Pharmacokinetics and pharmacodynamics of the short-acting sedative CNS 7056 in sheep

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Background. CNS 7056 is a new short-acting esterase-metabolized benzodiazepine. We report the first pharmacokinetic (PK) and pharmacodynamic (PD) study of CNS 7056 and its inactive metabolite CNS 7054 in sheep.

Methods. The stability of CNS 7056 in blood samples was examined ex vivo. Six sheep were prepared with physiological instrumentation, and were given doses of 0.37, 0.74, and 1.47 mg kg\(^{-1}\) (2 min infusion) of CNS 7056 in alternating order on separate days.

Results. CNS 7056 was degraded in warm whole sheep blood (23% over 2 h), but not in plasma or blood stored on ice. Using non-compartmental analysis (NCA), CNS 7056 had a mean (SD) clearance of 4.52 (0.96) litre min\(^{-1}\) and a terminal half-life of 21.3 (10.9) min. There was a rapid conversion of CNS 7056 to its metabolite CNS 7054, which had a terminal half-life of 22.5 (3.4) min. The arterial kinetics of CNS 7056 could be described by a three-compartment model, with volumes of 1.9, 3.9, and 79 litre, a clearance of 4.2 litre min\(^{-1}\), and inter-compartmental clearances of 2.85 and 1.44 litre min\(^{-1}\), while the metabolite could be described by a two-compartment model. Cardiac output was an important covariate. Sedation as measured by the alpha power band of the EEG showed rapid onset and offset. The \(t_{1/2,\text{pre}}\) for sedation was 1.78 min, and the EC\(_{50}\) was 0.10 \(\mu\text{g ml}^{-1}\).

Conclusions. CNS 7056 has PK–PD properties compatible with its potential human use as a short-acting i.v. sedative.

Keywords: benzodiazepine; cardiac output; CNS 7056; pharmacodynamics; pharmacokinetics; sedation; sheep

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CNS 7056 is a methyl ester, and its hydrolysis product CNS 7054 has low activity at the GABA\(_A\) receptor.\(^1\) It is presumed that ester hydrolysis of CNS 7056 to CNS 7054 is an important mechanism, limiting the duration and intensity of sedative effect in vivo. However, the pharmacokinetic (PK) and pharmacodynamic (PD) properties of CNS 7056 have not been reported in animals. This paper describes a PK–PD study of CNS 7056 in sheep using three doses (0.37, 0.74, and 1.47 mg kg\(^{-1}\)) corresponding to low, medium, and high doses in the clinically useful range for this species.

The aims of the study were to develop methods for measuring CNS 7056 and its metabolite CNS 7054 in plasma and to develop methods of studying the PKs of CNS 7056 unaffected by ex vivo ester hydrolysis. In addition, we aimed to define the systemic PKs using non-compartmental analysis (NCA) of CNS 7056 and CNS 7054 in arterial blood and to develop a descriptive population-based
Methods

Ethical approval

The study was approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science and complied with the National Health and Medical Research Council of Australia Code of Conduct for the Care and Use of Animals.

Analysis of CNS 7056 in sheep plasma

In vitro studies and initial development of methods for conducting PK studies of CNS 7056 made use of an assay with solvent extraction and ultraviolet (UV) detection. This assay could not measure the metabolite CNS 7054. The final PK studies made use of a liquid chromatograph mass spectrometer (LCMS) assay with greater sensitivity and the ability to quantitate the CNS 7054 metabolite.

UV assay method

The high-pressure liquid chromatograph (HPLC) (Shimadzu VP series with an SPD-M10A detector) used a C18 column (LUNA, 150×4.6 mm, Phenomenex, California, USA) with a flow rate of 1 ml min⁻¹ and the detector wavelength set at 235 nm. The mobile phase was a 70:30 (v/v) mixture of A and B, where A was a 90:10 methanol:water solution and B was a 10:90 methanol:water 2 mM with ammonium acetate. Diazepam was used as the internal standard.

Plasma samples were processed as follows: 500 μl of blood or plasma were stored in 1.5 ml Eppendorf® tubes. To each tube was added 12 μl of diazepam internal standard solution, 200 μl of saturated sodium carbonate solution (pH 13), and 500 μl of dichloromethane. The tubes were vortex mixed for 1 min and centrifuged (at 4200 RCF). Each standard sample was prepared in a 1.5 ml Eppendorf® tube by spiking 100 μl of the blank sheep plasma with 20 μl of internal standard stock solution (10 μg ml⁻¹ alprazolam in acetonitrile as weight of freebase) and 20 μl of CNS 7056 or CNS 7054 calibration or quality control stock solutions prepared in acetonitrile. These samples were then diluted with 260 μl of acetonitrile for analysis. Each assay run also included a duplicate set of individually prepared quality control standards (0.7, 2, and 7 μg ml⁻¹), and a blank standard comprising 300 μl of acetonitrile and 100 μl of blank sheep plasma. The peak area output of the LCMS was non-linear. Plotting the output against log concentration produced a linear relationship. 4 Unknown concentrations from the study were interpolated from these transformed standard curves.

The plasma samples were processed by thawing at room temperature and 250 μl aliquots were transferred into 1.5 ml Eppendorf® tubes preloaded with 50 μl of internal standard stock solution and 700 μl of acetonitrile. The standard and sample preparations were vortex mixed for 1 min followed by centrifugation for 10 min at 3250 RCF. Supernatant (112 μl) was transferred into injection vials preloaded with 98 μl of Milli-Q H₂O. Injection order was randomized with the exception of a calibration standard (excluding the highest and lowest) placed at the beginning and end of each injection sequence. Each sample (40 μl) was injected onto a 2.0 × 150 mm C18 column with a mobile phase consisting of milli-Q H₂O:acetonitrile:formic acid (60:40:0.1) running at 0.15 ml min⁻¹ (total run time 11 min). MS detection utilized an APCI probe in SIM positive mode with these detection parameters: APCI probe: 400°C/4.5 kV, CDL: 250°C/−10 V, Block: 200°C, Detector: 1.5 kV, Q-array: 5.0 V (DC), nebulizing gas: N₂ (2.5 litre min⁻¹), and drying gas: N₂ (~10 litre min⁻¹). Target ions were acquired under these parameters according to the following mass:charge ratios, CNS 7054: 425.10, CNS 7056: 439.10, and alprazolam: 309.10.

In vitro stability studies

As CNS 7056 (but not CNS 7054) may be subject to ester hydrolysis ex vivo, particular care was taken to ensure that CNS 7056 did not degrade in blood samples obtained from animals during the time-course of a typical PK study (i.e. on the bench before the samples could be processed and frozen). It was also important to ensure that plasma samples stored in a freezer before analysis were not subject to degradation. The following studies tested these scenarios.
Bench stability
Fresh sheep blood was collected in Eppendorf® tubes and CNS 7056 added to give final concentrations of 0.5 μg ml⁻¹ (n=6) or 2 μg ml⁻¹ (n=6). The tubes were left at room temperature, then after 0, 60, or 120 min; duplicate samples were assayed immediately for CNS 7056 using the UV assay described above. Blank sheep blood was used to make standard curves and quality controls. A similar study was conducted using plasma rather than whole blood. A final study used whole blood but with the tubes stored on ice, and with the time-scale reduced to 0, 10, and 20 min. The data were analysed using a linear mixed-effect model with the ratio of observed to expected concentration as the dependent variable.

Freezer stability
Sheep blood was collected in heparinized tubes. The blood (1 ml) was placed into 32 Eppendorf® tubes and CNS 7056 added to give final concentrations of 0.5 μg ml⁻¹ (n=16) or 2 μg ml⁻¹ (n=16). The tubes were centrifuged to harvest plasma. For each concentration, half of the plasma samples were stored in a −20°C freezer (n=8), the other half in a −80°C freezer (n=8). After 1, 30, 60, or 90 days in the freezers, two tubes were taken from each group and assayed for CNS 7056 using the UV assay described above. Fresh sheep blood was used to make standard curves and quality controls for each new assay day. The data were analysed using a linear mixed-effect model with the ratio of observed to expected concentration as the dependent variable. Freezer type, expected concentration, and days in storage were independent variables and replicate number was a random factor (repeated measure).

In vivo studies
General methods for sheep preparation
The general methods for the sheep preparation have been described in detail previously.2 In brief, female Merino sheep of 1–3 yr age were prepared surgically under isoflurane anaesthesia with instrumentation for blood sampling and physiological measurements. No neuromuscular blocking agent was used, and the adequacy of anaesthesia was assessed by the absence of movement to stimuli. Each sheep was prepared with: two aortic catheters (for blood sampling and arterial pressure), one Swan–Ganz pulmonary artery thermocatheter (arterial temperature measurement), one sagittal sinus catheter (to sample effluent blood from the brain), and three catheters outside the right atrium (for central venous pressure, CO injectate, and drug administration). A Doppler flow probe for measuring an index of cerebral blood flow was secured above the dorsal sagittal sinus via a trephine hole.5 6 For EEG measurements, two silver electrodes were placed under the skull but above the dura on either side of the trephine hole, and a reference electrode was placed under the scalp. Studies commenced 2 days after surgery.

Sheep breathed room air with unassisted ventilation during the studies.

Experimental design and PK measurements
CNS 7056 besylate (MW 597.5, chemical purity 97.6%, chiral purity 98.3%) was supplied by PAION UK Limited, Cambridge, UK. Six sheep with an average weight of 45 kg (range 39–55.5 kg) were administered doses of 0.37, 0.74, and 1.47 mg kg⁻¹ CNS 7056 base infused i.v. over 2 min on consecutive days and in alternating order (e.g. high-dose first or high-dose last). The sixth sheep did not receive the 1.47 mg kg⁻¹ dose due to limited compound availability. CO was measured in triplicate using a thermocatheter method immediately before drug administration. During and after the infusion, blood was sampled rapidly from the aortic catheter at sample times of 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 45, 60, and 90 min. Whole-blood samples (1.5 ml) were placed into Eppendorf® tubes in ice, and were centrifuged to obtain plasma within 20 min of sampling. The plasma was transferred to a second set of Eppendorf® tubes, and stored at −80°C until assay using the LCMS method.

PD measurements
In all studies, the raw EEG was measured using a Datex-Ohmeda S/5 monitor for 5 min before the start of the drug infusion, and after the administration of the drug until EEG readings had returned to baseline for at least 10 min. The power spectrum of 8 s EEG epochs was calculated using the fast Fourier transform and used to calculate the average power in the alpha frequency band (8–13 Hz). The alpha power has previously been shown to be a reliable continuous measure of sedation for CNS 7056 in the sheep.2 EEG processing was performed using scripts written for the ‘R’ data analysis software, Version 2.4.1.7

Data analysis
Non-compartmental PK analysis
NCA PK analysis was performed using the R data analysis and statistical language.7 The integration of the area under the concentration–time curve (AUC) used the ‘linear up, log down’ method.8 The extrapolation of the terminal concentrations used between three and six data points, as determined by the linear regression of the log concentration that gave the highest $R^2$ value. PK parameters were calculated using standard equations.8

Compartmental PK modelling
The parent compound (CNS 7056) and metabolite (CNS 7054) were modelled concurrently using an NMQUAL (http://metruminstitute.org/index.html) validated installation of the NONMEM® (Version VI, level 2.0; GloboMax, Hanover, MD, USA). NONMEM was used in conjunction with the Wings For NONMEM® interface (http://wfn.sourceforge.net/). Population parameters were assigned log-normal distributions for between-subject variability (BSV). Separate
additive and proportional residual error models were used for parent and metabolite. The conversion ratio of CNS 7056 to CNS 7054 was assumed to be 100%. The YLO functionality of NONMEM was used to reduce the bias introduced by censoring of the data due to samples below the lower limit of quantification (LLOQ) of the assay. The Laplacian estimation method with the interaction option was used for the final models, but the faster first-order conditional method was used for initial model development.

Combined PK models with combinations of two or three compartments for CNS 7056 and one or two compartments for CNS 7054 were examined using a modified two-stage approach that allowed the parameters for each study to be estimated separately. Models were tried with and without a lag term for the central compartment of the parent drug representing the lag in appearance of drug in blood due to vascular transit times. The best configuration of compartments was carried forward to examine the need for between-occasion variability (BOV) in the parameters and the influence of covariates. The available covariates were: dose (to test linearity), the order of the studies in each sheep, body weight, and the CO measured immediately before the dose. Covariates were selected for examination in the model in one of the two ways. First, using a traditional statistically based approach, covariate relationships were assessed by graphical inspection of plots of the post hoc individual parameter estimates vs covariates and screening using a regression-based technique. A covariate relationship was carried forward if it produced a statistically significant $R^2$ value of >0.05 (i.e. the covariate explained at least 5% of the variability in the parameter). These selected covariates were then added to the parameter as a power model. The second approach used underlying mechanistic and allometric principles. The covariates body weight and CO were added to volume parameters (e.g. $V_1$, $V_2$, and $V_l$) and/or flow parameters (e.g. $Q_{12}$, and $Q_{12}$) based on power models, with the power coefficient being either 0.75, 1, or fitted from the data. For example, the following NONMEM code shows a power model for CO on clearance (CL) with a fitted power coefficient.

$$\text{CL} = \text{THETA(1)} \times \text{EXP(ETA (1))} \times (\text{CO/\text{COSTD}})^{\text{THETA(2)}}.$$  

COSTD is the standard reference CO taken to be the mean baseline CO across all studies, THETA(1) is the population value of clearance and THETA(2) the power coefficient.

For both methods of covariate selection, models with and without covariates were compared using the Bayesian information criteria (BIC). This adjusts the objective function of NONMEM for the number of parameters and observations in the model (lower BIC implies a better model fit and/or fewer parameters for an equally good fit). The BIC can be used to compare un-nested models, and models with the same number of parameters.

**PD modelling**

The concentration–effect (EEG alpha power) relationship was examined using a sequential PK–PD approach with NONMEM. This used the best Bayesian estimate of PK model parameter values for each individual subject (i.e. post hoc parameters) to generate the arterial concentration input for a separate population PD model. The base dynamic model used an effect compartment and a sigmoid concentration–effect relationship with the effect being either additive or proportional to the baseline EEG. Parameters for this model were examined for evidence that a reduced model would suffice:

(i) Effect compartment could be removed ($k_{e0}$ value tends to a large number).

(ii) An $E_{max}$ model preferred over a sigmoid $E_{max}$ model (Hill coefficient (n) tends to 1).

(iii) A linear model is preferred over an $E_{max}$ model (EC$_{50}$ tends to a large value).

**Statistical analysis**

For the final PK and PD models, the precision of the parameter estimates (se%) were obtained from the covariance step of NONMEM. If the model did not pass the covariance step, the parameter precision was determined from fitting 200 bootstrap samples of the original data.

Linear mixed-effect modelling was used where appropriate. The significance level was $P=0.05$. All data were analysed using the ‘R’ data analysis software, Version 2.4.1.

**Results**

**UV assay performance**

Regression coefficients ($R^2$) of the six validation standard curves had median value of 0.998 (range 0.993–0.999), and the median intercept value was 0.05 $\mu$g ml$^{-1}$. The median inter-assay variability at 0.5 $\mu$g ml$^{-1}$ was 3.9% (range 1.7–8.7%) and at 2.0 $\mu$g ml$^{-1}$ was 3.6% (range 0.2–13.1%). It was concluded that the assay was of sufficient quality to conduct initial studies of the stability of CNS 7056.

**LCMS assay performance**

The transformed standard curves for all LCMS assays had a median $R^2$ value of 0.999 (range 0.990–1.000) for CNS 7056, and a median $R^2$ value of 0.999 (range 0.993–1.000) for CNS 7054. For CNS 7056, the median and 95% confidence intervals of the deviation of the quality control samples from the expected values were 2.64% (–7.37 to 15.01), 5.50% (–7.92 to 12.75), and 6.49% (–3.68 to 13.99) at concentrations of 0.7, 2, and 7 $\mu$g ml$^{-1}$, respectively. The LLOQ for CNS 7054, these values were 2.64% (–10.78 to 14.68), 6.42% (–11.17 to 17.70), and 6.33% (–8.50 to 13.96) at concentrations of 0.7, 2, and 7 $\mu$g ml$^{-1}$, respectively. The LLOQ was set at 0.2 $\mu$g ml$^{-1}$ and the limit of detection (LOD) was $\sim$0.02 $\mu$g ml$^{-1}$.

**In vitro studies**

**Bench stability**

Blood samples to which CNS 7056 had been added showed a reduction ($P<0.001$) in CNS 7056 concentration with time.
The average loss after 120 min was 23%. However, plasma samples with CNS 7056 added showed no such loss ($P = 0.66$) under the same conditions (Fig. 1), suggesting that the degradation of CNS 7056 in blood samples could be attributed to cellular components of blood. The degradation could be minimized by storing blood samples on ice for short periods—there was minimal reduction in concentrations with time under these conditions ($P = 0.58$; Fig. 1).

The PK study protocol minimized ex vivo loss by rapidly storing samples on ice, and separating plasma into a second tube within 20 min of sampling.

**Freezer stability**

Freezer temperature ($P = 0.34$) and expected concentration (0.5 or 2 μg ml$^{-1}$, $P = 0.23$) did not affect the ratio of observed to expected concentration of CNS 7056 in the sample tubes after storage. The number of days in storage was not significant ($P = 0.78$). These data show that plasma samples containing CNS 7056 could be stored in a $-20^\circ$C or less freezer for up to 3 months with minimal degradation. The samples for the PK study were stored at $-80^\circ$C and the assays were performed within 3 months of the study.

**PK data**

The peak concentrations of CNS 7056 in arterial blood were achieved at the end of the 2 min infusion, and the concentrations thereafter declined rapidly (Fig. 2). Many samples (49.8%) had CNS 7056 concentrations between the LLOQ and medium dose, $n = 6$ low and medium dose, $n = 5$ for high dose).
and LOD of the assay, and the LOD was often reached before the last 90 min sample (Fig. 3). There was a rapid conversion of CNS 7056 to CNS 7054, with the peak concentrations of the metabolite being achieved at $\approx 5$ min after the start of the infusion of CNS 7056 (Fig. 2). The CNS 7054 concentrations declined less rapidly than the parent, with only 13.3% of samples having concentrations between the LLOQ and LOD, and there were generally quantifiable concentrations in the 90 min samples.

**Non-compartmental analysis**

The NCA summary of CNS 7056 in arterial blood after the 1.47 mg kg$^{-1}$ doses is shown in Table 1. The mean (sd)

<table>
<thead>
<tr>
<th>Compound</th>
<th>CNS 7054</th>
<th>CNS 7056</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.37 mg kg$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.74 mg kg$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.47 mg kg$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time after dose (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log Concentration ($\mu$g ml$^{-1}$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1 NCA of CNS 7056 and CNS 7054 in arterial blood. Data are for the highest (1.47 mg kg\(^{-1}\)) dose. NCA variables are the maximum concentration (\(C_{\text{max}}\)), the time of the maximum concentration (\(t_{\text{max}}\)), the area under the concentration–time curve to the last measurable sample (AUC\(_{\text{last}}\)), the AUC extrapolated to time infinity (AUC\(_{\text{INF}}\)), the per cent contribution of the extrapolation to AUC\(_{\text{INF}}\) (AUC\(_{\text{ext}}\)), the \(R^2\) value of the extrapolated concentrations, the area under the moment curve extrapolated to time infinity (AUMC\(_{\text{INF}}\)), the clearance (CL), the volume of distribution at steady state (\(V_{\text{ss}}\)), MRT, and the terminal half-life (\(t_{1/2}\)). CV\% is coefficient of variation as a percentage.

<table>
<thead>
<tr>
<th></th>
<th>CNS 7056 Mean</th>
<th>CNS 7056 sd</th>
<th>CNS 7056 CV%</th>
<th>CNS 7054 Mean</th>
<th>CNS 7054 sd</th>
<th>CNS 7054 CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{\text{max}}) ((\mu)g ml(^{-1}))</td>
<td>5.48</td>
<td>1.89</td>
<td>34</td>
<td>7.91</td>
<td>2.12</td>
<td>27</td>
</tr>
<tr>
<td>(t_{\text{max}}) (min)</td>
<td>1.85</td>
<td>0.34</td>
<td>18</td>
<td>3.20</td>
<td>1.30</td>
<td>41</td>
</tr>
<tr>
<td>AUC(_{\text{last}}) ((\mu)g ml(^{-1}) min)</td>
<td>15.5</td>
<td>5.2</td>
<td>34</td>
<td>152.9</td>
<td>22.8</td>
<td>15</td>
</tr>
<tr>
<td>AUC(_{\text{INF}}) ((\mu)g ml(^{-1}) min)</td>
<td>15.7</td>
<td>5.3</td>
<td>34</td>
<td>161.8</td>
<td>27.6</td>
<td>17</td>
</tr>
<tr>
<td>AUC(_{\text{ext}}) (%)</td>
<td>3.24</td>
<td>1.97</td>
<td>61</td>
<td>5.26</td>
<td>2.16</td>
<td>41</td>
</tr>
<tr>
<td>(R^2)</td>
<td>0.94</td>
<td>0.11</td>
<td>12</td>
<td>0.96</td>
<td>0.08</td>
<td>8</td>
</tr>
<tr>
<td>AUMC(_{\text{INF}}) ((\mu)g ml(^{-1}) min(^2))</td>
<td>129.3</td>
<td>70.3</td>
<td>54</td>
<td>4808</td>
<td>1505</td>
<td>31</td>
</tr>
<tr>
<td>CL (ml min(^{-1}))</td>
<td>4521</td>
<td>958</td>
<td>21</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(V_{\text{ss}}) (litre)</td>
<td>36.4</td>
<td>13.5</td>
<td>37</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>8.11</td>
<td>2.98</td>
<td>37</td>
<td>29.2</td>
<td>4.2</td>
<td>14</td>
</tr>
<tr>
<td>(t_{1/2}) (min)</td>
<td>21.3</td>
<td>10.9</td>
<td>51</td>
<td>22.5</td>
<td>3.4</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 2 Bootstrap parameter values for the final combined population model for CNS 7056 and CNS 7054. The final model was a three-compartment model for the parent drug CNS 7056 and a two-compartment model of the metabolite CNS 7054. 100% metabolic conversion was assumed. PPV is the inter-individual variability in parameter (population parameter variability) expressed as per cent coefficient of variation (CV\%). The numbers in square brackets are the precision of the parameter estimate as given by the SD of the bootstrap replicates. Additive error was fixed at a small positive value to enhance model stability. The etabar P-values of NONMEM were all >0.74.

<table>
<thead>
<tr>
<th></th>
<th>CNS 7056 Population value</th>
<th>CNS 7056 PPV (CV%)</th>
<th>CNS 7056 Covariate</th>
<th>CNS 7054 Population value</th>
<th>CNS 7054 PPV (CV%)</th>
<th>CNS 7054 Covariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag (min)</td>
<td>0.199 fixed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (ml min(^{-1}))</td>
<td>4199 [6.5%]</td>
<td>18.8 [19.8]</td>
<td>*(CO/COSTD)(^1)</td>
<td>422 [4.5%]</td>
<td>16.4 [17.4%]</td>
<td>*(CO/COSTD)(^1)</td>
</tr>
<tr>
<td>(V_1) (ml)</td>
<td>1952 [6.5%]</td>
<td></td>
<td></td>
<td>251 [29.3%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Q_{12}) (ml min(^{-1}))</td>
<td>2851 [14.8%]</td>
<td>*(CO/COSTD)(^1)</td>
<td></td>
<td>6841 [12.1%]</td>
<td>43.4 [22.1%]</td>
<td>*(CO/COSTD)(^1)</td>
</tr>
<tr>
<td>(V_2) (ml)</td>
<td>3994 [7.7]</td>
<td></td>
<td></td>
<td>6397 [6.1%]</td>
<td>14.7 [23.6%]</td>
<td></td>
</tr>
<tr>
<td>(Q_{13}) (ml min(^{-1}))</td>
<td>1438 [10.5%]</td>
<td>16.4 [42.2%]</td>
<td>*(CO/COSTD)(^1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_3) (ml)</td>
<td>79 510 [38.3%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual error</td>
<td>Proportional error (%CV)</td>
<td>24.9% [8.7%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive error ((\mu)g ml(^{-1}))</td>
<td>0.001 fixed</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

clearance was 4.52 (0.96) litre min\(^{-1}\), the \(V_{\text{ss}}\) was 36.4 (13.5) litre, the mean residence time (MRT) was 8.1 (3.0) min, and the terminal half-life was 21.3 (10.9) min. For CNS 7054, the MRT was 29.2 (4.2) min, and the terminal half-life was 22.5 (3.4) min (Table 1). The maximum concentration of the metabolite was 143%, 108%, and 144% of the maximum concentration of the parent drug, for the 0.37, 0.74, and 1.47 mg kg\(^{-1}\) doses, respectively.

**Compartmental PK modelling**

The preferred configuration of the combined PK model was three compartments for the parent (with a lag term for \(V_1\)) and two compartments for the metabolite (Table 2). The model was characterized by a high systemic clearance for CNS 7056 (~4.2 litre min\(^{-1}\)) and moderate distribution volumes (1.9, 2.8, and 79 litre), and was an adequate description of the concentration data as evident in the diagnostic plots (Fig. 4). There was a clear preference for models with individual studies rather than individual sheep as the population unit [difference in minimum objective function value (delta MOF) = −264], suggesting BOV within a sheep was high for all parameters. As BOV could not be isolated to one or two parameters, for the sake of simplicity, the final model used individual studies as the population unit, implying that BOV was equal to BSV for all parameters.

Using a traditional statistical covariate selection process, the best model had CO as a covariate on the clearance of the metabolite (delta BIC = −39.5 vs base model), and

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**Table 1**
more complex covariate models using up to five of the best covariate relationships determined from the post hoc parameters were not supported by objective function changes when included in the model. However, covariate models embodying mechanistic and allometric principles were generally superior to those based on the traditional statistical approach. The best model overall had CO as a covariate for the flow parameters (e.g. CL or Q) with a fitted power coefficient (delta BIC = −70.3 vs base model). However, this model was only marginally better than a model in which the power coefficient for CO on flow parameters was fixed at 1 (delta BIC = −66.4 vs base model). As this latter model embodies...
the underlying physiological principle that changes in systemic clearance (CL) and inter-compartmental clearance (Q) are directly proportional to CO for a high clearance drug, this model was chosen as the final model. There was no support for body weight, dose, or study order as covariates for the model parameters. There was no support for non-linear elimination. This together with the fact that dose was not a covariate imply that the kinetics of CNS 7056 were linear.

Compartmental PD modelling

A sigmoid E\textsubscript{max} dynamic model was an adequate description of the data (Table 3). The observed and fitted time-courses of the EEG alpha power for the three dose groups are summarized in Figure 5, and the model diagnostic plots in Figure 6. Increasing the dose increased the duration of action, but all three doses were characterized by rapid onset and offset of sedation. The effect compartment rate constant was 0.39 min\textsuperscript{-1}, giving a t\textsubscript{1/2},k\textsubscript{e0} of 1.78 min. The model was characterized by a steep concentration–effect relationship (Hill factor = 5.2); however, the magnitude of the maximum effect (E\textsubscript{max}) was highly variable between animals. As the onset and offset times for sedation were similar between sheep, this may reflect between-animal variability in the EEG response or measurement rather than the underlying level of sedation.

Discussion and conclusion

We present the first report of the PKs and PDs of CNS 7056 and its major metabolite CNS 7054 in a large animal species. While a relatively rapid decline in the blood concentration of CNS 7056 was anticipated, it was found that the rate of decline and standard curve values chosen for this initial study was such that a disproportionate number of blood samples had CNS 7056 (but not CNS 7054) concentrations between the LLOQ and LOD of the assay, particularly for the lower doses. This could be rectified to some extent in subsequent studies by using a lower range for the standard curve. CNS 7056 concentrations from this ‘uncalibrated’

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Population value</th>
<th>PPV (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>k\textsubscript{e0} (min\textsuperscript{-1})</td>
<td>Effect compartment rate constant</td>
<td>0.392 [7.1%]</td>
<td>21.0 [30.8%]</td>
</tr>
<tr>
<td>Base (μV²)</td>
<td>Baseline (pre-drug) EEG alpha power</td>
<td>1.51 [5.7%]</td>
<td>58.4 [39.8%]</td>
</tr>
<tr>
<td>E\textsubscript{max} (μV²)</td>
<td>Maximum EEG alpha power</td>
<td>4.19 [12.0%]</td>
<td>419 [27.7%]</td>
</tr>
<tr>
<td>EC\textsubscript{50} (μg ml)</td>
<td>Concentration corresponding to half-maximum EEG alpha power</td>
<td>0.10 [7.2%]</td>
<td>50.0 [40.9%]</td>
</tr>
<tr>
<td>n</td>
<td>Hill (steepness) coefficient</td>
<td>5.18 [2.8%]</td>
<td>1.08 [2.8%]</td>
</tr>
<tr>
<td>Proportional error (%CV)</td>
<td></td>
<td>38.2 [25.5%]</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Parameter values for the population PD model for CNS 7056. The final equation describing the time-course of the EEG alpha power was: EEG\textsubscript{alpha} = base × [1 + (E\textsubscript{max} × C\textsubscript{eff})/(EC\textsubscript{50} + C\textsubscript{eff})], where C\textsubscript{eff} is the effect compartment concentration. PPV is the inter-individual variability in parameter (population parameter variability) expressed as per cent coefficient of variation (CV%). The numbers in square brackets are the precision of the parameter estimate (μ%) returned by the covariance step of NONMEM. An omega block was used in NONMEM to allow correlation between all population parameters. The etabar P-values of NONMEM were all >0.80.
Fig 6 Diagnostics plots for the final PD model. The solid line is a line with slope 1 or 0, and the dashed line is a loess smoothed line for the data. Upper left: observed EEG alpha power plotted against population predicted (PRED) EEG alpha power. Data are evenly distributed about the line of identify, indicating no major bias in the population component of the model. Upper right: observed EEG alpha power plotted against individual population predicted (IPRED) EEG alpha power. Data are evenly distributed about the line of identify, indicating an appropriate structural model could be found for each individual. Middle: conditional weighted residual (CWRES) plotted against time. Data are evenly distributed about zero, indicating no major bias in the structural model. Lower: conditional weighted residual (CWRES) plotted against the population predicted (PRED) EEG alpha power. Data are evenly distributed about zero, indicating no major bias in the residual error model.
part of the standard curve were included in the analysis as post hoc investigation showed the response of the transformed standard curve was linear over these lower ranges of concentrations. However, two steps were taken to ensure that the uncalibrated CNS 7056 concentrations did not unduly influence the derived PK parameters. First, the NCA analysis was restricted only to the highest dose given (1.47 mg kg$^{-1}$), so that the extrapolated AUC favoured CNS 7056 concentrations in the calibrated range of the standard curve. Secondly, the PK model was developed for parent drug and metabolite concurrently. For the model to simultaneously describe the data for both CNS 7056 and CNS 7054, the measured concentrations needed to be internally consistent between the two concentration sets (i.e. the inferred rate of formation of CNS 7054 at any given time must match the inferred rate of loss of CNS 7056). The fact that an unbiased model could be developed suggests that the lower uncalibrated CNS 7056 concentrations did not deviate greatly from those implied by all other calibrated measurements.

The development of the PK model of CNS 7056 and CNS 7054 presented an opportunity to examine the role of pre-dose CO on model parameters. There is growing recognition of the influence of CO on the kinetics of high clearance drugs. A traditional covariate selection process based on statistical principles favoured a model with CO as a covariate on only one parameter (CL of the metabolite). While statistically plausible, this isolated influence of CO is difficult to rationalize if it is considered that CO can affect drug distribution and elimination in many organs of the body. However, covariate models based on underlying physiological principles were found to be a better fit of the data, and needed fewer fitted parameters. The final model (Table 2) used CO as a parameter on the ‘flow’ related parameters CL and Q. Although the physiological identities of compartments in traditional mamillary models are ambiguous, this configuration of the covariate model is consistent with the concept of CO affecting the rate of delivery of drugs to sites of drug distribution and elimination. The present data suggest that there is merit in incorporating the relationship between blood flow and compartmental model parameters using underlying physiological principles—a process that has been termed ‘bottom-up’ rather than ‘top-down’ covariate model building.

The ideal PK profile of drugs given i.v. for sedation and anaesthesia is different from that of most other drugs. Drugs given by the oral route ideally have a low hepatic clearance for enhanced bioavailability and a long persistence in the body. In contrast, it is recognized that the key to effective i.v. sedation and anaesthesia is having a drug that is rapidly removed from the blood, so that titration of effect can be achieved through rapid dose adjustment. Persistence of action can be readily achieved by the use of a continuous i.v. infusion.

For drugs given as an i.v. bolus, the post-injection decline in blood concentration occurs due to a combination of metabolic elimination (as described by clearance) and redistribution into peripheral tissues (as described by the peripheral distribution volumes). While the two processes are functionally indistinguishable after a single dose, the redistribution process diminishes with repeated doses or longer infusions as the peripheral tissues become ‘full’ of drug. Drugs that rely on redistribution (high distribution volumes) to lower their blood concentration and terminate their effect suffer from the problem that the longer the drug is infused, the slower the recovery at the end of an infusion. This phenomenon has been described by the concept of ‘context sensitive half-times’. The present study suggests that the high clearance of CNS 7056 is responsible for its short duration of action. In sheep, the total body clearance was very high (~4 litre min$^{-1}$) and was much higher than the hepatic blood flow of sheep (~2.6 litre min$^{-1}$), indicating significant extra-hepatic metabolism. The in vitro studies showed that some of this metabolism occurred directly in the blood (but not plasma), suggesting a role for the red blood cell in metabolism. High extra-hepatic clearance also confers the theoretical advantages of kinetics unduly influenced by hepatic and renal failure, and little scope for metabolic interactions with other drugs in the liver.

We conclude that in sheep CNS 7056 showed the PK and PD properties of a useful i.v. sedative: high metabolic clearance, small distribution volumes, rapid onset and offset of sedative effect, and predictable effects over a range of doses. Collectively, these properties would favour its clinical use as an i.v. sedative if they were also displayed in humans. Initial clinical data suggest that rapid recovery after sedation with CNS 7056 is also seen in men.

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Conflict of interest
None declared.

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