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ORIGINAL RESEARCH ARTICLE

Population Pharmacokinetics of Intravenous Paracetamol and Its Metabolites in Extreme Preterm Neonates in the Context of Patent Ductus Arteriosus Treatment

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Abstract

Aims Our aim was to describe the pharmacokinetics of paracetamol and its metabolites in extreme preterm neonates in the context of patent ductus arteriosus treatment. Factors associated with inter-individual variability and metabolic pathways were studied. The association between drug exposure and clinical outcomes were investigated.

Methods Preterm neonates of 23–26 weeks' gestational age received paracetamol within 12 h after birth. Plasma concentrations of paracetamol and its metabolites were measured throughout 5 days of treatment. Clinical success was defined as ductus closure on two consecutive days or at day 7. Aspartate aminotransferase and alanine aminotransferase levels were used as surrogates for liver damage.

Results Data from 30 preterm neonates were available for pharmacokinetic analysis. Paracetamol pharmacokinetics were described using a two-compartment model with significant positive effects of weight on clearance and of birth length on peripheral compartment volume. Paracetamol was mainly metabolised into sulphate (89%) then glucuronide (6%), and the oxidative metabolic pathway was reduced (4%). The glucuronidation pathway increased with gestational age, whereas the sulfation pathway decreased. No difference was observed in drug exposure between successful and unsuccessful patients. No increase in aspartate aminotransferase and alanine aminotransferase levels were observed during treatment, and no association was found with either paracetamol or oxidative metabolite exposures.

Conclusion The relative proportions of the metabolic pathways were characterised with gestational age. In the range of observed drug exposures, no association was found with clinical response or liver biomarkers. These findings may suggest that paracetamol concentrations were within the range that already guarantee a maximum effect on ductus closure.

1 Introduction

Patent ductus arteriosus (PDA) is a common complication in preterm neonates. In the foetus, the ductus arteriosus is a blood vessel connecting the aorta to the pulmonary artery. The right atrium of the heart received oxygenated blood from the placenta, which then partially passes through the ductus arteriosus to detour away from the lungs and be distributed throughout the body. The ductus arteriosus is open in all newborns at birth and normally constricts itself within the first few days of birth. However, in many extremely preterm infants, the ductus arteriosus remains open. The persistence of the opening is associated with mortality and morbidity and can lead to the development of pulmonary arterial hypertension or heart failure, resulting in the death of the child [1].

Ductal constriction can be obtained with inhibition of prostaglandin synthesis, through inhibition of the cyclooxygenase (COX) enzymes. The two most frequently used COX inhibitors for the closure of a hemodynamically significant PDA are indomethacin and ibuprofen. However, these treatments may cause numerous adverse effects such as bleeding, immune disorders, renal impairment, gastrointestinal

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Key Summary Points

In extreme preterm neonates of 23–26 weeks' gestational age, paracetamol was mainly metabolised into sulfate (89%) then glucuronide (6%), and the oxidative metabolic pathway was reduced (4%).

The glucuronidation pathway increased with gestational age, whereas the sulfation pathway decreased and other pathways remained stable.

In the range of observed drug exposures, no association was found with clinical response or liver biomarkers.

Paracetamol concentrations may fall within the range that already guarantee a maximum effect on ductus closure.

haemorrhage, necrotising enterocolitis, intestinal perforation, and, in some cases, pulmonary hypertension [2, 3]. Treatments intended to close the PDA have failed to demonstrate significant long-term clinical benefits [4], and prophylactic use of indomethacin or ibuprofen is currently not recommended.

Paracetamol could be a safe pharmacological alternative to COX inhibitors for early prophylactic treatment of PDA. According to a recent meta-analysis, paracetamol appears to be as effective as indomethacin and ibuprofen for closure of PDA and with less renal and gastrointestinal harmful effects [5]. However, the number of extremely preterm infants included in this meta-analysis was very low. Therefore, additional research on the efficacy and safety of paracetamol for PDA treatment in this population is required.

Paracetamol is metabolised mainly in the liver by glucuronidation and sulfation and then eliminated in the urine. A minor metabolic pathway of oxidation catalysed by cytochrome P450 (CYP) leads to the formation of a toxic metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI), which can rapidly be detoxified by conjugation with glutathione stored in the liver. The rapid and subsequent metabolism of glutathione produces cysteine and mercapturate conjugates. A small amount (less than 5%) is excreted unchanged in the urine [6].

To date, no pharmacokinetics of paracetamol for the high-risk preterm population born at 23–26 weeks' gestation are specifically available.

The objectives of this study were to describe the population pharmacokinetics (pop-PK) of intravenous paracetamol and its metabolites in extreme preterm neonates and identify factors associated with inter-individual variability. Association between drug exposure and clinical response as well as liver biomarkers were also investigated.

2 Methods

2.1 Study Participants

The Prophylactic Treatment of the Ductus Arteriosus in Preterm Infants by Acetaminophen (TREOCAPA) study was approved by the ethics committee of Centre Hospitalier La Chartreuse (SI 20.03.09.40128) for France and by the regional medical research ethics committee of North Ostrobothnia (68/06.00.00/20 19) for Finland. This phase II trial was registered in the clinicaltrials.gov database under the reference NCT04459117.

Preterm neonates with a gestational age 23–26 weeks were included in the first 12 h after birth and received treatment with paracetamol. This study was conducted in two countries (France and Finland) and eight neonatal intensive care units. Patients with birth defects or congenital anomalies, twin-to-twin transfusion syndrome, suspicion of pulmonary hypoplasia, or clinical instability that can lead to rapid death were not included in the study.

This pharmacokinetic study was part of a dose-escalation trial with the Bayesian continual reassessment method that evaluated the efficacy, safety, and tolerability profile of prophylactic paracetamol to close the ductus [7]. In this study, patients were assigned to intravenous paracetamol with predefined doses. The first level was 20 mg/kg followed by 7.5 mg/kg four times daily (QID) for 5 days (total 20 doses). The second, third, and fourth predefined level doses stand for 25 mg/kg followed by 10 mg/kg QID, 30 mg/kg followed by 12 mg/kg QID, and 35 mg/kg followed by 15 mg/kg QID, respectively. Escalation stopped at the second level because analysis showed that no efficacy gains would be observed with further increases.

2.2 Data Collection and Sampling

Baseline data such as gestational age, birth weight, birth length, head circumference, and sex were collected at inclusion, as were data on pregnancy, antenatal care, delivery, and care in the delivery room. Bodyweight and body length were also collected every day during treatment. Systemic blood pressure was measured before the loading dose and 30, 60, 90, and 120 minutes after each dose.

For the study, a blood sample was collected after the end of the loading dose and after dose 10 (ideally between 15 min and 4 h after the start of infusion) to measure aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and paracetamol and its metabolites plasma

levels. A blood sample was also collected after the last dose (ideally 6 h after the start of the last infusion) to analyse paracetamol and metabolite plasma levels. Opportunistic blood samples collected for routine care during treatment were also analysed to determine paracetamol and metabolite plasma levels.

Echocardiography was performed each day until day 7 after birth. Echocardiograph were reviewed by two experts in paediatric cardiology, in Nantes University Hospital, to confirm the primary outcome (ductus arteriosus closure or non-closure). If the two experts disagreed, a third expert gave his opinion to decide.

2.3 Analytical Method

Paracetamol, acetamidophenyl β-D-glucuronide, paracetamol-sulfate, 3-cysteinylacetaminophen, and paracetamol-mercapturate plasma concentrations were quantified using a validated method on an ACQUITY ultra-performance liquid chromatography system coupled with a triple quadrupole mass spectrometer (XEVO-TQD) with electrospray ionisation source (UPLC-MS/MS) (Waters, Milford, USA).

Paracetamol-d3, paracetamol-d3 sulfate, 3-cysteinylacetaminophen-d5, paracetamol-mercapturate-d5, and 4-acetamidophenyl-β-D-glucuronide-d3 were used as internal standards (IS).

Sample preparation consisted of a protein precipitation and phospholipid removal on column. We mixed 40 µL of sample with 100 µL methanol containing the IS and agitated for 5 min. The purification was realised through Phree phospholipid removal tubes (Phenomenex, Le Pecq, France) by centrifugation at 2500 rpm for 5 min at room temperature. The eluates were evaporated to dryness, and reconstituted with 100 µL of mobile phase, composed of H_2O (95%) with ammonium formate (10 mM) and methanol (5%). Finally, we injected 20 µL of each sample into the UPLC-MS/MS system. The separation was conducted on an Atlantis T3 C18 analytical column (100 × 2.1 mm, 3 µm; Waters®). The mobile phase consisted of water with ammonium formate (10 mM) and methanol. The analytes were eluted using a linear gradient for 3.5 min. Detection was operated in positive ion mode using multiple reaction monitoring. The following transitions were monitored: m/z 151.968 → 109.96 for paracetamol, m/z 154.946 → 111.368 for paracetamol-d3, m/z 312.946 → 208.014 for paracetamol-mercapturate, m/z 318.096 → 212.192 for paracetamol-mercapturate-d5, m/z 231.968 → 152.05 for paracetamol-sulfate, m/z 234,818 → 110.967 for paracetamol-d3-sulfate, m/z 271.01 → 140.001 for 3-cysteinylacetaminophen, m/z 276.01 → 142.8 for 3-cysteinylacetaminophen-d5, m/z 328.096 → 152.03 for paracetamol-glucuronide, and m/z 330.968 → 155.056 for acetamidophenyl-β-D-glucuronide-d3.

The technique was validated according to US Food and Drug Administration and European Medicines Agency guidelines in the range of 20–20 000 ng/mL for paracetamol and paracetamol-mercapturate, of 40–40 000 ng/mL for 3-cysteinylacetaminophen, and 100–100,000 ng/mL for paracetamol-sulfate and acetamidophenyl β-D-glucuronide.

2.4 Pharmacokinetic Modelling

The paracetamol parent-metabolite modelling was achieved after converting doses and concentrations to micromoles per litre (µmol/L) using paracetamol and metabolite molecular weights [8]. The metabolite distribution volumes are not identifiable in parent-metabolite models, so they were fixed to the parent volume. Oxidative metabolites were considered as the molar sum of paracetamol-cysteine and paracetamol-mercapturate.

A population approach was used to analyse the concentrations of paracetamol and its metabolites. We tested both one- and two-compartment models and analysed data using the nonlinear mixed-effect modelling software program Monolix version 2021R2 (http://lixoft.com). Parameters were estimated by computing the maximum likelihood estimator using the stochastic approximation expectation maximisation algorithm combined with a Markov chain Monte Carlo procedure. Confidence intervals for population pharmacokinetic parameters were estimated using the bootstrap method ($n = 100$). For each bootstrap sample, we estimated the population pharmacokinetic parameters, and then obtained parameter statistics from the whole bootstrap set. We obtained individual pharmacokinetic parameter estimates from a Bayesian estimation.

The between-subject variability was ascribed to an exponential model:

$$\theta_i = \theta_{\text{pop}} \times e^{\eta_i},$$

where θ_i is the parameter for the ith individual, θ_{pop} is the population estimate of the parameter θ, and η_i is a random variable for the ith individual normally distributed with a mean of zero and an estimated variance of ω^2.

To describe the residual variability, additive, proportional, and combined error models were investigated. Data below the lower limit of quantification were handled as left-censored data by Monolix.

The parent-metabolite model was developed by setting the parameters of the previously constructed parent model. Total clearance of paracetamol was divided into three metabolic pathways (glucuronidation, sulfation, and oxidation) and one unchanged elimination pathway.

To estimate the different metabolic pathways, the metabolisation fractions were calculated using the parameters t_1, t_2, and t_3 estimated by the parent-metabolite model as follows:

$$f_{\text{gluc}} = \frac{t_1}{t_1 + t_2 + t_3 + 1}$$

$$f_{\text{sulf}} = \frac{t_2}{t_1 + t_2 + t_3 + 1}$$

$$f_{\text{ox}} = \frac{t_3}{t_1 + t_2 + t_3 + 1}$$

$$f_{\text{unchanged}} = \frac{1}{t_1 + t_2 + t_3 + 1}.$$

The clearance associated with each elimination pathway is obtained by multiplying total clearance of paracetamol (CL) by the corresponding metabolisation fraction. Thus, the sum of the clearances associated with each elimination pathway should not exceed the total clearance of paracetamol:

$$\text{CL} = \text{CL} \times f_{\text{gluc}} + \text{CL} \times f_{\text{sulf}} + \text{CL} \times f_{\text{ox}} + \text{CL} \times f_{\text{unchanged}}.$$

Covariates such as gestational age, weight, height, sex, 5-min Apgar score, total and conjugated bilirubin level, and blood pressure measurements (systolic and diastolic) were tested in the model. A power function centred on the median was used for continuous covariates:

$$\theta = \theta_{\text{pop}} \times \left(\frac{\text{cov}}{\text{Me(cov)}} \right)^{\beta^{\theta}_{\text{cov}}},$$

where θ is the parameter to estimate, θ_{pop} is the typical value of the parameter for a patient with the median covariate value, cov is the value of the covariate, Me(cov) is the median value for the covariate, and $\beta^{\theta}_{\text{cov}}$ is the estimated influential factor for the continuous covariate. Effects of the covariates were tested using a stepwise procedure.

The corrected Bayesian information criterion was used to test different hypotheses regarding the structural model, residual variability model, and structure of the variance–covariance matrix for the between-subject variance parameters. A covariate was retained in the model if its effect was biologically plausible, if it reduced the variability of the pharmacokinetic parameters, and if the objective function value dropped by at least 3.84 (i.e. χ^2 with one degree of freedom, $p < 0.05$).

2.5 Evaluation and Validation

The quality of each model was evaluated by visual inspection of the observed versus predicted (population and individual) concentrations and the normalised prediction distribution error (NPDE) metrics versus population predicted concentrations and time scatter plots.

The prediction corrected visual predictive checks (pcVPC) plots were also used to evaluate the predictive performance of the models. Plasma concentrations of paracetamol and its metabolites were simulated by the model, and the 95% confidence intervals of the 10th, 50th, and 90th percentiles of the simulated concentrations were overlaid to the 10th, 50th, and 90th percentiles of the observed concentrations.

2.6 Statistical Analysis and Clinical Outcomes

Statistical analysis was performed using R software version 4.2. Categorical data were summarised in numbers and percentages, and continuous data were described as median (interquartile range [IQR]) and range.

Cumulative areas under the concentration curves (AUCs) of paracetamol and its metabolites were computed for each patient from the start of treatment to time of AST/ALT measurement. Furthermore, AUC over the last 24 h before AST/ALT measurement was also computed. Associations between drug exposure and both AST and ALT levels were investigated to assess liver toxicity.

Clinical success was defined as closure of ductus on 2 consecutive days or closure at day 7. Associations between exposure and clinical success were investigated by analysing the mean concentration over the duration of treatment, AUC during the first 24 h of treatment, predicted peak concentration at loading dose, and predicted minimal concentration at steady state.

Spearman's rank correlation was used to evaluate the association between drug exposure and liver biomarkers, and Wilcoxon's test was used to evaluate associations with clinical success.

3 Results

3.1 Population Characteristics

Between November 2020 and September 2021, a total of 31 preterm neonates were enrolled in the study. One patient was excluded from analysis because no paracetamol plasma level was available. A total of 30 preterm neonates (17 boys and 13 girls) with a median weight of 800 g (range 470–920) and a median height of 32.8 cm (range 28–36.5) were included in this pharmacokinetic study. In total, 21 patients received the first dose level and nine patients received the second dose level. The characteristics of patients are summarised in Table 1. A total of 121 paracetamol and 484 metabolite plasma concentrations were available for pharmacokinetic modelling. The median number of samples per patient was 4 (range 2–6).

3.2 Population Pharmacokinetic Modelling

The pharmacokinetics of paracetamol were best described by a two-compartment model with first-order elimination. Between-subject variability was estimated for both clearance and volume of distribution of the peripheral compartment. The most significant covariate effect was that of bodyweight on clearance, which dropped the objective function by 15.9 units, and then the effect of birth length on peripheral volume of distribution, with an additional decrease of 8.8 units. Clearance increased significantly with bodyweight, and peripheral volume of distribution increased significantly with birth length. Bodyweight effect decreased the unexplained between-subject variability on clearance from 0.494 to 0.409, and birth length decreased the unexplained between-subject variability on peripheral volume of distribution from 0.515 to 0.325. Residual variabilities were best described by a proportional error model. The results are summarised in Table 2.

The final parent-metabolite model was a five-compartment model (two compartments from the parent model and three compartments for the glucuronide, sulfate, and oxidative metabolites). Paracetamol was mainly metabolised into sulfate, then glucuronide, and oxidative metabolites, and a small amount was eliminated unchanged. The corresponding structural model is shown in supplemental Figure 1. Apparent metabolisation fractions were estimated at 89.45% for sulfate, 6.07% for glucuronide, 3.67% for oxidation, and

Table 1 Characteristics of the study population at inclusion ($n = 30$)

Characteristics	Total
Inclusion data	
Gestational age (weeks)	
23	2 (6.7)
24	9 (30.0)
25	7 (23.3)
26	12 (40.0)
Birth weight (g)	800 (672.5–857.5; 470–920)
Birth length (cm)	32.75 (31.5–34.0; 28.0–36.5)
Head circumference (cm)	22.8 (21.6–23.5; 20.5–25.0)
Sex (M)	17 (56.7)
Dose level 1[a]	21 (70)
Dose level 2[a]	9 (30)
Apgar at 5 min	8 (7–9; 4–10)
Minimal mean arterial pressure (mmHg)	28 (24.25–37.5; 19–55)
Minimal diastolic arterial pressure (mmHg)	19.5 (15.25–29; 11–57)
Systemic blood pressure (mmHg)	Systolic: 50.5 (44–57; 39–78) Diastolic: 29.5 (25.25–33; 13–47)
Laboratory results	
Total bilirubin (µmol/L)	46 (38–50.5; 18–91)
Conjugated bilirubin (µmol/L)	6 (5–10; 4–12)
Aspartate aminotransferase (IU/L)	51.55 (35–67; 22–330)
Alanine aminotransferase (IU/L)	5.95 (5–9; 0–22)
Antenatal care and birth	
Type of pregnancy	Singleton: 22 (73.3) Twins: 8 (26.7)
Age of mother (years)	32 (30–34; 19–41)
Diagnosis of IUGR noted in medical records	3 (10.0)
Preeclampsia	3 (10.0)
Use of steroids before delivery	30 (100)

Categorical data are expressed as *n* (%), and continuous data are expressed as median (interquartile range; range)

IUGR intrauterine growth retardation, *M* male

[a]Dose level 1 = 20 mg/kg loading dose then 7.5 mg/kg/6 h for 5 days; dose level 2 = 25 mg/kg loading dose then 10 mg/kg/6 h for 5 days

Table 2 Population pharmacokinetic parameter estimates of the final paracetamol parent and parent-metabolite models from 30 preterm neonates

Parameter	Estimate	RSE (%)	5th–95th percentile derived from 100 bootstraps
Parent model estimates			
CL (L/h)	0.0785	8.49	(0.0656–0.0920)
β_{Cl_W}	1.81	0.302	(1.21–3.40)
V_1 (L)	0.124	1.11	(0.0181–0.575)
Q (L/h)	3.84	0.386	(0.616–32.4)
V_2 (L)	0.672	10.8	(0.246–0.976)
β_{V2_BL}	6.46	24.8	(1.90–10.6)
Between-subject variability			
ω_{Cl}	0.409	15.8	(0.318–0.477)
ω_{V2}	0.325	39.1	(0.0985–0.847)
Error model			
Proportional error	0.349	8.18	(0.290–0.381)
Parent-metabolite model estimates			
V_1 (L)	0.124	–	–
CL (L/h)	0.0785	–	–
β_{Cl_W}	1.81	–	–
t_1	7.53	19.4	(3.30–9.44)
β_{t1_GA}	6.88	1.33	(2.64–12.2)
t_2	111	20.1	(36.8–128)
t_3	4.56	2.13	(2.42–5.77)
$CL_{E\ gluc}$ (L/h)	0.0324	26.3	(0.0168–0.0823)
$CL_{E\ sulf}$ (L/h)	0.0173	7.66	(0.0132–0.0217)
$\beta_{CLE\ sulf\_GA}$	6.26	1.78	(2.22–10.8)
$CL_{E\ ox}$ (L/h)	0.00445	26.5	(0.00232–0.0162)
Q (L/h)	3.84	–	–
V_2 (L)	0.672	–	–
β_{V2_BL}	6.46	–	–
Between-subject variability			
ω_{CL}	0.409	–	–
ω_{t1}	0.495	31.3	(0.183–0.789)
ω_{t2}	0.924	15.7	(0.552–1.27)
$\omega_{CLE\ gluc}$	0.835	24.2	(0.149–1.15)
$\omega_{CLE\ sulf}$	0.278	30.2	(0.0915–0.472)
$\omega_{CLE\ ox}$	0.926	22.6	(0.194–1.37)
ω_{V2}	0.325	–	–
Error model			
Proportional error for paracetamol	0.349	–	–
Proportional error for glucuronide	0.443	12.5	(0.329–0.623)
Proportional error for sulfate	0.503	8.92	(0.420–0.593)
Proportional error for oxidative metabolites	0.47	10.9	(0.361–0.641)

RSE relative standard error, *CL* clearance, Q intercompartmental clearance, V_1 volume of distribution of the central compartment, V_2 volume of distribution of the peripheral compartment, ω inter-subject variability expressed as standard deviation, β effect of covariate on parameter.

Weight (W), birth length (BL), and gestational age (GA) were centred on median values: 800 g for weight, 33 cm for birth length, and 25 weeks for gestational age.

$CL_{gluc} = CL \times t_1/(t_1 + t_2 + t_3 + 1)$, $CL_{sulf} = CL \times t_2/(t_1 + t_2 + t_3 + 1)$, $CL_{ox} = CL \times t_3/(t_1 + t_2 + t_3 + 1)$, $CL_{unchanged} = CL/(t_1 + t_2 + t_3 + 1)$

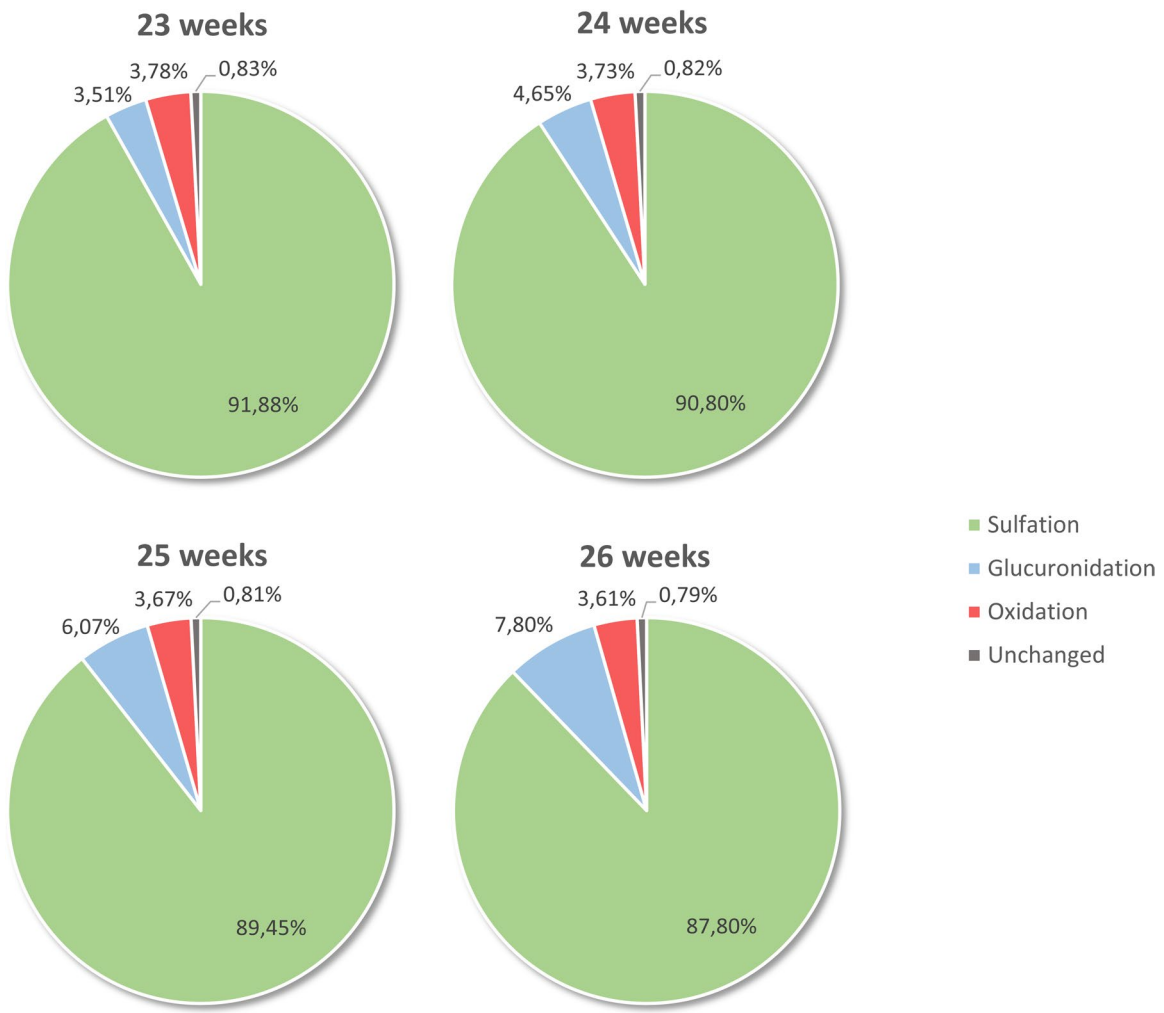

Fig. 1 Paracetamol elimination pathways according to gestational age (23–26 weeks)

0.81% for unchanged elimination for a typical preterm neonate of 25 weeks. The proportion of these different metabolic pathways varied according to gestational age. The apparent metabolisation fractions varied between 87.8 and 91.9% for sulfation, between 3.51 and 7.8% for glucuronidation, between 3.61 and 3.78% for oxidation, and between 0.79 and 0.83% for unchanged elimination. The metabolisation fraction according to gestational age is presented in Fig. 1. Table 2 summarises the specific elimination clearances for each compound. After inclusion of gestational age in the model, no other covariate was significant on elimination pathways.

Between-subject variability was estimated on the clearances associated with the different elimination, unchanged, and metabolic, pathways. A significant effect of gestational age was observed on the clearance of each metabolic and unchanged paracetamol elimination pathways. With increasing age, the glucuronide pathway increased and the sulfate pathway decreased. An additional effect of gestational age was observed on elimination clearance of sulfate metabolite.

Final population pharmacokinetic estimates for both parent and parent-metabolite models are summarised in Table 2. The parameters are well estimated given their low relative standard errors. Individual pharmacokinetic exposure parameters were provided in Table S1 in the supplementary material.

3.3 Model Evaluation

Diagnostic plots for paracetamol and metabolites are shown in Figures S2 and S3 in the supplementary material. The population and individual predictions match the observed concentrations, and the NPDE metrics are evenly distributed around zero.

The pcVPC plots show that the average prediction matches the observed concentration time-courses, and the variability is within the expected range for paracetamol and its metabolites. The 10th, 50th, and 90th percentiles of the observed data are well within the 90% confidence intervals of the 10th, 50th, and 90th simulated percentiles (Figure S4 in the supplementary material).

3.4 Clinical Outcomes

Median AST and ALT levels were 28.5 IU/L (IQR 18.8–36.4) and 7.0 IU/L (IQR 5.0–9.0), respectively. No significant correlation was found with paracetamol's cumulative AUC or with the paracetamol AUC during the last 24 h (Fig. 2). The oxidative metabolite's exposition was also compared with the liver biomarker levels, and no significant correlation was found (Figure S5 in the supplementary material).

Clinical response regarding ductus closure was available for 28 patients. One patient was excluded from analysis because they met the exclusion criteria, and one patient stopped the treatment earlier for suspicion of safety concerns. Treatment was considered successful for 14 patients (50%). The median number of days before ductus closure was 1.5 (IQR 1–3; range 1–7) days. No significant association was found between clinical success and the paracetamol exposure parameters considered (mean concentration over the duration of treatment and AUC over the first 24 h of treatment, predicted peak concentration after the loading dose, minimum predicted concentration at steady state). Boxplots are shown in Fig. 3.

4 Discussion

This is the first study to model the population pharmacokinetics of paracetamol and its metabolites in a population of extremely preterm neonates with a gestational age of 23–26 weeks. A two-compartment model best described the pharmacokinetics of paracetamol. The total clearance

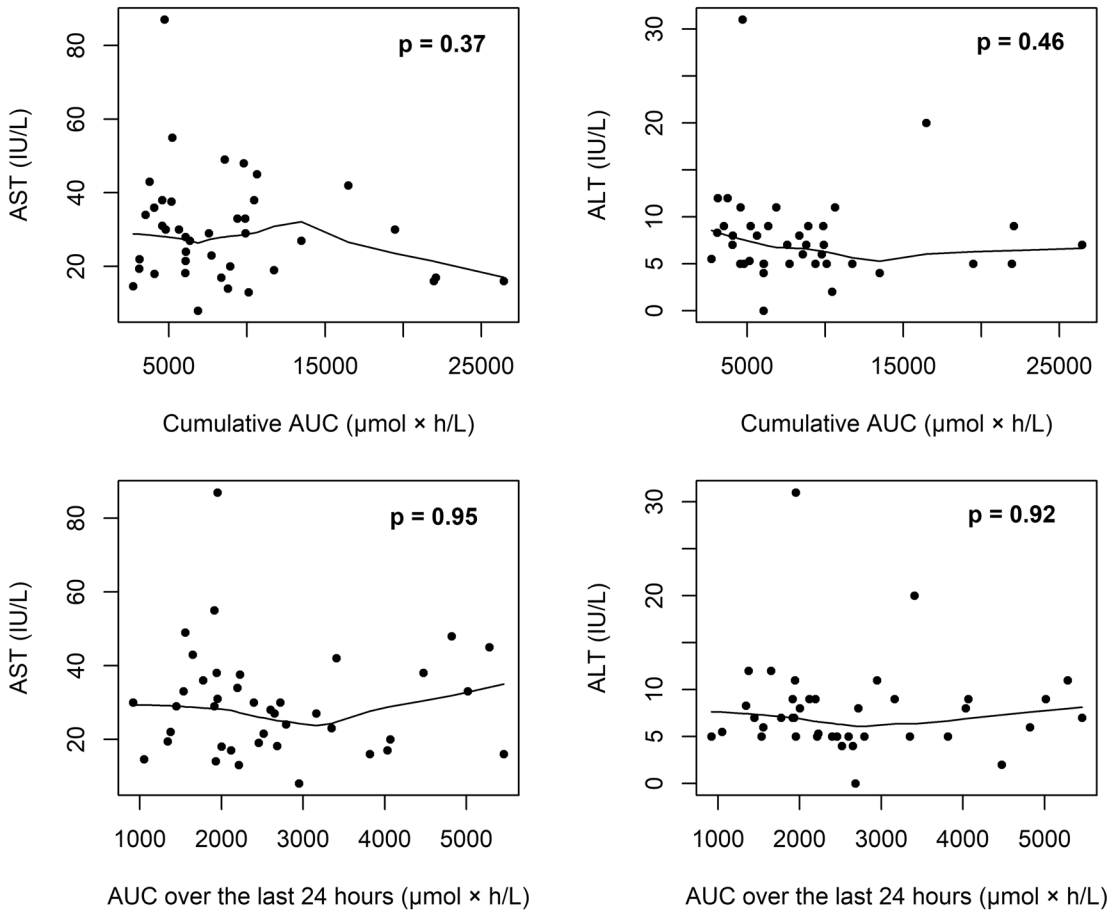

Fig. 2 Comparison of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels with area under the curve (AUC) of paracetamol plasma concentration from the start of treatment to time of blood sampling that was used to analyse AST and ALT levels (cumulative AUC) and over the last 24 h before this time. Spearman's rank correlation was used for comparison

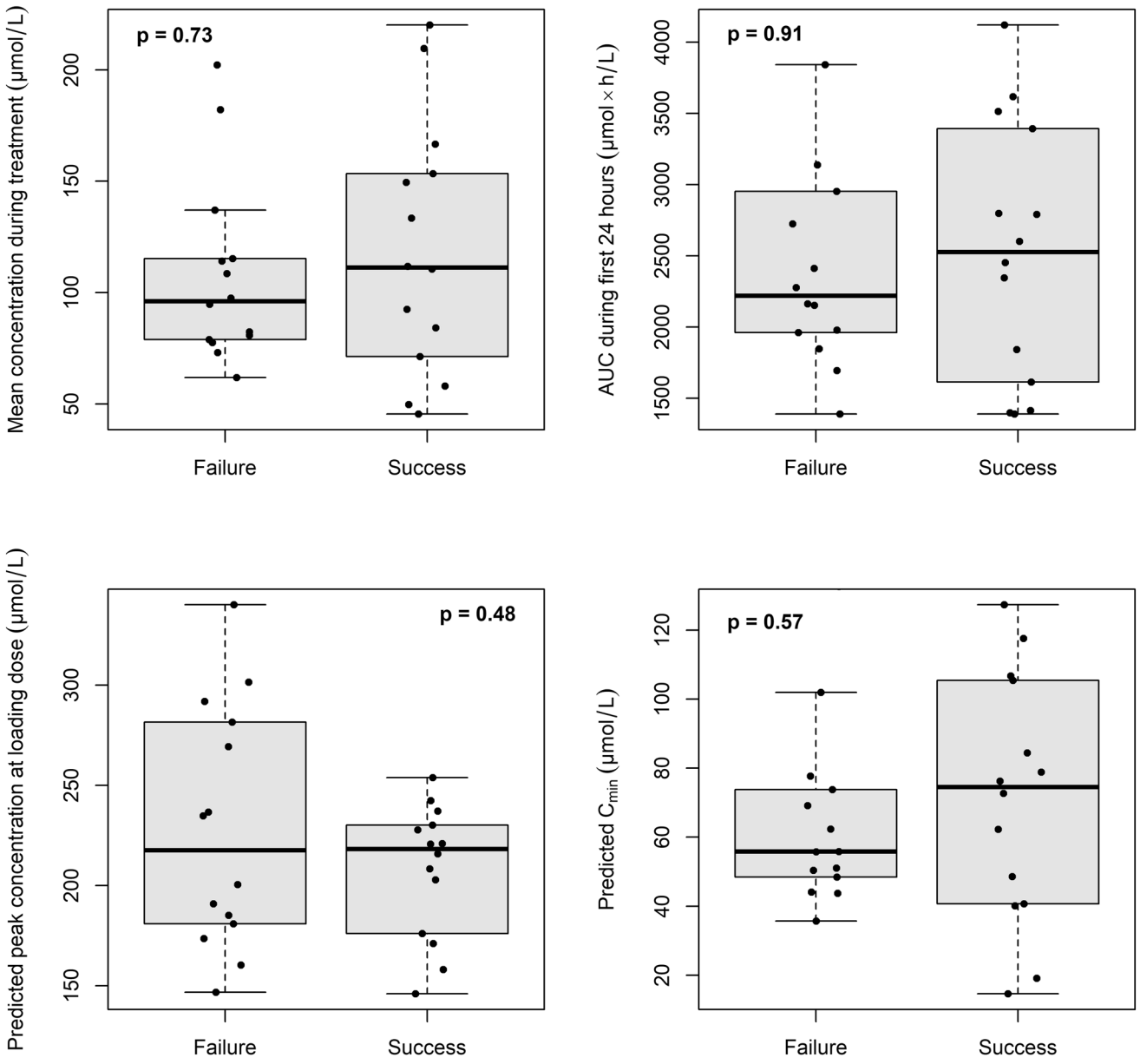

Fig. 3 Comparison of mean concentration during treatment, area under the curve (AUC) during the first 24 h of treatment, predicted peak concentration at loading dose, and minimal predicted concentration at steady state (C_{min}) with clinical success (closure of ductus on 2 consecutive days or closure at day 7). Wilcoxon's test was used for comparison

of paracetamol was estimated at 0.08 L/h with a significant effect of weight on this parameter. These results are comparable to those published in the literature. Currently, there are four studies with pharmacological data including extremely preterm infants [9–12]. By extrapolating the estimated clearances of these studies to a typical patient in our population (25 weeks of gestation and weight of 800 g), these ranged from 0.09 to 0.17 L/h on average. However, fewer than five patients under 26 weeks of gestation were included in these studies. The effect of weight on total clearance was also found in these publications [9, 11, 12]. After inclusion of bodyweight and height in the model, the unexplained inter-individual variability in this extremely preterm population remained high. Other covariates of interest could have been valuable, such as both fluid volume inputs and outputs, which could affect the volume of distribution of paracetamol.

Our results indicate that the main metabolic pathway of paracetamol in this population is sulfation, which is consistent with results from previous studies [9–11, 13]. A significant effect of gestational age was found on the different metabolic pathways, and a significant effect of weight was also found in another study [9]. Cook et al. present the weight-dependent evolution of the metabolisation fractions. The glucuronide metabolisation fraction increases with weight, whereas the sulfate metabolisation fraction decreases. As weight and gestational age are strongly correlated, these results are consistent with those found in our study. These results suggest a lower maturation process of the glucuronidation pathway.

A reversal between those two metabolisation fractions would occur by extrapolating to older ages according to our modelling. Miller et al [14] reported metabolisation rates of paracetamol in glucuronide and sulfate metabolites after oral administration of 10 mg/kg. Sulfate excretion of paracetamol was highest in neonates (0–2 days) and children (3–9 years), whereas glucuronide excretion was highest for children aged 12 years and adults. Other studies have demonstrated that the glucuronide excretion pathway is major in adults [6, 15–17].

The hepatotoxicity of paracetamol is a major concern in this population. It is attributed to the production of the highly reactive electrophile metabolite NAPQI by the hepatic CYP enzymes, specifically CYP2E1. NAPQI is rapidly detoxified in the liver by conjugation with glutathione and eliminated in the urine as cysteine and mercapturate metabolite conjugates. Excess of NAPQI depletes glutathione and causes liver damage [6].

In our study, the oxidative metabolic pathway of paracetamol seems remarkably reduced in extremely preterm neonates. It accounts for less than 4% of paracetamol metabolism. This is consistent with results from previous studies [9, 13]. A scoping review of drug metabolism in preterm newborns suggested that the CYP2E1 pathway is minimal. The degree of maturation of the CYP450 enzymes responsible for drug metabolism depends on both postnatal and gestational age. The adult level of CYP expression is generally reached at 2 years of age [18]. Moreover, glutathione concentrations are higher in preterm neonates at birth [19], suggesting rapid conjugation of NAPQI into nontoxic metabolites. However, the benefit–risk balance should always be considered with the aim of avoiding unnecessary overexposure to paracetamol in this vulnerable population.

A good tolerance profile for paracetamol within the range of exposures was observed in this study. The AST and ALT levels measured were within the normal range, and no association was found with the AUCs of paracetamol or its oxidative metabolites. This has also been reported in previous publications [10, 11].

No association was observed between predicted exposure levels and clinical response defined as success or failure. These findings may suggest that paracetamol concentrations were within the range that already guarantee a maximum effect, so further increasing the doses would not improve efficacy. However, more complex pharmacokinetic–pharmacodynamic modelling that simultaneously models the longitudinal data from both plasma drug concentrations and diameter of the ductus arteriosus may be considered to find a significant relationship [20].

5 Conclusion

This study is the first to describe the pharmacokinetics of paracetamol and its metabolites in a population of extremely preterm neonates with a gestational age of 23–26 weeks. Paracetamol total clearance increased with weight, and the volume of distribution of the peripheral compartment increased with birth length. Paracetamol was mainly metabolised in sulfate, and the oxidative metabolic pathway was reduced. Gestational age influenced the proportion of different metabolic pathways. In the range of observed drug exposures, no association was found with clinical response, defined as success or failure, or with liver biochemical parameters. These findings may suggest that paracetamol concentrations were within the range that already guarantee a maximum effect on ductus closure.

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Declarations

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Conflict of interest Faheemah Padavia, Jean-Marc Treluyer, Gilles Cambonie, Cyril Flamant, Aline Rideau, Manon Tauzin, Juliana Patkai, Géraldine Gascoin, Mirka Lumia, Outi Aikio, Frantz Foissac, Saïk Urien, Sihem Benaboud, Gabrielle Lui, Léo Froelicher Bournaud, Yi Zheng, Ruth Kemper, Marine Tortigue, Alban-Elouen Baruteau, Jaana Kallio, Mikko Hallman, Alpha Diallo, Léa Levoyer, Jean-Christophe Roze And Naïm Bouazza declare that they have no potential conflicts of interest that might be relevant to the contents of this manuscript.

Ethics approval The trial was approved by the ethics committee of Centre Hospitalier La Chartreuse (approval number SI 20.03.09.40128) for France and by the regional medical research ethics committee of North Ostrobothnia (approval number 68/06.00.00/20 19) for Finland.

Consent to participate Written informed consent was obtained from both parents of each infant.

Consent for publication Not applicable.

Data availability statement The data that support the findings of this study are available from Institut national de la santé et de la recherche médicale (Inserm) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. However, data are available from the authors upon reasonable request and with permission from Inserm.

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