1-2023

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Recommended Citation

Krzyzanski W, Stockard B, Gaedigk A, et al. Developmental pharmacokinetics of indomethacin in preterm neonates: Severely decreased drug clearance in the first week of life. CPT Pharmacometrics Syst Pharmacol. 2023;12(1):110-121. doi:10.1002/psp4.12881

Received: 13 July 2022 | Revised: 28 September 2022 | Accepted: 16 October 2022

DOI: 10.1002/psp4.12881

ARTICLE

Developmental pharmacokinetics of indomethacin in preterm neonates: Severely decreased drug clearance in the first week of life

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Funding information
Robert Wood Johnson Foundation, Grant/Award Number: 76230; National Institutes of Health, Grant/Award Number: 5K23HD091362 and T32 HD069038

Abstract

Indomethacin is used commonly in preterm neonates for the prevention of intracranial hemorrhage and closure of an abnormally open cardiac vessel. Due to biomedical advances, the infants who receive this drug in the neonatal intensive care unit setting have become younger, smaller, and less mature (more preterm) at the time of treatment. To develop a pharmacokinetics (PK) model to aid future dosing, we designed a prospective cohort study to characterize indomethacin PK in a dynamically changing patient population. A population PK base model was created using NONMEM, and a covariate model was developed in a primary development cohort and subsequently was tested for accuracy in a validation cohort. Postnatal age was a significant covariate for hepatic clearance (CL_H) and renal clearance (CL_R). The typical value of the total clearance (CL, the sum of CL_R and CL_H) was 3.09 ml/h and expressed as CL/WT_{median} = 3.96 ml/h/kg, where WT_{median} is the median body weight. The intersubject variability of CL_R and CL_H were 61% and 207%, respectively. The typical value of the volume of distribution V_p = 366 ml (V_p/WT_{median} = 470 ml/kg), and its intersubject variability was 38.8%. Half-life was 82.1 h. Compared with more mature and older preterm populations studied previously, indomethacin CL is considerably lower in this contemporary population. Model-informed precision dosing incorporating important covariates other than weight alone offers an opportunity to individualize dosing in a susceptible patient undergoing rapid change.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

With current weight-based dosing, indomethacin exposure is variable, and clinical response is unpredictable in preterm infants.

WHAT QUESTION DID THIS STUDY ADDRESS?

In modern preterm infants who are less mature and smaller, what covariates are important for indomethacin pharmacokinetics (PK)? Are the historical values for clearance, volume, and half-life still correct for modern-day patients?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Postnatal age is an important variable in indomethacin clearance and volume of distribution. In very preterm infants treated within the first week of life, indomethacin clearance is much less than previously reported in more mature and older cohorts, increasing the risk for toxic drug exposures with currently clinically accepted dosing protocols.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

Thorough understanding of drug PK in preterm infants, including ontogenic and genetic factors, can lead to precision dosing. PK models can be used to simulate drug exposures and recommend custom doses. With a rapidly maturing patient population, model-informed dosing may be a powerful tool to decrease variability in drug toxicity and drug response.

INTRODUCTION

Indomethacin is a nonsteroidal anti-inflammatory drug prescribed to preterm infants in the neonatal intensive care unit (NICU) for two primary purposes. One indication is immediately after birth to prevent intracranial hemorrhage (intraventricular hemorrhage [IVH])[1] and the second is pharmacologic closure of a symptomatic patent ductus arteriosus (PDA).[2] NICU care has advanced in the past 20 years due to technologic and knowledge advances,[3] resulting in a patient population that is increasingly less mature (viability at 22 weeks gestation).[4] Thus, conditions associated with increasing immaturity occur more frequently than in the past. Furthermore, knowledge of indomethacin disposition in NICU patients is derived from older, larger, relatively more mature patients, and the dosing guidelines based on clearance in those populations may not be relevant to less mature, contemporary patients. The challenge with current indomethacin dosing in preterm neonates is that treatment efficacy and toxicity are highly variable and highly unpredictable.[5–7] Because toxicities in preterm infants can be severe and life-threatening (renal failure, bowel perforation), it is imperative to improve our understanding of the dose–exposure relationship for this drug in the smallest patients.

Contemporary indomethacin dosing follows a weight-based paradigm (mg/kg) for both IVH prophylaxis and PDA treatment.[8] Despite this weight-based dosing, there is highly variable drug exposure, with plasma concentrations varying by at least 14-fold in a cohort of infants 25–34 weeks gestational age (GA) and 1–77 days old at treatment.[9] This variability is partially explained by rapidly maturing clearance mechanisms, captured by variables such as postnatal age (PNA) and postmenstrual age. In addition, ontogeny and genetic variation in drug metabolizing enzymes which have been shown to contribute to indomethacin biotransformation, may explain to the observed variability.

Indomethacin is biotransformed by cytochrome P450 (CYP) 2C9[10] and uridine diphosphate glucuronosyltransferase 1 family polypeptide A9 (UGT1A9), uridine diphosphate glucuronosyltransferase 1 family polypeptide A1 (UGT1A1), and uridine diphosphate glucuronosyltransferase 2 family polypeptide B7 (UGT2B7),[11,12] and metabolites are renally cleared. CYP2C9 expression,[13] uridine diphosphate glucuronosyltransferase (UGT) expression,[14] and renal function[15] are low at birth and increase at variable rates during the first weeks to months of life in neonates and infants. Prior pharmacokinetics (PK) analysis of indomethacin plasma concentrations following intravenous (i.v.) administration in neonates shows biexponential disposition and a second peak attributed to enterohepatic recirculation.[16] Historically, quantification of PK parameters in neonates is challenging because of ethical and safety concerns resulting in limited sampling. Thus, the population PK approach that combines sparse data from multiple infants is optimal.[9,17] Utility of urine indomethacin concentrations in PK analysis has been implied recently.[18] To our knowledge, a population PK model combining dense plasma and urine indomethacin data in neonates has not been developed.

Given current dosing results in variable exposure with the attendant risks of excessive toxicity at one end of the spectrum and treatment failure (inadequate exposure) at the other end, we aimed to describe important demographic and genetic variables that influence indomethacin

PK in the most preterm patients studied to date. The goal was to develop a model to be used in the future to individualize dose to a target exposure and investigate exposure–response relationships. Because of the novel study design collecting both scavenged plasma and frequent dried blood spots (DBS), we had access to very rich data for this model development stage.

METHODS

Patients

The Children's Mercy institutional review board approved the study prior to patient enrollment. Preterm infants <34 weeks GA at birth treated with indomethacin per routine clinical care (IVH prophylaxis in the first 72 h or PDA closure at day of life 3 onward) were eligible. Infants remained in the study from the first dose of indomethacin through 7 days after the last dose for prolonged PK sample collection.

Study design

This was a prospective cohort study. All enrolled preterm infants received i.v. indomethacin per standard guidelines (0.1 mg/kg every 24 h for three doses for IVH prophylaxis and every 12 h for three doses for PDA closure). If the PDA failed to close, a second course of indomethacin was given. Three types of biological samples were collected for PK analysis. First, if the infant had blood drawn for a clinical laboratory, a corresponding DBS was collected. Second, plasma was scavenged from the core laboratory for analysis. Third, infants had urine collected with each diaper change for up to 7 days after the last dose (cotton ball in diaper, total weight of each diaper recorded for entire urine collection timeframe). Urine samples from diapers were proportionally combined into 12 h intervals prior to drug quantification. Demographic data, dosing data, and clinical outcomes were collected from the electronic medical record. The time of each sample collected relative to the immediately prior dosing event was recorded.

Bioanalytical methods

The method validation procedures were based on US Food and Drug Administration guidelines for bioanalytical methods.[19] Linear calibration curves consisted of plotting the peak area of the analyte divided by that of the internal standard (IS) versus the analyte concentration. Indomethacin and indomethacin-D4 were purchased from Toronto Research Chemicals. Chromatography was performed on a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system (Waters). A Cortecs C18 column (2.1 mm × 100 mm) was used for separation. All analyses were performed on Waters Xevo TQ-XS (DBS, urine) and TQ-S (plasma, urine) triple quadrupole instruments equipped with electrospray ion (ESI) sources in multiple reaction monitoring (MRM) mode. The following MRM transitions were used to quantify the analytes in positive ESI mode: 358.1➔139 for indomethacin and 362.1➔143 for indomethacin-D4 (IS).

Seven-point calibration curves in matrix were prepared over the following ranges: urine, 1–1000 nM; plasma/DBS, 100–10,000 nM. To prepare each urine sample, 50 µl of urine (calibration standard, quality control (QC), or patient sample) and 200 µl of acetonitrile with 50 nM IS were added to a microcentrifuge tube, vortexed, and centrifuged. A total of 200 µl of the supernatant were transferred to a 96-well plate and diluted with 300 µl of 1:1 methanol/water for analysis. For the plasma sample preparation, 10 µl of plasma and 10 µl of 1 µM IS were added to a 96-well Hybrid SPE-phospholipid removal plate (Sigma Aldrich). A total of 480 µl of 1% formic acid in acetonitrile was added to each well and vortexed. The samples were then pulled through the Hybrid SPE plate into a collection plate using a microplate vacuum manifold. The final sample was diluted with 500 µl of water for analysis. Standards and QCs for DBS analysis were prepared by pipetting spiked blood onto Perkin Elmer sample collection sheets and air dried. Then, discs were punched from the DBS into a vial, extracted with acetonitrile/ammonium formate/internal standard, and diluted with water for analysis. Initially, DBS concentrations were converted to plasma concentrations using measured hematocrit values and the following equation (IND represents Indomethacin): plasma[IND] = DBS[IND]/(1 – hematocrit).[20] A correction factor (1.608, mean of the ratio of plasma:DBS concentrations) was used to calculate the theoretical plasma concentrations from the hematocrit-corrected DBS concentration. The final equation to convert DBS to plasma was plasma[IND] = DBS[IND]/(1 – hematocrit) * 1.608. The theoretical plasma concentrations from DBS compared with the paired plasma data showed good agreement with a Pearson correlation coefficient of 0.97. The theoretical plasma concentrations were compared with the direct plasma analysis via a Bland–Altman plot. The bias calculated from the Bland–Altman analysis was 2.9%, which was not statistically significant (95% confidence intervals [CIs] were −49 to +45%). The 95% CIs reflect the significant variability in using DBS data to predict plasma data; however, the Pearson coefficient indicates good accuracy.

Drug concentration units, although quantified as molar, were converted to nanograms per milliliter for

indomethacin in the plasma and DBS and to nanograms for urine.

Genotype analysis

Subjects were sequenced at the CMH Genomic Medicine Center using ADMEseq, a custom gene panel from Integrated DNA Technologies targeting 289 genes relevant to drug absorption, disposition, metabolism, and excretion, and variants were retrieved as described in detail elsewhere.[21] Variants were manually reviewed. Haplotype (star allele) calls for *CYP2C8* and *CYP2C9* are according to PharmVar (https://www.pharmvar.org/),[22,23] and those for *UGT1A1, UGT1A9* and *UGT2B7* are according to UGT nomenclature (https://www.pharmacogenomics.pha.ulaval.ca/ugt-alleles-nomenclature/).

For each gene, subjects were grouped into two genotype categories: Category 1, assigned if no variants were found indicating the presence of two normal function alleles, and Category 0, assigned if one or two variant alleles were identified.

Data for population analysis

Two subjects were excluded from the data analysis. One was missing genotype data. Another had the cumulative indomethacin detected in the urine more than 10-fold higher than an average subject. The remaining subjects were divided into two subpopulations for model development and validation. The observed covariates that were tested in the model included PNA, GA at birth, weight, sex, race, and genotype category.

For each patient, indomethacin plasma concentrations (C_p) and total cumulative amount in the urine (A_u) were measured at various timepoints after the first dose. The time of the first dose was considered to be $t = 0$. The PNA was updated at each observation. The body weight (WT) was updated after each dose based on actual weights recorded in the health record. The remaining covariates were time invariant.

Model development and evaluation

Sparseness and variability of the individual plasma concentrations permitted a one-compartment model. Because the indomethacin output in the urine was also measured, we divided the total clearance (CL) into renal clearance (CL_R) and nonrenal (presumably hepatic clearnace [CL_H]). The model equations were:

$$\frac{dA_p}{dt} = \sum_{i=1}^{n_{dose}} Dose_i \delta(t - t_i) - (CL_R + CL_H) C_p \quad (1)$$

$$\frac{dA_u}{dt} = CL_R C_p \quad (2)$$

where the term $\sum_{i=1}^{n_{dose}} Dose_i \delta(t - t_i)$ represents the bolus doses $Dose_1$, ..., $Dose_{n_{dose}}$ given to a patient at times t_1, ..., $t_{n_{dose}}$. Here, A_p is the amount of indomethacin in the plasma compartment and

$$C_p = \frac{A_p}{V_p} \quad (3)$$

where V_p is the indomethacin volume of distribution. In the presence of the bolus input, the initial conditions for A_p and A_u were set to 0.

We assumed the lognormal distribution of V_p, CL_R, and CL_H among patients without parameter correlations:

$$P = \theta_P \exp(\eta_P) \text{ and } \eta_P \sim \mathcal{N}(0, \omega_P^2) \quad (4)$$

where $P = V_p$, CL_R, and CL_H. θ_P is the typical value of P, and ω_P^2 is the variance. The impact of continuous covariates on the model parameters was modeled using the power relationship

$$\theta_P = \theta_{P^*} \left(\frac{Cov}{Cov_{mean}} \right)^{P\_Cov} \quad (5)$$

where Cov = PNA, WT, GA, and Cov_{mean} is the mean of Cov at the time of first dose. Here, θ_{P^*} is the value of θ_P when $Cov = Cov_{mean}$, and P_Cov is the power coefficient quantifying the effect of the covariate Cov on the parameter P. For the dichotomous covariates $Cov \in \{0, 1\}$, the relationship was:

$$\theta_P = \theta_{P\_0}{}^{1-Cov} \theta_{P\_1}{}^{Cov} \quad (6)$$

where θ_{P_0} is the typical value of the parameter P for subjects with Cov = 0, and θ_{P_1} is the typical value of the parameter P for subjects with Cov = 1.

The observed values for C_p and A_u were log-transformed, and the constant residual error model was applied to the transformed data:

$$\log(C_{pij}) = \log(C_p(t_{ij})) + DBS\varepsilon_{DBSij} + (1 - DBS)\varepsilon_{C_{pij}} \text{ and}$$

$$\varepsilon_{C_{pij}} \sim \mathcal{N}\left(0, \sigma_{Cp}^2\right) \text{ and } \varepsilon_{DBSij} \sim \mathcal{N}\left(0, \sigma_{DBS}^2\right) \quad (7)$$

$$\log(A_{uij}) = \log(A_u(t_{ij})) + \varepsilon_{A_{pij}} \text{ and } \varepsilon_{A_{uij}} \sim \mathcal{N}(0, \sigma^2_{Au}) \quad (8)$$

where C_{pij} and A_{uij} are observed values for ith subject at jth time t_{ij}. DBS = 1 if C_{pij} was determined from the dry blood spot sample and DBS = 0 otherwise. The residual errors $\varepsilon_{C_{pij}}$, ε_{DBSij}, and $\varepsilon_{A_{uij}}$ were uncorrelated and normally distributed with the means 0 and variances σ^2_{Cp}, σ^2_{DBS}, and σ^2_{Au}, respectively.

Evaluations of model performance were done by assessing change in the objective function value (OFV), standard errors of parameter estimates, goodness-of-fit plots, and visual predictive checks (VPCs). The plots were obtained by R 4.0.4 packages (ggplot2, lattice, vpc)[24] using RStudio 1.1.456.[25]

Model validation

For model validation, we used the validation dataset. A total of 200 datasets of individual indomethacin plasma concentrations and urine amounts were simulated using the population parameter estimates obtained from the model development step. The 95% prediction intervals were calculated for the observed 5th, 50th, and 95th percentiles using the vpc package.

Statistical methods

Model parameters were estimated by maximizing the likelihood of observation using the importance sampling with interaction method implemented in NONMEM 7.4 (ICON Clinical Research LLC). The data below the limit of quantification (BLQ) were handled using the Beal M3 method for which the likelihood objective function is corrected by the probability of observations falling below the limit.[26] The forward-inclusion backward-elimination technique for covariate selection was applied.[27] We used the log-likelihood ratio test of the change in the objection function value with the significance level 0.005 for inclusion and elimination. The linear regression and two-tailed t-tests were used to determine the correlation between individual parameter estimates and covariates.

RESULTS

A total of 38 infants were included in the development cohort, and 15 infants were included in the validation cohort. Demographic and genotypic characteristics are displayed in Table 1. This cohort was the most preterm and youngest (PNA at dosing) group of infants in an indomethacin PK study to date. The time courses of the observed C_p and A_u for each patient are shown in Figure 1 (development cohort) and Figure S1 (validation cohort). In total, the development dataset consisted of 502 C_p observations (25 BLQ) and 584 A_u observations. The validation data set consisted of 127 C_p observations (24 BLQ) and 209 A_u observations.

A model diagram is shown in Figure 2. The base model (without covariates) was fitted to the observed plasma and urine indomethacin data. Estimates of CL_R, CL_H, and V_p, are shown in Table 2. CL_H was 198-fold higher than CL_R, implying that only 0.5% of indomethacin is

TABLE 1 Demographic and genotypic baseline characteristics of subjects in the model development and validation populations

	Definition	Development cohort	Validation cohort
N	Number of patients	38	15
PNA, days	Postnatal age at first dose, median (IQR)	0.26 (0.19, 0.42)	0.25 (0.20, 3.6)
WT, g	Body weight at first dose, median (IQR)	779 (680, 908)	790 (695, 925)
GA, weeks	Gestational age at birth, median (IQR)	26.1 (25.0, 27.1)	25.4 (25.1, 26.7)
SEX	Sex, female/male	16/22	6/9
RACE	Race, African American/Caucasian	22/16	5/10
CYP2C9	*CYP2C9*, Category 1/Category 0[a]	24/14	8/7
CYP2C8	*CYP2C8*, Category 1/Category 0[a]	30/8	11/4
UGT1A1	*UGT1A1*, Category 1/Category 0[a]	15/23	7/8
UGT1A9	*UGT1A9*, Category 1/Category 0[a]	38/0	15/0
UGT2B7	*UGT2B7*, Category 1/Category 0[a]	31/7	3/12

Abbreviations: *CYP2C8*, cytochrome P450 2C8; *CYP2C9*, cytochrome P450 2C9; IQR, interquartile range; PNA, postnatal age; *UGT1A1*, uridine diphosphate glucuronosyltransferase 1 family polypeptide A1; *UGT1A9*, uridine diphosphate glucuronosyltransferase 1 family polypeptide A9; *UGT2B7*, uridine diphosphate glucuronosyltransferase 2 family polypeptide B7; WT, body weight.

[a]Category 1, no variants (two normal function alleles); Category 0, one or two variant alleles.

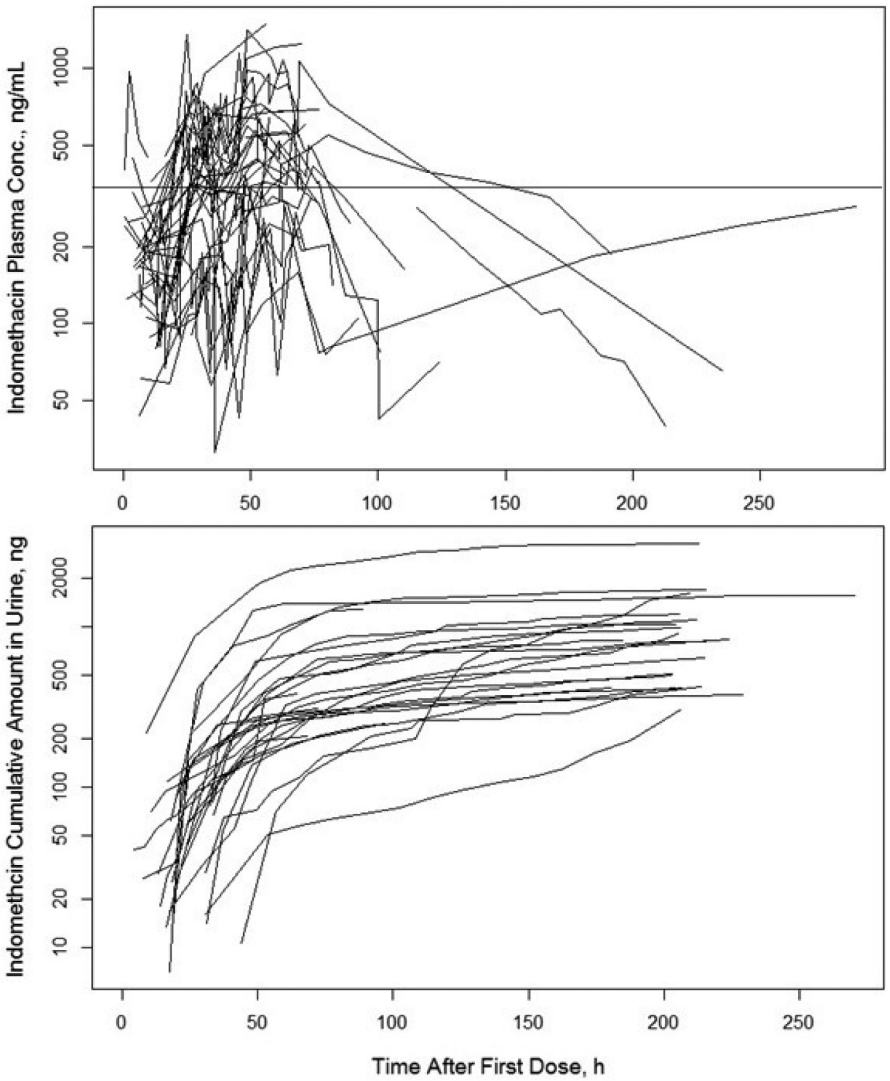

FIGURE 1 Individual time courses of indomethacin plasma concentrations in the plasma (top) and cumulative amount in the urine (bottom) for patients from the model development dataset. The black horizontal line at 400 ng/ml represents concentration at which 75% patent ductus arteriosus closure was observed in a prior publication.[9] Conc., concentration.

eliminated renally in this every preterm cohort treated in the first weeks of life. This aligns with 0.5%–6% reported by Friedman et al. (mean GA 28 weeks and PNA ranging from 1–34 days).[28] The total clearance CL = $CL_R + CL_H$ was 3.09 ml/h and expressed as CL/WT_{median} = 3.96 ml/h/kg. The V_p = 366 ml (V_p/WT_{median} = 470 ml/kg). The half-life calculated as $t_{1/2} = \ln(2)/(CL/V_p)$ was 82.1 h.

The estimates of the individual PK parameter were used to determine a possible correlation with the observed covariates. The R^2 values for all continuous covariates at the baseline (first dose) (WT, PNA, GA) were >0.1 only for CL_H versus WT, CL_H versus PNA, an CL_H versus GA. A *t*-test was applied to determine the effect of dichotomous covariates on the model parameters. The significant differences ($p < 0.05$) were detected for V_p versus RACE. The impact of genetic covariates on V_p, CL_R, and CL_H are presented in Figure 3. A significant effect was detected only for the *CYP2C9* genotype category on CL_R ($p = 0.013$). To confirm these observations, we developed covariate model Equations (5) and (6) for inclusion in the population model.

The selection of significant covariates was based on the log-likelihood ratio test with the forward-inclusion followed by the forward-elimination process applied to all observed covariates. The results of the steps are listed in Table S1. PNA significantly affected CL_H and CL_R, and the *CYP2C9* genotype category impacted CL_R. Because *CYP2C9* genotype was treated as a dichotomous variable, we report CL_{R1} as a typical value of CL_R for neonates classified as genotype Category 1, that is, having two normal function alleles (or a *CYP2C9*1/*1* genotype), and CL_{R0} as a typical value of CL_R for patients with at least one

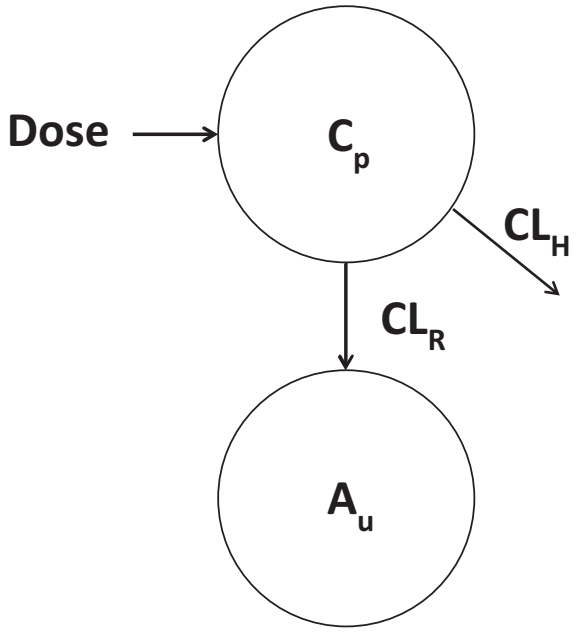

FIGURE 2 Diagram of the pharmacokinetic model of indomethacin. A_u, cumulative amount in the urine; CL_H, hepatic clearance; CL_R, renal clearance; C_p, plasma concentration.

variant allele. Weight was not included in the covariate model as weight did not significantly impact the OFV after PNA was included. The significant covariates (PNA and *CYP2C9* genotype category for CL_R) reduced the between-subject variability for V_p and CL_R, but increased it for CL_H.

The estimates of the typical values of model parameters, covariate parameters, interindividual variability (IIV), and residual variability for the final model are presented in Table 2. The values of V_p and CL_H for a typical subject of PNA_{mean} = 1.25 days were 468 ml and 0.624 ml/h, respectively. The estimates of CL_R for patients with *CYP2C9* Category 1 versus Category 0 genotype were 0.0133 and 0.0274 ml/h, constituting 2.1% and 4.4% of the total CL, respectively. The estimated intersubject variabilities of parameters were relatively high, with 207% coefficient of variation for the CL_H. The precision of estimates for all model parameters was good to moderate with relative standard errors (RSEs) not exceeding 54% except for IIV for CL_H, where the percentage RSE (%RSE) = 119%.

The final model performance was evaluated using diagnostic plots (observed vs. predicted) shown in Figure S2, VPCs presented in Figure 4, and %RSEs of parameter

TABLE 2 Basic and final model parameter estimates and their %RSE

		Estimate (%RSE)	
Parameter	**Definition**	**Base model**	**Final model**
V_p, L	Volume of distribution	0.366 (8.7)	0.438 (6.8)
CL_R, L/h	Renal clearance	0.0000156 (11.7)	NA
CL_{R0}, L/h	CL_R subject with *CYP2C9* = 1[a]	NA	0.0000133 (19.5)
CL_{R1}, L/h	CL_R subject with *CYP2C9* = 0[a]	NA	0.0000274 (49.6)
CL_H, L/h	Hepatic clearance	0.00307 (19.2)	0.000624 (53.2)
CL_H_PNA	PNA effect on CL_H	NA	0.906 (10.4)
CL_R_PNA	PNA effect on CL_R	NA	−0.172 (22.8)
IIV V_p, %	Interindividual variability of V_p	50.4[b] (33.2)	38.8[b] (34.0)
IIV CL_R, %	Interindividual variability of CL_R	71.5[b] (58.4)	61.4[b] (36.6)
IIV CL_H, %	Interindividual variability of CL_H	134[b] (20.6)	207[b] (119)
σ^2_{Cp}	Variance of residual C_p	0.172 (12.2)	0.1 (12.4)
σ^2_{DBS}	Variance of residual C_{DBS}	0.262 (10.1)	0.272 (9.5)
σ^2_{Au}	Variance of residual A_u	0.0927 (6.5)	0.0977 (6.5)

Abbreviations: A_u, total cumulative amount indomethacin in urine; C_p, indomethacin plasma concentration; *CYP2C9*, cytochrome P450 2C9; DBS, dried blood spots; NA, not applicable; PNA, postnatal age; %RSE, percentage residual standard error.

[a]Category 1, no variants (two normal function alleles); Category 0, one or two variant alleles.

[b]Interindividual variability is expressed as $100\,\% \sqrt{\exp(\omega_P^2) - 1}$, where ω_P^2 is an estimated variance of the parameter P.

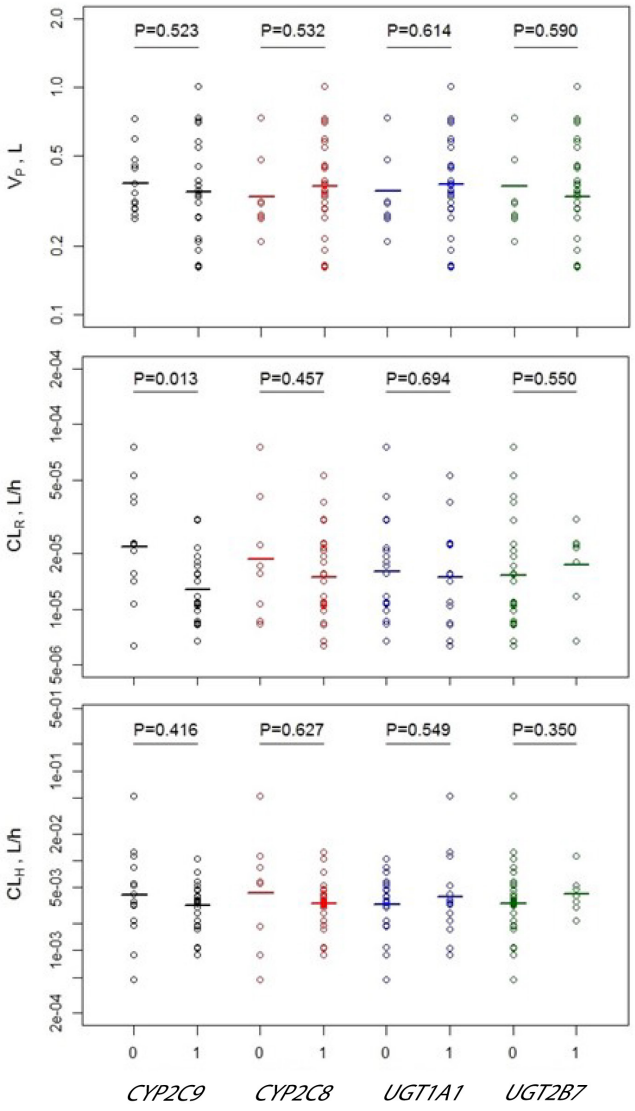

FIGURE 3 Difference between the geometrical means of the base model individual estimates of V_p, CL_R, and CL_H for Category 1 (two normal function alleles) and Category 0 (one or two variant alleles) for the interrogated genes. The indicated *p* values were calculated based on the two-tailed *t*-test. CL_H, hepatic clearance; CL_R, renal clearance; *CYP2C8*, cytochrome P450 2C8; *CYP2C9*, cytochrome P450 2C9; *UGT1A1*, uridine diphosphate glucuronosyltransferase 1 family polypeptide A1; *UGT2B7*, uridine diphosphate glucuronosyltransferase 2 family polypeptide B7; V_p, volume of distribution.

estimates listed in Table 2. The observed data correlated well with the model-predicted population and individual values. The noticeable autocorrelation of A_u results from accumulation of the residual error of the urine amount measurements that were added up to calculate the cumulative A_u. The VPCs show the observed median, 5th, and 95th percentiles of C_p and A_u embedded in the model generated 5th–95th percent prediction intervals for these observed percentiles, implying that the random-effect models properly described the IIV of the observed data. Finally, the %RSE of the parameter estimates are moderate with an exception of the variance for CL_H (119% RSE, reflecting wide distribution of individual CL_H values).

To assess the impact of significant covariates PNA and *CYP2C9* genotype category on indomethacin PK, we simulated C_p and A_u time courses of typical subjects at PNA of first dose for 1 and 14 days (Figure 5). We set indomethacin dosing of 0.1 mg once a day for 3 days. We observed a dramatic difference in indomethacin exposure affected by PNA. When dosed on Day 1, the area under the concentration-time curve between 0 hours and 120 hours ($AUC_{0–120}$) was 20,188 ng/ml h, whereas for Day 14, the $AUC_{0–120}$ was 16,357 ng/ml h, a 23.4% decrease. *CYP2C9* genotype category did not have an impact on indomethacin plasma concentrations. However, *CYP2C9* genotype category affected the cumulative amount of indomethacin cleared renally to the urine. The values of A_u after 120 h for Day 1 were 318 ng (*CYP2C9* = 1) and 155 ng (*CYP2C9* = 0). The analogous values for Day 14 were 240 and 117 ng. The small values of A_u excreted in the 5-day period after the first dose correspond to 0.11%, 0.05%, 0.08%, and 0.04% of the total dose of 0.3 mg, respectively. These are comparable with the lower limit of the range 0.5%–6% of indomethacin amount cleared by the kidneys reported by Friedman et al.[28]

The final model was validated using a portion of the original dataset that was not used for model development. Figure S3 shows the observed validation data together with their median, 5th, and 95th percentiles were overlaid with the 5th–95th percent prediction intervals for these observed percentiles calculated by the final model. Because the observed percentiles lie with the prediction bands, the final model accurately predicts the variability of observed C_p and A_u in the validation cohort.

DISCUSSION

Although indomethacin PK have been previously reported in preterm infants, this study is the first to include infants treated in the first few days of life who are more premature than historical cohorts. The current PK analysis represents modern-day use of indomethacin in the NICU. Knowledge of PK ontogeny will allow for optimized dosing in this population. Our study shows that in very young preterm infants, born at 26 weeks GA and administered indomethacin within 24 h of birth, clearance is drastically reduced (typical value 3.96 ml/kg/h vs. 10.5–23.9 ml/kg/h in infants born at 28 weeks gestation and treated at 7 days PNA[28] and 7.11 ml/kg h in infants born at 29 weeks gestation and treated at 14 days PNA[9]). Correspondingly, the half-life observed in our cohort was 82 h as opposed to 10–20 h[28] and 29 h.[9] In adults, the typical clearance is 45 ml/

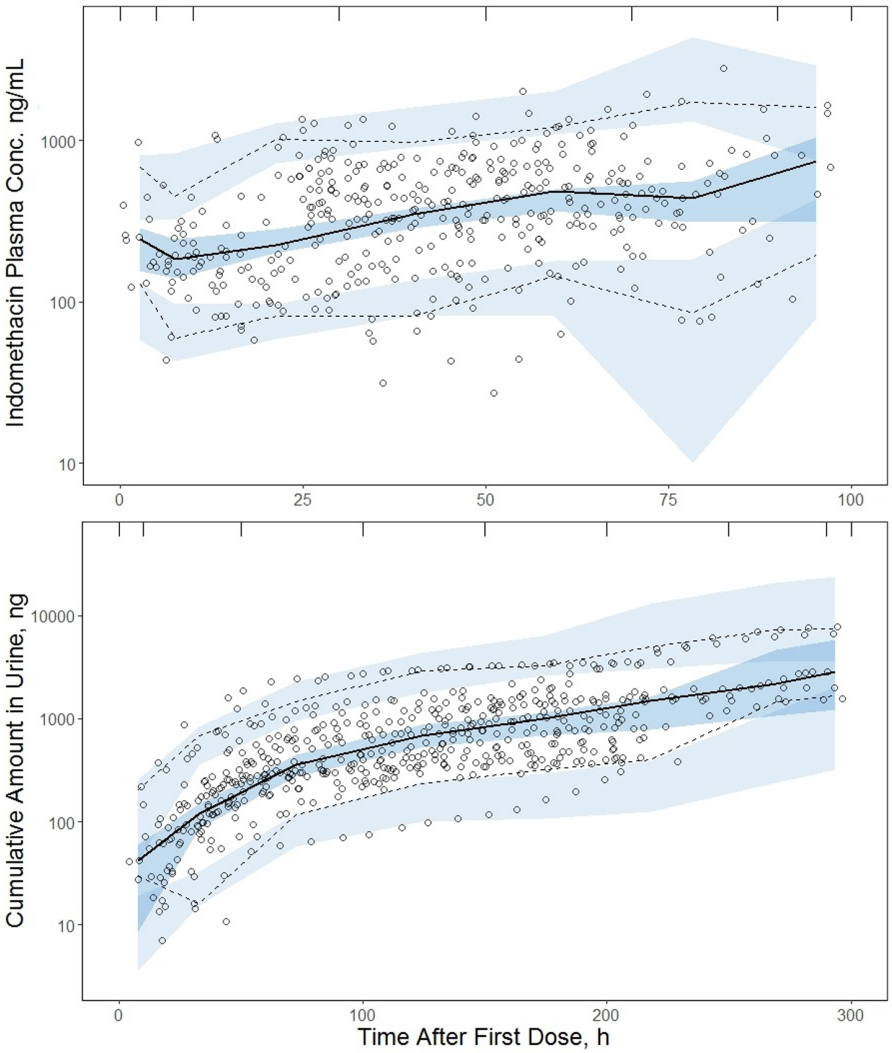

FIGURE 4 Visual predictive check plots for indomethacin plasma concentrations (top) and cumulative amounts in urine (bottom) for patients from the development dataset. The plots are limited by 100 and 300 h, respectively, due to sparseness of observations beyond these times. Symbols represent the observed plasma concentrations, continuous line is the median, and dashed lines are 5th and 95th percentiles of observed values. The shaded regions are model-predicted confidence intervals for these percentiles. Conc., concentration.

kg/h and half-life is approximately 4 h. Taken together, these data help us understand why current weight-based dosing strategies may lead to toxicities (renal failure, bowel perforations) and why PK model-informed precision dosing has the potential to improve outcomes. With the limit of viability decreasing for preterm infants, many accepted dosing guidelines may need revisiting.

The indomethacin plasma concentrations collected in this study exhibit high within- and between-subject variability. The availability of the DBS data, increasing timepoints per subject, was crucial for the resolution of model parameters. Urine indomethacin concentrations allowed separation of the total clearance into renal and hepatic. Despite rich urine data, the standard errors of estimates of the population means of both clearances were relatively high, owing to the variability in the plasma concentrations. The IIV of CL_R values were much smaller than CL_H, with the latter reflecting the high inherent individual variability of indomethacin plasma concentrations. The V_p was estimated with good precision and showed moderate IIV.

There were multiple unique challenges to analyzing the PK data from this study conducted during routine clinical care of sick neonates. Indomethacin doses were administered per kilogram of WT. We converted the dose to absolute amounts to avoid confounding of the WT in the clearances and volume. The weight and PNA were highly correlated as seen in Figure S4. Also, a high correlation was observed between GA and weight (data not shown). Another factor contributing to data complexity was the time variance of weight and PNA. Weight was updated at each dosing event and carried forward. PNA was updated at each observation event.

Because weight correlates with CL and V_p, weight is historically included in the covariate relationships with power coefficients of CL_WT = 0.75 and V_p_WT = 1.0, as determined by principles of allometric scaling.[8] This approach, however, favors weight over other covariates and excludes it from the process of covariate selection. We chose to test the effect of weight like any other covariate in the selection process.[27] Ultimately, PNA was selected as a significant covariate contributing to CL_H and CL_R.

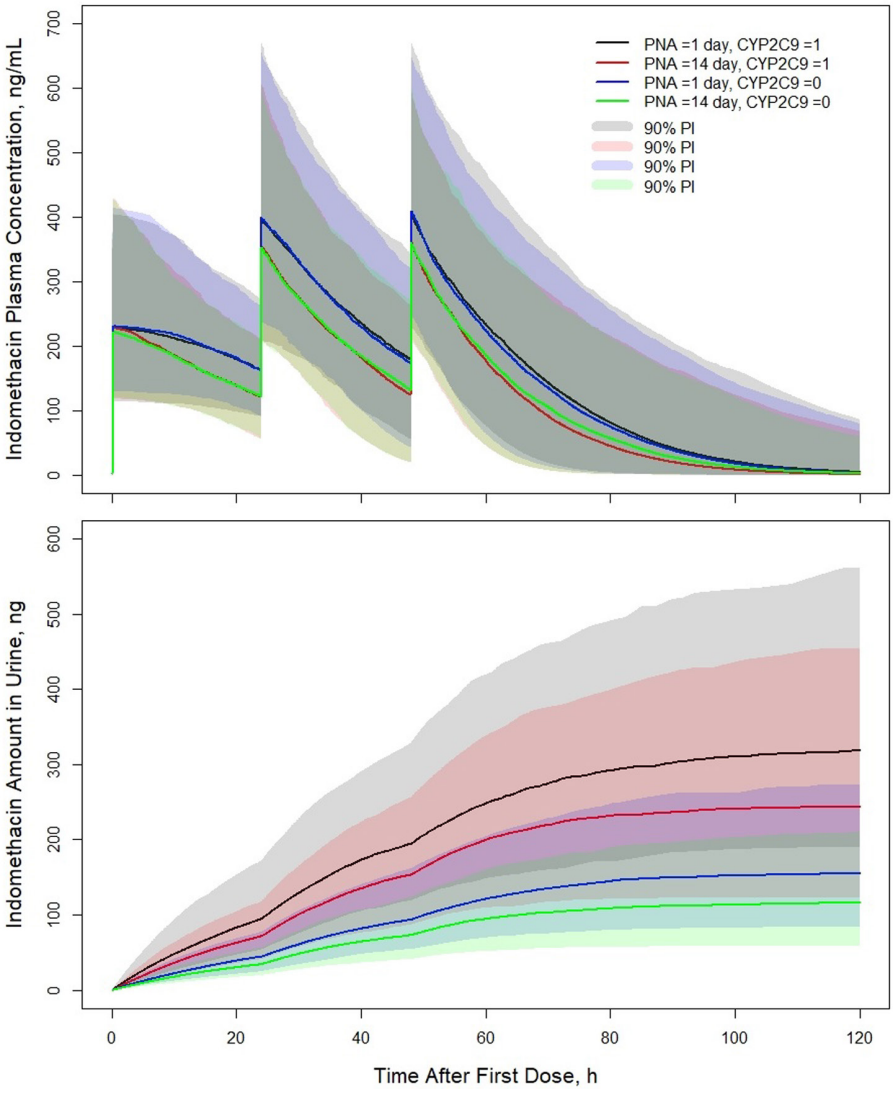

FIGURE 5 Simulated time courses of indomethacin plasma concentration (top) and cumulative amount in urine (bottom) for a typical patient who received 0.1 mg intravenous bolus doses at 0, 24, and 48 h. PNA refers to the postnatal age at the time of first dose. *CYP2C9* = 1 indicates the presence of two normal function alleles, whereas *CYP2C9* = 0 indicates the presence of one or two variant alleles. The shaded areas represent the 90% PIs. *CYP2C9*, cytochrome P450 2C9; PI, prediction interval.

Weight was slightly less influential (Table S1) as when combined with PNA, it did not significantly change the OFV. This atypical finding of weight being nonsignificant can be explained by PNA being a more dynamic time-dependent covariate than WT (see Figure S4). PNA increased at every observation point, whereas WT was updated only at the time of dosing. Also, WT decreased in many neonates during the first few days after birth, which is a common behavior attributed to a loss of body fluid.[29] We propose that maturation of body organs (age) in neonates is the predominant expression of their growth with respect to the impact on CL and V_p. In previous studies of indomethacin in preterms,[9,30] PNA was found not significant or significant when WT was considered as an allometric covariate. There, both covariates were evaluated at the first dose as opposed to our time-varying analysis.

The intersection of ontogeny and pharmacogenetics in drug clearance in preterm neonates is poorly understood. We tested whether known sequence variants in the major drug metabolizing enzymes (DMEs) contributed to the variability of PK parameters. None of the subjects had variant *UGT1A9* alleles, and thus it was not included in our analysis. Of the other interrogated genes, only *CYP2C9* variation significantly impacted CL_R. Infants with *CYP2C9*1/*1* genotypes (no variants) showed decreased CL_R. *CYP2C9* genotype did not significantly affect CL_H, which may be explained because of high variability and low hepatic enzyme expression in neonates,[31] potentially masking a correlation between *CYP2C9* genetic variation and CL_H. Similar reasons may also explain the lack of associations between CL_H variability and the other genes interrogated. On the contrary, CL_R was a parameter well informed by the rich urine data, which allowed us to detect even weak correlations with covariates such as *CYP2C9*. Because a small amount of drug was renally cleared in this population and significance on CL_R was borderline for *CYP2C9* genotype category (p = 0.005;

Table S1), this finding needs further investigation. A plausible mechanism includes indomethacin (via prostaglandin modulation) affecting afferent renal blood flow and thus glomerular filtration rate.[6]

Our estimate of indomethacin V_p is consistent with the values reported elsewhere.[9,30] According to Equation (5), the CL_H value at PNA = 14 days is 8.9 ml/h, which is in the range reported by Smyth et al. in a cohort of infants 25–34 weeks GA and 1–77 days PNA (CL = 7.4 ml/h)[9] and Al Za'abi et al. in a cohort of infants 23–32 weeks GA and 1–40 days PNA (CL = 16.6 ml/h).[30] Our estimate of V_p being 438 ml is similar to these publications of 484 and 266 ml. When expressed per kilogram of a typical subject V_p/WT_{median} = 0.47 L/kg, the V_p agrees with the values reported for healthy adults 0.34–1.57 L/kg,[32] largely exceeding the plasma volume. This can be explained by the high binding of indomethacin to plasma proteins and tissues.[33] We also found that the CL_R minimally contributes to the total clearance with only about 0.1% of the dose eliminated to the urine in this population. Therefore, CL_H is a good approximation of the total clearance reported elsewhere. We observed that CL_H is strongly dependent on PNA with an almost proportional relationship. We believe that this is reflective of the maturation of hepatic enzymes in the first days after neonate birth, and prior published urine metabolite data support this.[18] Low CL_H is caused by the relatively young age of our patients compared with other studies (PNA_{median} = 0.26 days). Preterm infants have an immature hepatic microsomal enzyme system with diminished metabolic activity after birth.[34] Our simulations show that indomethacin exposure in 1-day-old preterm infants is about 20% higher than at 2 weeks. This ontogenic profile may warrant indomethacin dose adjustments based on the PNA and not weight alone.

In summary, a one-compartment model confirmed that the renal clearance minimally contributes to the total clearance of indomethacin in the first days of life. PNA better explains variability in the total clearance than weight, which implies that the maturation is relatively more important than the growth of body mass. Finally, simulations show a large difference in indomethacin area under the curve between 1- and 2-week-old preterm neonates, which warrants future studies aiming at indomethacin model-informed dose individualization based on age and not only traditional weight-based dosing. In future analyses, we plan to add our metabolite quantification data, providing future insight into the hepatic clearance. In reality, few patients in the NICU are actually described by the characteristics of a model's "typical" patient, and incorporating relevant covariates for custom dosing will lead to improved outcomes.

AUTHOR CONTRIBUTIONS

W.K., B.S., and T.L. wrote the manuscript. T.L., A.G., and J.S.L. designed the research. T.L., A.G., A.S., W.N., and K.G. performed the research. T.L., W.K., B.S., A.G., W.N., and J.S.L. analyzed the data. W.N., K.G., and A.G. contributed new reagents/analytical tools.

ACKNOWLEDGMENTS

The authors thank Erin C. Boone, PhD, for managing the ADMEseq data and preparing variants for analysis.

FUNDING INFORMATION

Dr. Lewis is supported by The Robert Wood Johnson Foundation (76230) and the National Institutes of Health (5K23HD091362). Dr. Stockard was supported by the National Institutes of Health (T32 HD069038).

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Krzyzanski W, Stockard B, Gaedigk A, et al. Developmental pharmacokinetics of indomethacin in preterm neonates: Severely decreased drug clearance in the first week of life. *CPT Pharmacometrics Syst Pharmacol*. 2023;12:110-121. doi:10.1002/psp4.12881