Population pharmacokinetics of NNZ-2566 in healthy subjects

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Introduction

Rett syndrome is one of the most common causes of mental retardation in females (Hagberg et al., 1985). Rett syndrome was first described by Andreas Rett in 1966 (Rett, 1966). It is a severe genetically determined neurological disorder in which individuals are intellectually handicapped and suffer a variety of psychiatric symptoms. Loss of function mutations of the MeCP2 gene is thought to be the primary cause (Amir et al., 1999; van Karnebeek et al., 2016). The disorder is characterized by normal early growth followed by a slowing of development, loss of purposeful use of the hands, distinctive hand movements, slowed brain and head growth, problems with walking, seizure and intellectual disability and many features of autism (Colvin et al., 2003; Weaving et al., 2005). The annual mortality rate of 1.2 percent in Rett syndrome is higher than that of the general population (Kerr et al., 1997), of which 26 % of mortality is accounted for by sudden death. The cause of sudden death appears to be related to the presence of epilepsy or autonomic cardio-respiratory difficulties.

Unfortunately, here are no effective treatment options for these patients (Djukic et al., 2016; Percy, 2016). Pharmacological and non-pharmacological interventions are symptomatic and supportive, with medication being used for breathing irregularities, cardiovascular issues, motor difficulties and seizures. Some children may require special equipment, including braces to stop scoliosis, splints to modify hand movements and dietary programs to maintain weight.

NNZ-2566 (trofinetide) is a novel, small molecule with the structure glycine-2-methylproline-glutamate and a molecular weight of 315.3 g/mol. NNZ-2566 is an analogue of the N-terminal tripeptide of insulin-like growth factor 1, (IGF-1[1-3]), modified to enhance

its systemic stability and increase plasma half-life. Despite the differences in molecular weight, NNZ-2566 and IGF-1[1-3] are very similar structurally and in terms of biological effect. Pre-clinical data have shown that treatment with insulin-like growth factor 1 (IGF1) and its active peptide (1–3) IGF1 significantly ameliorates disease symptoms in a mouse model of the disease. In addition, both moieties cross the blood brain barrier, making it an ideal candidate for the treatment of neurodevelopmental disorders, such as Rett syndrome (Pini et al., 2014).

From a physiological perspective, IGF-1 is released in response to the production of growth hormone, and deficiencies of IGF-1 result in a phenotype that overlaps with that of growth hormone deficiency. The effects of IGF-1 appear to be mediated by the IGF-1 receptor, where it acts as a stimulator of the Akt pathway (Vahdatpour et al., 2016). In Mecp2-null (Mecp2(-/y)) mice, which have low IGF-1 levels, systemic treatment with recombinant human IGF1 (rhIGF1) improves lifespan, locomotor activity, heart rate, respiration patterns, and social and anxiety behaviour (Castro et al., 2014).

Recently, Phase I studies have demonstrated the safety and tolerability of NNZ-2566 in healthy subjects. In these studies, blood samples have also been collected for the characterization of the pharmacokinetics of NNZ-2566 after oral and intravenous administration. Here we aim to develop a population pharmacokinetic model to establish the linearity in absorption and disposition of the compound and explore the potential effect of demographic factors on the pharmacokinetics of NNZ-2566. The main justification for the use of model-based meta-analytical approach is the availability of concentration vs. time data from different routes, treatment duration and regimens tested across various cohorts, which make it difficult to summarise the pharmacokinetic parameters of interest, such as

AUC, Cmax, and C_τ. Most importantly, the use of a parametric approach allows further evaluation and prediction of the systemic exposure in subsequent clinical trials in patients, for which a sparse blood sampling scheme is envisaged. Our approach is also aimed at exploring other clinically relevant measures of exposure (e.g., cumulative exposure [AUC], steady-state concentration [Css]), which may be relevant for the assessment of the pharmacokinetic-pharmacodynamic relationships of NNZ-2566.

1 Material and methods

1.1 Analysis Populations

All of the studies included in this analysis were conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, as embodied in the Australian National Statement on Ethical Conduct in Research Involving Humans and were conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP). The clinical study protocols, amendments to the protocols, and versions of the subject informed consent forms (ICF) used in the studies were reviewed and approved by the Alfred Ethics and Research Committee; Alfred Research and Ethics Unit, Alfred Hospital, Commercial Road, Melbourne, VIC 3004, Australia.

The population pharmacokinetic analysis comprised data from three studies of single and multiple doses of NNZ-2566 in healthy subjects. NNZ-2566 doses ranged from 0.1 to 20 mg/kg in 10 minute I.V. infusions and 6 mg/kg to 100 mg/kg through oral administration, including multiple doses. In total 61 healthy subjects who received the NNZ-2566, and from whom blood samples were collected, were considered evaluable for the purposes of this

analysis. An overview of the design, dose, dosing regimen and sampling schedule used for the assessment of pharmacokinetics in each study is presented in table 1.

2.2. Bioanalytical methods.

Blood concentrations of NNZ-255 were analysed by high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS). The calibration curves were constructed using the ratios of areas vs. concentration of NNZ-2566 (0.100 µg/mL - 100 µg/mL in whole blood) showed linearity within the levels observed in the study. The limit of quantification in whole blood was 0.100 µg/ml.

1.2 Pharmacokinetic Modelling

Population pharmacokinetic modelling and simulation was performed using a nonlinear mixed effects approach using NONMEM version 7.3 (Beal, 2009). First, a base model (no covariates) was built. This was accomplished by first identifying the appropriate structural model parameters. Next, the appropriate stochastic models of between-subject and inter-occasion variability were identified. Selected covariates were identified using a stepwise forward inclusion backward deletion approach. Covariate effects were considered for the primary model parameters only (e.g., Ka, CL, V2). Biologically plausible factors identified prospectively to be included in the covariate analysis included age, weight, route of administration, dose and food intake. Data preparation and graphical summaries were created using R version 3.1.3 (R Core Team, 2015).

1.3 Stochastic model

Inter-individual variability (IIV) in the pharmacokinetic parameters was assumed to be log-normally distributed. Given subject i, the individual parameter value is given by:

$$\theta_i = \theta_{tv} \times e^{\eta_i}$$

where θ_{tv} is the typical value in the population and η_i is a random variable with a mean or zero and variance ω^2. Residual variability was described with an exponential error model. Inter-occasion variability (IOV) was tested on absorption parameters, distribution volumes, and clearance (CL) and was included as follows:

$$\theta_i = \theta_{tv} \times e^{\eta_i + \kappa_o}$$

where κ_o represents occasion *o* normally distributed with mean 0 and variance ω^2.

Given the j^{th} measurement of individual i the modelled value of the concentration (Y_{ij}) was given by:

$$Y_{ij} = F_{ij} \times e^{\varepsilon_{ij}}$$

where F_{ij} is the predicted concentration and ε_{ij} a random variable with a mean of zero and variance σ^2.

1.4 Model building, covariate selection, and evaluation

PK model building was done in a stepwise fashion. First, a base model (no covariates) was built after identifying the appropriate structural model, without regard to error model structure. Next, the appropriate error models of between-subject variability had been identified to complete the base model. Selected covariates were then added to the base model. Initially, each factor has been included individually in the base model to identify significant covariates where significance

is a reduction in the objective function value (OFV) of ≥3.84, $\chi^2 < 0.05$ for 1 degree of freedom (df) using the FOCE-I estimation method. The following covariate models were investigated:

- Linear model:

$$\theta_i = \theta_{tv} + \theta_{cov} \times (COV_i - COV_{med})$$

- Exponential model:

$$\theta_i = \theta_{tv} \times e^{\theta_{cov} \times (COV_i - COV_{med})}$$

- Power model:

$$\theta_i = \theta_{tv} \times \left(\frac{COV_i}{COV_{med}}\right)^{\theta_{cov}}$$

where θ_{cov} is the covariate specific effect, COV_i the individual covariate value and COV_{med} the population median.

During the final modelling steps, only the factors (inter-individual variability, covariates) which resulted in OFV reduction of ≥7.88 ($p<0.005$) have been kept in. Covariate analysis was performed to explore measurable sources of PK variability for NNZ-2566.

Model performance was assessed by numerical, graphical and statistical procedures, including visual predictive checks, bootstrapping, normalized prediction discrepancy error and mirror plots. Final parameter estimates were summarised along with their confidence intervals when appropriate. Goodness-of-fit was assessed by graphical methods, including population and individual predicted versus observed concentrations, conditional weighted residuals versus observed concentration or time, and the correlation between parameters.

Visual predictive check (VPC) was used to evaluate the adequacy of the final model parameter estimates, including the effects of statistically significant covariates, to produce simulated data that were similar to the original observed data. In the VPC, 1000 replicates of the original dataset were simulated and a 90% prediction interval was computed based on the simulated datasets. The observed concentration-time data were plotted on the prediction interval to visually assess the concordance between simulated and observed data.

Bootstrapping was performed to identify bias, stability, and accuracy of the parameter estimates and generate standard errors and confidence intervals on the parameter estimates. For bootstrapping, PsN was used to generate 1000 new datasets by sampling individuals with replacement from the original dataset and then fitting the model to each new dataset.

Further evaluation of the variance-covariance structure and overall random effects in the model was performed using mirror plots and NPDE diagnostics. To generate mirror plots, the population PK parameters estimates were used to simulate blood concentrations in patients with similar demographic characteristics, dosing regimens, and sampling scheme as the original clinical studies. Mirror plots of individual predicted versus observed concentration were created to evaluate the degree of similarity between the original fit and the pattern obtained from the simulated datasets. Finally, the normalized prediction distribution error (NPDE) was estimated. Plots to evaluate whether the discrepancies between observed and predicted values were normally distributed included a quantile-quantile (QQ) plots of NPDE vs. the expected standard normal distribution, a histogram of

NPDE with the density of the standard normal distribution overlaid, a scatter plot of the NPDE vs. observed values, and a scatter plot of NPDE vs. predicted values.

1.5 Secondary pharmacokinetic parameters

Secondary parameters were calculated based in individual predicted concentrations simulated every 0.1 h. AUC was calculated by using the trapezoidal rule. Cmax was the maximum predicted concentration observed per individual, whereas half-life was calculated by dividing ln(2) by the elimination rate constant.

2 Results

The age of patients across all studies included in the current analysis ranged from 19 to 38 years, whereas weight ranged between 53.4 and 95 kg. Weight, height and BMI were all positively correlated with one another. A summary of the demographic characteristics and studied covariates is presented by study and overall in table 2. The majority of subjects were female (69%). Neu-2566-HV-001 included only male subjects (n=20) while Neu-2566-HV-004 included only female subjects (n=29).

In total 1435 samples were analysed. Of the total number of samples available, 20 were excluded as they had concentrations below the limit of detection. In addition, data from 6 individuals receiving doses of 6 mg/kg doses of NNZ-2566 had to be excluded from the analysis due to unexplained variability in drug levels, which seemed to increase throughout the sampling interval. A sensitivity analysis showed that the exclusion of these individuals had no further implications for model development and parameter estimation. Of all the

population parameter estimates on the absorption constant deviated more than 10%, the IIV parameters Ka, V2 and CL were respectively 3.5, 2.5 and 1.5 times higher when the 6 mg/kg individuals were included in the analysis. The blood NNZ-2566 concentration-time profiles were consistent across studies and regimens (figure 1).

2.1 Modelling Results

The pharmacokinetics of NNZ-2566 following intravenous administration to healthy subjects was best described by a 2-compartment model with first order elimination (figure 2). Oral administration of NNZ-2566 was characterised by two thetas ($F1_{AM}$, $F1_{PM}$) describing diurnal variation in bioavailability, with lower systemic exposure observed after the afternoon/ evening doses, most likely reflecting the impact food effect. IIV was identified for CL, V2 (table 3). No covariate effects were found on any of the model parameters. The residual error was described using an exponential error model.

All parameters were well estimated without significant correlations between parameters and with good precision (% relative standard error (RSE)<16%). The IIV effects were also estimated with reasonable precision (%RSE<11%). The diagnostic plots for the population PK model (figure 3) showed the model adequately described the data and produced unbiased population and individual predictions. No relationships were noted between the conditional weighted residuals and predicted concentration or covariate (body weight). Inter-individual random effects were close to normally distributed and data were found to be uncorrelated.

The non-parametric bootstrap estimates of the model parameters were similar to the NONMEM estimates (table 3) and the mirror plots showed that the final population PK model accurately replicated the data of healthy subjects in the available studies (figure 4).

From the VPC plots (figure 5), it seems that the median concentration is slightly overestimated. Given that the IIV for CL and V2 is <10%, such overestimation may be explained by the estimated residual variability, The NPDE plots (figure 1S) did not reveal any additional bias for any of the dose levels included in the analysis (See supplemental material).

Estimates of the secondary pharmacokinetic parameters (table 4) seem consistent across studies and dose levels, indicating linear pharmacokinetics and dose proportionality. However, results from study HV-005 showed a slightly lower normalized AUC.

3 Discussion

Our analysis has allowed the characterisation of the pharmacokinetic properties of NNZ-2566, a synthetic analogue of a naturally occurring neurotrophic peptide derived from insulin-like growth factor-1 (IGF-1), in healthy subjects (Bickerdike et al., 2009; Tropea et al., 2009). Created as a non proteinogenic analogue to IGF-1[1-3] to increase its protease resistance, NNZ-2566 has shown an increase in plasma half-life (49 minutes) in rats compared to its naturally occurring tripeptide glypromate (4.95 minutes) (Batchelor et al., 2003; Bickerdike et al., 2009). The pharmacokinetic profiles estimated in healthy volunteers show an even higher half-life of 1.4 hours, a significant improvement considering its route of administration.

Our results contrasts with published data on mecasermin, a recombinant IGF-1, which has been recently evaluated in Rett syndrome patients (Pini et al., 2014). Mecasermin was found to have a considerably longer half-life (between 12.9 to 21 hours) (Camacho-Hubner et al., 2006; Khwaja et al., 2014) than NNZ-2566, but it requires intravenous administration.

NNZ-2566 has a clear therapeutic advantage in that it can be delivered orally. In addition, IGF-1 is believed to have limited blood-brain-barrier permeability (EMA, 2007), unlike the IGF-1 tripeptide (Tropea et al., 2009) on which NNZ-2566 is based.

NNZ-2566 showed linear pharmacokinetics across the dose range evaluated in the clinical trials included in this analysis. From a pharmacokinetic perspective, there was no accumulation, metabolic inhibition, or induction observed during treatment. Zero-order processes did not explain some of the deviation in model predictions observed for some of the samples within the high concentration range. Moreover, good oral bioavailability and low inter-individual variability were observed after oral administration. The population estimate for the volume of distribution at steady-state (i.e., sum of the volumes of distribution for the central and peripheral compartments) was 61.7 L, i.e. larger than total body water. This indicates that NNZ-2566 does distribute into fat tissue and that treatment effects observed in pre-clinical species may indeed result from drug distribution into the brain. The lack of identifiable covariates effects such as weight on the pharmacokinetics of NNZ-2566 in healthy volunteers is not unexpected, as such an effect is usually observed in groups with more wildly ranging demographic characteristics (Jusko and Chiang, 1982; Kersting et al., 2012; Piana et al., 2014) instead of a relatively homogenous group of healthy subjects.

The availability of a population pharmacokinetic model will facilitate the evaluation of drug exposure in future clinical trials in patients suffering from neurodevelopmental disorders. In these patients, sparse sampling schemes will have to be used, with only a few samples are taken per patient. Estimating secondary parameters such as Cmax, AUC and half-life or even developing a comprehensive pharmacokinetic model will be difficult. In addition to

addressing the limitations of sparse sampling, we envisage the possibility of applying Bayesian priors to facilitate with the estimation of inter-individual variability on key pharmacokinetic parameters such as clearance (Dansirikul et al., 2006; Kashuba et al., 1996). This will allow the comparison of drug exposure versus drug effect measures, taking into account the inter-individual variability of the pharmacokinetics.

Moreover, our approach provides the basis for study design optimisation. Sampling schemes can be far more informative if sampling times and sampling frequency are defined taking into account ED-optimality concepts and relevant sources of variability (Bellanti et al., 2016).

4 Conclusions

In summary, we have shown that the population pharmacokinetics of NNZ-2566 can be best described by a two-compartment model with first order oral absorption and first order elimination. Administration of different doses and dosing regimens of NNZ-2566 to healthy subjects yielded dose proportional changes in exposure. Despite the homogeneous cohort of healthy subjects, inter-individual variability and inter-occasion variability were identified for CL, V, and Ka. Estimates of the volume of distribution suggest that the compound distributes into tissue, beyond total body water, making it a suitable candidate for the treatment of neurodevelopmental disorders.

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