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Pharmacokinetics and Safety of Prolonged Paracetamol Treatment in Neonates: An Interventional Cohort Study

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ORIGINAL ARTICLE

Pharmacokinetics and safety of prolonged paracetamol treatment in neonates: An interventional cohort study

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Aims: To investigate the pharmacokinetics and safety of prolonged paracetamol use (>72 h) for neonatal pain.

Methods: Neonates were included if they received paracetamol orally or intravenously for pain treatment. A total of 126 samples were collected. Alanine aminotransferase and bilirubin were measured as surrogate liver safety markers. Paracetamol and metabolites were measured in plasma. Pharmacokinetic parameters for the parent compound were estimated with a nonlinear mixed-effects model.

Results: Forty-eight neonates were enrolled (38 received paracetamol for >72 h). Median gestational age was 38 weeks (range 25–42), and bodyweight at inclusion was 2954 g (range 713–4750). Neonates received 16 doses (range 4–55) over 4.1 days (range 1–13.8). The median (range) dose was 10.1 mg/kg (2.9–20.3). The median oxidative metabolite concentration was 14.6 μmol/L (range 0.12–113.5) and measurable >30 h after dose. There was no significant difference ($P > .05$) between alanine aminotransferase and bilirubin measures at <72 h or >72 h of paracetamol treatment or the start and end of the study. Volume of distribution and paracetamol clearance for a 2.81-kg neonate were 2.99 L (% residual standard error = 8, 95% confidence interval 2.44–3.55) and 0.497 L/h (% residual standard error = 7, 95% confidence interval 0.425–0.570), respectively. Median steady-state concentration from the parent model was 50.3 μmol/L (range 30.6–92.5), and the half-life was 3.55 h (range 2.41–5.65).

Conclusion: Our study did not provide evidence of paracetamol-induced liver injury nor changes in metabolism in prolonged paracetamol administration in neonates.

KEYWORDS
acetaminophen, neonates, pain, paracetamol, pharmacokinetics

Sissel Haslund-Krog, Tine Brink Henriksen and Susanne Poulsen are the principal investigators of this study.

1 | INTRODUCTION

Neonates admitted to neonatal intensive care units (NICUs) are frequently exposed to painful procedures between 7 and 17 times per day,[1] such as surgery, heel lancing for blood sampling, lumbar punctures, insertion of chest tubes or central lines, laryngoscopy and endotracheal intubation.[2] Studies exploring the long-term consequences of untreated exposure to painful procedures during the neonatal period have shown a negative impact on sensorimotor, cognitive outcomes and impaired neurodevelopment later in life.[3–5] This is due to an immature nervous system and extensive neuroplasticity compared to older children and adolescents.[6] In the NICU, pain treatment primarily includes opioids. As opioid exposure in the absence of pain leads to neuronal degeneration as demonstrated in juvenile tox studies[7] it becomes prudent that neonates receive effective and safe pain treatment regimen that reduces the amount of opioids to ensure an optimal risk benefit balance.[2,8]

Paracetamol[9] (N-acetyl-p-aminophenol or acetaminophen) is frequently used as an add on analgesic in NICUs due to its clinically significant opioid-sparing effect.[10] The proposed target concentration for pain management is 9–11 mg/L (60–73 μmol/L) at steady-state.[11–14] As Paracetamol has a variable absorption in neonates and a higher volume of distribution (Vd) per kg compared to older children and adults[15] a loading dose is recommended at treatment initiation.[13,15]

Paracetamol is primarily metabolized in the liver and undergoes sulfation by SULT1A1, SULT1A3 and SULT1C4, the major pathway in neonates.[16] The parent compound, paracetamol, undergoes glucuronidation by 4 **UDP-glucuronosyltransferases**[17] (UGTs), namely UGT1A1, 1A6, 1A9 and 2B15.[18,19] Several UGT1A enzymes are only expressed postnatally,[20] which results in lower glucuronide formation in neonates relative to sulfation.[16] Oxidation by **CYP2E1**[17] to the highly reactive **N-acetyl-p-benzo quinonimine**[9] (NAPQI) is a minor pathway. NAPQI is rapidly detoxified in the form of two **glutathione**[9]-derived conjugates (cysteine- and N-acetylcysteine-paracetamol, i.e., the oxidative metabolites), both surrogate measures for NAPQI.[21,22] CYP2E1 is expressed at low levels in the third trimester and gradually increases during the first 90 postnatal days.[23] Repeated dosing with paracetamol in children may shift drug metabolism towards oxidation by CYP2E1.[24] Neonates can form NAPQI but appear to have a lower incidence of liver failure than adults.[25] Clinical trials examining the safety and pharmacokinetics of paracetamol in neonates have primarily focused on short-term use (up to 72 h).[26–29] Studies have assessed paracetamol and metabolite concentrations and liver biomarkers for a limited time.[26,28] Other studies did not include liver biomarkers[28] nor metabolite concentrations.[27] Previous studies have administered 1 dose of paracetamol[29] or examined propacetamol.[25,30] Comprehensive information, which combines liver biomarkers, paracetamol concentrations and metabolites from prolonged clinical administration, are missing. Our study included plasma parent and metabolite concentrations, measurements of liver biomarkers (alanine aminotransferase [ALT] and bilirubin) to assess paracetamol treatment in neonates.

What is already known about this subject

- Due to frequent painful procedures and conditions associated with continuous pain, paracetamol is often administered to neonates treated in the neonatal intensive care unit.
- The pharmacokinetic profile for paracetamol has been examined before in short-term use (<72 h), and some studies have included liver biomarkers.

What this study adds

- This study examined liver biomarkers and the pharmacokinetics of prolonged use of paracetamol (>72 h) for neonatal pain.
- No differences were found in liver biomarkers between short (<72 h) and prolonged (>72 h) dose administration and the oxidative metabolism remained active. Thus, this study did not provide evidence for paracetamol-induced liver injury or changes in metabolism in prolonged paracetamol administration.

2 | METHODS

2.1 | Study population

This interventional cohort study investigated the prolonged use of paracetamol administered for pain control in neonates. Between 10 April 2018 and 30 November 2019, patients were enrolled from 2 Danish NICUs at Aarhus University Hospital and Rigshospitalet, which have around 800–1100 neonatal admissions annually. All neonates up to 44 weeks postmenstrual age (PMA) who were intended to be treated with intravenous paracetamol for any indication or with oral paracetamol for fractures, intra-and extracranial haemorrhages, chest tubes, postoperative pain or painful skin lesions were included. Both intravenous and oral formulations of paracetamol were included as per the standard of care. Exclusion criteria included suspected allergy to paracetamol, failure to obtain consent or the neonate being considered unsuitable for participation in the clinical trial by the treating physician. The Intravenous and Oral Paracetamol in Neonates: Safety and Ethanol-Drug Interactions (PARASHUTE) study targeted the enrolment of 60 patients, as outlined in previously published work.[31]

2.2 | Dosing and sampling schedule

Paracetamol treatment typically consisted of a loading dose of 20 mg/kg followed by a maintenance dose depending on PMA: 7,5-mg dose/kg every 8 h for neonates with a PMA of 28–32 weeks;

10-mg dose/kg every 8 h (PMA = 33–36 weeks); 10-mg dose/kg every 6 h (PMA = 37–44 weeks). Intravenous doses were administered as an infusion over 2 min. Baseline ALT and bilirubin measurements were collected prior to treatment, within 24 h after the initial dose, and within 24 h after treatment discontinuation. This extended timeslot for baseline sampling was a pragmatic approach to allow more time for obtaining parental consent. Paracetamol parent and metabolite concentrations were measured 18–36 h after initial dosing (at steady-state as outlined by Allegaert et al.[11]) hereafter every second day. In total, 10 samples deviated from this schedule. ALT, bilirubin and drug concentrations were collected opportunistically during the remaining treatment to limit skin punctures.[31,32]

2.3 | Data collection and analysis

Data collected on each subject included weight, head circumference, length, postnatal age (PNA), PMA, gestational age (GA), Apgar score (1, 5 and 10 min), birth method (e.g., vaginal, c-section), sex, diagnosis, plasma creatinine (start and end trial), number of paracetamol doses and administration time, treatment length, and pain scores. Comedications were collected for all patients without dosing details at the beginning and end of the trial.

The liver biomarkers were analysed in the respective hospitals, in the Departments of Clinical Biochemistry, which use comparable analytical equipment accredited by the same standards. Normal neonatal ranges used for ALT were 1–40 U/L. The limit was individually determined for bilirubin using weight, gestational age and comorbidities.

2.4 | Analytical methods for paracetamol

Blood samples were centrifuged at 1500 $\times g$ for 9 min at 20°C within 2 h of collection. Plasma was transferred to a cryovial and stored at a minimum of −60°C before shipment to the laboratory. The plasma samples were analysed by a validated assay by high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry for simultaneous quantification of paracetamol paracetamol-glucuronide, paracetamol-sulfate, paracetamol-glutathione, paracetamol-cysteine, and paracetamol-N-acetylcysteine in 10 µL human plasma.[33] Parent or metabolite concentrations above the upper limit of quantification ($n = 33$) were diluted and re-analysed. The lower limit of quantification (LLOQ) was defined as 0.05 mg/L for all analytes. The molar sum of paracetamol-cysteine and paracetamol-N-acetylcysteine are the *oxidative metabolites*. Paracetamol-glutathione was not included in the model as only 10 (7.8%) samples had concentrations above the LLOQ. One subject was excluded due to improper sampling schedule.

2.5 | Model development

The base structural model, not including covariate relationships, was developed based on a previously published model by Cook et al.[28,34] Model development for the parent compound was conducted using a nonlinear mixed-effects model (NONMEM, version 7.4, ICON plc, Dublin, Ireland) interfaced with PsN 5.0.0. The first-order conditional estimation with the interaction method was used for parameter estimation. Processing and visualization of the NONMEM output was conducted in R 4.0.1. Data below the limit of quantification (BQL) were considered categorical and the likelihood of the BQL sample value was quantified using the probability that an observation was below LLOQ, a common technique for handling BQL data.[35] Weight (WT) was included in the base model as in Cook et al.,[28,34] centred on the mean WT and with a power relationship, where the effect of WT on clearance (CL) and WT on V_d were estimated. Typically, allometric scaling is inappropriate for patients younger than 2 years due to immature kidneys and development maturation and was not used.[36]

A 1-compartment model was tested.[28,34] The addition of a lag time, absorption and bioavailability component for oral doses or a second compartment for paracetamol distribution were tested. Bioavailability was assumed to be 100%, which is consistent with previous adult literature.[37] Parameters were estimated using the ADVAN1 and TRANS2 subroutines and the first-order conditional estimation method with interaction. Akaike information criterion (AIC) was used to compare non-nested models. Prediction corrected, compartment-stratified and binned visual predictive checks were performed for the observed data to plasma concentrations from 1000 model-simulated datasets.[38] Nonparametric bootstrap was also performed.

Random effects were considered either unexplained residual variability (RUV) or between-subject variability (BSV). Three RUV models were evaluated, additive, proportional and combined. RUV was estimated as a standard deviation.[39,40] In addition, BSV was modelled exponentially, as BSV was assumed to be log-normally distributed (Equation 1). Therefore, BSV estimation was attempted on total parent CL.

$$\theta_{ind} = \theta_{pop} \times e^{\eta_i} \quad (1)$$

where θ_{ind} is the pharmacokinetic parameter of an individual, θ_{pop} is the typical value for the parameter and η_i is normally distributed with a mean of 0 and a variance of ω. Half-life was calculated using estimates of volume and clearance for the parent drug (Equation 2).

$$t_{1/2} = \frac{0.693 * V}{CL} \quad (2)$$

2.6 | Covariate analysis

Covariates were assessed with a stepwise forward addition and backward elimination procedure. Potential covariates included GA, PNA, PMA, sex, ALT levels, total bilirubin levels at four different time points and creatinine on the first and last days of the trial, Apgar score at 1 min and birthing method. Serum creatinine values on the first and last days of the trial were used to estimate the glomerular filtration

rate using the modified Schwartz equation. Subjects with missing information for a covariate undergoing evaluation had their values imputed to population medians or the subject's previous measurement, depending on the covariate. The objective function value (OFV) was used to compare the nested models, and covariate–parameter plots were used to visually select potential covariates for further testing. During forward addition, changes in OFV were considered significant below an alpha value of 0.05 (χ^2 distribution, 1 degree of freedom, ΔOFV > 3.84), while during backward elimination, changes below an α value of 0.01 (χ^2 distribution, 1 degree of freedom, ΔOFV > 6.63) were considered significant. The goodness of fit plots are stratified by compartment, including DV *vs*. IPRED, DV *vs*. PRED, CWRES *vs*. TAD, CWRES *vs*. PRED, logDV *vs*. logPRED and logDV *vs*. logIPRED were also used to assess model fit. Categorical covariates were tested for inclusion via a linear relationship (Equation 3), where θ_{ind} is the individual pharmacokinetic parameter, θ_{pop} is the population level value for the pharmacokinetic parameter when COVi is 0, WT is the individuals weight, WT_{mean} is the mean weight for the sample population, $\theta_{WT\sim\theta_{pop}}$ is the estimated effect of WT on the population level pharmacokinetic parameter and θ_{cov} is the proportional change in θ_{pop} when COVi is 1. Clearances were scaled with the effect of weight on CL fixed to 1.1, as estimated by the parent model and similar to the previously published model.[28]

$$\theta_{ind} = \theta_{pop} \times \left(\frac{WT}{WT_{mean}}\right)^{\theta_{WT\sim\theta_{pop}}} \times (1 + \theta_{cov} COV_i) \quad (3)$$

Continuous covariates were assessed for inclusion with an exponential relationship (Equation 4), where θ_{ind} is the individual pharmacokinetic parameter, θ_{pop} is the population level value for the pharmacokinetic parameter when COV_i is equal to the population covariate value COV_{avg}, WT is the individual's weight, WT_{mean} is the mean weight for the sample population and θ_{cov} is the covariate effect and was included as part of the base model as described above.

$$\theta_{ind} = \theta_{pop} \times \left(\frac{WT}{WT_{mean}}\right)^{\theta_{WT\sim\theta_{pop}}} \times \exp\left(\frac{\theta_{cov} COV_i}{COV_{mean}}\right) \quad (4)$$

2.7 | Statistical analyses

Study data were managed using Research Electronic Data Capture (REDCap) tools hosted in the Capital Region of Denmark.[41,42] Data were analysed in R 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria) for further analysis and visualization. Demographic data were presented as median (range) or numbers (percentages). Model estimates were reported with confidence interval, residual standard error (RSE) and shrinkage. To present the change in paracetamol concentrations throughout the study, a rolling median using sample windows of 15 was used to construct Figure 3. In addition, the Wilcoxon Rank test was used to compare the liver biomarkers via an unpaired 2-sample *t*-test.

2.8 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[9,17]

3 | RESULTS

3.1 | Patient characteristics

Sixty-two neonates were screened for inclusion, with a total of 48 being completed (Figure 1). No serious adverse events correlating to the paracetamol treatment were reported during the study. The characteristics of the study population are shown in Table 1. A total of 126 plasma concentration samples were available for measurement of the parent and the 3 metabolites in each of the 48 neonates who received intravenous or oral paracetamol. Nine observations were below the LLOQ of 0.05 mg/L. The number of samples per patient was median 3, range (1–6; Table 2). Thirty-eight neonates received paracetamol for ≥72 h. In total, 865 doses of paracetamol were administered with a median of 16 doses (range 4–55) per participant over a treatment length of 4.1 days (range 1–13.8), Table 2. The median (range) dose was 10.1 mg/kg (2.9–20.3) Paracetamol was administered orally ($n = 218$), intravenous ($n = 642$) or via an intravenous formulation is given orally ($n = 5$). The median observed concentration of oxidative metabolite was 14.6 μmol/L (range 0.12–113.5), the glucuronide metabolite concentrations was 18.7 μmol/L (range 0.12–123.1) and sulfate metabolite concentration was 63.1 μmol/L (range 0.04–261.2). The metabolite concentrations over time are shown in Figure 2. The majority of patients were neonates in need of gastrointestinal surgery, e.g., for gastroschisis, oesophageal or anal atresia. The complicated surgical patients and severe asphyxia patients received most paracetamol doses (Figures S1 and S2). For information on the birthing method and APGAR scores, see Table 1.

3.2 | Liver biomarkers

ALT and total bilirubin did not change over time, and there was no significant difference ($P > .05$) between measures at <72 or >72 h of paracetamol treatment or the start and end of the study (Figure 3). ALT values increased >3 times the normal range for 9 patients with values above 100 U/L. Bilirubin median was 67 μmol/L (range 20–256) at inclusion and 39 μmol/L (range 5–296) at the end of the study, with no difference <72 or >72 h of paracetamol treatment ($P > .05$).

3.3 | Comedication

Out of 48 neonates, 44 received at least 1 comedication at inclusion. By the end of the study, 32 received comedication. The most

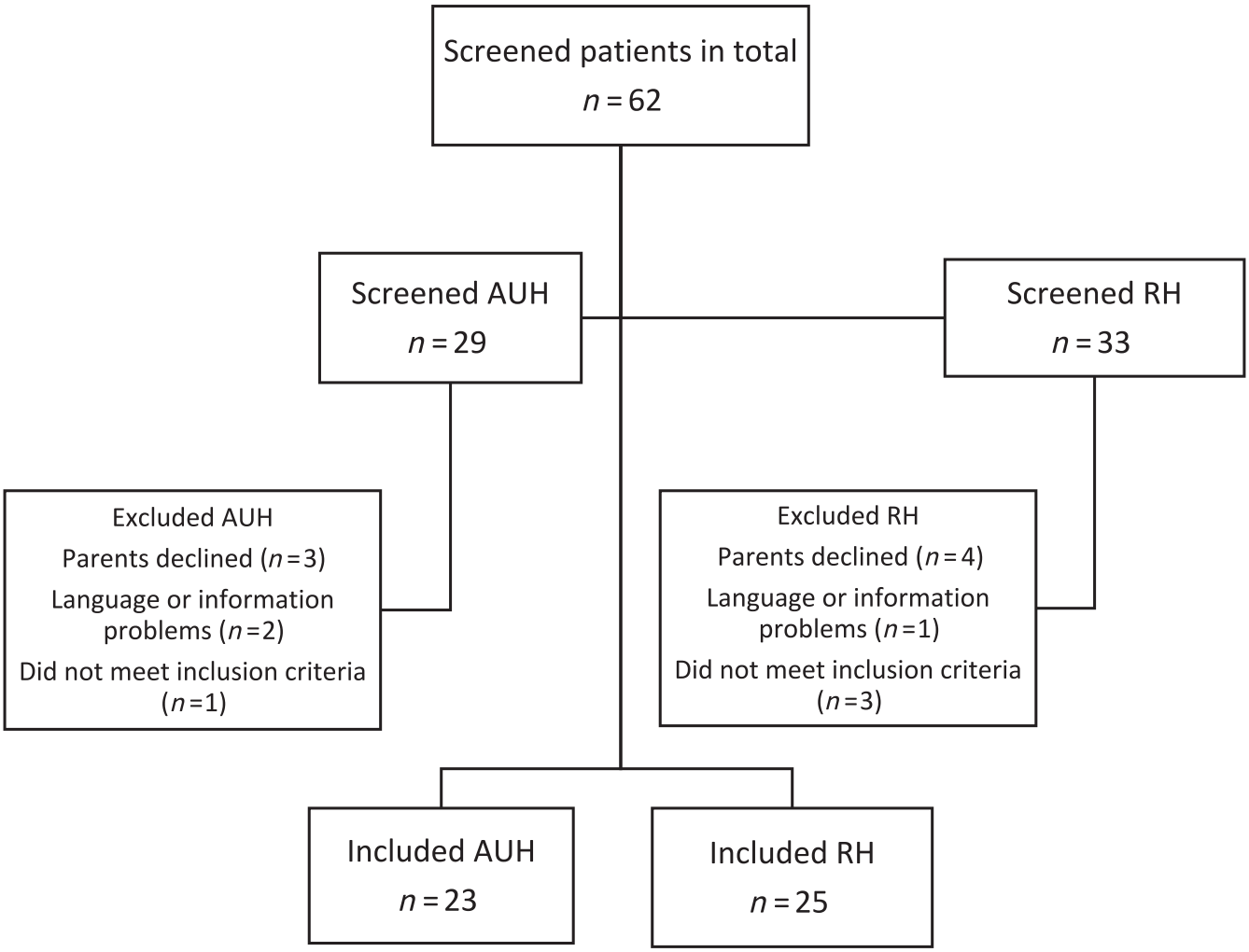

FIGURE 1 Flow chart of the screening and inclusion of patients. AUH, Aarhus University Hospital; RH, Rigshospitalet.

frequently coadministered medicine at inclusion was morphine (62.5%), gentamicin (47.9%), metronidazole (41.7%), fentanyl (37.5%), ampicillin (37.5%), total parental nutrition (TPN; 35.4%) and caffeine citrate (29.2%; Figure 4). No medications with a known inducing or inhibitory influence on paracetamol were coadministered.[43]

3.4 | Population estimates

For the paracetamol parent model, a 1-compartment intravenous model fit the data well. Population estimates for the parent drug for a 2.81 kg (mean WT) neonate for paracetamol were Vd = 2.99 L (% RSE = 8) and CL = 0.497 L/h (%RSE = 7). Total paracetamol clearance increased with weight and PMA (Figure 5). Formation and renal clearances estimated from the final parent model are presented in Table 3. The estimate of BSV for the formation of the sulfate metabolite was calculated with a shrinkage of 11%. A proportional error model was superior to additive and combined error models. Only BSV on CL was estimated in the model. The median steady-state concentrations as estimated by the model (50.3 μmol/L, range 30.6–92.5), were outside of the reported therapeutic range. Half-life was 3.55 h (range 2.41–5.65). Weight was the only significant covariate. No additionally covariates were found to be statistically significant.

3.5 | Model evaluation

The goodness of fit plots were qualitatively assessed for model fit. RSE and shrinkage in the final model were within acceptable limits for a model developed in a neonatal population (Table 3). Nonparametric bootstrap ($n = 1000$) showed that the model's stability, uncertainty and bias were acceptable (Figure S3).

4 | DISCUSSION

This study investigated the pharmacokinetics and safety of prolonged paracetamol use (>72 h) for pain associated with procedural or postoperative pain treatment in neonates. The median duration of treatment was 4.1 days (range 1–13.8), making this the most prolonged

TABLE 1 Population characteristics of included neonates.

Characteristics	*n* (%)	Median (range)
Male	31 (64.6)	-
Female	17 (35.4)	
Site		
Rigshospitalet	25 (52.1)	-
Aarhus University Hospital	23 (47.9)	
Age, weight and length		
Gestational age (weeks)	48 (100)	37.9 (24.9–42)
Postmenstrual age (weeks)	48 (100)	38.5 (25–42)
Postnatal age (days)	48 (100)	1.5 (0–51)
Weight, inclusion (g)	48 (100)	2953.5 (713–4750)
Weight, end trial (g)	48 (100)	3061 (830–4610)
Length, inclusion (cm)	47 (97.9)	51 (32–56)
Length, end trial (cm)	48 (100)	51 (33–56)
Head circumference, inclusion (cm)	45 (93.8)	34 (22.7–39.5)
Birthing method and Apgar score		
Vaginal birth	21 (43.8)	-
Assisted vaginal birth	9 (18.8)	
Acute C-section	9 (18.8)	
Planned C-section	4 (8.3)	
Subacute C-section	5 (10.4)	
Apgar 1 min	47 (97.9)	8 (0–10)
Apgar 5 min	46 (95.8)	9 (0–10)
Apgar 10 min	35 (72.9)	9 (0–10)

TABLE 2 Biomarkers and paracetamol doses.

	n (%)	Median (range)
Number of paracetamol doses	865 doses	16 (4–55)
mg/kg	-	10.1 (2.9–20.3)
Intravenous	642	-
Oral	218	
Intravenous given as oral	5	
Treatment length (days)	48 (100)	4.1 (1–13.8)
No of blood samples	126[a]	3 (1–6) per patient
Diagnosis		
Gastrointestinal surgery	21 (43.7)	
Pulmonary disease	9 (18.7)	
Central nervous system	12 (25)	
Others	6 (12.5)	
Alanine aminotransferase (U/L)		
Total	169 (88)	17 (5–431)
Inclusion	48 (100)	15 (6–431)
End of study	47 (98)	18 (7–338)
Bilirubin (μmol/L)		
Total	156 (81)	60.5 (5–316)
Inclusion	48 (100)	67 (20–256)
End of study	48 (100)	39 (5–296)
Creatinine (μmol/L)		
Inclusion	48 (100)	99 (28–147)
End of study	48 (100)	64 (17–116)

[a]One subject was excluded from the model due to improper sample timing due to hospital transfer. In total we collected 128 samples, however 126 was included in the model.

exposure data published to date. In addition, the study included liver biomarkers and the concentration measurements of both paracetamol parent and metabolite compounds in plasma, allowing examination of elimination pathways. Overall, this study reported steady-state concentration, half-life and surrogate safety measures for this specialized patient population.

One of the most significant findings in this study was that the median steady-state concentration was below the target concentration of 9–11 mg/L (60–73 μmol/L), as proposed in the literature[11,13] and below that reported in similar published studies.[26,27] One possible explanation is that the mean dose of 10.6 ± 2.3 mg/kg was lower than in the Palmer et al. and Cook et al. studies with comparable populations with respect to GA/PMA and weight.[27,28] Likewise, the suggested dosing for this population would be a loading dose of 20 mg/kg followed by 10 mg/kg for 6 h for neonates GA 32–44 weeks,[12,13] whereas some neonates in our study received treatment every 8 h. Our reported half-life of 3.55 h was longer than the 1.5–2.5 h reported in adults[44] and 0.9–2.6 h reported by Wang et al. in a population with a weight range of 0.5–50 kg.[45] Similar to other studies, the increased Vd and prolonged half-life in this study supports the use of a loading dose to attain a given target concentration earlier.[13,15] Total paracetamol CL increased with PMA and weight as in Cook et al.,[28] although they used PNA. The metabolite concentration was not directly quoted in the study by Cook et al.[28]; however, the results show an oxidative metabolite 50th percentile concentration between 1 and 2 mg/L (~3–6 μmol/L) with observed 5th and 95th percentiles of 0–6 mg/L (0–20 μmol/L) compared to the median 14.6 μmol/L (range 0.12–113.5) found in our study. When comparing the visual plots for the observed metabolite concentrations, both Cook et al. and our study have outliers, and the reported 50th and median concentrations are comparable for the sulfate metabolite but with a higher median and wider range for the glucuronide metabolite in our study. Oxidative metabolites were measurable > 30 h after the dose. Being surrogate measures for the detoxification of NAPQI, the oxidative metabolite concentration show that the metabolism remains active in prolonged paracetamol administration.

Reporting of liver biomarkers in relation to paracetamol treatment differs between studies. Palmer et al. reported that 4 out of 50 neonates had an elevated ALT range of 86–174 U/L. Two were associated with hyperbilirubinaemia, 1 with hypoproteinaemia and 3 of 4 received TPN.[27] Ganzewinkel et al. found no ALT levels above 50 U/L in 15 neonates.[26] It is difficult to assess the elevated ALT results since neonates may have multiple causes of ALT elevations, e.g., asphyxia, therapeutic hypothermia and TPN (35.4% received TPN

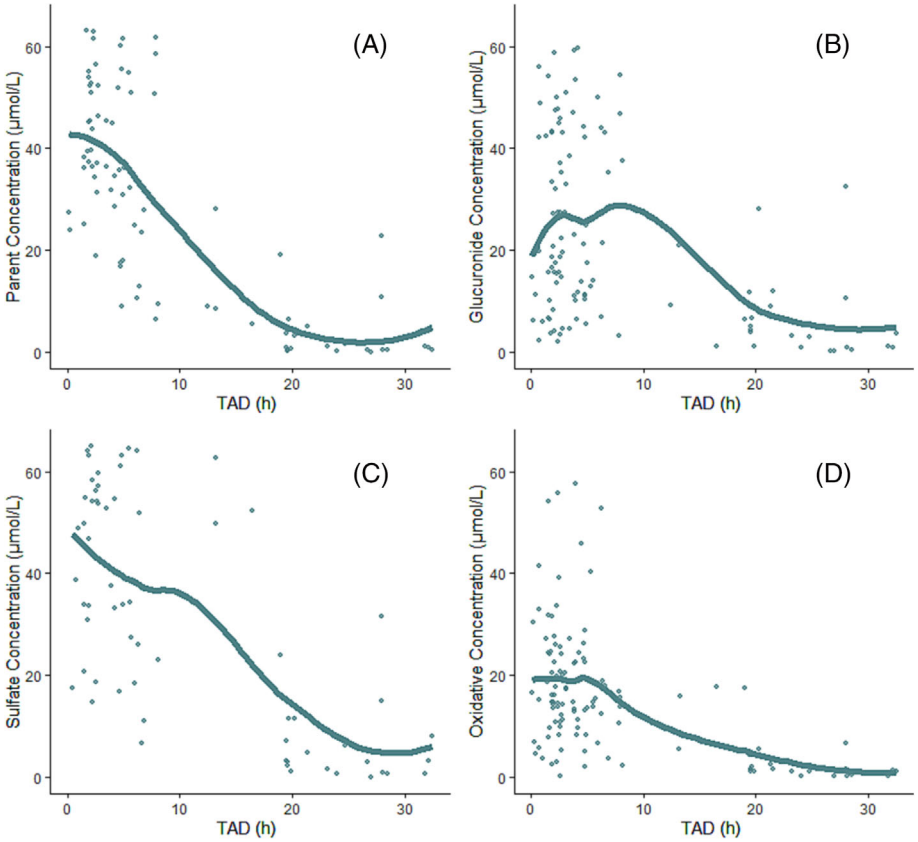

FIGURE 2 Observed parent and metabolite plasma molar concentrations in relation to time after dose. A loess curve was used to visualize changes in concentration over time of parent and each metabolite. (A) Parent concentration, (B) glucuronide concentration, (C) sulfate concentration, (D) oxidative metabolite concentration. One patient was excluded from this figure for readability, due to a time after most recent dose (TAD) of 80 h.

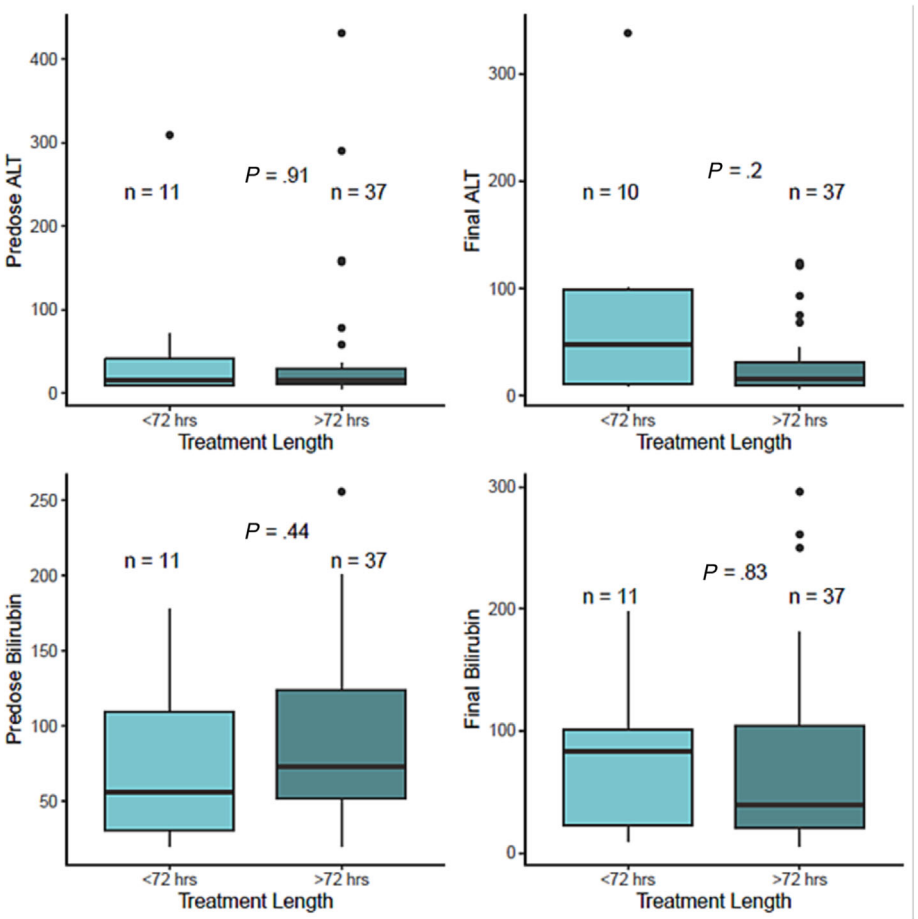

FIGURE 3 Comparison of alanine aminotransferase (ALT) and bilirubin at trial initiation and end of study in patients treated with observed paracetamol plasma concentrations for <72 or >72 h. Wilcoxon rank test was used for comparison.

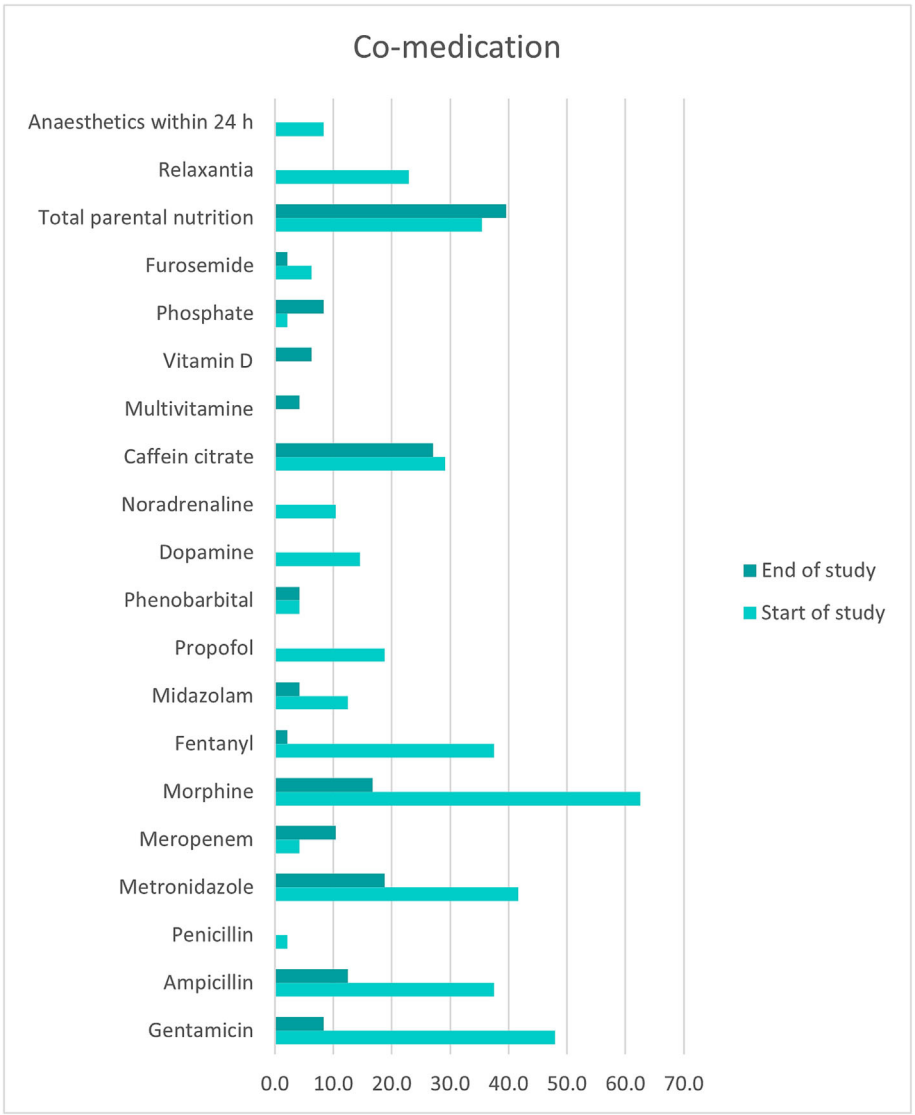

FIGURE 4 Comedication. Percentage of patients receiving comedication at 2 time points. Information on comedication was collected at start of study (at inclusion) and at the end of study (after last paracetamol treatment). *Possible interaction with the anaesthetics such as sevoflurane, halothane, enflurane and isoflurane since they are also substrates for CYP2E1. No inducers or inhibitors to paracetamol have been coadministered. According to Drug Interactions Flockhart Table.

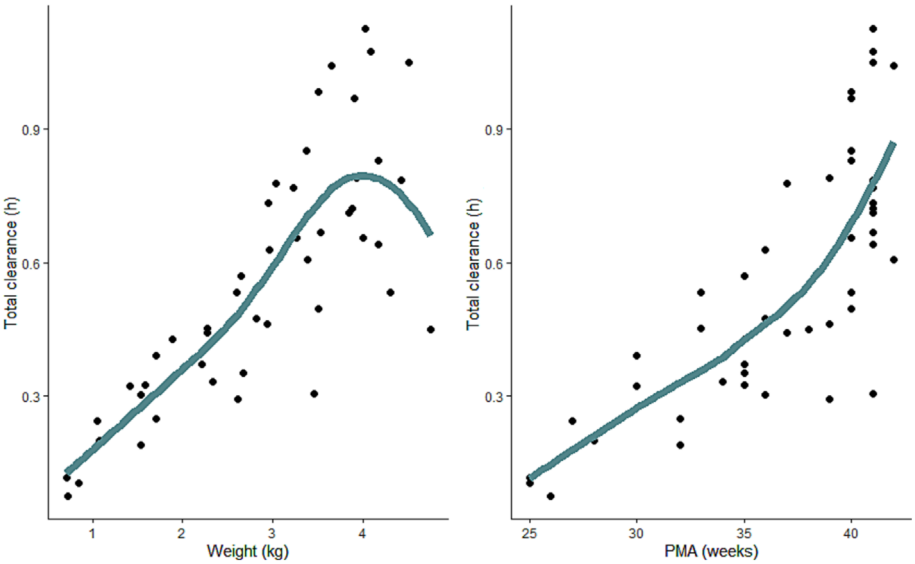

FIGURE 5 The relationships between total paracetamol clearance and weight and postmenstrual age (PMA).

TABLE 3 Model estimates for the pharmacokinetic model for the parent compound.

Parent model results			Bootstrap results ($n = 1000$) (997 successful)
Parameter[a]	**Estimate**	**RSE (%)**	**Estimate median [95% CI]**
V_d (L)	2.99	8	2.988 [2.44–3.55]
CL_{total} (L/h)	0.498	7	0.498 [0.425–0.570]
WT ~ CL[b]	1.1	15	1.116 [0.764–1.452]
WT ~ V[b]	0.8	21	0.779 [0.313–1.24]
Residual variability, proportional[c]	0.429	8	0.425 [0.361–0.497]
Parameter	**Estimate**	**CV (%)**	**Estimate median [95% CI]**
BSVCL	0.316	11	0.305 [0.226–0.386]

Abbreviations: BSV, between-subject variability; CI, confidence interval; CL, clearance; CV, coefficient of variation; RSE, residual standard error; Vd/V, volume of distribution; WT, weight.

[a]Volume was assumed constant for all compounds.

[b]The effects of WT on CL and V were included in the model as $\theta_{pop} \times \left(\frac{WT}{WT_{mean}}\right)^{\theta_{WT\sim\theta_{pop}}}$ where θ_{pop} is CL or V.

[c]The unexplained residual variability is presented as the square root of the residual variance.

at inclusion and 39.6% at the end of this study). We found that 1 patient had a 3-fold increase in ALT during the trial period and received TPN. However, the increase happened post-trial when the paracetamol treatment was already discontinued. One patient had an ALT >300 U/L with a primary diagnosis of cranial haemorrhages and meningitis, and the ALT decreased to 24 U/L post-trial. Three patients with severe neonatal asphyxia had ALT values >200, 1 received TPN and 2 received therapeutic hypothermia. All ALT values fell below 75 U/L during the trial. The other 5 outliers were briefly above 100 U/L but below 200 and fell during the trial, 1 received TPN and 1 had therapeutic hypothermia.

TPN and caffeine citrate are administered almost equally at the inclusion and end of the trial, which is logical given the inclusion of premature neonates and neonates in need of surgery. Anaesthetics such as sevoflurane, halothane, enflurane and isoflurane are also substrates for CYP2E1 and could theoretically compete with the metabolism of paracetamol. However, these substrates have a noticeably short half-life and are used within an extremely limited timeframe.

4.1 | Limitations

In general, this population of neonates were of an acceptable size (compared to previous studies, see Table S1), with a similar number included from each site, 23 and 25, respectively. The amount of intravenous and oral dosing was unequal, with 3 times as many intravenous doses as oral. A randomized (intravenous/oral) treatment design is optimal; however, this was not feasible due to extensive set up and resources. We included the oral treatment in an amendment because this was the clinical practice at 1 site. An oral dose compartment was included in the model but did not improve the fit of the data. A few samples were available per individual; however, previous studies were of shorter duration, and we had to consider the total blood volume taken in the design. ALT and bilirubin might not be the most sensitive biomarkers for liver injury. We discussed the possibility of measuring protein adducts to detect paracetamol-induced liver injury.[46] However, we did not have sufficient plasma remaining to measure protein adducts. Indeed, there are newer biomarkers, e.g., microRNAs, mitochondrial and nuclear DNA.[47] We did not collect dosing or administration details for the comedication.

5 | CONCLUSION

This study included 48 neonates and is the largest study to date measuring both paracetamol parent and metabolite concentrations and liver biomarkers. The observed median steady-state concentration was below the target concentration reported in the literature, possibly explained by longer time between doses and lower dosing regimen compared to other studies. Notably, oxidative metabolites were measurable >30 h after the dose, showing that the metabolism remains active in prolonged paracetamol administration. In addition, liver biomarkers did not differ from inclusion to end of the study nor between short-term and prolonged paracetamol treatment. Accordingly, this study did not find evidence for paracetamol-induced liver injury nor changes in metabolism.

AUTHOR CONTRIBUTIONS

Helle Holst, Kim Dalhoff, Tine Brink Henriksen, John van den Anker and Sissel Haslund-Krog participated in the conception of the study, outlined the study design and contributed to the protocol. Sissel Haslund-Krog, Helle Holst, Tine Brink Henriksen and Kim Dalhoff applied for all funding. Helle Holst, Sissel Haslund-Krog, Tine Brink Henriksen and Diana Wilkins applied for all permissions, including data agreements in Denmark and between countries. Helle Holst was the sponsor of the trial. Tine Brink Henriksen and Susanne Poulsen were principal investigators, Ulla Christensen was an investigator and Sissel Haslund-Krog were the coordinating principal investigator. Jessica Barry and Angela Birnbaum were primarily responsible for the pharmacokinetic model development, and Charul Avachat was involved in data management, Rory Remmel consulted on the metabolite data. Catherine Sherwin consulted on the model and was the

initial contact for the collaboration. Diana Wilkins provided the sample analysis. Sissel Haslund-Krog drafted the first version of the manuscript. All authors participated in writing different sections in the revision and approval of the final version of the manuscript prior to submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Upon reasonable request to the corresponding author data can be procured for future research.

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SUPPORTING INFORMATION

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

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