Title of study

A double-blind, randomised, single dose, crossover study to investigate the plasma clearance of two different doses of CLF (NRL972) in 24 volunteers with stable non-cholestatic liver cirrhosis

Principal investigator

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Publication (reference)

Not applicable

Study period	**Phase of development**
Screening date of first patient in: 12 NOV 2001 Date of last patient completed: 20 JUL 2002	Phase IIa

Objectives

The primary study objective was to determine the pharmacokinetics and clearance of NRL972 in patients with hepatic impairment due to stable non-cholestatic liver cirrhosis.

The secondary study objectives were (1) to calculate the correlation of NRL972 clearance to the histological fibrosis score and the correlation of NRL972 clearance Child-Pugh score; (2) to compare the pharmacokinetic and clinical sensitivity of the 2 and 5 mg dose levels and (3) to evaluate the clinical value of the most sensitive sampling points to be used in further studies in patients with liver disease.

Methodology

This study was a double-blind single dose, crossover study in patients with biopsy proven hepatic cirrhosis. Patients received 2 and 5 mg NRL972 in randomised order, separated by at least seven days. Urine was collected for the pharmacokinetic (PK) analysis and a pregnancy test (if applicable). Blood samples were drawn for PK analysis and laboratory safety tests (serum biochemistry, haematology, coagulation). Local tolerability and blood pressure were assessed at scheduled times up to 360 minutes after dosing. Adverse Events (AE(s)) were assessed until discharge 360 minutes after dosing and unresolved AEs were followed by a phone call up to 72 hours after last dosing.

Number of patients (planned and analysed)

Twenty-four (24) patients were to be enrolled. To ensure 24 evaluable PK data sets, it was later decided to dose 25 (18 males and 7 females). All 25 dosed patients participated in the safety analysis and only patients with biopsy proven hepatic cirrhosis and evaluable PK parameters (23) were included in the PK analysis.

Main criteria for in- and exclusion

To participate in this study, individuals were to be willing to sign the Informed Consent Form (ICF), be aged between 18 and 70 years (inclusive) and have a recent biopsy result proving non-cholestatic hepatic cirrhosis. During the study, the protocol was amended to allow the inclusion of patients aged between 18 and 75 years and patients whose biopsies were taken less than three years instead of less than two years before the study. Patients were to have a Child-Pugh score of A or B and were to be considered fit to participate by the Investigator.

Individuals were to be excluded if they had known or suspected allergy to fluorescein, were unable to communicate with the Investigator, were pregnant or lactating, participated in another clinical study or donated ≥450 mL blood within the previous 12 weeks.

Test product, dose and mode of administration, batch number

2 mg NRL972 (5 mL intravenous (IV) injection of 0,4 mg/ mL NRL972 in water); batch no 01H16

5 mg NRL972 (5 mL IV injection of 1 mg/ mL NRL972 in water); batch no 01H09

Reference therapy, dose and mode of administration, batch number

Not applicable

Duration of treatment

The study consisted of two periods of approximately seven hours each, separated by at least seven days. Two patients were asked to repeat one of their periods and thus completed three instead of two periods.

Criteria for evaluation – Pharmacokinetics

The PK of NRL972 was evaluated by the following parameters: C_{max}, t_{max}, $AUC_{0\text{-}tlast}$, $t_{½z}$, $AUC_{0\text{-}\infty}$, CL and V_z.

The correlation between the NRL972 clearance and the Child-Pugh score, respectively the histological fibrosis score, was assessed.

For future studies, the most sensitive sampling points and the dose with the best characteristics regarding pharmacokinetics and clinical sensitivity were to be assessed.

Criteria for evaluation – Safety

Safety was evaluated by assessing the AEs, serum biochemistry, haematology, coagulation, blood pressure and local tolerability of the study drug.

Statistical methods

All 25 patients participated in the safety analysis. All safety data were analysed with SAS®, version 6.12. All safety data were listed individually and for safety data, only descriptive statistics were foreseen. Scheduled safety assessments performed ≥20% off-schedule were flagged in the listings and were to be excluded in the summary tables.

Pre-dose AEs were listed but not included in the frequency tables. Frequency tables of all (post-dose) AEs and RAEs (possibly, probably and almost definitely related AEs) were to be generated per system organ class, per preferred term, and per treatment group. In addition, a frequency table of Adverse Events by relationship and severity was to be generated. Frequencies of the AEs were to be counted both as the number of times an event was reported as well as the number of patients that reported the event at least once. Serum biochemistry, haematology, coagulation and blood pressure values outside of reference ranges were flagged in the listings.

Statistical methods (continued)

All abnormal and clinically significant values for serum biochemistry, haematology and coagulation were listed separately. Safety summary tables were generated by treatment, and summary tables of change from baseline values by treatment were generated for serum biochemistry, haematology and blood pressure.

During the analysis of the blood pressure data, it became clear that the pre-dose blood pressures (which should have been recorded 30 minutes before dosing) were taken ≥20% off-schedule in most cases. It was decided to include these off-schedule pre-dose assessments.

Induration, erythema and thrombosis (local tolerability) were summarised in a frequency table.

For the two patients who had a repeat period, the numerical data (serum biochemistry, haematology, coagulation, blood pressure) of the repeated period were summarised (but all data from both periods were listed). For these patients, all clinically significant findings during both the initial and the repeated period (AEs, serum biochemistry, haematology, coagulation, blood pressure and local tolerability) were described in this report.

Twenty-three (23) patients participated in the PK analysis. All PK data were analysed with WinNonlin, edition 3.3.
Plasma and urine concentrations were listed individually. Scheduled PK assessments performed ≥5% off-schedule were flagged. Individual plasma concentrations were presented graphically. Summary statistics of plasma concentrations per time-point were tabulated per treatment group and presented graphically.
All PK parameters were listed individually. Summary statistics (mean, standard deviation/coefficient of variation, median, minimum, maximum) were used to summarise each derived pharmacokinetic variable. $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, $t_{½}$, CL and CL_R were subjected to statistical analysis. $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$, were tested for dose-proportionality, with Analysis of Variance using a model with patient, treatment and period as main factors. 90% confidence intervals for the ratio between the two doses were calculated on the original scale. CL and $t_{½}$ were tested for dose-independence.

For each treatment group scatter plots were produced of the CL values versus histological fibrosis scores and Child-Pugh scores, respectively. Pearson correlation coefficients were calculated together with 95% confidence intervals between the 5 above mentioned pharmacokinetic variables (after appropriate log-transformation) and the histological (fibrosis) score and Child-Pugh score, respectively.

Pharmacokinetic results

The plasma concentrations showed a fast and continuous decline after the IV bolus injection, and the pattern of decline was independent of the dose level administered. In most individuals there were at least two phases of elimination, and possibly more as concluded from visual analysis of the plasma concentration vs. time plots.

The geometric mean C_{max} values were 512 and 1263 ng/mL after the 2 and 5 mg doses, respectively.
The geometric mean $AUC_{0-\infty}$ values were 19.3 and 49.3 min∗µg/mL [∗=multiply-sign] after the 2 and 5 mg doses, respectively. The geometric mean dose-normalized $AUC_{0-\infty}$ values for the two dose levels (9.66 and 9.86 min∗µg/mL/mg) were remarkably similar, indicating that the increase in AUC was proportional with the dose increase. The half-life values in the terminal elimination phase were 60 and 68 min after the 2 and 5 mg doses. The geometric mean clearance values were 6.21 and 6.09 L/h after the 2 and 5 mg doses, respectively, clearly indicating that the total body clearance was dose-independent. There was considerable inter-patient variation for all pharmacokinetic parameters; the intra-patient variation was relatively small; clearance values after the two dose levels were very similar on an individual basis.
No effect of dose was demonstrated for dose-normalised $AUC_{0-tlast}$ and $AUC_{0-\infty}$ and CL. The estimated difference between t½ after the 5 and 2 mg dose was 8.7 minutes (P=0.0078). This finding might be explained as follows.

In most individuals there were at least two phases of elimination and possibly more. After the 2 mg dose, the plasma concentrations reached the lower determination limit earlier than after the 5 mg dose, and thus the terminal elimination rate was possibly estimated from one or more data points in an earlier elimination phase. This may have resulted in slightly shorter estimated t½ values for the 2 mg dose level. The mean total body clearance values were 6.9 and 6.8 L/h in the group of patients (n=20) with Child-Pugh score A and 3.1 and 3.0 L/h in patients with Child-Pugh score B (n=3), for the 2 and 5 mg dose levels, respectively. The clearance values for two of the three patients with Child-Pugh score B were lower than any individual value seen in the patients Child-Pugh score A, but the value for the third fell in the range of CL values seen in patients with Child-Pugh score A.

The ratios of the NRL972 plasma concentrations at 30 and 10 min post-dose were independent of dose; for most individuals the ratios were quite similar after the two dose levels. At 30 minutes post-dose the mean plasma concentration was about half (47-48%) of the plasma concentration at 10 minutes post-dose. Individual minimum and maximum values ranged from 16 to 73 % in this group. The 5 values noted in the patients with Child-Pugh score B were higher (mean ratios of 65 and 70% vs. 47 and 48% in Child-Pugh score A patients), which would indicate a slower elimination from the plasma, but the ranges for the two groups overlapped.
An exploratory analysis was performed to investigate the presence of a correlation between the ratios of plasma concentrations and total body clearance, to evaluate the clinical value of the most sensitive sampling points to be used in further studies in patients with liver disease. After the 5 mg dose, the 45/10, 30/10 and 20/10 min ratio represented the best values for clearance, while after the 2 mg dose, the correlation closest to 1 was observed for the 45/30, 30/10 and 20/10 min ratio. The number of observations used to calculate the Pearson correlation coefficients varied due to missing values and this may have affected the outcome. The results showed that the concentration ratios can serve as a measure for total body clearance.

Patients with higher Child-Pugh scores tended to have a lower clearance, although there was considerable overlap. The Pearson correlation coefficients for the relation between log-transformed $AUC_{0-tlast}$, AUC_{0-inf} and Cl values, and $t_{½,z}$, respectively, ranged between –0.4853 and 0.4144, indicating that the correlation was not very good, even though statistical significance was reached for most tests. There was no correlation between NRL972 clearance and the typical histological fibrosis scores for liver cirrhosis with F5 and F6, as expected. Renal clearance of NRL972 was a negligible route of elimination(<1 mL/min). The renal clearance values were quite similar for the two dose levels.

Safety results

Nineteen AEs occurred in 14 of 25 patients (56%). None of the AEs were considered severe, there were no deaths, SAEs or other significant AEs. All patients recovered without sequelae and, except for two patients, no concomitant medication was used to treat an AE. One AE (moderate hearing impairment) was considered possibly related to the study drug by the investigator.

The number of AEs was similar in the two treatments; 10 AEs in 7 patients (28%) during the 2 mg treatment and 9 AEs in 9 patients (36%) during the 5 mg treatment. The most frequently reported AEs (AEs, number of patients with corresponding AE) were injection site irritation (4, 4), dizziness (3, 2) and headache (2, 2).

During the whole study, total protein and albumin were low, while AST, ALT and GGT were high, due to the clinical state (hepatic cirrhosis) of the study population. Other than that, no remarkable changes in serum biochemistry were observed over the study periods or between the different doses.

No remarkable changes in haematology were observed over the study periods or between the different doses, except for a slight drop in neutrophils six hours post-dose (both doses).

Coagulation times were generally increased due to the hepatic impairment of the study population. Other than that, no remarkable changes in coagulation were observed over the study periods or between the different doses.

Blood pressure slightly decreased after one to six hours post-dose at both doses, which may have been caused by the fact that patients were restricted to their beds until at least four hours after dosing. The decrease in blood pressure was slightly more distinct with the 5 mg dose. Except for some patients with a history of hypertension, patients only had occasional values outside of the reference range. None of the individual abnormalities were considered clinically significant.

Conclusions

NRL972, given as single doses of 2 and 5 mg each, was safe and well tolerated in the present study.

NRL972 was eliminated rapidly after the IV bolus, with a mean terminal elimination half-life of about one hour. At least two elimination phases were detected. Most of the drug was eliminated at 6 hours after dosing. Renal clearance of NRL972 was negligible. The primary PK parameter $AUC_{0\text{-tlast}}$ and $AUC_{0\text{-}\infty}$ responded proportionally to the dose, i.e. CL was dose-independent. The pharmacokinetic behaviour of NRL972 was highly variable between patients, but showed very little variation within patients.

Patients with higher Child-Pugh scores tended to have a lower clearance, although there was some overlap. The Pearson correlation coefficients for the relation between log-transformed $AUC_{0\text{-tlast}}$, $AUC_{0\text{-inf}}$ and CL values, and $t_{½,z}$ ranged between –0.4853 and 0.4144, indicating that the correlation was not very good, even though statistical significance was reached for most tests. The statistical significance indicated there was a relation between these PK parameters and the Child-Pugh score, but due to wide variation of individual values and the small (n=3) number of patients with Child-Pugh score B, the correlation coefficients were well below 1. There was no correlation between NRL972 clearance and histological fibrosis scores associated with liver cirrhosis, as expected.

There was a good correlation between ratios of plasma concentrations and the total body clearance, for both dose levels. Ratios of samples obtained at any time between 30 and 10 minutes represent the absolute clearance quite well for both dose levels. The results indicate that the plasma concentration ratios can serve as a measure for total body clearance of NRL972.

Determining ratios rather than the full clearance of NRL972 eliminates the effect of dosing errors, as the ratios are clearly dose-independent. In addition there is the benefit of having to take only two samples during a relatively short interval after the dose administration.

Date of report

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