ORIGINAL RESEARCH ARTICLE

Clin Pharmacokinet 2005; 44 (8): 863-878
0312-5963/05/0008-0863/$34.95/0

Population Pharmacokinetic Modelling and Estimation of Dosing Strategy for NXY-059, a Nitrone Being Developed for Stroke

Siv Jönsson,[1] *Yi-Fang Cheng*,[2] *Charlotte Edenius*,[3] *Kennedy R. Lees*,[4] *Tomas Odergren*[2] and *Mats O. Karlsson*[1]

1 Division of Pharmacokinetics and Drug Therapy, Uppsala University, Uppsala, Sweden
2 AstraZeneca R&D, Södertälje, Sweden
3 Biolipox AB, Stockholm, Sweden
4 University Department of Medicine and Therapeutics, Western Infirmary, Glasgow, UK

Abstract

Background and objectives: NXY-059 (disufenton sodium, Cerovive®), a nitrone with neuroprotective and free radical trapping properties (in experimental stroke) is under development for the treatment of acute stroke. The objectives of this study were to develop a population pharmacokinetic model for NXY-059 in acute stroke patients and to estimate individualised dosing strategies for NXY-059 using preclinical pharmacological and clinical pharmacokinetic information and knowledge of characteristics of the patient population.

Methods: NXY-059 was given as a continuous intravenous infusion for 72 hours, including a 1-hour loading infusion. Maintenance infusion rates were individualised based on creatinine clearance (CL_{CR}). Population pharmacokinetic models were derived using NONMEM software. Optimal dosing strategies, individualised based on CL_{CR} or bodyweight, were estimated using the population pharmacokinetic models, empirical covariate distributions relevant for the target population, and a target definition. Dosing strategies were selected based on target fulfilment criteria and parsimony.

Patients: Pharmacokinetic data from 179 patients with acute ischaemic or haemorrhagic stroke, included in two clinical studies, were used for the analyses. Patients were aged 34–92 years with varying degrees of renal impairment (estimated CL_{CR} 20–143 mL/min).

Main outcome measures and results: The final population model based on data from both studies comprised a two-compartment model with unexplained interpatient variability for clearance (23% coefficient of variation [CV]) and central volume of distribution (40% CV). Part of the variability in clearance and volume of distribution was explained by CL_{CR} and bodyweight, respectively. Typical clearance was estimated to 4.54 L/h in a patient with CL_{CR} of 70 mL/min. The preferred dosing strategy for NXY-059 comprised an initial loading infusion (the same for all patients) followed by an individualised maintenance infusion on the basis of CL_{CR} (three dosing categories) with cut-off values (at which infusion rates are incremented or decremented) of 50 and 80 mL/min.

Conclusion: The results illustrate how an individualised dosing strategy, given a pharmacokinetic target, for NXY-059 was successfully optimised through estimation using the increasing pharmacokinetic and pharmacodynamic knowledge during a clinical drug development programme. The chosen dosing strategy of NXY-059 provides an easily adapted treatment regimen for acute stroke, resulting in early achievement of target plasma concentrations.

Background

NXY-059 (disodium 4-[(*tert*-butylimino)-methyl]-benzene-1,3-disulfonate N-oxide; disufenton sodium; Cerovive® [1], AstraZeneca) a nitrone with neuroprotective and free radical trapping properties, is under development for the treatment of acute stroke. The chemical structure of NXY-059 is shown in figure 1. NXY-059 is neuroprotective in transient focal ischaemia in rats,[1] and in permanent focal ischaemia in both rats[2,3] and monkeys.[4] Free radical mediated toxicity has been implicated as a major mechanism involved in neuronal death following an ischaemic insult; therefore, free radical trapping may be useful as a therapeutic approach in the treatment of ischaemic stroke.[5,6]

NXY-059 appears to be well tolerated by healthy volunteers[7] and by patients with ischaemic or haemorrhagic stroke[8,9] following 72 hours of continuous intravenous infusions. In the most recent study of patients with acute ischaemic stroke, the average unbound plasma concentration at steady state was 260 μmol/L in the highest dose group.[9]

NXY-059 is moderately bound to primarily albumin in plasma, fraction unbound in plasma (f_u) being 0.61, and is eliminated mainly by renal excretion, mostly via filtration.[7] Unbound clearance (CL_U) has been demonstrated to be linearly related to creatinine clearance (CL_{CR}) in healthy elderly subjects, and it was concluded that the ability to eliminate NXY-059 could be approximately predicted from CL_{CR}.[7] This was confirmed in patients with renal impairment.[10] Biomarkers predicting the effect of NXY-059 are not available; therefore, the unbound plasma concentration of NXY-059 was considered the most suitable variable to use as a target for dose individualisation. To increase the probability of safe and effective treatment with NXY-059, regardless of renal function, dosing would aim at reaching a target unbound concentration of the drug in all patients. This is of particular importance as acute stroke patients display a high incidence of associated renal impairment. Therefore, an individualised CL_{CR}-based dosing strategy is needed to reach target exposures of NXY-059 in most patients without producing an exposure that is too high in patients with moderate-to-severe renal impairment. In addition, it is likely that treatment response is optimised if drug administration is initiated as early as possible after stroke onset (the thrombolytic alteplase, for example, must be administered within 3 hours of stroke onset)[11] and if target concentrations are achieved rapidly. Hence, a practical and adaptive dosing strategy is required.

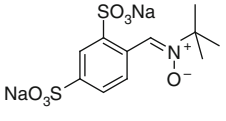

Fig. 1. Chemical structure of NXY-059, molecular weight 381.3.

This paper reports on the population pharmacokinetic characteristics of NXY-059 in acute stroke patients, and on the methods for estimating dosing strategies for NXY-059 using pharmacokinetic information and knowledge of patient characteristics. The results are based on data from two clinical studies (SA-NXY-0003 [study 1] and SA-NXY-0004 [study 2]) for which safety and tolerability have been reported previously.[8,9] The aim of this paper was to describe how the progressive accumulation of pharmacokinetic information at each step in drug development, and the methods of analyses were used to optimise, through estimation, an individualised dosing strategy for NXY-059. The studies and analyses were performed consecutively over time as follows: (i) design and execution of study 1; (ii) population pharmacokinetic analysis of

1 The use of trade names is for product identification purposes only and does not imply endorsement.

study 1 data; (iii) dose estimation for study 2 based on population pharmacokinetic analysis of study 1 data, followed by performance of study 2 using the estimated dosing strategy; (iv) population pharmacokinetic analysis of the results from study 2; (v) population pharmacokinetic analysis of the combined data from studies 1 and 2; and (vi) dose estimation for future studies based on population pharmacokinetic analysis of the combined data from studies 1 and 2.

Methods

The primary objective of the two double-blind, placebo-controlled, randomised, parallel-group, multinational, multicentre studies was to evaluate the safety and tolerability of NXY-059 at various dose levels. The secondary objective was to study the pharmacokinetic characteristics of NXY-059 in patients with acute stroke. The studies were performed in accordance with the Declaration of Helsinki, Good Clinical Practice and applicable regulatory requirements. The study protocols, including the patient information and consent forms, together with protocol amendments, were reviewed and approved by Independent Ethics Committees in the UK, Sweden and Germany.

This section describes the methods used during the course of development. As far as possible, information has been condensed but, when necessary, specific procedures for studies 1 and 2, respectively, have been pointed out. Initially, justification of target levels and description of establishment of infusion rates for studies 1, 2 and the future studies are given. Thereafter, the design of studies 1 and 2 are described. Finally, drug analysis and population pharmacokinetic modelling methods for both studies are given.

Justification of Targets and Estimation of Dosing Strategies

Study 1

At the time of planning study 1, an unbound plasma concentration of 40 μmol/L NXY-059 had been shown to be neuroprotective in a rat transient focal cerebral ischaemia model.[1] This exposure level had also been shown to be well tolerated in healthy volunteers.[7] Therefore, in study 1 the objective was to reach an unbound plasma concentration of NXY-059 at steady state ($C_{u,ss}$) of ≥40 μmol/L in most patients (>90%) in the high-dose group. The mean and distribution of CL_{CR} in a stroke patient database extracted from a previous clinical study[12] and other observations (AstraZeneca, unpublished data), together with the known CL_{CR}–CL_u relation,[7] were used to derive a maintenance infusion rate that would result in the target exposure. Various infusion rates were evaluated and the one fulfilling the desired target (≥40 μmol/L in >90% of the patients) was chosen. The lower dose was set to 50% of the dose given in the high-dose group, i.e. with a target unbound plasma concentration of ≥20 μmol/L in most patients. Furthermore, as the pharmacokinetics of NXY-059 in renally impaired patients were unknown at the start of this study, a dose adjustment (50% reduction) was recommended for patients with a CL_{CR} of <60 mL/min.

Study 2

Results from study 1[8] confirmed that doses administered were well tolerated. However, clearance (CL) of NXY-059 in patients with acute stroke was somewhat higher than expected, resulting in lower exposure of NXY-059 than predicted. Additional preclinical studies in rats,[2,3] where NXY-059 was administered as a loading dose (intravenously or subcutaneously) followed by a continuous infusion (intravenously or subcutaneously) for 24 hours, demonstrated that unbound plasma concentrations of 50–150 μmol/L (assuming f_u in rat plasma is 0.3) were required for efficacy in a permanent middle cerebral artery occlusion model. Furthermore, in this model, the neuroprotective effect was linearly related to plasma concentration.[3] The permanent focal brain ischaemia model is the most relevant model of stroke in humans, as many patients do not spontaneously reperfuse in the first few days following a stroke. A target concentration exceeding the concentration required for neuroprotection in the rat permanent focal ischaemia model was therefore desired. The available information at this point of development suggested that initially increasing the target unbound plasma concentration to 100 μmol/L would be both well tolerated and therapeutically valuable. However, from the efficacy point of view, considering the locally linear relationship between neuroprotection and plasma concentrations in the

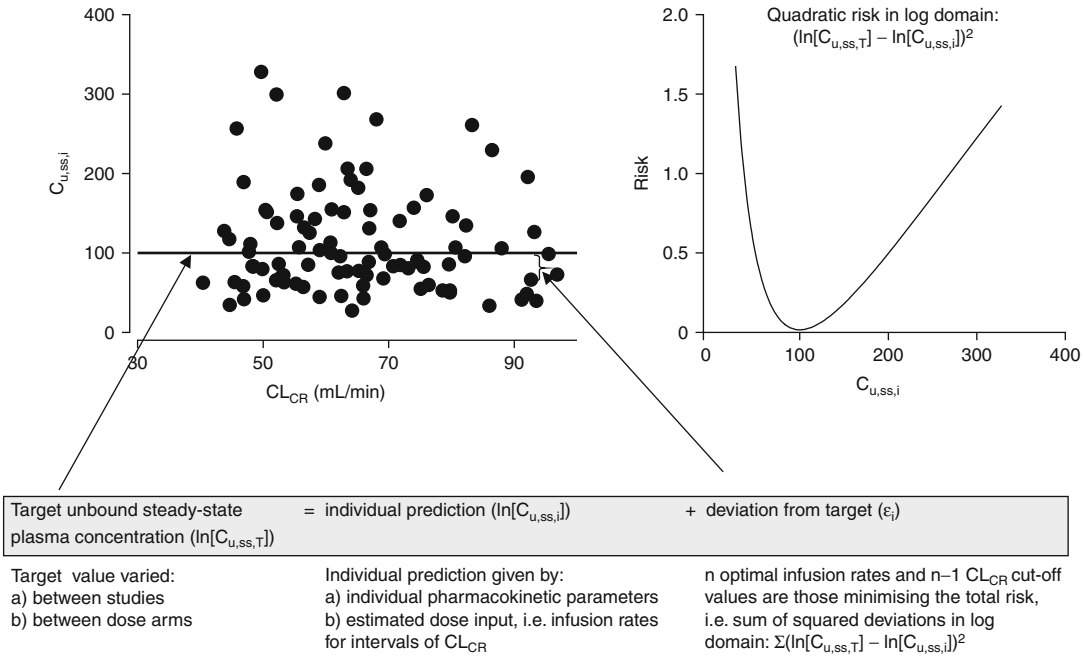

Fig. 2. Summary of dose estimation approach exemplified for the situation where target is individual prediction of unbound steady-state plasma concentration ($C_{u,ss,i}$) and individualisation is based on creatinine clearance (CL_{CR}). **n** = number of dosing categories.

permanent focal ischaemia model, an increased exposure to target unbound plasma concentrations of 200 μmol/L was needed. This was accomplished by performing the study in two steps, with the doses aimed at the lower target concentration administered initially to one group of patients. An evaluation of safety was planned based on safety and tolerability in this group of patients and on information from a complementary tolerability study in healthy volunteers (AstraZeneca, unpublished data) receiving infusions aiming at unbound concentrations of up to 300 μmol/L. If the safety evaluation was acceptable, the higher infusion rates could be commenced.

Once target concentrations were determined (100 and 200 μmol/L), infusion rates for study 2 were estimated using a previously described method.[13] This method allowed optimal (given restrictions and assumptions) dosing strategies to be estimated as infusion rates for different subpopulations and CL_{CR} cut-off values at which infusion rates are incremented or decremented, i.e. implying the advantage of not having to choose CL_{CR} cut-off values beforehand. This method is graphically presented in figure 2. The target variables were unbound plasma concentration of NXY-059 at 1 hour for the loading infusion and $C_{u,ss}$ for the maintenance infusions. The seriousness of deviations from the target was based on preclinical data, and reaching effective exposure of the drug was considered more important than risking plasma levels that were too high and resulting in subsequent undesired effects. Therefore, a risk function that penalises a concentration at half the target as much as one at twice the target (quadratic risk function in the log domain) was judged to be appropriate. The optimal dosing strategy was estimated by minimising the overall risk (figure 2).

The expected individual outcome, given the estimated dose input, was based on the final population pharmacokinetic model from study 1, together with relevant empirical covariate distributions obtained from previous clinical studies in the target population[12] and other observations (AstraZeneca, unpublished data). The empirical distribution of CL_{CR} (n = 902) had a median of 63 mL/min, a coefficient of variation (CV) of 38%, and ranged from 30 to 183 mL/min. Corresponding figures for the empirical

bodyweight distribution were 72kg, 19% and 36–153kg, respectively. CL_{CR} was calculated according to the Cockcroft and Gault method.[14]

Individualisation based on renal function was evaluated for the maintenance infusion. For the loading infusion, individualisation based on renal function and/or bodyweight was initially investigated. Up to two and four dosing categories were assessed for the loading and maintenance infusions, respectively. Patients with a CL_{CR} of <30 mL/min were excluded from the study. To select between rival dosing strategies, e.g. two strategies with different numbers of dosing categories, a criterion was defined based on the practicality of as few doses as possible and the therapeutic requirement of a target fulfilment, expressed as less than certain percentages of the population outside an interval around the target. The most practical dosing strategy resulting in at least 90% of the target population reaching unbound concentration >70/150 μmol/L (low/high target) and <5% of the patients reaching unbound concentrations >150/300 μmol/L (low/high target), was chosen as the most appropriate. Based on the measured unbound concentrations in study 1, these limits were considered reasonable.

The estimation of optimal infusion rates was based on an f_u of 0.61 in all patients; the value obtained from patients in study 1 as well as in healthy elderly volunteers.[7] Estimations for loading and maintenance infusions were done separately. The optimal infusion rate was determined using a stepwise search for the optimal dosing strategy, allowing CL_{CR} cut-offs to increase in multiples of 5 mL/min, which was thought to result in practical dosing strategies. The estimations were done using the NONMEM software (Globomax, Hanover, MD, USA) and the optimal dosing strategy was considered as the one giving the lowest objective function value (OFV), which corresponds to minimising the overall risk.

Future Studies (SAINT-I and SAINT-II)

Apart from using population pharmacokinetic models for the combined analysis and another target concentration, dosing strategies for future studies (SAINT-I and SAINT-II) were estimated as for study 2. Considering the linear relationship between neuroprotection and plasma concentrations in the permanent focal ischaemia model,[3] increasing the exposure is expected to be therapeutically beneficial. The highest dose in study 2 was well tolerated and without dose-limiting adverse events.[9] Hence, based on the exposure at the highest dose in study 2, the target was set to 250 μmol/L of unbound concentration of NXY-059. A dosing strategy resulting in at least 90% of the target population reaching unbound concentrations >150 μmol/L and <5% of the patients reaching unbound concentrations >400 μmol/L was chosen as the most appropriate. This upper limit was guided by the limited experience from subjects attaining such exposure in previous studies during drug development (n = 16).

Patient Group Studied

In study 1 and 2, a total of 184 patients with acute ischaemic or haemorrhagic stroke were treated with NXY-059 (low or high dose) within 24 hours after onset of symptoms of stroke. Details on inclusion/exclusion criteria have been reported previously.[8,9] Two major differences to be observed were that patients with haemorrhagic stroke were excluded from study 2 and the lower limit for inclusion with respect to calculated CL_{CR} was <50 mL/min and <30 mL/min in studies 1 and 2, respectively.

Treatment

The study drug was given as a continuous intravenous infusion for 72 hours, including a 1-hour loading dose. Treatment duration was primarily guided by brain-imaging data indicating that ischaemic but still viable brain tissue can be detected for several days in stroke patients.[15] Both studies comprised a low- and high-dose group. Infusion rates were individualised based on the calculated CL_{CR} using the Cockcroft and Gault method,[14] as summarised in table I. The dosing strategies used in the studies are those estimated as described in this paper. The infusion site was a peripheral vein in the forearm and the infusion was delivered by the pump and device routinely used at each hospital. Special light-protective bags were used during the infusion to cover the bags containing the solution for infusion. NXY-059 concentrates for infusion (200 mg/mL or 400 mg/mL in 10mL vials) were manufactured and distributed by AstraZeneca. To obtain

Table I. Infusion rates of NXY-059 used in studies 1 and 2[8,9]

Study	Target unbound concentration (μmol/L)[a]	Creatinine clearance (mL/min)	Loading infusion (μmol/h)[a]	Maintenance infusion (μmol/h)[a]
Study 1[b]	20/40[c]	≥60	656/1311	223/446
	20/40[c]	50–59	50% reduction	50% reduction
Study 2[b]	100/200	>80	2400/4774	1102/2212
	100/200	51–80	2400/4774	688/1360
	100/200	30–50	2400/4774	433/854

a Values expressed are for low-/high-dose group.

b Once a dose reduction had been performed the dose was not increased again, regardless of subsequent creatinine clearance values.

c The aim was for >90% of the patients to achieve unbound concentrations ≥20 and 40 μmol/L for the low- and high-dose group, respectively.

concentrations of NXY-059 suitable for infusion (2.65 and 5.3 mg/mL in study 1; 7.5 and 14.8 mg/mL in study 2), solutions for infusion were prepared at the clinic by further dilution of the concentrate in commercially available sodium chloride solution (9 mg/mL) for infusion.

Pharmacokinetic Sampling

Venous blood samples of 5mL were collected into heparinised Venoject tubes (Terumo®) from an antecubital or other peripheral vein in the arm not currently used for study drug infusion. In both studies, samples were collected before the start of infusion and 0.5, 1 (or end of loading infusion), 24 and 72 (or end of maintenance infusion) hours after the start of treatment. In study 2 and at selected centres in study 1, samples were also collected at 2, 73, 74, and 76–78 hours after the start of treatment (equivalent to 1 hour after the end of loading infusion and 1, 2 and 4–6 hours after the end of the maintenance infusion). One extra sample (5mL) to be analysed for unbound concentration of NXY-059 was collected at 24 hours in both studies. Owing to the short half-life of NXY-059, the observed concentrations at 24 hours after the start of drug treatment were assumed to reflect plasma concentration of NXY-059 at steady state (C_{ss}) and $C_{u,ss}$. Within 30 minutes of collection, the blood samples were centrifuged at 2000–2500 × *g* for 15 minutes. The plasma was transferred into a Nunc® tube and frozen at −20°C until analysed. Plasma samples were protected from light during handling and storing.

Plasma Drug Analysis

All analyses were performed at AstraZeneca R&D, Södertälje, Sweden.

Study 1

Concentrations of NXY-059 were determined in plasma and plasma ultrafiltrate (unbound concentration determined by use of ultrafiltration) using a validated column-switching high-performance liquid chromatographic method.[7] The ultraviolet absorbance at 299nm was used for detection and quantification of the substance. The limit of quantification (LOQ) was 0.05 μmol/L, with a CV of <6.5% in the concentration range of 0.365 to 139 μmol/L for plasma samples. For the unbound concentrations the LOQ was 0.03–0.06 μmol/L, with a CV of <9% for the 4.47 μmol/L concentration (low-dose group) and <4.5% for the 126 μmol/L concentration (high-dose group).

Study 2

In study 2 the method was modified using an improved, more time-efficient sample preparation procedure as follows. In a Centricon YM-30 ultrafilter (Amicon®; Millipore, Billerica, MA, USA), 200μL plasma was added to 400μL sodium caprylate solution (5 mmol/L in phosphate buffer pH7). After mixing and incubating at room temperature for 10 minutes, the samples were centrifuged for 20 minutes at 20°C. The centrifugate (10 or 50μL) was injected into the chromatographic system. The LOQ was between 0.08 and 0.2 μmol/L, with a CV of 16.1% at 0.605 μmol/L and a CV of <5.0% in the concentration range of 76.1–501 μmol/L for plasma samples. The LOQ for ultrafiltrate samples was 0.08

μmol/L, with a CV of <2.44% at a total plasma concentration of 400 μmol/L.

Data Analysis

This paper reports work performed over a time period of 5 years, meaning that some methods used may not be the choice of today; however, the purpose is to report what was actually done.

Population Pharmacokinetic Modelling

The population pharmacokinetic analyses were carried out using NONMEM version V,[16] using the first-order estimation method in study 1 and the first-order conditional estimation method/first-order conditional estimation method with interaction in study 2 and the combined analysis. The statistical package S-Plus version 5 (Insightful Corporation, Seattle, WA, USA), together with Xpose versions 2 and 3, a model building aid for population analysis using NONMEM, was used for dataset checkout, exploration and visualisation, model diagnostics, and model comparison.[17] Model diagnostics included graphical evaluation, OFV and the precision of parameter estimates. The main tool used for selection between hierarchical models was the difference in OFV between models. The OFV is proportional to minus twice the log likelihood and the difference in OFV for the two models is approximately χ^2-distributed. If the models differ by one parameter, a difference in OFV of >3.84 (one degree of freedom) is significant at the 5% level. Corresponding values (changes in OFV) for $p = 0.01$ and $p = 0.001$ are 6.63 and 10.83, respectively.

The population pharmacokinetic models based on data from studies 1 and 2, respectively, were built in a stepwise procedure. First, the pharmacokinetic model (structural model) was established, then the random effect models were refined and established and, finally, the covariate model was added and established. Based on previous knowledge,[7] a two-compartment open pharmacokinetic model was used as a starting point. Exponential models were used to account for inter-individual variability, and covariance between inter-individual random effects was considered. Different models were evaluated for the description of the residual error, including an additive, a proportional, or a combined additive and proportional model on untransformed data and an additive model on log-transformed data.

Covariate Model Building

Covariate models were built applying a stepwise, generalised additive modelling procedure[18,19] for identifying covariate-parameter relations, followed by stepwise forward inclusion (of selected relations) and backward elimination within NONMEM in study 1. In study 2, a stepwise, covariate model building procedure within NONMEM (including forward inclusion and backward elimination)[20] was used. The methods were implemented as previously described.[21] For retention of the covariate-parameter relationship in the backward elimination, a p-value below 0.001 or 0.01 was required in studies 1 and 2, respectively.

Covariates used in the analysis in study 1 were age, bodyweight, body mass index (BMI), CL_{CR}, sex and the study centre. In study 2, age, bodyweight, height, CL_{CR}, BMI, serum creatinine, f_u and sex were considered. Graphical inspection showed strong collinearities between age and CL_{CR}, bodyweight and CL_{CR}, bodyweight and BMI, and bodyweight and height. Age, BMI and height were not included in the analysis in study 2 for the following reasons: (i) CL_{CR} was chosen as the covariate reflecting the kidneys' capability of eliminating the drug as being mechanistically closer related to the elimination function than age; and (ii) when choosing one measure to describe body size, bodyweight was chosen instead of BMI and height as this is the covariate used more and as results from the earlier analysis had indicated that this covariate may have an influence on the volume of distribution.

Patients potentially driving or masking a covariate relation were identified by a method that uses the contribution of the individual to the OFV for two competing models.[22]

Main Outcome Measures and Results

Data Sources

Table II shows demographic data for patients treated with NXY-059 with pharmacokinetic observations. Samples were excluded (<5% of all samples) for use in population analysis for any of the following reasons: (i) plasma concentrations record-

Table II. Demographic characteristics at baseline for patients treated with NXY-059 with pharmacokinetic observations in studies 1 and 2[8,9]

Variable	Study 1	Study 2
No. of patients used for population analysis	92	87 (84)[a]
No. of plasma samples	558	667 (642)[a]
No. of patients on low-dose infusion	44	48
No. of patients on high-dose infusion	48	39
Age (y)[b]	71 (37–85)	70 (34–92)
Bodyweight (kg)[b]	77 (40–125)	76 (42–108) [n = 85]
Height (cm)[b]	169 (136–190)	170 (150–185) [n = 81]
Body mass index (kg/m^2)[b]	26 (16–44)	26 (18–44) [n = 81]
Serum creatinine (μmol/L)[b]	88 (57–133)	97 (66–155) [n = 85]
Creatinine clearance (mL/min)[cb]	71 (40–141)[d]	61 (22–129)[e] [n = 83]
Male	57	53
Female	35	34
Caucasian	90	86
Black	1	0
Oriental	0	1
Other	1	0

a No. of patients after those with extreme observations were omitted.

b Values are expressed as median (range).

c Calculated according to the Cockcroft and Gault method.[14]

d The estimate was a mean of values at admission and at 1, 24, 48 and 72h after the start of treatment. In addition, the estimates were truncated upwards at 140 mL/min, as the formula may give unrealistically high values in patients with high bodyweights.

e Creatinine clearance varied over study duration; minimum and maximum values observed, taking all values into account, were 20 and 143 mL/min, respectively.

ed as below LOQ; (ii) measurable plasma concentration before the start of infusion; (iii) sample collected from the same arm as the infusion was given in; or (iv) unreliable records for the rate of infusion. In addition, further samples from study 2 were omitted during model building – three patients exhibited plasma concentration profiles that did not correspond with the dosing information and two additional patients had one sample each with an extremely high plasma concentration. After establishment of the final model for study 2, the data were restored to the dataset and the final model re-estimated. The observed total plasma concentrations in studies 1 and 2 are given in figure 3.

Target Fulfillment: Unbound Plasma Concentrations of NXY-059

Unbound plasma concentrations measured at approximately 24 hours after the start of treatment, i.e. $C_{u,ss}$, were available in 156 patients (76 and 80 patients in studies 1 and 2, respectively). In study 1, unbound concentrations were 25 (12–52) μmol/L [mean (range)] and 46 (19–90) μmol/L in the low- and high-dose groups, respectively. Sixty-eight percent and 63% of patients had levels of >20 and >40 μmol/L in the low- and high-dose groups, respectively. In study 2, the observed unbound concentrations were 109 (54–177) μmol/L and 260 (126–424) μmol/L for each group, respectively. For the low-target group the criteria were met (92% of patients >70 μmol/L; 7% >150 μmol/L), but for the high-target group a larger proportion of patients than expected had unbound concentrations >300 μmol/L (92% >150 μmol/L; 25% >300 μmol/L), also illustrated in figure 4. A less pronounced deviation from the expectation was indicated for total plasma concentrations; corresponding total plasma concentrations in the high-target group were 374 (196–682) μmol/L. The f_u in studies 1 and 2 was 0.61 (0.51–0.71) and 0.68 (0.56–0.94), respectively. No strong deviation from linearity was evident over the studied concentration range, although a slightly higher f_u was indicated in the study arms with higher concentrations (averages of 0.67 and 0.70 in the

low- and high-dose groups, respectively). On the basis of the estimated f_u and total plasma concentrations measured following the loading infusion, unbound concentrations could be calculated at early timepoints. For study 2, it was shown that target concentrations were achieved at the end of the loading infusion and were acceptably within the predefined criteria.

Population Pharmacokinetic Models

Parameter estimates of the three final population pharmacokinetic models are presented in table III. Basic goodness-of-fit plots (figure 5) revealed good correlation between model predictions and observations.

Analyses of Studies 1 and 2

The final population pharmacokinetic models based on studies 1 and 2 were similar and comprised a two-compartment model with interpatient variability for CL and central volume of distribution (V_c) described with exponential models. Linear covariate relations were established for CL_{CR}-CL and body-weight-V_c. In study 1, a combined additive and proportional error model described the residual variability, while in study 2 a proportional error model was sufficient (additive model with log-transformed

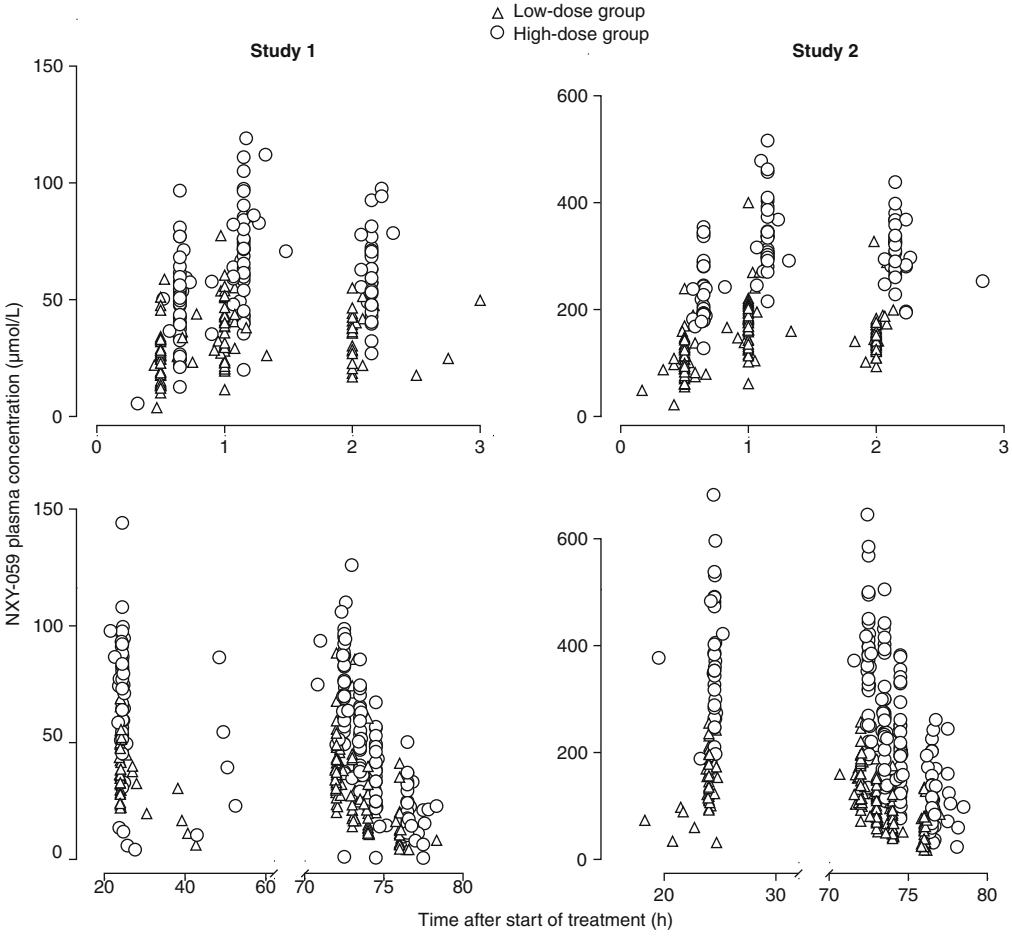

Fig. 3. Observed NXY-059 plasma concentration vs time after the start of infusion in studies 1 and 2.[8,9] The upper panels show the plasma concentration during the first 3 hours and the lower panels show the plasma concentration around the 24-hour sample and following termination of the maintenance infusion. The data from the high-dose infusion group are displaced on the time axis by 0.15 and 0.5 hours in the upper and lower panel, respectively. Data observations in study 2 omitted from the analysis are not included.

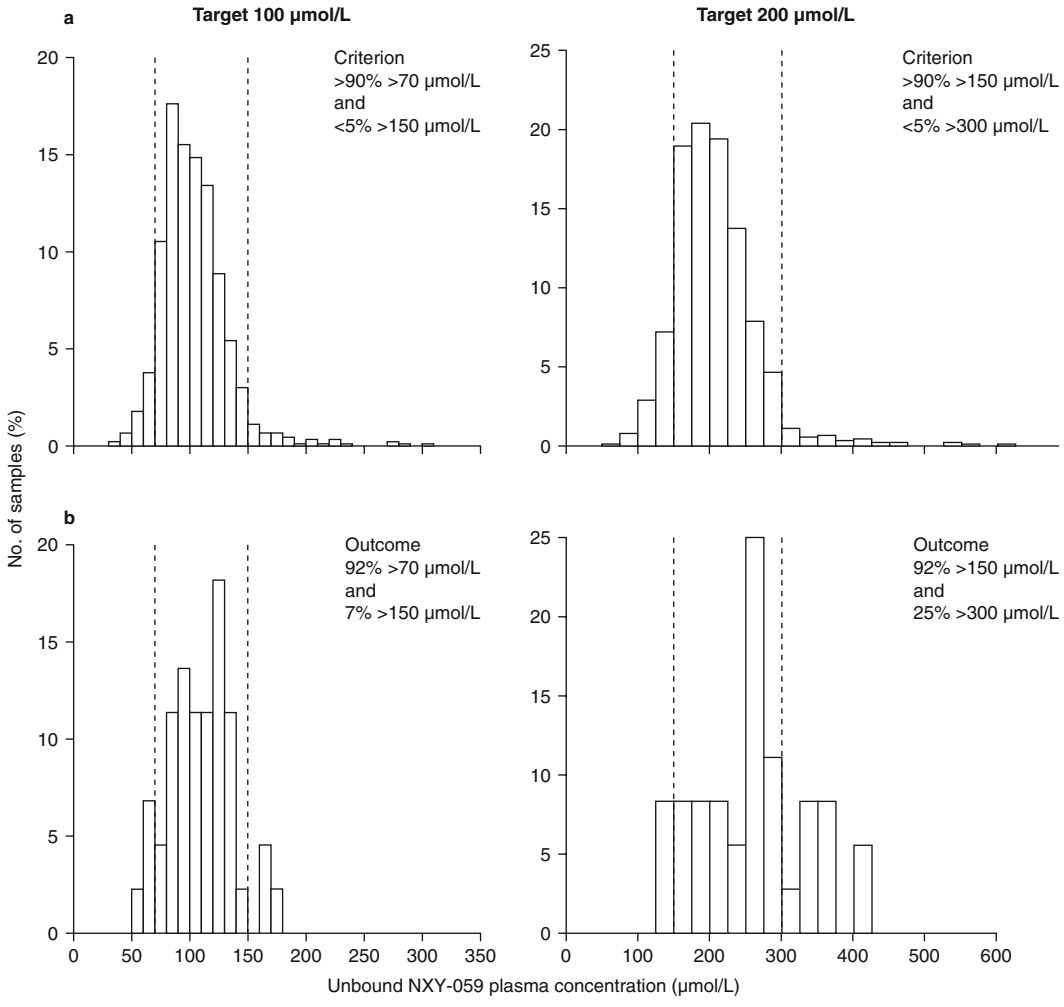

Fig. 4. Distribution of (**a**) expected and (**b**) observed unbound plasma concentrations of NXY-059 in study 2 for the low and high target groups. Observed values were measured at approximately 24 hours after the start of drug treatment. The vertical dotted lines correspond to the fulfilment criterion.

data). Re-estimation of the population pharmacokinetic model for study 1 with the first-order conditional estimation method with interaction resulted in similar parameter estimates.

Few candidate covariate relations were identified by generalised additive modelling in study 1 (CL_{CR} on CL, bodyweight and sex on V_c), of which two were kept following NONMEM evaluation. The OFV decreased by 106 by including the effect of CL_{CR} on CL, and a further drop of 27 was seen with inclusion of bodyweight-V_c relation. No new covariate relations were identified in study 2, and the drops in OFV were 34 and 23 with inclusion of CL_{CR}-CL and bodyweight-V_c relationships, respectively. Although typical CL for a patient with median CL_{CR} was similar for the two models based on the different studies, a difference was observed with respect to the relationship between typical CL and CL_{CR} as the slope was less steep in study 2.

Restoration of suspect datapoints (study 2) resulted in high imprecision in some parameter estimates, an increased residual error and somewhat steeper slopes in covariate relationships.

Combined Analysis

The final population pharmacokinetic model, based on the combined studies, differed from the final model for study 2 in that CL was independent of CL_{CR} in patients with CL_{CR} ≤40 mL/min and, above this threshold, the CL_{CR}-CL relation was linear. In addition, covariance between inter-individual random effects in CL and V_c was included (table III).

Initially, the final model from study 2 was applied to the combined data. To explain the higher than expected total and unbound NXY-059 concentrations at the highest infusion rate in study 2, nonlinear (Michaelis-Menten), or combined linear and nonlinear, elimination was included in the model, but the data did not support this. Furthermore, unbound concentrations were included in the dataset, enabling simultaneous modelling of nonlinear protein binding and nonlinear elimination. Although models including both nonlinear features could be estimated, inclusion of further complexity into the model resulted in uncertain parameter estimates, and improvements with respect to OFV were not statistically significant.

The study-related difference in the relationship between CL and CL_{CR} was promoted by one patient in study 1, as indicated by the likelihood-based diagnostic method.[22] Further analyses were performed excluding this patient. Visual inspection of empirical Bayes estimates of CL versus CL_{CR} suggested a nonlinear relationship (figure 6) and was supported by a decrease in OFV. A model with a constant CL when CL_{CR} ≤40 mL/min and a linear relation when CL_{CR} >40 mL/min, was chosen.

As a final refinement of the model, a correlation between individual random effects on CL and V_c was found to improve the model.

Table III. Population pharmacokinetic parameter estimates[a] for final models

Parameter	Study 1	Study 2	Combined
No. of patients	92	84	175[b]
No. of datapoints	558	642	1196
Structural model parameters			
CL_{70} [L/h][c]	4.59 (2.5)	4.34 (3.2)	4.54[d] (1.8)
$V_{c,75}$ [L][e]	6.96 (16)	8.79 (13)	7.76 (18)
Inter-compartmental CL [L/h]	15.9 (24)	7.41 (50)	13.1 (46)
V_p [L]	8.37 (13)	5.82 (15)	7.17 (19)
Fractional change in CL with CL_{CR}[c]	0.0147 (8.5)	0.00829 (13)	0.0120 (6.4)
Fractional change in V_c with bodyweight[e]	0.0185 (20)	0.0140 (24)	0.0198 (27)
Intersubject variability parameters			
CL (%CV)	22.0 (20)[f]	26.0 (19)[f]	23.0 (13)[f]
V_c (%CV)	38.0 (40)[f]	31.0 (39)[f]	40.0 (52)[f]
Correlation between inter-individual random effects in CL and V_c	NE	NE	0.27 (47)[f]
Residual variability parameters			
Additive residual error (SD) [μmol/L]	5.8 (19)	NE	NE
Proportional residual error (%CV)	9.70 (25)	14.0 (8.9)	16.5 (7.5)

a Values expressed as mean (%RSE), unless specified otherwise.

b Patient promoting the study-related difference in the relationship between CL and CL_{CR} is not included.

c CL_{70} refers to a patient with a CL_{CR} of 70 mL/min where CL = CL_{70} • (1 + fractional change in CL with CL_{CR} • [CL_{CR} −70]).

d CL = 2.91 if CL_{CR} ≤40 mL/min.

e $V_{c,75}$ refers to a patient with a bodyweight of 75kg where V_c = $V_{c,75}$ • (1 + fractional change in V_c with bodyweight • [bodyweight −75]).

f The %RSE for the corresponding variance or covariance term.

CL = total body clearance; **CL_{CR}** = creatinine clearance; **CV** = coefficient of variation; **NE** = not estimated; **RSE** = relative standard error; **SD** = standard deviation; **V_c** = central volume of distribution; **V_p** = peripheral volume of distribution.

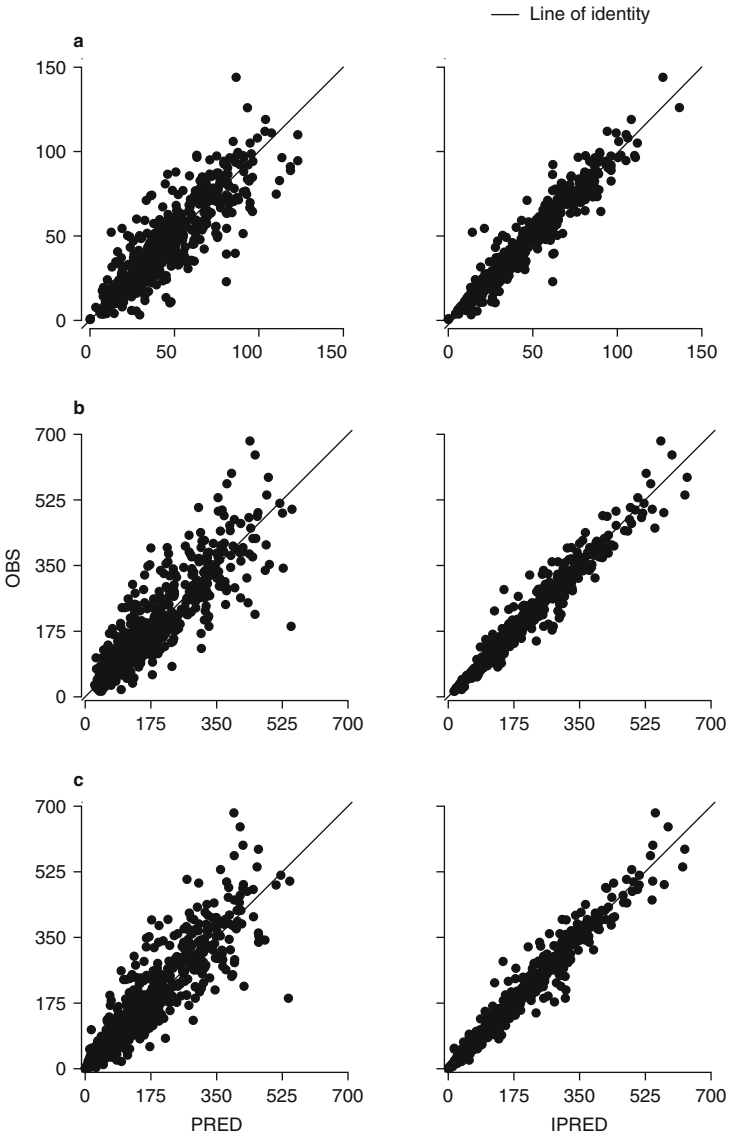

Fig. 5. Goodness-of-fit plots for the final models: (**a**) study 1,[8] (**b**) study 2[9] and (**c**) combined analysis. The left panels show the observed plasma concentrations (OBS) vs the predictions based on the population pharmacokinetic parameter estimates (PRED). The right panels show the OBS vs the predictions based on individual empirical Bayes estimates (IPRED).

Estimation of Dosing Strategies

Study 2

Individualising the loading infusion based on bodyweight or CL_{CR} was predicted to have little impact on the overall variability in plasma concentrations at 1 hour (24% and 21% CV using one or two dosing categories, respectively). In addition, the criteria regarding percentage of patients expected to have unbound concentrations >70 and >150 μmol/L were met using the same dose for all patients. Hence, individualisation of the loading dose was not considered in further estimations using other target concentrations. However, for the maintenance infu-

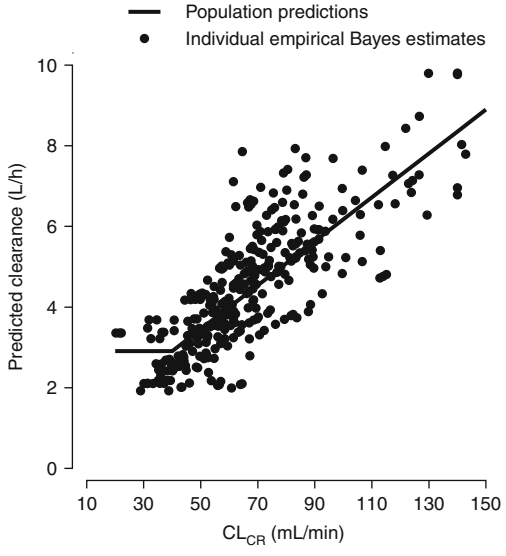

Fig. 6. Predicted clearance vs creatinine clearance (CL_{CR}) based on final model for the combined analysis.

sion, individualisation based on CL_{CR} was required to satisfy the criteria. Three dosing categories, with CL_{CR} cut-offs at 50 and 80 mL/min, were found to be sufficient to fulfill the defined criteria. Overall variability in plasma concentrations at steady state was decreased from 40% CV, using one dose to all, to 26% CV. There was little benefit from the introduction of a fourth dosing category (overall variability in plasma concentrations at steady state decreased from 26% to 24% CV). Increasing the target concentration resulted in the infusion rates being scaled based on the same model and with the same number of dosing categories; CL_{CR} cut-off remained the same.

Future Studies (SAINT-I and SAINT-II)

The optimal dosing strategy for future studies, based on the population pharmacokinetic model from the combined studies, involved slightly different CL_{CR} cut-offs (55 and 85 mL/min) than the ones used in study 2. The outcome for a dosing strategy fixing the cut-off values to those used in study 2 (50 and 80 mL/min) was assessed and found to be similar to that for the optimal strategy, and the increase in overall variability was small (from 24% to 25% CV). Because of the defined risk function, the mean of the expected unbound concentrations (258 μmol/L) was somewhat higher than the predefined target concentration and coincided with the average unbound concentrations obtained in study 2 at the highest dose level. The final dosing strategy established for future studies (efficacy and safety studies) is given in table IV.

For the best treatment response, it is likely that NXY-059 administration should be initiated early following stroke onset. In the clinical situation, the calculated CL_{CR} value may not be available until about 4 hours after the patient has arrived at the hospital. Hence, treatment may need to be started with an inappropriate rate of infusion with respect to CL_{CR}. A simulation was performed to predict a worst case; 100 patients with a low CL_{CR} (30 mL/min) received the highest maintenance infusion (2500 μmol/h) for up to 4 hours after the start of treatment, followed by the correct infusion rate (1250 μmol/h) for the remaining time. At 4 hours, 25% of the patients had unbound concentrations >400 μmol/L. The reduction of the infusion rate resulted in exposure levels <400 μmol/L within the following 4 hours for 80% of the patients with initial high exposure levels. Hence, even in the worst case scenario, the risk of obtaining an unbound plasma concentration >400 μmol/L is predominantly short-term. The risk is considered acceptable based on the findings in safety pharmacology and toxicology studies at similar and higher exposure levels, and on the clinical experience, although limited, showing the compound to be well tolerated at this exposure.

Discussion

Population pharmacokinetic models were estimated for NXY-059. Separate analysis of studies 1

Table IV. Final dosing strategy of NXY-059 established for future studies

Variable	Rate of infusion		
	μmol/h	mg/h	mL/h[a]
Loading infusion (1h)			
All subjects	5940	2270	151
Maintenance infusion (71h)			
CL_{CR} >80 mL/min	2520	960	64
CL_{CR} 51–80 mL/min	1730	660	44
CL_{CR} 30–50 mL/min	1260	480	32

a Concentration of infusion solution is 15 mg/mL.
CL_{CR} = creatinine clearance.

and 2, and analysis of the studies combined, resulted in similar models. Deviations in the models, mainly related to the shape of the CL_{CR} relationship, were explained by further analysis of the data and found to be partly due to one individual. The population pharmacokinetic models were applied successfully in the establishment of individualised dosing strategies, with the observed outcomes being reached satisfactorily and being in agreement with expectations.

Following the highest infusion rate in study 2, the average of the observed $C_{u,ss}$ and C_{ss} (unbound and total plasma concentration of NXY-059 approximately 24 hours after the start of drug treatment) were 30% and 14% higher, respectively, than expected based on models derived from study 1. The assumption, used in dose estimations, of f_u being 0.61 could partly explain the results as there was a tendency for a higher f_u in the patient group receiving the highest infusion; however, this alone cannot explain the results. A potential explanation for the observations would be a nonlinear elimination process in conjunction with nonlinear protein binding, thereby accounting for the larger deviation in $C_{u,ss}$ compared with C_{ss}. Such a modelling approach was supported by the fact that active tubular secretion of NXY-059 has been shown to contribute to total renal CL by one-third[7] and a saturation of this process is probable with increasing unbound concentrations. In addition, the unbound molar concentrations of NXY-059 in study 2 correspond to levels where saturation of protein binding can be observed. Accordingly, complex models including both nonlinear features could also describe the data, but not with a statistically significant improvement (OFV). This may reflect the fact that the signal in the data was too low or that the results suggesting a nonlinear elimination process in conjunction with nonlinear protein binding were obtained by chance owing to large variability and a small sample. The latter explanation is endorsed by the percentage of patients expected to be above certain concentration levels being based on predictions made without taking residual variability into account. Furthermore, pharmacokinetic studies performed in young and elderly healthy volunteers in the exposure range of 50–300 μmol/L ($C_{u,ss}$) could not detect nonlinear pharmacokinetics (AstraZeneca, unpublished data).

The final population pharmacokinetic model based on the combined datasets contained a nonlinear relationship between CL_{CR} and CL (for CL_{CR} ≤40 mL/min, CL was constant; above this threshold the CL_{CR}-CL relationship was linear) in comparison to previous studies[7,10] and the population pharmacokinetic models based on the separate studies, where linear relations were described. However, general trends, i.e. estimates of typical CL depending on CL_{CR}, are similar to previous results. In study 2 patients with severe renal impairment have been included, which, in combination with the relatively large total number of patients in the combined studies, may explain the detection of the nonlinear relationship. However, it is well known that the correlation between renal function and CL_{CR} calculated by the Cockcroft and Gault method, which is by far the most commonly used predictor for glomerular filtration rate (GFR), may be an imprecise estimate of GFR, particularly in patients with low renal function.[23-25] Hence, the shape of the nonlinear relationship between CL_{CR} and CL should not be overinterpreted.

The dosing strategy used in study 1 was produced by combining knowledge of the characteristics of NXY-059 and the target population. Two dosing categories were predefined and the CL_{CR} cut-off used for dosage adjustment was set to 60 mL/min, which is very similar to the one estimated later for a dosing strategy with two categories. The dosing strategy did not entirely satisfy predefined aims regarding target concentration (<90% of the patients were above 40 μmol/L), probably owing to the CL in the target population being higher than expected based on available information. In the subsequent studies, stricter criteria were defined regarding target fulfilment for a dosing strategy involving limitations for an upper concentration. This meant that the dosing strategy may need more than two dosing categories to lower variability. Therefore, it was desirable to use another approach and to estimate dosing strategies, rather than simulate the outcome following specific scenarios. The estimation of a dosing strategy aims to find the optimal dosing strategy, given certain restrictions. However, there will often be several alternative dosing strategies that are almost as good with respect to outcome. This was illustrated for the future studies, where the

optimal dosing strategy was compared with an alternative where the CL_{CR} cut-offs were set to those used in study 2. The expected results were almost identical and the chosen dosing strategy can be considered as optimal given the defined target, thereby demonstrating the application of the method to determine the possible loss using an alternative dosing strategy.

There are several contrasts between the method for dose individualisation strategy determination used in this work and more commonly employed methods, in that the latter often: (i) use CL_{CR} cut-off values that are pre-defined based on standard grouping of renal impairment rather than adapted to the drug in question; (ii) use standard dose decrements, usually a halving of the dose,[13] for each group of decreased renal function; (iii) seldom incorporate the distribution of CL_{CR} in the target population into the dosing strategy decision; and (iv) do not attempt to obtain optimal conditions for dose individualisation. It is further unusual to: (i) compare, using formal prespecified measures, the quality of therapy that can be expected when different numbers of discrete doses are made available; and (ii) update the dose individualisation strategy during the development process.

Conclusions

This paper exemplifies how it is feasible to integrate relevant information and apply rational estimation methods in order to determine the most appropriate dosing strategy for a compound during clinical drug development. The variables that formed the basis for the dosing strategy estimation were preclinical pharmacology, clinical pharmacokinetics, preclinical and clinical safety, and patient characteristics both in the studied groups and in a larger stroke population. The ultimate purpose was to estimate dosing strategies resulting in unbound concentrations of NXY-059 in all patients being reasonably close to the defined target concentration. The paper also shows how, as the information increased, the individualisation strategy was updated, e.g. the target value increased based on preclinical efficacy and clinical safety data.

The final population pharmacokinetic model for NXY-059 comprised a two-compartment model with part of the variability in CL and V_c explained by CL_{CR} and bodyweight, respectively. Individualised dosing strategies were successfully estimated based on the population models and characteristics of the patient population, comprising an initial loading infusion (same for all patients) followed by a maintenance infusion individualised on CL_{CR} with cut-off values of 50 and 80 mL/min. This strategy resulted in reaching target concentrations rapidly following the end of the loading infusion, and in achieving unbound plasma concentrations within the predefined target range in the majority of the patients. The emergency situation contingent on the treatment of acute ischaemic stroke represents specific challenges. The results presented indicate that it appears appropriate to administer NXY-059 with a common maintenance dose for all patients for a short time period, followed by a subsequent dosage adjustment according to the three strata of renal function based on the easily accessible calculation of CL_{CR} according to the Cockcroft and Gault method. Hence, the chosen dosing strategy of NXY-059 satisfies the need for an easily adapted treatment regimen for acute stroke.

Acknowledgements

Professor K.R. Lees is the principal investigator for the phase III clinical trial of NXY-059, SAINT-I.

The authors thank AstraZeneca Research and Development, Södertälje, Sweden, for providing financial support for Siv Jönsson. The following investigators involved in the clinical studies are gratefully acknowledged: N.G. Wahlgren, Department of Neurology, Karolinska Hospital, Stockholm, Sweden; V. Kostulas, Department of Neurology, Huddinge Hospital, Huddinge, Sweden; H-G. Hårdemark, Department of Neurology, Uppsala Akademiska Hospital, Uppsala, Sweden; A. Terent, Department of Medicine, Uppsala Akademiska Hospital, Uppsala, Sweden; S. Bornhov, Helsingborg Hospital, Helsingborg, Sweden; P. Palmqvist, Department of Medicine, Kalmar Hospital, Kalmar, Sweden; T. Olsson, Department of Medicine, Östersund Hospital, Östersund, Sweden; K.R. Lees, University Department of Medicine and Therapeutics, Western Infirmary, Glasgow, UK; A.K. Sharma, Department of Medicine for the Elderly, Fazakerley Hospital, Liverpool, UK; D. Grosset, Southern General Hospital, Glasgow, UK; D. Barer, Stroke Research Team, Queen Elizabeth Hospital, Gateshead, UK; G.A. Ford, Clinical Pharmacology and Therapeutics, Freeman Hospital, Newcastle, UK; L. Kalra, Department of Medicine, King's College, London, UK; P. Tyrrell, Department of Geriatric Medicine, Hope Hospital, Manchester, UK; T.G. Robinson, Leicester General Hospital, Leicester, UK; J. Barrett, Wirral Hospital, Wirral, UK; O. Busse, Department of Neurology, Minden

Hospital, Minden, Germany; H.C. Diener, Department of Neurology, University of Essen, Essen, Germany; W. Hacke, Department of Neurology, University of Heidelberg, Heidelberg, Germany.

In addition, we would like to thank Kerstin Lanbeck Vallén, BSc, for her excellent work undertaking the bioanalyses. AstraZeneca is developing NXY-059 under a licence agreement with Renovis, Inc., South San Francisco, CA, USA.

Y.-F. Cheng is an employee of AstraZeneca. C. Edenius was an employee at AstraZeneca and is a shareholder in AstraZeneca. K.R. Lees has received fees and expenses for work on the Steering Committee for the SAINT trial programme but has no financial or related interests in AstraZeneca or Renovis. T. Odergren is an employee and shareholder in AstraZeneca. M.O. Karlsson has received research grants from AstraZeneca.

References

1. Kuroda S, Tsuchidate R, Smith ML, et al. Neuroprotective effects of a novel nitrone, NXY-059, after transient focal cerebral ischemia in the rat. J Cereb Blood Flow Metab 1999 Jul; 19: 778-87
2. Zhao Z, Cheng M, Maples KR, et al. NXY-059, a novel free radical trapping compound, reduces cortical infarction after permanent focal cerebral ischemia in the rat. Brain Res 2001 Aug 3; 909: 46-50
3. Sydserff SG, Borelli AR, Green AR, et al. Effect of NXY-059 on infarct volume after transient or permanent middle cerebral artery occlusion in the rat; studies on dose, plasma concentration and therapeutic time window. Br J Pharmacol 2002 Jan; 135: 103-12
4. Marshall JW, Duffin KJ, Green AR, et al. NXY-059, a free radical-trapping agent, substantially lessens the functional disability resulting from cerebral ischemia in a primate species. Stroke 2001 Jan; 32: 190-8
5. Facchinetti F, Dawson VL, Dawson TM. Free radicals as mediators of neuronal injury. Cell Mol Neurobiol 1998 Dec; 18: 667-82
6. Schulz JB, Henshaw DR, Siwek D, et al. Involvement of free radicals in excitotoxicity in vivo. J Neurochem 1995 May; 64: 2239-47
7. Edenius C, Strid S, Borgå O, et al. Pharmacokinetics of NXY-059, a nitrone-based free radical trapping agent, in healthy young and elderly subjects. J Stroke Cerebrovasc Dis 2002 Jan-Feb; 11: 34-42
8. Lees KR, Sharma AK, Barer D, et al. Tolerability and pharmacokinetics of the nitrone NXY-059 in patients with acute stroke. Stroke 2001 Mar; 32: 675-80
9. Lees KR, Barer D, Ford GA, et al. Tolerability of NXY-059 at higher target concentrations in patients with acute stroke. Stroke 2003 Feb; 34: 482-7
10. Strid S, Borga O, Edenius C, et al. Pharmacokinetics in renally impaired subjects of NXY-059, a nitrone-based, free-radical trapping agent developed for the treatment of acute stroke. Eur J Clin Pharmacol 2002 Sep; 58: 409-15
11. Marler JR, Tilley BC, Lu M, et al. Early stroke treatment associated with better outcome: the NINDS rt-PA stroke study. Neurology 2000 Dec 12; 55: 1649-55
12. Wahlgren NG, Ranasinha KW, Rosolacci T, et al. Clomethiazole acute stroke study (CLASS): results of a randomized, controlled trial of clomethiazole versus placebo in 1360 acute stroke patients. Stroke 1999 Jan; 30: 21-8
13. Jonsson S, Karlsson MO. A rational approach for selection of optimal covariate-based dosing strategies. Clin Pharmacol Ther 2003 Jan; 73: 7-19
14. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31-41
15. Markus R, Reutens DC, Kazui S, et al. Topography and temporal evolution of hypoxic viable tissue identified by 18F-fluoromisonidazole positron emission tomography in humans after ischemic stroke. Stroke 2003 Nov; 34: 2646-52
16. Beal SL, Sheiner LB. NONMEM users guides (I-VIII). Hanover (MD): GloboMax, 1989-1998
17. Jonsson EN, Karlsson MO. Xpose: an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed 1999 Jan; 58: 51-64
18. Mandema JW, Verotta D, Sheiner LB. Building population pharmacokinetic-pharmacodynamic models. In: D'Argenio DZ, editor. Advanced methods of pharmacokinetic and pharmacodynamic systems analysis. New York: Plenum Press, 1995: 69-86
19. Chambers JM, Hastie TJ, editors. Statistical models in S. London: Chapman & Hall, 1993
20. Jonsson EN, Karlsson MO. Automated covariate model building within NONMEM. Pharm Res 1998 Sep; 15: 1463-8
21. Wahlby U, Jonsson EN, Karlsson MO. Comparison of stepwise covariate model building strategies in population pharmacokinetic-pharmacodynamic analysis. AAPS PharmSci 2002; 4: E27
22. Sadray S, Jonsson EN, Karlsson MO. Likelihood-based diagnostics for influential individuals in non-linear mixed effects model selection. Pharm Res 1999 Aug; 16: 1260-5
23. Lam YW, Banerji S, Hatfield C, et al. Principles of drug administration in renal insufficiency. Clin Pharmacokinet 1997 Jan; 32: 30-57
24. Brater DC. Measurement of renal function during drug development. Br J Clin Pharmacol 2002 Jul; 54: 87-95
25. Comstock TJ. Quantification of renal function. In: Dipiro JT, Talbert RL, Hayes PE, editors. Pharmacotherapy: a pathophysiological basis. 3rd ed. Stamford (CT): Appleton-Lange, 1996: 867-85

Correspondence and offprints: Dr *Siv Jönsson*, Division of Pharmacokinetics and Drug Therapy, Department of Pharmaceutical Sciences, Uppsala University, Box 951, Uppsala, SE-751 24, Sweden.
E-mail: siv.jonsson@farmbio.uu.se