Safety and pharmacokinetics of multiple dose *myo*-inositol in preterm infants

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BACKGROUND: Preterm infants with respiratory distress syndrome (RDS) given inositol had reduced bronchopulmonary dysplasia (BPD), death and severe retinopathy of prematurity (ROP). We assessed the safety and pharmacokinetics of daily inositol to select a dose providing serum levels previously associated with benefit, and to learn if accumulation occurred when administered throughout the normal period of retinal vascularization.

METHODS: Infants ≤ 29 wk GA (n = 122, 14 centers) were randomized and treated with placebo or inositol at 10, 40, or 80 mg/kg/d. Intravenous administration converted to enteral when feedings were established, and continued to the first of 10 wk, 34 wk postmenstrual age (PMA) or discharge. Serum collection employed a sparse sampling population pharmacokinetics design. Inositol urine losses and feeding intakes were measured. Safety was prospectively monitored.

RESULTS: At 80 mg/kg/d mean serum levels reached 140 mg/l, similar to Hallman's findings. Levels declined after 2 wk, converging in all groups by 6 wk. Analyses showed a mean volume of distribution 0.657 l/kg, clearance 0.058 l/kg/h, and half-life 7.90 h. Adverse events and comorbidities were fewer in the inositol groups, but not significantly so.

CONCLUSION: Multiple dose inositol at 80 mg/kg/d was not associated with increased adverse events, achieves previously effective serum levels, and is appropriate for investigation in a phase III trial.

Retinopathy of prematurity (ROP) is a common problem worldwide among preterm infants, often leading to vision impairment or blindness (1). Hallman reported two trials of postnatal inositol treatment of preterms with RDS to support phosphatidylinositol in surfactant synthesis, and both trials demonstrated improved RDS and a lower incidence of death or BPD, and ROP (2,3). Inositol is an important component of surfactant, and essential intracellularly as phosphoinositides. Howlett concluded in a Cochrane metaanalysis of inositol in preterm infants, "that a multi-center, randomized controlled trial of appropriate size is warranted to confirm these findings" (4). We reported the pharmacokinetics (PK) of a single dose of i.v. inositol in preterm infants at doses of 60 or 120 mg/kg, and found the half-life was 5.22 h, with large urine losses, particularly in the first 12 h after dosing (5). Our three goals were to identify a daily dose to achieve serum levels similar to those reported by Hallman, [170 mg/l (994 μmol/l) at 8–9 d for infants given 160 mg/kg/d, and an approximate mean value over the first week of life of 135 mg/l (750μmol/l) when receiving 80 mg/kg/d (2,6)]; to learn if divided doses would reduce urine losses; and to assure safety with up to 10 wk of treatment. We examined the safety and pharmacokinetics (PK) of inositol given at three dose levels compared with placebo for up to 10 wk, both i.v. and enteral (#NCT01030575). This time frame was chosen to support inositol levels throughout the postpreterm delivery period when most retinal vessel growth normally occurs within the high inositol, *in-utero* environment (7,8).

RESULTS

From January to October 2010, 125 infants ≤29 wk gestation were randomized and 122 received treatment during the time

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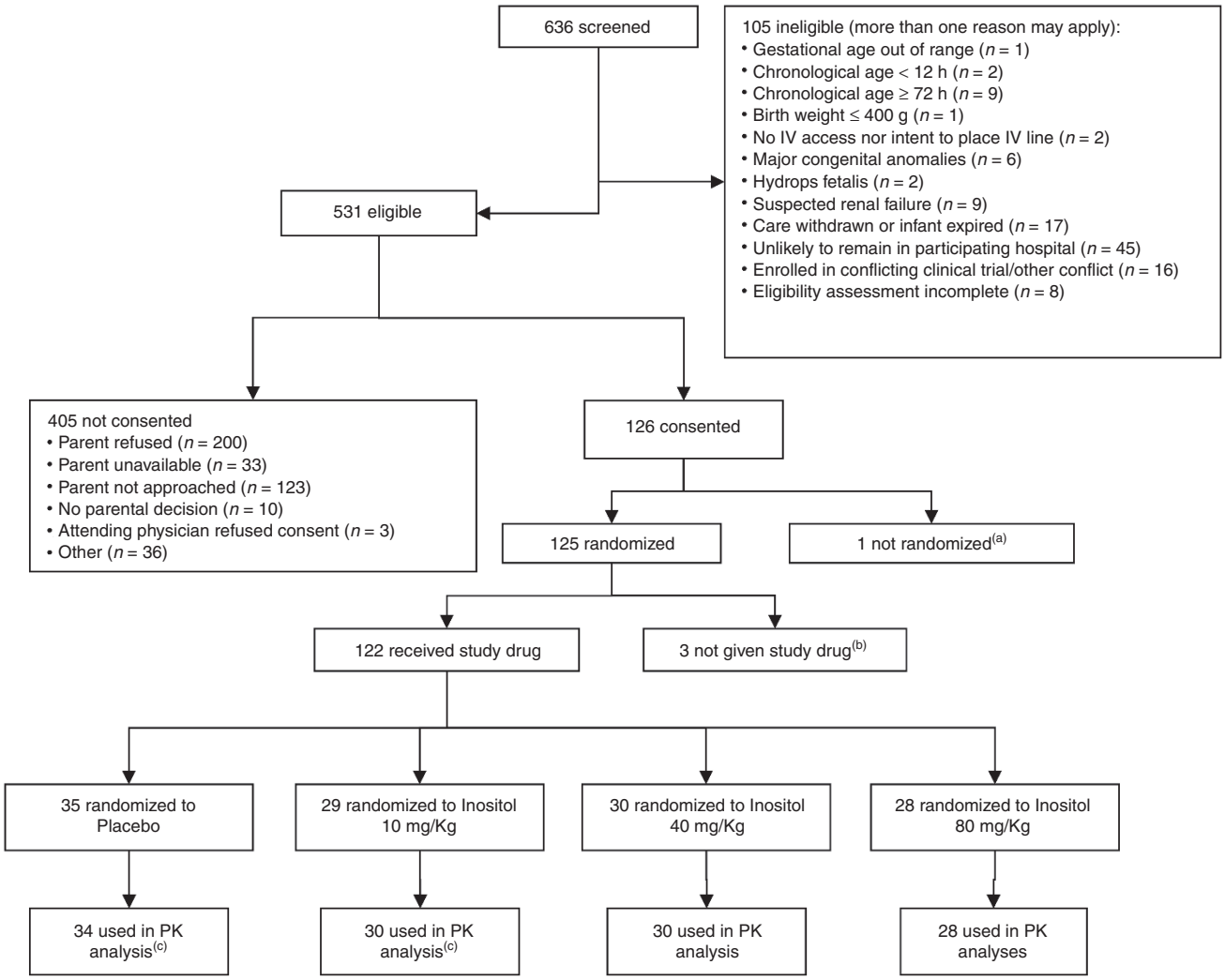

Figure 1. Consort flow sheet of study subjects. (**a**) Reason unknown. (**b**) One infant identified as ineligible postrandomization; two infants died. (**c**) One placebo subject incorrectly received 10 mg/kg/d of inositol for 7 d before it was discovered and stopped, and is considered to be part of the 10 m/kg/d group only for the pharmacokinetics analysis. However, other data from this subject are analyzed, as randomized, with the placebo infants.

for the designed 96 infants to complete the protocol (Figure 1). The mean gestation was 26 wk, and baseline characteristics were similar across groups (Table 1). On average, study drug was received for 42–51 d, and 43–57% of doses were i.v. The number of missed or held doses was similar across groups (average of 1–3 per subject).

Safety Outcomes

At least one adverse event of moderate or greater severity occurred in 104 infants, and the average number/subject (5.5–5.7) was similar across treatment groups (**Supplementary Table S1** online). No specific type of event occurred more frequently in the inositol groups compared with the placebo group. Per protocol, inositol doses were held for severe oliguria (renal losses of inositol are large enough that oliguria could have led to high serum inositol levels). This occurred in five infants on placebo and two, four, and three infants in the 10, 40, and 80 mg/kg/d groups, respectively. Of these, eight infants resumed study drug after recovery, four discontinued study drug permanently (one, placebo; 1, 10 mg/kg/d; and two, 80 mg/kg/d), and two expired (both in the 40 mg/kg/d group). Comparing adverse event rates in placebo vs. all inositol, or across the dose groups, P values were all > 0.05 with most > 0.10.

Serious adverse events (severe, life threatening or fatal) were common in this population (Table 2), but no specific types occurred more frequently in the inositol groups compared with placebo. Clinical diagnoses are listed in Table 3, and all 15 deaths occurred in the 23–26 wk GA stratum. Infection was reported as a primary cause of death in the 40 mg/kg group for 17% of subjects; compared with 0–3% as the cause of death for other dose groups ($P < 0.01$ for comparing across all dose groups). No diagnoses had P-values < 0.05 when comparing across the treatment groups (Table 3). However, intraventricular hemorrhage (any intraventricular hemorrhage, as well as grades III/IV) occurred more frequently in the 0 and 40 groups than in the 10 and 80 groups ($P = 0.05$ for intraventricular hemorrhage III/IV). Failing the discharge

Table 1. Baseline characteristics and demographics

Characteristic		Inositol dose group (mg/kg/d)				*P*- value[a]
		0 (*N* = 35)	10 (*N* = 29)	40 (*N* = 30)	80 (*N* = 28)	
GA (wk)	Mean (SD)	26.5 (1.6)	26.6 (1.8)	26.7 (1.8)	26.7 (1.9)	0.95
Lower GA stratum	23-26 wk *n* (%)	19 (54%)	15 (52%)	16 (53%)	14 (50%)	0.99
Birth weight (g)	Mean (SD)	884 (224)	897 (272)	939 (245)	921 (286)	0.83
Head circumference (cm)	Mean (SD)	23.8 (2.0)	24.1 (2.0)	25.1 (2.5)	24.6 (1.9)	0.10
Sex	Female	17 (49%)	15 (52%)	14 (47%)	16 (57%)	0.87
Race	North American Native	0	3 (10%)	0	0	0.12
	Asian	0	1 (3%)	1 (3%)	0	—
	Black	18 (51%)	15 (52%)	12 (40%)	13 (46%)	—
	More than one race	0	1 (3%)	0	0	—
	White	17 (49%)	9 (31%)	17 (57%)	15 (54%)	—
Ethnicity	Hispanic or Latino	9 (26%)	3 (10%)	7 (23%)	8 (29%)	0.35
Antenatal steroids		32 (91%)	24 (83%)	24 (80%)	26 (93%)	0.37
Chorioamnionitis[b]		5 (14%)	4 (14%)	4 (13%)	3 (11%)	0.97
Cesarean delivery		21 (60%)	16 (55%)	19 (63%)	14 (50%)	0.75
Early onset sepsis		0	0	1 (3%)	0	0.38
Apgar-1 min	Median (range)	3 (1–9)	5 (1–8)	3 (1–8)	5 (1–8)	0.97
Apgar-5 min	Median (range)	7 (1–9)	8 (1–9)	7 (1–8)	7 (3–9)	0.97

[a]*P*-values calculated by testing the null hypothesis of equality across all four treatment groups using ANOVA techniques for continuous measures; Mantel–Haenszel mean score tests using modified ridit scores for ordinal measures, and chi-square or Fisher's exact test for nominal measures. [b]Chorioamnionitis, as documented in the maternal record.

Table 2. Severe adverse events of interest through 7 d post-last dose of inositol

Category	Preferred term	Inositol dose group (mg/kg/d)				*P*-values	
		0 (*N* = 35)	10 (*N* = 29)	40 (*N* = 30)	80 (*N* = 28)	INS vs. placebo[a]	Across doses[b]
Any	Any	28(80%)	17(59%)	20(67%)	17(61%)	0.09	0.24
Cardiopulmonary	Poor perfusion or hypotension	8 (23%)	5 (17%)	3 (10%)	6 (21%)	0.44	0.54
Gastrointestinal	Delayed gastric emptying	7 (20%)	3 (10%)	7 (23%)	5 (18%)	0.80	0.62
	Other	4 (11%)	3 (10%)	0 (0%)	2 (7%)	0.28	0.26
Hematologic	Anemia	13 (37%)	5 (17%)	6 (20%)	7 (25%)	0.07	0.28
	Thrombocytosis	5 (14%)	4 (14%)	1 (3%)	2 (7%)	0.32	0.39
Metabolic	Hyperglycemia	4 (11%)	1 (3%)	1 (3%)	1 (4%)	0.10	0.52
Renal	Other[c]	2 (6%)	0 (0%)	0 (0%)	0 (0%)	0.08	0.25
Respiratory	Apnea	6 (17%)	4 (14%)	6 (20%)	9 (32%)	0.63	0.38

[a]*P*-values calculated by testing the null hypothesis of equality between placebo and all active doses combined. [b]*P*-values calculated by testing the null hypothesis of equality separately across all the four treatment groups using Fisher's exact tests.[c]Other renal events were anuria and possible or definite hydronephrosis.

hearing screening in either ear occurred more often in the 40 or 80 mg/kg/d inositol groups (20 and 14%, respectively) than in the 0 and 10 mg/kg/d groups (4%), ($P = 0.25$).

Severe ROP meeting criteria for surgery, or receiving intervention for ROP occurred among the surviving infants examined in 19% of the placebo group and in 12, 8, and 9% of the 10, 40, and 80 mg/kg/d inositol groups, respectively ($P = 0.72$). The planned phase III study primary outcome of meeting criteria for ROP surgery, or death before ROP outcome, among those infants eligible for that trial (< $28^{0/7}$ wk GA) was highest in the placebo group and lower in the inositol groups (44, 23, 36, and 19%, for the placebo, 10, 40, and 80 mg/kg/d groups, respectively, $P = 0.29$). Results including the six adjudicated ROP outcomes were similar: 41, 22, 36, and 16%, respectively.

Growth (weight, head circumference, and length) was examined using z-scores to adjust for PMA, and these parameters did not significantly differ across groups. An average of 15 concomitant medications were received while on study drug (range 3–34) and the number of courses of medication was similar across the four dose groups (average 19 courses, range 3–67). The 10 most frequent concomitant drug exposures by study group did not reveal a particular pattern (**Supplementary Table S2** online).

Table 3. Clinical diagnoses

Comorbidities	Dose group (mg/kg/d) 0 (*N* = 35)	10 (*N* = 29)	40 (*N*=30)	80 (*N* = 28)	*P*-value[a]
Death (through NRN status[b])	6 (17%)	2 (7%)	6 (20%)	1 (4%)	0.16
[c]BPD (O_2 at 36 wk[d] PMA or prior death from BPD)	11 (38%)	7 (26%)	7 (30%)	8 (30%)	0.81
Respiratory distress syndrome	34 (97%)	29 (100%)	30 (100%)	27 (96%)	0.72
[e]PDA	13 (37%)	14 (48%)	14 (47%)	10 (36%)	0.68
PDA (received surgery)	3 (9%)	3 (10%)	1 (3%)	3 (11%)	0.75
[f]IVH (any)	13 (38%)	4 (14%)	10 (34%)	5 (18%)	0.08
IVH (grade III/IV)	10 (29%)	2 (7%)	6 (21%)	2 (7%)	0.05
Seizures (Rx for > 72 h)	2 (6%)	0	1 (3%)	0	0.62
Cystic areas in parenchyma (within 28 d of birth)	2 (15%)	0	1 (10%)	1 (14%)	1.00
Sepsis (early onset)	0	0	1 (3%)	0	0.71
Sepsis (late onset)	4 (11%)	6 (21%)	7 (23%)	5 (18%)	0.63
[g]NEC (suspected or proven)	5 (14%)	1 (3%)	4 (13%)	1 (4%)	0.28
NEC (requiring surgery)	3 (9%)	0	2 (7%)	0	0.17
Spontaneous [h]GI perforation	2 (6%)	0	2 (7%)	1 (4%)	0.72
Severe [i]ROP	5/27(19%)	3/26(12%)	2/24 (8%)	2/23(9%)	0.72
Hearing screen failed (either ear)	1 (4%)	1 (4%)	4 (20%)	3 (14%)	0.25

[a]*P*-values calculated by testing the null hypothesis of equality across all four treatment groups using Fisher's exact tests. [b]NRN status = Neonatal Research Network definition: age of earliest of death, discharge, transfer, or 120 days after birth. [c]BPD, bronchopulmonary dysplasia; [d]PMA, postmenstrual age (=chronologic age + gestational age at birth); [e]PDA, patent ductus arteriosus; [f]IVH, intraventricular hemorrhage; [g]NEC, necrotizing enterocolitis; [h]GI, gastrointestinal; [i]ROP, retinopathy of prematurity, severe ROP = meeting criteria for treatment or treated with laser, cryotherapy or anti-vascular injection, followed up to 55 wks PMA if needed for outcome and denominators include all infants with available data for that outcome. With the six adjudicated ROP outcomes included, rates were similar (17%, 11%, 8%, and 8% respectively). All comorbidities are captured through NRN status unless otherwise specified.

Serum Inositol

Mean serum levels were elevated in a dose-related manner in the early weeks (**Figure 2a**); however by 6 wk, the differences were minimal. To explore an effect of changing from i.v. to enteral drug, serum levels were plotted separately for samples obtained while infants were on i.v. drug (**Figure 2b**), and while on enteral drug (**Figure 2c**). Serum levels continued to decrease with age with both drug routes, and once i.v. doses ended, mean levels over 75 mg/l (416 μmol/l) were rare.

Inositol intake from feedings (calculated from the measured inositol levels and daily volumes of each type of feeding) rose from an average of 4 mg/kg/d in week 1, to 40–50 mg/kg/d by wk 6, and did not differ significantly across groups (data available from authors). There was no evidence of inositol accumulation in the serum with continued treatment at 80 mg/kg/d, despite the additional intake of inositol from full enteral feeds.

Pharmacokinetics

The PK analysis initially considered a three-part model with components for the (i) combined effects of endogenous synthesis of inositol and inositol from feeding, (ii) initial i.v. administration, and (iii) the shift to enteral administration. The enteral administration portion of the analysis used a multiple-administration, first-order absorption with linear elimination model including terms for bioavailability and a lag time prior to the start of absorption. However, it was not possible to estimate the third part of the model related to enteral administration. As noted in relation to **Figure 2**, it appears that as an infant matures, and is more likely to receive enteral inositol, the serum concentration is less affected by exogenous administration. The remainder of the PK analysis focused on the first two parts of the inositol serum concentration model.

The final PK analysis used data from two sources. The data from the current study were limited to observations obtained prior to the first enteral administration of inositol (**Figure 2b**). The data thus correspond to observations related to the multiple i.v. administrations and are referred to as the multiple-administration dataset. The single-dose data previously analyzed were included in parts of the analysis and will be referred to as the single-administration dataset (5). Both studies were conducted by the same investigators in the same research network using protocols consistent across both studies except for the repeated dosing.

For both datasets, a constant variance for the residual error fit best. Also, 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 (see definitions in Methods section). A strong linear relationship was observed between the random-effects estimates for clearance (*Cl*) and endogenous production rate (*R*) with no apparent relationship between the other two combinations of random effects. The random effects were then modeled only with the correlation between *Cl* and *R*.

Table 4 presents the population PK (Pop-PK) estimates for the i.v. administration model including the apparent endogenous infusion rate. Derived values for the elimination rate, the half-life, and the apparent concentration associated with endogenous synthesis are also shown. This is done for three available sets of data, the single i.v. administration column as

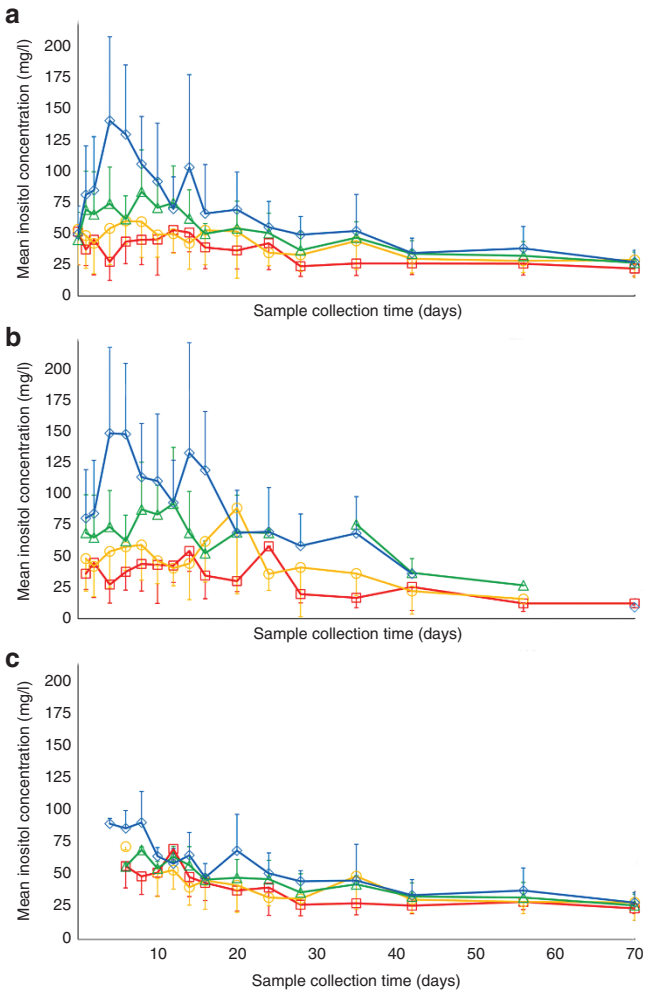

Figure 2. Serum inositol levels. Mean ± SD, by dose, clustered by days on study. Symbols: 80 mg/kg/d = blue diamond, 40 mg/kg/d = green triangle, 10 mg/kg/d = orange circle, and placebo = red square. Panel (**a**) includes all samples; panel (**b**) values only while subjects were receiving i.v. doses; and panel (**c**) values obtained only when subjects were receiving enteral dosing. Timed samples were collected within scheduled windows (see Methods), plus additional scavenged laboratory residual samples as available and if exact timing after the previous dose was known for the sample. For presentation, collection days are clustered in mean values to simplify display: Study day 0 = baseline before first infusion; day 2 = first sample after first infusion; day 3 = third study day; day 4 = 4–5 d; day 6 = 6–7 d; day 8 = 8–9 d; day 10 = 10–11 d; day 12 = 12–13 d; day 14 = 14–15 d; day 16 = 16–18 d; day 20 = 19–22 d; day 24 = 23–26 d; day 28 = 27–31 d; day 35 d = 32–38 d; day 42 = 39–48 d; day 56 = 49–63 d; day 70 = 64–77 d. Plotting only peak values, or only trough levels did not assist in displaying the data.

published earlier, the multiple i.v. administration column from fitting the model to the new multiple-administration dataset, and the last column from fitting the model to the combined datasets (5). The three sets of results are very consistent, with the combined results intermediate to the single and multiple-administration results. The half-life estimates range from the 5.22 h for the single-administration data to 7.90 h for the multiple-administration data, with the combined data estimate being 6.31 h. The random effect variance and correlation estimates are shown in **Table 5** for the combination single- and multiple-administration data. Plots of the actual vs. individual predicted values were examined (not shown) and the values were well aligned, indicating the model provided a good fit to the data. In addition, plots comparing the individual predicted residuals vs. the actual values did not indicate any major model deficiencies.

Twenty four hour urine inositol losses were determined at the end of weeks 1, 2, 4, and 5–6 (**Figure 3**). At week 1, mean urine losses were close to, or greater than the dose received, despite dividing doses q 12 h to lower peak serum levels. Week 1 excretion rates in the 80 mg/kg group were similar to the observed 24 h excretion following a single dose of 120 mg/kg in week 1, as previously published (5). At all ages, the mean inositol excretion was highest in the 80 mg/kg/d group, falling from 107 mg/kg/24 h at week 1, to 68 mg/kg/24 h at weeks 5–6. There was no evidence of a diuretic effect of inositol as urine volumes measured between 3 and 5 ml/kg/ h and did not vary significantly by group (data not shown).

DISCUSSION

Inositol at 80 mg/kg/d in low gestation infants was effective in reaching serum levels similar to those achieved during previous trials, and importantly, these levels did not continue to rise with dosing throughout the period of rapid retinal vascular development up to 10 wk (~34 wk PMA) (9). There was no significant evidence of harm at any dose during the study, but prospective monitoring of hearing and infection should be conducted in future trials. Although not statistically significant with these small sample sizes, several comorbidities appeared less frequent in the treated groups, which is reassuring in consideration of a phase III trial.

The PK were best described by a one-compartment multiple dose i.v. infusion model with linear elimination combined with apparent endogenous production for the periods of time when infants were receiving i.v. administration. Whereas an expanded model was considered that included both i.v. infusion and enteral administration, we were unable to get a single model to converge for both the i.v. and enteral portions of the PK study. Brown *et al.* reported the turnover rate of inositol using dual labeled stable isotopes to be ~150–250 mg/kg day in 33–34 wk GA infants, a value consistent with the data that inositol is endogenously synthesized, as well as catabolized in the kidney (10,11). The inositol oxidase enzyme, unique to the renal cortex, catabolizes inositol to glucuronic acid and becomes active in the weeks after birth in the term newborn, likely contributing our finding of decreasing inositol in the urine despite ongoing treatment plus increasing inositol from feeds (12).

Inositol is necessary for phosphatidylinositol surfactant synthesis, which predominates over phosphatidylglycerol in preterm infants. Infants unable to receive human milk or formula feedings experience falling serum inositol levels. Whereas supplementation may improve respiratory distress syndrome (RDS) and reduce both bronchopulmonary dysplasia (BPD) and ROP, the effect on ROP was unexpected, and it is possible inositol was sufficiently effective in reducing pulmonary

Table 4. Population pharmacokinetic parameter estimates for a typical infant (fixed effects)

Parameter	Units	Estimates (SE)		
		Single i.v. administration dataset	Multiple i.v. administration dataset	Combined dataset
Model parameters				
V– volume	l/kg	0.5115 (0.0345)	0.6572 (0.0707)	0.5610 (0.0341)
Cl– clearance	(l/kg)/h	0.0679 (0.0064)	0.0577 (0.0061)	0.0616 (0.0048)
R– endogenous infusion rate	(mg/kg)/h	2.666 (0.2762)	2.369 (0.3151)	2.449 (0.2336)
SD of residual error	mg/l	18.71 (1.048)	24.77 (0.971)	22.96 (0.739)
Derived Values				
k – elimination rate (= *Cl*/*V*)	1/h	0.1327 (0.0154)	0.0878 (0.0137)	0.1098 (0.0109)
$t_{1/2}$ – half-life (=.693/k)	h	5.22 (0.605)	7.90 (1.229)	6.31 (0.631)
E – concentration due to endogenous infusion (= *R*/*Cl*)	mg/l	39.26 (1.655)	41.06 (1.777)	40.71 (1.255)

Table 5. Population pharmacokinetic random effect variances and correlations for the combined dataset

	Volume (u_v)	Clearance (u_{Cl})	Endogenous infusion rate (u_R)
Volume (u_{vi})	0.1181	—	—
Clearance (u_{Cl})	0.0[a]	0.3508	—
Endogenous infusion rate (u_R)	0.0[a]	0.9349	0.4899

Random effect variances are displayed on the diagonal and correlations between the random effects on the off diagonal.[a]Correlation set to 0.0 (zero) based on review of plots of u_{vi} vs. u_{Cl} and u_{vi} vs. u_R.

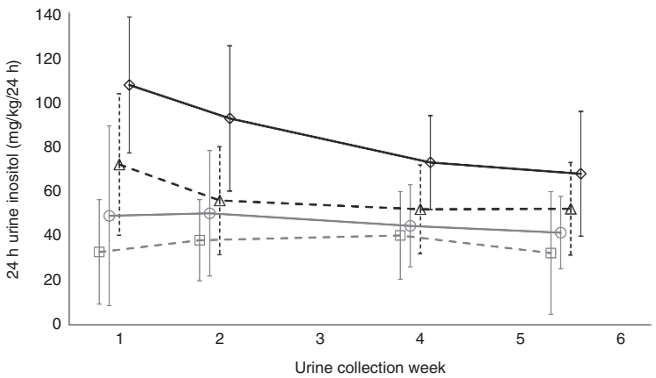

Figure 3. Inositol urine losses. Inositol in the urine from each diaper (Concentration × Volume) was summed over 24 h to determine the urine losses, at weekly intervals. Data are mean ± SD, and slightly offset for better visualization. Symbols: Square = placebo, circle = 10 mg/kg/d, triangle = 40 mg/kg/d, and diamond = 80 mg/kg/d.

morbidity that it lowered the risk for ROP as a secondary effect by reducing oxygen exposure (2,3). However, phosphoinositides, inositol polyphosphates, and inositol phosphoglycerols serve as signaling molecules in a number of intracellular events, these are essential factors in the chain of mediators leading to vascular growth, including vascular endothelial growth factor and insulin-like growth factor-I (7,8,13). Therefore, inositol or its derivatives may be rate limiting in the roles of vascular endothelial growth factor and insulin-like growth factor-I, or other factors critical to retinal vascular development which normally occurs *in utero* from 14 to 36 wk gestation. If inositol is permissive for sustaining the health of retinal endothelial cells during this time period, it may explain why infants with low inositol levels in the early neonatal period are at higher risk for ROP (14). Metabolism of inositol is complex and not fully understood, changing *in utero*, with birth, and affected by enteral intake and complex endogenous controls that are likely developmentally regulated (7). Nonetheless, in early trials, inositol treatment appears safe and beneficial in preterm infants.

Conclusions

These data add to the evidence that inositol at doses up to 80 mg/kg/d for 7–10 wk is well tolerated and does not increase adverse events. As recommended in the Cochrane Review, the data on inositol supplementation warrants a large phase III clinical trial to test its safety and efficacy to improve survival without severe ROP (4).

METHODS

Study Design

A randomized double-masked phase II clinical trial was conducted in 14 centers of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN). The study design was approved by the Food and Drug Administration and registered on clinicaltrials.gov (#NCT01030575). The protocol was approved by the NRN Data Safety Monitoring Committee and the Institutional Review Board from each institution, and each subject's parent or guardian provided written informed consent.

Population

Subjects were $23^{0/7}$ to $29^{6/7}$ wk GA who weighed at least 400 g, and could receive study drug by 72 h after birth. Exclusions included death before 12 h, major congenital anomalies, severe oliguria, or a moribund state. Eligible infants were randomized and stratified within center by gestational strata ($23^{0/7}$–$26^{6/7}$ vs. $27^{0/7}$–$29^{6/7}$wk), to placebo or one of three daily doses of inositol.

Study Drug

Myo-inositol 5% Injection was provided by Abbott Nutrition, Columbus, OH as an isotonic, preservative and pyrogen-free, sterile, 5% solution of *myo*-Inositol i.v. at neutral pH. Doses were 10, 40, or 80 mg/kg/d, divided q12 h given over 20 min. Placebo was 5% glucose (United States Pharmacopeia) for i.v. infusion and dispensed at the equivalent various volumes to maintain masking. Randomization was by central computer, communicated to the research pharmacists who prepared doses of inositol or placebo in the pharmacy, dispensed as unit doses. Thus research and clinical personnel, and families

remained masked to treatment assignment. When enteral feedings reached at least 100 ml/kg/d (or the infant no longer was receiving i.v. fluids), the study drug was given enterally as the same formulation, at the same per kg dose. Study drug continued until 10 wk chronologic age, 34 wk PMA, death, or discharge.

Individual infants contributed 8–10 scheduled serum samples over the 10 wk (**Supplementary Table S3** online). Sampling times were assigned so that ~ 8 samples were collected in each of the specified time frames for each dose group. Collection times were divided within each time window to collect peak, trough, and mid-dose samples. The time of each sample and the starting time of the preceding drug infusion were recorded. Additional measurements from scavenged serum or plasma with known times of collection (left over from laboratory studies ordered for usual care) were also processed, if available, with consent.

A 24 h urine collection was obtained at the end of wk 1, 2, 4, and 6 (or 5 if being discharged). The change of diaper weight (dry to wet) was used as the best estimate of void volume where 1 g = 1 ml. Urine was expressed from each wet diaper separately during the collection period, and an aliquot frozen (15). The time of each diaper-on and diaper-off were recorded.

Enteral Feeds

Volume and sources (human milk, fortified human milk, or specific formulas) were recorded daily until intake was 100 ml/kg/d for at least 7 d, and later were recorded weekly. Inositol concentrations were sampled from human milk actually given to the infant in the same week, unless insufficient volume was available. Formula inositol concentrations were measured twice from the stock of each type of formula the infant received, and reassayed if the formula was then fortified. Inositol from milk feedings was calculated by summing the daily inositol intake from the volume ingested of each milk, multiplied by the measured inositol concentration for that milk.

Assay

myo-Inositol was measured with a validated assay on 25 or 50 µl samples of serum, plasma, urine, and milks utilizing a multiple-column, multiple mobile phase liquid chromatographic system with electrochemical detection (15).

Clinical Outcomes

Adverse events were prospectively monitored from 24 h prior to the start of study drug until 7 d following the final dose (unless discharged sooner) and judged according to a neonatal toxicity table developed for the study. Concomitant medications were recorded from 24 h prior to the start of study drug until 7 d following its final dose, unless the infant was discharged sooner. Weight, length, and head circumferences were measured prospectively.

Retinopathy outcomes were determined from the clinical eye examinations (16). The primary examining ophthalmologist at each center was trained and certified on the International Classification of ROP as used in 2006 (17). An unfavorable outcome was defined as either Type I ROP or worse, in either eye, or surgical intervention for severe ROP in either eye (18). A favorable ROP outcome was assigned if the retinal vessels progressed to full vascularization in both eyes without meeting criteria for severe ROP, or if on two consecutive examinations the retinal vessels were in zone III (16). Examinations were continued, if necessary, up to 55 wk PMA (3 mo after term due date) (19,20). Infants who did not meet either criterion had all available examinations reviewed by an adjudication committee.

Comorbidities were recorded prospectively using the established definitions of the NRN Generic Database Protocol (21). At 18–22 mo corrected age, infants received a set of standardized examinations of neurologic function and development according to the NRN Follow Up Protocol (to be reported separately) (22).

Statistical Analyses

Sample size. Enrollment was continued until at least 48 infants in each of the two GA strata had completed a minimum of 28 d on study drug and contributed at least five serum samples. Infants were enrolled and randomized during the time when waiting to document that 96 infants had completed the protocol. All infants beginning treatment were permitted to complete the protocol, and all available data are included in the final analyses. Pop-PK studies typically target six to eight samples at each of the time points to describe the change in serum concentrations (23,24). No formal power calculations were conducted because no formal hypotheses were to be tested in this phase II study, as the analyses are exploratory and descriptive in nature.

Data obtained within the study assessment windows were all used. Whereas the primary analyses were conducted without imputation for missing ROP data due to loss to follow-up or indeterminate final status, additional exploratory analyses were conducted to assess the impact of the missing data on the estimates of ROP. Adjudication was conducted by a committee of three experienced ophthalmologists not involved with the study and masked to study group assignment. They were provided data on the infant's GA, birth weight, and each available eye examination including age (chronologic and PMA) at each exam. The final ROP status was judged separately in each eye as "probably favorable", "probably unfavorable" or "cannot be determined", and the majority classification was assigned as the adjudicated outcome.

Baseline characteristics and comorbidities for all randomized and treated infants were compared by testing the null hypothesis of equality across all four dose groups using ANOVA for continuous measures, Mantel–Haenszel mean score tests (using modified ridit scores) for ordinal measures, and chi-square or Fisher's exact test for nominal measures.

Methods for Pharmacokinetic Analyses

In recognition of the relatively sparse sampling design for the collection of serum samples, Pop-PK models were fit to the data using the nonlinear mixed effects approach in Monolix 3.2 (LIXOF, Antony, France). This approach accounts for the variability between infants in the model parameters, the correlation between measurements in the same infant at different occasions, as well the residual unexplained variability in serum concentrations (25). Two issues dictated the structure of the Pop-PK models that were considered for modeling the data from the study: (i) endogenous synthesis of inositol by the infants and inositol contained in feedings of human milk or infant formula; and (ii) initial i.v. administration of inositol.

Endogenous synthesis and feeding intake of inositol were modeled in the same way as the single dose PK analyses (5). The steady state endogenous concentration for the ith infant is modeled as $E_i = R_i/Cl_i$ where R_i is the apparent rate of inositol infusion due to the combination of endogenous synthesis and feeding, and Cl_i is the clearance. It is not possible to separate endogenous synthesis and feeding intake of inositol since enteral feeding intake was measured as the total amount fed over a day and not the amount fed at each occasion. In addition, as discussed in the Results section, the estimation of R_i will be from data from the period of time prior to the establishment of full enteral feeds.

As was used previously, the Pop-PK model for the initial i.v. administration period is a 1-compartment i.v. infusion model with linear elimination (5). For this study the model was expanded to account for multiple administrations of inositol rather than a single administration used with the previous single-dose study. The model for serum concentrations resulting from endogenous synthesis and feeding of inositol and from i.v. administration is then

$$C_i(t) = \frac{R_i}{Cl_i} + \begin{cases} \sum_{k=1}^{n-1} \frac{D_i}{T \times Cl_i}\left[1 - e^{-\frac{Cl_i \times T}{V_i}}\right]\left[e^{-\frac{Cl_i \times (t - t_{Dik} - T)}{V_i}}\right] \\ \quad + \frac{D_i}{T \times Cl_i}\left[1 - e^{-\frac{Cl_i \times (t - t_{Din})}{V_i}}\right] \qquad \text{if } t - t_{Din} \le T \\ \sum_{k=1}^{n} \frac{D_i}{T \times Cl_i}\left[1 - e^{-\frac{Cl_i \times T}{V_i}}\right]\left[e^{-\frac{Cl_i \times (t - t_{Dik} - T)}{V_i}}\right] \text{if } t - t_{Din} > T \end{cases} + \epsilon_{it}$$

where $C_i(t)$ is the serum concentration for the ith infant at time t, in hours, with time measured since the start of the first i.v. administration. The time of the kth i.v. administration to the ith infant is t_{Dik} and T is the duration of the infusion period common to all infants and

administrations (1/3 h). The summations are over the i.v. administrations for the *i*th infant up to time *t* with *n* such that $t_{Din} \le t < t_{Din+1}$. D_i is the dosage administered to the *i*th infant at each administration in mg of inositol per kg of body weight. V_i is the apparent volume of distribution. Finally, ε_{it} is the residual error at time *t*.

The between infant variability in the Pop-PK model parameters, R_i, Cl_i, V_i, are modeled using random effect variables (u_R, u_{Cl}, and u_V) that approximate the individual trajectory over time of each infant's serum inositol 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 $Cl_i = Cl \times e^{u_{Cli}}$ where *Cl* is the fixed-effect common to all infants and u_{Cli} is the random effect unique to the *i*th infant. Similarly 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, also modal, values of the parameters and are often 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 (zero) and variance that is estimated from the data. The quality of fit of the Pop-PK model was judged by visual examination of plots of observed vs. individual predicted concentrations and of residuals vs. individual predicted concentrations.

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. The US Food and Drug Administration approved the protocol which was conducted under an Investigational New Drug Application, and the trial was registered with ClinicalTrials.gov (NCT01030575). Data collected at participating centers and inositol assay results were transmitted to the data coordinating center (DCC), RTI International, which stored, managed and analyzed it. Dr Abhik Das (DCC Principal Investigator) and Dr Tracy Nolen (DCC Statistician) had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. Rick Williams, Michael Goedecke, and Timothy Fennell had full access to all data in the study and were responsible for conducting the pharmacokinetic analyses.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

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