Title:
A randomised, open, controlled crossover study in male and female healthy volunteers to describe the plasma pharmacokinetics of cholyl-lysyl-fluorescein (NRL972) during and after a constant rate 2 hour iv-infusion of 5 and 15 mg NRL972 per hour.
Norgine Study №: NRL972-06/2008 (IVCO)

Investigators:
Dr E. Peterfai

Study centre(s):
Balatonfüred, Hungary

Publication (reference):
n.a.

Study period:	**Clinical Phase:** I
26 Nov 2008 (screening of first subject) – 19 Dec 2008 (end-of-trial in last subject)	

GCP-compliance:
The study was planned, conducted, analysed and reported in accordance with the pertinent GCP Guidelines.

Objectives of the study:
To describe and compare the plasma pharmacokinetics of NRL972 during and after 2-hour iv infusions of 5 and 15 mg per hour NRL972; in addition, the study was to provide further information on the safety and tolerability of NRL972 administered under these conditions.

Study design:
Single-centre, open, controlled, two-way crossover with randomly assigned period balanced sequences investigating 2-hour iv infusions of 5 and 15 mg per hour; periods were separated by a washout of at least 1 week. Each subject was studied on both occasions; on each occasion a single dose of NRL972 was administered (time 0:00 = start of the infusion which lasted for 2 hours) after an overnight fast and rest.

Number of subjects:
Twelve evaluable subjects were intended to be investigated. A subject was to be considered evaluable when he/she provided evaluable data for both investigational treatments.

Diagnosis and criteria for inclusion:
Male and female (of non-childbearing potential or while taking medically appropriate contraception), Caucasian subjects, 25 – 40 years of age, Body Mass Index (BMI) of 22 to 26 $kg.m^{-2}$ and body weight (BW) of 50 to 100 kg who were confirmed to be healthy on the basis of extensive screening investigation (medical history, physical examination, vital functions, 12-lead ECG, clinical laboratory safety tests [haematology, clinical chemistry, urinalysis, serology, screening tests for substances of abuse and alcohol]), and who were able and willing to provide informed consent.

Test product, dose, batch N°:
Cholyl-L-lysine-fluorescein (synonyms: CLF, cholyl-lysyl-fluorescein; INN Fluorescein lisicol tri-sodium salt; Development Code: NRL972), Norgine Ltd., 2 mg NRL972 in 5 mL solution for iv injection), 10 mg and 30 mg were administered by 2-hour iv infusion on two occasions in each subject according to randomly assigned period-balanced within-subject crossover sequences. IMP Batch-N°: NOR-p004

Reference product, batch N°:
Not applicable

Duration of treatments:

Two single administrations of 10 mg and 30 mg doses of NRL972 by 2-hour iv infusion on the morning of Day D01 on each occasion; both study periods were at least one week apart for washout purposes.

Schedule:

- SCR: screening visit within 14 and 2 days before hospitalisation: assessment of eligibility
- Two study periods separated by a washout of at least one week; for each period, the subject was admitted from the evening of Day D-1 until 12:00 h after the start of the infusion (evening of D01)
- EOT: end-of-trial evaluation within 1 week after study day D01

CLINICAL PHARMACOLOGY FINDINGS

Subject disposition:

- Twenty-one (21) subjects were screened for enrolment. Eight (8) subjects were not enrolled.
- Thirteen (13) subjects were enrolled. One subject (R005) was found to have a positive pregnancy test upon admission for PE2. The subject was discontinued from the trial after PE1 (see safety section below). She was replaced by subject R013 who was assigned to the same treatment sequence. Twelve subjects completed the study in accordance with the protocol with evaluable data for both treatment periods All subjects were included in the safety analyses and ITT-dataset for the PK-evaluations; subject R005 was excluded from the PP-dataset for PK-analyses.

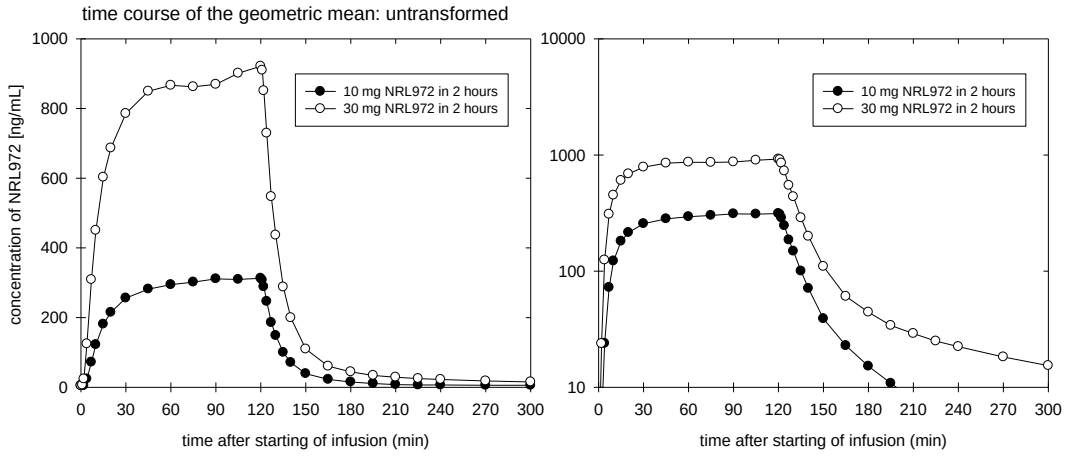
Demographics

All 13 subjects (six males, seven females) were Caucasian: mean ± SD [range], age: 31.8 ± 4.0 years [26 to 38 years]; mean body weight: 69.0 ± 10.0 kg [53 to 88 kg]; mean BMI: 24.2 ± 1.4 $kg.m^{-2}$ [22.3 to 25.9 $kg.m^{-2}$]. All subjects were judged to be healthy upon in-depth evaluation at the screening visit.

Pharmacokinetics – Time courses of the plasma concentrations

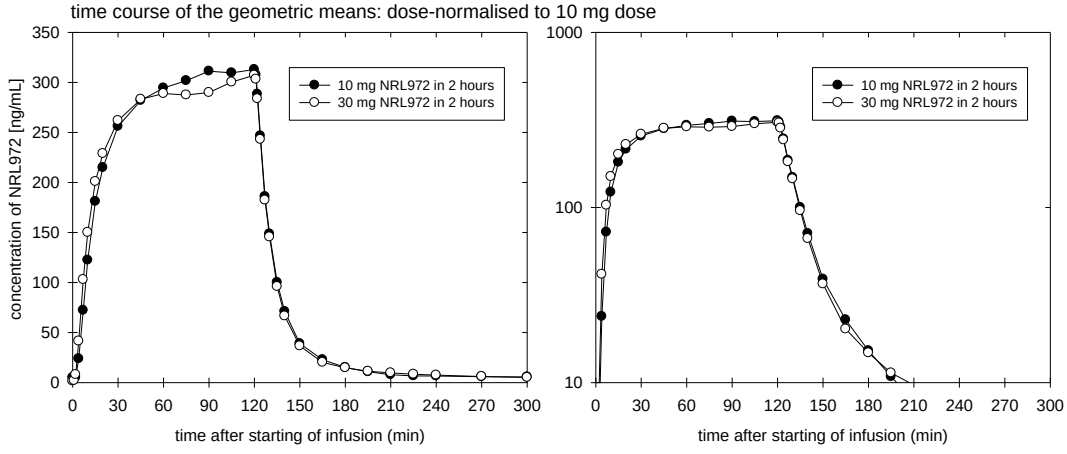
The time courses of the observed geometric mean plasma concentrations during and after the infusion of 5 mg and 15 mg NRL972 per hour for 2 hours were as follows:

Untransformed



BLQ-data replaced by 1/2 LoQ

Dose-normalised



BLQ-data replaced by 1/2 LoQ

Pharmacokinetics – PK methods & variables

Ceq: variables based on the assumed equilibrium at the end of the infusion: equilibrium concentration (CEQ); total clearance (CL); CL by kg body weight (CL/BW)

2P-ANA: fractional concentration recovery in the post-infusion course: C(30):C(10)- and C(30):C(15)-ratio

I-NCA: non-compartmental analysis (NCA) of the full course of the concentrations during and after the infusion: maximum observed plasma concentration (C_{max}); time of C_{max} (t_{max}); CL, CL/BW; apparent terminal disposition half-life (t½)

PI-Full: NCA of the post-infusion course including all values as quantifiable: t_{max}; C_{max}; t½

PI-Short: NCA of the post-infusion course up to one hour after the end of infusion: t_{max}; C_{max}; t½ ;

FIT-2C: fitting the full course during and after infusion to an open two-compartmental model: estimated volume of the central compartment (V_1); estimated volume of the peripheral compartment (V_2); rate constant for the transfer from V_1 to V_2 (k_{12}); rate constant for the transfer from V_2 to V_1 (k_{21}); elimination rate constant outside of V_1 (k_{10}); estimated total volume of distribution ($V_{dss}=V_1 + V_2$); half-lives of the 1st (fast) and 2nd (slower) exponential disposition phase (t½ (λ_1), and t½ (λ_2)); CL and CL/BW

Pharmacokinetics – Descriptive statistics

The descriptive statistics of the main variables (N: number of quantifiable data, MX: arithmetic mean, aCV: arithmetic coefficient of variation, gM: geometric mean; gCV: geometric coefficient of variation; MIN: minimum value; MED: median; MAX: maximum observed value) were:

Low-dose: 2-hour infusion of 5 mg/hour NRL972 (N:13)

METH	Variable	Unit	N	MX	aCV	gM	gCV	MIN	MED	MAX
Ceq	CEQ	ng/mL	13	322.6	0.282	311.3	0.283	191.9	287.0	486.6
Ceq	CL	mL/min	13	277.3	0.272	267.7	0.283	171.3	290.4	434.4
Ceq	CL/BW	mL/min per kg	13	4.00	0.203	3.92	0.221	2.63	4.15	5.09
2P-ANA	2P-10/30	1/1	13	0.268	0.227	0.262	0.209	0.204	0.243	0.402
2P-ANA	2P-15/30	1/1	13	0.392	0.117	0.389	0.115	0.324	0.382	0.489
I-NCA	tmax	min	13	108	0.205	105	0.246	60	120	121
I-NCA	Cmax	ng/mL	13	330.6	0.294	318.3	0.291	195.0	291.8	528.9
I-NCA	CL	mL/min	13	279.1	0.279	268.5	0.303	156.8	296.1	430.0
I-NCA	CL/BW	mL/min per kg	13	4.03	0.218	3.93	0.244	2.41	4.17	5.29
I-NCA	t½	min	13	48.83	0.567	42.39	0.594	20.84	38.82	105.89
PI-Full	tmax	min post-inf.	13	1	0.258	1	0.194	1	1	2
PI-Full	Cmax	ng/mL	13	320.7	0.281	309.5	0.282	191.5	281.0	479.3
PI-Full	t½	min	13	47.94	0.557	41.9	0.577	20.84	38.82	105.89
PI-short	tmax	min post-inf.	13	1	0.258	1	0.194	1	1	2
PI-short	Cmax	ng/mL	13	320.7	0.281	309.5	0.282	191.5	281.0	479.3
PI-short	t½	min	13	22.45	0.107	22.34	0.107	17.83	21.6	26.43
FIT-2C	V1	L	13	4.407	0.138	4.369	0.139	3.606	4.27	5.157
FIT-2C	k12	/h	13	1.088	0.688	0.911	0.653	0.367	0.917	2.928
FIT-2C	k21	/h	13	0.082	0.713	0.066	0.818	0.016	0.067	0.242
FIT-2C	k10	/h	13	2.885	0.347	2.638	0.541	0.634	3.232	4.115
FIT-2C	V2	L	13	150.74	1.772	60.31	2.162	6.92	50.16	963.09
FIT-2C	Vdss	L	13	155.15	1.723	67.77	1.842	11.47	53.86	968.23
FIT-2C	t½ (1)	min	13	10.89	0.235	10.64	0.222	7.82	9.89	16.48
FIT-2C	t½ (2)	min	13	2054.5	1.941	933.1	1.520	202	862.2	14993
FIT-2C	CL	mL/min	13	208.8	0.356	192.1	0.500	54.4	203.0	300.8
FIT-2C	CL/BW	mL/min per kg	13	3.07	0.349	2.81	0.537	0.67	3.29	4.76

High-dose: 2-hour infusion of 15 mg/hour NRL972 (N:12)

METH	Variable	Unit	N	MX	aCV	gM	gCV	MIN	MED	MAX
Ceq	CEQ	ng/mL	12	929.7	0.209	911.4	0.211	654.5	855.6	1233.8
Ceq	CL	mL/min	12	279.8	0.206	274.3	0.211	202.6	292.2	382
Ceq	CL/BW	mL/min per kg	12	3.98	0.167	3.93	0.166	3.11	3.94	5.28
2P-ANA	2P-10/30	1/1	12	0.254	0.169	0.251	0.171	0.187	0.251	0.325
2P-ANA	2P-15/30	1/1	12	0.383	0.118	0.381	0.114	0.329	0.373	0.469
I-NCA	tmax	min	12	117	0.081	116	0.089	90	120	121
I-NCA	Cmax	ng/mL	12	953.0	0.217	932.8	0.218	657.4	886.1	1294.4
I-NCA	CL	mL/min	12	271.6	0.221	265.5	0.225	188.3	281.3	379.0
I-NCA	CL/BW	mL/min per kg	12	3.86	0.174	3.80	0.176	2.90	3.79	5.12
I-NCA	t½	min	12	137.13	0.328	131.45	0.299	96.07	118.38	233.76
PI-Full	tmax	min post-inf	12	1	0.266	1	0.202	1	1	2
PI-Full	Cmax	ng/mL	12	929.2	0.211	910.6	0.212	651.3	886.1	1294.4
PI-Full	t½	min	12	135.41	0.346	128.96	0.326	80.22	118.38	233.76
PI-short	tmax	min post-inf	12	1	0.266	1	0.202	1	1	2
PI-short	Cmax	ng/mL	12	929.2	0.211	910.6	0.212	651.3	886.1	1294.4
PI-short	t½	min	12	22.94	0.252	22.4	0.221	17.15	22.49	39.11
FIT-2C	V1	L	12	4.061	0.218	3.971	0.226	2.781	3.965	5.277
FIT-2C	k12	/h	12	0.389	0.234	0.38	0.232	0.277	0.365	0.546
FIT-2C	k21	/h	12	0.296	0.472	0.269	0.489	0.13	0.282	0.638
FIT-2C	k10	/h	12	4.029	0.249	3.923	0.242	2.662	3.954	6.246
FIT-2C	V2	L	12	7.09	0.848	5.61	0.736	2.38	5.09	21.83
FIT-2C	Vdss	L	12	11.15	0.598	9.83	0.525	5.16	9.21	27.11
FIT-2C	t½ (λ_1)	min	12	9.8	0.213	9.58	0.227	6.07	9.79	13.69
FIT-2C	t½ (λ_2)	min	12	190.53	0.501	171.2	0.510	71.45	160.92	374.53
FIT-2C	CL	mL/min	12	265.5	0.222	259.6	0.223	186.4	268.0	377.6
FIT-2C	CL/BW	mL/min per kg	12	3.77	0.177	3.720	0.178	2.840	3.64	5.10

Pharmacokinetics – Comparative statistics

For selected variables the treatments were contrasted parametrically (ANOVA) estimating the true ratio and difference of the treatments means (plus 95% CI) for the high-dose (HD) relative to the low-dose (LD). In the following, the estimated treatment ratios are presented (least-square adjusted means for low- [LD] and high-dose [HD], estimate of the ratio of the true means for HD:LD, 95% CI [CI-LL to CI-UL], and the residual coefficient of variation [CVr]):

		estimated means			95% confidence interval		
method	variable	HD	LD	HD:LD	95-LL	95-UL	CVr
Ceq	CEQ	911.4	303.1	3.007	2.821	3.204	7.01
Ceq	CL	3.93	3.94	0.998	0.936	1.063	7.01
Ceq	CL/BW	274.3	274.9	0.998	0.936	1.063	7.01
2P-ANA	2P-10/30	0.251	0.260	0.966	0.854	1.094	13.69
2P-ANA	2P-15/30	0.381	0.387	0.984	0.929	1.043	6.36
I-NCA	tmax	116	110	1.056	0.929	1.200	15.13
I-NCA	Cmax	932.8	310.4	3.005	2.799	3.227	7.84
I-NCA	CL	265.5	276.4	0.961	0.889	1.038	8.55
I-NCA	CL/BW	3.8	3.96	0.961	0.889	1.038	8.54
I-NCA	t½	131.5	39.9	3.294	2.546	4.262	28.9

		estimated means			95% confidence interval		
method	**variable**	**HD**	**LD**	**HD:LD**	**95-LL**	**95-UL**	**CVr**
FIT-2C	V1	4.0	4.4	0.895	0.830	0.964	8.19
FIT-2C	V2	5.6	67.4	0.083	0.036	0.190	113.08
FIT-2C	k10	3.923	2.617	1.499	1.124	1.999	32.47
FIT-2C	k12	0.380	0.958	0.397	0.275	0.573	45.28
FIT-2C	k21	0.269	0.063	4.260	2.646	6.859	56.17
FIT-2C	t ½ (λ_1)	9.58	10.53	0.910	0.821	1.009	11.39
FIT-2C	t ½ (λ_2)	171.2	994.9	0.172	0.083	0.355	93.95
FIT-2C	CL	259.6	193.6	1.341	0.995	1.806	33.67
FIT-2C	CL/BW	3.72	2.77	1.341	0.995	1.806	33.67

SAFETY

Wellbeing and adverse events (AEs)

For each period, the subjects were admitted from the evening of D-1 until the evening of D01 at least 12:00 hours after administration of NRL972. During this time the subjects were under close surveillance in the study clinic.

There were no adverse events. One subject (R005) was found to have a positive pregnancy test upon admission for the second study period, after having been exposed to the low-dose infusion in the first period. The pregnancy was uneventful and delivery by caesarean section was at term and without concerning findings.

Physical examination

A thorough physical examination took place at SCR and EOT. There were no relevant safety findings at the end of the study compared with the examination at SCR.

Vital functions

Recumbent blood pressure (SBP/DBP) and pulse rate (PR) were recorded at SCR and EOT and before each dosing with NRL972 and at scheduled times after each dosing. There was no indication of clinically relevant noteworthy average or individual SBP/DBP- or PR-findings over the course of the study that were attributable to the investigational clinical trial medication.

12-lead ECG

A 12-lead digital ECG was recorded at SCR and EOT and at regular times before, during and after each NRL972 infusion. There was no indication of relevant average or individual ECG-findings (HR, PQ, QT, QTc[Bazett], QTc[Fridericia], and QTc[Framingham]: untransformed and changes from baseline) over the course of the study.

Continuous 3-lead ECG HOLTER-recording

A 3-lead ECG was recorded continuously from about one hour before up to 12:00 hours after the start of the infusion; there was no indication of relevant average or individual HOLTER-findings over the course of the study.

CLINLAB: Clinical laboratory tests

Routine safety laboratory testing took place at SCR, prior to each NRL972 infusion, at 12:00 hours after the start of the infusion and at the EOT. The results were compared with the laboratory defined range of normality. Values outside the range of normality were highlighted and evaluated by the Investigators in terms of their clinical relevance.

In subject R005, there was a relatively low RBC count, haemoglobin and haematocrit at 12:00 hours after the start of the infusion in PE1; this was considered clinically relevant by the Investigator; however, this was not seen as a change that might have related to the trial medication; more likely it was thought to reflect a particularly high sensitivity to blood loss by pharmacokinetic sampling.

Increases in blood glucose were frequently observed at 12:00 hours after the start of the infusion; these were not considered to be of clinical relevance by the Investigator. However, such increases had also been seen in many subjects in the previous infusion study NRL972-03/2005 (IV [ACPS528]). A meal (snack) had been taken (in both studies) three hours before the clinical laboratory test; however, the level of the increase in blood glucose appears too high to have been caused by a meal taken three hours earlier. In the absence of a placebo control there is no conclusive evidence to attribute this change to the infusion of NRL972. However, although a postprandial change is possible it cannot be excluded that a medication effect might be involved.

There were no further relevant safety findings on clinical laboratory testing.

CONCLUSIONS

- Intravenous infusions of 5 mg/hour and 15 mg/hour NRL972 yielded readily rising plasma concentrations of NRL972 reaching relatively stable values at the end of the 2-hour infusion.
- The courses of the dose-normalised concentrations during the infusion and over the first hour after the infusion were superimposable. This is in accordance with a dose-proportional i.e. three times higher C_{eq} (estimated true treatment ratio: 3.007; 95% CI: 2.821 to 3.204) and C_{max} (estimated true treatment ratio: 3.005; 95% CI: 2.799 to 3.227). Accordingly, there was also very good agreement between the low- and the high-dose infusion with regard to the C_{eq}-derived CL (estimated true treatment ratio for high- to low-dose: 0.998; 95% CI: 0.936 to 1.063), the concentration ratios in the early post-infusion decay i.e. the C(30):C(10)-ratio (estimated true treatment ratio: 0.966; CI: 0.854 to 1.094) and C(30):C(15)-ratio (estimated true treatment ratio: 0.984; CI: 0.929 to 1.043), t_{max}, and the I-NCA-derived CL (estimated true treatment ratio: 0.961; CI: 0.889 to 1.038). Accordingly, the pharmacokinetics of NRL972 were well proportional with the dose administered by 2-hour constant rate infusion (total doses of 10 and 30 mg NRL972).
- There were differences in compartmentalisation between the low- and the high-dose infusion that mainly related to the assay's constraints in "capturing" the later i.e. slower λ_2-disposition phases with the lower-dose. There is no evidence that there is a dose-related difference compartmentalisation, albeit that the expression thereof is more accurate at higher doses.
- Although the data were well concordant between different doses within the trial, they were distinctly lower than previously observed in trial NRL972-03/2005 (IV [ACPS528]) and the predictions from trial NRL972-02/2003 (ACPS [ACPS391]).
- Two-hour iv infusions of 5 mg/hour and 15 mg/hour NRL972 (Part B) were very well tolerated. There is a signal that these infusions might be associated with a rise in blood glucose at 12:00 hours after the start of the infusion. In the absence of a placebo control there is no conclusive evidence to attribute this change to the infusion of NRL972. However, although a postprandial change is possible it cannot be excluded that a medication effect might be involved, which has not been seen in other studies of NRL972.

18 Nov 2009

Norgine discontinued the development of NRL972 in July 2013.