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Pharmacokinetics and Safety of a Single Intravenous Dose of *myo*-Inositol in Preterm Infants of 23 to 29 weeks

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Abstract

Background—*Myo*-inositol given to preterm infants with respiratory distress has reduced death, increased survival without bronchopulmonary dysplasia (BPD) and reduced severe retinopathy of prematurity (ROP) in 2 randomized trials. Pharmacokinetic (PK) studies in extremely preterm infants are needed prior to efficacy trials.

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Methods—Infants of 23–29 weeks gestation were randomized to a single intravenous (IV) dose of inositol at 60 or 120 mg/kg or placebo. Over 96 h, serum levels (sparse sampling population PK) and urine inositol excretion were determined. Population PK models were fit using a nonlinear mixed effects approach. Safety outcomes were recorded.

Results—A 1-compartment model that included factors for endogenous inositol production, allometric size based on weight, gestational age (GA) strata and creatinine clearance fit the data best. The central volume of distribution was 0.5115 l/kg, the clearance 0.0679 l/kg/h, endogenous production 2.67 mg/kg/h and the half life 5.22 h when modeled without the covariates. During the first 12 h renal inositol excretion quadrupled in the 120 mg/kg group, returning to near baseline after 48 h. There was no diuretic side-effect. No significant differences in adverse events occurred between the 3 groups ($p > 0.05$).

Conclusions—A single compartment model accounting for endogenous production satisfactorily described the PK of IV inositol.

INTRODUCTION

Inositol is ubiquitous in living organisms where it is largely present as a free sugar alcohol and also as a headgroup of membrane lipids. In addition, phosphoinositides and glycosyl-phoshatidylinositols have specific roles in signal transduction and in lipid-protein interactions (1–4). In its free form, inositol is a sugar alcohol present in human milk, widely available in the diet and classified as “Generally Regarded as Safe” for enteral administration by the Food and Drug Administration (FDA). In utero, early fetal serum inositol levels are 2–10 times higher than adult levels, and decrease gradually towards term (5–7). Inositol has been supplemented in infant formulas since the late 1990s at approximately 44 mg /100 kcal (350 mg/l), yet its clearance has not been studied in extremely preterm newborns. If an infant is not receiving enteral milk feedings, serum levels fall to levels substantially below those that would have been present in utero (5,6).

Hallman et al administered *myo*-inositol intravenously (IV) and/or enterally to preterm infants with respiratory distress syndrome (RDS) in two randomized trials and found increased survival, increased survival without bronchopulmonary dysplasia (BPD), reduced severe retinopathy of prematurity (ROP), reduced severe intraventricular hemorrhage (IVH) and no observed toxicity (8–11). An additional partially randomized trial of inositol supplemented formula in preterm infants (12) was also considered in the Cochrane review of inositol that concluded these findings warrant further study, particularly in today's younger gestation infants who remain at highest risk for these morbidities (13).

The endogenous production and metabolism of inositol, combined with dietary intake add complexity to a pharmacokinetic (PK) analysis. However, to guide dosing of IV inositol as a potential treatment in extremely preterms for further study, we need to better understand its disposition in this population.

RESULTS

Population Demographics

Figure 1 shows the number of infants screened, eligible and enrolled between June 2006 and December 2007 at 10 participating centers. Consent was obtained for 79, 76 were randomized, and 74 received study drug. Two infants did not complete the minimum of 4 specified blood samples (3 post drug infusion) and their randomizations were replaced with two additional enrollees from the same center and gestational age (GA) stratum, per protocol. Available data from the 2 replaced infants were included in the PK and safety analyses. One infant received placebo instead of the assigned 120 mg/kg dose, and for the

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PK analysis, this infant's serum and urine data were included in the placebo group. However, this subject's data on adverse events and clinical outcomes were included as randomized (intention to treat). The baseline characteristics of the enrolled infants were similar across all three groups, and the median ages at study drug infusion by group were between 2.4 and 3.2 days (Table 1).

Safety

During the infusions, heart rate, blood pressure, and respiration did not differ between placebo and inositol infants at either dose (data not shown). The incidence of at least one adverse event was lowest for the higher dose of inositol: 80% in the placebo, 84% in the 60 mg/kg, and 54% in the 120 mg/kg group (p=0.05), (Table 2). The frequencies of sepsis, IVH, need for supplemental oxygen, need for mechanical ventilation and use of the specified medications did not differ significantly among the groups, nor did early specified clinical diagnoses during the first 4 days after infusion (Table 2). Serious adverse events occurred in 29% of all subjects, and were lowest in the 120 mg/kg group (17%, p=0.11), (data not shown, tables available from the authors). During hospitalization there were no significant differences between groups in the rates of expected preterm diagnoses as a whole (Table 3), nor between groups in the upper or lower GA strata. Clinical event rates were similar to historic data observed in this population in the NICHD Neonatal Research Network (14).

Pharmacokinetic Analyses

The raw mean serum levels rose in proportion to the dose given, gradually returned to baseline (Figure 2) and appeared consistent with the compartmental model under consideration. The pharmacokinetic analysis, which included endogenous production, revealed no improvement between 1 and 2 compartment models (p=0.38), so a 1 compartment model was used. In the measurement of residual error, a constant variance best fit the data. The relationships between the random effects were graphically studied by plotting u_{vi} vs. u_{Cli}, u_{Vi} vs. u_{Ri} and u_{Cli} vs. u_{Ri} for all infants. A strong linear relationship was observed between the random effect estimates for clearance (*Cl*) and endogenous production (*R*) with no apparent relationship between the other two combinations of random effects. The random effects were then modeled only with correlation between *Cl* and *R*.

Table 4 presents the population-PK (Pop-PK) estimates for the 1-compartment model excluding any covariates with the associated random effect variance and correlation estimates in Table 5. Derived values for the elimination rate, the half-life and the apparent concentration associated with endogenous production are also shown in Table 4. The model appears to provide a good fit to the data as shown in Figure 3 with the observed and individual predicted values being well aligned. The mass of data points at the low values are primarily comprised of pre-dosing and placebo measurements combined with a smaller number of late time point measurements, all of which are expected to have low inositol concentrations. Residual plots comparing predicted and actual values, not included here, did not indicate any major model deficiencies.

This model was used to assist the investigators to project the effect on serum levels of inositol from repeated dosing with a q12 h or q 24 h schedule using several different daily doses. As an example, Figure 4 shows the model's prediction for 80 mg/kg/day divided into two doses q12 h, over 36 h. While this study did not directly evaluate multiple dose administrations, the model's estimate represents probable serum levels over a short time period for planning future studies of repeated administration.

The effect of the following covariates on each of the model parameters was tested: infant birth weight, allometric size at birth, GA strata at birth (23–26 weeks vs. 27–29 weeks),

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postmenstrual age, postnatal age, creatinine clearance and sex (15). Allometric size, a function of birth weight, entered the model for a parameter as a multiplicative scaling factor given by $F_{size} = (w_i/\tilde{w})^{\beta size}$ where w_i is the birth weight, in grams, for the *i*-th infant, $\tilde{w}$ is the median birth weight of the infants in the study (997.5 g) and β_{size} is the estimated coefficient. The remaining covariates had an exponential multiplicative effect given by $F_z = e^{\beta z \times z}$ where *z* is one of the covariates. Table 6 presents the mean and standard deviations for the continuous covariates while the two categorical covariates were both equally divided with 50% in each of their two categories.

Each covariate was tested individually against the base model. Those covariates found to have a statistically significant effect on any of the three Pop-PK model parameters, *R*, *Cl* and *V*(V = volume of distribution), were then combined into a single model. Birth weight, postmenstrual age, postnatal age and sex had no significant effects on the model parameters. Allometric size, GA strata and creatinine clearance were found to have a statistically significant effect on one or more of the three model parameters (Table 7). The final model including all of the statistically significant covariates indicates that infants with a GA of 27–29 weeks had a lower volume of distribution than infants with a lower GA of 23–26 weeks, while clearance increased with increasing allometric size. The pathway of influence for creatinine clearance was less clear. When its effect was considered separately on each of the model parameters, *V*, *Cl* and *R*, it was statistically significant for *Cl* and *R* (p=.044 and p=.014, respectively) with a direction of effect that was physiologically plausible, increasing for *Cl* and decreasing for *R*. However, when creatinine clearance was allowed to simultaneously affect both *Cl* and *R*, then its direction of effect on *Cl* was the opposite of that expected with increased creatinine clearance leading to a reduction in *Cl*. In addition, the model with creatinine clearance affecting *R* alone was not significantly different from the model with creatinine clearance affecting both *Cl* and *R* (p=.292). Thus, the final covariate model included the effect of creatinine clearance solely on *R*. The estimated typical value for each of the three model parameters were affected as follows by the final covariate model:

$$V^* = 0.5924 \times \exp[-0.2777 \times I(GA)] \quad Cl^* = 0.0549 \times \left(\frac{Birthwgt}{997.5}\right)^{0.2158} \quad R^* = 3.040 \times \exp[-0.1898 \times CrCl]$$

where *V**, *Cl** and *R** are the covariate adjusted model parameters and *I*(*GA*) is an indicator function that equals 0 (zero) for GA stratum 23–26 weeks and 1 (one) for stratum 27–29 weeks.

Urine excretion of inositol: Excretion in the placebo infants had a baseline rate of 26.6 ± 12.3 mg/kg/24 h (m ± sd) and showed an upward trend with age over the 96 h study to 36.8 ± 28.8 mg/kg/24 h on study day 4. Following inositol administration, excretion rose during the first 12 h collection and decreased in the second 12 h. Thereafter, excretion approached levels in the placebo group. Following the 120 mg/kg dose, average excretion was 67.3 ± 35.8 mg/kg in the first 12 h and 25.3 ± 14.4 mg/kg in the second 12 h. For the 60 mg/kg subjects 31.9 ± 13.4 mg/kg was excreted in the first 12 h and 18.0 ± 9.6 mg/kg in the second 12 h.

We examined the volume of urine output for evidence of a potential diuretic effect of the excreted inositol and found none. The mean volume of urine in the first 12 h after drug dosing when excretion was the highest was 40.7 ml/kg/12 h in placebo infants, 37.8 ml/kg/12 h in the 60 mg/kg group and 46.2 ml/kg/12 h in the 120 mg/kg group respectively, (p-value=0.1242 Kruskal-Wallis test).

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DISCUSSION

These findings demonstrate among preterm infants of 23–29 weeks gestation that a single IV dose of 60 mg/kg or 120 mg/kg inositol given over 20 minutes results in an initial dose proportional increase in serum concentrations that persists between 24 to 36 h. Accommodating a parameter for endogenous production, the PK analysis showed a central volume of distribution of 0.5115 l/kg with a half life of 5.22 h when excluding any covariate effects. While increased urine inositol losses initially occurred, this did not result in a diuresis.

Previous controlled trials suggest supplemental inositol is safe and beneficial for preterm infants. The serum levels achieved in the key study demonstrating benefit was 126–153 mg/l during the first week of treatment (11). In this study inositol also appeared safe, however this study's power to detect differences in uncommon adverse events is limited by the small sample size, and infants received only a single dose. While the higher rates of BPD and sepsis in the highest dose group were unexpected, have not been previously reported, and are not statistically significant, it will be important to monitor these event rates during future studies. To date, reported studies do not describe IV inositol supplementation for more than 8 days.

Hallman et al observed an apparent steady state serum concentration after the first of 5 days of 80 mg/kg/day dosing (11). The fall in serum levels in the 'wash out' period in the Hallman study suggested a half-life of 4–5 days (11), which differs substantially from our estimate (5.22 h) and is not explained, although fully established enteral intake of human milk in the Hallman studies may have contributed to supporting the serum levels despite discontinuing IV dosing. A PK study conducted with repeated dosing and a wider range of doses should help resolve these findings.

Brown and colleagues found that the plasma rate of appearance of inositol in term and late preterm infants was 121.7 mg/kg/day, well in excess of the usual daily enteral intake from preterm formulas or human milk (approximately 54 mg/kg/day) (16). Endogenous production from glucose occurs in many tissues, and enterally received inositol is actively transported across the intestinal mucosa where it is absorbed almost completely (17,18). The catabolic enzyme for inositol, *myo*-inositol oxidase, is localized almost exclusively to the renal cortex (19), but the activity of this enzyme is very low in the fetal and newly born renal cortex. It rises rapidly after birth as the renal blood flow redistributes from the renal medulla to the cortex. Thus, urinary losses of intact inositol are high in both the term and preterm newborn, but gradually fall to very low levels in the weeks following birth (4,20). Despite these developmental physiologic mechanisms that affect inositol concentrations, serum levels remain responsive to dietary intake decreasing in both tissue and serum when diets are low in inositol, and rising with supplemented diets (4).

We speculate that immature renal tubular transport at these stages of development is inadequate to reabsorb the amount of inositol filtered by the glomerulus, similar to glucose in extremely low birth weight newborns. The amount of filtered inositol reaching the renal tubules over a short period of time exceeded renal tubular reabsorption so the higher dose caused a greater renal loss. This pattern of renal excretion is expected to change rapidly postnatally, and could lead to reabsorption of a greater percentage of the dose and higher circulating concentrations rather than to faster clearance and shorter half-lives which occurs with most drugs administered to preterm newborns during maturation. With the expected increase in the catabolic *myo*-inositol oxidase enzyme in the renal cortex in the weeks after birth, remaining filtered inositol will likely be converted to d-glucuronic acid and no longer appear in the urine (19). These observations and remaining uncertainties are important to the

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design of future multi-dose efficacy trials. We plan to examine daily administration of inositol over longer periods of time. Different doses will be evaluated, and divided doses will be used in an attempt to reduce peak serum levels and therefore reduce urine losses. As we increase our understanding of how to influence serum levels, we can better test inositol's safety, and potential to benefit extremely preterm infants.

METHODS

Design

A randomized, double-masked, placebo-controlled pharmacokinetic trial with sparse blood sampling was conducted by the *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development Neonatal Research Network (NRN). Ten of the NRN Centers participated and enrolled subjects between June 2006 and December 2007. Randomization was performed centrally via computer within two pre-specified GA strata ($23^{0/7}$–$26^{6/7}$ weeks, and $27^{0/7}$–$29^{6/7}$ weeks), and infants were monitored prospectively for toxicity and clinical outcomes during hospitalization.

Population

Eligible subjects were $23^{0/7}$–$29^{6/7}$ weeks gestation, ≥ 600 g birth weight, had no major congenital anomalies, were between 12 h and 6 days of age at randomization, and had received no human milk or formula feedings since birth.

Intervention

Inositol was given as a single low (60 mg/kg) or high (120 mg/kg) dose of 5% *myo*-inositol (referred to as 'inositol' in the remaining text) intravenously over 20 minutes in a 1:1:1 randomization with placebo delivered in one of two volumes to maintain masking (5% glucose, USP, for IV administration). Drug or placebo was dispensed from the respective pharmacies in unit doses labeled as "inositol study drug", and all clinical and research personnel except for the pharmacist were masked to the study group. Inositol (hexahydroxycyclohexane) is manufactured from corn or rice bran and was provided as an isotonic, pyrogen and preservative-free, sterile 5% solution of *myo*-inositol in water containing 0.5 g sodium chloride per liter (8.55mM), pH 6.5–7.5.

Outcome Variables

Infants were continuously observed during the infusion by study personnel and vital signs recorded every 5 minutes for the first 30 minutes and thereafter at 15–30 minute intervals for 2 h. Six pre-specified clinical conditions and 7 concomitant medications were recorded for 4 days following infusion (culture positive sepsis, documented patent ductus arteriosus (PDA), clinically diagnosed PDA, supplemental oxygen, mechanical ventilation, (IVH); and surfactant, dopamine/dobutamine, antibiotics, indomethacin as prophylaxis for IVH, indomethacin as a treatment for PDA, systemic steroids, and diuretics).

Adverse events were recorded for 7 days and severity determined using a toxicity table modified for neonatal use from the National Cancer Institute. Baseline characteristics and demographic data as well as neonatal morbidities from birth through hospital discharge (or 120 days if sooner) were recorded from the medical record using the definitions of the Neonatal Research Network generic database. BPD was defined as receiving oxygen at 36 weeks post menstrual age (or at discharge if discharged before 36 weeks postmenstrual age (PMA));

IVH was classified according to Papile (21); late onset sepsis was defined as a positive culture from a normally sterile body fluid obtained after 72 h of age; and necrotizing

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enterocolitis (NEC) was defined as either Modified Bell's IIA or worse not needing surgery, or requiring surgery (IIIB) (22).

Blood Sampling

Each subject contributed 4 blood samples (predose plus three post dose samples) according to a sparse sampling, Pop PK design (23,24). The pre-dose sample was within 4 h before infusion; and the 3 post dose samples were distributed among 9 sample windows: within 4 minutes of the end of infusion, 4±1, 8±1, 12 ±1, 24±2, 36±2, 48±2, 72±2 and 96±2 h after the start of the infusion. In addition, some scavenged serum samples from the clinical laboratories were obtained, with consent, to supplement the scheduled samples. The exact time of sampling in relation to the end of drug infusion was recorded for all samples. Blood (200 μL) was collected in serum separator micro-tubes, refrigerated until centrifuged, up to 60 h after sampling, and frozen at −70°C to −80°C until analysized. (Stability data at room temperature for up to a week are available from the authors upon request.)

Urine Collections

Six consecutive urine collections starting with the 8–12 h before the study drug were collected continuously for 96 h post infusion (the second two for 12 h each, then three for 24 h each). Urine volume was determined by the change in weight of each pre-weighed non-gel containing diaper/cotton balls within the given 12 or 24 h time period, assuming 1 g = 1ml. Urine was expressed from the diapers/cotton balls and pooled within each timed collection, and inositol and creatinine were determined on an aliquot of the pooled sample. Serum creatinine was measured at least twice during the 96 h by each institution's clinical labs.

Enteral Inositol Intake

Following drug infusion, enteral intake was permitted when the infant was considered ready to begin feedings by the clinical team. Enteral intake (type and volume each day) was recorded for 4 days beginning with the day of study drug infusion. Samples of ingested milk were assayed for inositol to permit estimation of additional inositol intake during the sampling period.

Assay

Inositol isomers (*myo*-inositol, 1,5-anhydro-D-sorbitol, and d-chiro-inositol) were quantified from 25 or 50 μL samples using high performance liquid chromatography with electrochemical (pulsed amperometry) detection (method and validation documents available upon request from http://www.ttuhsc.edu/sop/research/internalgrants/CPET.aspx., Leff RD). Results of validation experiments showed the following assay performance parameters: lower limit of quantitation 1.0 mg/l; coefficient of variation from low to high concentrations was 10.2% to 13.4% between days, and 1.9% to 2.3% within a day. Validated test samples included serum; plasma collected using EDTA, sodium heparin, or lithium heparin; urine extracted from any of 5 common non-gel preterm infant diaper types with or without cotton balls; human milk and infant formulas. Serum, human milk and formulas contained only non-detectable or only barely detectable levels of the D-sorbitol and d-chiro-inositol isomers. Therefore, only *myo*-inositol levels are reported. The concentration of inositol in formulas was 356 (67) mg/l (m (±sd)), and in human milk was 287 (107) mg/l.

Statistical Analysis

The planned sample size was at least 36 infants in each GA stratum who received study drug and completed 4 blood samples. Baseline characteristics and clinical outcomes for all

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randomized infants were analyzed by study group using Chi Square tests, Fisher's exact tests, ANOVA, or Kruskal-Wallis tests, where appropriate.

Pharmacokinetic Analysis

Pop-PK models were fit to the data using the nonlinear mixed effects approach in Monolix (Monolix Version 3.2. LIXOFT http://www.lixoft.com/, Paris, France, 2010). This approach accounts for the variability between infants in the model parameters, the correlation between measurements on the same infant at different occasions, as well the residual unexplained variability in serum concentrations (25).

As noted, inositol is produced endogenously and is present in human milk and infant formulas (26). Hence, the Pop-PK model had to account for an endogenous serum concentration of inositol not related to treatment. Apparent endogenous inositol production was included in the Pop-PK model similarly to Hayashi (27). The final model combines apparent endogenous production with a one-compartment IV-infusion model with linear elimination. The model for serum concentration is then

$$C_i(t)=\frac{R_i}{Cl_i}+\left\{\begin{array}{ll}\frac{D_i}{T\times Cl_i}\left[1-e^{-\frac{Cl_i\times t}{V_i}}\right] & \text{if} t\le T\\ \frac{D_i}{T\times Cl_i}\left[1-e^{-\frac{Cl_i\times T}{V_i}}\right]\left[e^{-\frac{Cl_i\times(t-T)}{V_i}}\right] & \text{if} t>T\end{array}\right\}+\varepsilon_{it}$$

where, for the *i*-th infant, $C_i(t)$ is the serum concentration at time *t*, R_i the apparent rate of inositol infusion due to the combination of endogenous production and feeding, Cl_i the clearance and V_i the apparent volume of distribution. *T* is the duration of the infusion period and *t* is the time after the start of the infusion, both in h; finally, ε_{it} is the residual error at time *t*. The steady-state endogenous concentration is then given by $E_i = R_i/Cl_i$ for the *i*-th infant, which is used to measure the combined effect of endogenous inositol production and inositol intake from enteral feeding. It was not possible to separate these two sources of inositol since enteral intake was measured as a total amount fed over a day and not the amount fed at each occasion (feedings are given 8 times throughout the 24 h day). In addition, the enteral intake of inositol was very low during the 4 day study. There was minimal enteral intake of inositol in the first 12 h after the IV dose, and a maximum of 3.6 mg/kg in the first 24 h. Only 49% of subjects received any enteral intake in the 2nd 24 h period (mean intake of inositol 1.8 mg/kg/d, range 0–20) and the mean intakes on days 3 and 4 were 4.5 and 5.5 mg/kg, respectively. Because the calculated enteral intake of inositol from human milk or formula among those able to receive some feedings was so low during the first two study days, measured enteral intake was not included as a separate source in these models.

The between infant variability in the Pop-PK model parameters, R_i, Cl_i and V_i, is modeled using random effect variables (u_R, u_{Cl} and u_V) that approximate the individual trajectory over time of each infant's concentration. The random effects are assumed to be normally distributed with means of 0 (zero) and variances and correlations that will be estimated. For example, the clearance for the *i*-th infant is modeled as $Cl_i = Cl \times e^{uCli}$ where *Cl* is the fixed-effect common to all infants and u_{Cli} is the random effect unique to the *i*-th infant. A similar formulation is used for R_i and V_i. Thus, the three model parameters are log-normal. Individual specific parameter estimates were obtained as the conditional modes, or the maximum *a posteriori*, of the Bayes estimates of the parameters. The fixed effects, *R*, *Cl* and *V*, are the median values of the parameters and are called the typical values for the population from which each infant's parameters are derived. The residual error, ε_{it}, is assumed to be uncorrelated with the random effects and normally distributed with mean 0

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(zero) and a variance that will be estimated. The quality of fit of a Pop-PK model was judged by visual examination of plots of observed vs. individual predicted concentrations and of residuals vs. individual predicted concentrations. Nested models were compared by referencing the improvement in the objective function (−2 log-likelihood) against the chi-square distribution with degrees of freedom equal to the difference in the number of estimated parameters between two models. Statistical significance was assessed at the level of $p \leq 0.05$.

Ethical oversight

The institutional review boards of each center approved the protocol, and written informed consent was obtained for each participant. An independent Data Safety Monitoring Committee approved the protocol and monitoring plan before the study began and monitored the accumulating safety data at regular intervals. The FDA approved protocol (IND # 70510) was registered with ClinicalTrials.gov (NCT00349726).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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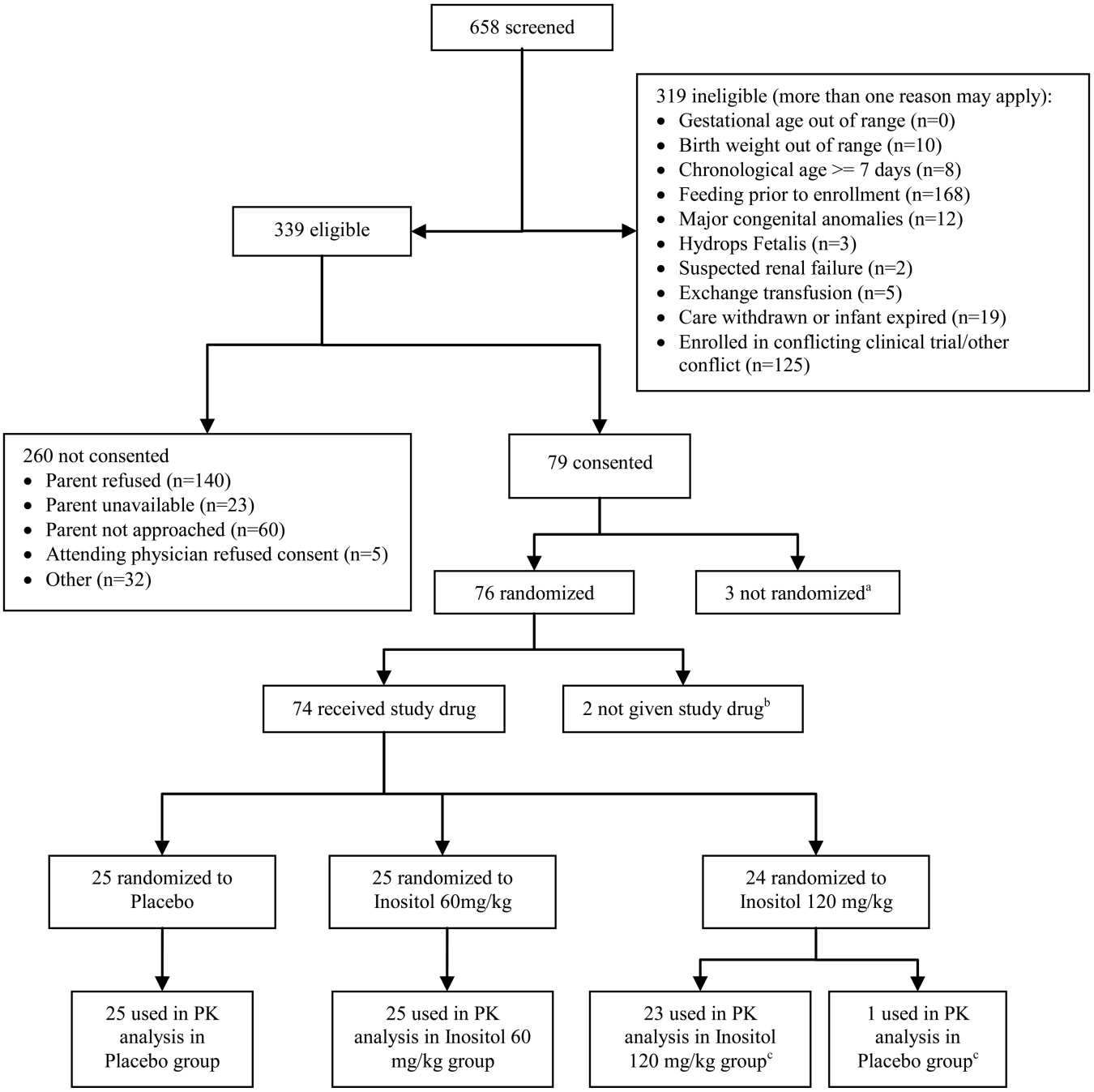

Figure 1. Consort Diagram: Flow of enrolled, randomized and analyzed subjects

[a] Two infants started feeding prior to randomization; 1 infant's condition worsened prior to randomization resulting in loss of vascular access required for drug administration.

[b] Two infants identified as ineligible post-randomization (1 had < 600 grams birth weight, 1 developed severe IVH post-consent)

[c] One infant received placebo in error, samples included as placebo in the pharmacokinetic (PK) analyses, however clinical outcomes included as intention to treat.

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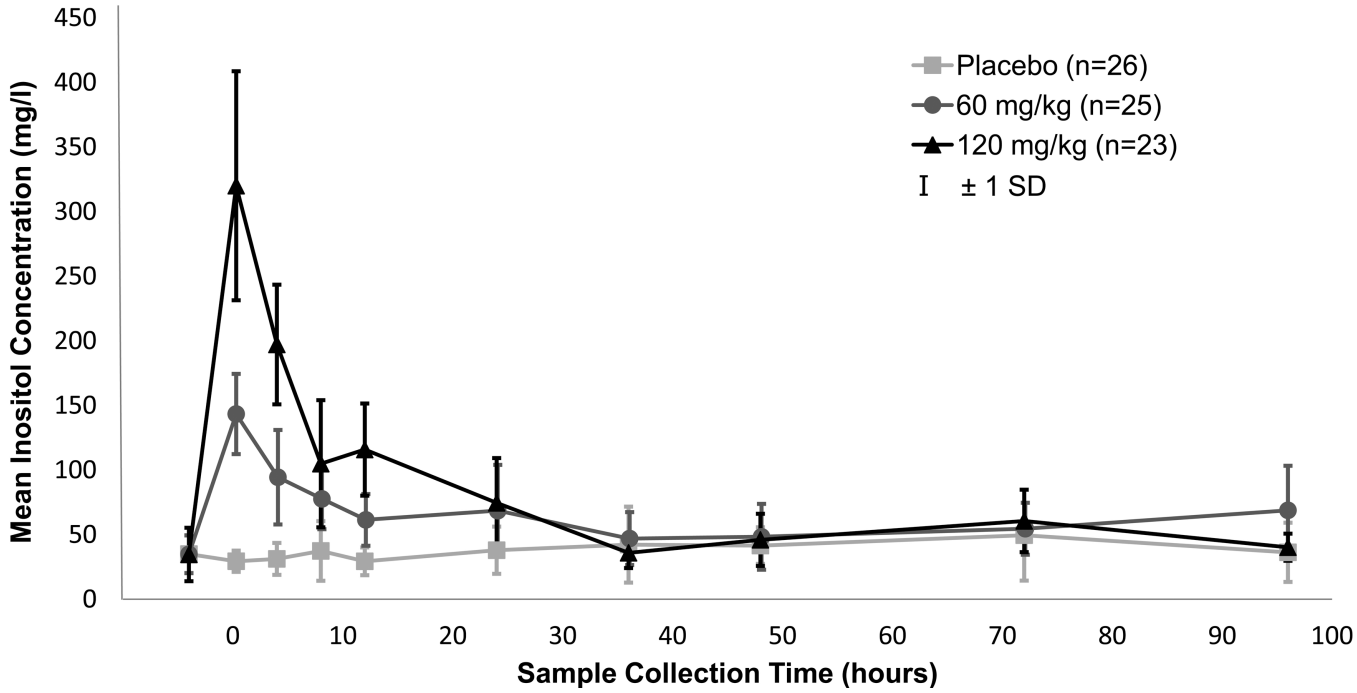

Figure 2. Serum Inositol Levels by Dose Group

Samples were collected within scheduled windows, plus additional scavenged samples as available. For this graph, collection times were clustered as follows to obtain mean values: 0 h (baseline) = obtained prior to infusion; 0.3 h (end of infusion) = 0–2 h post-infusion; 4 h = 2–6 h post-infusion; 8 h = 6–10 h post-infusion; 12 h = 10–14 h post-infusion; 24 h= 14–30 h post-infusion; 36 h = 30–42 h post-infusion; 48 h = 43–60 h post-infusion; 72 h = 60–82 h post-infusion; 96 h = >82 h post-infusion. Square = placebo, circle = 60mg/kg, triangle= 120mg/kg, vertical bar = ± 1 SD.

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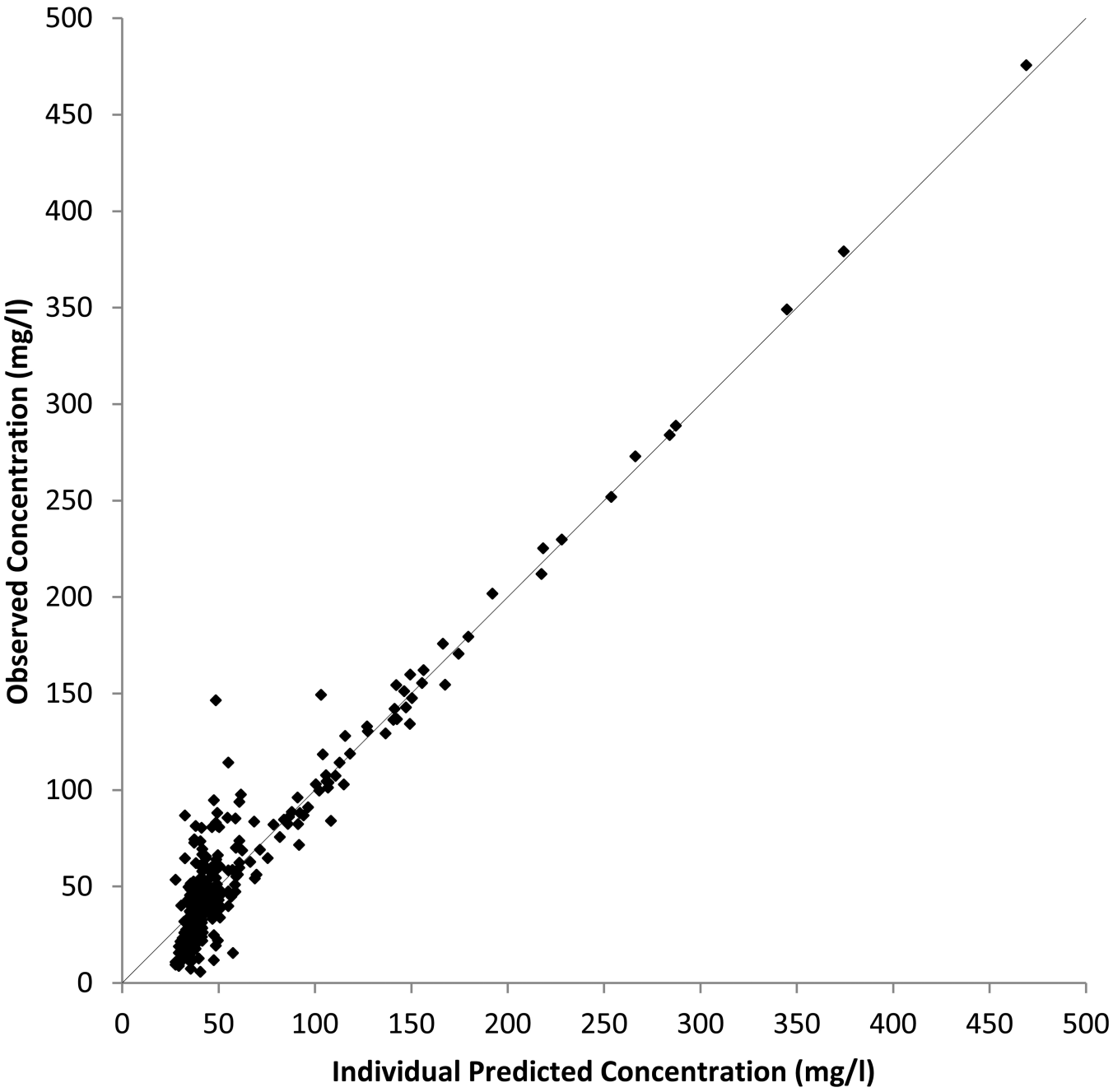

Figure 3. Observed vs. Individual Predicted Inositol Concentrations from the Pop-PK Analysis
Predicted values were calculated for each observed data point using the individual characteristics in the model described in the PK section of the text.

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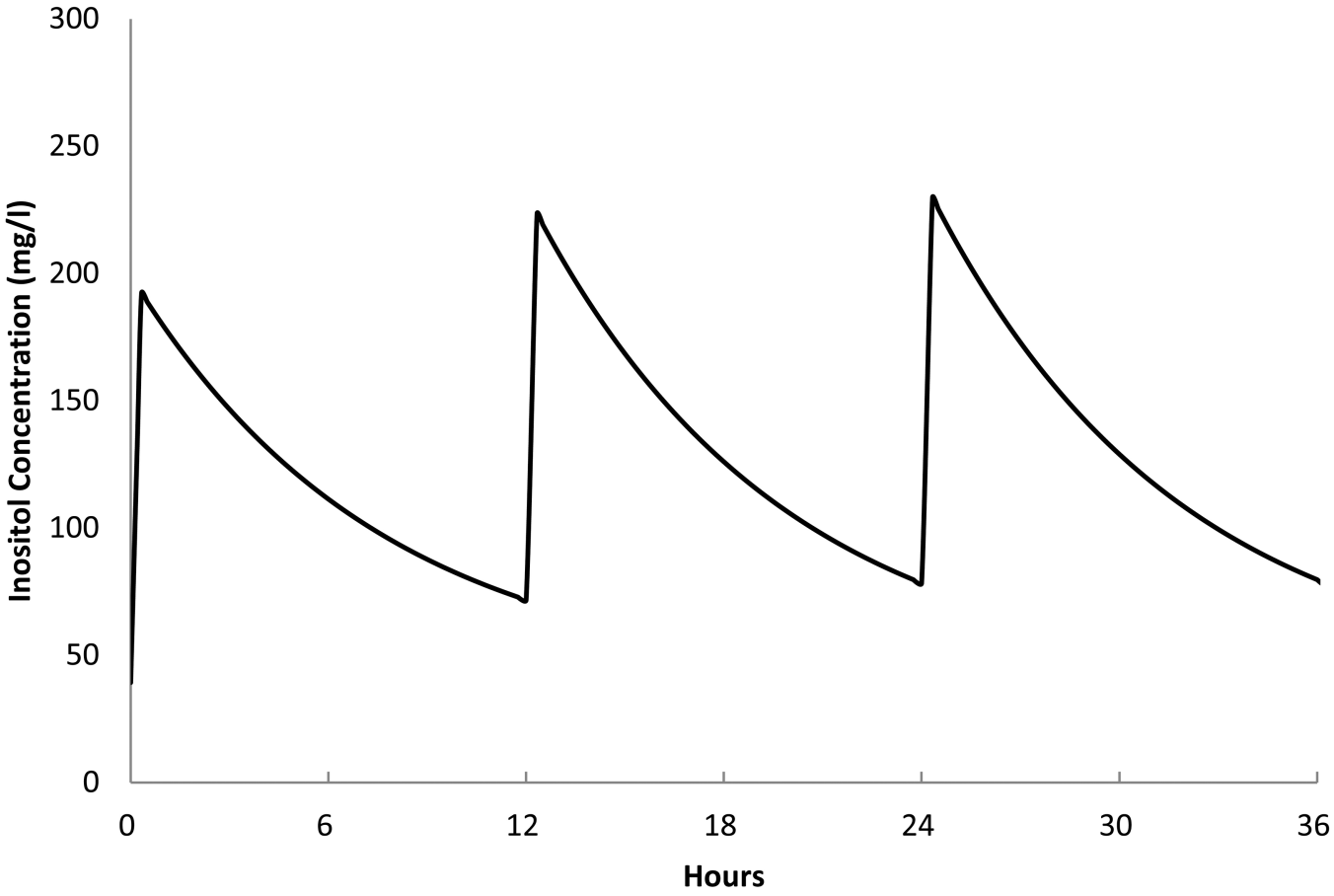

Figure 4. Predicted Serum Inositol Model

The model described in the PK section (before covariates) was used to predict the pattern of serum levels for a typical infant given repeated doses of 80 mg/kg/day divided into 40 mg/kg every 12 h for 36 h.

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Table 1

Baseline Characteristics of Enrolled Infants by Treatment Group

Characteristic		**Placebo (N=25)**	**Inositol 60 mg/Kg (N=25)**	**Inositol 120 mg/Kg (N=24)**	**P-value**
Gestational Age (weeks)	Median (range)	26.9 (24.0–29.3)	26.7 (23.7–29.7)	27.1 (23.7–29.7)	0.98
Gestational Age Stratum	27–29 weeks, N (%)	12 (48%)	12 (48%)	13 (54%)	0.88
Birth Weight (g)	Median (range)	995 (610–1470)	1020 (640–1340)	968 (690–1495)	0.95
Gender	Female, N (%)	12 (48%)	14 (56%)	11 (46%)	0.75
Race, Non-Hispanic White	N (%)	11 (44%)	12 (48%)	12 (50%)	0.91
Antenatal steroids	N (% yes)	20 (80%)	21 (84%)	20 (83%)	0.92
Chorioamnionitis	N (% yes)	8 (32%)	5 (20%)	5 (21%)	0.54
Apgar score, 1 minute[a]	Median (range)	4.5 (1–9)	6.5 (0–9)	6 (1–9)	0.51
Apgar score, 5 minute[a]	Median (range)	7 (4–9)	7 (0–9)	8 (1–9)	0.47
Age (days) at infusion	Median (range)	3.2 (1.4–5.2)	3.1 (1.4–5.1)	2.4 (1.4–4.9)	0.22

[a] missing Apgar scores: infant born in ambulance (n=1); infant born in Mexico (n=1)

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Table 2

Clinical outcomes through Day 7 after infusion by treatment group

Characteristic		Placebo (N=25)	Inositol 60 mg/Kg (N=25)	Inositol 120 mg/Kg (N=24)	P-value
Severe Adverse Events					
Died within 7 days of infusion	Yes (%)	0	1 (4%)	0	>0.99
Adverse events					
During Infusion	N	25	25	24	
At least 1 event	Yes	4 (16%)	3 (12%)	1 (4%)	0.52
Total # events per infant	Median (range)	0 (0–2)	0 (0–2)	0 (0–1)	0.38
After Infusion	N	25	25	24	
At least 1 event	Yes	20 (80%)	21 (84%)	13 (54%)	0.05
Total # events	Median (range)	2 (0–12)	3 (0–16)	1 (0–14)	0.11
Within 2 hours					
Apnea	Yes (%)	1 (4%)	1 (4%)	2 (8%)	0.69
Perfusion change	Yes (%)	3 (12%)	1 (4%)	1 (4%)	0.52
Clinical conditions within 4 days of infusion:					
Sepsis	n (%)	2 (8%)	1 (4%)	3 (13%)	0.52
PDA[a]	n (%)	8 (32%)	5 (20%)	7 (29%)	0.65
IVH[b], clinical	n (%)	1 (4%)	2 (8%)	1 (4%)	>0.99
IVH[b], imaged	n (%)	3 (12%)	4 (16%)	4 (17%)	0.92
On supplemental oxygen	n (%)	20 (80%)	21 (84%)	20 (83%)	>0.99
On ventilator	n (%)	16 (64%)	19 (76%)	15 (63%)	0.56
Concurrent Medications in 4 days after infusion (Study days 1–4):					
Surfactant	n (%)	4 (16%)	1 (4%)	3 (13%)	0.42
Dopamine or Dobutamine	n (%)	2 (8%)	5 (20%)	5 (21%)	0.42
Antibiotics	n (%)	21 (84%)	22 (88%)	18 (75%)	0.48
Indomethacin, for IVH[b] prophylaxis	n (%)	6 (24%)	6 (24%)	6 (25%)	>0.99
Indomethacin, PDA[a] treatment	n (%)	7 (28%)	3 (12%)	6 (25%)	0.41

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Characteristic		Placebo (N=25)	Inositol 60 mg/Kg (N=25)	Inositol 120 mg/Kg (N=24)	P-value
Systemic Steroids	n (%)	2 (8%)	1 (4%)	1 (4%)	>0.99
Diuretics	n (%)	3 (12%)	7 (28%)	5 (21%)	0.37

[a] PDA patent ductus arteriosus

[b] IVH intraventricular hemorrhage

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Table 3

Clinical Outcomes and Adverse Events by Treatment group

Characteristic [a]		**Placebo (N=25)**	**Inositol 60 mg/Kg (N=25)**	**Inositol 120 mg/Kg (N=24)**	**P-value**
Died prior to discharge	n Yes (%)	25 3 (12%)	25 4 (16%)	24 3 (12%)	>0.99
BPD [b] (On O_2 at 36 weeks PMA [c])	n Yes (%)	23 3 (13%)	21 6 (29%)	21 9 (43%)	0.09
Days on Oxygen	n	25	25	24	0.22
	Median (range)	32 (1–108)	32 (1–120)	53 (0–120)	
PDA [d]	n Yes (%)	25 13 (52%)	25 7 (28%)	24 11 (46%)	0.20
If PDA [d], Indomethacin Rx	n Yes (%)	13 7 (54%)	7 4 (57%)	11 9 (82%)	0.36
If PDA [d], surgical ligation	n Yes (%)	13 2 (15%)	7 3 (43%)	11 4 (36%)	0.35
IVH[e] grade 3,4	n Yes (%)	25 3 (12%)	24 5 (21%)	23 1 (4%)	0.22
Seizures treated > 72 hours	n Yes (%)	25 0	25 1 (4%)	24 2 (8%)	0.31
Sepsis, late onset	n Yes (%)	25 7 (28%)	25 7 (28%)	24 13 (54%)	0.09
NEC [f] IIA or worse	n Yes (%)	25 5 (20%)	25 2 (8%)	24 2 (8%)	0.49
If NEC [f], had surgery	n Yes (%)	5 2 (40%)	2 1 (50%)	2 1 (50%)	>0.99
Spontaneous GI perforation, no NEC [f]	n Yes (%)	25 0	25 2 (8%)	24 0	0.32
Hearing test, failed both ears	n Yes (%)	21 2 (10%)	17 0	19 1 (5%)	0.77
ROP [g]	Final Status Known Received Surgery for ROP (%)	8 4 (50%)	8 2 (25%)	9 1 (11%)	0.21

[a] number of subjects in the denominator shown because of variation

[b] BPD bronchopulmonary dysplasia,

[c] PMA postmenstrual age (=gestational age + chronologic age),

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[d] PDA patent ductus arteriosus,

[e] IVH intraventricular hemorrhage,

[f] NEC necrotizing enterocolitis,

[g] ROP retinopathy of prematurity.

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Table 4

Population Pharmacokinetic Parameter Estimates for a Typical Infant (Fixed Effects)

Parameter	Units	Estimate	Standard Error
Model Parameters			
V (volume)	L/kg	0.5115	0.0345
Cl (clearance)	L/kg/h	0.0679	0.0064
R (endogenous infusion rate)	mg/kg/h	2.666	0.2762
Standard deviation of the residual error	mg/L	18.71	1.048
Derived Values			
k (elimination rate; *Cl*/*V*)	1/h	0.133	0.0154
$t_{1/2}$ (half-life; 0.693/*k*)	h	5.22	0.605
E (endogenous concentration; *R*/*Cl*)	mg/L	39.25	1.655

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Table 5

Population Pharmacokinetic Random Effects Variances and Correlations

	Volume (u_V)	**Clearance (u_{Cl})**	**Endogenous Infusion Rate (u_R)**
Volume (u_V)	0.08506	-	-
Clearance (u_{Cl})	0.0[a]	0.22636	-
Endogenous Infusion Rate (u_R)	0.0 [a]	0.87606	0.14492

Random effect variances on the diagonal and correlations between the random effects are displayed on the off diagonal.

[a] Correlation set to 0.0 (zero) based upon review of plots of u_{Vi} vs. u_{Cli}, and u_{Vi} vs. u_{Ri}.

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Table 6

Covariate Means and Standard Deviations

Covariate	Mean	Standard Deviation
Birth weight (grams)	986.8	245.0
Size at birth $(w_i/w)^{\sim}$	0.989	0.246
Postmenstrual age (weeks)	27.3	1.72
Postnatal age (weeks)	0.44	1.15
Creatinine clearance (ml/min)	0.69	0.34

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Table 7

PK Model Parameter Estimates for a Typical Infant (Fixed Effects)

Model Label	Parameter Name [a]	Parameter Estimate (std. error)	Covariate Estimate (standard error)			−2 log likelihood (p-value [e])
			log(Size)[b]	GA Strata[c]	Creatinine Clearance[d]	
Base Model	Volume (V)	0.5115 (0.0345)				2708.2 (NA)
	Clearance (Cl)	0.0679 (0.0064)				
	Endogenous Production (R)	2.666 (0.2762)				
log(Size)	Volume (V)	0.5177 (0.0359)				2702.3 (0.02)
	Clearance (Cl)	0.0661 (0.0059)	0.3544 (0.1460)			
	Endogenous Production (R)	2.542 (0.2566)				
GA Strata	Volume (V)	0.5937 (0.0546)		−0.2776 (0.1286)		2704.1 (0.04)
	Clearance (Cl)	0.0660 (0.0059)				
	Endogenous Production (R)	2.572 (0.2566)				
Creatinine Clearance	Volume (V)	0.5096 (0.0341)				2701.1 (0.03)
	Clearance (Cl)	0.0671 (0.065)				
	Endogenous Production (R)	3.273 (0.430)			−0.3252 (0.1260)	
All Covariates	Volume (V)	0.5924 (0.0539)		−0.2777 (0.1257)		2697.4 (0.03)
	Clearance (Cl)	0.0549 (0.0120)	0.2158 (0.1963)			
	Endogenous Production (R)	3.040 (0.457)			−0.1898 (0.1583)	

[a] Units – V in L/kg, Cl in L/kg/h, R in mg/kg/h; where L: liters of serum, kg: kg's of body weight, mg; mg's of inositol, h: hours

[b] log(Size): Natural logarithm of $w_i/\tilde{w}$ where w_i is the birth weight, in grams, for the i-th infant, $\tilde{w}$ is the median birth weight of the infants in the study (997.5 gm);

[c] Gestational Age Strata: Reference cell 23–26 weeks; Comparison cell 27–29 weeks;

[d] Creatinine Clearance: clearance in ml/min

[e] p-value for testing the model including the covariates versus the base model without the covariates.