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RESEARCH PAPER

An Observational Study of Blood Concentrations and Kinetics of Methyl- and Propyl-Parabens in Neonates

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ABSTRACT

Purpose Systemic exposure to parabens in the neonatal population, in particular propyl-parabens (PPB), remains a concern. Blood concentrations and kinetics of methyl-parabens (MPB) and PPB were therefore determined in neonates receiving medicines containing these excipients.

Methods A multi-centre, non-interventional, observational study of excipient-kinetics in neonates. 'Dried Blood Spot' samples were collected opportunistically at the same time as routine samples and the observations modelled using a non-linear mixed effects approach.

Results A total of 841 blood MPB and PPB concentration data were available for evaluation from 181 pre- and term-neonates. Quantifiable blood concentrations of MPB and PPB were observed in 99% and 49% of patients, and 55% and 25% of all concentrations were above limit of detection (10 ng/ml), respectively. Only MPB data was amenable to modelling. Oral bioavailability was influenced by type of formulation and disposition was best described by a two compartment model with clearance (CL) influenced by post natal age (PNA); $CL_{PNA<21\ days}$ 0.57 versus $CL_{PNA>21days}$ 0.88 L/h.

Conclusions Daily repeated administration of parabens containing medicines can result in prolonged systemic exposure to the parent compound in neonates. Animal toxicology studies of PPB that specifically address the neonatal period are required before a permitted daily exposure for this age group can be established.

KEY WORDS excipient · kinetics · methyl-parabens · neonates · propyl-parabens

BACKGROUND

Children born prematurely (<32 weeks gestation) are at high risk of death due to cardiovascular compromise and infection and of neurodevelopmental disorders resulting from damage to the developing brain. They are also at risk of developing a variety of other problems with important impact ranging from anaemia of prematurity and neonatal bone disease to patent ductus arteriosus and chronic lung disease. It is common practice to prevent and/or treat these complications with medicines such as iron, vitamin and mineral supplements as

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well as drugs such as antibiotics, steroids and diuretics. Such medicines are commonly administered as oral liquids or intravenous formulations, since it is not possible or practical to administer solid oral dosage forms to babies. Whilst the risk-benefit profile for many of the active ingredients have been adjudged to be favourable in neonates based on accumulated evidence, similar data for the excipients that are also present in oral liquid and intravenous formulations is lacking.

Excipients are utilised in many medicines and justifiably so in the majority of products as it would otherwise be impossible to formulate the active drug (1). For example, some excipients are essential for enhancing drug solubility (e.g. ethanol, propylene glycol) or to prevent microbial contamination (e.g. parabens) or to buffer the pH of the liquid (sodium phosphates). Excipients are regarded as pharmacologically inactive, and the risk-benefit profile of excipients has overwhelmingly been concluded to be positive on the basis of their widespread use in the adult population. However, excipients are not completely inert and adverse effects have been reported (2–6). Moreover, the risk-benefit profile may not be so clear cut when certain excipients are used in formulations intended for children. Indeed, children may be exposed to unacceptable excipient risks when a formulation specifically designed for the adult population is used in vulnerable young children, a frequently unavoidable and relatively common practice (7,8). The European Medicines Agency (EMA) has drawn attention to the need to consider excipients in the assessment of safety of medicines in newborns (9).

Pharmaceutical dosage forms such as multidose oral liquid preparations and parenteral injections must be protected by an efficient antimicrobial preservation system to minimise microbial growth and contamination during storage and removal of doses throughout the intended in-use shelf life. Methyl hydroxybenzoate (methyl-parabens, MPB) and propyl hydroxybenzoate (propyl-parabens, PPB) are widely used in pharmaceutical formulations and usually in combination up to strengths of 0.2%*w/v* (10). Reports of weak *in vitro* oestrogenic activity of a series of parabens (PB), between10,000- to 100,000-fold less potent than that of oestradiol, first surfaced in 1998 (11). Other studies suggested that PPB and butyl-parabens, but not MPB or ethyl-parabens, may produce adverse reproductive effects in young male rats (lower mean epididymides and seminal vesicle weights, lower sperm production, lower testosterone levels) when given orally via the diet for 8 weeks (12–15). However, subsequent studies failed to reproduce these results and found no adverse reproductive effects suggesting a lack of endocrine disrupting effects of PB (16,17).

In a recent reflection paper, the EMA concluded that based on the totality of the *in vitro* and *in vivo* data, MPB seems to be devoid of adverse effects on reproduction and development and considered the excipient to be safe to use in medicinal products intended for children of all ages. For PPB, the EMA concluded that based on the divergent *in vivo* data, a 'no effect level' (NOEL) of 250 mg/kg could be determined, but acknowledged that since the studies did not involve juvenile rats corresponding to the human neonatal period, the data as yet was not fully reassuring for children below the age of 2 years (18). Physiology in neonates and infants differs considerably from that of adults and hence their ability to metabolise or eliminate an excipient may not be equivalent to that in adults (19). Hence altered systemic exposure to PB, particularly following repeated administration, could exist and may be a cause for concern.

The pharmacokinetic profiles of MPB and PPB have been investigated in animal models. A single 100 mg/kg dose of MPB and PPB administered via oral, dermal, or subcutaneous routes to Sprague–Dawley rats, produced a single peak in plasma, corresponding to that of the metabolite para-hydroxybenzoic acid (PHBA), and which according to the authors suggested no significant exposure of mammalian organisms to the parent PB. PHBA is considered to be non-toxic, ubiquitous in human nutrition, and an essential and natural constituent of plant and mammalian organisms, possessing no or negligible oestrogenic activity (20). Conversely, in a recent toxicology study of oral PPB administered to male Wistar rats in doses ranging 3–1,000 mg/kg, concentrations of PPB conjugated to sulphate was quantifiable in plasma. Free (parent) PPB concentrations were however low and only quantifiable at doses in excess of 100 mg/kg (17). In human plasma, intact PB compounds have been reported in adult male volunteers and in women using personal care products (21,22). To date, there are no reported data on the extent to which neonates are exposed systemically to PB.

The aims of this investigation were therefore to determine blood concentrations of MPB and PPB in neonates administered medicines known to contain these excipients and, if possible, estimate excipient-kinetic (EK) parameters. In the past, such studies in infants have not been possible, not least because of limits on blood sample volume. The EMA recommends that sampling is limited to 1% of circulating volume any one time and a total of 3% of circulating volume from all research activity over a 28 day period. Recent advances in bio analysis have made it possible to overcome some of these challenges. It is now possible to quantify drugs and other chemicals using microvolume whole blood samples (5–50 μl) spotted and dried on a filter paper to give a dried blood spot (DBS) sample. This technique reduces the amount of blood required for single time-point measurements and hence DBS is particularly suited for studies involving babies in whom large-volume samples are not appropriate (23). By using the dual approach of DBS and sparse sampling, observed whole blood concentrations of MPB and PPB from a study population can be pooled and EK parameters estimated using a population mixed effects modelling approach (24).

METHODS

Study Design and Subject Population

The study was a multi-centre, non-interventional, observational, population EK study in pre-term and term neonates. The study was conducted in neonatal units at 4 sites in the UK (Chester, Leighton, Liverpool Womens Hospital, and Arrowe Park) and 1 site in Estonia (Tartu). Appropriate multi-centre ethics approval and site approvals were obtained and the investigation was conducted in line with the Declaration of Helsinki. All neonates prescribed or likely to be prescribed a medicinal product containing methyl- or propyl-parabens were eligible for inclusion. Neonates were excluded if the parents or guardians refused or were unable to give a valid, written informed consent.

Administration of Medicinal Products

Neonates were prescribed medicines for a variety of indications based on clinical judgement and according to unit protocols. All oral medicines were administered with feeds (milk) and given via a naso/ oral gastric tube or mixed into a bottle with milk. Nystatin (for oral candida prophylaxis) was administered locally into the mouth using a swab. Gentamicin was administered intravenously over 15 min.

Each 'dose' of MPB or PPB that the child received was derived and based on knowledge of the quantitative amounts of MPB and PPB within each medicinal product. Since MPB and PPB are present as a 'dual-system' preservative in many oral and intravenous medicines, the majority of neonates recruited into the study received multiple 'doses' of both preservatives each day from a range of formulations. The dosing history for every medicinal product that the child received for the duration of the study was diligently recorded on the case report form (CRF), and hence a complete record of the doses of MPB and PPB that each child received was able to be compiled for the analysis dataset.

Sample Collection and Analysis

Since the study was observational, blood samples were taken opportunistically from neonates at the same time as routine samples. Whole blood samples were collected using 15 μL Microsafe collection tubes and spotted on to Guthrie cards (Ahlstrom 226). The number of spots per card ranged between 2 and 4. The cards were dried for 4 h and then individually bagged with a silica sachet, placed in a grease proof envelope and stored in a −20°C freezer until analysis.

Analytical Methodology

A selective and sensitive HPLC–MS/MS assay for the parent PB molecules (MPB and PPB) was developed and validated using DBS samples. An 8 mm disc was punched (whole spot) from each DBS and extracted with methanolic solution of the internal standard (IS) benzyl-parabens. This was further subjected to solid phase extraction (SPE) using the Oasis HLB columns. The PB were separated by reversed phase HPLC separation, using a XBridge™ C18 column and combined with multiple reaction monitoring (MRM) mass detection using negative electrospray ionisation (ESI). This LC-MS/MS assay was validated according to US FDA guidelines with a limit of detection (LOD) of 10 ng/mL and a limit of quantification (LOQ) of 20 ng/mL. The calibration curve for both the PB was found to be linear over the range 0 to 1,000 ng/mL ($r2 = 0.995$) and the blood plasma ratio 1.0, which showed that the PB distributed equally in plasma and the whole blood. Inter- and intra-day variations for both the PB were established by the analysis of five replicate sets of each of the 5 concentrations (20, 40, 200 and 800 ng/mL) of the QC samples on five separate occasions. The inter-day precision was between 1.1 and 3.4% for MPB and 1.1 to 4.3% for PPB. The intra-day precision for MPB was between 0.05 and 0.1%, for PPB it was 0.02 and 0.14%. The accuracy for both the PB was well within ±15%.

Modelling Methodology

Data from each individual patient's CRF, comprising the parabens dosing pattern (arising from multiple formulations), sampling times for DBS collection and covariates of interest were reconciled with blood parabens concentrations and then pooled into a modelling dataset for kinetic modelling analysis. The population non-linear mixed effects modelling programme, NONMEM (v 7.2; Icon) and a gfortran compiler was used to undertake the model development. Post processing of NONMEM output was conducted using the software Perl-speaks-NONMEM (v 3.7.6), R(v 3.0.1) and Xpose (v 4.4.0).

The observed blood concentrations were log transformed prior to analysis. Concentrations between the LOD and LOQ were reported as actual values and therefore included in the modelling dataset. Parabens concentrations below the limit of detection (LOD) were reported as < 10 ng/ml. This data was also retained in the modelling dataset and contributed to the likelihood calculation by employing the M3 method in NONMEM as previously described (25). This method applies conditional likelihood estimation to the observations above and below LOD with the data below LOD being treated as categorical (aribtarily set to LOD/2 in the dataset). By simultaneous modelling of continuous and categorical data, the likelihood for below LOD data to be indeed below LOD

can be maximized with respect to the model parameters. All modelling was carried out using the LAPLACE method, adding the options 'numerical' and 'slow'.

Model development was progressed through four phases: (1) selection of appropriate structural model (2) selection of appropriate 'between-subject and residual variability (random effects) models (3) identification of influential covariates and (4) model evaluation. Model selection involved both statistical and graphical methods but ultimately the most parsimonious model which best explains the data was to be selected. Only models that minimised successfully were considered, whereas a successful covariance step was not required *a priori*. The objective function value, calculated using minus twice the log likelihood of the data, details the amount of variation explained in the model. If there was a difference of more than 3.84 when two nested models were compared then this was to be considered to be a statistically significant ($p<0.05$, 1 df) and relevant change. Visual inspection of diagnostic plots and individual concentration plots were also used to support model selection.

Structural Model

The observed PB blood concentrations revealed significant variability at all time points and in a small proportion of patients in whom multiple samples were available within dose intervals, it was clear that the rate and extent of absorption was substantially variable with often a lag phase. Hence, as well as initial attempts to model this part of the profile using first order, zero order or mixed first/zero order with or without lag phase, a transit compartment model where the absorption delay is modelled by passage of drug through a series of hypothetical transit compartments was attempted, to improve the goodness of fits (26). All subjects from Tartu, and selected subjects from other sites, received MPB and PPB intravenously (via gentamicin therapy) and hence bioavailability (F) for the oral medicines could be estimated. Systemic disposition was modelled using 1 and 2 compartment models.

Model for Random Effects

The between-subject variability and between-occasional variability in all parameters were modelled as exponential variance parameters. Since the observed data was log transformed, residual variability comprising unspecified within subject variability, model misspecification and experimental error was described using an additive error structure. A separate residual variability model was estimated for concentrations above and below the LOQ 20 ng/ml.

Covariate Selection

Once the structural and random effects model describing the EK characteristics of the parabens was developed, selected covariates were tested for their impact on the model parameters: weight, gestational age (GA), post menstrual age (PMA), post natal age (PNA), type of oral medicine (source of parabens), creatinine, liver function tests and haematocrit. Scatterplots of covariates against initial parameter estimates were examined to identify those factors that may have a potential influence in the model. A multivariable analysis was then performed in a forward addition and backward elimination fashion. For the forward addition step, a change in the OFV > 3.84 ($p<0.05$, 1df) was accepted as statistically significant whereas during the backward elimination step, a change in the OFV > 10.84 ($p<0.005$) was required for retaining a covariate in the model.

Model Evaluation

The software tool Perl-speaks-NONMEM was used to generate 500 replicates of the data by bootstrap (i.e. resampling from the original data with each individual subject as a sampling unit) for NONMEM analysis and to provide mean and 95% confidence intervals (95%CI) of the fixed-effect and random-effect parameters, and between subject variability estimates. The predictive performance of the model was evaluated by performing the prediction corrected-visual predictive check. The final model was used to simulate blood concentrations (500 replicates) of PB and the distribution compared with the distribution of observations.

RESULTS

A total of 202 neonates were recruited in to the study; in 12 subjects no bloods were taken following recruitment and in 9 subjects samples were rejected or failed bio-analysis. Whole blood PB concentration data were available for evaluation from 181 neonates. Table I provides a summary of the population demography.

Table II shows the range of medicines administered and the PB content. The mean (range) number of MPB and PPB doses administered per subject were 32.5 (1 – 254) and 20.3 (1 – 174) of which 95.5% and 92.8% were administered orally, respectively. The mean (range) duration of administration of PB containing medicines was 14 (1 – 50) days. The mean (sd) dose of MPB and PPB administered at any given time point was 0.58 (0.31) and 0.14 (0.1) mg/kg respectively.

A total of 841 blood MPB and PPB concentrations were available for analysis. The mean (range) number of MPB and PPB blood samples per subject were 4.6 (1–10) and 4.8 (1–10)

Table I Demography of study population

	Median (Range)
Number of Subjects	181
Gestational age at birth (days)	222 (169–292)
Postnatal age at recruitment (days)	5 (1–120)
Birth weight (kg)	1.6 (0.6–5.0)
Apgar scores at 5 mins	9 (1–10)
Maximum bilirubin (μM)	191 (17–290)
Serum creatinine (μM)	40 (14–156)
Haematocrit	0.4 (0.2–0.9)

respectively. Blood concentrations of MPB and PPB versus 'time after dose' plots are shown in Fig. 1. For both analytes, the range of observed concentrations was wide. The central tendency and dispersion of the observed PB concentrations are shown in Table III. Median concentration of MPB was only just above the LOD (10 ng/ml), whereas for PPB this value was below LOD. At least one quantifiable MPB blood level was observed in all but one subject and 55% of all MPB blood concentrations were above LOD. In contrast, blood PPB concentrations above the LOD were only observed in 87 (49%) patients and only 25% of all PPB blood concentrations were above LOD.

Excipient Kinetic Model

Since only a fraction (25%) of PPB observations were above LOD, preliminary attempts at modelling this data using the M3 method proved futile with lack of convergence, extreme sensitivity to initial estimates (suggesting numerous local minima), large 'Eta-shrinkage' and biased diagnostic plots. The analysis of the observed PPB blood concentration data was therefore limited to a descriptive nature.

The MPB data was amenable to modelling and disposition was best described by a two compartment open model. The use of a simpler model (i.e. one compartment) resulted in significantly worse fits ($p<0.05$). MPB absorption was described by a fixed first order rate constant (Ka). An attempt to describe the latency and slow, erratic absorption observed in the limited number of patients with more intense sampling, by implementing lag parameters and subsequently transit compartment absorption models was not supported by the data.

The inclusion of between-subject variability in total blood clearance (CL), volume of distribution in the central compartment (V2) and bioavailability (F) parameters decreased the objective function significantly and improve model fit. Covariance between random effects associated with CL and V2 were significant. The data did not support the inclusion of inter-occasion variability in drug absorption or disposition. No difference in the residual variability was identified in MPB concentrations above or below LOQ during model development and hence in the final model a single error model was estimated.

It was apparent during model development that there was a bimodal distribution of F. A substantially lower F could be attributed to 2 of the 10 oral products; nystatin and sodium feredate. Postnatal age, introduced as a categorical covariate (>21 days<), was the only covariate that showed a statistically significant effect on CL, whereas for V2, only bodyweight was a significant covariate. Table IV lists the parameter estimates obtained from the final population PK model together with the corresponding 95% CI which did not include the zero value for any parameter. Eta-shrinkage (%) was 31.06 (CL), 38.4 (V2), and 57.9 (F); ε-shrinkage was 6.9%.

Plots of observed versus model-predicted blood MPB concentrations indicated good correlation. Plots of weighted residuals versus time and predicted concentrations revealed no

Table II Parabens Containing Medicines Administered to the Study Population

Medicine	Indication	MPB content % (*w/v*)	PPB content (%*w/v*)
Alfacalcidol	Neonatal hypocalcaemia	0.15	–
Caffeine	Apnoea of permaturity	0.08–0.12	0.02–0.03
Domperidone	Gastric motility	0.18	0.02
Folic acid	Folate supplementation	0.07	0.014
Gentamicin[a]	Prophylaxis / Treatment neonatal sepsis	0.13	0.02
Hydrochlorthiazide	Diuretic for fluid overload	0.1	0.05
Multivitamins	Prevention of deficiency	0.15	–
Nystatin	Candida prophylaxis	0.1–0.137	0.2–0.46
Paracetamol	Analgesia	0.12–0.18	0.02–0.03
Potassium chloride	Replacement therapy	0.04	0.01
Ranitidine	Gastric acid reduction	–	0.015
Sodium feredetate	Prophylaxis/treatment of iron deficiency anaemia	0.1	0.02

a. Administered intravenously. All other medicines were administered orally

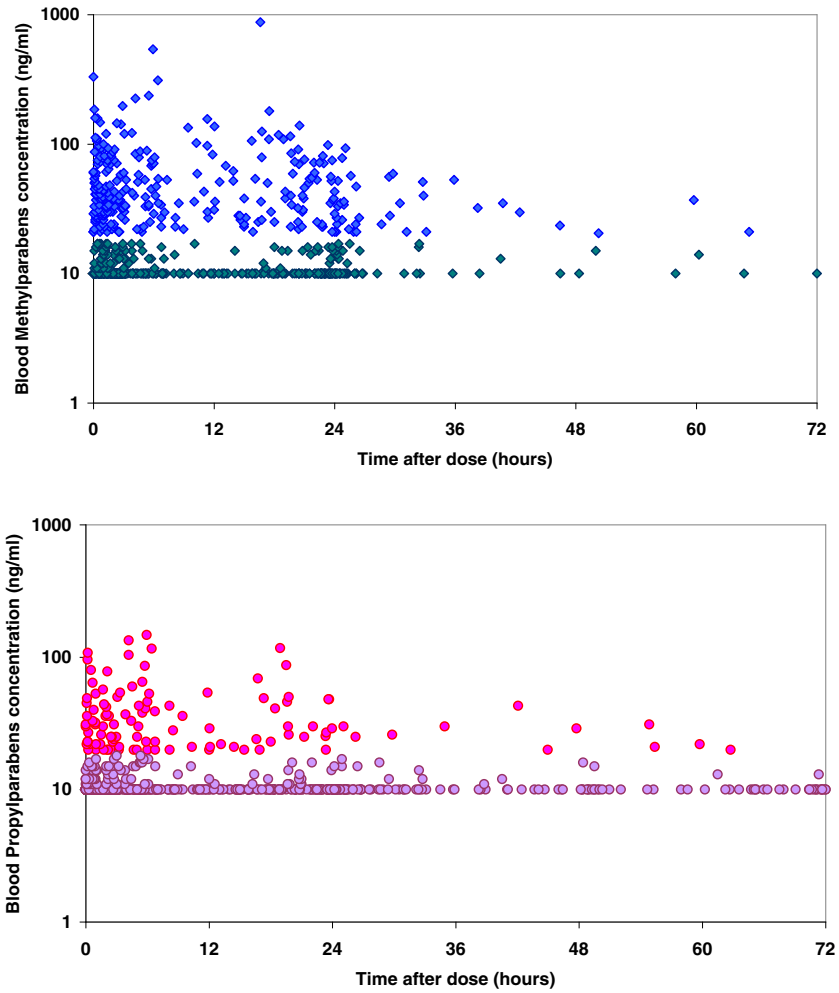

Fig. 1 Raw data plot: blood methylparabens (*diamonds*) and propylparabens (*circles*) concentrations versus time after dose. Data above and below the LOQ (20 ng/ml) are in contrasting colors. The LOD was 10 ng/ml.

systematic error (not shown). Figure 2 shows the results from the visual predictive check corresponding to 72 h post-dose. Both typical profiles and data dispersion were captured reasonably well. The 50th percentile of the observed data, apart from the first 6 h post-dose, is well contained within the 95% confidence intervals of the simulated 50th percentile. The relative scarcity of samples in the absorption phase and consequent simplification of the absorption model explains the disagreement between simulation and observed data.

Evaluation of the Covariate Effects

The impact of type of medicine and PNA on the MPB blood concentration profiles was explored by simulating a 1 kg neonate receiving regular 8 hourly doses of oral and intravenous MPB for 15 days. Figure 3 show that there is accumulation of blood MPB levels on repeated exposure with steady state peak concentrations in the range 40–125 ng/ml. The covariate impact of PNA and formulation type is evident; highest

Table III Median and Percentiles of Blood Parabens Concentrations (ng/ml)

Parabens	GA at birth(days)	N	% < LOD	Percentiles			
				25th	Median	75th	Maximum
Methylparabens	<210	407	47.3	<LOD	12.0	30.0	311
	>210	435	42.2	<LOD	15.0	30.0	874
	overall	842	45.4	<LOD	13.0	29.0	874
Propylparabens	<210	407	67.4	<LOD	<LOD	14.0	134
	>210	447	79.4	<LOD	<LOD	<LOD	147
	overall		73.9	<LOD	<LOD	11.0	147

Table IV Population Excipient Kinetic Model Estimates for Methylparabens

Parameter	Estimate	95% CI	BSV (CV%)	95% CI
$CL_{PNA<21}$ (L/h)	0.570	0.490, 0.650	43.5	39.2, 47.3
$CL_{PNA>21}$ (L/h)	0.880	0.715, 1.05	30.3	18.0, 38.9
V2 (L/1.6 kg)	1.84	1.57, 2.11	30.9	20.6, 38.5
Q (L/h)	12.2	4.63, 19.8	NE	
V3 (L)	35.4	27.1, 43.7	NE	
Ka (h^{-1})	0.10 fix		NE	
Infusion duration	0.25 fix			
F	0.0777	0.0277, 0.128	25.1	4.6, 35.2
F*	0.445	0.342, 0.548	70.2	28.5, 95.1
Additive residual error	0.44	0.38, 0.50		

Parameters are estimates with 95% confidence intervals (95% CI) from 500 bootstrap datasets. CV%: percentage coefficient of variation; *NE* not estimated

CLPNA <21, CLPNA >21 are total blood clearance in neonates of post natal age less than and greater than 21 days, respectively; Q is the intercompatmental distribution clearance between central and peripheral compartments; V2 and V3 are apparent volumes of distribution of the central and peripheral compartments, respectively; Ka, is the oral absorption rate constant; Infusion duration is the infusion period for intravenous gentamicin administration; F, is the oral bioavailability for nystatin and sodium iron feredetate; F*, is the oral bioavailability of all other oral medicines administered. Estimates of between subject variability (BSV) are shown as CV%. Additive residual error is expressed on the natural logarithmic scale

exposures are simulated in neonates of PNA<21 days who receive MPB intravenously.

DISCUSSION

Parabens are antimicrobial preservatives used in pharmaceutical dosage forms to minimise microbial contamination and growth during storage and use (10). In recent years, as a consequence of concerns about oestrogenic activity and reproductive toxicity, there has been an ongoing debate about their safety, particularly in the context of medicines for children. Concerns about potential endocrine disrupting properties of parabens have recently been assuaged to an extent by robustly conducted animal studies suggesting the lack of toxic effects (17,20). The EMA in a subsequent reflection paper concluded that the there is no substantive evidence to suggest that MPB is toxic and stated that it is safe to use in medicinal products for children of all ages (18). For PPB too, they deemed its use in children older than 2 years to be safe, with a permitted daily exposure, 'PDE', of 5 mg/kg. Judgement however was reserved for children less than 2 years since the toxicology studies did not include juvenile animals corresponding to the neonatal period. The ontogeny and maturation of liver and renal metabolic enzymes and xenobiotic elimination pathways have been

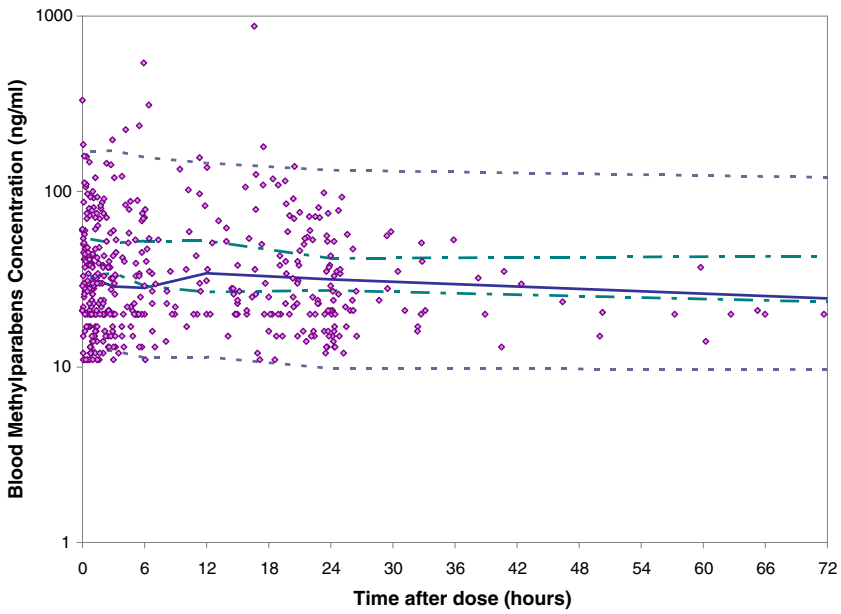

Fig. 2 EK model: visual predictive check for final model. Results from 500 simulations. Points, raw data. Solid line is the median raw data profile. Interrupted lines represent the 95% confidence intervals for the simulated 50th percentile. Dotted lines are the simulated medians for the 5th and 95th simulated percentiles.

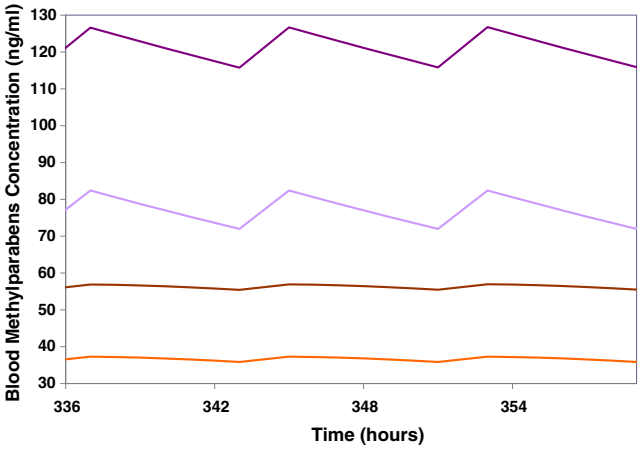

Fig. 3 Evaluation of covariate effects on MPB profiles: solid lines describe the EK profiles at steady state in a simulated 1 kg neonate administered 0.58 mg/kg MPB every 8 h for 15 days (**a**) iv dose, PNA <21 days (*purple*) (**b**) iv dose, PNA >21 days (*lavendar*) (**c**) oral dose, PNA <21 days (*brown*) (**d**) oral dose, PNA >21 days (orange).

shown to occur gradually over the first two years of life. Hence, the concern that systemic exposure to PPB could be significantly different in this vulnerable age group lingers and safety concerns relating to the use of PPB in the neonatal age group are yet to be fully resolved.

This study reports, for the first time, on systemic exposure to PB in pre-term and term neonates. Our findings indicate that routine administration of oral and intravenous medicines containing MPB and PPB results in not insignificant systemic exposure. In contrast to animal studies, where relatively little or no parent compound could be quantified, the present study quantified the presence of MPB and PPB in blood despite administering less than 1/200th of the pre-clinical doses.

Preservative efficacy of PB is optimised by combining hydroxybenzoates of short alkyl chains, and it is common for formulations to contain both MPB and PPB. Unsurprisingly therefore, only 2 of the 12 products administered to neonates recruited into this study were formulated with a single PB excipient. Although, median levels of both analytes were low (below LOD for PPB), a wide range of exposures were observed in the population. The maximum and overall dispersion of MPB was considerably higher than PPB and is explained by the difference in mean dose, 0.58 versus 0.14 mg/kg respectively. Even so, levels of PPB (parent compound only) in this group of infants are significantly higher than in healthy adult volunteers (maximum 147 vs 5.5 ng/ml respectively). This is particularly significant considering the study in adults reported "total" PPB levels i.e. parent and conjugated PPB fractions (21). The median and maximum levels of both MPB and PPB are also higher than in women from the general population who routinely used topical personal care products containing PB (22). Thus, despite the overall low PB exposure, blood levels should not be considered insignificant.

This is also the first clinical report to determine EK parameters for any PB. The EK model parameters were reasonably precisely estimated despite the complexity of the dataset. Specifically, there were multiple inputs into the model as a result of multiple-doses and multiple-formulations being administered to patients every day. The developed EK model for MPB contains significant unaccounted variability in MPB exposure. This variability is most likely due to a combination of DBS sampling and spotting errors and model misspecification (27). It is also possible that babies were exposed to PB from non-medicinal sources such as milk feeds or topical moisturisers, neither of which could be quantified and so included in the analysis dataset.

An interesting finding during model development was that oral bioavailability of MPB is significantly influenced by the type of oral medicine. This is not surprising since nystatin is generally applied topically to the oral mucosa using a swab. Hence, some of the drug may be retained on the swab and any ingested drug is likely to be absorbed very slowly, if at all. Bioavailability of MPB from a formulation of sodium feredetate was also found to be significantly lower for unknown reasons. The estimate of a large peripheral volume of distribution suggests that MPB distributes extensively in neonates, a not altogether surprising finding given that MPB (like all PB) is a lipophilic compound (log P value of 1.9). It suggests that PB compounds have the potential to be exposed to a wide range of tissues. Indeed, PB have been reported in human breast milk, seminal plasma and breast cancer tissue (21,28,29).

Blood clearance of MPB was found to be significantly correlated with PNA. Similarly in animal studies, a decrease in plasma PPB concentrations was observed in the exposure period during which juvenile rats grew into adulthood (17). While it is not possible to speculate the fate of the parent molecules in this study since metabolite profiles were not conducted, data from preclinical and human studies suggests that PB is largely excreted in urine as the non-specific PHBA or sulphate- or glucuronide conjugates (17,20,21,30). In animal models, PB compounds have shown to undergo extensive phase 1 metabolism by carboxylesterases to generate PHBA as the main metabolite (31). Carboxylesterases are major hydrolytic enzymes, ubiquitous in most organs including the intestines and liver, and are responsible for the metabolism of a range of therapeutic drugs and detoxification of xenobiotics (32). *In vitro* data suggests that the expression and activity of carboxylesterases in both mice and humans is age-related with significantly low levels in very early developmental stages (33).

Results from this study reveal that daily, repeated administration of pharmaceutical dosage forms containing MPB and PPB can result in prolonged systemic exposure to the parent compound in neonates. Simulations from the final model show that regular three times a day administration of oral and intravenous MPB to neonates results in accumulation and

population steady state peak blood concentrations up to 125 ng/ml. In contrast, toxicokinetic studies of PPB in rats have either been unable to identify the parent compound or only after doses in excess of 100 mg/kg suggesting rapid and extensive metabolic transformation (17,20). However, as previously identified, the period studied in the rats corresponded to the human development period from approximately 2 years of age and did not address the neonatal period (18). Age related activity of carboxylesterases could have significant influence on the kinetics of PB compounds (33). Hence, premature babies with less than mature carboxylesterase activity and therefore reduced metabolic capacity may indeed experience higher systemic exposure to the parent PB compound than older children, adolescents or adults. Whilst the current evidence suggests that the use of MPB as a product preservative is not a concern for humans regardless of age, a PDE of 5 mg/kg has been proposed for PPB based on divergent reports on the effects on the male and female reproductive systems (18). The proposed PDE for PPB is limited to children over the age of 2 years in the absence of animal data corresponding to the neonatal period. The results of this observational EK study therefore tends to support the EMA view that toxicological studies of PPB in a relevant animal model and covering the neonatal period is required before a PDE for this age group can be established (18). Further clinical studies of the relationship between blood PPB concentration, tissue exposure and clinical endpoints (effects on reproductive organs) are also warranted.

In conclusion, we report the first clinical study of systemic PB exposure in neonates. The study reveals that routine administration of medicines containing PB results in not insignificant blood concentrations of the parent molecules, MPB and PPB. An EK model of MPB was developed, revealing that bioavailability is influenced by the type of oral medicine. A 2-compartment disposition model with a large peripheral volume suggests MPB distributes extensively and blood clearance is correlated with PNA. Simulations using the final model reveal that continuous dosing with MPB results in significant accumulation. The results of this study support the view that animal toxicological studies of PPB that specifically address the neonatal period are urgently required before a PDE for this age group can be established.

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