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Bayesian Population Pharmacokinetic Modeling of Ondansetron for Neonatal Opioid Withdrawal Syndrome

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ABSTRACT

Ondansetron is an anti-emetic 5-HT3 receptor antagonist being investigated for treating neonatal opioid withdrawal syndrome (NOWS). Sparse PK data were analyzed from a multicenter, double-blind clinical trial with 98 mother/neonate dyads. Pregnant women with opioid use disorder were randomized to receive either placebo or ondansetron 8 mg intravenously within 4 h of delivery. Neonates born to mothers who were randomized to ondansetron received 0.07 mg/kg orally once every 24 h for up to five doses. Using current PK data, model parameters from a two-compartmental structural model from the literature (i.e., a priori model) were updated with the Metropolis-Hastings Markov-chain Monte Carlo estimation algorithm in NONMEM. The updated Bayesian model indicated a slower absorption rate (KA) but no differences in model parameters (CL, V, V2, Q) after including body weight and postmenstrual age. Sensitivity analyses on CL prior revealed statistical improvement favoring larger body weights, but not changes in postmenstrual age. However, further model development using larger body weights did not illustrate superior performance through visual inspection of diagnostic plots. Overall, a cumulative AUC of at least 1000 ng*h/mL appears to be the threshold for reductions in symptom severity. Exposure-response analyses suggest the total number of doses to be the primary driver for efficacy with respect to AUC, which reasonably aligns with the literature. Overall, it is suggested that at least three doses of the current oral ondansetron regimen are required to reduce symptom severity in neonates.

1 | Introduction

The consequences of the opioid epidemic in the United States are a public health challenge. Neonatal opioid withdrawal syndrome (NOWS) is a condition where withdrawal signs develop in newborns with in utero opioid exposure [1]. The increased incidence of NOWS has paralleled the increase in opioid use disorder, with 6.2 cases of NOWS per 1000 live births in 2020 [2–4]. The American Academy of Pediatrics endorsed nonpharmacologic measures as the basis of therapy, with the use of an opioid (morphine, methadone, or buprenorphine) as the first-line pharmacologic agent for treatment of severe signs of withdrawal [5]. Prolonged hospital stays for pharmacologic treatment of NOWS impairs maternal bonding and is associated with substantially increased health care costs [6]. While inpatient use of opioids in NOWS is generally safe, concerns about the impact that opioids can have on neurodevelopment have been raised [7]. Thus, treatment approaches that minimize opioid exposure would provide a therapeutic advantage.

Analysis of murine models identified alleles within the *Htr3a* gene, which encodes the 5-hydroxy tryptamine 3a (5-HT3a) receptor, as associated with opioid withdrawal severity [8]. The 5-HT3 antagonists ondansetron and palonosetron reduced

Summary

- What is the current knowledge in the topic?
  - Ondansetron pharmacokinetics in healthy volunteers including pregnant women, infants at least 1 month of age, and elderly patients have been reported. However, there is potential for optimization of ondansetron dose for the treatment of NOWS.
- What question did this study address?
  - This substudy characterized ondansetron disposition in neonates with NOWS as well as investigated characteristics of exposure-response relationships with mean Finnegan scores that could aid in dose optimization.
- What does this study add to our knowledge?
  - The updated Bayesian model derived from the literature revealed a slower rate of absorption in neonates. Exposure-response analyses suggest dose to be a significant driver of ondansetron efficacy in reducing mean Finnegan scores from baseline. At least three doses of ondansetron 0.07 mg/kg once daily are needed to decrease symptom severity in neonates with NOWS.
- How might this change clinical pharmacology or translational science?
  - The description of ondansetron disposition and exposure-response characteristics will allow the generation of an optimized dosing regimen. The analysis suggests that increased ondansetron systemic exposure may reduce NOWS symptom severity, which could impact clinical outcomes of length of stay and reduce the number of infants requiring treatment with an opioid such as morphine or buprenorphine.

opioid withdrawal severity in mice and in human volunteers [8–10]. Given its widespread use and established safety in pregnancy [11, 12], the ability of ondansetron to reduce NOWS severity and the number of neonates that require opioid treatment was investigated in a multicenter study. Earlier simulations based on healthy pregnant women and their newborns informed ondansetron dosing in the efficacy study, which demonstrated a statistically significant decrease in symptom severity but a non-statistically significant decrease in the mean length of hospitalization and median dose of morphine [10, 13]. The simulations were limited as external oral doses were not administered to neonates and the assumption that ondansetron absorption in neonates was similar to adults. Moreover, a recent murine pharmacokinetic analysis revealed that ondansetron concentrations in the brain are 1000-fold lower than the blood concentrations and brain concentrations decrease rapidly after administration [14]. These results suggest that there is an opportunity to optimize ondansetron dosing to enhance its treatment effect on NOWS, which could be determined using a pharmacometric approach. Ondansetron pharmacokinetics (PK) were previously characterized in healthy volunteers, but not in pregnant women with opioid use disorders, nor in neonates that were experiencing NOWS. The goal of this substudy was to characterize ondansetron disposition in newborns using a population PK approach. The primary intent was to predict ondansetron exposures based on neonate dosing while accounting for maternal transfer. Then, an exploratory exposure-response analysis was performed to evaluate the relationship between plasma exposures and NOWS severity (as assessed using mean total Finnegan scores). The overarching goal is to define ondansetron exposure-response relationships of the current dosing regimen which could ultimately support dose optimization for NOWS in future investigations and clinical use.

2 | Methods

2.1 | Study Protocol

This was a substudy of a double-blind, placebo-controlled, multicenter clinical trial (NCT01965704) of 98 healthy pregnant women who had at least 3 weeks of daily opioid exposure prior to delivery and their newborns. Study details are documented in a previous publication [13]. This study was approved by the Institutional Review Board at each participating site and an informed consent was obtained for each mother and neonate enrolled in the study. Study procedures were performed in accordance with the Declaration of Helsinki.

Pregnant women and their newborns were randomized 1:1 to receive either ondansetron or placebo (normal saline for intravenous (IV) dosing or simple syrup for oral). Pregnant women assigned to the ondansetron group received a single dose of ondansetron 8 mg IV. An additional dose was allowed one time if delivery did not occur within 4 h of the first study dose. Neonates born to mothers in the ondansetron group received ondansetron 0.07 mg/kg by mouth once every 24 h starting the day of birth and up to five doses maximum. Regardless of the group assignment, the first neonatal study dose was administered within 4–8 h of delivery. All neonates received non-pharmacologic therapy according to institutional policies. If neonates met the threshold for NOWS treatment as per the local institutional protocol, they were administered pharmacologic therapy (i.e., morphine sulfate). Maternal blood samples for ondansetron PK were collected within 30 min of delivery by needle stick. Between one and five 0.1 mL blood samples were collected via heel stick from each neonate after birth. These were obtained during clinically indicated blood sampling to minimize trauma to neonates. The first sample was generally collected during the first 12 h of life and prior to the first study dose of ondansetron. If there was no clinically indicated blood sampling within this period, a heel stick for research PK was obtained. One blood sample was then collected approximately every 24 h.

2.2 | PK Model Development

Plasma ondansetron quantification was adapted from a similar study [10] and described in the methods of the primary study report [13]. Plasma ondansetron concentration data from neonates randomized to ondansetron were analyzed using nonlinear mixed-effects modeling with NONMEM version 7.5.1 (ICON, Ellicott City, MD). Any observed plasma concentration below the limit of quantification (BLQ) of 1.0 ng/mL was excluded from the analysis. The Metropolis-Hastings Markov-chain

Monte Carlo estimation algorithm (METHOD = BAYES) was utilized for all model runs. Log-transformed parameter estimates were supplied as priors with the bbr.bayes (Metrum Research Group, Boston, MA) package in R version 4.2.1 or higher (R Foundation, Vienna, Austria) [15, 16]. Ondansetron was assumed to follow a two-compartmental model as documented in the literature [17]. Informative neonate priors for clearance (CL), central volume (V), peripheral volume (V2), and intercompartmental clearance (Q) were calculated while accounting for allometric scaling and maturation effects since these were previously estimated in a model with infants, which served as the a priori model. Ondansetron absorption rate constant (KA) in neonates had not been previously characterized, so a weakly informative prior was used for KA. Theta priors for CL, V, V2, Q, and KA were assumed to be normally distributed with a mean and variance defined by $THETAP and $THETAPV, respectively. Bioavailability (F) was fixed to 0.62 for all model runs [10, 18].

Given the available data, only the interindividual variability (IIV) on CL was estimated. The IIV on CL from the infant reference model was retained as an informative prior using the following structure:

$$\mathrm{CL}_i = \exp\left(\theta_{\mathrm{CL}} + \eta_{\mathrm{CL}i}\right)$$

where CL_i is the estimated CL for a given individual i, θ_{CL} is the typical population CL value, and η_{Cli} is the IIV estimate in CL for individual i. An inverse Wishart distribution was assumed for CL prior with the mode and degrees of freedom defined by $OMEGAP and $OMEGAPD, respectively. The degrees of freedom for CL omega prior were set using the following relationship [15, 16, 19]:

$$\mathrm{df} = 2 * \left(\frac{\omega}{\mathrm{SE}_{\omega}}\right)^2$$

where df is the degrees of freedom, ω is the omega estimate from the infant reference model, and SE_{ω} is the standard error of the omega estimate.

Similarly, an additive residual error model structure was retained as an informative prior:

$$Y_{ij} = \hat{Y}_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the observed ondansetron concentration j for a given individual i, $\hat{Y}_{ij}$ is the individual predicted concentration, and ε_{ij} is the residual random error for measurement j for individual i. An inverse Wishart distribution for sigma prior was assumed with the mode and degrees of freedom defined by $SIGMAP and $SIGMAPD, respectively. The degrees of freedom for sigma prior were calculated similar to omega prior using the same relationship defined above.

The posterior R package was used to obtain effective sample size (ESS) and $\hat{\mathrm{R}}$ as well as all posterior medians with 95% credible intervals. Additionally, model convergence was assessed using trace and density plots with ggplot2. Model selection and sensitivity analyses were aided by Pareto smoothed importance sampling leave-one-out (PSIS-LOO) cross-validation via the loo R package. General model diagnostics (i.e., observed vs. population and individual predicted concentrations, normalized prediction distribution errors vs. population predicted concentrations and time, individual concentration vs. time, and individual posterior predictive check plots) were generated using NONMEM, bbr.bayes, mrgsolve (Metrum Research Group, Boston, MA), and ggplot2 in R.

2.2.1 | Infant Reference Model

The reference model was a two-compartment model describing intravenous ondansetron PK in 124 infants aged 1–48 months with allometric scaling on CL, V, V_2, and Q [17]. There were 745 plasma samples. All parameters were normalized to 10.4 kg and a maturation effect was incorporated to account for decreased clearance observed in infants:

$$\mathrm{CL} = \theta_{\mathrm{CL}} * \left(\frac{\mathrm{WT}_i}{10.4}\right)^{0.75} * \left(1 - \beta_{\mathrm{CL}} * \exp(-(\mathrm{AGE} - 1)) * \left(\frac{ln2}{T_{\mathrm{CL}}}\right)\right)$$

where WT_i is the individual infant's body weight, AGE is the infant's age in months, β_{CL} is the fractional change in CL, and T_{CL} is the half-life for CL maturation. V, V_2, and Q were described using a standard allometric model:

$$P = \theta_{\mathrm{P}} * \left(\frac{\mathrm{WT}_i}{10.4}\right)^{\theta_{\mathrm{allo}}}$$

where P is the typical parameter value, θ_{P} is the parameter estimate for a typical infant weighing 10.4 kg, and θ_{allo} is the fixed allometric exponent of 0.75 for clearance and 1 for volumes.

2.3 | Exploratory Exposure-Response Analyses

Following the final model selection and sensitivity analyses, mrgsolve was used to simulate $\mathrm{AUC}_{0-\mathrm{tl}}$ and $C_{\max}$ for each neonate. Due to a maturation effect being considered on neonate CL, ondansetron concentrations in the central compartment were integrated:

$$\mathrm{AUC}_{0-\mathrm{tl},i} = \frac{A_{\mathrm{central,t}}}{V_i}$$

where $\mathrm{AUC}_{0-\mathrm{tl}}$ is the cumulative area under the curve from time of birth to time tl (up to 75 h following receipt of last study dose) for individual i, $A_{\mathrm{central,t}}$ is the amount of ondansetron present in the central compartment at time t, and V_i is the estimated central volume of distribution for individual i. Given the prolonged elimination half-life observed in neonates, tl was extended up to 75 h following the last dose (five half-lives) [10]. Steady-state was not assumed as not all neonates had received the maximum number of study doses of ondansetron. Simulated $C_{\max}$ for each neonate was simply determined as the maximum concentration until time tl calculated with mrgsolve. All non-BLQ plasma observations as well

as response data (i.e., Finnegan scores) up until time tl were included in the analyses.

Finnegan score is a sum of all individual scores assessing symptom severity of opioid withdrawal (e.g., central nervous system (CNS), vasomotor, metabolic, respiratory, and gastrointestinal disturbances) on an ordinal scale. Standardized scoring was done as per standard of care at each institution, which was approximately every 3–4 h. As the parent efficacy trial had found a statistically significant difference in mean Finnegan scores compared to placebo [13], average scores for each neonate were calculated between time of birth to time of first study dose of ondansetron, each dosing interval thereafter, and the dosing interval from time of last dose to time tl. Following, the average change in mean Finnegan scores from baseline (defined as the interval between birth and time of first study dose) to timepoint tl was calculated for each neonate. Lastly, the average change in mean Finnegan scores from baseline to timepoint tl was plotted against quartiles of summary ondansetron exposures using ggplot2 in R. The relationships between Finnegan scores and ondansetron exposures stratified by select covariates of interest (i.e., receipt of morphine sulfate, gestational age, neonate sex, and total number of study doses administered) were graphically evaluated. Gestational age was categorized into designations (early term, 37 0/7–38 6/7 weeks; term, 39 0/7–40 6/7 weeks; late-term, 41 0/7–41 6/7 weeks; post-term, 42 0/7 weeks and beyond) as recommended by a joint working group [20].

TABLE 1 | Patient demographics and summary statistics of included data.

	Neonates ($n = 36$)
Gestational age (weeks)	38.6 (1.1)
Birth weight (kg)	3.1 (0.4)
Height or length at birth (cm)	49.7 (2.7)
Sex, *n* (%):	
Male	18 (50)
Female	18 (50)
Summary statistics	
Number of ondansetron doses received, median (min, max)	2 (1, 5)
Number of neonate plasma observations, median (min, max)	3 (1, 5)
Neonate ondansetron dose (mg)	0.21 (0.03)
Number of maternal doses received, median (min, max)	1 (1, 2)
Maternal plasma ondansetron concentration (ng/mL)	28.2 (23.5)

Note: Data are expressed as mean (SD) unless otherwise specified.

3 | Results

Thirty-six neonates with in utero opioid exposure who received at least one dose of ondansetron were included in this analysis. The following exclusions were made in the dataset due to: one mother-neonate pair being assigned placebo, but the mother received IV ondansetron; one mother delivered at home and no study drug was administered to mother or neonate; one neonate had only BLQ measurements; two neonates never received ondansetron due to QTc prolongation, four neonates received at least one IV dose of ondansetron,

TABLE 2 | PSIS-LOO cross-validation for model selection and sensitivity analyses on CL prior.

Model	ELPD	Standard error	Difference	Standard error of difference
Final model	591.5	38.94	0	0
CL model selection				
No covariate structures with PNA on CL prior	676.9	39.84	85.37	8.50
Full covariate structures with PMA on CL prior	594.3	38.61	2.85	3.64
Full covariate structures with PNA on CL prior	723.4	39.42	131.95	8.06
CL prior adjustments				
Decreased PMA (37 0/7 weeks)	605.2	38.79	13.71	5.31
Increased PMA (42 4/7 weeks)	591.9	38.93	0.40	0.26
Extremely low birth weight (0.5 kg)	591.5	39.00	0.03	0.35
Extremely high birth weight (5 kg)	648.7	39.53	57.20	8.23
50% decreased birth weight (1.595 kg)	596.1	39.65	4.60	4.28

Note: CL prior adjustments were calculated based on a median PMA and birth weight of 39 1/7 weeks and 3.19 kg, respectively. Models with full covariate structures were based on the infant reference model where allometric scaling with or without maturation was used for CL, V, V2, and Q.
Abbreviations: ELPD, expected log predictive density; PMA, postmenstrual age; PNA, postnatal age.

TABLE 3 | Parameter estimates from the final neonate PK model.

Parameter	Posterior median	95% CDI	$\hat{R}$	Bulk ESS	Tail ESS
CL (L/h)	0.58	(0.51, 0.67)	1.00	21,908	33,743
V (L)	0.29	(0.26, 0.32)	1.00	4444	6080
V2 (L)	0.91	(0.75, 1.11)	1.00	2246	3864
Q (L/h)	6.15	(5.63, 6.74)	1.00	4903	6105
KA (h^{-1})	0.19	(0.15, 0.23)	1.00	1565	3101
F	0.62 (FIXED)	—	—	—	—
B_{CL}	0.76 (FIXED)	—	—	—	—
T_{CL} (months)	3.82 (FIXED)	—	—	—	—
Interindividual					
Variability (% CV): CL	0.54 (84.2)	(0.32, 0.92)	1.00	11,340	21,055
Residual error: Additive error (SD)	80.9 (9.2)	(59.3, 114.6)	1.00	9431	16,373

Note: All parameters that were estimated in the log domain were back transformed for clarity. 95% CDI was calculated from Bayesian posteriors. CL, V, V2, and Q were previously estimated on a per-kilogram basis based on a reference weight of 10.4 kg. The median postmenstrual age and median birth weight of the current cohort were 39 1/7 weeks and 3.19 kg, respectively, which were used to calculate informative theta priors. % CV of omega $= \sqrt{\exp(\text{posterior median}) - 1} * 100$.
SD of sigma $= \sqrt{\text{posterior median}}$.
Abbreviations: CDI, credible interval; CV, coefficient of variation; ESS, effective sample size, $\hat{R}$, Gelman-Rubin diagnostic, SD, standard deviation.

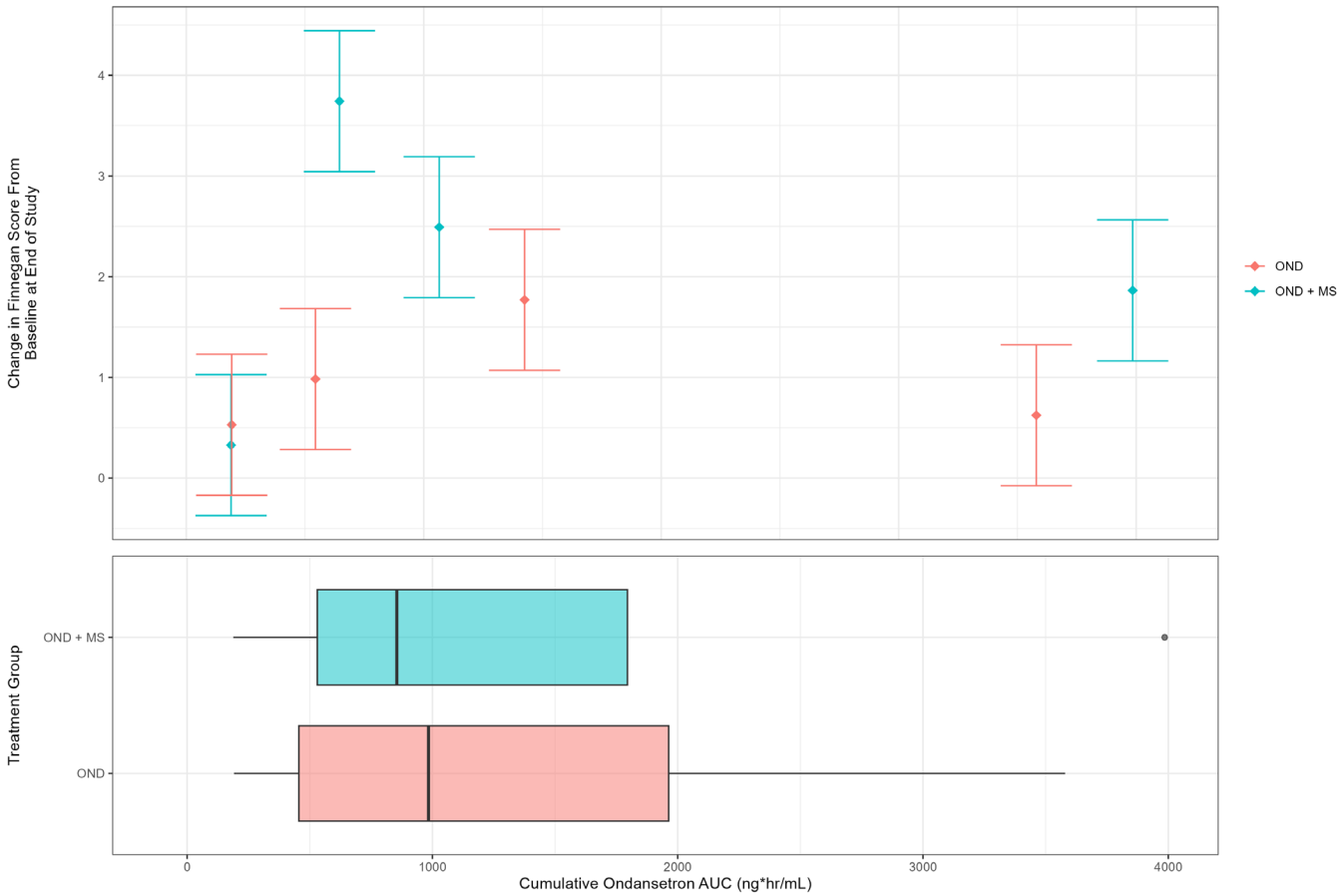

FIGURE 1 | Exploratory exposure-response analyses in neonates with NOWS with cumulative AUC stratified by receipt of morphine sulfate. Diamonds with error bars represent average difference in mean Finnegan scores with 90% confidence intervals. The red and teal boxplots represent the distribution of cumulative AUC values in neonates. AUC, cumulative area under the curve from time of birth up to 75 h after the receipt of the last ondansetron study dose; NOWS, neonatal opioid withdrawal syndrome; OND, ondansetron; OND + MS, ondansetron with morphine sulfate.

which was not enough for robust analysis; and one neonate was born prematurely. There were 109 plasma samples from neonates that were included in the current analysis. An additional 29 plasma samples were included from pregnant mothers. Demographics of subjects as well as summary statistics of the data included are shown in Table 1.

3.1 | Updated Bayesian PK Model

More than half of the neonates had pre-dose concentrations due to maternal transfer, which were captured by initializing the neonatal central compartment to the observed maternal concentrations. The degrees of freedom for the prior omega estimate on CL and additive residual error were set to 14 and 5, respectively. Since infant CL was estimated using allometric scaling with a maturation component, the use of postmenstrual age (PMA) or postnatal age (PNA) (median of 2 days) as a time-varying covariate for the calculation of CL prior was informed using PSIS-LOO cross-validation. Given the similarity in covariates such as birth weight, PMA, and PNA, it was not expected that these would impact clearance processes and overall organ ontogeny between days. Thus, a similar model without any covariate effects applied at the individual level was examined. As shown in Table 2, models that had CL prior calculated with PNA had higher expected log predictive density (ELPD) values than their PMA counterparts, indicating better statistical performance. However, diagnostic plots depicted misspecification with the PNA-based models, thus PMA was utilized for the calculation of CL prior. The covariate model based on PMA had a marginally higher ELPD value than the model without any covariate effects. Despite having the lowest ELPD value, the model without covariate effects was selected as the covariate model had underpredicted individual concentrations and showed similar performance in individual fits and individual posterior predictive checks. Low Pareto $\hat{k}$ values were obtained for all model runs, indicating ELPD estimates are reliable ($\hat{k} < 0.7$). Additionally, all model runs demonstrated convergence as all $\hat{R}$ and ESS values were approximately 1.00 and above 1000, respectively. Figures S1–S3 also confirms adequate convergence.

Sensitivity analyses on the distribution of CL prior revealed that the final model was insensitive to changes in PMA but not changes in weight. More specifically, the model indicated statistical improvement in ELPD favoring larger birth weights (Table 2). However, the diagnostic plots (observed vs. population predicted concentrations, individual fits, and individual posterior predictive checks) did not indicate superior model performance. Thus, there was no compelling reason to utilize an alternative CL prior distribution. The NONMEM control file for the final PK model is provided at the end of the Appendix S1.

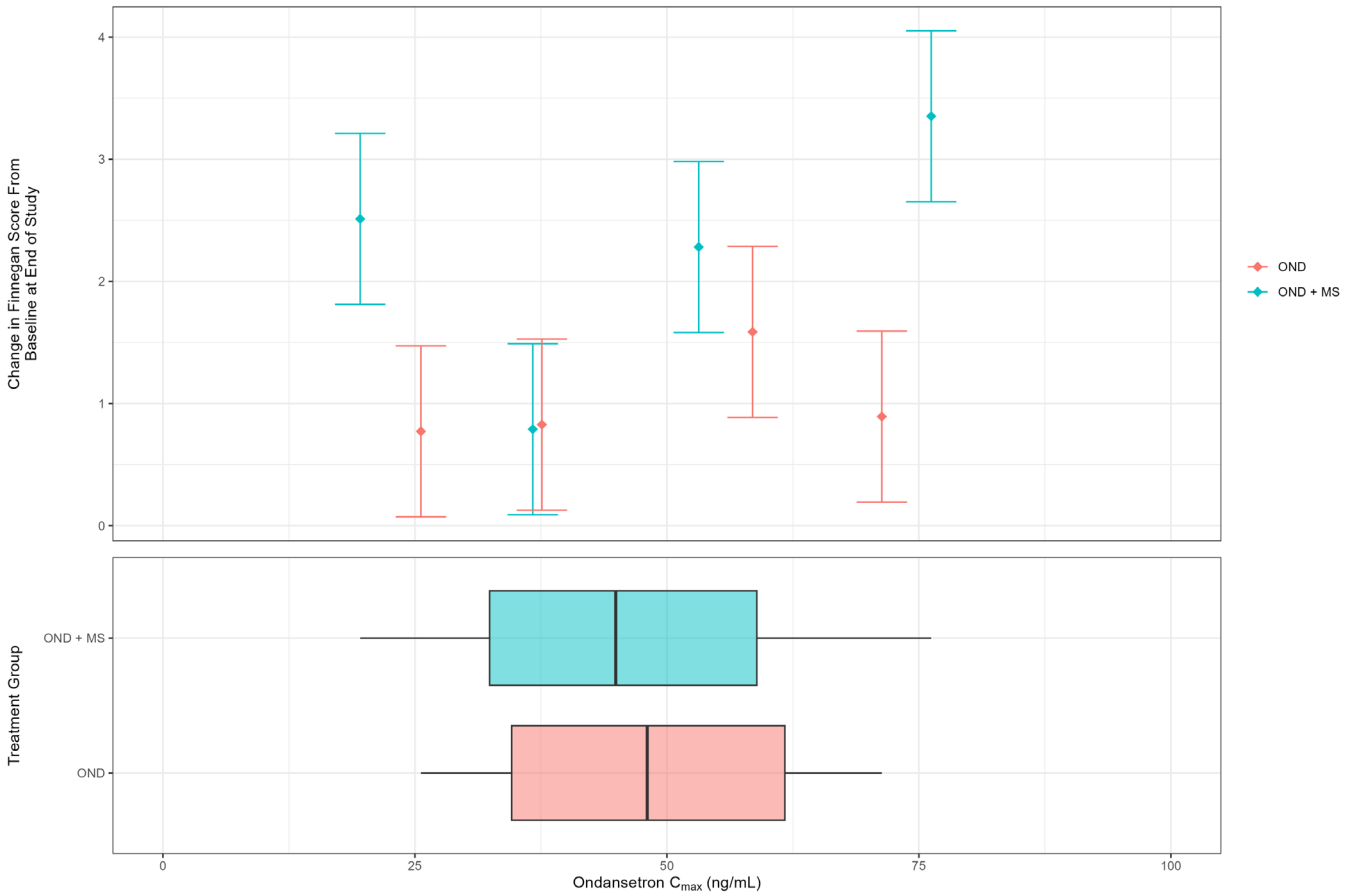

FIGURE 2 | Exploratory exposure-response analyses in neonates with NOWS with C_{max} stratified by receipt of morphine sulfate. Diamonds with error bars represent average difference in mean Finnegan scores with 90% confidence intervals. The red and teal boxplots represent the distribution of C_{max} values in neonates. C_{max}, maximum plasma concentration; NOWS, neonatal opioid withdrawal syndrome; OND, ondansetron; OND + MS, ondansetron with morphine sulfate.

The diagnostic plots for the final model are also shown in Figures S1–S15. The final parameter estimates of the updated Bayesian model are summarized in Table 3.

3.2 | Exploratory Exposure-Response Analyses

Thirty-five neonates randomized to ondansetron were included in the exploratory exposure-response analyses, examining the average change in mean Finnegan scores and simulated AUC_{0-tl} and C_{max} values. One neonate was excluded for not having any Finnegan scores. The final Bayesian model was used to plot the average change in mean Finnegan scores against exposure quantiles stratified by select covariates as seen in Figures 1–4 and S16–S19. Overall, an AUC_{0-tl} of at least 1000 ng*h/mL appears to be the threshold for reducing mean Finnegan scores. Neonates who had received both ondansetron and morphine had a sharper decrease (−1.25 units) in mean Finnegan scores when the median cumulative AUC_{0-tl} was approximately 645 ng*h/mL (Figure 1). These neonates continued to observe a reduction in symptom severity with increasing exposures. The ondansetron-only group did not see a reduction in mean Finnegan scores until the AUC_{0-tl} was approximately 1420 ng*h/mL, where the mean difference was −1.15 units through the last quantile. Regarding C_{max}, mean Finnegan scores were relatively stable even at higher exposures for neonates with ondansetron only (Figure 2). The addition of morphine saw a decrease in mean Finnegan scores by 1.72 units when the median ondansetron C_{max} was between 19.6 and 37 ng/mL.

When stratifying the exposure-response profiles by the total number of ondansetron doses received (Figure 3), neonates who had received at least three doses saw a decrease in mean Finnegan scores with the greatest effect seen with five doses (−3.59 unit change between a median of 700 and 1650 ng*h/mL). Receiving one or two doses of ondansetron did not improve mean Finnegan scores. As the number of doses increased, the median AUC_{0-tl} generally appeared to increase. Similar trends could be seen in Figure 4 with C_{max}. While the number of doses did not generally appear to change the median C_{max}, neonates who had received five doses saw the largest drop in mean Finnegan scores by 3.59 units between a median of 19.6 and 37.5 ng/mL.

Term neonates experienced a drop in mean Finnegan scores by 2.44 units when the median AUC_{0-tl} was approximately between 1250 and 3579 ng*h/mL (Figure S16). Early-term neonates saw a change in mean Finnegan scores by 1.09 units between the second and third quantiles. Gestational age did not appear to affect AUC_{0-tl}. Similarly, term neonates had a −1.33 unit change in mean Finnegan scores with a median C_{max} between 53.2 and 73.1 ng/mL (Figure S17). Early term neonates saw a sharper decrease at lower exposures (2.38-unit change),

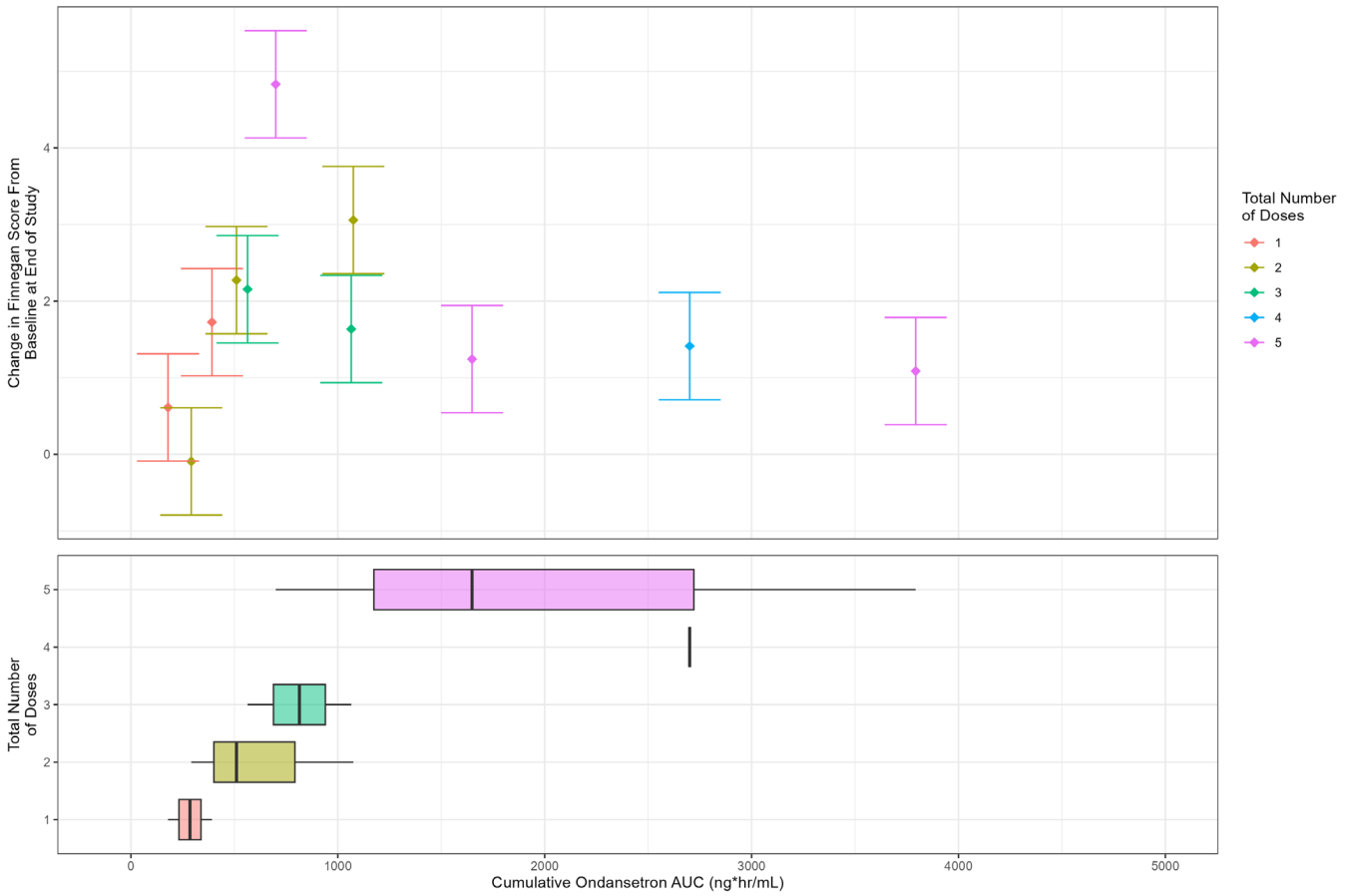

FIGURE 3 | Exploratory exposure-response analyses in neonates with NOWS with cumulative AUC stratified by the total number of doses received. Diamonds with error bars represent average difference in mean Finnegan scores with 90% confidence intervals. The red and teal boxplots represent the distribution of cumulative AUC values in neonates. Only one neonate received a total of four doses of ondansetron. AUC, cumulative area under the curve from time of birth up to 75 h after the receipt of the last ondansetron study dose; NOWS, neonatal opioid withdrawal syndrome.

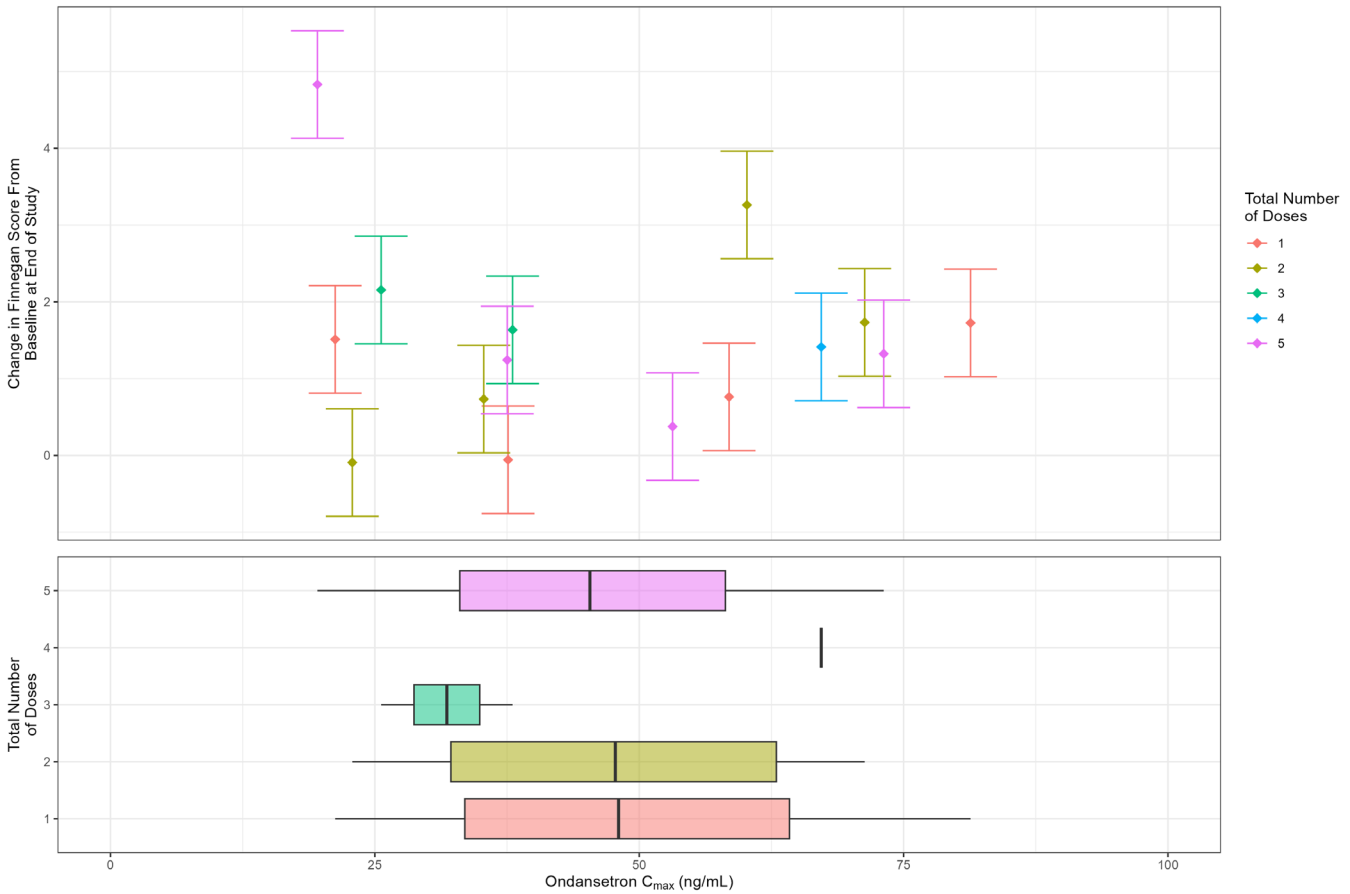

FIGURE 4 | Exploratory exposure-response analyses in neonates with NOWS with C_{max} stratified by the total number of doses received. Diamonds with error bars represent average difference in mean Finnegan scores with 90% confidence intervals. The red and teal boxplots represent the distribution of C_{max} values in neonates. Only one neonate received a total of four doses of ondansetron. C_{max}, maximum plasma concentration; NOWS, neonatal opioid withdrawal syndrome.

but did not maintain this trajectory at higher exposures. Lastly, male neonates had higher mean Finnegan scores and did not have a similar change in mean Finnegan scores compared to females (Figure S18). As seen in Figure S19, the mean Finnegan scores between males and females were relatively stable despite increasing exposures.

4 | Discussion

An infant reference model was used to enable PK parameter estimation aside from KA and F. For all model runs, KA was approximately 0.2. Ondansetron has an approximate T_{max} of 3 h and bioavailability of 60% in healthy volunteers [18], but several factors may limit its absorption in neonates as indicated in the current analysis. Gastric pH varies from a neutral pH at birth due to in utero ingestion of amniotic fluid, smaller stomach volumes, and the composition and timing of feeds, but gradually declines to an acidic pH over weeks or years [21–24]. While the consensus is unclear regarding the effects of ontogeny, elevated gastric pH may support the use of a smaller absorption rate constant for ondansetron considering the treatment duration and impaired dissolution as a weak base. Additionally, prior simulations had assumed that the rate of absorption was similar to adults which is not supported in the current analysis [10].

Ondansetron is metabolized by multiple isoforms of cytochrome P450 including CYP1A1/2, CYP3A, and CYP2D6 [18]. These isoforms are poorly expressed at birth but gradually increase during development, which can be explained using allometric scaling and maturation processes on CL [25–27]. PNA and PMA were explored as a time-varying age covariate for CL maturation as both consider the amount of time passed since birth. PMA is superior to PNA since maturation processes start in utero, which may explain earlier misspecification among PNA-based models. The updated Bayesian model showed marginal improvement when considering a full covariate model structure, which aligns with the consensus that significant changes in organ development are not expected within days but rather weeks or months [21, 22, 28].

The parent trial demonstrated that ondansetron treatment could reduce NOWS symptoms, but its efficacy was viewed as suboptimal since it did not reduce the incidence of NOWS [10]. This may be due to inadequate exposure in the brain with the doses used [14]. Due to the presence of p-glycoprotein (p-gp) on the surface of the blood–brain barrier (BBB), most drugs slowly accumulate in the brain even with sufficiently small size and lipophilicity. Ondansetron is no exception as a lipophilic p-gp substrate, which may explain the low concentrations observed in the brain [14]. Other 5-HT3 receptor antagonists such as granisetron and

palonosetron are similar in lipophilicity [29, 30] and can be expected to follow the same fate. In contrast, neonates may have higher concentrations as the BBB is immature and more permeable prior to maturational changes [22, 28, 31, 32]. Cerebrospinal fluid (CSF) is typically used as a surrogate for CNS drug disposition to avoid invasive sampling, especially in neonates. However, CSF levels may be misleading since they can underestimate the impact of p-gp and do not reflect the drug concentration in brain parenchyma [33–35]. Indeed, subsequent analysis of a mouse model showed that ondansetron concentration in the brain was 1000-fold lower than in blood and rapidly declined after ondansetron administration, and that CNS ondansetron was located largely in the ventricular fluid [14]. Since CNS samples were not collected nor analyzed in this study, optimal CNS concentrations in neonates for the prevention of NOWS could not be ascertained. The observed plasma concentrations were generally associated with anti-emetic efficacy (10–200 ng/mL) [14].

Mean Finnegan scores were used as a proxy for drug action in the CNS as they reflect symptom severity and have been successfully used in buprenorphine PKPD models [36, 37]. Given the results of the parent trial, there is interest in understanding the relationship between exposures and symptom severity in order to optimize ondansetron dosing for NOWS. AUC_{0-tl} and C_{max} were chosen as summary exposure metrics given that ondansetron dose was found to be a significant covariate in its disposition, quick absorption time, and no safety issues were identified in the parent trial even at higher exposures [10, 13, 18]. An optimized regimen would provide infants quick relief from opioid withdrawal symptoms, as this will ultimately reduce the duration of hospitalization and minimize disruptions in bonding time with their mothers. In the current analysis, the number of doses was identified as a relevant covariate in driving efficacy relative to AUC_{0-tl}. This aligns with an earlier report stating doubling the dose from 4 to 8 mg decreased clearance and steady-state volume by approximately 30% each [10]. Furthermore, a 3.59 unit decrease in mean Finnegan scores regardless of the receipt of morphine is deemed clinically significant. It should be noted that while weight-based dosing was utilized, multiple dose levels beyond 0.07 mg/kg once daily were not studied. Instead, the total number of doses received was used as a proxy for the total amount of ondansetron administered. While increased plasma AUC_{0-tl} through higher dosages may yield better outcomes, this could not be ascertained in the current analysis. The observed trends in AUC_{0-tl} may be due to ondansetron only addressing gastrointestinal and locomotor disturbances [38]. While 5-HT3 receptor antagonism was found to decrease opioid severity, it may not be addressing the hyperadrenergic state which is thought to be the primary mechanism of NOWS. Given the relative lack of any consistent exposure-response trends and sparse sampling, C_{max} may not be a reliable exposure surrogate for efficacy.

Several study limitations include a small sample size and sparse PK data which may limit the model's robustness. The current model was a Bayesian model updated from the literature to circumvent this issue. The impact of feeds such as breast or formula milk and plasma protein binding was not studied. Forty percent of neonates received at least a single feeding of breast milk during their hospitalization in the parent study. Despite encouragement to mothers, continued and dense breastfeeding for newborns was low. Neonates fed with formula as opposed to breast milk were reported to have quicker maturation and higher expression of CYP1A2 in addition to their effects on drug absorption [21, 31, 39], which may contribute to the large IIV as seen here. Additionally, breastfeeding is recommended as part of the initial strategy to mitigate symptoms of NOWS. Due to even fewer, sparse data and quality control issues, the study did not incorporate the umbilical cord as an intermediate compartment between the maternal and neonatal central compartments. Maternal concentrations were instead used to initialize the neonatal central compartment although this may oversimplify drug transfer due to placental transporters. Lastly, as gastrointestinal symptoms are a cardinal manifestation of NOWS, the variability in Finnegan scores may not have been adequately accounted for due to potential local effects mediated by peripheral 5-HT3 receptors and/or other pharmacologic therapies as per standard of care.

In conclusion, the rate of ondansetron absorption in neonates is decreased relative to adults. With the current dosing regimen, at least three doses are needed to reduce mean Finnegan scores. Additionally, a cumulative AUC from the time of birth to up to 75 h from receipt of the last dose may be a relevant surrogate marker for efficacy. While not approved for this indication, there is an interest in ondansetron as an additional therapeutic for NOWS to attenuate symptom severity and the need for opioid replacement therapy.

Author Contributions

K.L. wrote the manuscript; G.P. and W.K.K. designed and performed the research; K.L. and J.T.M. analyzed the data; M.W. contributed new reagents/analytical tools.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. S. M. Jilani, H. E. Jones, M. Grossman, et al., "Standardizing the Clinical Definition of Opioid Withdrawal in the Neonate," *Journal of Pediatrics* 243 (2022): 33–39.e1, https://doi.org/10.1016/j.jpeds.2021.12.021.

2. A. H. Hirai, J. Y. Ko, P. L. Owens, C. Stocks, and S. W. Patrick, "Neonatal Abstinence Syndrome and Maternal Opioid-Related Diagnoses in the US, 2010-2017," *Journal of the American Medical Association* 325, no. 2 (2021): 146–155, https://doi.org/10.1001/jama.2020.24991.

3. M. Mascarenhas, E. M. Wachman, I. Chandra, R. Xue, L. Sarathy, and D. M. Schiff, "Advances in the Care of Infants With Prenatal Opioid Exposure and Neonatal Opioid Withdrawal Syndrome," *Pediatrics* 153 (2024): e2023062871, https://doi.org/10.1542/peds.2023-062871.

4. C. L. Mattson, L. J. Tanz, K. Quinn, M. Kariisa, P. Patel, and N. L. Davis, "Trends and Geographic Patterns in Drug and Synthetic Opioid Overdose Deaths—United States, 2013–2019," *Morbidity and Mortality Weekly Report* 70, no. 6 (2021): 202–207, https://doi.org/10.15585/mmwr.mm7006a4.

5. S. W. Patrick, W. D. Barfield, B. B. Poindexter, Committee on Fetus and Newborn, and Committee on Substance Use and Prevention, "Neonatal Opioid Withdrawal Syndrome," *Pediatrics* 146, no. 5 (2020): e2020029074, https://doi.org/10.1542/peds.2020-029074.

6. G. Peltz and K. J. Anand, "Long-Acting Opioids for Treating Neonatal Abstinence Syndrome: A High Price for a Short Stay?," *Journal of the American Medical Association* 314, no. 19 (2015): 2023–2024, https://doi.org/10.1001/jama.2015.13537.

7. L. A. Devlin, L. W. Young, W. K. Kraft, et al., "Neonatal Opioid Withdrawal Syndrome: A Review of the Science and a Look Toward the Use of Buprenorphine for Affected Infants," *Journal of Perinatology* 42, no. 3 (2022): 300–306, https://doi.org/10.1038/s41372-021-01206-3.

8. L. F. Chu, D. Y. Liang, X. Li, et al., "From Mouse to Man: The 5-HT3 Receptor Modulates Physical Dependence on Opioid Narcotics," *Pharmacogenetics and Genomics* 19, no. 3 (2009): 193–205, https://doi.org/10.1097/FPC.0b013e328322e73d.

9. M. J. Erlendson, N. D'Arcy, E. M. Encisco, et al., "Palonosetron and Hydroxyzine Pre-Treatment Reduces the Objective Signs of Experimentally-Induced Acute Opioid Withdrawal in Humans: A Double-Blinded, Randomized, Placebo-Controlled Crossover Study," *American Journal of Drug and Alcohol Abuse* 43, no. 1 (2017): 78–86, https://doi.org/10.1080/00952990.2016.1210614.

10. M. H. Elkomy, P. Sultan, B. Carvalho, et al., "Ondansetron Pharmacokinetics in Pregnant Women and Neonates: Towards a New Treatment for Neonatal Abstinence Syndrome," *Clinical Pharmacology and Therapeutics* 97, no. 2 (2015): 167–176, https://doi.org/10.1002/cpt.5.

11. O. Mansour, R. G. Russo, L. Straub, et al., "Prescription Medication Use During Pregnancy in the United States 2011-2020: Trends and Safety Evidence," *American Journal of Obstetrics and Gynecology* 231, no. 2 (2023): 250.e1–250.e16, https://doi.org/10.1016/j.ajog.2023.12.020.

12. M. M. Werler, S. M. Kerr, E. C. Ailes, et al., "Patterns of Prescription Medication Use During the First Trimester of Pregnancy in the United States, 1997-2018," *Clinical Pharmacology and Therapeutics* 114, no. 4 (2023): 836–844, https://doi.org/10.1002/cpt.2981.

13. G. Peltz, L. M. Jansson, S. Adeniyi-Jones, et al., "Ondansetron to Reduce Neonatal Opioid Withdrawal Severity a Randomized Clinical Trial," *Journal of Perinatology* 43, no. 3 (2023): 271–276, https://doi.org/10.1038/s41372-022-01487-2.

14. M. Wu, Z. Cheng, A. T. Le, Y. Tan, and G. Peltz, "Optimizing a Therapy for Opiate Use Disorders: Characterizing Ondansetron Pharmacokinetics in Blood and Brain," *Clinical and Translational Science* 16, no. 2 (2023): 216–223, https://doi.org/10.1111/cts.13440.

15. A. H. P. Chan Kwong, E. A. M. Calvier, D. Fabre, F. Gattacceca, and S. Khier, "Prior Information for Population Pharmacokinetic and Pharmacokinetic/Pharmacodynamic Analysis: Overview and Guidance With a Focus on the NONMEM PRIOR Subroutine," *Journal of Pharmacokinetics and Pharmacodynamics* 47, no. 5 (2020): 431–446, https://doi.org/10.1007/s10928-020-09695-z.

16. C. K. Johnston, T. Waterhouse, M. Wiens, J. Mondick, J. French, and W. R. Gillespie, "Bayesian Estimation in NONMEM," *Clinical Pharmacology and Therapeutics* 13, no. 2 (2023): 192–207, https://doi.org/10.1002/psp4.13088.

17. J. T. Mondick, B. M. Johnson, L. J. Haberer, et al., "Population Pharmacokinetics of Intravenous Ondansetron in Oncology and Surgical Patients Aged 1-48 Months," *European Journal of Clinical Pharmacology* 66, no. 1 (2010): 77–86, https://doi.org/10.1007/s00228-009-0730-8.

18. R. Huddart, R. B. Altman, and T. E. Klein, "PharmGKB Summary: Ondansetron and Tropisetron Pathways, Pharmacokinetics and Pharmacodynamics," *Pharmacogenetics and Genomics* 29, no. 4 (2019): 91–97, https://doi.org/10.1097/FPC.0000000000000369.

19. S. L. Beal, L. B. Sheiner, A. J. Boeckmann, and R. J. Bauer, "NONMEM 7.5 Users Guide," ICON plc; 1989–2020, https://nonmem.iconplc.com/nonmem750.

20. C. Y. Spong, "Defining 'Term' Pregnancy: Recommendations From the Defining "Term" Pregnancy Workgroup," *JAMA* 309, no. 23 (2013): 2445–2446, https://doi.org/10.1001/jama.2013.6235.

21. B. D. Chapron, A. Chapron, and J. S. Leeder, "Recent Advances in the Ontogeny of Drug Disposition," *British Journal of Clinical Pharmacology* 88, no. 10 (2022): 4267–4284, https://doi.org/10.1111/bcp.14821.

22. J. van den Anker, M. D. Reed, K. Allegaert, and G. L. Kearns, "Developmental Changes in Pharmacokinetics and Pharmacodynamics," *Journal of Clinical Pharmacology* 58, no. 10 (2018): S10–S25, https://doi.org/10.1002/jcph.1284.

23. H. Lu and S. Rosenbaum, "Developmental Pharmacokinetics in Pediatric Populations," *Journal of Pediatric Pharmacology and Therapeutics* 19, no. 4 (2014): 262–276, https://doi.org/10.5863/1551-6776-19.4.262.

24. C. Tayman, M. Rayyan, and K. Allegaert, "Neonatal Pharmacology: Extensive Interindividual Variability Despite Limited Size," *Journal of Pediatric Pharmacology and Therapeutics* 16, no. 3 (2011): 170–184, https://doi.org/10.5863/1551-6776-16.3.170.

25. B. T. McPhail, C. Emoto, D. Butler, T. Fukuda, H. Akinbi, and A. A. Vinks, "Opioid Treatment for Neonatal Opioid Withdrawal Syndrome: Current Challenges and Future Approaches," *Journal of Clinical Pharmacology* 61, no. 7 (2021): 857–870, https://doi.org/10.1002/jcph.1811.

26. F. Tang, C. M. Ng, H. S. Bada, and M. Leggas, "Clinical Pharmacology and Dosing Regimen Optimization of Neonatal Opioid Withdrawal Syndrome Treatments," *Clinical and Translational Science* 14, no. 4 (2021): 1231–1249, https://doi.org/10.1111/cts.12994.

27. K. Allegaert, M. van de Velde, and J. van den Anker, "Neonatal Clinical Pharmacology," *Paediatric Anaesthesia* 24, no. 1 (2014): 30–38, https://doi.org/10.1111/pan.12176.

28. K. O. Zimmerman, D. K. Benjamin, and M. L. Becker, "Neonatal Therapeutics: Considerations for Dosing," *American Journal of Perinatology* 36, no. S 02 (2019): S18–S21, https://doi.org/10.1055/s-0039-1691772.

29. A. Dallmann, I. Ince, K. Coboeken, T. Eissing, and G. Hempel, "A Physiologically Based Pharmacokinetic Model for Pregnant Women to Predict the Pharmacokinetics of Drugs Metabolized via Several Enzymatic Pathways," *Clinical Pharmacokinetics* 57, no. 6 (2018): 749–768, https://doi.org/10.1007/s40262-017-0594-5.

30. J. Gilmore and A. Bernareggi, "Complementary Pharmacokinetic Profiles of Netupitant and Palonosetron Support the Rationale for Their Oral Fixed Combination for the Prevention of Chemotherapy-Induced Nausea and Vomiting," *Journal of Clinical Pharmacology* 59, no. 4 (2019): 472–487, https://doi.org/10.1002/jcph.1338.

31. L. C. Ku and P. B. Smith, "Dosing in Neonates: Special Considerations in Physiology and Trial Design," *Pediatric Research* 77, no. 1–1 (2015): 2–9, https://doi.org/10.1038/pr.2014.143.

32. J. Lam, S. Baello, M. Iqbal, et al., "The Ontogeny of P-Glycoprotein in the Developing Human Blood-Brain Barrier: Implication for Opioid Toxicity in Neonates," *Pediatric Research* 78, no. 4 (2015): 417–421, https://doi.org/10.1038/pr.2015.119.

33. M. Chiang, H. M. Back, J. B. Lee, et al., "Pharmacokinetic Modeling of the Impact of P-Glycoprotein on Ondansetron Disposition in the Central Nervous System," *Pharmaceutical Research* 37, no. 10 (2020): 205, https://doi.org/10.1007/s11095-020-02929-2.

34. M. D. Chiang, K. Frey, C. Lee, et al., "Plasma and Cerebrospinal Fluid Pharmacokinetics of Ondansetron in Humans," *British Journal of Clinical Pharmacology* 87, no. 2 (2021): 516–526, https://doi.org/10.1111/bcp.14412.

35. J. M. Nicolas and E. C. M. de Lange, "Mind the Gaps: Ontogeny of Human Brain P-Gp and Its Impact on Drug Toxicity," *AAPS Journal* 21, no. 4 (2019): 67, https://doi.org/10.1208/s12248-019-0340-z.

36. T. Mizuno, B. T. McPhail, S. Kamatkar, et al., "Physiologic Indirect Response Modeling to Describe Buprenorphine Pharmacodynamics in Newborns Treated for Neonatal Opioid Withdrawal Syndrome," *Clinical Pharmacokinetics* 60 (2020): 249–259, https://doi.org/10.1007/s40262-020-00939-2.

37. J. N. Moore, M. R. Gastonguay, C. M. Ng, et al., "The Pharmacokinetics and Pharmacodynamics of Buprenorphine in Neonatal Abstinence Syndrome," *Clinical Pharmacology and Therapeutics* 103, no. 6 (2018): 1029–1037, https://doi.org/10.1002/cpt.1064.

38. K. D. Alex and E. A. Pehek, "Pharmacologic Mechanisms of Serotonergic Regulation of Dopamine Neurotransmission," *Pharmacology & Therapeutics* 113, no. 2 (2007): 296–320, https://doi.org/10.1016/j.pharmthera.2006.08.004.

39. K. O'Hara, I. M. Wright, J. J. Schneider, A. L. Jones, and J. H. Martin, "Pharmacokinetics in Neonatal Prescribing: Evidence Base, Paradigms and the Future," *British Journal of Clinical Pharmacology* 80, no. 6 (2015): 1281–1288, https://doi.org/10.1111/bcp.12741.

Supporting Information

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