Population Pharmacokinetic Modeling in Very Premature Infants Receiving Midazolam during Mechanical Ventilation

Midazolam Neonatal Pharmacokinetics

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Background: Midazolam is used widely as a sedative to facilitate mechanical ventilation. This prospective study investigated the population pharmacokinetics of midazolam in very premature infants.

Methods: Midazolam (100 µg/kg) was administered as a rapid intravenous bolus dose every 4–6 h to 60 very premature neonates with a mean (range) gestational age of 27 weeks (24–31 weeks), a birth weight of 965 g (523–1,470 g), and an age of 4.5 days (2–15 days). A median (range) of four (one to four) blood samples, 0.2 ml each, were drawn at random times after the first dose or during continuous treatment, and concentrations of midazolam in serum were assayed by high-performance liquid chromatography. A population analysis was conducted using a two-compartment pharmacokinetic model using the NONMEM program.

Results: Average parameter values (interpatient percent coefficient of variation) for infants with birth weights 1,000 g or less were total systemic clearance (Cl) = 0.785 ml/min (83%), intercompartmental clearance (Cl12) = 6.53 ml/min (116%), volume of distribution of the central compartment (V1) = 473 ml (70%), and volume of distribution of the peripheral compartment (V2) = 513 ml (146%). For infants with birth weights more than 1,000 g they were as follows: Clr = 1.24 ml/min (78%), Cl0 = 9.82 ml/min (98%), V1 = 823 ml (43%), and V2 = 1,040 ml (193%). The intrapatient variability (percent coefficient of variation) in the data was 4.5% at the mean concentration midazolam in serum of 121 ng/ml.

Conclusions: Serum concentration-time data were used in modeling the population pharmacokinetics of midazolam in very premature, ventilated neonates. Clearance of midazolam was markedly decreased compared with previous data from term infants and older patients. Infants weighing less than 1,000 g at birth had significantly lower clearance than those weighing more than 1,000 g. (Key words: Clearance; low birth weight; neonates; simulation; volume of distribution.)

MIDAZOLAM is often used as a sedative in children and adults who are being mechanically ventilated.1-5 The importance of effective sedation in critically ill, ventilated infants also is well recognized.6-7 Respiratory support is associated with unpleasant stimuli, which may lead to hormonal, metabolic, and cardiorespiratory changes and, subsequently, possibly to long-term modification of behavior.8 Further, infants who actively resist ventilation may be at increased risk of development of barotrauma8 and intraventricular hemorrhage.9

Although midazolam has been used increasingly in neonatal intensive care nurseries,10 previous studies show that there is marked variability in the elimination of midazolam in premature, newborn infants.11-13 High concentrations of midazolam in serum may cause hypotension10,12 and decreased cerebral blood flow veloc-
ity. The targeting of appropriate doses of midazolam depends on a sound knowledge of its disposition in premature infants who have altered processes of drug distribution and markedly compromised clearances. Until now, there has been little information available regarding the magnitude and variability of the pharmacokinetic parameters for midazolam in very premature neonates. The purpose of the current study was to determine the population pharmacokinetics of midazolam in 60 very-low-birth-weight infants who were being mechanically ventilated.

Materials and Methods

Patients
The study was performed in the Neonatal Intensive Care Unit of the Mater Mothers' Hospital (Brisbane, Australia). Selection of the infants was dependent on one of the investigators obtaining informed parental consent. The study received previous written approval from the ethics committees of the Mater Mothers' Hospital and The University of Queensland. Mechanically ventilated, preterm infants with birth weights less than 1,500 g requiring midazolam for sedation were eligible for enrollment. The indication for sedation was determined according to the clinical opinion of the attending physician and nurse who were not otherwise associated with the study. Infants were ineligible if they were younger than 21 h of age, had cardiovascular instability (use of an inotrope or volume expander for blood pressure support in the previous 4 h) or neurologic instability (abnormal clinical signs, suspicion of seizure activity, abnormality on cranial ultrasonography), or had hepatic or renal dysfunction. Characteristics of the study infants are presented in Table 1.

Administration of Midazolam and Blood Collection
Midazolam (HCl salt) was administered intravenously (100 μg/kg) over 2 min as a single dose or as multiple doses every 4–6 h when necessary. The frequency of administration of midazolam was determined according to the clinical opinion of a physician and nurse who were not otherwise associated with the study.

Blood (0.25 ml) was collected via heel prick (arterial) or umbilical catheter (venous) as follows: one sample before administration of midazolam; three samples drawn randomly at predetermined times within each of the postdose intervals 5–15 min, 20–60 min, and 60–120 min; and one sample drawn randomly at a predetermined time after 2 h. Samples were collected either during the first dose interval or during subsequent intermittent administration. Sera were collected and stored at −75°C before analysis.

Determination of Concentrations of Midazolam in Serum
Midazolam was assayed by a published high-performance liquid chromatographic method. Serum (100 μl) was extracted with 10% vol/vol isopropryl alcohol in dichloromethane containing 25 ng/ml climazolam (internal standard) followed by back extraction into phosphoric acid (0.02 M). A mobile phase of acetonitrile:tetrahydrofuran:phosphate buffer (0.01 M, pH 6.7, 350:50:600 vol/vol/vol) was pumped at 1 ml/min through a C8 Symmetry® (150 × 3.9-mm) column (Waters, Milford, MA). The eluent was monitored at 220 nm. Midazolam and climazolam were eluted at 7.4 and 8.4 min, respectively. Recoveries were more than 70%. Calibration plots in drug-free serum were linear (r > 0.999) from 12.5–800.0 ng/ml. Within-day and between-day imprecisions (percent coefficient of variation [CV%]) were 1.8–6.5% and 4.1–8.8%, respectively. Inaccuracy, defined as the difference between target and measured concentrations.

Table 1. Characteristics of Study Infants (n = 60)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>965 (523–1470)</td>
</tr>
<tr>
<td>&lt;1,000 g</td>
<td>33 (55)</td>
</tr>
<tr>
<td>Current weight (g)</td>
<td>963 (513–1,650)</td>
</tr>
<tr>
<td>Age (wk)</td>
<td>27.1 (24–31)</td>
</tr>
<tr>
<td>Study entry (day)</td>
<td>4.5 (2–15)</td>
</tr>
<tr>
<td>Gender</td>
<td>Female (60), Male (40)</td>
</tr>
<tr>
<td>Race</td>
<td>White (54), Other (10)</td>
</tr>
<tr>
<td>Apgar score†</td>
<td>1 min: 5 (1–9), 5 min: 9 (2–10)</td>
</tr>
<tr>
<td>Route of delivery</td>
<td>Caesarian (42), Vaginal (18)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Singleton (38), Multiple (22)</td>
</tr>
<tr>
<td>Serum midazolam concentration (ng/ml)*</td>
<td>121 (0.0–583)</td>
</tr>
<tr>
<td>Post-dose sample time (h)*</td>
<td>3.3 (0.0–24.0)</td>
</tr>
<tr>
<td>Total samples</td>
<td>199</td>
</tr>
<tr>
<td>Samples per patient†</td>
<td>4 (1–4)</td>
</tr>
</tbody>
</table>

Data are number of infants (% of group total), unless otherwise stated: *mean (range), †median (range)
expressed as a percentage of target concentration, was 12.3% or less. The lowest quantifiable concentration was 12.5 ng/ml.

**Population Modeling**

**Fixed Effects.** All modeling was performed with NONMEM (version 4.2)** using a two-compartment linear pharmacokinetic model. The parameters of this model are the total systemic clearance (Clr), intercompartmental clearance (Clq), and volumes of distribution of the central (V1) and peripheral (V2) compartments. Model building was conducted as described previously. A baseline model (Clr = θ1; V1 = θ2; Clq = θ3; V2 = θ4) was obtained using all 60 patients; then candidate covariates were screened statistically by adding these, in turn, according to a slope-intercept model (e.g., Clr = θ1 + θ2 · WT, where WT = total body weight) or using indicator variables (e.g., Clr = θ1 · GEN + θ2 · [1 − GEN], where GEN [gender] = 1 for male and 0 for female). Thus, for example, in the slope-intercept model, the typical Clr in the population of infants might be described by a baseline component (θ1) plus the product of current weight (WT) multiplied by a coefficient (θ2). The covariates screened were current weight, birth weight, birth of a single/multiple pregnancy, and sex of the infant. The difference in the objective function (OF; a NONMEM-calculated global goodness-of-fit indicator equal to −2 log likelihood value of the data) between a full/reduced pair (e.g., Clr = θ1 + θ2 · WT; Clr = θ1) approximates the χ² value with one degree of freedom.**

The level of significance (α) was set to 0.01, which corresponds to a required change in the OF value of 6.6. Changes in the OF value and plots of residuals and weighted residuals (weighted in NONMEM by the standard deviation) versus predicted concentration of midazolam in serum were recorded.

**Population Modeling**

**Random Effects.** Deviations of Clr, V1, Clq, and V2 of the jth individual from the estimated population average values were estimated according to an exponential interpatient error model,**

\[ \eta_{ij} = \text{TVPK} \times \text{EXP} (\eta_{ij}) \],

where PK is the required pharmacokinetic parameter in the jth infant, EXP is the exponentiation operator, and \( \eta_{ij} \) is a random variable distributed with zero mean and variance \( \omega^2 \) about the average value (TVPK) in the population. NONMEM also estimates the residual variance among pairs of observed and model-predicted data. For pharmacokinetic data, these differences, \( \varepsilon_{ij} \), are attributable to interpatient variability introduced, for example, in the timing of blood collections, dose times, drug assay, and by misspecification of the pharmacokinetic model. The following additive interpatient error model was used:

\[ C_{ij} = C_{\text{pred},ij} + \varepsilon_{ij} \],

where \( C_{ij} \) is the ith observed concentration for the jth individual, \( C_{\text{pred},ij} \) is the concentration of midazolam in serum predicted by the pharmacokinetic model, and \( \varepsilon_{ij} \) (the difference between \( C_{ij} \) and \( C_{\text{pred},ij} \)) is a randomly distributed variable with zero mean and variance \( \sigma^2 \).

**Simulations**

Using the fixed and random effects parameter values of the final population model, Monte Carlo simulations were performed in NONMEM** to obtain the estimated concentrations of midazolam in serum that might be expected in 40 infants (in each of the two birth-weight categories) up to 152 h after the introduction of midazolam.

**Statistical Analysis**

Unpaired \( t \) tests of boys versus girls and birth of a single-birth pregnancy versus a multiple-birth pregnancy were performed using birth weight as the dependent variable. The level of significance was 0.05. A normal probability plot for the analysis of weighted residuals was constructed. These statistical analyses were obtained using the STATISTICA software package (version 5.1H; StatSoft, Tulsa, OK).

**Results**

A scatterplot of concentrations of midazolam in serum versus postdose sampling time is shown in figure 1. There were 199 serum concentrations ranging from 0.0–583.0 ng/ml; the lowest quantifiable concentration was 19 ng/ml. The data also were fitted to a one-compartment model, but the results were inferior to the two-compartment model in that they showed larger errors of estimation of the parameters and lack of random scatter in the plots of residuals. The inclusion of a birth-weight
category (1,000 g) reduced the OF value by a significant amount ($\chi^2_{0.01} > 6.6$) for all kinetic parameters. Birth from a single/multiple-birth pregnancy and male/female gender were identified as significant categorical factors modifying ClT. Table 2 contains examples of factors that caused the largest decrease in the OF value of the baseline model. Attempts to combine the effect of each of these factors, in turn, into a combined model was unsuccessful. For example, incorporation of single/multiple-birth pregnancy (model 3, table 2) with gender (model 2, table 2) resulted in an increase in the OF value of 490.

Plots of observed concentrations in serum versus predicted concentrations in serum and weighted residual versus predicted concentrations in serum are shown in figures 2A and 2B, respectively. The latter plot shows that most weighted residuals lay within two units of the zero ordinate of perfect agreement. Furthermore, the weighted residuals were normally distributed as shown by the linear normal probability plot (fig. 2C).

Summary results of the population pharmacokinetics of midazolam and the interpatient variability in the parameters are presented in table 3. Infants with birth weights more than 1,000 g had average values of ClT, ClG, V1, and V2 that were approximately 1.5- to 2.0-fold greater than for infants weighing 1,000 g or less at birth. The interpatient variability (CV%) for these four parameters was considerably and ranged from 43-193% (table 3). The intrapatient SD was 5.4 mg/ml, which translates to CV% values of 28%, 4.5%, and 0.93%, respectively, at the lowest (19 mg/ml), arithmetic mean (121 mg/ml), and highest (583 mg/ml) concentrations of midazolam in serum measured. The uncertainty (CV%) in estimating each population parameter value was determined by expressing the standard error of estimation (calculated in NONMEM) as a percentage of the estimated value.** As expected for a population analysis, the fixed effects parameters (θ values) were estimated with better precision (11.5-31.1%) than the random effects parameters (σ = 32.8-83.9%; σ = 59.0%).

Plots of simulated concentrations of midazolam in serum generated for a 24-h loading dose infusion (0.025 mg/h), followed by a continuous infusion (0.0125 mg/h), are shown in figures 3A (n = 40, birth weight ≤ 1,000 g) and 3B (n = 40, birth weight > 1,000 g).

### Discussion

Very premature infants frequently have a range of breathing disorders that require mechanical ventilation. To facilitate intubation, the sedating agent midazolam often is prescribed, and in the current study, we aimed to investigate the pharmacokinetic disposition of this drug in 60 ventilated infants weighing less than 1,500 g at birth. The limited amount of previous kinetic data in very premature infants is attributable largely to the fact that serious ethical and logistical restrictions apply to the rate and extent of blood sampling required by traditional

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Table 2. Comparison of Objective Functions (OF) of Several Models Obtained during Model Building

<table>
<thead>
<tr>
<th>Number</th>
<th>Model</th>
<th>OF</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ClT = $\theta_1$; $V_1 = \theta_2$; ClG = $\theta_3$</td>
<td>1,858</td>
<td>Base model</td>
</tr>
<tr>
<td>2</td>
<td>ClT = $\theta_1$; $V_1$ = $\theta_2$; $\theta_3$(1-GEN); V2 = $\theta_4$</td>
<td>1,737</td>
<td>Significantly different ClT for males (GEN = 1) and females (GEN = 0)</td>
</tr>
<tr>
<td>3</td>
<td>ClT = $\theta_1$; MUL + $\theta_2$(1-MUL); $V_1$ = $\theta_3$; ClG = $\theta_4$; V2 = $\theta_5$</td>
<td>1,796</td>
<td>Significantly different ClT in multiple (MUL = 1) and singleton (MUL = 0) births</td>
</tr>
<tr>
<td>4</td>
<td>ClT = $\theta_1$, BWT + $\theta_2$(1-BWT); $V_2$ = $\theta_3$, BWT + $\theta_4$(1-BWT); ClG = $\theta_5$, BWT + $\theta_6$(1-BWT); V2 = $\theta_7$, BWT + $\theta_8$(1-BWT)</td>
<td>1,756</td>
<td>Final model; significantly different ClT, $V_1$, ClG, V2 for birth weight &gt;1,000 g (BWT = 0), and birth weight ≤1,000 g (BWT = 1)</td>
</tr>
</tbody>
</table>
two-compartment kinetic parameters $C_l_1$, $V_1$, $C_l_2$, and $V_2$ together with small, normally distributed (weighted) residuals. The differences between our results and those of the study by Burtin et al.\textsuperscript{13} may be attributable partly to the fact that most of the patients in the latter study were much less premature than ours, and it included term infants up to a birth weight of 5,200 g. Furthermore, the influence of coadministered drugs was not considered in the current study because of the large imbalance in the numbers of infants who were receiving other drugs.

Substitution of current body weight for birth weight gave OF values that were considerably higher than for the baseline model, indicating an inferior fit to the data (particularly in estimating $V_1$ and $V_2$). Moreover, the application of a slope-intercept model, $C_l_1 = \theta_1 + \theta_2 \cdot BWT$ (where BWT = birth weight), produced rounding errors and substantially higher OF values compared with those obtained from the baseline model. This strongly suggested that the data did not support a model in which a component of the $C_l_1$ could be identified that was independent of the degree of prematurity. More than one third of the 60 study infants were born of either twin (n = 15), triplet (n = 6), or quintuplet (n = 1) pregnancies. It is noteworthy that, of the covariates screened, the greatest change (−121) in the OF value resulted when the infants were subdivided on the basis of whether they were a child from a multiple-birth or a single-birth pregnancy. The population average $C_l_1$ of those born of a single-birth pregnancy was greater than that from multiple-birth pregnancies (1.05 ml/min vs. 0.783 ml/min, respectively). Initially, it was assumed that multiple-birth pregnancy could be acting as a surrogate marker for the degree of prematurity (i.e., birth weight). Using an unpaired t test analysis, however, the mean ± SD birth weight of infants born of a single-birth pregnancy (937 ± 233 g) was statistically insignificant ($P >$

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**Table 3. Estimated Typical Values and Interpatient Variability of Population Parameters for Midazolam**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Birth Weight ≤1000 g</th>
<th>Birth Weight &gt;1000 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>CV (%)</td>
</tr>
<tr>
<td>$C_l_1$ (ml/min)</td>
<td>0.783</td>
<td>83</td>
</tr>
<tr>
<td>$C_l_2$ (ml/min)</td>
<td>6.53</td>
<td>116</td>
</tr>
<tr>
<td>$V_1$ (ml)</td>
<td>473</td>
<td>70</td>
</tr>
<tr>
<td>$V_2$ (ml)</td>
<td>513</td>
<td>146</td>
</tr>
</tbody>
</table>

* Interpatient variability (coefficient of variation) of parameter.
The population mean $\text{Cl}_T$ of 0.938 ml/min $\cdot$ kg$^{-1}$ calculated over all patients was markedly lower than the average value of 12.0 ml/min $\cdot$ kg$^{-1}$ reported in children from 0.25-8.00 yr old$^{23}$ and in young, healthy adults ($4.05-7.80$ ml/min $\cdot$ kg$^{-1})^{2,24}$ This trend is almost certainly attributable to the markedly compromised hepatic and renal function in very premature neonates.$^{15}$ The total $V$ (i.e., $V_1 + V_2$) calculated over all patients averaged 1.15 l/kg, which agreed closely with the mean $V$ of 1.10 l/kg in term and preterm infants$^{15}$ but which was smaller than the 1.3-2.0 l/kg determined in adults.$^2$ Exploration of interpatient variability is an important aspect of population modeling, and in this study we determined that infants varied considerably among each other regarding each of the pharmacokinetic parameters of midazolam. This finding agreed with the general conclusion of Burtin et al.$^{13}$ and is consistent with our own studies of the population pharmacokinetics of other drugs in very premature infants, including theophylline,$^{17}$ caffeine,$^{18}$ and amoxicillin.$^{19}$ Variation in $\text{Cl}_T$ is attributable to either variable hepatic or renal elimination, but, unlike the situation in adults, the renal clearance of midazolam in very premature infants probably makes a significant contribution to the variability in $\text{Cl}_T$ because of deficient hepatic mixed-function oxidase activity.$^{15}$ Furthermore, the glomerular filtration rate of unmetabolized drug in the premature newborn may be affected by hemodynamic instability$^{25}$ and several other factors, notably mechanical ventilation,$^{20}$ a procedure received by all infants in the current study. Unfortunately, the renal clearance of midazolam could not be measured because of logistical problems and concerns about the risk of infection with catheterization of the urinary tract.

In adults, concentrations in serum of approximately 50-500 ng/ml in healthy volunteers$^{27}$ and approximately 75-175 ng/ml in mechanically ventilated patients undergoing surgery$^{28}$ reportedly produce sedation (with arousability), whereas in children (aged 0.50-8.75 yr), sedation during artificial ventilation required 250-500 ng/ml.$^{1}$ Simulations obtained using the final population model parameter values indicated that an initial 24-h infusion of 0.025 mg/h followed by 0.0125 mg/h continuously up to 152 h theoretically should produce average steady state concentrations in serum of 265 ng/ml and 170 ng/ml in infants with birth weights of 500-1,000 g and 1,001-1.500 g, respectively. The interpatient variability in the population kinetic parameters was reflected in the considerable spread of the simulated concentrations in serum. Nonetheless, most infants should
have concentrations between 50 ng/ml and 500 ng/ml within 6 h of starting the loading infusion, although we emphasize that the dose recommendations mentioned have not been tested prospectively. More studies using objective outcome measures are necessary to establish therapeutic windows for midazolam when prescribed alone or in combination with other drugs (e.g., fentanyl, morphine).

The results of this study confirm and extend the small amount of pharmacokinetic data of midazolam that exists for very premature neonates. Our results and those of Burtin et al. established the feasibility of using a population approach in which only a limited amount of data is contributed by each patient, using a two-compartment population pharmacokinetic model in NONMEM. The $\text{CL}_F$, $\text{CL}_D$, $V_1$, and $V_2$ values were lower in infants with birth weights of 500–1,000 g compared with those with birth weights of 1,001–1,500 g. There was marked interpatient variability in all pharmacokinetic parameters.

References


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