Pharmacokinetics of melatonin in preterm infants

Nazakat M. Merchant,^{1,2,3,4} Denis V. Azzopardi,^{1,2,3,4}
Ahmed F. Hawwa,⁶ James C. McElroy,⁶ Benita Middleton,⁵ J. Arendt,⁵
Tomoki Arichi,^{1,2,3,4} Pierre Gressens^{3,7} & A. David Edwards^{1,2,3,4}

¹Centre for the Developing Brain, King's College London, ²Centre for the Developing Brain, Imperial College, ³Division of Neonatology, Imperial College Healthcare NHS Trust, ⁴Division of Neonatology, Guy's and St Thomas' NHS Trust, London, ⁵Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, ⁶Clinical and Practice Research Group, School of Pharmacy, Medical Biology Centre, Queen's University, Belfast, UK and ⁷Inserm, U676, Paris, France

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Preterm infants are deprived of the normal intra-uterine exposure to maternal melatonin.
- Normal plasma melatonin concentration is presumed to be low in the preterm population based on measurements of urinary metabolites.
- No pharmacokinetic data are available to guide therapeutic trials of melatonin.

WHAT THIS STUDY ADDS

- Preterm infants have no or minimal circulating plasma melatonin.
- The half-life of melatonin in the preterm population is approximately 15 h.
- Melatonin concentrations closest to adult concentrations were achieved with a 2 h infusion of 0.1 $\mu\text{g kg}^{-1} \text{ h}^{-1}$ of melatonin.

Correspondence

Professor David Edwards, Centre for the Developing Brain, King's College London, St Thomas' Hospital, Westminster Bridge Road, London SE1 7EH, UK.
Tel.: +020 7188 8364
Fax: +020 7188 9154
E-mail: ad.edwards@kcl.ac.uk

Key words

6-sulfatoxymelatonin, melatonin, neuroprotective agents, pharmacokinetics, preterm infants

Received

21 November 2012

Accepted

7 February 2013

Accepted Article Published Online

21 February 2013

AIMS

Preterm infants are deprived of the normal intra-uterine exposure to maternal melatonin and may benefit from replacement therapy. We conducted a pharmacokinetic study to guide potential therapeutic trials.

METHODS

Melatonin was administered to 18 preterm infants in doses ranging from 0.04–0.6 $\mu\text{g kg}^{-1}$ over 0.5–6 h. Pharmacokinetic profiles were analyzed individually and by population methods.

RESULTS

Baseline melatonin was largely undetectable. Infants receiving melatonin at 0.1 $\mu\text{g kg}^{-1} \text{ h}^{-1}$ for 2 h showed a median half-life of 15.82 h and median maximum plasma concentration of 203.3 pg mL^{-1} . On population pharmacokinetics, clearance was 0.045 L h^{-1} , volume of distribution 1.098 L and elimination half-life 16.91 h with gender ($P = 0.047$) and race ($P < 0.0001$) as significant covariates.

CONCLUSIONS

A 2 h infusion of 0.1 $\mu\text{g kg}^{-1} \text{ h}^{-1}$ increased blood melatonin from undetectable to approximately peak adult concentrations. Slow clearance makes replacement of a typical maternal circadian rhythm problematic. The pharmacokinetic profile of melatonin in preterm infants differs from that of adults so dosage of melatonin for preterm infants cannot be extrapolated from adult studies. Data from this study can be used to guide therapeutic clinical trials of melatonin in preterm infants.

Introduction

In recent decades the survival rate of very premature infants has increased significantly but the incidence of neurological impairment and disability has remained rela-

tively constant or even increased [1–3]. In babies with a birth weight less than 1500 g or less than 28 weeks gestation approximately 10% later exhibit cerebral palsy and 50% cognitive or behavioural deficits [4,5]. These morbidities are associated with significant psychological and

economic costs both to the families and society [6, 7]. Currently, apart from neonatal intensive care there are no specific neuroprotective therapies available for these vulnerable infants.

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring hormone with potent neuroprotective properties in animal models [8–11] and favourable pharmacological and toxicological properties that make it an attractive candidate for clinical studies of neuroprotection in the preterm. Normally secreted with a marked diurnal variation by the pineal gland, melatonin influences the sleep–wake cycle [12] and is important for normal neurodevelopment and embryonic growth [12, 13]. The fetus does not secrete melatonin but receives melatonin by rapid trans-placental transfer according to the maternal circadian secretion [14]. In contrast preterm infants are deprived of the maternal circadian melatonin secretion. Preterm birth does not induce melatonin secretion, and circadian pineal hormone production is only apparent approximately 12 weeks after delivery [15]. The onset of pineal secretion appears even more delayed when infants are exposed to neurological insults [16].

Although the pharmacokinetic profile of melatonin has been clearly documented in adults [17, 18], there is a lack of pharmacokinetic data in neonates. This impedes the planning of therapeutic trials. The aims of the study were thus to conduct a dose-ranging study, to determine a general pharmacokinetic model for melatonin administration in preterm infants and to determine a specific dosage scheme that would allow replication of adult and thus fetal melatonin concentrations for studies of replacement therapy.

Methods

The study had approval from Hammersmith and Chelsea Research Ethics Committee and the UK Medicines and Healthcare Products Regulatory Agency and was registered in the International Standard Randomized Controlled Trial Number Register (ISRCTN 01115788, EUDRACT no. 2007-007156-33). All procedures in the study were in accordance with the Helsinki Declaration of 1975 (as revised in 1983). Written parental consent for participation was obtained for all trial participants.

We conducted an open label dose ranging study between May 2010 and December 2010 in three neonatal intensive care units in the UK. Infants born less than 31 weeks gestation and less than 7 days old were eligible for the study, although those with major congenital malformation or cystic periventricular leucomalacia or haemorrhagic parenchymal infarcts on cranial ultrasonography prior to enrolment were excluded. A maximum of four infants were recruited at each dosing concentration. Indications to stop the infusion of melatonin were withdrawal of parental consent or any suspected unexpected serious

adverse reaction, defined as unexplained deterioration in baby's condition such as severe worsening of respiratory and cardiovascular functions, development of seizures or acute gastrointestinal complications.

Formulation

There is no licensed intravenous formulation available in the UK. Consequently a melatonin injection 0.1 µg in 1 ml was formulated (Stockport Pharmaceuticals, Stockport, UK). The formulation used consisted of melatonin (0.1 µg), sodium chloride (9 mg) and water for injection as solvent (to 1 ml). Stability was assessed by the West Midlands Regional Quality Control Laboratory (Birmingham, UK). The product was terminally sterilized using a standard pharmaceutical cycle 121°C for 18 min.

Treatment regime

Each infant received one dose of melatonin as an intravenous infusion. Extrapolating from adult pharmacokinetic data, the estimated starting rate of infusion of melatonin was $0.174 \mu\text{g kg}^{-1} \text{h}^{-1}$, based on a clearance of $0.05 \text{ l min}^{-1} \text{kg}^{-1}$ and a concentration of 58 pg ml^{-1} [19]. The first dose administered was $0.1 \mu\text{g kg}^{-1} \text{h}^{-1}$ for 6 h. Successive groups received varying doses depending on the melatonin blood concentrations until mean adult physiological concentrations were achieved (approximately $46\text{--}58 \text{ pg ml}^{-1}$). The dose regimens given were $0.1 \mu\text{g kg}^{-1} \text{h}^{-1}$ intravenously for 6 h, $0.1 \mu\text{g kg}^{-1} \text{h}^{-1}$ for 2 h, $0.02 \mu\text{g kg}^{-1} \text{h}^{-1}$ for 2 h, $0.01 \mu\text{g kg}^{-1} \text{h}^{-1}$ for 2 h and $0.04 \mu\text{g kg}^{-1}$ over 30 min.

Investigations

Capillary, venous or arterial blood was collected for melatonin concentrations at various time points. The samples were centrifuged within 30 min at $3000 \text{ rev min}^{-1}$ for 10 min and frozen to -20°C until assay. Urine samples were collected as 2 hourly samples for melatonin and its primary metabolite 6-sulfatoxymelatonin (aMT6s) and frozen to -20°C until assay. Melatonin and aMT6s were analyzed by specific radioimmunoassay (RIA) at the University of Surrey, Guildford, UK based on a method described by Arendt and coworkers [18, 20]. All plasma samples were assayed at 1:5 dilution (100 µl volume) then diluted 1:10 whenever possible and checked twice at both dilutions and the mean value was taken. The urine samples were assayed at 1:250 dilutions and some at 1:25 dilution. The detection limit of the plasma melatonin assay was 3 pg ml^{-1} ; that of urine aMT6s was 0.2 ng ml^{-1} and of urine melatonin was 0.03 ng ml^{-1} .

Pharmacokinetic modeling

The elimination half-life, clearance (CL), volume of distribution (V) and area under the curve (AUC) were calculated for each individual using STATA 11 (Stata Corporation, College Station Tx).

Population modelling was carried out by means of non-linear mixed-effect modelling using first order conditional estimation with interaction (NONMEM version VII, level 2.0, ICON Development Solutions, Ellicott City, MD) [21].

Step 1: Determination of the basic pharmacokinetic model Infants were allocated into two groups which had least variable data ($0.1 \mu\text{g kg}^{-1} \text{h}^{-1}$ vs. $0.01\text{--}0.02 \mu\text{g kg}^{-1} \text{h}^{-1}$) and the pharmacokinetic parameters of melatonin in each group were estimated and compared. In addition, the combined dataset was used for development of a population pharmacokinetic model that described the influence of patient variables on melatonin disposition. Potential models considered were classical linear one and two compartment models. Inter individual variability (IIV) in the estimated parameters CL and V were modelled using an exponential error model, as their distribution was often right skewed [22]. The covariance of CL and V was also incorporated into the model. However, when each group was analysed separately, variability between individuals was combined with the residual variability. Proportional and additive components of residual variability were estimated as follows: $C_{ij} = C_{predij} \times (1 + \epsilon_{pij}) + \epsilon_{Aij}$, where C_{ij} is the measured and C_{predij} is the model predicted melatonin concentration of the i^{th} individual at the j^{th} sampling time and ϵ_{ij} is the residual error term, which is a random variable with zero mean and variance of σ^2 .

However, simplification was considered during the model building by deleting the additive residual variance component as its value was close to zero. Body weight was included as an *a priori* covariate on CL and V and was scaled to the median weight of the studied population as follows [22, 23].

$$CL_i = \theta_{CL} \times \left(\frac{WT_i}{WT_{\text{median}}} \right)^{0.75} \quad V_i = \theta_V \times \left(\frac{WT_i}{WT_{\text{median}}} \right)$$

where CL_i and V_i are the CL and V of the i^{th} individual with weight (WT_i) and θ_{CL} and θ_V are the mean population estimates of CL and V in a standard individual with median weight (WT_{median}).

Step 2: Selection of the influential covariates The following covariates were tested for inclusion into the model: gestational age, postnatal age, gender, race, serum creatinine, bilirubin and urea concentrations. Visual examination of scatter plots (or Box and Whisker plots in the case of categorical covariates) of empirical Bayesian estimates and IIV variability obtained for each pharmacokinetic parameter from Step 1 vs. each covariate were used to help identify whether the pharmacokinetic parameter might be significantly related to the covariate. Direct covariate testing was then performed to see if this relationship was significant.

Step 3: Development of the final covariate population pharmacokinetic model The final model was established using the forward inclusion–backward elimination method [24]. Forward inclusion of a covariate required a reduction in the minimum value of the objective function (MOFV) of at least 3.84 ($P < 0.05$, d.f. = 1). However, the level of significance for retaining a covariate in the model during backward elimination was set at a more stringent ΔMOFV of >10.83 ($P < 0.001$, d.f. = 1). Only covariates that showed a significant contribution were kept in the final model. Graphical inspection of goodness-of-fit was used throughout model building and evaluation [25] by examining the residuals (RES), conditional weighted residuals (CWRES) and measured melatonin concentrations plotted separately against predicted concentrations.

Results

Demographic and clinical data (Table 1)

Eighteen preterm infants (nine male, nine female) born before 31 weeks gestation and less than 7 days of post-natal age were enrolled into the study. The median (range) birth weight was 867.5 g (610–1430 g) and gestation at birth was 26.63 weeks (24.71–30.28 weeks). The median age at melatonin infusion was 2 days (range 1–6 days). All infants were receiving some human donor or maternal milk and none received artificial milk formula. All the preterm infants were treated with caffeine (12.5 mg kg^{-1} loading dose, followed by 6 mg kg^{-1} once a day) and 16 out of 18 were receiving total parenteral nutrition. Six of 18 infants were receiving intravenous co-amoxiclav but none had confirmed sepsis. No infant was withdrawn from the study or required the infusion to be stopped early. No adverse events related to the melatonin infusion occurred.

Individual pharmacokinetic data

Table 2 and Figure 1 give the measured concentrations of melatonin in plasma. Pre-infusion median plasma melatonin concentrations were not detectable except in one infant who had a pre-infusion concentration measured as 38.5 pg mL^{-1} . At the starting dose of $0.1 \mu\text{g kg}^{-1} \text{h}^{-1}$ for 6 h, the median (range) plasma melatonin concentrations at the end of the 6 h infusion were 393.3 pg mL^{-1} ($339.3\text{--}554.5 \text{ pg mL}^{-1}$) but steady-state concentrations had not

Table 1

Patient characteristics

Patient characteristics (n = 18)	Median (range)
Post-menstrual age at birth (weeks)	26.63 (24.71–30.28)
Birth weight (g)	867.5 (610–1430)
Post natal age (days)	2 (1–6)
Gender (M : F)	1:1

Table 2

Plasma melatonin concentrations at different doses

Dose (number of patients)	Time (h)	Median plasma melatonin (range) (pg ml ⁻¹)
0.1 µg kg ⁻¹ h ⁻¹ for 6 h (n = 4)	0	0 (0–38.5)
	1	31 (0–77)
	3	153.3 (145.5–317.5)
	6	393.3 (339.3–554.5)
0.1 µg kg ⁻¹ h ⁻¹ for 2 h (n = 4)	0	0 (0–0)
	2	203.3 (160–220)
	6	145.4 (110.8–156.8)
	24	71.6 (51.8–79)
0.02 µg kg ⁻¹ h ⁻¹ for 2 h (n = 6)	0	0 (0)
	2	48.7 (38.8–71)
	4	52 (41–65)
	5	64 (49.5–67)
	6	63 (0–71)
0.01 µg kg ⁻¹ h ⁻¹ for 2 h (n = 2)	2	54.8 (43.5–66)
	4	110 (110)
	5	103.8 (103.8)
	6	97.5 (97.5)
	24	28 (28)
	48	38.8 (38.8)
0.04 µg kg ⁻¹ for 0.5 h (n = 2)	1	58.5 (42–75)
	72	120.3 (110.5–130)
	96	108 (97–118.5)
	336	74.8 (66.5–83)

Lower doses of melatonin infusion (0.02 µg kg⁻¹ h⁻¹ for 2 h, 0.01 µg kg⁻¹ h⁻¹ for 2 h and 0.04 µg kg⁻¹ over 30 min) gave highly variable plasma melatonin concentrations and unreliable estimates of pharmacokinetics in individuals. Not detectable concentrations were taken as 0 pg ml⁻¹.

been reached. Melatonin concentrations achieved with a 2 h infusion of 0.1 µg kg⁻¹ h⁻¹ were median (range) of 203.3 pg ml⁻¹ (160–220 pg ml⁻¹). At this infusion dose, the median (range) half-life was 15.82 h (11.3–21.0 h) and AUC was 1665.08 pg ml⁻¹ h (1392.06–1992.14) while the CL was 0.06 l h⁻¹ kg⁻¹. Lower doses of melatonin infusion (0.02 µg kg⁻¹ h⁻¹ for 2 h, 0.01 µg kg⁻¹ h⁻¹ for 2 h and 0.04 µg kg⁻¹ over 30 min) gave highly variable plasma melatonin concentrations and unreliable estimates of pharmacokinetics in individuals. Urine melatonin and urine aMT6s concentrations were negligible at all doses.

Population pharmacokinetic modelling

Data from 16 infants were used in the population pharmacokinetic modelling. Two infants who were given infusion rates of 0.04 µg kg⁻¹ over 30 min were not included as the results were highly variable.

Base model The one compartment model with first order elimination adequately described the disposition of melatonin in plasma. The resulting estimates of CL and V when the base model was fitted to each group of preterm neonates receiving either 0.1 µg kg⁻¹ h⁻¹, 0.02 µg kg⁻¹ h⁻¹ or 0.01 µg kg⁻¹ h⁻¹ melatonin dose are shown in Table 3. In

Figure 1

Plasma (log) melatonin concentrations vs. time curve. Data fitting was performed using a robust non-linear fitting algorithm as implemented in the curve fitting toolbox in MATLAB (2011, The Mathworks, Natick, MA, USA). Blue circles show the data points for the dose of melatonin at 0.1 µg kg⁻¹ h⁻¹ for 2 h. Red crosses show the data points for the dose of melatonin at 0.1 µg kg⁻¹ h⁻¹ for 6 h. On giving melatonin as a continuous infusion of 0.1 µg kg⁻¹ h⁻¹ for 6 h, steady-state concentrations were not reached. On giving melatonin at the dose of 0.1 µg kg⁻¹ h⁻¹ for 2 h there was a time dependent decrease in plasma melatonin concentrations suggesting first order kinetics. The graph also demonstrates that melatonin has a long half-life in preterm infants as compared with adults again highlighting the fact that the pharmacokinetic profile in preterm infants differs significantly from adults. As the dose is the same for the first 2 h, data were used in both to make the model fit more accurately, although data points have been plotted separately

Table 3

Pharmacokinetic parameter estimates of melatonin

Parameter estimates	0.1 µg kg ⁻¹ h ⁻¹	0.02 µg kg ⁻¹ h ⁻¹ or 0.01 µg kg ⁻¹ h ⁻¹
Observations	23	23
Number of patients	8	8
CL (l h ⁻¹ /0.867 kg)	0.045 (7.7%)	0.012 (15.4%)
V (l/0.867 kg)	1.098 (11.5%)	0.364 (32.1%)
t _{1/2} (h)	16.91	21.02
Residual variability (CV%)	30.24 (43.0%)	62.93 (17.5%)

Obtained from fitting the base model to two subsets of patients receiving the 0.1 µg kg⁻¹ h⁻¹ (over 2 h and 6 h) or the lower doses of melatonin infusion (0.02 µg kg⁻¹ h⁻¹ for 2 h, 0.01 µg kg⁻¹ h⁻¹ for 2 h). Pharmacokinetic parameter estimates are presented as mean (RSE%) and are scaled to a median weight of 0.867 kg. Variance terms are presented as CV% of the estimate (%RSE), where %RSE = $\frac{\text{standard error of estimate}}{\text{mean estimate}}$.

the combined dataset, the covariance matrix showed high correlation ($p = 0.99$) between CL and V random variables and, as a result, the model was reduced to a shared variance model:

$$CL_i = \theta_{CL} \times \exp(\eta_{i,CL}) \text{ and}$$

Table 4

Stages of the model-building process to reach the final pharmacokinetic model of melatonin in the combined dataset

Parameter	Base model (WT-power allometric; full covariance matrix)	Reduced base model (shared variance model)	Intermediate model (Gender-prop on V)	Final model (Gender-prop on V; Race-exp on CL and V)
Observations	46	46	46	46
Patients	16	16	16	16
MOFV	381.97	381.97	378.05	360.91
P value	–	–	0.047*	3.48×10^{-5} *
CL (l h ⁻¹)	0.02	0.020 (0.39)	0.021 (0.37)	0.021 (0.63)
V (l)	0.75	0.75 (0.14)	0.64 (0.14)	0.74 (0.08)
t _{1/2} (h)	26.10	26.10	21.10	24.30
θ _{gender}	–	–	0.47 (0.67)	1.73 (0.20)
θ _{race}	–	–	–	1.09 (0.13)
ω _{CL} (CV%)	111.80	111.90 (0.47)	103.0 (0.48)	111.90 (0.75)
Corr _{CL,V}	0.999	–	–	–
ω _V (CV%)	51.70	–	–	–
θ _{corr}	–	0.46 (0.26)	0.47 (0.26)	0.215 (0.57)
σ _{prop} (CV%)	24.90	24.90 (0.29)	24.40 (0.30)	22.90 (0.29)

Parameters are presented as mean parameter estimate (CV% in the estimate). MOFV: Minimum value of objective function; ΔMOFV indicates a change in MOFV relative to the previous model; *ΔMOFV of –3.84 is significant at $P < 0.05$ (1 d.f.). Weight (WT) is scaled to a median of 0.867 kg; θ_{race}: fractional difference from θ_{CL} and θ_V at a certain value of Race; θ_{gender}: fractional difference from θ_V at a certain gender value; ω: Inter-individual variability; Corr_{CL,V}: correlation between CL and V variability parameters; θ_{corr}: shared variance between CL and V; σ: residual variability.

$$V_i = \theta_V \times \exp(\theta_{\text{corr}} \cdot \eta_{i,\text{CL}})$$

where $\eta_{i,\text{CL}}$ is a random variable (zero mean and a variance of ω_{CL}^2) that distinguishes the i th individual's parameter (CL _{i}) from the population mean values. θ_{corr} is the shared variance between CL and V as described by the following equation:

$$\theta_{\text{corr}} = \omega_V / \omega_{\text{CL}}$$

Covariate screening and selection of the final model During forward selection of covariates, inclusion of gender as a covariate affecting V in the model, as shown below, resulted in significant decrease in the MOFV (3.92 unit; $P = 0.047$). Inclusion of race as a covariate affecting both CL and V using an exponential model resulted in further 17.1 unit decrease in the MOFV ($P < 1 \times 10^{-4}$) and significant improvement in goodness of fit and was therefore included in the final model (Table 4). In the backward elimination step, the effect of both covariates was confirmed since the MOFV increased by more than 10.83 units ($P < 0.01$, d.f. = 1) when either of them was removed from the model. None of the other tested covariates, including serum creatinine, urea and bilirubin concentrations resulted in any significant improvement of the model predictions. The final model for melatonin was, therefore, as follows:

$$\text{CL (L h}^{-1}\text{)} = \theta_{\text{CL}} \times \left(\frac{\text{WT}}{0.867} \right)^{0.75} \times \exp(-\theta_{\text{race}} \times \text{Race})$$

$$V (\text{l}) = \theta_V \times \left(\frac{\text{WT}}{0.867} \right) \times (1 + \theta_{\text{gender}}) \times \exp(-\theta_{\text{race}} \times \text{Race})$$

where θ_{CL} and θ_V are the population estimates for CL and V, respectively, standardized to a median weight of 0.867 kg using allometric models. θ_{race} is a parameter estimating the fractional difference from θ_{CL} and θ_V at a certain value of race. θ_{gender} describes the fractional difference from θ_V at a certain gender value.

According to this final model, the estimates for CL and V in a typical individual who weighs 867 g, are 0.021 l h⁻¹ and 0.74 l, respectively, resulting in an elimination half-life of 24.3 h. The IIV associated with the shared variance between CL and V was reduced from 46.2% (base model) to 20.5% (final model), i.e. a reduction of 55.6% of the initial value. The residual variability (CV%) in final model was 29.8%.

Graphical evaluation

Plots of observed against population and individual predicted melatonin concentrations in the final model are shown in Figure 2A, B.

Discussion

Although melatonin has been administered to newborn infants in a variety of doses [26–33], a systematic analysis of the pharmacokinetic profile in preterm infants has not previously been available.

Figure 2

Plots of measured vs. population predicted (A) and individual predicted (B) melatonin concentrations from the final model as well as plots of residuals (C) and conditional weighted residuals (D) vs. population predicted melatonin concentrations

In adults, the melatonin pharmacokinetic profile has been well defined [17, 18]. There is a circadian secretion of endogenous melatonin with a marked diurnal variation. Secretion usually starts by 21.00 h and peaks at 03.00 h, then plasma concentrations decline to negligible levels by 09.00 h [17, 34]. The adult peak melatonin concentration is reported to be 44.3 pg ml⁻¹ but can range from ≤8–275 pg ml⁻¹ [34, 35]. Circulating melatonin is predominantly metabolized by hydroxylation at the C-6 position in the liver and then conjugated with sulfate to form aMT6s or to a lesser extent with glucuronide (6%). Less than 1% of circulating melatonin is excreted unchanged into urine [19, 36]. The elimination half-life of melatonin in adults is 45–60 min [37]. Urine melatonin concentrations in adults are variable but usually within the range of 38.2–179.1 (ng) in 24 h samples [38] and, for example, a reported mean excretion between 18.00 h–10.00 h of 29.7 ng (range 9–59.1 ng) [39]. Mean aMT6s excretion in adults was 11100 ng (range 4100–24200) in a 16 h period 18.00 h–10.00 h [33, 35]. Endogenous plasma melatonin concentrations generally correlate well with urinary melatonin and aMT6s, although after administration of 3 mg mela-

tonin to adults there was good correlation between plasma melatonin and urinary melatonin but not aMT6s [39].

Based on adult pharmacokinetic profiling we chose a starting dose of 0.1 µg kg⁻¹ h⁻¹ for 6 h. This resulted in plasma melatonin concentrations higher than predicted and did not achieve steady-state at 6 h. Reliable pharmacokinetic data and melatonin concentrations closest to physiological adult concentrations were achieved with a 2 h infusion of 0.1 µg kg⁻¹ h⁻¹ with a time dependant decrease in plasma melatonin concentrations as seen in Figure 1, suggesting first order kinetics. Lower doses of melatonin infusion (0.02 µg kg⁻¹ h⁻¹ for 2 h, 0.01 µg kg⁻¹ h⁻¹ for 2 h and 0.04 µg kg⁻¹ over 30 min) gave unreliable estimates of pharmacokinetics in individuals, most likely due to the very small quantities of melatonin involved.

The population pharmacokinetic one compartment model with first order elimination adequately described the disposition of melatonin in plasma. The results shown in Figure 2C, D indicate that the assumption of random effects was appropriate because both residuals and conditional weighted residuals were evenly distributed around 0

and almost all conditional weighted residuals were contained within ± 2 units of the null ordinate indicating absence of any influential observations. The limited number of samples collected shortly after melatonin administration did not permit accurate evaluation of the distribution phase and hence the two compartment model did not provide a better fit to the data. The model revealed considerable variation in CL and V which was reduced when race and gender were incorporated as covariates, reducing the shared variance of CL and V by over 50%. A plausible explanation for the effect of race on melatonin CL could be the variation between races in the allele frequency of polymorphisms affecting melatonin 6-hydroxylation by CYP1A2 (hepatic clearance) and CYP1B1 (extra-hepatic clearance) [40, 41]. Allometric size adjustment, with fixed exponents of 0.75 for CL and 1 for V , was used for *a priori* inclusion of weight. Investigation of models where the power values were not fixed but included as additional θ s, did not result in any significant improvement in model fit.

Compared with adults and older children, in preterm infants melatonin half-life and CL were prolonged and V decreased. This could be related to several factors. Melatonin is extremely lipophilic and the lower body fat content in preterm infants (10%) as compared with adults and children (20–25% and 15–20%) would affect the V and may contribute to higher than expected plasma concentrations [42]. Some drug interactions can increase melatonin concentrations. Caffeine is known to increase the concentrations of melatonin in the blood by competing for the same metabolic pathway in adults and acts as a substrate for CYP1A2 (43). Caffeine improves outcomes for preterm infants and is routine in neonatal care [43]. All infants in this study received caffeine. Plasma melatonin concentrations assayed may be higher due to heparin in the circulation, but in our study venous or capillary blood samples were obtained in all except one infant where samples were obtained from a heparinized arterial line. Plasma melatonin may also be higher if there is a delay in extraction of the plasma resulting in drug diffusing out of the erythrocytes into the plasma.

However, the most likely cause of the observed pharmacokinetic profile is immature liver metabolism and poor renal excretion in preterm infants [44]. Urine aMT6s and melatonin concentrations were very low, suggesting that preterm infants do not excrete melatonin as rapidly as expected from adult studies. This does not appear to be linearly related to post-menstrual age, as we found no significant difference in the plasma melatonin and urine aMT6s concentrations at different gestations, although the numbers in the study were small. It would have been useful to confirm that the observed pharmacokinetic profile was related to immature metabolism and renal excretion. Unfortunately it was not possible to measure aMT6s in the blood due to limitations in the amount of blood that can ethically be collected from this vulnerable population

group according to international guidelines [45]. Whatever the cause, the long half-life of melatonin in the preterm infants is unlikely to reflect the normal fetus where transplacental equilibration is likely to control blood concentrations more than renal or hepatic drug handling. It may thus be difficult for experimentalists to recreate the fetal diurnal rhythm in preterm infants.

Previous studies have suggested that preterm infants do not secrete melatonin until at least 52 weeks post conception [15] and in the present study melatonin was undetectable before administration in all infants except one. All subjects received maternal or human donor milk which was a potential source of exogenous melatonin, being present in human milk with a peak concentration of $23 \pm 6 \text{ pg mL}^{-1}$ [46]. However since the infants were studied at a median 2 (1–6) days of age the volume of milk feeding was small and milk was unlikely to account for the detectable melatonin in this single baseline sample. The high melatonin concentration in one infant on day 2, prior to the infusion could reflect maternal melatonin and given the prolonged clearance there may have been some transplacental melatonin but his twin had undetectable melatonin concentrations suggesting that the result was spurious.

We administered melatonin by intravenous infusion because previous studies suggested that oral melatonin has variable bioavailability [47, 48] and 30–60% is immediately metabolized to aMT6s [46]. All the infusions were given during daytime, and the long half-life detected suggests that melatonin given once daily would be adequate to attain physiological melatonin concentrations i.e. the same as adult concentrations.

In conclusion, a 2 h infusion of $0.1 \text{ } \mu\text{g kg}^{-1} \text{ h}^{-1}$ increased plasma melatonin from undetectable to approximately peak adult concentrations. Slow clearance makes replacement of a typical maternal circadian rhythm problematic. The pharmacokinetic profile of melatonin in preterm infants differs from that of adults so dosage of melatonin for preterm infants cannot be extrapolated from adult studies. Data from this study can be used to guide therapeutic clinical trials of melatonin in preterm infants.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare the authors had support from the Medical Research Council as part of the grant for the submitted work and Alliance Pharmaceuticals for providing the drug and support for regulatory compliance, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

Author contributions

N. Merchant contributed to conception and design, acquisition of data and analysis, interpretation of data, and drafting of the article.

D. Azzopardi contributed to conception and design, interpretation of data, and drafting of the article.

A. Hawwa contributed to analysis and interpretation of data, and drafting of the article.

J. McElnay contributed to analysis and interpretation of data, and drafting of the article.

B. Middleton contributed to analysis and interpretation of the data.

J. Arendt contributed to analysis and interpretation of data, and drafting of the article.

T. Arichi contributed to analysis of data and drafting of the article.

P. Gressens contributed to conception and design of the study, interpretation of the data and drafting of the article.

A.D. Edwards contributed to conception and design, analysis and interpretation of data, drafting of the article and helped in revising it critically and in the final approval of the version.

This study was carried out with support from the Medical Research Council, UK. The sponsor of the study was Imperial College, London. We are grateful for the support of the National Institute for Health Research through the Medicines for Children Network (MCRN), King's College London Biomedical Research Centre and Imperial College Biomedical Research Centre. We are extremely grateful to Alliance Pharmaceuticals Limited for providing the melatonin and support for achieving regulatory compliance. We thank Stockport Pharmaceuticals for the manufacture and formulation of melatonin and Evelyne Jacqz-Aigrin, pharmacologist at INSERM, Paris for her valuable advice.

We thank the parents and staff at Queen Charlotte's and Chelsea Hospital, Royal Preston Hospital (Dr R. Gupta) and Royal Salford Hospital (Dr N. Maddock) and the MCRN (J Nichols & A Cook) who helped facilitate this study.

REFERENCES

- 1 Wilson-Costello D, Friedman H, Minich N, Fanaroff AA, Hack M. Improved survival rates with increased neurodevelopmental disability for extremely low birth weight infants in the 1990s. *Pediatrics* 2005; 115: 997–1003.
- 2 Hintz SR, Kendrick DE, Wilson-Costello DE, Das A, Bell EF, Vohr BR, Higgins RD. Early-childhood neurodevelopmental outcomes are not improving for infants born at <25 weeks' gestational age. *Pediatrics* 2011; 127: 62–70.
- 3 Claas MJ, Bruinse HW, Koopman C, van Haastert IC, Peelen LM, de Vries LS. Two-year neurodevelopmental outcome of preterm born children $\leq$ 750 g at birth. *Arch Dis Child Fetal Neonatal* Ed 2011; 96: F169–77.
- 4 Volpe JJ. Cerebral white matter injury of the premature infant-more common than you think. *Pediatrics* 2003; 112: 176–80.
- 5 Doyle LW, Roberts G, Anderson PJ. Outcomes at age 2 years of infants < 28 weeks' gestational age born in Victoria in 2005. *J Pediatr* 2010; 156: 49–53. e1.
- 6 Mathiasen R, Hansen BM, Nybo Anderson AM, Greisen G. Socio-economic achievements of individuals born very preterm at the age of 27 to 29 years: a nationwide cohort study. *Dev Med Child Neurol* 2009; 51: 901–8.
- 7 Petrou S, Eddama O, Mangham L. A structured review of the recent literature on the economic consequences of preterm birth. *Arch Dis Child Fetal Neonatal* Ed 2011; 96: F225–32.
- 8 Skaper SD, Floreani M, Ceccon M, Facci L, Giusti P. Excitotoxicity, oxidative stress, and the neuroprotective potential of melatonin. *Ann N Y Acad Sci* 1999; 890: 107–18.
- 9 Welin AK, Svedin P, Lapatto R, Sultan B, Hagberg H, Gressens P, Kjellmer I, Mallard C. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. *Pediatr Res* 2007; 61: 153–8.
- 10 Husson I, Mesples B, Bac P, Vamecq J, Evrard P, Gressens P. Melatoninergic neuroprotection of the murine periventricular white matter against neonatal excitotoxic challenge. *Ann Neurol* 2002; 51: 82–92.
- 11 Olivier P, Fontaine RH, Loron G, Van Steenwinckel J, Biran V, Massonneau V, Kaindl A, Dalous J, Charriaut-Marlangue C, Aigrot MS, Pansiot J, Verney C, Gressens P, Baud O. Melatonin promotes oligodendroglial maturation of injured white matter in neonatal rats. *PLoS ONE* 2009; 4: e7128.
- 12 Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr Rev* 1991; 12: 151–80.
- 13 Guzman-Marin R, Suntsova N, Methippara M, Greiffenstein R, Szymusiak R, McGinty D. Sleep deprivation suppresses neurogenesis in the adult hippocampus of rats. *Eur J Neurosci* 2005; 22: 2111–6.
- 14 Okatani Y, Okamoto K, Hayashi K, Wakatsuki A, Tamura S, Sagara Y. Maternal-fetal transfer of melatonin in pregnant women near term. *J Pineal Res* 1998; 25: 129–34.
- 15 Kennaway DJ, Stamp GE, Goble FC. Development of melatonin production in infants and the impact of prematurity. *J Clin Endocrinol Metab* 1992; 75: 367–9.
- 16 Jan JE, Wasdell MB, Freeman RD, Bax M. Evidence supporting the use of melatonin in short gestation infants. *J Pineal Res* 2007; 42: 22–7.
- 17 Arendt J. Melatonin and the Mammalian Pineal Gland, 1st edn. London: Chapman & Hall, 1995.
- 18 Bojkowski CJ, Arendt J. Factors influencing urinary 6-sulphatoxymelatonin, a major melatonin metabolite, in normal human subjects. *Clin Endocrinol (Oxf)* 1990; 33: 435–44.
- 19 Paakkonen T, Mäkinen TM, Leppaluoto J, Vakkuri O, Rintamäki H, Palinkas LA, Hassi J. Urinary melatonin: a

- noninvasive method to follow human pineal function as studied in three experimental conditions. *J Pineal Res* 2006; 40: 110–5.
- 20 Arendt J, Bojkowski C, Franey C, Wright J, Marks V. Immunoassay of 6-hydroxymelatonin sulfate in human plasma and urine: abolition of the urinary 24-hour rhythm with atenolol. *J Clin Endocrinol Metab* 1985; 60: 1166–73.
 - 21 Beal SL, Sheiner LB, Boeckmann AJ. *NONMEM Users Guides*. Ellicott City, MD: Icon Development Solutions, 1989; 2006.
 - 22 Bonate PL. *Pharmacokinetic-Pharmacodynamic Modeling and Simulation*, 2nd edn.. New York: Springer, 2006.
 - 23 Holford NH. A size standard for pharmacokinetics. *Clin Pharmacokinet* 1996; 30: 329–32.
 - 24 Meibohm B, Laer S, Panetta JC, Barrett JS. Population pharmacokinetic studies in pediatrics: issues in design and analysis. *AAPS J* 2005; 7: E475–87.
 - 25 Mandema JW, Verotta D, Sheiner LB. Building population pharmacokinetic – pharmacodynamic models. I. Models for covariate effects. *J Pharmacokinet Biopharm* 1992; 20: 511–28.
 - 26 Gitto E, Romeo C, Reiter RJ, Impellizzeri P, Pesce S, Basile M, Antonuccio P, Trimarchi G, Gentile C, Barberi I, Zuccarello B. Melatonin reduces oxidative stress in surgical neonates. *J Pediatr Surg* 2004; 39: 184–9. discussion 84–9.
 - 27 Gitto E, Reiter RJ, Sabatino G, Buonocore G, Romeo C, Gitto P, Bugge C, Trimarchi G, Barberi I. Correlation among cytokines, bronchopulmonary dysplasia and modality of ventilation in preterm newborns: improvement with melatonin treatment. *J Pineal Res* 2005; 39: 287–93.
 - 28 Gitto E, Reiter RJ, Karbownik M, Xian-Tan D, Barberi I. Respiratory distress syndrome in the newborn: role of oxidative stress. *Intensive Care Med* 2001; 27: 1116–23.
 - 29 Gitto E, Reiter RJ, Cordaro SP, La Rosa M, Chiurazzi P, Trimarchi G, Gitto P, Calabro MP, Barberi I. Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin. *Am J Perinatol* 2004; 21: 209–16.
 - 30 Gitto E, Reiter RJ, Amodio A, Romeo C, Cuzzocrea E, Sabatino G, Buonocore G, Cordaro V, Trimarchi G, Barberi I. Early indicators of chronic lung disease in preterm infants with respiratory distress syndrome and their inhibition by melatonin. *J Pineal Res* 2004; 36: 250–5.
 - 31 Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre- and post-natal period and the clinical utility of melatonin. *J Pineal Res* 2009; 46: 128–39.
 - 32 Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, Cordaro S, Corona G, Trimarchi G, Barberi I. Effects of melatonin treatment in septic newborns. *Pediatr Res* 2001; 50: 756–60.
 - 33 Fulia F, Gitto E, Cuzzocrea S, Reiter RJ, Dugo L, Gitto P, Barberi S, Cordaro S, Barberi I. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. *J Pineal Res* 2001; 31: 343–9.
 - 34 Arendt J. Mammalian pineal rhythms. *Pineal Res Rev* 1985; 3: 161–213.
 - 35 Arendt J. Melatonin. *Clin Endocrinol (Oxf)* 1988; 29: 205–29.
 - 36 Vaughan GM. Melatonin in humans. In: *Pineal Research Reviews*, ed. Reiter RJ. New York: Liss, 1984; 141–201.
 - 37 Fourtillan JB, Brisson AM, Fourtillan M, Ingrand I, Decourt JP, Girault J. Melatonin secretion occurs at a constant rate in both young and older men and women. *Am J Physiol Endocrinol Metab* 2001; 280: E11–22.
 - 38 Lang U, Kornemark M, Aubert ML, Paunier L, Sizonenko PC. Radioimmunological determination of urinary melatonin in humans: correlation with plasma levels and typical 24-hour rhythmicity. *J Clin Endocrinol Metab* 1981; 53: 645–50.
 - 39 Kovacs J, Brodner W, Kirchlechner V, Arif T, Waldhauser F. Measurement of urinary melatonin: a useful tool for monitoring serum melatonin after its oral administration. *J Clin Endocrinol Metab* 2000; 85: 666–70.
 - 40 Ma X, Idle JR, Krausz KW, Gonzalez FJ. Metabolism of melatonin by human cytochromes p450. *Drug Metab Dispos* 2005; 33: 489–94.
 - 41 CDC. U.S. Genome Variation Estimates. CYP1B1 Allele and Genotype Frequencies. Available polymorphism(s). In, edOffice of Surveillance E, and Laboratory Services [online]. Available at <http://www.cdc.gov/genomics/population/genvar/frequencies/CYP1B1.htm> (last accessed 30 November 2009).
 - 42 Smits A, Allegaert K. Perinatal pharmacology: applications for neonatal neurology. *Eur J Paediatr Neurol* 2011; 15: 478–86.
 - 43 Schmidt B, Roberts RS, Davis P, Doyle LW, Barrington KJ, Ohlsson A, Solimano A, Tin W. Long-term effects of caffeine therapy for apnea of prematurity. *N Engl J Med* 2007; 357: 1893–902.
 - 44 Lane EA, Moss HB. Pharmacokinetics of melatonin in man: first pass hepatic metabolism. *J Clin Endocrinol Metab* 1985; 61: 1214–6.
 - 45 European Commission. Ethical considerations for clinical trials on medicinal products conducted with the paediatric population. Recommendations of the ad hoc group for the development of implementing guidelines for Directive 2001/20/EC relating to good clinical practice in the conduct of clinical trials on medicinal products for human use. In: EU Legislation - Eudralex, 2008. Available at http://ec.europa.eu/health/files/eudralex/vol-10/ethical_considerations_en.pdf (last accessed 4 April 2013).
 - 46 Illnerova H, Buresova M, Presl J. Melatonin rhythm in human milk. *J Clin Endocrinol Metab* 1993; 77: 838–41.
 - 47 Waldhauser F, Waldhauser M, Lieberman HR, Deng MH, Lynch HJ, Wurtman RJ. Bioavailability of oral melatonin in humans. *Neuroendocrinology* 1984; 39: 307–13.
 - 48 Aldhous M, Franey C, Wright J, Arendt J. Plasma concentrations of melatonin in man following oral absorption of different preparations. *Br J Clin Pharmacol* 1985; 19: 517–21.