Contribution of midazolam and its 1-hydroxy metabolite to preoperative sedation in children: a pharmacokinetic-pharmacodynamic analysis

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Background. Oral midazolam is widely used for preoperative sedation in children. We have studied the pharmacokinetics (PK) of both midazolam and its active 1-hydroxy metabolite and their contributions to sedative effect in 45 children attending for day surgery.

Methods. Blood samples (two per individual) were collected at the beginning and end of the surgical procedure. Plasma midazolam and 1-hydroxymidazolam (1-OHMDZ) were measured by HPLC. Sedation score (score: 1=awake, 2=drowsy/asleep) was recorded at the same time as the first blood sample. The population-PK software P-Pharm was used to analyse the data. Age, weight, sex, concomitant drugs, and the metabolic ratio, 1-OHMDZ/midazolam were investigated as co-variates of the PK of midazolam and 1-OHMDZ. The pharmacokinetic-pharmacodynamic (PK-PD) modelling of the score in relation to plasma midazolam and 1-OHMDZ was performed using logistic regression analysis.

Results. A median dose of 0.5 mg kg^{-1} was given to the children, median age 5 yr (range from 9 months to 12 yr) and weight 21 kg (range 8–75 kg). Average concentrations of midazolam 150 ng ml^{-1} and 1-OHMDZ 90 ng ml^{-1} were observed in the first plasma samples. These concentrations resulted in an odds ratio of 4 in favour of score 2 *vs* 1. The best PK-PD model included both midazolam and 1-OHMDZ as active moieties and predicted correct scores in 86% of cases.

Conclusion. Studies of midazolam should evaluate the contribution of 1-OHMDZ to the overall PD effect. The metabolite 1-OHMDZ has approximately half the activity of the parent drug and can compensate for at least part of the decreased effect due to increased midazolam metabolism.

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Despite the lack of a product licence for the oral route of administration and associated indications, oral midazolam is commonly used for procedural sedation in children. However, presumably because of the difficulties in obtaining multiple blood samples from a paediatric population, the PK-PD characteristics of midazolam for this indication are poorly characterized. Payne and colleagues[1] found that midazolam 0.45 mg kg^{-1} produced a sedative effect associated with plasma midazolam concentrations of 40–100 ng ml^{-1}, which is in agreement with more recent studies reporting average plasma midazolam concentrations of between 42 and 93 ng ml^{-1} after a 0.5 mg kg^{-1} dose.[2 3]

A feature of the oral administration of midazolam is a low bioavailability of around 27–36%[1 4] as a result of first-pass metabolism in both the small intestine and the liver. Thus, midazolam is hydroxylated by intestinal and hepatic cytochrome P450 3A (CYP3A) in both the 1- and 4-positions (1-OHMDZ, 4-OHMDZ). These metabolites are

subsequently glucuronidated and excreted in the urine. 1-OHMDZ accounts for 50–70% of the metabolism of midazolam and is reported to be pharmacologically active.[5 6] Data obtained using saccadic eye movement and electroencephalographic changes as surrogate effects after separate i.v. injections of midazolam 0.1 mg kg^{-1} and 1-OHMDZ 0.15 mg kg^{-1} suggest that 1-OHMDZ is at least as potent as the parent compound, midazolam.[6]

Studies of age-related changes in i.v. midazolam kinetics have shown a prolonged half-life and a decreased weight-corrected clearance in neonates,[7 8] an increased weight-corrected clearance to a maximum of 0.78 litre kg^{-1} h^{-1} between 1 and 2 yr of age, and then a decline to adulthood values of 0.38–0.66 litres kg^{-1} h^{-1}.[9]

We have studied the kinetics of both midazolam and 1-OHMDZ in relation to sedative effect in 45 children (from 9 months to 12 yr of age) who had received the drug orally for pre-procedural sedation. Age, weight, sex, concomitant drug therapy, and the plasma metabolic ratio (1-OHMDZ/midazolam=MR) were investigated as co-variables of PK parameters. Logistic regression analysis was used to define the PK-PD relationship between plasma midazolam and 1-OHMDZ concentrations and a simplistic but clinically relevant sedation score (score). A sparse blood sampling strategy with subsequent population-PK analysis was used to overcome the problems associated with taking multiple blood samples from children.

Methods

Patients

Forty-five children attending Sheffield Children's Hospital for day-case surgery were studied. Informed consent was obtained from each child's parent or legal guardian, and the study was approved by the South Sheffield Research Ethics Committee. Operative procedures included circumcision, inguinal hernia repair, insertion of suprapubic catheter, plastic surgery (skin tags, ear correction, extra digits), orchidopexy, in-growing toenails, and hyperspadia.

Data collection

Oral midazolam syrup (Special Products, Addlestone, UK) was administered to the patients as a pre-medication to alleviate preoperative anxiety. Age, sex, weight, biochemical renal and hepatic function tests, concurrent drug therapy, anaesthetic drugs administered, the dose of midazolam, and time of administration were recorded.

Blood samples (5 ml) were collected from an i.v. cannula into lithium-heparin tubes and labelled with the time of collection. Samples were collected at the beginning of the procedure, before any anaesthetic drugs were administered, and at the end when the i.v. cannula had been cleared with saline, thus avoiding contamination of the samples by other drugs. Using a simple rating system (1=awake, 2=drowsy/

asleep), the anaesthetist recorded a sedation score (score) at the beginning of the procedure as the first blood sample was taken. The blood samples were centrifuged at 2000 g and 4°C for 10 min and the plasma transferred into Eppendorf tubes for storage at −80°C pending assay.

Drug and metabolite assay

The HPLC assay was based on that of Carrillo and colleagues.[10] Briefly, to 1 ml of plasma in a 15 ml screw top tube, 180 ng/50 µl of internal standard (diazepam), 1 ml of buffered glycine (0.75 M, pH 9.0) and 4 ml of methyl *tert* butyl ether were added. The samples were extracted for 30 min on a horizontal shaker and then centrifuged for 10 min at 2500 g. The organic phase was transferred into conical glass tubes and evaporated to dryness at 37°C in a vortex evaporator (Buchler, New Jersey, USA). The residue was dissolved in 70 µl of mobile phase and 50 µl injected onto the column.

The HPLC system consisted of a Spectroflow 400 pump (Kratos Analytical Systems, New Jersey, USA), a Rheodyne valve with a 200 µl injection loop, a Waters C18 pre column, an Ultrasphere ODS, 3 µm particle size, 75×4.6 mm reverse-phase column (Beckman Instruments, California, USA), a Spectromonitor lll variable wavelength detector (Laboratory Data Control, Florida, USA) and a RE571.2 chart recorder (Venture Smith Industries, California, USA). A Hewlett Packard 931 integrator (Hewlett Packard, Waldbron, Germany) was also used alongside the chart recorder. The mobile phase was acetonitrile 40% v/v, methanol 5% v/v, and buffered acetate (pH 4.2) 55% v/v. HPLC was performed isocratically with a flow rate of 1.5 ml min^{-1} and the eluant was monitored by UV absorbance at 240 nm. The retention times of parent compound and metabolites were: 4-OHMDZ (2.2 min), 1-OHMDZ (2.7 min), midazolam (3.8 min), and diazepam (4.7 min). Standard curves for the assay of 4-OHMDZ, 1-OHMDZ, and midazolam passed through the origin and were linear over the range 0–200 ng ml^{-1} with r^2 values of 0.99. The coefficient of variation at 25 ng ml^{-1} ($n=6$) was better than 8% for all compounds and the limit of detection was 20 nmol (approximately 7 ng ml^{-1}).

Data analysis

As only two (and in one case three) data points were collected for each subject in the present study it was not possible to use a classical analysis to obtain PK parameter values. Therefore, a population-PK approach was applied. In the population approach all data from different individuals are fitted simultaneously and *post hoc* individual kinetic parameters can be calculated with as few samples as one per individual. We used the software package P-Pharm (version 1.5, InnPhase, France). The algorithm in P-Pharm is Bayesian in nature and utilizes an expectation-maximization-like algorithm[11 12] for population parameter estimation
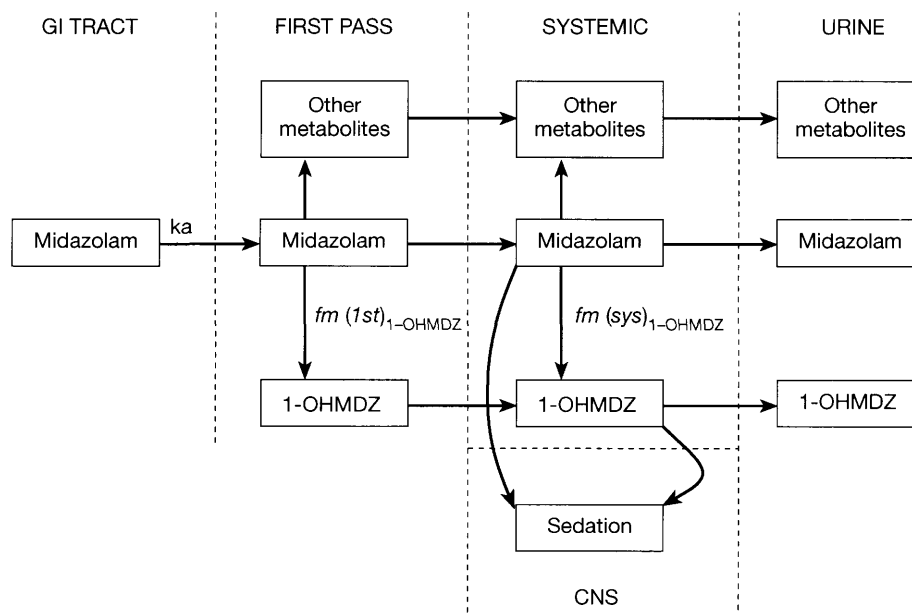
Fig 1 PK-PD model for midazolam and 1-OHMDZ.

and subsequent estimation of *post hoc* values of individual parameters.

Both one- and two-compartment models with first-order input were investigated to identify which model best described the midazolam data. The time-course of plasma midazolam was best described by a one-compartment model with first-order input. A non-uniform weighting ($1/C^2$) on measurement error together with log normal population distributions of all PK parameters were assumed following an initial inspection of different statistical models. The oral clearance (*CL/F*), volume of distribution (*V/F*), absorption rate constant (ka) and absorption lag time (t_{lag}) were considered as primary parameters; secondary parameters (such as half-life) were calculated from the primary model parameters. Initial parameter estimates for fitting procedure were obtained from the literature.[1 6 13]

Age, weight, sex, concomitant drugs (carbamazepine, propofol, thiopentone, and inhalation anaesthetics), and the metabolic ratio MR, were investigated as co-variables for each of the primary parameters. MR was determined using the following equation:

$$MR = \frac{(C(t_1)_{1-\text{OHMDZ}} + C(t_2)_{1-\text{OHMDZ}}) \times \frac{1}{2}}{(C(t_1)_{\text{MDZ}} + C(t_2)_{\text{MDZ}}) \times \frac{1}{2}}$$

where $C(t_1)_{1-\text{OHMDZ}}$, $C(t_2)_{1-\text{OHMDZ}}$, and $C(t_1)_{\text{MDZ}}$, $C(t_2)_{\text{MDZ}}$ are the concentrations of 1-OHMDZ and midazolam in the first and second plasma samples, respectively. This ratio was used as a marker of combined intestinal and hepatic CYP3A activity. The PK data for 1-OHMDZ were evaluated by a two-step link model for drug and metabolite.[14] The model accounts for first-pass formation of 1-OHMDZ (fm

(1st)) and subsequent systemic formation (fm (sys)). The co-variate effects were investigated for each of the primary parameters related to the PK of 1-OHMDZ, namely fm(1st), fm(sys), and k(1-OHMDZ) (the elimination rate constant for 1-OHMDZ). A representation of the PK-PD model is shown in Figure 1.

Assessment of 'best fit' among rival models was based on the Akaike Information Criteria[15] and visual inspection of the predicted *vs* measured concentration plots. The validity of the model was checked by inspection of residual distributions. The significance of co-variates was determined from stepwise multiple regression analysis (*F* values ⩾5).

Considering previous reports of fast onset of action and hence rapid transfer of both midazolam and 1-OHMDZ to the CNS (transfer half lives of around 1 min[6]), no transfer function was assumed between the systemic circulation and the CNS. The effects of midazolam and 1-OHMDZ on the score were analysed by logistic regression analysis using SPSS version 10.0 (SPSS Inc., Chicago, USA). The success of the model in predicting score was tested using the chi-squared test on the number of correct scores predicted.

Results

The median age of the patients was 5 yr (range from 9 months to 12 yr), median weight was 21 kg (range 8–75 kg), and there were 22 females and 23 males. Midazolam dosage varied from 0.2 to 1.37 mg kg^{-1} (median 0.5 mg kg^{-1}). The timing of the first blood sample was between 20 and 122 min (median 49 min) and the second between 40 and 200 min (median 85 min) after dose.

Pharmacokinetics

Individual measured plasma midazolam concentrations (normalized for a dose of 100 μg kg^{-1}) are shown in
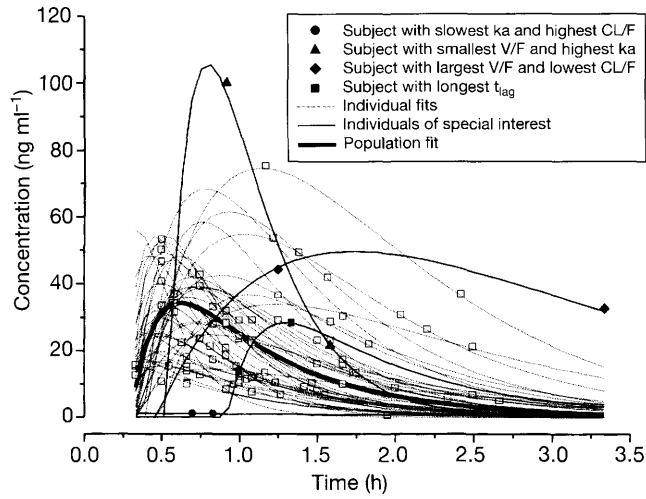

Fig 2 Individual (lines) and population (solid line) model fits to plasma midazolam concentrations following oral midazolam administration to 45 children (data are normalized for a 100 μg kg^{-1} dose).
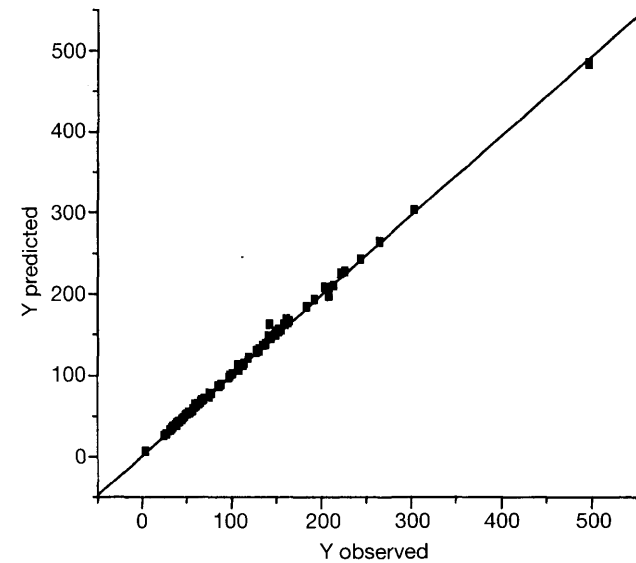

Fig 3 Relationship between plasma midazolam concentrations generated by the model (Y predicted) and those measured in individual patients (Y observed) (r^2=0.996).

Figure 2, along with the Bayesian model predictions of the full concentration–time profiles and the mean profile for the population. The profiles for patients who had extreme values for one or more PK parameters are highlighted. The goodness of fit of the model to the experimental data is indicated in Figure 3. The residual concentrations against time were scattered randomly about zero indicating no systematic error. The population parameter values for midazolam PK are summarized in Table 1.

Individual measured plasma 1-OHMDZ concentrations (normalized for a midazolam dose of 100 μg kg^{-1}) are shown in Figure 4, along with the model predictions of the full concentration–time profiles and the mean profile for the population. The best model was generated with log normal distributions for fm(1st), fm(sys), and k(1-OHMDZ). Predicted *vs* observed values for 1-OHMDZ concentrations are shown in Figure 5, and indicate the goodness of fit of the link model. The correlation between observed and predicted values for 1-OHMDZ was less than that for the parent drug. Similarly, the residual 1-OHMDZ concentration against time showed greater random scatter around zero than for parent drug, midazolam. However, no systematic error was apparent. The population parameter values for 1-OHMDZ are summarized in Table 1. It should be noted that, in the absence of information on the volume of distribution of 1-OHMDZ, the values of fm(1st) and fm(sys) are not
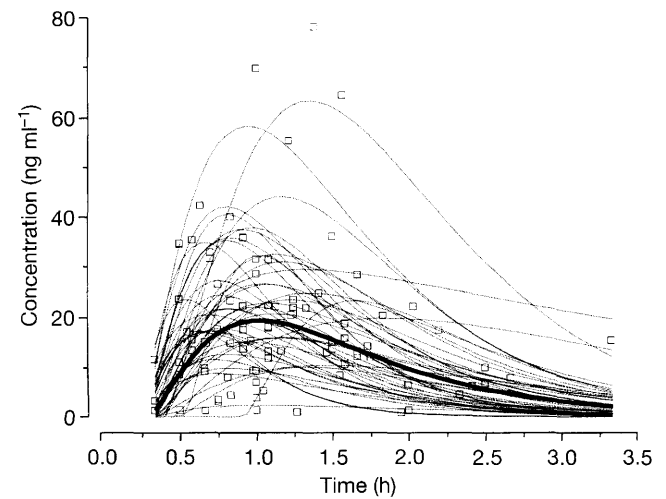

Fig 4 Individual (lines) and population (solid line) model fits to plasma 1-OHMDZ concentrations following oral midazolam administration to 45 children (data are normalized for a 100 μg kg^{-1} dose of midazolam).

Table 1 Median (upper and lower quartile) values of PK parameters for midazolam and 1-OHMDZ. CL/F=oral clearance, V/F=volume of distribution, ka=absorption rate constant, t_{lag}=absorption lag time, kel=elimination rate constant for midazolam, fm(sys)=proportion of 1-OHMDZ generated from the systemic circulation, fm(1st)=proportion of 1-OHMDZ produced from the first-pass metabolism of midazolam, and k(1-OHMDZ)=elimination rate constant for 1-OHMDZ

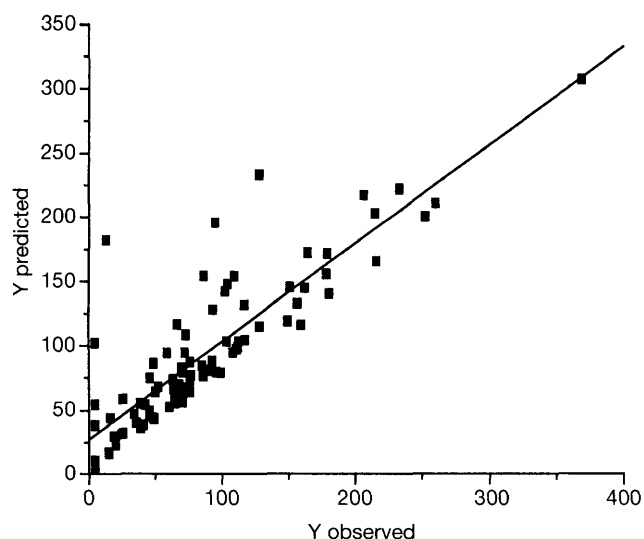| CL/F (litre kg^{-1} h^{-1})	V/F (litre kg^{-1})	ka (h^{-1})	t_{log} (h)	kel (h^{-1})	fm(sys)	fm(1st)	k(1-OHMDZ)
2.56 (1.90–4.04)	2.2 (1.9–3.8)	6.11 (3.94–9.43)	0.29 (0.26–0.32)	1.41 (1.16–1.81)	0.269 (0.187–0.374)	0.178 (0.140–0.214)	3.02 (2.58–3.51)

Fig 5 Relationship between plasma 1-OHMDZ concentrations generated by the model (Y predicted) and those measured in individual patients (Y observed) (r^2=0.94).

Table 2 Co-variate analysis by stepwise multiple regression ($F \geqslant 5$). CL/F=oral clearance, V/F=volume of distribution, ka=absorption rate constant, and MR_{AUC}=metabolic ratio

Kinetic parameter	Co-variable	% Variation explained
CL/F	MR_{AUC}	84
V/F	Propofol	9
ka	Propofol, thiopentone	16

identifiable. However, their ratio is. Accordingly it can be calculated that 37% of exposure to 1-OHMDZ is related to the metabolite formed during the first-pass metabolism of midazolam.

Co-variates for PK parameters

Stepwise multiple regression analysis ($F \geqslant 5$) of the co-variables revealed a number of correlations as summarized in Table 2. The MR was directly related to CL/F and accounted for 84% of the variability in oral midazolam clearance. Propofol administration was inversely correlated with V/F and directly with ka. The latter was also correlated with thiopental administration. There were no significant co-variables for the metabolite PK parameters.

Weight, age, and sex were not significant co-variables for weight-corrected oral midazolam clearance, weight-corrected volume of distribution, ka or t_{lag}. The data for weight-corrected midazolam oral clearance were also analysed independently by weight assuming no effects from other co-variables. This relationship is shown in Figure 6. The change in clearance with body weight (BW) was best described by a non-linear function:
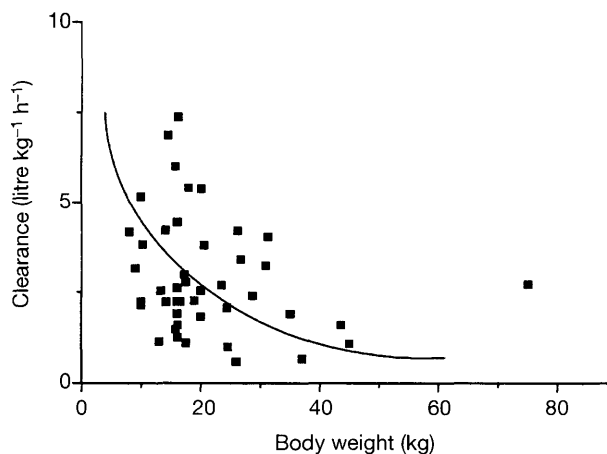
$$[(CL/F)/BW=7.73*BW^{-0.37}].$$

Fig 6 Relationship between weight-normalized oral clearance of midazolam and body weight in 45 children (r^2=0.063; P=0.1).

However, the r^2 value (0.063) did not reach statistical significance (P=0.10).

Pharmacodynamics

Twenty-eight patients had scores recorded at the same time as the first blood sample as follows: score 1=11, score 2=17. Neither patient characteristics nor PK parameters were different between the 28 patients where a sedation score was recorded and the remaining 17 out of 45 patients where no score was recorded. Results of the logistic regression analysis of score in relation to plasma midazolam concentration alone and score and plasma 1-OHMDZ concentration alone are shown in Figure 7A and B, respectively. The combined influence of midazolam and 1-OHMDZ concentration on score is shown in Figure 8.

The logistic regression PK-PD models were compared using the −2 log likelihood values. A number of additional co-variables (age, weight, and MR) were introduced into the PK-PD analysis in order to ascertain the most parsimonious model. The results are summarized in Table 3. The prediction of score from 1-OHMDZ alone appeared to be a slightly better than for midazolam alone, but inferior to the model that assumed midazolam and 1-OHMDZ together contribute to the sedative effect. Age, weight, and MR were not significant co-variates in the PD analysis. The regression curves in Figure 8 give the probability of a response at a given drug/metabolite concentration. As seen in Figure 8, higher plasma concentrations of both midazolam and 1-OHMDZ increase the probability of a higher sedation score.

According to the parameter values derived from the logistic regression analysis describing the influence of midazolam (0.021) and 1-OHMDZ (0.048) on sedation score, it appears that 1-OHMDZ has a greater potency than midazolam. However, after considering the higher unbound fraction of 1-OHMDZ (0.106) *vs* midazolam (0.024),[6] the
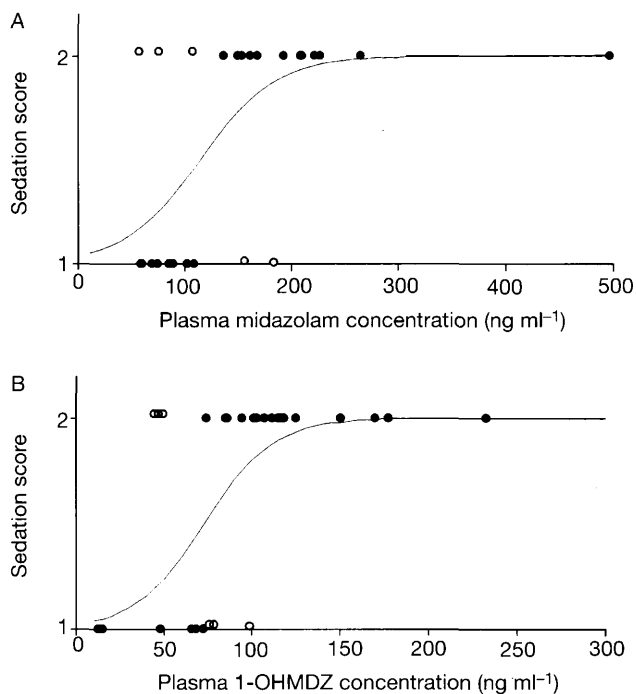
Fig 7 Observed sedation score (symbols) and predicted probability of acore (line) with respect to plasma concentration of (A) midazolam and (B) 1-OHMDZ. Closed symbols indicate those patients for whom the model successfully predicted score.
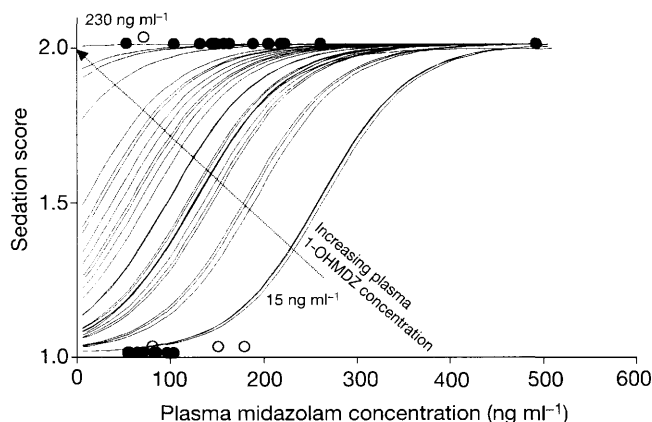

Fig 8 Observed sedation score (symbols) and predicted probability of score (line) with respect to plasma concentrations of both midazolam and 1-OHMDZ. Closed symbols indicate those patients for whom the model successfully predicted score.

Table 3 Comparison of the logistic regression models. Score=sedation score

Model	−2 LL
Score and midazolam	26.25
Score and 1-OHMDZ	24.25
Score and midazolam+1-OHMDZ	20.04

relative potency of midazolam was calculated to be 1.9 times that of 1-OHMDZ.

Overall, the PK-PD model predicted the correct sedation score successfully in 86% of cases (P=0.01 and P=0.05 for scores 1 and 2, respectively). For the observed average plasma concentrations of 150 ng ml^{-1} (midazolam) and 90 ng ml^{-1} (1-OHMDZ) the odds ratio was 4 in favour of score 2 vs 1. A simulation based on the PD parameters showed that a 50% increase in the current mean dose (from 0.5 to 0.75 mg kg^{-1}) would result in an odds ratio of 275 in favour of score 2.

Discussion

The random nature of the sampling times in this study necessitated the use of sparse data analysis. Sparse data analysis from as few as 20–30 patients may be sufficient to perform a reasonable population PK study provided that the sampling times are not fixed but random.[16 17] This methodology is well suited to clinical practice as it reduces the inconvenience to patients, particularly neonates and children and can thus be considered more ethical.

The previous applications of population-PK analysis to midazolam have used a two-compartment model to fit i.v. data from neonates in intensive care.[8 18] Within the confines of the present study a one-compartmental model was sufficient and produced the best fit. It is a common observation that following oral administration of many

drugs that display multi-compartment characteristics after i.v. injection, a single compartmental model is observed. This is because the absorption phase overlaps with the distribution phase.[19] Our population PK estimates for midazolam using sparse data population PK analysis were in broad agreement with those from other paediatric studies where more intensive blood sampling was performed (Table 4). The estimated oral clearance in our study (2.7 litre kg^{-1} h^{-1}) was higher than values recorded in adult studies of between 0.51 and 1.2 litre kg^{-1} h^{-1}.[10 24]

Despite large variations between individual patients, predicted plasma midazolam concentrations from the final model were very close to the actual data. This large inter-patient variation was consistent with the observations of Marshall and colleagues.[3] The variability could not be explained by concurrent drug therapy or disease state but a direct relationship between MR and midazolam oral clearance was established. The latter was expected as the MR is a reasonably robust measure of gut and liver CYP3A activity. Rogers and colleagues[25] found that MR values derived from concentrations at 60 min after dosage explain 46% of the variability of midazolam clearance, and Lin and colleagues[26] found that a 4 h plasma MR could explain between 80 and 91% of the inter-individual variability in the area under the curve of midazolam. Our median sample time was 70 min and we were able to explain 84% of the variability in post hoc estimates of oral midazolam clearance. In another study in 20 children, de Wildt and

Table 4 Clearance, volume of distribution, and elimination half-life of midazolam in children. *Estimated from i.v. clearance assuming an oral bioavailability of 0.27. CL/F=oral clearance, V/F=volume of distribution, and $t_{1/2}$=elimination half-life

Study	Age range (yr)	CL/F (litre kg^{-1} h^{-1})	V/F (litre kg^{-1})	$t_{1/2}$ (h)	Route
Present	9 months to 12 yr	2.7 (1.5)	2.2 (1.5)	0.5 (0.45)	Oral
Tolia and colleagues[20]	6–18	2.2 (1.1)*	2.2 (0.4)*	0.75 (0.4)	i.v.
Payne and colleagues[1]	3–10	3.5 (1.2)	1.3 (0.6)	1.2 (0.3)	Oral
de Wildt and colleagues[13]	3–16	2.0 (1.6)*	4.0 (4.4)*	2.5 (2.3)	i.v.
Jones and colleagues[21]	5–9	3.4 (0.7)*	–	1.8 (0.5)	i.v.
Rey and colleagues[22]	1–5	–	–	2.4	i.v.
Reed and colleagues[23]	6 months to 15 yr	2.3 (1.1)	11 (5.5)	3.5	Oral
Carillo and colleagues[10]	Adults	0.51 (0.18)		2.39 (0.9)	Oral

Table 5 Reported variability in hepatic and intestinal CYP3A content. *Relative units. **From quantitative PCR analysis (transcripts per microgram of total RNA). All intestinal data are normalized per unit of villin

Study	n	Mean expression (pmol (mg protein)$^{-1}$)	SD	Range	Fold difference
Liver					
Shimada and colleagues[27]	30	96	51		
Shimada and colleagues[28]	8	77	34	37.8–143.0	3.8
Guengerich and colleagues[29]*	36	248	37		
Snawder and colleagues[30]	40	142	105	19.7–440.0	22.3
Gentest Corp (Pelkonen and colleagues)[31]	12	109	20		
Iribarne and colleagues[32]	20	2.3*	1.2	0.4–4.9	10.2
Forrester and colleagues[33]	12				60
Transon and colleagues[34]	42				13
Iyer and colleagues[35]	21				18
Intestine					
Taipalensuu and colleagues[36]	13	1.4	0.3	0.8–1.8	2.2**
Paine and colleagues[37]	20	30.6 (duodenum)		3.0–91	30.3
		22.6 (jejunum)		2.1–91	43.2
Johnson and colleagues[38]	74	10.8	4.85	1.3–23	19

colleagues[13] failed to show a relationship between MR and midazolam clearance, although there was a correlation between MR and age.

Assuming that MR is a valid marker of CYP3A4 activity, a 10-fold inter-individual variability was observed within the children that we studied. This is in agreement with the majority of values reported in the literature (Table 5). The variability measured in MR observed after oral administration of midazolam reflects variability in both gut and hepatic CYP3A4, which appear to be independently regulated.[39] Inspection of our previous data on the development of enterocytic CYP3A4 in children,[38] indicates an 11-fold range in expression in biopsy samples from 48 children aged 1.25–12 yr (19 female). This is again in good agreement with the range of variability observed in MR.

The correlations between propofol and V/F and propofol/thiopental and ka, albeit low, are difficult to explain and may be spurious. Although propofol can decrease the systemic clearance of midazolam by 30–40%,[40] no effect was detected in this study as the first sample was taken before administering propofol. Age and weight were not significant co-variates of weight-adjusted oral midazolam clearance. Two previous studies have found no age-related changes in midazolam weight-corrected clearance.[13 41]

Another study performed in a paediatric intensive care setting has shown a decreased weight-corrected midazolam clearance in children below 2 yr of age and an increased clearance from 3 yr.[9]

When analysed independently of other co-variables, weight-adjusted oral midazolam clearance appeared to decrease exponentially with body weight (Fig. 6); a trend also observed for other CYP3A4 substrates such as carbamazepine.[42] A recent study has also shown decreasing oral midazolam clearance with age in children 6 months to 16 yr old with age-related weight-adjusted clearance values comparable with our results.[23] The trend is confirmed by decreased oral midazolam clearance in adults.[10 24] An explanation for increased weight-normalized drug clearance in children of lower weight could be their greater liver volume relative to body weight. Thus, the liver represents 4% of the body in 1-yr-old children compared with 2.5% in adults.[43 44] This alone could account for a 1.5-fold increase in clearance in children. An alternative explanation could be a higher concentration of catalytically active CYP3A4 per gram liver weight in children. However, Blanco and colleagues[45] have shown that this is not the case. As oral midazolam is given at a standard dose of 0.5 mg kg^{-1} to children up to 30 kg body weight, younger children may be

underdosed, although this may be partly offset by increased plasma concentrations of 1-OHMDZ in these patients.

While Blumer [46] considers that 1-OHMDZ shows only minimal biological activity, there is overwhelming evidence against this notion.[5 6 47–49] Indeed our results indicate that at the same plasma concentrations 1-OHMDZ contributes 1.9-fold more to sedation than midazolam. However, after considering the 4-fold higher unbound fraction of 1-OHMDZ in plasma[6] the relative potency of 1-OHMDZ compared with midazolam decreased to 0.5. Thus, high oral midazolam clearance (resulting in the formation of the less active 1-OHMDZ metabolite) coupled with a decreased benzodiazepine receptor density in younger infants[50] may lead to therapeutic failure in some paediatric patients.

According to our analysis, about 37% of the 1-OHMDZ formed appeared to be generated during the first-pass of parent drug through the gut wall and liver. Thummel and colleagues[24] in a cross-over study of both intravenous and oral midazolam PK in 20 healthy adults, showed that both liver and gut contributed equally to the first-pass metabolism each removing 40% of available parent drug. The low value for first-pass generation of 1-OHMDZ in this study could be a result of lower intestinal expression of enterocytic CYP3A4 and subsequent intestinal first-pass in some of the younger children.[38] Higher weight-adjusted liver volume in children[43 44] will have a disproportionate effect on the systemic (liver alone) compared with the first-pass production of 1-OHMDZ (sum of gut and liver). Therefore, the relative value for the proportion of metabolite generated on first-pass calculated from $fm(1st)/(fm(1st)+fm(sys))$ will be reduced.

The separate i.v. administration of active metabolites, such as 1-OHMDZ, is the gold standard technique for assessing the *in vivo* activity relative to parent compound. However, these studies are difficult to justify in children. Other approaches include inhibition of the metabolic pathway that is responsible for the formation of the metabolite,[51 52] or investigation of the pharmacological effect in sub-populations who are genetically deficient with respect to the biotransformation pathway that produces the active metabolite.[53] Alternatively, PK-PD modelling, based on either inter-individual or intra-individual differences in the plasma concentration–time profiles of parent drug and metabolite, can be used without disruption of drug metabolism or the use of phenotypic groups. We have used such an approach to estimate the contribution of dihydromorphine to the analgesic effect of dihydrocodeine,[14] and now extend it to assessing the relative sedative activity of midazolam and 1-OHMDZ.

In developing the PD model we omitted a delay function between concentrations in plasma and the effect compartment (CNS). However, it has been shown that this transfer half-life for both midazolam and 1-OHMDZ is very short (0–5 min)[6 54 55] and will not have a significant impact on the 2-level sedation endpoint. A similar logistic regression model for sedation score was recently applied to data for

adult patients receiving a continuous i.v. infusion of midazolam.[56] However, the latter study failed to account for the effects of active metabolites.

For the 28 patients who were assigned a sedation score after administration of midazolam, 11 patients had a score of 1 (awake) and 17 a score of 2 (drowsy/asleep). Previous reports on response rates to similar oral midazolam doses have related the effects to ablation of anxiety.[3 57–59] Our scoring system was not directly comparable with those used in these reports as the authors combined all patients who were asleep, drowsy, or awake but not anxious to form one single group of 'patients with some response'. These reports indicated a response rate of between 80 and 90%, albeit response varying from being calm to asleep.[3 57–59] By subdividing score 1 from our records into those who were anxious (n=2) and those who were calm (n=9) it appears that an overall 93% (26/28) achieved some type of response in our study. This is entirely consistent with previous reports.[3 57–59]

Higher scores correlated significantly with higher plasma concentrations of midazolam and 1-OHMDZ, in agreement with the findings of Marshall and colleagues.[3] Nevertheless, if the aim is not to have any patients fully awake before the start of the operative procedure then a 50% increase in current dosage might be required. This would increase the odds ratio from 4 to 275 in favour of score 2 (drowsy, asleep).

Children often present with anxiety pending an invasive procedure, and require rapid sedation without the discomfort of i.v. administration. Accordingly, oral midazolam is the agent of choice used by many paediatric anaesthetists. However, a current issue with oral midazolam is the lack of a licensed formulation for oral administration to children, although a cherry flavoured formulation has recently been evaluated for use in the USA (Versed Syrup, Roche Laboratories Inc., Nutley, NJ). This has been assessed in a multi-centre randomized, double-blind, parallel group, dose ranging trial and found to be effective in sedating 80% of patients within 30 min.[3] Although an oral midazolam preparation is now commercially available in the UK as a 'special', this does not have a product licence.

Acknowledgements

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