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Pharmacokinetics of Midazolam in Neonates Undergoing Extracorporeal Membrane Oxygenation

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Background: **Although the pharmacokinetics of midazolam in critically ill children has been described, there are no such reports in extracorporeal membrane oxygenation.**

Methods: **The pharmacokinetics of midazolam and 1-hydroxy midazolam after continuous infusion (50–250 $\mu g \cdot kg^{-1} \cdot h^{-1}$) were determined in 20 neonates undergoing extracorporeal membrane oxygenation. Patients were randomized into two groups: group 1 (n = 10) received midazolam extracorporeally (into the circuit), and group 2 received drug *via* central or peripheral access. Blood samples for determination of plasma concentrations were taken at baseline, 2, 4, 6, 12, 18, and 24 h, then every 12 h. Population pharmacokinetic analysis and model building was conducted using WinNonMix (Pharsight Corporation, Mountain View, CA). The 1-hydroxy midazolam/midazolam metabolic ratio was determined as a surrogate marker of cytochrome P450 3A activity.**

Results: **The parameter estimates (n = 19) were based on a one-compartment model with time-dependent change in volume of distribution. Volume (mean ± standard error) expanded monoexponentially from the onset of extracorporeal membrane oxygenation to a maximum value, 0.8 l ± 0.5 and 4.1 ± 0.5 l/kg, respectively. Consequently, plasma half-life was substantially prolonged (median [range]) from onset to steady-state: 6.8 (2.2–39.8) and 33.3 (7.4–178) h, respectively. Total body clearance was determined as (mean ± standard error) 1.4 ± 0.15 ml $\cdot kg^{-1} \cdot min^{-1}$. The median metabolic ratio was 0.17 (0.03–0.9). No significant differences were observed between the two groups with respect to parameter estimates. Simulations of plasma concentration profiles revealed excess levels at conventional doses.**

Conclusions: **These results reveal significantly increased volume of distribution and plasma half-life in neonates receiving extracorporeal membrane oxygenation. Altered kinetics may reflect sequestration of midazolam by components of the extracorporeal membrane oxygenation circuit.**

ADEQUATE sedation of neonates receiving extracorporeal membrane oxygenation (ECMO) is essential to allay the physical, emotional, and psychologic distress experienced during intensive care. Inadequate sedation with excessive patient movement can also affect cannula position and hence blood drainage into the ECMO circuit, thereby reducing gas exchange.[1] Midazolam, a benzodiazepine derivative, is our sedative of choice and is preferred over other benzodiazepines because of its water solubility and perceived rapid elimination.[2] It undergoes extensive metabolism by the cytochrome P450 3A (CYP3A) subfamily to a major hydroxylated metabolite (1-hydroxy-midazolam) and several minor metabolites (4-hydroxy and 1,4-hydroxy midazolam) before glucuronidation and excretion in the urine. In neonates, hepatic CYP3A activity is decreased, resulting in significantly reduced clearance when compared with adults.[3]

The effective and appropriate use of midazolam during ECMO requires an understanding of its pharmacokinetics. Although there have been several studies of preterm neonates or neonates of wide gestational age range,[3–6] it may not be appropriate to relate these studies to neonates on ECMO, who tend to be term or near term. Moreover, the disposition of drugs is known to be altered during ECMO. Physiologic changes as a result of the expanded circulating volume, intrinsic increase in intracellular and extracellular water, nonpulsatile blood flow during veno-arterial ECMO and reduced plasma protein concentrations may significantly affect pharmacokinetics and pharmacodynamics.[7] Sequestration of many drugs, including midazolam, by the polymeric components of the ECMO circuit has also been demonstrated.[8,9]

We recently reported midazolam doses administered and plasma concentrations achieved during neonatal ECMO, revealing attenuation of plasma levels during the first 24 h of ECMO and then a subsequent and significant increase suggestive of a reduced rate of elimination.[10] We believe this may be attributable to altered midazolam pharmacokinetics secondary to binding to the ECMO circuit. In the current investigation we use dose and concentration data to determine the pharmacokinetics of midazolam and 1-hydroxy midazolam. Our goals were to improve the accuracy of midazolam administration by determination of pharmacokinetic parameters and through simulations to suggest an appropriate dosing approach.

Materials and Methods

Patient Recruitment

After local research and ethics committee approval, neonates undergoing ECMO were recruited in the study with written informed parental consent. Twenty neonates were randomized into two groups: group 1 (n = 10) received midazolam extracorporeally, prereservoir, *via* a pigtail catheter, whereas group 2 received the drug *via* a central or peripheral venous catheter. Patients

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were excluded from the study if midazolam was administered to them before initiation of ECMO.

Study Design

Midazolam was administered as a continuous infusion, at a rate usually between 50–250 $\mu g \cdot kg^{-1} \cdot h^{-1}$, initiated as soon as cannulation was achieved and extracorporeal blood flow was established. Infusion rates were titrated according to the unit sedation scoring guidelines: 1 = wide awake, 2 = awake but sleepy, 3 = asleep but moved spontaneously, 4 = asleep, but responded to stimulation, and 5 = hard to rouse. The target sedation score in most cases was 4. When levels of sedation were not satisfactory, rates of infusion were increased or decreased as indicated and additional bolus injections of 50–100 μg/kg were given if necessary. Infusions continued for the duration of ECMO, and weaned postdecannulation and before extubation. Infusions were stopped during ECMO when deemed clinically necessary by the responsible clinician (for assessment of neurologic status or to avoid oversedation).

Along with continuous heparinization to activated clotting times of 160–200 s, various drugs such as antibiotics, inotropes, diuretics, and H_2 blockers were coadministered during the study as indicated. ECMO circuits used in all neonates consisted of Tygon® S-65-HL (Norton Performance Plastics, Corby, Northants., U.K.) and a silicone membrane oxygenator (0800, Avecor, Cardiovascular Inc., Minneapolis, MN). The priming volume of the circuit was approximately 500 ml. The clear prime consisted of 100 ml of human albumin solution, 20%, and 400 ml Plasmalyte A, whereas the blood prime consisted of 500 ml donor blood, 120 units heparin, 15 ml sodium bicarbonate, 8.4%, and 2.5 ml calcium chloride, 10%.

Blood Sampling and Analysis

Blood samples (1 ml) for assay were collected from indwelling arterial lines. Samples were drawn at baseline (before cannulation for ECMO), at 2, 4, 6, 12, 18, and 24 h and every 12 h thereafter. Samples were immediately stored at −20°C until analysis. Concentrations of midazolam and the free unconjugated form of its major metabolite 1-hydroxy midazolam were determined using gas chromatography–mass spectrometry.[10] The assay was validated over the concentration range 10–10,000 ng/ml. The within and between day coefficient of variation was less than 10%.

Pharmacokinetic Analysis: Model Development

Population pharmacokinetics of midazolam were estimated using WinNonMix, a nonlinear mixed effects regression program (WinNonMix, Pharsight Corporation, Mountain View, CA). This approach estimates not only the structural parameters of the model but also the interindividual (population) variability in parameters and residual error (difference between observed and predicted midazolam concentrations).[11] In a preliminary analysis, the parameters of one-, two-, and three-compartment models were fitted to the data by use of this approach. The models were parameterized in terms of volume of distribution and clearance. The interindividual variability in parameters was modeled as a proportional deviation from the "true" parameter values (assuming a log normal distribution). Residual error was estimated with a combined proportional and additive model.

The influence of demographic and clinical variables on parameters of the model were also analyzed and, when significant, were added to the model to determine whether the overall variability in the model could be reduced. These included gestational and postnatal age, weight, gender, creatinine, urine output, continuous veno-venous hemofiltration, liver function tests, plasma albumin concentrations, comedication, cannulation mode, duration of ECMO, and ECMO pump-flow rates.

Pharmacokinetic Analysis: Cross-Validation and Predictive Performance

Because it was not practical to collect new data prospectively, the ability of our final model to perform in prospective tests was estimated using the cross-validation technique.[12] Although cross-validation is not a truly prospective validation method, it is a recognized and established approach to estimating model performance, assuming identical experimental conditions.[13–15]

The study group was divided into eight smaller groups of two patients each and one group of three patients. The pharmacokinetic model was fitted to the data while excluding one group. The estimated structural parameters from the submodels were then used to predict the concentrations in the excluded group. This process was repeated nine times, excluding each group in turn. A measure of the predictive performance of models can be determined by calculating the median prediction error, *i.e.*, observed minus predicted concentrations, and the absolute median prediction error.[16] The median prediction error describes the bias and absolute median prediction error, the precision (variability) of the predictions. Because the excluded group is not used to develop the model, the median prediction error and the absolute median prediction error are almost unbiased estimates of the predictive capabilities of the model.[14] In addition, the area under the curve plasma metabolic ratio (MR) (1-hydroxymidazolam/midazolam MR) was determined using the following equation[17]:

$$MR = \frac{(C_{(t1)\text{1-hydroxyMDZ}} + C_{(t2)\text{1-hydroxyMDZ}})}{(C_{(t1)\text{MDZ}} + C_{(t2)\text{MDZ}} \times 0.5} \times 0.5$$

Table 1. Patient Demographics

Gestational age, wk	39.5 ± 1.9
Postnatal age at cannulation, d	3.8 (0.5–18)
Weight, kg	3.4 ± 0.6
Gender, M/F	11/9
Cannulation type, VV/VA	17/3
ECMO duration, d	6.2 ± 2.9
Length of ICU stay, d	11.8 ± 8.6
No. of patients on CVVH	7
No. patients with elevated ALP/ALT	7
Diagnoses, no. of patients	
MAS/PPHN	12
Sepsis	1
CDH	4
Postcardiac surgery	2
Metabolic	1
Survival, %	80

Data are expressed as mean ± SD (range).

ALP = alkaline phosphatase; ALT = alanine transaminase; CVVH = continuous veno-venous hemofiltration; CDH = congenital diaphragmatic hernia; ECMO = extracorporeal membrane oxygenation; MAS = meconium aspiration syndrome; PPHN = persistent pulmonary hypertension of the newborn; VA = veno-arterial; VV = veno-venous.

where $C_{(t1)\text{1-hydroxy MDZ}}$, $C_{(t2)\text{1-hydroxy MDZ}}$, and $C_{(t1)\text{ MDZ}}$, $C_{(t2)\text{ MDZ}}$ are the concentrations of 1-hydroxy midazolam and midazolam, respectively, at two time points. This ratio has been used as a marker of CYP3A activity.[17]

Statistical Analysis

Data are expressed as mean ± SD, mean ± standard error for population parameter estimates, and median (range). The MR was determined as the median MR from all patients. Selection of the optimal model was based on examination of graphic residuals plots and the objective function (−2 times the logarithm of the likelihood of the results). WinNonMix minimizes the objective function in performing nonlinear regression analysis. A model with a lower objective function value (OFV) offers an improvement in the goodness of fit. The difference in the OFV obtained before and after the addition of parameters is approximately chi-square distributed with degrees of freedom equal to the number of parameters that are set to the null hypothesis value. A change in the OFV greater than 7.88 ($P < 0.005$) was considered statistically significant. The impact of significant covariates was also evaluated using this approach.

Results

Demographic data of the study population are shown in table 1. There were no significant differences in characteristics between the two randomized groups. Three patients died during ECMO, one patient postdecannulation. In three of the cases, deaths were as a result of pulmonary or cardiopulmonary failure, whereas myophosphorylase deficiency was the cause in another. From a total of 210 midazolam plasma concentrations analyzed, 45 (21%) observations were excluded from the pharmacokinetic analysis. Excluded were 13 observations from the patient with myophosphorylase deficiency who exhibited no decrease in plasma concentrations despite no drug being administered for 50 h; 15 observations collected during the post-ECMO period; and 17 observations that confounded normal physiologic principles (*e.g.*, increase in plasma midazolam concentrations when no drug had been administered).

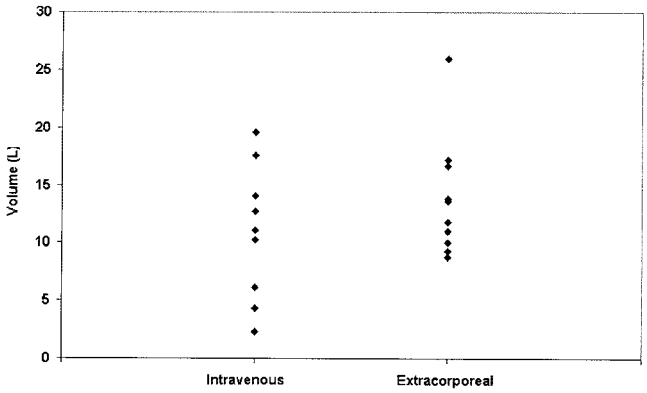

Fig. 1. Volume of distribution *versus* route of administration of midazolam.

The one-compartment model was selected for further analysis of midazolam because it demonstrated a more appropriate structural model on examination of the graphical residual plots. Furthermore, the OFV was not significantly lower with the two- and three-compartment models and estimates of the pharmacokinetic parameters were less precise, which is probably a reflection of our limited sampling (particularly in the initial phase of ECMO) and inability to discern peripheral compartments. Plots of model parameters *versus* clinical and demographic covariates revealed no significant influences apart from randomization allocation (*i.e.*, whether the drug was administered extracorporeally or intravenously) with volume of distribution. The latter suggested that those patients administered midazolam extracorporeally had a higher volume of distribution (mean ± SD: 13.8 ± 5.2 *vs.* 10.9 ± 5.9 L) (fig. 1). Although this improved the model by reducing the variability on the volume and the OFV by 3.1 U, this was not significant at the $P = 0.005$ level (table 2) and thus was excluded from the final model.

A closer inspection of the residuals against time plot (fig. 2, *top*) suggested that the model underpredicted concentrations in the early phase of ECMO. Therefore, the influence of various time covariates added categorically to both clearance and volume (range, 2–24 h) were tested in the model. The model improved measurably with the inclusion of a 12-h time covariate on volume, producing a drop in the OFV of 16.6 U (table 2). This time-dependent change in volume was attributed to reversible sequestration of midazolam by components of the circuit. Thus, after detecting time dependency as a significant covariate for volume, the phenomenon was

Table 2. Model Development and Covariate Analysis

Model	OFV*,†	Comments	Bias, %§	Precision, %§
One compartment, base model	242.3		−11.9 (−23.0, 0.8)	36.7 (30.3, 43.5)
Randomization on volume	239.2	Administration method does not significantly influence volume	0.2 (−7.6, 7.0)	19.8 (16.3, 25.3)
12-hour time covariable on clearance	241.9	Clearance is not time-dependent	0.3 (−6.4, 6.2)	22.0 (18.2, 25.4)
12-hour time covariable on volume	225.7	Volume is time-dependent	−1.4 (−5.2, 3.8)	20.9 (15.8, 25.0)
Time dependent volume,† final model	204.7	Volume expansion with time (defining midazolam sequestration) improves model	−0.3 (−5.2, 5.0)	20.0 (16.2, 23.1)
Cross-validation of final model			−0.3 (−5.6, 4.8)	19.3 (15.1, 23.3)

* Volume (L) = $V_{ss} - (V_{ss} - V_0)e^{kseq.time}$. † Assuming a chi-squared distribution, a change in the OFV > 7.88 ($P < 0.005$) was accepted as statistically significant. ‡ −2 * log likelihood. § Figures in parenthesis are 95% confidence intervals for the median.

incorporated into the structural model. Midazolam uptake by the circuit was accommodated by allowing the initial value of volume (V_0) to increase mono-exponentially to an asymptotic value, V_{max}. This approach was analogous to that previously described by Rostami-Hodjegan *et al.* for time-dependent change in clearance due to enzyme induction[18] (Volume (L) = $V_{max} - (V_{max} - V_0)e^{kseq.time}$, where K_{seq} = the rate constant for uptake of midazolam into the circuit and time = duration of infusion).

The revised model was considerably better at fitting plasma midazolam concentrations, abolishing the early trend in residuals (fig. 2, *bottom*), and reducing OFV by 21 U, although it explained only 4% of the variance. The mean individual Bayesian posterior volumes increased approximately fivefold from the onset of ECMO to the steady-state value (median [range]): 3.1 (1.0–8.0) *versus* 14.2 (4.7–35.8) L, respectively (fig. 3). These changes were accompanied by a significant increase in the initial to steady-state half-life (median [range]): 6.8 (2.2–39.8) *versus* 33.3 (7.4–178) h, respectively (fig. 3). Interpatient variability in clearance and volume was 73 and 53%, respectively, whereas residual error corresponded to a proportional error of 26% and an additive error of 0.17 μg/ml (table 3).

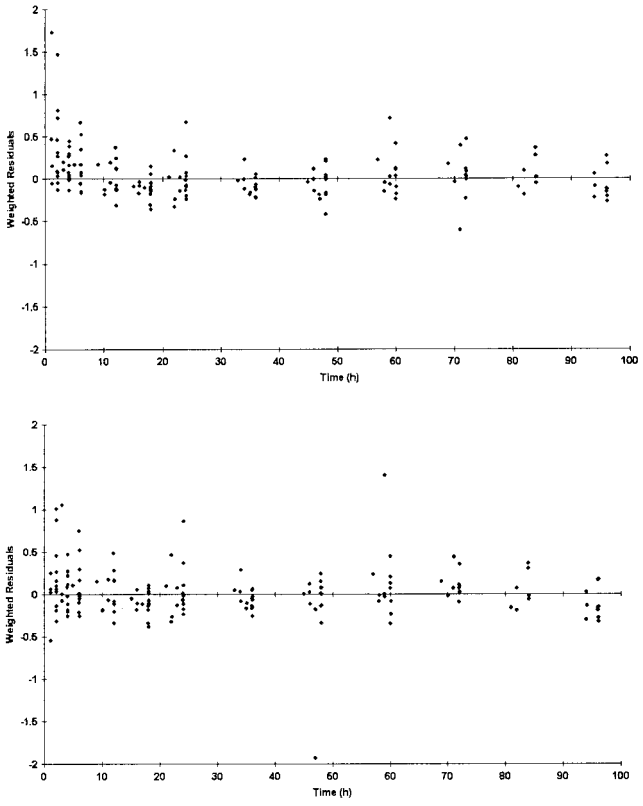

Fig. 2. Weighted residuals as a function of time. (*Top*) A simple one-compartment model. The model seems to underpredict in the early phases of extracorporeal membrane oxygenation. (*Bottom*) With the inclusion of a time-dependent increase in volume, the early trend in the residuals diminishes.

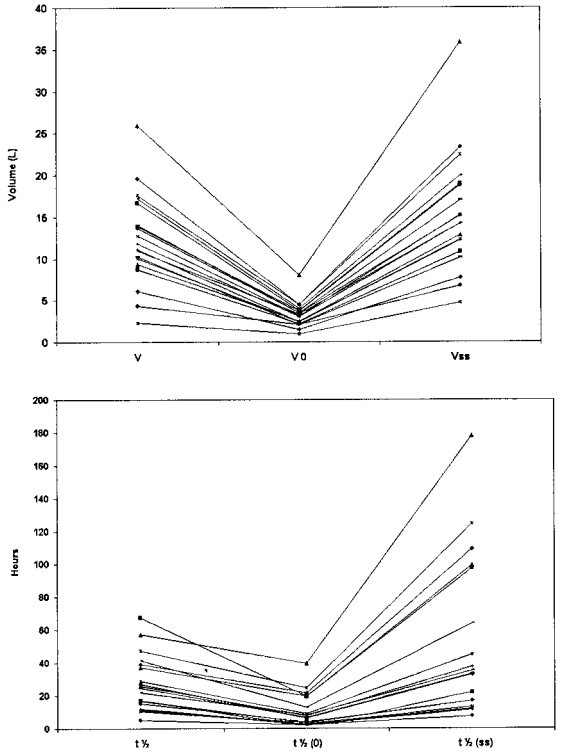

Fig. 3. Individual estimates of (*top*) volume of distribution (V) and (*bottom*) half-life ($t_{1/2}$) obtained using the simple one-compartment and time-dependent volume models. 0 = initial values; ss = steady-state values.

Table 3. Pharmacokinetic Parameters for Midazolam and 1-hydroxy Midazolam in Neonatal Extracorporeal Membrane Oxygenation Patients

V_0 (L)*	V_{max}(L)*	Kseq (hr^{-1})*	CL ($L.hr^{-1}$)*	$t_{1/2\ (0)}$ (hr)†	$t_{1/2(ss)}$ (hr)†	MR
2.8 ± 1.7	13.9 ± 1.6 53%§	−0.19 ± 0.07	0.28 ± 0.03 73%§	6.8 (2.2–39.8)	33.3 (7.4–178)	0.17 (0.03–0.9)

* Population parameter estimates, expressed as mean ± standard errors. † Half-lives were not primary structural model parameters and were calculated as median (range) *a posteriori* from individual Bayesian parameter values. ‡ Data from 12 patients. § Interindividual variability in parameter estimates, expressed as coefficients of variation.

Constant CL = clearance; K_{seq} = midazolam sequestration rate; MR = 1-hydroxy midazolam/midazolam metabolic ratio; $t_{1/2\ (c0)}$ = initial half-life; $t_{1/2(ss)}$ = terminal half-life; V_0 = initial volume; V_{max} = maximum volume.

The relationship between observed and model predictions and selected individual patient profiles before and after the addition of the time dependency model are shown in figures 4 and 5, respectively. The individual Bayesian posterior parameters estimated from the model described the data well with bias of −0.3% and precision of 20%. The cross-validation exercise revealed an overall bias and predictive precision for the submodels of −0.3 and 19.3%, respectively, indicating that the full model is likely to perform well in a truly prospective trial during similar conditions (table 2).

Plasma concentrations of the free unconjugated 1-hydroxy midazolam were assayed in 12 patients (106 observations with no exclusions). The median MR was 0.17 with a large interindividual variability (range, 0.03–0.9). Seven neonates (35%) exhibited biochemical signs of liver dysfunction with raised alkaline phosphatase (ALP) and alanine transaminase (ALT), though no correlation was observed with MR. However, the lowest MR (0.03) was observed in a patient with significantly deranged liver function tests (ALT 281, ALP 922, bilirubin 100 mM), suggesting impaired metabolism of midazolam. The highest MR (0.9) was observed in the patient with highest estimate of midazolam clearance (3.8 $ml \cdot kg^{-1} \cdot min^{-1}$). Midazolam and 1-hydroxy midazolam pharmacokinetics are summarized in table 3.

Discussion

The most significant observations of this study are the exhibition of markedly altered midazolam pharmacoki-

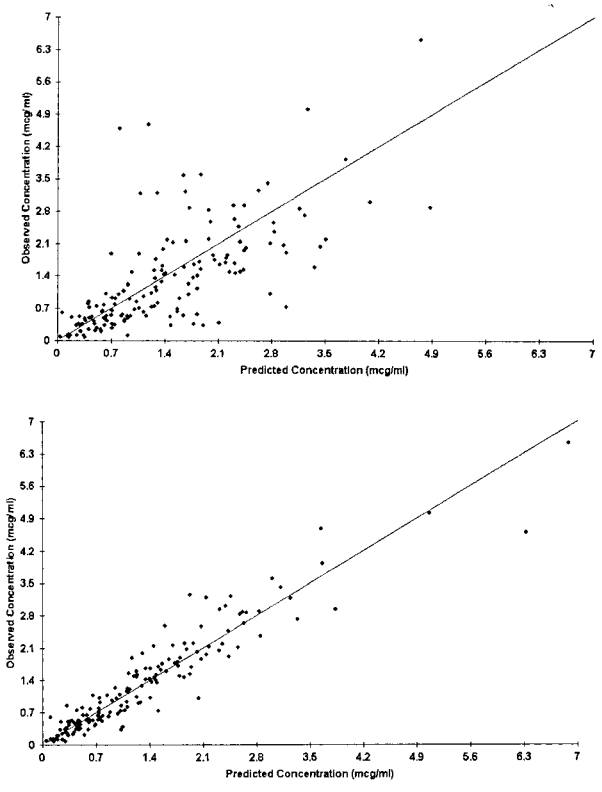

Fig. 4. Observed *versus* (*top*) population and (*bottom*) individual Bayesian posterior predictions from the final time-dependent model. *Solid line* represents unity.

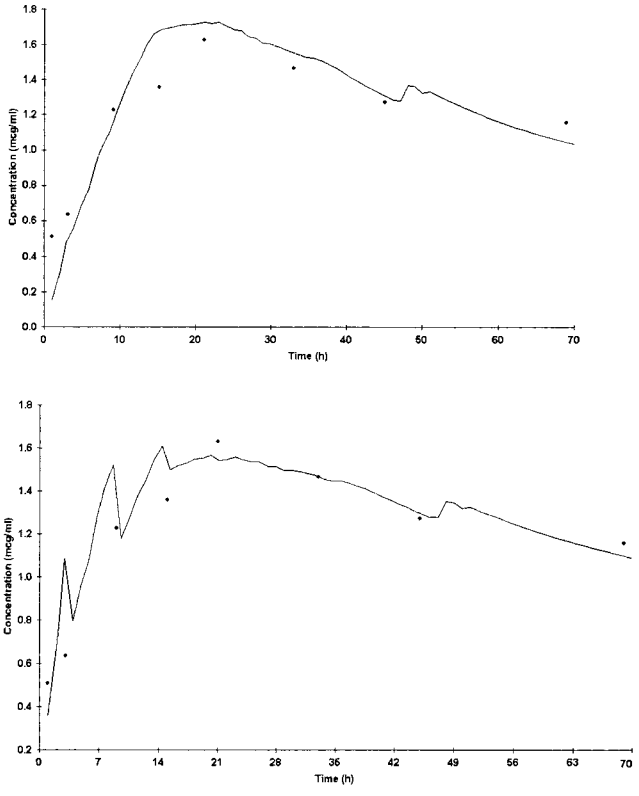

Fig. 5. Model fits to the same patient. (*Top*) A simple one-compartment model, (*bottom*) with time-dependent volume. *Dots* = observed concentrations; *line* = individual Bayesian posterior predictions.

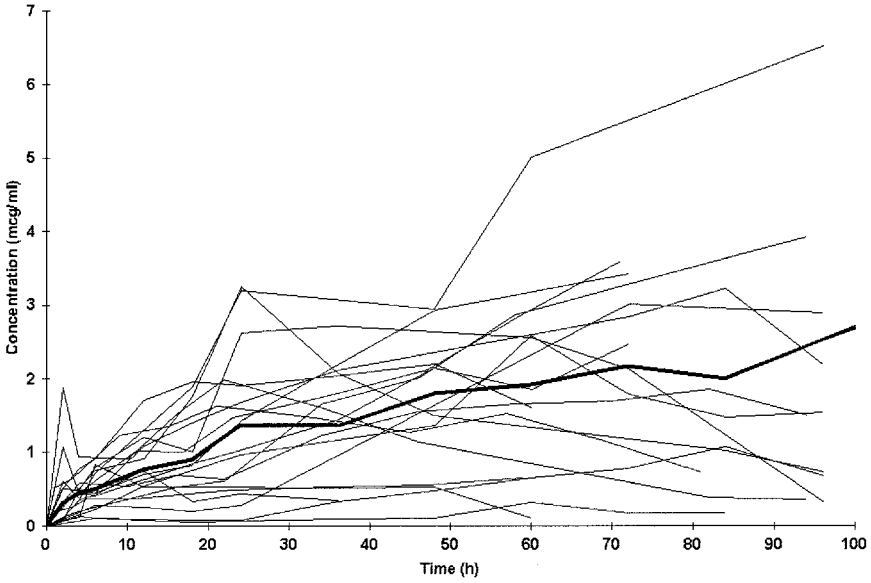

Fig. 6. Time-plasma concentration profiles of midazolam from the study population, with the mean profile in *bold*. The mean dose administered over this period was 259 $\mu g \cdot kg^{-1} \cdot h^{-1}$.

netics by neonates treated with ECMO compared with previously reported values for such patients. Previous investigators have reported that a two-compartment model is optimal for describing the pharmacokinetics of midazolam, in contrast to our one-compartment model.[2–6] This may be a reflection of our study design, which used continuous infusions of midazolam with limited sampling in the distribution phase and few samples captured in the elimination phase.

Our parameter estimates are based on a model in which volume of distribution expands with time as ECMO support progresses. In this population, the mean initial volume on ECMO was estimated at 0.8 l/kg, similar to that previously reported by Burtin *et al.* in non-ECMO neonates of gestational ages of 26–42 weeks (1 l/kg).[5] This initial volume is perhaps lower than expected considering the expanded circulating volume for a child on ECMO. The normal intravascular volume for a neonate of 80 ml/kg would be significantly diluted at cannulation with the addition of a circuit volume of 500 ml. However, this parameter was imprecisely estimated with a coefficient of variation of almost 63%. A time-dependent increase in volume produced a maximum mean population value of 4.1 l/kg, three to four times greater than previously reported in neonates.[2,3,5,6] The mechanism for this change may be attributable to sequestration of midazolam by the ECMO circuit. The uptake of drugs by polymeric components of the ECMO circuit has previously been demonstrated through *in vitro* studies.[1,9] This is an exponential, concentration-driven process eventually reaching a state of equilibrium. Many physicochemical factors will affect the time to equilibrium and the extent of drug loss, including lipid solubility (octanol:water coefficient or log *P* values) of drugs, dosing rate, plasma concentration of drug, presence of co-drugs, and ECMO flow rates. Inflation in volume resulted in a mean terminal half-life of 33.3 h, substantially longer than previous reports in neonates (12 ± 4.9 h),[2] older

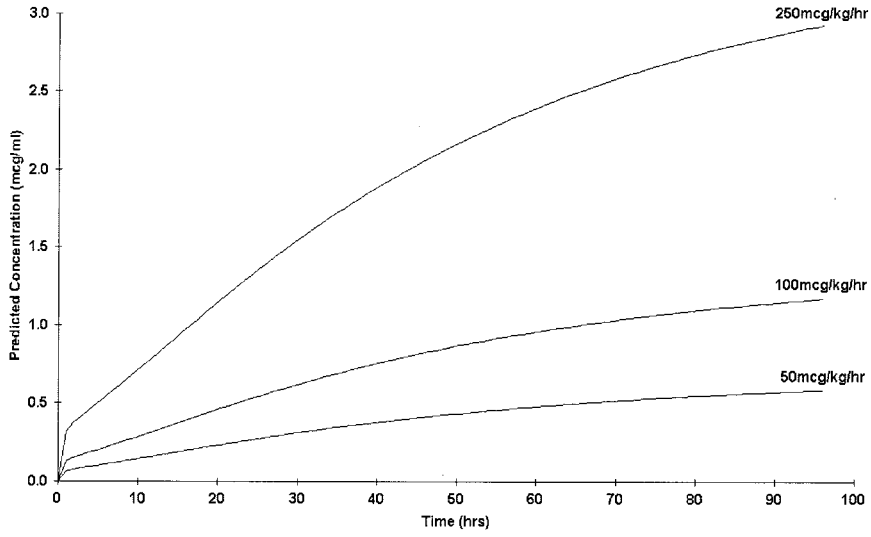

Fig. 7. Simulated time-concentration profiles of midazolam at various dose rates using the final model pharmacokinetic parameters.

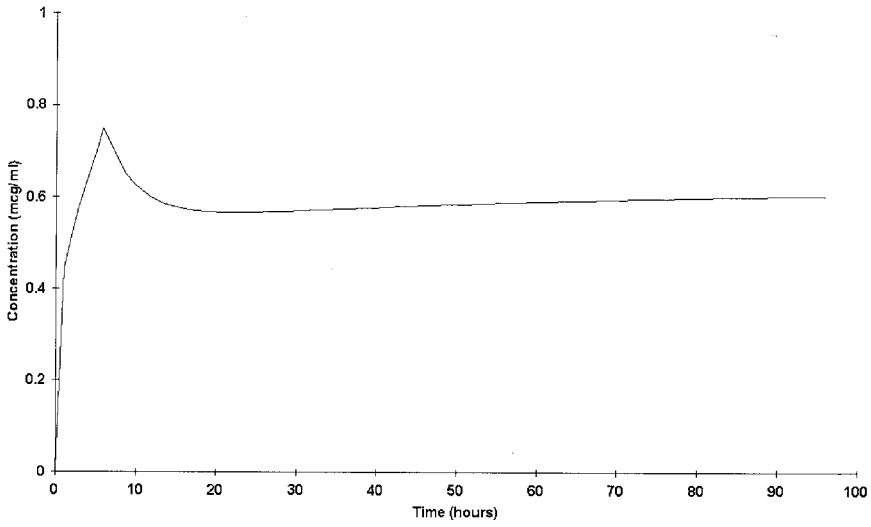

Fig. 8. Simulated time-concentration profile using parameters from the final model. A regimen consisting of a continuous infusion rate of 350 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 6 h and then 50 $\mu g \cdot kg^{-1} \cdot h^{-1}$ thereafter was used to maintain therapeutic plasma levels.

infants, children and adults (range, 1–7 h).[19–22] This accounts for raised plasma concentrations of midazolam observed during the later phases of ECMO in our study population (fig. 6) with levels far in excess of those required for adequate sedation (< 1 μg/ml).[10] Similar increases in plasma fentanyl levels and the development of tolerance during ECMO have been reported.[23–25] Analogous to midazolam (log *P*, 2.7; Roche FH-L: Midazolam (base) Safety Data Sheet. Basel, Switzerland, 2000), fentanyl, a highly lipophilic molecule with a log *P* value of 2.9[26] has frequently shown to be sequestered by polymeric components of the cardiopulmonary bypass circuit.[27–31] Interestingly, the group that received midazolam extracorporeally had a tendency toward a higher volume of distribution. Although the addition of this covariate did not significantly improve the fit, it is plausible that a higher volume of distribution during extracorporeal drug administration exists because of greater distribution of the drug into the polymeric components of the circuit. This implies that midazolam should ideally be administered directly *via* peripheral or central venous access.

For ECMO neonates, the metabolic clearance of midazolam is similar to that previously reported in non-ECMO neonates. Population estimates of clearance in our cohort of term or near-term neonates (1.4 $ml \cdot kg^{-1} \cdot min^{-1}$) was comparable with values previously reported in population studies of neonates by Burtin *et al.* (1.2 $ml\ kg^{-1} \cdot min^{-1}$) and Lee *et al.* (0.9 $ml \cdot kg^{-1} \cdot min^{-1}$), but significantly lower than those reported in older infants and children (5.8–13.6 $ml \cdot kg^{-1} \cdot min^{-1}$) and adults (6.4–11.1 $ml \cdot kg^{-1} \cdot min^{-1}$).[5,6,32,33] Reduced midazolam elimination in our population was not unexpected, reflecting the pattern for the ontogeny of CYP3A4.[34] In adults, plasma clearance of midazolam has been delineated and correlated with CYP3A4/5 activity.[35] Hepatic CYP3A4 is abundantly expressed in adults being responsible for catalyzing the biotransformation of approximately 50% of currently used drugs. CYP3A5 expression shows large interindividual differences and has been shown to be present in only 10–30% of liver samples tested.[36] In contrast, CYP3A7 is the major isoform expressed in the fetal liver, with probably only marginal contribution to midazolam clearance in the postnatal period. CYP3A4 expression is activated during the first few weeks after birth, increasing thereafter to reach adult values at 1 yr of age, with a simultaneous decrease in CYP3A7 activity.[34]

We determined the 1-hydroxy midazolam/midazolam MR as a surrogate marker for CYP3A4/5 activity. The median (range) values in our patients (0.17 [0.03–0.9]) appear to be significantly higher than previously reported in preterm infants (0.09) and similar to those in older children and adults (0.13–0.25).[37,38] Reports indicate that CYP3A4 expression is activated irrespective of gestational age[39] and that enzyme activity increases only marginally during the first 2 weeks of life. Though gestational age range in our group was higher than previous reports (33–42 *vs.* 26–34 weeks), postnatal age ranges were similar (0.5–18 *vs.* 3–11 days) (reference ranges). Thus, the higher MR in our group does not necessarily represent higher CYP3A activity. Rather, it may be indicative of reduced glucuronidation of 1-hydroxy midazolam by uridine-diphosphate glucuronosyl transferases and thus reduced renal elimination. The developmental ontogeny of uridine-diphosphate glucuronosyl transferases has not yet been delineated. The wide interpatient variability in elimination of midazolam and metabolite and MR observed in our population corresponds with previous reports[3] and reflects not only the heterogeneous and critically ill nature of the ECMO population but also indicates that CYP3A and glucuronidation activity in the neonate exhibits large variability.

The clinical implications of our findings are illustrated in figure 7. The figure depicts simulated concentrations for a hypothetical neonate on ECMO receiving continuous infusions of midazolam at various dose rates, using

the estimated population parameters. These simulations closely resemble the mean observed concentration profile in this population (fig. 6) revealing attenuation of plasma levels during the early phase of ECMO followed by a significant rise. The results also suggest that a higher initial dose rate in the early phase of ECMO needs to be followed by a significant decrease in the dose rate to prevent excessive plasma concentrations. For optimum sedation, plasma concentrations of midazolam up to 1.0 μg/ml are required.[10] This was simulated using an initial infusion rate of 350 $\mu g \cdot kg^{-1} \cdot h^{-1}$)for 6 h followed by a drop in the dose rate to 50 $\mu g \cdot kg^{-1} \cdot h^{-1}$ (fig. 8). Cross-validation provides a measure of the predictive ability of the model in the absence of prospectively collected test data. The exercise revealed an insignificant change in bias or precision, suggesting that the estimated model is likely to perform well in truly prospective trials.

In summary, we have analyzed the pharmacokinetics of midazolam in neonates undergoing ECMO, deriving parameters and their variability using a population approach. The midazolam model reveals a significantly altered volume of distribution in ECMO patients compared with reports in non-ECMO patients, probably through reversible binding to the circuit. Consequently, half-life was significantly prolonged and hence midazolam must not be considered a sedative with a short half-life in neonatal ECMO patients. We also determined the MR, a surrogate measure of CYP3A activity, as being higher than previous reports in preterm neonates and attribute this to a reduced clearance of the metabolite. Using final model parameters, we simulated plasma midazolam concentration profiles at various dose rates and a strategy to achieve adequate plasma levels. An understanding of the pharmacokinetics of midazolam during ECMO helps promote the development of rational dosing approaches and titration to sedation scoring systems. Appropriate sedation levels reduce the incidence of hemodynamic side effects associated with oversedation, expediting return to normal mental status following cessation of the drug.

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