Tramadol disposition in the very young: an attempt to assess in vivo cytochrome P-450 2D6 activity

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Background. Tramadol is potentially a very useful pain relief medication in neonates and infants. It is primarily metabolized into O-demethyl tramadol (M1) by CYP2D6. Data concerning tramadol disposition and CYP2D6 activity in young infants are not available.

Methods. A population pharmacokinetic analysis of tramadol and M1 time–concentration profiles was undertaken using non-linear mixed-effects models (NONMEM), based on newly collected data on tramadol and M1 time–concentration profiles in neonates and young infants (n=20) and published studies on intravenous tramadol in children and adults. M1 formation served as a surrogate for CYP2D6 activity.

Results. Tramadol clearance was described using a two-compartment linear model with zero-order input and first-order elimination. Clearance increased from 25 weeks post-conception age (PCA) (5.52 litre h⁻¹ [70 kg]⁻¹) to reach 84% of the mature value by 44 weeks PCA (standardized to a 70 kg adult using allometric ‘1/4 power’ models). The central volume of distribution decreased from 25 weeks PCA (256 litre [70 kg]⁻¹) to reach 120% of its mature value by 87 weeks PCA. Formation clearance to M1 contributed 43% of tramadol clearance, but had no relationship with PCA. There was a weak non-linear relationship between PCA and M1 metabolite clearance.

Conclusions. Maturational clearance of tramadol is almost complete by 44 weeks PCA. A target concentration of 300 μg litre⁻¹ is achieved after a bolus of tramadol hydrochloride 1 mg kg⁻¹ and can be maintained by infusion of tramadol hydrochloride 0.09 mg kg⁻¹ h⁻¹ at 25 weeks PCA, 0.14 mg kg⁻¹ h⁻¹ at 30 weeks PCA, 0.17 mg kg⁻¹ h⁻¹ at 35 weeks PCA, 0.18 mg kg⁻¹ h⁻¹ at 40 weeks, 0.19 mg kg⁻¹ h⁻¹ at 50 weeks PCA to 1 yr, 0.18 mg kg⁻¹ h⁻¹ at 3 yr and 0.12 mg kg⁻¹ h⁻¹ in adulthood. CYP2D6 activity was observed as early as 25 weeks PCA, but the impact of CYP2D6 polymorphism on the variability in pharmacokinetics, metabolism and pharmacodynamics of tramadol remains to be established.

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Tramadol is an aminocyclohexanol derivative or 4-phenyl piperidine analogue of codeine. Its analgesic effect is mediated through norepinephrine reuptake inhibition, increased release and decreased reuptake of serotonin in the spinal cord and a weak μ-opioid receptor effect based on a 6000 times weaker affinity for opioid receptors compared with morphine. Tramadol is metabolized by either O-demethylation in the liver (CYP2D6) to O-demethyl tramadol (M1) or by N-demethylation (CYP3A) to N-demethyl tramadol. Since the active O-demethyl tramadol (+)-M1
metabolite has an μ-opioid affinity ~200 times greater than that of tramadol, CYP2D6 isoenzyme activity is important for the analgesic effect attributable to tramadol.\textsuperscript{1–4}

In general, the phenotypic variation in drug metabolism is based on constitutional, genetic and environmental factors.\textsuperscript{5,6} However, age-related tramadol and O-demethyl tramadol metabolite pharmacokinetics in neonates and young children have not been previously quantified. In addition, it is anticipated that phenotypic variation reflects isoenzyme-specific ontogeny to a greater degree, but observations on phenotypic CYP2D6 activity in the first year are very limited and mainly based on \textit{in vitro} studies.\textsuperscript{7,10}

Therefore tramadol disposition was used to assess maturation of \textit{in vivo} CYP2D6 activity in early neonatal and paediatric life, based on a population-based approach that included size as the primary covariate in an effort to disen-tangle age-related from size-related factors.

\section*{Methods}

\textbf{Neonatal pharmacokinetic study}

The study in 20 neonates and young infants (0–3 months postnatal age) was conducted in the Gasthuisberg Neonatal Intensive Care Unit. The study protocol was approved by the local ethical board of the University Hospital, Gasthuisberg, Leuven, Belgium. Infants were included after informed written consent from parents. The decision to prescribe tramadol (Contramal\textsuperscript{18}, Grünenthal, Aachen, Germany) or any other analgesic was made by the attending neonatologist and was based on standardized evaluation and treatment of pain after a variety of surgical or medical interventions.\textsuperscript{11}

Tramadol was administered by the intravenous route (loading dose 2 mg kg\textsuperscript{-1} over 30 min, followed by continuous administration of tramadol hydrochloride 5 mg kg\textsuperscript{-1} [24 h]\textsuperscript{-1}). Infants with associated renal dysfunction (>1 mg dl\textsuperscript{-1} creatinemia), hepatic dysfunction (direct bilirubinaemia >2 mg dl\textsuperscript{-1}) or peripartal asphyxia (blood lactate >2 mg dl\textsuperscript{-1}) were excluded from this pharmacokinetic study. Clinical characteristics and indications to initiate treatment were registered prospectively. The serum–time profiles for one neonate included in the present study were recently described as part of the evaluation of the blood–brain barrier for tramadol.\textsuperscript{12}

Blood samples (0.2 ml) were taken from an arterial line 0.5, 1, 2, 4, 6, 9, 12, 15, 18 and 24 h after initiation of intravenous administration. The number of samples taken from the smallest infants was lower because the cumulative blood volume collected in a single infant was limited to 1 ml kg\textsuperscript{-1}. Blood samples were centrifuged (3 min, 10 000 r.p.m., 4°C) shortly after collection and plasma samples were stored at −20°C until analysis. Plasma concentrations of tramadol and O-demethyl tramadol were determined by high-performance liquid chromatography (HPLC) in low volume plasma samples (see Appendix).\textsuperscript{13,14}

\noindent \textbf{Paediatric pharmacokinetic study}

Concentration–time profiles from the study involving neonates and young infants were combined with data on the pharmacokinetics of intravenous tramadol in nine children with mean weight 13.2 (SD 4.8) kg and age 2.4 (range 1.17–6.6) yr following single i.v. bolus administration (tramadol hydrochloride 2 mg kg\textsuperscript{-1}) as reported by Murthy and colleagues.\textsuperscript{15}

Children received this drug after either elective limb or thoracic surgery. Venous blood samples were collected for up to 20 h after i.v. bolus administration. Samples were centrifuged and stored at −20°C until assay. Serum concentrations of tramadol and its metabolite O-demethyl tramadol (M1) were measured simultaneously by non-stereoselective gas chromatography with nitrogen-selective detection.\textsuperscript{15}

\noindent \textbf{Adult pharmacokinetic study}

To assess the maturational aspects of tramadol disposition further, concentration–time profiles for 20 healthy adults, as reported by Lintz and colleagues\textsuperscript{16} following single i.v. bolus administration (tramadol hydrochloride 100 mg), were also included. The mean (SD) weight was 70 (10.5) kg and mean age was 40.4 (23–57) yr. Venous blood samples were collected up to 24 h after tramadol administration. Samples were centrifuged and stored at −20°C until assay. Serum concentrations of tramadol were determined twice by means of gas chromatography–mass spectrometry.\textsuperscript{16,17}

\noindent \textbf{Population pharmacokinetics}

A two-compartment linear model (central compartment V1 and peripheral compartment V2) with zero-order input and first-order elimination fitted the data better than a single-compartment pharmacokinetic model. The model parameters were central volume (V1), peripheral volume (V2), clearance (CL) and intercompartment clearance (Q). Population parameter estimates were obtained using non-linear mixed-effects modelling (NONMEM).\textsuperscript{18}

There were three sources of data for this population analysis and between-study variability was accounted for by giving each study a separate residual error. The quality of fit of the pharmacokinetic models to the data was assessed by visual examination of plots of observed vs predicted concentrations. Models were nested, and an improvement in the objective function was referred to the \(\chi^2\) distribution to assess significance; for example, an objective function change (\(\Delta OBJ\)) of 3.84 is significant at \(\alpha=0.05\).

Parameter values were standardized for a body weight of 70 kg using allometric models in order to compare neonatal estimates with those from adults. While body weight is used most commonly in the clinical setting, it is recognized that there is a non-linear relationship between weight and dose. Therefore an allometric ‘\(3/4\) power’ model might be a more appropriate scaling to study maturational aspects of drug...
clearance, based on the observation that the logarithmic plot of basal metabolic rate against weight produces a straight line with a slope of 3/4 in homeotherms, poikilotherms and unicellular organisms. This allometric ‘3/4 power’ model can be used to scale metabolic processes such as drug clearance.19–24

Covariate analysis included a model investigating age-related changes for parent tramadol clearance and volume of distribution using an exponential function.

Population parameter estimations for metabolite (O-demethyl tramadol) pharmacokinetics
Data for studying O-demethyl tramadol (M1) metabolism were available in neonates, infants and children only because no adult metabolite data were collected.15–17 M1 tramadol metabolite data were converted to tramadol milligram equivalents using a molecular weight of 249.38 mg mmol\(^{-1}\) for M1 and 263.38 mg mmol\(^{-1}\) for tramadol (molar ratio 0.947). A two-compartment model (parent drug and metabolite compartments) with zero-order input and first-order elimination was used with NONMEM. Differential equations were used to describe the pharmacokinetics of tramadol and O-demethyl tramadol (see Appendix).

Results
Clinical characteristics and indications for administration of tramadol in neonates and young infants are presented in Table 1.

The pooled parent drug (tramadol) pharmacokinetic (PK) analysis comprised 49 subjects and 507 drug assay samples. Parameter estimates for the two-compartment analysis are shown in Tables 2 and 3. Figure 1 shows the quality of fit for parent tramadol pharmacokinetic data. Individual concentration predictions are based on values of maximum a posteriori Bayesian estimates of the parameters using the post hoc option while predicted typical (population) concentrations are based on population parameters and covariate information. Predictions from NONMEM’s post hoc (posterior individual) step are based on values of the parameters for the specific individual using his or her observed data.

The correlation of parameter variability for CL, V1 and V2 was low and is shown in Table 4. Changes in clearance and central volume of distribution with age are shown in Figure 2A and 2B. Clearance increased from 25 weeks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>%PPV</th>
<th>%SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLstd (litre h(^{-1}) [70 kg](^{-1}))</td>
<td>24</td>
<td>43.6</td>
<td>7.0</td>
</tr>
<tr>
<td>V1std (litre [70 kg](^{-1}))</td>
<td>149</td>
<td>26.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Qstd (litre h(^{-1}) [70 kg](^{-1}))</td>
<td>19.3</td>
<td>–</td>
<td>35.4</td>
</tr>
<tr>
<td>V2std (litre [70 kg](^{-1}))</td>
<td>40.2</td>
<td>42.5</td>
<td>17.1</td>
</tr>
<tr>
<td>Err (neonates and infants)</td>
<td>0.1%</td>
<td>–</td>
<td>27.3</td>
</tr>
<tr>
<td>Err (children)</td>
<td>0.12%</td>
<td>–</td>
<td>12.7</td>
</tr>
<tr>
<td>Err (adults)</td>
<td>0.08%</td>
<td>–</td>
<td>20.6</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>%SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>βcl</td>
<td>0.23</td>
<td>46.5</td>
</tr>
<tr>
<td>Tcl (weeks)</td>
<td>9.9</td>
<td>29.4</td>
</tr>
<tr>
<td>βvol</td>
<td>1.72</td>
<td>8.4</td>
</tr>
<tr>
<td>Tvol</td>
<td>34.2</td>
<td>35.1</td>
</tr>
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</table>
PCA (5.52 litre h\(^{-1}\) [70 kg]\(^{-1}\)) to reach 84% of the mature value by 44 weeks PCA (standardized to a 70 kg adult using allometric ‘1/4 power’ models). The central volume of distribution decreased from 25 weeks PCA (256 litre [70 kg]\(^{-1}\)) to reach 120% of its mature value by 87 weeks PCA. The peripheral volume of distribution (V2) (Fig. 2c) and the intercompartment clearance (Q) did not change with age.

Mean age-related PK predictions based on the covariate models are shown in Table 5. This table also expresses PK parameters as per kilogram, based on an estimated weight for each age group.

Based on the PK estimates observed, age-dependent dose regimens can be suggested. A target concentration of 300 \(\mu\)g litre\(^{-1}\) is achieved after a bolus of tramadol

### Table 5

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight (kg)</th>
<th>CL</th>
<th>V1</th>
<th>Q</th>
<th>V2</th>
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<tr>
<td>25 weeks PCA</td>
<td>0.6</td>
<td>5.52</td>
<td>4.32</td>
<td>256</td>
<td>3.66</td>
</tr>
<tr>
<td>30 weeks PCA</td>
<td>1.3</td>
<td>11.0</td>
<td>7.08</td>
<td>246</td>
<td>3.51</td>
</tr>
<tr>
<td>35 weeks PCA</td>
<td>2.5</td>
<td>14.8</td>
<td>8.12</td>
<td>237</td>
<td>3.38</td>
</tr>
<tr>
<td>40 weeks PCA</td>
<td>3.3</td>
<td>17.5</td>
<td>8.96</td>
<td>228</td>
<td>3.26</td>
</tr>
<tr>
<td>45 weeks PCA</td>
<td>4.5</td>
<td>19.4</td>
<td>9.19</td>
<td>221</td>
<td>3.15</td>
</tr>
<tr>
<td>50 weeks PCA</td>
<td>5.5</td>
<td>20.7</td>
<td>9.4</td>
<td>214</td>
<td>3.05</td>
</tr>
<tr>
<td>60 weeks PCA</td>
<td>7.5</td>
<td>22.4</td>
<td>9.32</td>
<td>202</td>
<td>2.88</td>
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<tr>
<td>1 yr postnatal</td>
<td>10</td>
<td>23.8</td>
<td>9.22</td>
<td>178</td>
<td>2.54</td>
</tr>
<tr>
<td>3 yr postnatal</td>
<td>14</td>
<td>24</td>
<td>8.54</td>
<td>153</td>
<td>2.18</td>
</tr>
<tr>
<td>Adult</td>
<td>70</td>
<td>24</td>
<td>5.71</td>
<td>149</td>
<td>2.13</td>
</tr>
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</table>
A two-compartment model produced a better fit than the single-compartment model commonly used for this drug. 12 Hydrochloride 1 mg kg⁻¹. This serum concentration can be maintained by infusing tramadol hydrochloride 0.09 mg kg⁻¹ h⁻¹ at 25 weeks PCA, 0.14 mg kg⁻¹ h⁻¹ at 30 weeks PCA, 0.17 mg kg⁻¹ h⁻¹ at 35 weeks PCA, 0.18 mg kg⁻¹ h⁻¹ at 40 weeks, 0.19 mg kg⁻¹ h⁻¹ at 50 weeks PCA to 1 yr, 0.18 mg kg⁻¹ h⁻¹ at 3 yr and 0.12 mg kg⁻¹ h⁻¹ in adulthood.

The O-demethyl tramadol (M1) time–concentration profiles were not determined in the adult population; consequently the pooled paediatric metabolite PK analysis comprised 29 subjects and 545 drug assay samples. Parameter estimates for the two-compartment analysis are shown in Tables 6 and 7. Figure 3 shows the quality of fit for the M1 metabolite data. Figure 4 shows the relationship between PCA and M1 metabolite formation (CL2M1), tramadol clearance by other routes (CL other) and M1 metabolite elimination clearance (CLM1). We were able to demonstrate a non-linear relationship between PCA and CLM1 only, but parameter estimates had high SE values (Table 6), suggesting a poor relationship. There was no relationship between PCA and M1 metabolite formation clearance (CL2M1) or tramadol clearance by other routes (CL other).

### Discussion

This is the first report concerning the pharmacokinetics of intravenous tramadol in neonates and young infants. The same data were also used to study the maturation of phenotypic CYP2D6 activity.

A two-compartment model produced a better fit than the single-compartment model commonly used for this drug. 12 The current study included sick neonates and young infants after surgical interventions that included cardiac surgery, and it is possible that surgery may contribute to...

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**Table 6** Standardized metabolite population pharmacokinetic parameter estimates. PPV, population parameter variability; se, standard error of the estimate; CL other, tramadol clearance by other routes; CL2M1, formation clearance to O-demethyl tramadol (M1); CLM1, elimination clearance of O-demethyl tramadol; VM1, volume of distribution of M1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>%PPV</th>
<th>%SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL other std (litre h⁻¹ [70 kg]⁻¹)</td>
<td>8.58</td>
<td>63.9</td>
<td>16.4</td>
</tr>
<tr>
<td>CL2M1 std (litre h⁻¹ [70 kg]⁻¹)</td>
<td>6.42</td>
<td>84.8</td>
<td>21.5</td>
</tr>
<tr>
<td>CLM1 std (litre h⁻¹ [70 kg]⁻¹)</td>
<td>117</td>
<td>55.5</td>
<td>129.1</td>
</tr>
<tr>
<td>V std (litre [70 kg]⁻¹)</td>
<td>222</td>
<td>33.7</td>
<td>6.5</td>
</tr>
<tr>
<td>VM1 std (fixed)</td>
<td>224</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dclm (weeks)</td>
<td>164</td>
<td>–</td>
<td>309.8</td>
</tr>
<tr>
<td>Err additive CM1</td>
<td>0.004</td>
<td>–</td>
<td>31.4</td>
</tr>
<tr>
<td>Err proportional CM1</td>
<td>0.006</td>
<td>–</td>
<td>118.6</td>
</tr>
<tr>
<td>Err additive CS</td>
<td>0.0009</td>
<td>–</td>
<td>29.2</td>
</tr>
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</table>

**Table 7** The correlation of parameter variability for metabolite parameters

<table>
<thead>
<tr>
<th>CL other</th>
<th>CLM</th>
<th>V</th>
<th>CL2ODT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLM1</td>
<td>-0.0176</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.113</td>
<td>0.0278</td>
<td>1</td>
</tr>
<tr>
<td>CL2M1</td>
<td>0.102</td>
<td>0.108</td>
<td>-0.00732</td>
</tr>
</tbody>
</table>

Pooled data were from three protocols; sampling techniques and tramadol assay methods varied. 12,15–17 These differences were monitored by allocating separate residual errors to each study. We were reassured to note that these residual errors were similar in each study. Total clearance was only 23% that of the adult value at 25 weeks PCA but the maturation half-time was 10 weeks and therefore clearance was 84% of the mature value by 44 weeks PCA (Fig. 2B). The estimates of tramadol clearance observed in the present population PK analysis of 24 (CV 43.5%) litre h⁻¹ (70 kg)⁻¹ (5.7 ml min⁻¹ kg⁻¹) in adults and 2.7 ml min⁻¹ kg⁻¹ in children are similar to those reported by others. 12,15–17

The central volume of distribution had a longer maturation half-time (34.2 weeks) than the clearance. These findings reflect the slower changes in body composition compared with clearance enzyme maturation that occur with age.

There was no relationship between PCA and M1 metabolite formation (CL2M1) or tramadol clearance by other routes (CL other).

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*Fig 3* Quality of fit of M1 metabolite data. (a) Comparison of predicted and observed populations. The line x=y is the line of identity. (b) Comparison of the individual Bayesian concentration predictions based on values of the parameters for the specific individual with observed values.
Morphine clearance in neonates is reduced after cardiac surgery. A target tramadol concentration of 300 mg litre\(^{-1}\) has been suggested in adult patients given fentanyl 5 µg kg\(^{-1}\) intraoperatively or 600 (590) mg litre\(^{-1}\) in adults not given supplementary analgesics. Based on this suggestion, an age-dependent dose regimen has been developed, although the minimal effective analgesic serum tramadol concentration in adults is uncertain because of differences in tramadol metabolism since the M1 metabolite (CYP2D6 mediated) is a much more potent analgesic. Postulated infusion rates in this current study achieve a mean tramadol concentration only and do not take into account the effect of CYP2D6 polymorphism and activity that has a dramatic influence on the analgesic effect mediated through M1 metabolite production.

The pharmacodynamic impact of CYP2D6 polymorphism is not only limited to adulthood. Abdel-Rahman and colleagues recently described the impact of the number of functional CYP2D6 alleles on tramadol metabolism in a...
paediatric population (n=21, mean 6.8 yr, SD 1.6) of extensive metabolizers. We had anticipated that we could use M1 metabolite formation as a reliable marker of in vivo phenotypic CYP2D6 activity since plasma pharmacokinetics of tramadol have been used as a rapid and simple CYP2D6 genotyping assay in adults.28

We were unsuccessful in describing a maturational relationship between M1 metabolite formation (CYP2D6 activity) and PCA in this cohort of neonates and infants (Fig. 4A), but observed that significant CYP2D6 activity is already present in early neonatal life. It is likely that CYP2D6 polymorphism contributes to the analgesic effect in neonatal life.5,4

In vitro CYP2D6 activity has been evaluated in fetal, neonatal, infant, paediatric and adult liver tissue to study the ontogeny of CYP2D6.7–10 Treluyer and colleagues7 detected limited CYP2D6 protein and activity in 30% of fetal livers.7 CYP2D6 activity was more frequently documented after spontaneous abortion than after medically induced abortion.7 In the first month of life, CYP2D6 protein and activity increased further, and between 1 month and 5 yr of age protein levels were reported to be approximately two-thirds of adult levels.7 Another study reported no additional significant differences in protein levels of CYP2D6 in infants older and younger than 1 yr, suggesting that CYP2D6 ontogeny is complete by age 1 yr.7–10 This is consistent with the rapid maturation of total clearance reported here, to which CL2M1 contributed 43%.

Data on in vivo phenotypic CYP2D6 activity are mainly based on the dextromethorphan/dextrophan (DM/DX) ratio but such data are only reported in adult and paediatric populations and have not yet been reported in neonates. In a large adult population, the DM/DX ratio was 0.01 (SD 0.022) in extensive metabolizers compared with 0.014 (0.021) in heterogeneous genotypes and 3.6 (3.8) in poor metabolizers.29 In a paediatric population (n=21, 6.8 yr, SD 1.6) of extensive metabolizers, the DM/DX ratio was 0.01 (0.011), suggesting CYP2D6 activity at an adult level.29,30

In conclusion, this is the first report on the disposition of tramadol in neonates. By comparing neonatal data with PK data for tramadol in children and adults, we were able to demonstrate rapid maturation of tramadol clearance. These estimates were used to calculate age-dependent dose suggestions. The impact of CYP2D6 polymorphism on the variability in pharmacokinetics and dynamics of tramadol in the first years of life remains to be established, but we were able to document in vivo CYP2D6 activity in early neonatal life.

Appendix

Tramadol and O-demethyl tramadol assay
Plasma concentrations of tramadol and O-demethyl tramadol were determined by HPLC in low-volume plasma samples using a method based on modifications and improvements to methods described earlier.13,14 Ten μl of standard dilutions of tramadol and O-demethyltramadol (to obtain a standard range of 0.05–5 μg ml⁻¹), 10 μl of the internal standard D617 (2-(3,4-dimethoxy-phenyl)-2-isopropyl-5-methylamino-pentane nitrite, a metabolite of verapamil), 0.2 ml of 0.2 M sodium carbonate buffer pH 10.5 and 2 ml of tert-butyl-methylether were added to 0.1 ml of plasma. After the mixture had been shaken for 10 min and centrifuged for 5 min at 4°C and 1286g, the organic layer was transferred to conical glass tubes. After evaporation of tert-butyl-methylether at 40°C in a water bath with an airstream, the residues were dissolved in 200 μl of the mobile phase. These mixtures were transferred to Eppendorf tubes (1.5 ml) and centrifuged at 9300g for 8 min and finally pipetted into microvials for automatic injection. A Waters 600E pump was used in combination with a Merck–Hitachi fluorescence detector F-1000, set at excitation and emission wavelengths of 280 nm and 310 nm, respectively. A stainless steel column (250 mm×4.6 mm internal diameter) packed with Spherisorb CN 5μ (Alltech Associates, Deerfield, IL, USA) was used. The mobile phase consisted of a mixture of acetonitrile and 15 mM potassium phosphate buffer pH 4.0 with 0.05% triethylamine (10:90 v/v) and pumped at a flow rate of 0.9 ml min⁻¹. Good chromatographic separation between tramadol and O-demethyl tramadol was obtained. Linearity of the calibration curves for tramadol and O-demethyl tramadol in plasma was found in the range 0.05–5 μg ml⁻¹ (y=−0.018+2.05x, r=0.9921 and y=0.031+3.06y, r=0.9949, respectively). The lower limit of quantification for tramadol and O-demethyl tramadol was 0.05 μg ml⁻¹, which was the lowest concentration of the standard curve with a coefficient of variation <20%.

Population pharmacokinetic modelling

Population parameter estimations for parent tramadol
A two-compartment linear model (central compartment V1 and peripheral compartment V2) with zero-order input and first-order elimination was used, parameterized in terms of central volume (V1), peripheral volume (V2), clearance (CL) and intercompartment clearance (Q). Population parameter estimates were obtained using non-linear mixed-effects modelling (NONMEM).18 This model accounts for population parameter variability (between and within subjects) and residual variability (random effects) as well as parameter differences predicted by covariates (fixed effects). The population parameter variability in model parameters was modelled by a proportional variance model. The residual unknown variability was characterized by a proportional term. The population mean parameters between subject variance and residual variance were estimated with the first-order conditional interaction estimate method using ADVAN3 TRANS4 of NONMEM V. The convergence criterion was three significant digits. A Compaq Digital Fortran
The parameter values were standardized for a body weight of 70 kg using an allometric model:\(^2^0^2^1\)

\[
P_i = P_{std} \times (W_i/W_{std})^{PWR}
\]

where \(P_i\) is the parameter for the \(i\)th individual, \(W_i\) is the weight of the \(i\)th individual and \(P_{std}\) is the parameter for an individual with a standard weight of 70 kg. The exponent PWR was 0.75 for the clearance volume and 1 for the distribution volume. This standardization has a strong theoretical and empirical basis and allows the neonatal parameter estimates to be compared with those reported for adults.\(^2^2^–^2^4\)

Covariate analysis included a model investigating age-related changes for parent tramadol clearance and volume of distribution using an exponential function:

\[
V1 = [V1std \times (Wt/70)] \\
\times [1 - (1 - \beta v ol) \times \exp\{-[\text{PCA} - 25] \times \text{Ln}(2)/\text{Tvol}\}] \times [1 - (1 - \beta c l) \times \exp\{-[\text{PCA} - 25] \times \text{Ln}(2)/\text{Tcl}\}] \times V
\]

where \(V1std\) and \(CLstd\) are the population estimates for \(V1\) (central volume) and \(CL\), respectively, standardized to a 70 kg person using allometric models, PCA is the post-conception age in weeks, \(\beta v ol\) and \(\beta c l\) are parameters estimating the fractional difference from \(V1std\) and \(CLstd\) at PCA weeks, and \(Tvol\) and \(Tcl\) describe the maturation half-lives of the age-related changes of \(V1\) and \(CL\).

Similar models were used for the metabolite analysis.

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