabaan AE, Nour, Caffeine therapy in preterm infants, World J Clin Pediatr. 2015 Nov 8; 4(4):81-93.
45. Gary Ginsberg , Dale Hattis , Abel Russ & Babasaheb Sonawane Physiologically Based Pharmacokinetic (PBPK) Modeling of Caffeine and Theophylline in Neonates and Adults: Implications for Assessing Children's Risks from Environmental Agents, Journal of Toxicology and Environmental Health, Part A: Current Issues, 2004, 67:4, 297-329

APPENDIX

Appendix I – Part of aminophylline data

ID	RATE	AMT	duration	durPER	TIME	DV	PNA	MDV
1	15	7.5	30	0.5	0	.	4	1
1	15	2.5	10	0.166667	8	.	5	1
1	15	2.5	10	0.166667	16	.	5	1
1	15	2.5	10	0.166667	24	.	5	1
1	15	2.5	10	0.166667	32	.	6	1
1	15	2.5	10	0.166667	32.17	.	6	1
1	.	.	.	.	41	13.02	6	0
2	10	5	30	0.5	0	.	1	1
2	12	2	10	0.166667	8	.	2	1
2	12	2	10	0.166667	16	.	2	1
2	12	2	10	0.166667	24	.	2	1
2	12	2	10	0.166667	40	.	3	1
2	12	2	10	0.166667	48	.	3	1
2	12	2	10	0.166667	64	.	3	1
2	12	2	10	0.166667	72	.	4	1
2	12	2	10	0.166667	80	.	4	1
2	12	2	10	0.166667	88	.	4	1
2	12	2	10	0.166667	96	.	5	1
2	12	2	10	0.166667	104	.	5	1
2	12	2	10	0.166667	112	.	5	1
2	12	2	10	0.166667	120	.	6	1
2	12	2	10	0.166667	128	.	6	1
2	12	2	10	0.166667	136	.	6	1
2	12	2	10	0.166667	144	.	7	1

Appendix II – Part of Caffeine data

CID	DUR	RATE	AMT	TIME	DV	LNDV	AMTNRM	MDV
1	30	0.5	27	0	.	.	.	1
1	60	1	7	24	.	.	.	1
1	60	1	7	48	.	.	.	1
1	60	1	7	73	.	.	.	1
1	.	.	.	74.16	17.94811	2.887485	2.564	0
1	60	1	7	96	.	.	.	1
1	60	1	7	120	.	.	.	1
1	60	1	7	144.5	.	.	.	1
1	.	.	.	168.5	14.82606	2.696386	2.118	0
3	30	0.5	20	0	.	.	.	1
3	30	0.5	5.5	24	.	.	.	1
3	30	0.5	5.5	48.16	.	.	.	1
3	.	.	.	49.98	8.194747	2.103493	1.49	0
3	30	0.5	5.5	72	.	.	.	1
3	30	0.5	8	96.25	.	.	.	1
3	30	0.5	8	120.25	.	.	.	1
3	.	.	.	143.5	12.34445	2.513206	1.543	0
5	30	0.5	17	0	.	.	.	1
5	30	0.5	4.3	24	.	.	.	1
5	30	0.5	7	48	.	.	.	1
5	30	0.5	7	72	.	.	.	1
5	30	0.5	9	96	.	.	.	1
5	30	0.5	9	120	.	.	.	1
5	30	0.5	9	168	.	.	.	1

Plagiarism Report

PBPK of aminophylline and caffeine

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1	Gary Ginsberg, Dale Hattis, Abel Russ, Babasaheb Sonawane. "Physiologically Based Pharmacokinetic (PBPK) Modeling of Caffeine and Theophylline in Neonates and Adults: Implications for Assessing Children's Risks from Environmental Agents", Journal of Toxicology and Environmental Health, Part A, 2004 Publication	%2
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