A novel, palatable paediatric oral formulation of midazolam: pharmacokinetics, tolerability, efficacy and safety

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Short title: Novel paediatric formulation of midazolam
Keywords: Midazolam: paediatrics oral dosage; midazolam: bio-availability versus Route; pre-operative anxiety
Summary

Midazolam is one of many bitter drugs where provision of a suitable paediatric formulation, particularly in the pre-anaesthetic setting, remains a challenge. To overcome this problem a novel chocolate-based tablet formulation has been developed with positive pre-clinical results. To further investigate the potential of this formulation, 150 children aged 3-16 years who were prescribed midazolam as a pre-medication were randomised to receive 0.5 mg.kg⁻¹ either as the novel formulation or an intravenous solution given orally, which is the current standard at our institution. Tolerability was assessed by each child, parent and nurse using a five-point facial hedonic scale and efficacy was determined as the time to onset of sedation. Blood samples for midazolam and 1-hydroxymidazolam values were analysed using high-performance liquid chromatography. Population pharmacokinetics were evaluated using non-linear mixed effects modelling. The novel formulation had significantly improved tolerability scores from children, parents and nurses (all p<0.001). Time to effect was not different between the groups (p=0.140). The pharmacokinetics of midazolam and 1-hydroxymidazolam were able to be suitably modelled simultaneously. The novel formulation was subject to a higher estimated first pass metabolism compared with the intravenous solution (8.6 vs 5.0 %) and a significantly lower relative bioavailability of 82.1% (p=0.013), with no other significant differences. Exposure relative to dose was in the range previously reported for midazolam syrup. We conclude that the novel chocolate-based formulation of midazolam provides improved tolerability while remaining efficacious with suitable pharmacokinetics when used as a premedicant for children.
Introduction

The importance of palatability for paediatric medicines, a previously neglected aspect of drug development, has recently been recognised by regulatory authorities and the pharmaceutical industry [1-3]. Midazolam, a short-acting benzodiazepine with sedative, amnesic and anxiolytic effects, is one example where the importance of this has been highlighted. Oral midazolam is commonly prescribed for children as premedication prior to the induction of anaesthesia. Alone it has a bitter taste, limiting its clinical utility in the paediatric population. Only one commercially available syrup is available in some regions, so it is common practice to use the intravenous (iv) formulation orally. To improve tolerability, the iv formulation has been mixed with various palatable liquids, however masking its taste has only been moderately successful [4,5].

As an alternative solution to this problem a novel chewable Chocolate-based tablet Delivery System (CDS) was developed [6]. Pre-clinical data from both in-vitro and animal studies have suggested an improved acceptability of the novel formulation. Rodent data showed the CDS matrix to be effective at masking the bitter taste of midazolam. The midazolam CDS tablet did not require refrigerated storage and was stable for at least 18 months when wrapped in foil and maintained at room temperature. In vitro drug dissolution experiments showed complete release of the midazolam load from the tablet into simulated gastric fluid in 15 min for pre-crushed tablets (to simulate mastication) and 35 min for intact tablets. Based on these data the CDS formulation was then trialled in a paediatric population who were prescribed midazolam prior to general anaesthesia at our centre. We decided to assess tolerability, efficacy and safety, as well as comparative pharmacokinetics of the novel CDS formulation compared with the iv formulation administered orally.
Methods

We performed a prospective, open-label, single centre, randomised, single treatment trial at Princess Margaret Hospital, the only paediatric tertiary referral centre in Western Australia. Institutional research ethics approval was obtained from the Princess Margaret Hospital and the University of Western Australia (2014102EP and RA/4/1/7610, respectively). Written informed parental or guardian consent and assent from the child (where appropriate) was obtained prior to enrolment in the study.

Computer-generated block randomisation, stratified according to age (< 7 years old or >7 years old) and gender, was produced by the clinical trials pharmacy in order to assign patients, and was performed independently from the study team. Children received either the midazolam CDS tablet or iv midazolam solution (Pfizer, Australia) orally using 1:1 randomisation. Both formulations were dosed according to local institutional guidelines, in a target dose of 0.5 mg.kg\(^{-1}\), as prescribed by the treating anaesthetist. Midazolam CDS tablets used in the clinical trial were manufactured within the Department of Pharmacy, Princess Margaret Hospital. We excluded children allergic to midazolam or chocolate (CDS base is nut free) or when informed consent could not be obtained.

During administration of the study drug, a member of the research team recorded whether the whole dose was swallowed, partially expelled or totally refused by the patient. For tolerability, the child was asked immediately after administration to record how much he or she liked the sample by putting a mark on a five-point facial hedonic scale (ranging from 1; dislike very much, to 5; liked very much) [7] and whether they would be happy to take the drug again if required. The parent and accompanying nurse were also asked independently to give a score, using separate five-point hedonic scales, based on their perception of how the child reacted to the taste of the assigned formulation. If the child expelled the dose immediately, the treating anaesthetist was free to decide whether a repeat dose was required, in line with current routine management. If a second dose was recommended the treating anaesthetist, together
with the parent and/or child, decided whether to repeat the dose or administer the alternative form. The
time to sedation onset (clinical effect of midazolam) was recorded for all patients.

Monitoring as recommended by the AAGBI and Australian and New Zealand College of Anaesthetists
(ANZCA) (electrocardiography, non-invasive blood pressure measurements, capnography and pulse
oximetry) was commenced and general anaesthesia was induced either by a consultant anaesthetist or
under their direct supervision. The choice of anaesthetic agents and analgesia was left to the discretion
of the anaesthetist. Oxygen saturation was continuously monitored throughout surgery and in the post
anaesthesia care unit until patient discharge. One to one nursing was guaranteed at all times in the post
anaesthesia care unit with at least one additional circulating nurse present at all time.

Given the paediatric study population undergoing otherwise usual care, a flexible, sparse venous blood
sampling approach was utilised for PK sampling. The first sample was collected as soon as possible after
the child lost consciousness (approximately 30 minutes after the midazolam premedication had been
administered). The second and third samples were collected between 45-60 minutes and 90-120 minutes,
respectively. The final sample was collected as late as possible before the procedure was completed whilst
the patient was still anaesthetised. At each time point 3ml venous blood was withdrawn from a cannula
and emptied into an EDTA tube. The number of samples varied according to the length of the procedure,
with a maximum of four samples, and total blood volume not exceeding 12ml. After collection blood
samples were centrifuged at 2,000 rpm at 4°C for 10 minutes before the plasma was separated into
Eppendorf tubes for storage at -80°C until analysis was performed.

The method for quantifying midazolam and 1-hydroxymidazolam in plasma was adapted from Juřica et al.
[8] with minor modifications. Differences in the pre-analytical step were that the stock solutions were
prepared at a lower concentration (10 µg.ml⁻¹ for diazepam, 25 µg.ml⁻¹ for midazolam and 1-
hydroxymidazolam), dilutions were made with methanol alone, the dynamic range for both analytes was
17 – 333 ng.ml$^{-1}$, 20 µl of 0.5 M NaOH was used for alkalisation, samples were spiked with 30 µl of internal
standard, a second reconstitution/evaporation step was performed and final dissolution utilised 100 µl
methanol, of which 30 µl was injected for analysis.

The HPLC system comprised an Agilent 1260 Infinity HPLC (Agilent Technologies Australia, NSW, Australia)
with a Hypersil BDS column (250 × 4.6 mm, 5 µm, Thermo Fisher Scientific Australia, WA, Australia). The
mobile phase consisted of 20 mM potassium phosphate buffer, pH 5.0, and acetonitrile. A double gradient
elution method was applied as follows: 35% acetonitrile ramping to 45% in 12 min, held isocratic for 9
min, followed by another ramp from 45% acetonitrile to 100% in 7 min, held isocratic for 5 min before
bringing the acetonitrile concentration down to 35% in 2 min, and holding for at least 7 min prior to next
sample injection. The flow rate was 1 ml.min$^{-1}$, at ambient temperature, and eluent was monitored at 245
nm.

The retention times for midazolam, 1-hydroxymidazolam and diazepam (internal standard) were 13.9,
10.2 and 17.0 min, respectively. Calibration curves for midazolam and 1-hydroxymidazolam were linear
from 17 to 333 ng.ml$^{-1}$ ($R^2 \geq 0.99$). Inter- and intra-day accuracy and precision were suitable within this
range with bias <9% and coefficient of variability <15%. The lower limit of quantification was 13 ng.ml$^{-1}$
for both analytes.

Log$_e$ plasma concentration-time datasets for midazolam and 1-hydroxymidazolam were analysed by
nonlinear mixed effects modelling using NONMEM (v 7.2.0, ICON Development Solutions, Ellicott City,
MD, USA) with an Intel Visual FORTRAN 10.0 compiler. The first order conditional estimate with
interaction (FOCE with INTER) method was used. The minimum value of the objective function (OFV) and
visual predictive checks were used to choose suitable models during the model-building process. A
significance level of $p<0.05$ was set for comparison of nested models. Allometric scaling for body weight
(WT) was employed a priori, with volume terms multiplied by \((WT/70)^{1.0}\) and clearance terms by \((WT/70)^{0.75}\) [9]. Residual variability (RV) was estimated as additive error for the log-transformed data. Base models were parameterised using \(V_C\) (central volume of distribution), \(CL\) (clearance), \(V_P\) and \(Q\) (peripheral volumes of distribution and their respective inter-compartmental clearances). A time to event (onset of effect) was planned, however this could not be established due to a lack of concentration data prior to onset of effect.

Given the primary purpose of the analysis was to compare the two formulations, different bioavailability and absorption parameters were estimated in the model. The bioavailability of the CDS formulation relative to the iv formulation was included as a parameter while different absorption parameters for each of the formulations were included, where supported by available data.

Initial modelling was performed on the midazolam data set alone and one-, two- and three-compartment models (ADVAN 2, 4 and 12, respectively) were assessed. Given that the absorption profile between subjects varied, several absorption models were tested, including single- and double-phase absorption with zero- and first-order rates, with and without an initial lag time, as well as a transit compartment model. In this model, the dose passes through a series of transit compartments before entering the absorption compartment to model the delay often associated with drug absorption. A single rate constant \((k_{tr})\) describes the entry and exit for all transit compartments. Using a previously described implementation of the transit compartment model in NONMEM [10], the number of transit compartments \((NN)\) and the mean transit time \((MTT = (1+NN) / k_{tr})\) were estimated as continuous variables. Once a suitable structural model for midazolam was established, 1-hydroxymidazolam plasma concentration-time data were added and custom general linear disposition models were constructed using ADVAN5. Modelling of midazolam and 1-hydroxymidazolam was then performed simultaneously.

To allow PK identifiability in the parent drug-metabolite model, complete conversion of midazolam to 1-
hydroxymidazolam was assumed. Although this assumption is not biologically correct, it only represents a scaling factor for the 1-hydroxymidazolam modelling. Therefore, all midazolam parameters were relative to bioavailability (F) while all 1-hydroxymidazolam parameters were relative to F x metabolic conversion (F*). One and two additional compartments were tested for 1-hydroxymidazolam. Models with first-pass metabolism, estimated separately for each of the formulations, were also assessed. Once the base structure of the models was established, inter-individual variability (IIV) as well as correlations between IIV terms, were evaluated for each suitable parameter and included where supported by the data. Inter-individual variability was exponentially modelled for all parameters.

Relationships between model parameters and age, BMI and sex were assessed through inspection of scatterplots and boxplots of individual parameters vs covariate, and subsequently evaluated within NONMEM. A stepwise forward inclusion and backward elimination method was used with a significance level of p<0.05 required for inclusion of a covariate relationship and p<0.01 to retain a covariate relationship.

Once modelling for the primary PK population (those patients reported to have ingested the entire dose) was completed, a secondary analysis was performed to estimate the related absorption of the dose in those reported to have partially ingested the dose but who did not receive a further dose. For this analysis all other parameters in the model were fixed to the values in the primary final model. Details of model evaluation are provided in the supplementary material available online.

Statistical analysis was performed using R version 2.14.2 (R Foundation for Statistical Computing, Vienna, Austria) software. Two-sample comparisons for non-normally distributed variables were made using Mann Whitney U-test. Unless otherwise stated, all p values are two-tailed and unadjusted for multiple comparisons. Power analysis was performed using the Monte-Carlo Mapped Power (mcmap) method automated through Perl speaks NONMEM (PsN) using PK parameters from a previous study [11] to
determine if the proposed sampling schedule is sufficient to determine a 20% difference between the two formulations (a clinically relevant difference). Fifty patients in each group would achieve 80% power with an $\alpha$ value of 0.05, while 75 in each group corresponded to a power of 90%. Similar results were obtained when the $ka$ of the chocolate formulation was set 50% lower (54 and 80 in each group for a power of 80% and 90%, respectively). In order to account for drop outs and missing PK data points a target study population of 150 was set.
Results

One hundred and fifty children were included in the study and baseline characteristics are shown in Table 1. Twenty participants did not fully ingest the first dose, with 6 in the chocolate group and 12 in the iv formulation group partially ingesting the dose, and an additional 2 in the iv formulation group completely refusing the dose. Therefore approximately 8% in the chocolate group did not entirely ingest the dose compared with 18% in the iv formulation group, p=0.065. For three patients in the iv group who did not entirely ingest the first dose, a decision was made by the treating anaesthetist to give a second dose, and all were given the CDS tablets. Two of the three entirely ingested the CDS tablets while the other patient partially ingested this dose. Participants who did not entirely ingest the dose had a lower mean age than those who did (6.1 vs 7.7 years, p=0.046), and there was no difference with regards to the formulation they had been assigned.

Although all children were included in the taste and safety analysis, only the 130 children who completely ingested the dose were included in the primary efficacy analysis (Table 1). This consisted of 67 in the chocolate group and 62 in the iv formulation group. One participant in the chocolate group did not have any PK samples taken and was excluded from the primary PK analysis. An additional 17 children (6 in the chocolate group and 11 in the iv formulation group) who partially ingested the dose, but did not receive further doses, were included in the secondary PK analysis.

The CDS tablet had acceptable tolerability with significantly improved scoring compared with the iv formulation on the 5-point scale for children, parents and clinical staff, p<0.001 (Figure 1). Five children (all younger than 4.5 years, four of whom were in the iv formulation group) were unable to provide a score, and were scored low by the parent (≤2). Mean (SD) scores given by the children, parents and nurses for the CDS tablet were 3.16 (1.45), 3.52 (1.25) and 3.36 (1.29), compared with corresponding mean (SD) scores of 1.71 (1.13), 1.71 (1.00) and 1.97 (1.00) for the iv formulation. These significant differences were
noted across age and gender strata with p values <0.050. Significantly more children in the chocolate group than in the iv formulation group (62% vs 39%), indicated they would take the same formulation again, p = 0.007. Despite a higher mean (SD) administered dose for patients in the iv formulation group, there was no significant difference in sedation onset time between the groups. Median (interquartile range [range]) time to onset of sedation was 13 (10 – 17 [5-31]) min in the CDS group compared with 12 (9 – 16 [4-30]) min in the iv formulation group, p=0.140. We observed no serious adverse events during the study. From the 129 children included in the primary PK analysis there were 294 individual plasma midazolam concentrations (160 and 134 from the chocolate and iv formulation groups, respectively) and 317 1-hydroxymidazolam concentrations (172 and 145 from the chocolate and iv formulation groups, respectively) available for analysis. Of these, 3% and 9%, respectively, were measurable but below the lower limit of quantification. Given these were <10% of the total dataset they were kept at their measured values for the purposes of analysis [12]. There was an additional 45 midazolam and 48 1-hydroxymidazolam concentrations in the secondary analysis estimating the degree of absorption with partial dose ingestion. A two-compartment model for midazolam was most appropriate, with no benefit from additional compartments, p>0.05. The absorption was best represented with a transit compartment model with a different MTT parameter for the two formulations, MTT<sub>CDS</sub> for the chocolate formulation and MTT for the iv formulation. A single additional compartment was adequate to describe the disposition of 1-hydroxymidazolam. The inclusion of first-pass metabolism, with a separate parameter for each formulation, resulted in significantly lower objective function value and improved appearance of diagnostic plots. Inter-individual variability was estimable for CL/F<sub>MDZ</sub>, V<sub>c</sub>/F<sub>MDZ</sub>, V<sub>p</sub>/F<sub>MDZ</sub>, CL/F*<sub>OHMDZ</sub>, MTT and MTT<sub>CDS</sub>. Correlation between V<sub>c</sub>/F<sub>MDZ</sub> and V<sub>p</sub>/F<sub>MDZ</sub> was estimated to be close to one, therefore this was fixed to
unity. Otherwise a full covariance matrix was estimable between $\text{CL/F}_{\text{MDZ}}$, $\text{Vc/F}_{\text{MDZ}}$ and $\text{CL/F}_{\text{OHMDZ}}^*$. None of the tested covariate relationships improved the fit of the model.

The final model parameter estimates and the bootstrap results are summarised in Table 2. The relative bioavailability for the CDS tablets was lower than for the iv formulation, specifically, 82.1% with an empirical 95% confidence interval of 69.3 – 95.1% ($p=0.013$ for the difference between formulations), coincident with higher first-pass metabolism (8.6 % vs 5.0 %, respectively). A clinically insignificant trend for slower absorption of the CDS tablets (mean transit time estimated to be 2 minutes longer) with slightly larger interindividual variability (78 vs 68%) was also observed. When the children with partially ingested doses were added to the analysis, the estimated dose absorbed was 70% and 58% for the CDS tablet and iv formulation, respectively. The dose normalised AUCs for both formulations were within the range which has previously been reported for midazolam syrup (Figure 2) [13,14].
This study demonstrates that the novel paediatric formulation of midazolam as a chocolate-based tablet has improved tolerability, whilst maintaining similar efficacy, compared with the iv solution given orally, the current standard at our institution. A population PK model, using sparse sampling accommodating for usual care in this paediatric population, was successfully created. This model was used to estimate the relative bioavailability between the formulations as well as to investigate differences in the absorption profiles of the two formulations.

Consistent with in vitro and pre-clinical data [6], we found a significant improvement in tolerability of midazolam when incorporated into the CDS tablet. Taste scores using a 5-point facial hedonic scale were significantly better for the CDS tablet when scored by children, their parents and nursing staff caring for the child. This scale has been used for taste evaluation in children from 3 to 12 years old and for parents of children aged 4 to 16 years [7]. Although other methods can be used, such as a visual analogue scale, this method is more commonly used, particularly in younger children. The hospital environment can be stressful for the very young and it is not surprising that they may not comply with such scoring, particularly if they dislike the taste of the premedication. Significantly more children in the chocolate group were willing to have the same formulation again, further demonstrating an improved tolerability of this formulation.

There was no significant difference in the primary measure of efficacy between the two formulations. Although median time to onset of sedation was 1.5 minutes longer in the chocolate group there was reasonable variability within each group. This difference was not statistically significant and could not be considered clinically significant either. This was so despite a 13% lower average mg.kg⁻¹ dose for patients in the chocolate group. The population PK model mirrored these findings. The estimated mean transit time for the CDS tablets was slightly longer than the iv formulation (13 vs 11 min) with significant inter-
individual variability (>60% for both formulations). This difference was not significant in the PK model, with overlapping 95% confidence intervals.

Other differences between the two formulations identified in the PK model were with respect to absorption parameters. Relative bioavailability was estimated to be 82%. Considering the low reported absolute bioavailability of midazolam syrup in children (15-37%) [13,14], a reduced absorption for a solid formulation (CDS tablets) when compared with a high concentration liquid (iv formulation) is not surprising. Consistent with these differences there was lower estimated first pass metabolism for the iv formulation group (5.0% vs 8.6% in the chocolate group) possibly due to greater buccal absorption which avoids first pass effect. No other differences were noted between the two formulations. More importantly, there was no formulation effect on the clearance of midazolam or 1-hydroxymidazolam. The differences between the two tested formulations are of unclear clinical significance given there was no difference in the primary efficacy measure. In patients who partially ingested the dose there was a 42% reduced exposure in the iv solution group compared with 30% in the chocolate group. Although this difference was not statistically significant it does suggest that children are more likely to expel the iv formulation compared with the CDS tablets.

There have been two previously published reports on the PK of a midazolam syrup in paediatric patients. Payne et al [14] compared several different formulations of midazolam in anaesthetised children aged 3 to 10 years that included 3 different doses for an oral syrup compounded from the iv formulation. They reported an absolute bioavailability of 15-27% for the syrup administered via nasogastric tube, with lower bioavailability for 0.45 mg.kg⁻¹ and 1.0 mg.kg⁻¹ compared with 0.15 mg.kg⁻¹ dose. Reed et al [13] reported the PK of Versed® Syrup administered orally in children aged 6 months to 16 years old. The absolute bioavailability was estimated to be 37% with an interindividual variability of 28%. The range of dose-normalised AUC for both formulations in the present study was within the range reported in these
previous studies and had a similar spread to that of Reed et al. Therefore, with equivalent dosing it would be expected that the novel CDS formulation would result in similar overall exposure to midazolam.

Our study has some limitations. The use of a sparse sampling approach and avoiding discomfort to the child by delaying sampling until after they were anaesthetised resulted in poorer precision PK parameters relating to absorption. In particular, first pass metabolism and the number of transit compartments had a relative standard error (RSE) of >50%. Despite this, key PK parameters in this study, including relative bioavailability and clearance parameters, were well estimated with RSE <30%. A further potential limitation was the use of iv formulation as the comparator, given paediatric formulations of midazolam syrups exist. Use of the iv formulation is the standard of care within our institution, as the commercial midazolam syrup (Versed\textsuperscript{®} Syrup) is not available in Australia. Despite this we would expect the bioavailability of a high concentration, pH unadjusted, small volume liquid to be no worse and likely greater, given reduced first-pass metabolism, compared with the oral syrup formulation. Consistent with this, when a prepared mixture of the iv formulation was compared with the syrup formulation it resulted in a 45% higher serum concentration up to 90 minutes after administration [5]. Therefore, we would expect that the CDS tablets would have higher relative bioavailability when compared with the commercial midazolam syrup.

Developing suitable medications for children raises specific challenges, particularly with regard to ensuring tolerability and performing ethically appropriate studies in order to allow for their evaluation. The present study was designed to have minimal impact on our usual care of children while still providing key information to evaluate a new paediatric formulation. The novel CDS tablets were found to have favourable tolerability for midazolam, a drug with a bitter taste, while remaining equally efficacious, safe and with adequate relative bioavailability. Not only does this provide a better alternative for pre-medication but establishes a methodology that could be applied for other bitter medications in children.
Acknowledgements

The trial was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12615000225516). The authors thank all participating children and their families. The authors would like to acknowledge the contributions of the members of the anesthesia research team as well as the staff of the Department of Anaesthesia and Pain Management at Princess Margaret Hospital for Children, Perth, Australia.

Competing interests

This study was funded by grants from the Australian and New Zealand College of Anaesthetists, Perth Children’s Hospital Foundation and Pathfinder grant from the University of Western Australia. A provisional patent was filed in Australia on 17 November 2017 (# PCT/AU2017/051266) with L–Y. Lim and M.N. Nguyen as inventors, and B.S. von Ungern Sternberg and E.K.Y. Tang as contributors in the development of the CDS tablet formulation.


Table 1. Baseline characteristics for children administered midazolam orally as either CDS tablets or iv formulation. Data are mean (SD) or number (proportion).

<table>
<thead>
<tr>
<th>All randomised children (tolerability and safety analysis)</th>
<th>Primary efficacy</th>
<th>CDS formulation</th>
<th>iv formulation</th>
<th>p value</th>
<th>CDS formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n=76</td>
<td>n=74</td>
<td></td>
<td>n=67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight; kg</td>
<td></td>
<td>29 (14)</td>
<td>30 (17)</td>
<td>0.790</td>
<td>29 (14)</td>
</tr>
<tr>
<td>Age; years</td>
<td></td>
<td>7.4 (3.1)</td>
<td>7.5 (3.6)</td>
<td>0.860</td>
<td>7.5 (3.2)</td>
</tr>
<tr>
<td>Sex; male</td>
<td></td>
<td>36 (49%)</td>
<td>37 (49%)</td>
<td>0.870</td>
<td>32 (48%)</td>
</tr>
<tr>
<td>Height; cm</td>
<td></td>
<td>125 (19)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124 (22)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.660</td>
<td>125 (19)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dose; mg of midazolam base</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td>10.2 (3.2)</td>
</tr>
<tr>
<td>Weight adjusted dose; mg.kg&lt;sup&gt;-1&lt;/sup&gt; midazolam base</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td>0.38 (0.09)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Height missing for one participant in each group.

<sup>b</sup> One additional 6-year-old male with no evaluable PK data included in the efficacy population, inclusion of this participant did not alter the between group comparisons.

<sup>c</sup> Height missing for one participant.
Table 2. Final population pharmacokinetic estimates and bootstrap results for midazolam and 1-hydroxymidazolam in plasma from children prior to surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>RSE%</th>
<th>Bootstrap median [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective Function Value</td>
<td>-265.078</td>
<td>-300.214 [ -458.616 - 161.877 ]</td>
<td></td>
</tr>
<tr>
<td>Structural model parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F&lt;sub&gt;CDs&lt;/sub&gt; (%)</td>
<td>82.1</td>
<td>8</td>
<td>82.7 [69.3 - 95.1]</td>
</tr>
<tr>
<td>MTT (h)</td>
<td>0.186</td>
<td>26</td>
<td>0.187 [0.105 - 0.266]</td>
</tr>
<tr>
<td>MTT&lt;sub&gt;CDs&lt;/sub&gt; (h)</td>
<td>0.216</td>
<td>20</td>
<td>0.216 [0.123 - 0.300]</td>
</tr>
<tr>
<td>NN</td>
<td>2.45</td>
<td>53</td>
<td>2.45 [0.43 - 8.55]</td>
</tr>
<tr>
<td>CL/F&lt;sub&gt;MDZ&lt;/sub&gt; (l.h&lt;sup&gt;-1&lt;/sup&gt;70kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>90.4</td>
<td>30</td>
<td>90.8 [38.3 - 142]</td>
</tr>
<tr>
<td>V&lt;sub&gt;C&lt;/sub&gt;/F&lt;sub&gt;MDZ&lt;/sub&gt; (l.70kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>165</td>
<td>9</td>
<td>161 [126 - 213]</td>
</tr>
<tr>
<td>Q/F&lt;sub&gt;MDZ&lt;/sub&gt; (l.h&lt;sup&gt;-1&lt;/sup&gt;70kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>90.4</td>
<td>31</td>
<td>95.5 [52.1 - 156.3]</td>
</tr>
<tr>
<td>V&lt;sub&gt;P&lt;/sub&gt;/F&lt;sub&gt;MDZ&lt;/sub&gt; (l.70kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>290</td>
<td>63</td>
<td>279 [86.7 - 1,000]</td>
</tr>
<tr>
<td>FP (%)</td>
<td>4.99</td>
<td>57</td>
<td>4.99 [0.65 - 12.8]</td>
</tr>
<tr>
<td>FP&lt;sub&gt;CDs&lt;/sub&gt; (%)</td>
<td>8.63</td>
<td>50</td>
<td>8.76 [1.00 - 17.7]</td>
</tr>
<tr>
<td>CL/F*&lt;sub&gt;O&lt;/sub&gt;OHMDZ (l.h&lt;sup&gt;-1&lt;/sup&gt;70kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>127</td>
<td>28</td>
<td>129 [51.6 - 192]</td>
</tr>
<tr>
<td>V/F*&lt;sub&gt;O&lt;/sub&gt;OHMDZ (l.70kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>67.4</td>
<td>26</td>
<td>69.8 [24.8 - 127]</td>
</tr>
<tr>
<td>Variable model parameters [shrinkage%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIV in CL/F&lt;sub&gt;MDZ&lt;/sub&gt;</td>
<td>65 [19]</td>
<td>28</td>
<td>61 [27 - 99]</td>
</tr>
<tr>
<td>IIV in V&lt;sub&gt;C&lt;/sub&gt;/F&lt;sub&gt;MDZ&lt;/sub&gt;</td>
<td>50 [16]</td>
<td>31</td>
<td>55 [15 - 100]</td>
</tr>
<tr>
<td>IIV in CL/F*&lt;sub&gt;O&lt;/sub&gt;OHMDZ</td>
<td>58 [21]</td>
<td>20</td>
<td>56 [27 - 83]</td>
</tr>
<tr>
<td>IIV in V&lt;sub&gt;P&lt;/sub&gt;/F&lt;sub&gt;MDZ&lt;/sub&gt;</td>
<td>36 [16]</td>
<td>30</td>
<td>40 [3 - 137]</td>
</tr>
<tr>
<td>IIV in MTT</td>
<td>65 [54]</td>
<td>20</td>
<td>64 [39 - 91]</td>
</tr>
<tr>
<td>IIV in MTT&lt;sub&gt;CDs&lt;/sub&gt;</td>
<td>78 [50]</td>
<td>15</td>
<td>77 [50 - 108]</td>
</tr>
<tr>
<td>r(V&lt;sub&gt;C&lt;/sub&gt;/F&lt;sub&gt;MDZ&lt;/sub&gt;, V&lt;sub&gt;P&lt;/sub&gt;/F&lt;sub&gt;MDZ&lt;/sub&gt;)</td>
<td>1</td>
<td></td>
<td>FIXED</td>
</tr>
<tr>
<td>r(V&lt;sub&gt;C&lt;/sub&gt;/F&lt;sub&gt;MDZ&lt;/sub&gt;, CL/F&lt;sub&gt;MDZ&lt;/sub&gt;)</td>
<td>0.762</td>
<td></td>
<td>0.753 [0.112 - 0.947]</td>
</tr>
<tr>
<td>r(V&lt;sub&gt;C&lt;/sub&gt;/F&lt;sub&gt;MDZ&lt;/sub&gt;, CL/F*&lt;sub&gt;OHMDZ&lt;/sub&gt;)</td>
<td>0.614</td>
<td></td>
<td>0.594 [-0.199 - 0.922]</td>
</tr>
<tr>
<td>r(CL/F&lt;sub&gt;MDZ&lt;/sub&gt;, CL/F*&lt;sub&gt;OHMDZ&lt;/sub&gt;)</td>
<td>0.718</td>
<td></td>
<td>0.684 [-0.319 - 0.905]</td>
</tr>
<tr>
<td>RV MDZ (%)</td>
<td>29 [26]</td>
<td>10</td>
<td>30 [23 - 36]</td>
</tr>
<tr>
<td>RV OHMDZ (%)</td>
<td>30 [26]</td>
<td>13</td>
<td>29 [16 - 37]</td>
</tr>
</tbody>
</table>

F<sub>CDs</sub> (bioavailability of CDS formulation relative to iv formulation), MTT (mean transit time), NN (number of transit compartments), CL/F<sub>MDZ</sub> (clearance of midazolam), V<sub>C</sub>/F<sub>MDZ</sub> (central volume of distribution of midazolam), Q/F<sub>MDZ</sub> (intercompartmental clearance of midazolam), V<sub>P</sub>/F<sub>MDZ</sub> (peripheral volume of distribution of midazolam), FP (degree of first-pass metabolism), CL/F*<sub>O</sub>OHMDZ (clearance of 1-hydroxymidazolam), V<sub>C</sub>/F*<sub>O</sub>OHMDZ (volume of distribution of 1-hydroxymidazolam), r (correlation
coefficient), IIV (inter-individual variability) and RV (residual variability). IIV and RV is presented as

100% × \sqrt{\text{variability estimate}}.
Figure Legends:

Figure 1. Histogram of patient, parent and nurse scoring of tolerability for the CDS tablet IV solution (black bars) and iv solution CDS tablet (grey bars) formulations with the 5-point facial hedonic scale used for assessments.

Figure 2. Mean and standard deviation of dose normalised midazolam area under the curve for CDS tablet (black with star), IV solution given orally (black with triangle) and various dose levels in previously published reports [13] (grey with square) and [14] (grey with circle).
Supplemental material

Novel, palatable paediatric oral formulation of midazolam: pharmacokinetics, tolerability, efficacy and safety

Model evaluation method

For model evaluation, plots of observed vs individual- and population-predicted values, and time vs WRES, were first assessed. A bootstrap using Perl speaks NONMEM (PSN) with 1,000 samples was performed, and the parameters derived from this analysis summarised as median and 2.5th and 97.5th percentiles (95% empirical CI) to facilitate evaluation of final model parameter estimates. In addition, prediction corrected visual predictive checks (pcVPCs) stratified according to formulation, were performed with 1,000 datasets simulated from the final models. The observed 10th, 50th, and 90th percentiles were plotted with their respective simulated 90% CIs to assess the predictive performance of the model and to evaluate any major bias.

Shrinkage of population variability parameters and residual variability was assessed to help determine whether models were over-parameterised and to determine the reliability of diagnostic plots [1].

Model evaluation results

Bias was less than 9% for all fixed and random model parameters, except for IIV of \( V_P/F_{MDZ} \) for which it was less than 15%. Supplemental Figures 1 and 2 show goodness-of-fit plots for midazolam and 1-hydroxymidazolam respectively, with no bias evident. Supplemental Figure 3 presents the pcVPC plots. The actual 10th, 50th and 90th percentiles fell within their respective 95% CI for both midazolam and 1-hydroxymidazolam for both formulations, therefore there is
suitable predictive performance of the model.
**Supplemental figure captions.**

**Supplemental Figure 1.** Goodness-of-fit plots for midazolam given to children prior to surgery. The observed plasma concentration has been plotted against population (A) and individual (B) predicted plasma concentrations, and weighted residuals against time (C) and population predicted plasma concentrations (D). Concentrations are on a log$_{10}$ scale.

**Supplemental Figure 2.** Goodness-of-fit plots for 1-hydroxymidazolam after oral midazolam given to children prior to surgery. The observed plasma concentration has been plotted against population (A) and individual (B) predicted plasma concentrations, and weighted residuals against time (C) and population predicted plasma concentrations (D). Concentrations are on a log$_{10}$ scale.

**Supplemental Figure 3.** Prediction corrected visual predictive check for midazolam (A, B) and 1-hydroxymidazolam (C, D) (ng.ml$^{-1}$ log$_{10}$ scale) for children prior to surgery receiving oral midazolam either as IV solution formulation (A, C) or novel CDS formulation (B, D). Plots demonstrate observed 50th (solid line), 10th and 90th (dotted lines) percentiles within their simulated 95% CI (grey shaded areas) with overlying data points (○).
Dose normalised midazolam AUC
(mg.h.l\(^{-1}\) per mg.kg\(^{-1}\))

Dose (mg.kg\(^{-1}\))
A

Conditional weighted residuals
midazolam

B

Conditional weighted residuals
midazolam

C

Time (h)

D

Population predicted midazolam (ng.ml⁻¹)
A

B

C

D