

MEMORANDUM



**Department of Health and Human Services
Food and Drug Administration**

Center for Biologics Evaluation and Research

Toxicology Review – STN 12495/0- Ruconest- Recombinant (transgenic rabbit milk derived) human complement component 1 (C1) esterase inhibitor for the treatment of acute hereditary angioedema attacks.

Primary Reviewer: Jin H. Baek, Ph.D. Visiting Scientist, Laboratory of Biochemistry and Vascular Biology, Division of Hematology, OBRR, CBER

Through: Paul W. Buehler, Pharm.D, Ph.D., Pharmacologist, Laboratory of Biochemistry and Vascular Biology, Division of Hematology, OBRR, CBER

Through: Abdu I. Alayash, Ph.D. Laboratory Chief, Laboratory of Biochemistry and Vascular Biology, Division of Hematology, OBRR, CBER

Through: Basil Golding, M.D., Director, Division of Hematology, OBRR, CBER

Sponsor: Pharming Group NV

----- (b)(4) -----

Recommendation: Approvable

Executive Summary: Ruconest is supplied in glass vials containing 2100 IU of recombinant human complement component 1. Reconstitution is carried out by adding 14 mL sterile water for Injection per vial to obtain a final solution of 150 IU/mL. Ruconest is to be administered as a dose of 50 IU/kg for individuals < 84 kg or 4200 IU (2 vials) for individuals ≥ 84 kg. For pre-clinical studies all formulations contained 8% sucrose (8 g/100ml preparation).

The non-clinical section of STN 12495/0 contains individual study reviews of the primary pharmacokinetic/toxicokinetic, safety pharmacology and toxicology of Ruconest (rhC1-INH). This includes eleven studies in four different species. Primary findings

from the safety pharmacology (**study PCL-R-03-006**) in --(b)(4)-- dogs suggest a normal hemodynamic response (systolic/diastolic blood pressure, cardiac output and systemic vascular resistance), normal cardiac conductance (ECG), normal respiratory function and normal blood gas parameters following 2 hours of evaluation at a maximal dose of 625 IU/kg (6.25-12.5x the clinical dosing range).

Pharmacokinetic data are presented following a 125 U/kg dose in --(b)(4)-- rats (**study PCL-R-03-009**) with C_{max} values ranging between 2.5-3.0 U/kg. This concentration range was maintained for approximately 0.5 hours followed by a rapid circulatory clearance, determined to be 90 ml/hr/kg. A decreased exposure is not characteristic of plasma derived C1-INH in rats, where exposure is approximately four times greater for plasma derived versus rhC1-INH (based on C_{max} and total clearance). In addition, the pharmacokinetics of 250 U/kg single doses and 2000 U/kg single doses were evaluated in cynomolgus monkeys (**study PCL-R-03-009**). This study demonstrated a C_{max} of 6.41 ± 0.42 U/mL and 47.73 ± 2.49 U/mL following 250 U/kg and 2000 U/kg single doses, respectively. Total clearance was 15.1 mL/hr/kg and 5.04 mL/hr/kg after dosing with 250 U/kg and 2000 U/kg, respectively. This data suggests that higher doses may allow for longer duration of efficacious response. Combined with the high NOAELs observed in pivotal toxicology studies, higher doses could be evaluated to offset the differences in pharmacokinetic/toxicokinetic response observed with approved plasma derived products, Cinryze and Berinert.

Acute multiple dosing studies were carried out for two consecutive days in -----(b)(4)- rats (**study PCL -03-001**) at dose levels of 625, 1250 or 2500 U/kg/day. Minimal toxicity was observed, however all doses induced reversible injection site swelling after each treatment. At a dose level of 2500 U/kg/day there was a reversible increase in serum cholesterol concentrations in male rats. A 14 day repeat dose toxicity study evaluating 25, 125 and 625 U/kg of rhC1-INH in -----(b)(4)----- rats (**study PCL-03-003**) demonstrated that no test substance related effects were noted in rH-C1INH groups and a NOAEL of 625 U/kg could reasonably be established in this study. **PCL-R-03-001** is a Acute multiple dose infusion of C1-INH with a 14 day recovery in rat was carried out over 2 doses per group (625 U/kg, 1250 U/kg and 2500 U/kg). Administration was performed by the intravenous route for 2 consecutive days. This study served as a pilot for **PCL-03-003**. The primary toxicity observed was injection site swelling and inflammation associated with confirmatory findings of localized inflammatory response documented by histopathological examination of the injection site tissue. Additionally serum cholesterol increased in males at the highest dosing level and remained elevated at 14 days following the second day of treatment. **PCL-03-003** is a 14-day intravenous infusion toxicity study with rhC1-INH and C1-inhibitor TIM3 in the unrestrained rat followed by a 14-day observation period is the pivotal study in rodents. Dosing included 14 daily intravenous injections of 25, 125, 625 U/kg rhC1-INH. Male and females rats demonstrated increased spleen weight at 14 days in the 625 U/kg dosing cohort that resolved at 14 days. The C1-inhibitor, TIM-1 was used positive control in this study; however, TIM-1 dosing did not indicate demonstrably increased toxicity over rhC1-INH. **PCL-R-03-040.A01** is an rhC1-INH dose range finding study of 1000, 2000, 4000 and 6000 U/kg by the intravenous route in the cynomolgus monkey. There was no mortality noted and no clinical signs considered related to treatment throughout the study.

Hemorrhagic infiltration was noted at the injection sites as of the second day of treatment. The males tended to eat less than pretest after the third treatment day (4000 U/kg) and after the fourth treatment day (6000 U/kg). There was a decrease in red blood cell count, hemoglobin concentration and packed cell volume, in both genders and a slight increase in reticulocyte count for the females, compared with pretest. Total bilirubin and serum aspartate aminotransferase activity were increased in both sexes at the end of the treatment period compared with pretest. At necropsy, the only finding possibly treatment related was a mottled aspect of the liver, for the male. **PCL-R-03-043** is the pivotal study of rhC1-INH in a non-redent species. This is a 2-week intravenous (twice daily 15-minute infusion) toxicity study in the cynomolgus monkey followed by a 2-week treatment-free period evaluating the intravenous administration of 250, 500, 1000, 2000 U/kg. No mortality occurred during the study. There were neither clinical signs nor any local reaction that could be related to treatment with the test item. The body weight and food consumption were not affected by treatment with the test item. There were no relevant ophthalmological findings. The cardiovascular functions were not affected by treatment with the test item. The test substance induced an increase in serum ALP and ASAT levels at 500 and 1000 U/kg/adm (up to 2 fold pretest values) and at 2000 U/kg/adm (up to 5 fold pretest values). There were no histopathological correlates at any dose. This observation defines the NOAEL level in this species. At the end of the recovery period, day 26, ALP and ASAT values for all treated groups were similar to pretest values indicating the treatment-related effect on these parameters was reversible. Dose-related histopathological changes (microvacuoles in epithelial cells lining the renal tubules) were noted in the kidneys at 500 - 2000 U/kg/administration. The effects were minimal at 500 U/kg/adm but increased in severity and frequency at doses up to 2000 U/kg/adm. Following the treatment-free period, these changes were completely reversed at doses up to 1000 U/kg/administration. Given that only one high dose recovery animal (2000 U/kg/administration) had slight microvacuolation. Renal changes are considered to be the end result of high protein load experienced by the animals in this study. **PCL-R-03-016** is a study of rhC1-INH embryo toxicity of 625 U/kg by the intravenous route in rats the, rat-segment II. There were no significant outcomes from this study. **PCL-R-03-014** is a study of rhC1-INH performed to establish the dose range acceptable in the pregnant rabbit. At 625 U/kg no adverse effects on developing fetal rabbits or mothers were observed. **PCL-R-03-007** is the formal assessment of intravenous, intra-arterial and perivenous tolerance of rhC1INH in the rabbit after single administration. In this study 141 U/ml (total 5 ml) were administered by the ear vein. Slight erythema (grade 1) was noted in the majority of animals treated with any of the possible formulations (Saline vehicle control and rH-C1INH) and injected intravenously, perivenously or intra-arterially. In all cases, the erythema was no longer present on day 5.

The cumulative assessment of studies suggests a dose from 625 U/kg up to 1000 U/kg is acceptably safe in animals.

Roster of pre-clinical toxicology studies:

GLP (yes/no)	Protocol #	Study Title
yes	PCL-R-03-006	Cardiovascular and respiratory safety pharmacology rH-C1INH.
yes	PCL-R-03-009	Single dose pharmacokinetics of rhC1-INH after intravenous administration in the male --(b)(4)-- rat.
yes	PCL-R-03-009	Pharmacokinetic study in the cynomolgus monkey after a single intravenous administration of C1-INH.
yes	PCL-R-03-003	Single intravenous infusion of C1-INH with 14 day recovery in rat.
yes	PCL-R-03-001	Acute multiple dose infusion of C1-INH with 14 day recovery in rat
yes	PCL-03-003	14-Day intravenous infusion toxicity study with rhC1-INH and C1-inhibitor TIM3 in the unrestrained rat followed by a 14-day observation period
yes	PCL-R-03-040.A01	rhC1-INH Dose range finding study by the intravenous route in the cynomolgus monkey
yes	PCL-R-03-043	rhC1-INH- 2-week intravenous (twice daily 15-minute infusion) toxicity study in the cynomolgus monkey followed by a 2-week treatment-free period.
yes	PCL-R-03-016	rhC1-INH - Embryo toxicity study by the intravenous route in the rat-segment II.
yes	PCL-R-03-014	rhC1-INH: Dose range finding study by the intravenous route in the pregnant rabbit.
yes	PCL-R-03-007	Assessment of intravenous, intra-arterial and perivenous tolerance of rhC1INH in the rabbit after single administration

Review of Specialized Pharmacology/pre-clinical safety studies and pre-clinical pharmacokinetics/toxicokinetics.

Study PCL-R-03-006 – Cardiovascular and respiratory safety pharmacology rH-C1INH

Sponsor: Pharming Group NV

Product: Recombinant C1 esterase inhibitor (rH-C1INH)

Proposed use: Hereditary angioedema

Study Reviewer: Paul W. Buehler, Pharm.D., Ph.D.

Study summary: 4 female -(b)(4)- dogs were evaluated for cardiovascular and respiratory changes following intravenous administration of vehicle (20 mM citric acid buffer/8% sucrose) and rH-C1INH (625 U/kg). This dose represents approximately 6-fold the clinical dose. In general cardio-respiratory parameters remained unaltered over the duration of the study.

Toxicology Study Review:

Performing laboratory: -----(b)(4)-----

Conducted under GLP: Yes

Study initiation date: September 4th 2000

Final Report date: April 10th 2001

Test article batch/lot: rH-C1INH (97641 d-e-f-g)

Animal species and strain: Dog , purpose bred --(b)(4)--

Breeder/supplier: -----(b)(4)-----

Number of animal per group and sex: 4/female

Age: 11-12 months

Body weight range: 8.4-11.5 kg

Route and site of administration: intravenous, saphenous

Volume of injection: 5 ml

Frequency of administration and study duration: single bolus

Dose: 625 IU/kg

Stability: at least 8 weeks at 4 degrees Celsius.

Means of administration: Constant dose volume syringe pump

Report status: Final

Experimental design

Group	Treatment	Number of Animals (#/sex/group)	
		Treatment phase	Recovery phase
1	Vehicle	4 female	-
2	625 IU/kg	4 female	-

The following parameters were evaluated:

- Systolic/diastolic, mean arterial blood pressures
- Heart rate
- Left ventricular systolic pressure (LVSP)
- Left ventricular contractility (dp/dt min)
- Cardiac output
- II lead electrocardiogram (ECG)
- Calculated systemic vascular resistance (SVR)
- Respiratory rate
- Respiration minute volume
- Respiration tidal volume
- Blood gas parameters

Summary of results Vehicle and 625 IU/kg:

Effects on arterial blood pressure: No effects noted within a 2 hour post infusion period.

Effects on heart rate: No effects noted within a 2 hour post infusion period.

Effects on LVSP and LV dp/dt: Minor changes observed, however, no relevant effects noted within a 2 hour post infusion period.

Effects on cardiac output (CO) and systemic vascular resistance (SVR): No relevant effects noted within a 2 hour post infusion period.

Effects on femoral blood flow and systemic vascular resistance (SVR): No relevant effects noted within a 2 hour post infusion period.

Effects on respiratory parameters: Tidal volume and minute volume demonstrated a minor increase within a 2 hour post infusion period. However, no consistent effects on respiratory parameters were observed.

Effects on II lead ECG: Sporadic ectopic beats; however normal ECG waveforms predominantly observed. No changes in QTc_B interval were observed.

Effects on blood gas parameters: No relevant effects noted within a 2 hour post infusion period.

Reviewer conclusions: No significant findings with acute administration of 625 IU/kg on cardiovascular or respiratory function. This dose represents 6.25-fold greater than the clinical dose of 100 IU/kg.

Study PCL-R-03-009– Single dose pharmacokinetics of rhC1INH after intravenous administration in the male --(b)(4)-- rat

Sponsor: Pharming Group NV

Product: Recombinant C1 esterase inhibitor (rH-C1INH)

Proposed use: Hereditary angioedema

Study Reviewer: Paul W. Buehler, Pharm.D., Ph.D.

Study summary: Single dose pharmacokinetics of rH-C1INH after intravenous administration in the male --(b)(4)-- rat. AUC_{0-last} was the most reliable toxicokinetic parameter identified in this study. Other calculated toxicokinetic parameters were approximations since one or more of the parameters did not meet the requirements as set in the protocol. When $t_{1/2}$ and β are determined to be approximations derived parameters AUC_{0-inf} , Cl , $V_{d_{area}}$, $V_{d_{ss}}$, and MRT are also approximations. The mean clearance values were determined to be 1.741 ± 0.2844 , 1.612 ± 0.3331 and 1.395 ± 0.3351 ml/min/kg for the batches 04100013, 04100016 and 0410017, respectively. The volume of distribution of the respective batches was 0.04281 ± 0.002959 , 0.04297 ± 0.003044 and 0.03989 ± 0.005039 L/kg. When comparing the various toxicokinetic parameters and the concurring standard deviations on the parameters for all three batches tested, no differences could be observed for exposure, rate of elimination, clearance and volume of distribution.

Toxicology Study Review:

Performing laboratory: -----(b)(4)-----

Conducted under GLP: Yes

Study initiation date: December 18th, 2000

Final Report date: July 13th 2001

Test article batch/lot: rH-C1INH (97641, 106956/A, 106956/B)

Animal species and strain: --(b)(4)-- rat

Breeder/supplier: -----(b)(4)-----

Number of animal per group and sex: 6/group all male

Age: 7 weeks

Body weight range: 250-275

Route and site of administration: Intravenous injection into the tail vein

Volume of injection: 4 ml/kg body weight

Frequency of administration and study duration: Once daily

Dose: 125 U/kg

Stability: at least 8 weeks at 4 degrees Celsius.

Means of administration: Constant dose volume syringe pump

Report status: Final**Experimental design**

Group	Substance	Batch	Dose (U/kg)	Animals/group	Animal #
1	97641/J	04100013	125	6	1-6
2	106956/A	04100016	125	6	7-12
3	106956/B	04100017	125	6	13-18

Blood sampling schedule:

Proposed: -5, 2, 4, 6, 8, 10, 15 and 30 minutes, 1, 2, 4, 8, 24 hours.

Blood sample deviations:

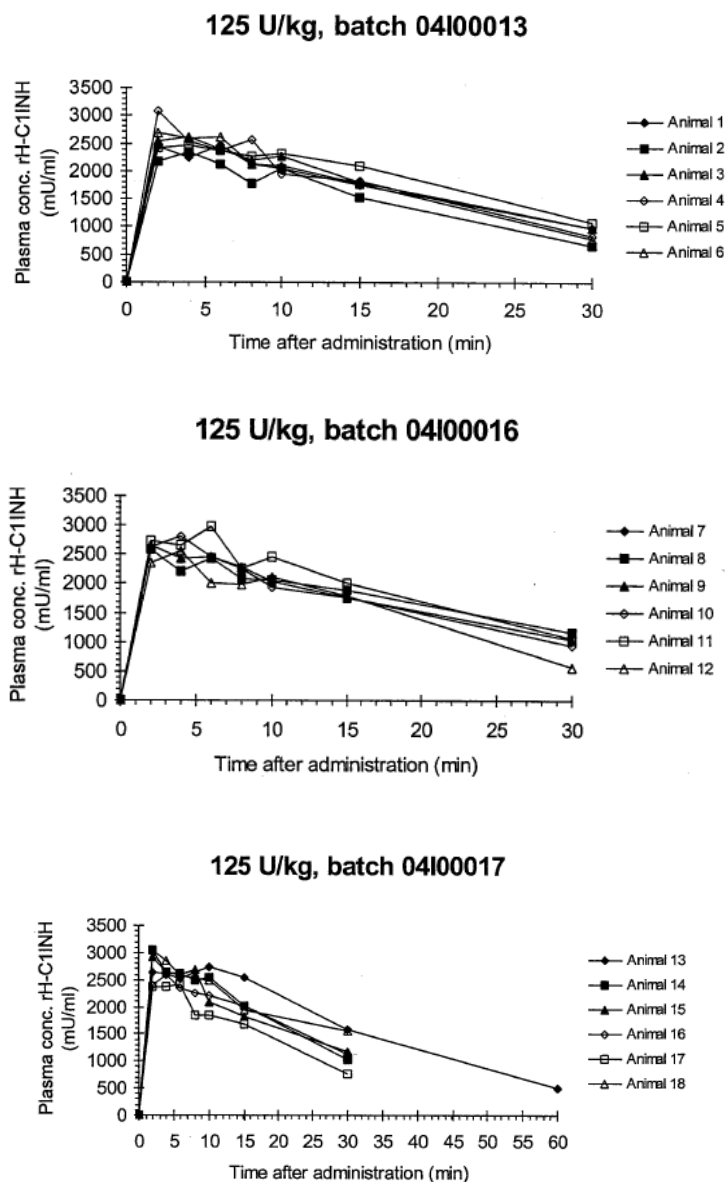
animal 1	animal 2	animal 3	animal 4	animal 5	animal 6
- 5 min.	- 5 min.	- 5 min.	- 5 min.	- 5 min.	- 5 min.
2 min	2 min	2 min	3 min	2 min	2 min
4 min	4 min	4 min	4 min	5 min	4 min
6 min	6 min	6 min	9 min	6 min	7 min
8 min	8 min	8 min	11 min	10 min	13 min
10 min	10 min	10 min	13 min	13 min	15 min
15 min	15 min	16 min	15 min	15 min	17 min
30 min	30 min	30 min	32 min	/	29 min
60 min	60 min	60 min	67 min	61 min	60 min
121 min	120 min	120 min	120 min	/	121 min
240 min	240 min	240 min	240 min	241 min	283 min
480 min	480 min	480 min	480 min	480 min	481 min
1440 min	1440 min	1440 min	1440 min	1440 min	1439 min

animal 7	animal 8	animal 9	animal 10	animal 11	animal 12
- 5 min.	- 5 min.	- 5 min.	- 5 min.	- 5 min	- 5 min.
2 min	2 min	2 min	2 min	2 min	2 min
4 min	4 min	4 min	4 min	5 min	4 min
6 min	6 min	6 min	7 min	6 min*	6 min
8 min	8 min	9 min	9 min	/	9 min
11 min	10 min	11 min	11 min	/	10 min
15 min	15 min	15 min	16 min	15 min	16 min
31 min	30 min	30 min	32 min	31 min	30 min
61 min	60 min	60 min	/	60 min	64 min
126 min	121 min	120 min	123 min	120 min	120 min
240 min	241 min	241 min	242 min	242 min	241 min
480 min	480 min	480 min	/	482 min	480 min
1440 min	/	1440 min	1440 min	1440 min	1440 min

Site of blood sampling: 200 microliters of blood were collected in EDTA from the tail vein.

Assay for rH-C1INH: Analysis of samples was performed by -----(b)(4)-----.
Assay validation reported in the see study section review of the BLA submission.

Summary of pharmacokinetic results 125 IU/kg:



Pharmacokinetic parameters:**Batch 04100013 at 125 IU/kg:**

		Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6	MEAN ± SD
Parameter								
Dose	U/kg	125	125	125	125	125	125	125 ± 0.0
t _{last}	min	30	30	30	30	30	30	30.0 (30-30) [#]
AUC _{0-last}	mU.min/ml	49896	43239	52277	51366	55391	50006	50362 ± 4026
Dose normalised AUC _{0-last}	mU.min/ml	399.2	345.9	418.2	410.9	443.1	400.0	402.9 ± 32.21
AUC _{0-24h}	mU.min/ml	49896	43239	52277	51366	55391	50006	50362 ± 4026
Dose normalised AUC _{0-24h}	mU.min/ml	399.2	345.9	418.2	410.9	443.1	400.0	402.9 ± 32.21
AUC _{0-∞}	mU.min/ml	76564*	56979*	76865*	70097*	93033*	67097*	73439* ± 12064*
Dose normalised AUC _{0-∞}	mU.min/ml	612.5*	455.8*	614.9*	560.8*	744.3*	536.8*	587.5* ± 96.52*
% extrapolated		34.83	24.12	31.99	26.72	40.46	25.47	30.60 ± 6.331
β	1/min	0.03682*	0.04803*	0.04055*	0.04447*	0.02901*	0.04622*	0.04085* ± 0.007071*
t _{1/2}	min	18.82*	14.43*	17.09*	15.59*	23.89*	15.00*	17.47* ± 3.523*
Corr. coeff.	r ²	0.9928	0.9643	0.9994	0.9666	0.9113	0.9811	0.9693 ± 0.03160
Cl	ml/min/kg	1.633	2.194	1.626	1.783	1.344	1.863	1.741 ± 0.2844
Vd _{area}	l/kg	0.04434	0.04567	0.04011	0.04010	0.04632	0.04030	0.04281 ± 0.002959
Vd _{ss}	l/kg	0.04611	0.04714	0.04245	0.04092	0.04554	0.04141	0.04393 ± 0.002655
MRT	min	28.33	21.57	26.19	23.03	33.98	22.31	25.90 ± 4.715

Batch 04100016 at 125 IU/kg:

		Animal 7	Animal 8	Animal 9	Animal 10	Animal 11	Animal 12	MEAN ± SD
Parameter								
Dose	U/kg	125	125	125	125	125	125	125 ± 0.0
t _{last}	min	30	30	30	30	30	30	30 (30-30) [#]
AUC _{0-last}	mU.min/ml	54408	53029	51676	50984	57077	45662	52139 ± 3843
Dose normalised AUC _{0-last}	mU.min/ml	435.3	424.2	413.4	407.9	456.6	365.3	417.1 ± 30.74
AUC _{0-24h}	mU.min/ml	54408	53029	51676	50984	57077	45662	52139 ± 3843
Dose normalised AUC _{0-24h}	mU.min/ml	435.3	424.2	413.4	407.9	456.6	365.3	417.1 ± 30.74
AUC _{0-∞}	mU.min/ml	82256*	97302*	82077*	74075*	87951*	55895*	79926* ± 14058*
Dose normalised AUC _{0-∞}	mU.min/ml	658.0*	778.4*	656.6*	592.6*	703.6*	447.2*	639.4* ± 112.5*
% extrapolated		33.85	45.50	37.04	31.17	35.10	18.31	33.50 ± 8.892
β	1/min	0.03638*	0.02659*	0.03398*	0.04054*	0.03488*	0.05521	0.03793* ± 0.009605*
t _{1/2}	min	19.05*	26.07*	20.40*	17.10*	19.87*	12.55	19.17* ± 4.421*
Corr. coeff.	r ²	0.9493	0.9586	0.9926	0.9887	0.9359	0.9304	0.9593 ± 0.02629
Cl	ml/min/kg	1.520	1.285	1.523	1.687	1.421	2.236	1.612 ± 0.3331
Vd _{area}	l/kg	0.04178	0.04832	0.04482	0.04163	0.04074	0.04050	0.04297 ± 0.003044
Vd _{ss}	l/kg	0.04182	0.04879	0.04573	0.04321	0.04098	0.04178	0.04372 ± 0.002996
MRT	min	27.60	38.06	30.11	25.69	28.92	18.77	28.19 ± 6.276

Batch 04100017 at 125 IU/kg:

		Animal 13	Animal 14	Animal 15	Animal 16	Animal 17	Animal 18	MEAN ± SD
Parameter								
Dose	U/kg	125	125	125	125	125	125	125 ± 0.0
t _{last}	min	60	30	30	30	30	30	30.0 (30-60) [#]
AUC _{0-last}	mU.min/ml	95013	57443	55720	54697	46293	61583	61792 ± 17030
Dose normalised AUC _{0-last}	mU.min/ml	760.1	459.5	445.8	437.6	370.3	492.7	494.3 ± 136.2
AUC _{0-24h}	mU.min/ml	95013	57443	55720	54697	46293	61583	61792 ± 17030
Dose normalised AUC _{0-24h}	mU.min/ml	760.1	459.5	445.8	437.6	370.3	492.7	494.3 ± 136.2
AUC _{0-∞}	mU.min/ml	109229	80404*	91544*	91062*	64207*	126685*	93855* ± 21851*
Dose normalised AUC _{0-∞}	mU.min/ml	873.8	643.2*	732.4*	728.5*	513.7*	1013*	750.8* ± 174.8*
% extrapolated		13.01	28.56	39.13	39.93	27.90	51.39	33.32 ± 13.18
β	1/min	0.03489	0.04490*	0.03286*	0.03094*	0.04343*	0.02396*	0.03516* ± 0.007898*
t _{1/2}	min	19.87	15.44*	21.10*	22.41*	15.96*	28.93*	20.62* ± 4.932*
Corr. coeff.	r ²	0.9931	1.000	0.9666	0.9764	0.9614	0.9493	0.9745 ± 0.01933
Cl	ml/min/kg	1.144	1.555	1.365	1.373	1.947	0.9867	1.395 ± 0.3351
Vd _{area}	l/kg	0.03280	0.03462	0.04156	0.04437	0.04483	0.04118	0.03989 ± 0.005039
Vd _{ss}	l/kg	0.03500	0.03714	0.04291	0.04499	0.04621	0.04279	0.04151 ± 0.004456
MRT	min	30.67	23.98	31.51	32.86	23.82	43.45	31.05 ± 7.206

Reviewer conclusions: C_{max} was not reported; however, based on batches tested, a single dose of rH-C1INH (125 IU/kg) generated a maximum plasma concentration ranging between 2.5-3.0 U/ml. A general interpretation of the circulation time required to maintain the concentration is approximately 0.5 hr. The clearance of rH-C1INH is quite rapid (approximately 1.5 ml/min/kg) making rH-C1INH significantly different in the rat response. Pre-clinical pharmacokinetics in the rat demonstrates significant differences between the recombinant C1INH when compared to the two existing plasma derived products (Cynrizo and Berinert-see labeling for each).

Study PCL-03-042– Pharmacokinetic study in the cynomolgus monkey after a single intravenous administration

Sponsor: Pharming Group NV

Product: Recombinant C1 esterase inhibitor (rH-C1INH)

Proposed use: Hereditary angioedema

Study Reviewer: Paul W. Buehler, Pharm.D., Ph.D.

Study summary: Minimal levels of test item were detected close to the lower limit of quantification (0.5 U/mL) in plasma taken before administration on day 0 from female nos. 2301, 2302, 2304 and 2306 (measured concentrations were between 0.514 and 0.560 U/mL). Primary pharmacokinetic parameters demonstrate dose dependence over the nearly 10-fold difference in dose administered. At 250 U/kg, rh-C1INH appeared to be eliminated more rapidly as suggested by the mean half-life of 1.47 hours. At 2000 U/kg, rh-C1INH was more slowly eliminated (mean half-life was 3.64 hours), as confirmed by the mean clearance, which was 3-fold higher at 250 U/kg than at 2000 U/kg. rh-C1INH appeared to be distributed in the plasma as suggested by the small volume of distribution of 31.7 and 26.5 mL/kg at 250 and 2000 U/kg, respectively. The systemic exposure increased greater than dose-proportionally between 250 and 2000 U/kg, but this finding was not confirmed by the C_{max}. rh-C1INH appeared to be rapidly eliminated at 250 U/Kg, more slowly at 2000 U/Kg, as suggested by the increase in half-life and the decrease in clearance with increasing dose. The systemic exposure increased greater than dose-proportionally between 250 and 2000 U/Kg.

Toxicology Study Review:

Performing laboratory: -----(b)(4)-----

Conducted under GLP: Yes

Study initiation date: March 30th, 2007

Final Report date: January 7th, 2008

Test article batch/lot: rH-C1INH (97641, 106956/A, 106956/B)

Animal species and strain: Cynomolgus monkeys (Macaca fascicularis)

Breeder/supplier: -----(b)(4)-----

Number of animal per group and sex: 6 animals

Calibration curves:

Title:		The determination of rhC1INH in cynomolgus monkey plasma and formulation samples											
Sponsor code:		PCL-03-042											
(b)(4) code:		BS0749											
Component:		rhC1INH											
		Back calculated concentrations									Regression parameters		
		Nominal value (U/mL)											
		0.500	0.400	0.300	0.250	0.200	0.150	0.100	0.0500	0.000			
Batch number	Analysis date <small>(dd.mm.yyyy)</small>	Std A (U/mL)	Std B (U/mL)	Std C (U/mL)	Std D (U/mL)	Std E (U/mL)	Std F (U/mL)	Std G (U/mL)	Std H (U/mL)	Std I (U/mL)	intercept (A)	slope (B)	correlation coefficient
Phar-002	13 Mar 2007	0.514	0.390	a	0.245	0.200	0.147	0.105	0.0483	a	-2.98E+04	8.31E-01	0.9993
Phar-004	19 Mar 2007	0.492	b	0.309	0.245	0.198	0.152	0.0994	c	d	2.74+04	2.41-00	0.9998
Mean (U/mL):		0.503	0.390	0.309	0.245	0.199	0.150	0.102	0.048		-2.98.E+04	8.31E-01	0.9996
SD (U/mL):		0.016			0.000	0.001	0.004	0.004					
CV%:		3.1			0.0	0.7	2.4	3.9					
Bias% ((x _{mean} -u)/u)*100:		0.6	-2.5	3.0	-2.0	-0.5	-0.3	2.2	-3.4				
Number		2	1	1	2	2	2	2	1	0	1	1	2

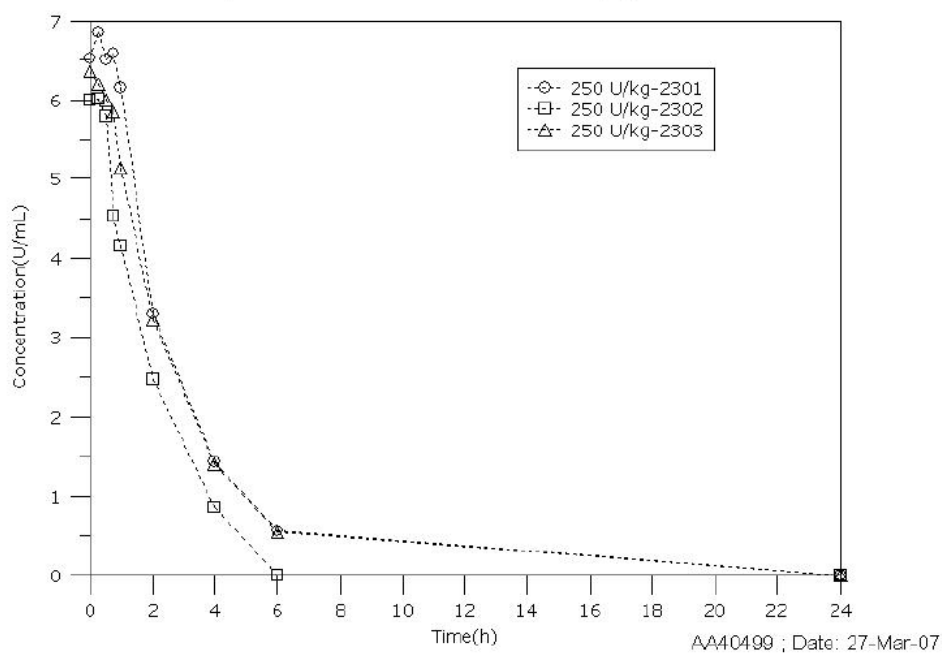
a: Std deleted for the purpose of curvefitting
b: Std rejected, BIAS% > 20%
c: Std rejected, CV% > 15%
d: The signal of the respective sample is lower than the asymptote min value

Quality control samples:

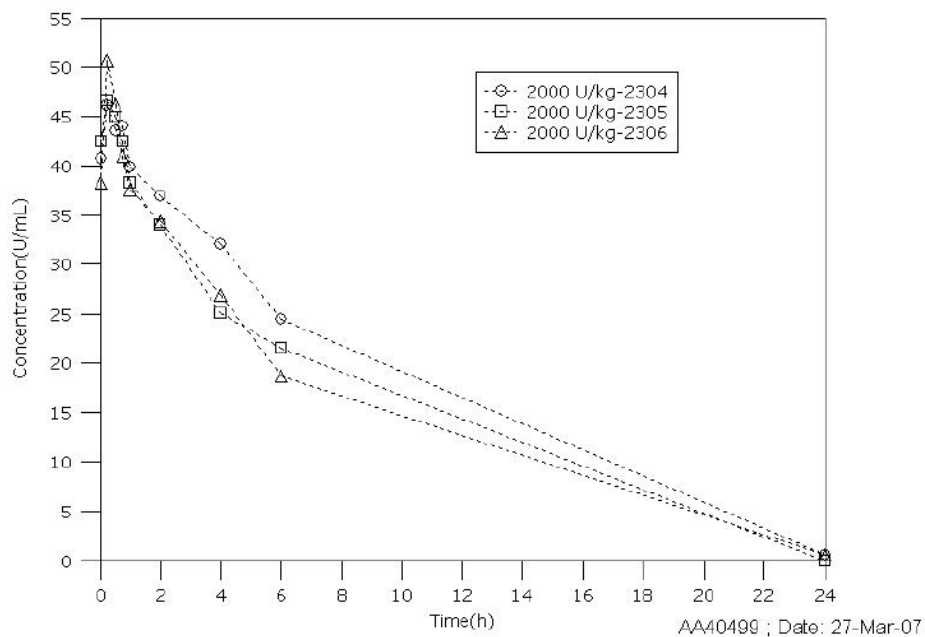
Title:		The determination of rhC1INH in cynomolgus monkey plasma and formulation samples					
Sponsor code:		PCL-03-042					
(b)(4) code:		BS0749					
Component:		rhC1INH					
Nominal or spiked concentration:		Low 1.37 U/mL		Medium 17.7 U/mL		High 32.6 U/mL	
Endogenous concentration:							
Batch number	Analysis date (dd.mm.yyyy)	x1 (U/mL)	x2 (U/mL)	x1 (U/mL)	x2 (U/mL)	x1 (U/mL)	x2 (U/mL)
Phar-002	13 Mar 2007	1.61	1.66	19.1	22.1	34.5	32.5
Phar-004	19 Mar 2007	1.42	1.45	19.3	19.1	31.6	31.8
Mean (U/mL):		1.54		19.9		32.6	
SD (U/mL):		0.12		1.5		1.3	
CV%:		7.7		7.4		4.1	
Bias% ((x _{mean} -u)/u)*100:		12.0		12.4		0.0	
Spike rec.% ((x _{mean} -x _{endo})/u)*100:		112.0		112.4		100.0	
Number		4		4		4	

Summary of pharmacokinetic results 250 and 2000 IU/kg:

rhC1INH plasma concentrations linear scale concentration versus time (250 IU/kg).



rhC1INH plasma concentrations linear scale concentration versus time (2000 IU/kg).



Pharmacokinetic parameters:

Dose (U/kg)	Female numbers	C _{max} (U/mL)	T _{max} (h)	t* (h)	AUC ₀₋₁ (U.h/mL)	AUC _{0-24h} (U.h/mL)	AUC _{extra} (%)	AUC _{0-inf} (U.h/mL)	t _{1/2} (h)	Cl (mL/h/kg)	Vd (mL/kg)
250	2301	6.85	0.25	6	18.03	19.30	6.6	19.30	1.57	12.95	29.24
	2302	6.01	0.25	4	12.00	13.61	11.8	13.61	1.31	18.37	34.68
	2303	6.36	0.00	6	16.69	17.87	6.6	17.87	1.54	13.99	31.06
	Mean	6.41	0.25	6	NA	16.93	NA	16.93	1.47	15.10	31.66
	SD	0.42	NA	NA	NA	2.96	NA	2.96	0.14	2.88	2.77
2000	2304	46.10	0.25	24	433.64	433.64	0.8	436.98	3.52	4.58	23.25
	2305	46.50	0.25	6	185.39	NA	49.3	NA	NA	NA	NA
	2306	50.60	0.25	24	360.03	360.03	1.0	363.64	3.75	5.50	29.76
	Mean	47.73	0.25	24	NA	396.83	NA	400.31	3.64	5.04	26.50
	SD	2.49	NA	NA	NA	19**	NA	18**	6**	18**	25**

Reviewer conclusions: The present study demonstrates that rh-C1INH is limited to the central compartment following both 250 and 2000 U/kg doses. C_{max} increases linearly with increasing dose, while 24 hour exposure increases more than linearly with increasing dose. The 2000 U/kg administration represents a 20-fold increase over the clinical dose and animals did not experience any observational adverse safety effects or mortality.

Study number PCL-03-001: rhC1-INH- Acute multiple dose toxicity study by the intravenous route in the -----(b)(4)----- rat.

Performing laboratory: Test facility: -----(b)(4)-----

Test site: -----(b)(4)-----

Study initiation date: 28 July 2006.

Final Report date: not provided

Test article batch/lot:

rh-C1INH - (04I02003 then 04I01002

Control Vehicle(103203) - Sterile 20 mM citric acid buffer with 6.5 % sucrose, pH 6.8.

Animal species and strain: -----(b)(4)----- rats: -----(b)(4)-----

Breeder/supplier: -----(b)(4)-----

Number of animal per group and sex: 120 (60 males and 60 females)

Age: 9 weeks.

Body weight range: males 318 to 384 g, females 210 to 259 g

Route and site of administration: Intravenous injection via the tail vein.

Volume of injection: 10 mL/kg/dose

Frequency of administration and study duration: daily for 4 consecutive days. After a 7-hour interval (\pm 5 minutes) the rats received an additional dose of test item (group 4) or an equivalent volume of vehicle (groups 1 to 3)

Dose: 0, 625, 1250, 2500 U/kg

Stability: Maximum 8 hours at room temperature.

Means of administration: Slow intravenous injection using a pump and a microflex infusion set introduced into a tail vein (0.5 mL/minute).

Report status: Final report

Summary

Under the conditions of this study, rhC1INH given intravenously at dose levels of 625, 1250 or 2500 U/kg/day induced reversible swelling after each treatment.

The dose level of 2500 U/kg/day was associated with minimal changes in red blood cell parameters, a reversible increase in cholesterol concentration in males, and slight body weight gain and food consumption effects.

The IgG titers against rhC1INH were slightly elevated in a few animals (ranging from 100 to 6400). The increase in titer was not dose-or gender-dependent.

Experimental design

Group	Daily dosing schedule		Dose (U/kg/)	Dose volume (mL/kg)	Dose		Number of animals			
	Dose 1 (U/kg)	Dose 2 (U/kg)			1 st dose (U/mL)	2 nd dose (U/mL)	Day 4		Day 14	
							M	F	M	F
1. Control	0	0	0	10	0	0	10	10	5	5
2. Low dose	625	0	625	10	62.5	0	10	10	5	5
3. Middle dose	1250	0	1250	10	125	0	10	10	5	5
4. High dose	2500	2500	2500*	10	125	125	10	10	5	5

Day 0: first day of treatment

M: male.

F: female.

(1) end of the treatment period.

(2) end of the treatment-free period.

(3) given as 2 doses of 1250 U/kg with a 7 hour interval.

Group 1 animals (control) received the vehicle (see section 3.2.) on each daily occasion and group 1 to 3 animals on the second daily occasion.

Methods:

Endpoint	Methodology
Hematology	------(b)(4)-----
Clinical chemistry	------(b)(4)----- -----
Coagulation	

Randomization procedure: Performed during the acclimatization period by computer-generated randomization. Mean body weights of the groups at allocation were not statistically significantly different from each other (analysis of variance), each sex being considered separately.

Statistical analysis plan: For each parameter (except terminal body weights and organ weights), Levene's test was used to test the equality of variances across groups and Shapiro-Wilk's test was used to assess the normality of the data distribution in each group. Data showing homogeneous variances across groups and normal distribution in all groups were analyzed using parametric procedures. Such analysis consisted of a one way analysis of variance (ANOVA) allowing for a group effect, followed, if the ANOVA test was significant, by Student's t-test (pre-treatment data) or Dunnett's test to assess the significance of any differences between treated and control groups. Data showing non-homogeneous variances across groups or a non-normal distribution in at least one group were analyzed using non-parametric methods. Such analysis consisted of the Kruskal-Wallis test, followed by the Wilcoxon's rank sum test if the Kruskal-Wallis test was significant. These analyses were performed using a SAS software package.

For terminal body weights and organ weights, statistical analysis was performed by the data acquisition system (------(b)(4)-----) as follows: Kolmogorov's test for normality of the data distribution in each group and Bartlett's test for homogeneity of variances across groups, followed by ANOVA and Dunnett's test for data showing equality of variances and normal distribution, or Kruskal-Wallis test and Dunn's test for data with non-homogeneous variances or non-normal distribution. Statistically significant results for pairwise comparisons are indicated on the tables next to the appropriate mean value, using the following abbreviations: *: $P(\text{probability}) \leq 0.05$ or * or # (non-parametric): $P < 0.05$, **: $P \leq 0.01$ ** or ##: $P < 0.01$, ***: $P \leq 0.001$ for terminal body weights and organ weights. The ANOVA results are not reported but are kept in the study file.

The following parameters were evaluated:

Parameters	Frequency of Testing
Cageside observation	Twice a day
Clinical observations	Daily, weekly full clinical examination
Body weight	at the time of allocation (data not reported), prior to dosing

Parameters	Frequency of Testing
	on day 0 and then twice weekly during the treatment and treatment-free periods
Food consumption	twice weekly
Body temperature	NC
Blood pressure	NC
Ophthalmological examination	Day 3
Clinical chemistry	days 4 and 14
Hematology	days 4 and 14
Coagulation	days 4 and 14
Immunological response	days 4 and 14
Evaluation of site of inoculation	NC
Necropsy	days 4 and 14
Tissues for histopathology	days 4 and 14
Urine	at least 14 hours (day 4) or at least 18 hours (day 14)

(NC = not collected)

The following tissues and organs were collected from all animals at necropsy

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
Digestive	<i>Large intestine (caecum, colon, rectum), small intestine (duodenum, jejunum, ileum), liver, salivary gland, mandibular, sublingual, pancreas, stomach, esophagus,</i>	<i>gall bladder,</i>
RESPIRATORY	<i>Lung (with main-stem bronchi), trachea,</i>	<i>nasal turbinate,s, trachea bifurcation</i>
CARDIOVASCULAR	<i>aorta, heart</i>	
IMMUNOLOGIC / HEMATOPOIETIC	<i>bone with marrow(sternum), bone with marrow (femur), lymph node (mandibular, mesenteric), spleen, thymus</i>	<i>lymph nodes(1 related to route of administration, and 1 from a distant location),</i>
UROGENITAL	<i>uterus, kidney, ovaries, prostate, seminal vesicle, testes, urinary bladder, uterus (with cervix), vagina, epididymis</i>	<i>fallopian tubes,</i>
NEUROLOGIC	<i>sciatic nerve, spinal cord (cervical, lumbar, mid-thoracic), optic nerves</i>	<i>Brain (cerebrum, cerebellum, medulla/pons), optic</i>

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
		<i>nerve,</i>
HORMONAL	<i>adrenals, thyroid (with parathyroid glands), pituitary glands, parathyroid gland, preputial gland, clitoral gland, Lacrymal gland (exorbital), mammary glands</i>	<i>Zymbal's Gland</i>
OTHER	<i>eyes, , skin, tongue, skeletal muscle, brain, Harderian gland, peyer's patches</i>	
GROSS LESIONS	<i>All gross lesions</i>	
INJECTION SITE OR SITE OF APPLICATION	No collected	

Results:

Morbidity and mortality: No treatment related mortality occurred. Group 3 female no. 103 died on day 2 at blood sampling 15 minutes after dosing. Group 2 female (no. 82) was injured tail accidentally on day 0 and was sacrificed for ethical reasons on day 1.

Clinical Signs: Most animal showed a dose-related effect, with a higher incidence of swelling of the muzzle and limbs in groups 3 and 4 compared with group 2. The number of animals affected by swelling 1 hour after treatment tended to decrease between days 1 and 3. There were no other clinical observations (hair loss, stripped tail, tooth abnormality)

Body Weight: Body weight change was comparable in all groups during to treatment period (days 0 to 3). However, the high dose rats tended to gain slightly less weight than controls during the 10-day treatment-free period.

Ophthalmology: There were no treatment-related ophthalmological findings.

Food consumption: Females previously given 1250 or 2500 U/kg/day groups ate slightly less than controls at the end of the treatment-free period (-9 and -14% respectively, $p \leq 0.05$). The difference in the high dose group was consistent with the slightly lower mean body weight gain.

CLINICAL CHEMISTRY

MEASUREMENT	PARAMETERS	NOTE
ELECTROLYTE BALANCE	Calcium, chloride, phosphorus potassium, sodium	Normal relative to control
CARBOHYDRATE METABOLISM	Glucose	Normal relative to control
A) HEPATOCELLULAR	Alanine aminotransferase (ALT or SGPT)	Normal relative to control
	Aspartate aminotransferase (AST or SGOT)	Normal relative to control
	Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids	NC
	Alkaline phosphatase (ALP)	Normal relative to control
	Gamma-glutamyl transferase (GGT)	NC
	Total bile acids	NC
B) HEPATOBILIARY	Total bilirubin	Normal relative to control
ACUTE PHASE REACTANTS	C-reactive protein	Normal relative to control
	fibrinogen (also under coagulation),	NC
KIDNEY FUNCTION	Creatinine	Normal relative to control
	Blood urea nitrogen	Normal relative to control
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Albumin (A)	Normal relative to control
	Globulin (G, calculated) or A/G Ratio	Normal relative to control
	Total cholesterol	dose-related increase in the treated males at day 4 and return to normal at day14
	Cholinesterase	NC
	Total protein	Normal relative to control
	Fasting triglycerides	NC
MUSCLE INJURY	CK-MB CK-MM CK-BB	NC

Table of Clinical Chemistry Result Comments: There was a dose-related increase in serum cholesterol concentration in the treated males at the end of the treatment period. At day 14, the total cholesterol level returned to normal in all groups.

HEMATOLOGY

MEASURE MENT RELATED TO	PARAMETERS	NOTE
RED BLOOD CELLS	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes	Normal relative to control Normal relative to control
WHITE BLOOD CELLS	lymphocyte count Neutrophil count Basophils, eosinophils count Monocyte/macrophage (day 24) Total leukocytes (WBC) Large unstained cells (LUC)	Normal relative to control Normal relative to control Normal relative to control Normal relative to control Normal relative to control
CLOTTING POTENTIAL	Fibrinogen Prothrombin time (PT) Activated partial thromboplastin time (APTT)	NC Normal relative to control Normal relative to control
OTHERS	Bone marrow cytology	NC

Table of Hematology Result Comments: There were no differences between groups at the end of the treatment-free period.

Urine analysis: No clear treatment related differences in urine analysis parameters were noted between treated and control animals.

Systemic toxicity: There were no changes at necropsy which were considered to be associated with the test item at the end of both periods.

Gross Pathology: There were no evident treatment-related microscopic findings during both treatment periods. Sporadic microscopic findings were noted with a similar incidence in the controls and treated groups.

Group	Findings
1(Control)	No significant findings
2 (625u/kg)	No significant findings
3 (1250U/kg)	No significant findings
4 (2500U/kg)	No significant findings

Study number PCL-03-003: 14-Day intravenous infusion toxicity study with rhC1INH and C1-inhibitor TIM3 in the unrestrained rat followed by a 14-day observation period

Performing laboratory: Test facility: -----(b)(4)-----

Study initiation date: 05 July 2000

Final Report date: not provided

Test article batch/lot:

rH-C1INH (04100011) - Control Vehicle(Notebook 04 PD 9902): Sterile 20mM citric acid buffer with 8% sucrose, pH 6.8.

Sucrose Solution (Notebook LIQ-PD-04-00/002): Sterile 80% sucrose solution in water, pH 7.0

Test Substance 103311 (790200B C1-INHIBITOR TIM3, 7007299F Water for injection): C1-INHIBITOR TIM3

Animal species and strain: -----(b)(4)----- rat ----(b)(4)----, outbred , SPF quality

Breeder/supplier: -----(b)(4)-----

Number of animal per group and sex: 10 males and 10 females in the Main groups, 3 males and 3 females in the Observation groups

Age: approximately 9 weeks

Body weight range: not provided

Route and site of administration: Intravenous injection via the tail vein.

Volume of injection: 4 ml/kg/hr

Frequency of administration and study duration: Animals were infused continuously with the test substance for 24 hours per day for 14 days.

Dose: 0, 25, 125, 625 U/kg

Stability: Not indicated

Means of administration: By intravenous infusion into the posterior vena cava via the femoral vein. (4 ml/kg/hr)

Report status: Final report

Summary

The test substance was administered continuously at a dose volume of 4 ml/kg/hr into the vena cava via the femoral vein for at least 14 days. All animals were subjected to daily clinical observation, Body weights and food consumption were measured twice weekly, Ophthalmoscopic examination took place during pre-test and in week 2. At termination, blood for toxicokinetic analyses and, blood and urine for clinical pathology were collected and macroscopic observations and organ weights were recorded. Histopathology was performed on a selection of tissues. No test substance related effects were noted in rH-C1INH groups. rH-C1INH TIM3 group showed hunched posture and piloerection. Body weight gain and (relative food) consumption were decreased, Clinical laboratory investigation revealed alterations in red blood cell parameters indicative of anemia, increases in white blood cell count, and changes in clinical biochemistry parameters. Furthermore enlargement of the spleen was noted, and increased liver, kidney, spleen and adrenal (females only) weight. A decrease in thymus weight (females only) was observed. These changes had all regressed after a 2-week observation period, with the exception of red blood cell count and hematocrit, which were still reduced in females, and liver and spleen weights, which were still elevated. No treatment-related microscopic findings were directly attributed to C1-INHIBITOR TIM3. There was a treatment-related exacerbation of procedure-related lesions which may be a result of slight irritation or stimulation of the immune system. On the basis of the above considerations, a NOEL of 625 U/kg is concluded for RH-C1INH. A similar dose of the reference compound, C1-INHIBITOR TIM3, produced clear treatment-related effects and proved to be less favorable in this 14-day continuous intravenous infusion study in the rat.

Experimental design

U/kg/day	Group 1 0 (vehicle)	Group 2 25 U/kg/day	Group 3 125 U/kg/day	Group 4 625 U/kg/day	Group 5 625 U/kg/day
Test Substance	—	97641/D	97641/D	97641/D	103311
Males Main Observation	01-10 11-13	14-23	24-33	34-43 44-46	47-56 57-59
Females Main Observation	60-69 70-72	73-82	83-92	93-102 103-105	106-115 116-118

Methods:

Endpoint	Methodology
Hematology	----(b)(4)-----
Clinical chemistry	----- (b)(4) ----- -----
Coagulation	----- (b)(4) -----
Urine analysis	----- (b)(4) ----- -----

Randomization procedure: By computer-generated random algorithm according to body weight, with all animals within: $\pm 20\%$ of the sex mean, A health inspection was performed prior to commencement of treatment to ensure that the animals are in a good state of health.

Statistical analysis plan: The following statistical methods were used to analyze the data: If the variables could be assumed to follow a normal distribution, the Dunnett-test (many-to-one test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex, The Steel-test (many-to-one rank test) was applied instead of the Dunnett-test if the data could not be assumed to follow a normal distribution. The exact Fisher-test was applied to frequency data.

The following parameters were evaluated:

Parameters	Frequency of Testing
Cage side observation	Twice a day
Clinical observations	Daily
Body weight	Twice weekly
Food consumption	twice weekly
Body temperature	NC
Blood pressure	NC
Ophthalmological examination	pre-test for all groups and in week 2 for groups 1 , 4 and 5.
Clinical chemistry	days 14 and 28
Hematology	days 14 and 28
Coagulation	days 14 and 28
Immunological response	days 14 and 28
Evaluation of site of inoculation	NC
Necropsy	days 14 and 28
Tissues for histopathology	days 14 and 28
Urine	days 14 and 28

(NC = not collected)

The following tissues and organs were collected from all animals at necropsy

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
DIGESTIVE	<i>Large intestine (caecum, colon, rectum), small intestine (duodenum, jejunum, ileum), liver, salivary gland, mandibular, sublingual, pancreas, stomach, esophagus,</i>	<i>gall bladder,</i>
RESPIRATORY	<i>Lung (with main-stem bronchi), trachea,</i>	<i>nasal turbinate, trachea bifurcation</i>
CARDIOVASCULAR	<i>aorta, heart</i>	
IMMUNOLOGIC / HEMATOPOIETIC	<i>bone with marrow(sternum), bone with marrow (femur), lymph node (mandibular, mesenteric), spleen, thymus</i>	<i>lymph nodes(1 related to route of administration, and 1 from a distant location),</i>
UROGENITAL	<i>uterus, kidney, ovaries, prostate, seminal vesicle, testes, urinary bladder, uterus (with cervix), vagina, epididymis</i>	<i>fallopian tubes,</i>
NEUROLOGIC	<i>eyes with optic nerve, Brain (cerebrum, cerebellum, medulla/pons), sciatic nerve, spinal cord (cervical, lumbar, mid-thoracic)</i>	
HORMONAL	<i>adrenals, thyroid (with parathyroid glands), pituitary glands, preputial gland, clitoral gland, Lacrymal gland (exorbital), mammary glands</i>	<i>Zymbal's Gland, Harderian gland, peyer's patches</i>
OTHER	<i>skin, tongue, skeletal muscle, brain,Vena cava, femoral vein</i>	
GROSS LESIONS	<i>All gross lesions</i>	
INJECTION SITE OR SITE OF APPLICATION		

Results:

Morbidity and mortality: One animal treated with rH-C1INH at 25 U/kg/day died after blood sampling.

Clinical Signs: No test substance related clinical signs were observed in the rH-C1INH groups, in animals treated with the C1-INHIBITOR TIM3, increased incidences of hunched posture and piloerection were noted.

Body Weight: No effects were observed on body weights or body weight gain in the rH-C1INH groups. Bodyweights improved during the observation period. In the group treated with the C1-INHIBITOR TIM3 a decreased body weight gain was observed during treatment in males (day 12, 14) and females (day 5, 12, 14).

Ophthalmology: There were no treatment-related ophthalmological findings.

Food consumption: Food consumption was increased between days 5-8 for group 4 males and relative food consumption (Le, after correction for body weight) was increased between days 1-5 for group 3 females and group 4 males and females. No further effects on (relative) food consumption were observed in the rH-C1INH treated groups.

Both parameters were decreased for animals treated with C1-INHIBITOR TIM3 between days 12 to 14 of treatment among both sexes. In addition, relative food consumption was decreased in females between days 5 and 8 of treatment. All values returned to normal during the observation period.

CLINICAL CHEMISTRY

Measurement related to	PARAMETERS	NOTE
ELECTROLYTE BALANCE	Calcium, chloride, phosphorus potassium, sodium	Normal relative to control
CARBOHYDRATE METABOLISM	Glucose	Normal relative to control
A) HEPATOCELLULAR	Alanine aminotransferase (ALT or SGPT)	Normal relative to control,
	Aspartate aminotransferase (AST or SGOT)	Lower at group 5 (rH-C1INH-TIM3) in both sex but return to normal at recovery groups.
	Glutamate dehydrogenase Sorbitol dehydrogenase	NC
	Alkaline phosphatase (ALP)	Normal relative to control
	Gamma-glutamyl transferase (GGT)	NC

Measurement related to	PARAMETERS	NOTE
B) HEPATOBILIARY	Total bile acids	NC
	Total bilirubin	Normal relative to control in HC1INH treated group, but higher in rH-C1INH-TIM3-treated group
ACUTE PHASE REACTANTS	C-reactive protein	Normal relative to control
	fibrinogen (also under coagulation),	NC
KIDNEY FUNCTION	Creatinine	Normal relative to control
	Blood urea nitrogen	Normal relative to control
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Albumin (A)	Normal relative to control
	Globulin (G, calculated) or A/G Ratio	Normal relative to control
	Total cholesterol	Normal relative to control
	Cholinesterase	NC
	Total protein	Normal relative to control
	Fasting triglycerides	Normal relative to control
MUSCLE INJURY	CK-MB CK-MM CK-BB	NC

Table of Clinical Chemistry Result Comments: No changes occurred in clinical biochemistry in groups treated with rH-C1INH. After 14 days, serum concentrations of total bilirubin (males and females), glucose, and total globulin (males) were increased in animals dosed with the C1-INHIBITOR TIM3. At the end of observation period all effects on clinical biochemistry parameters had resolved in the C1-INHIBITOR TIM3 group,

STN-12495

HEMATOLOGY

MEASURE MENT RELATED TO	PARAMETERS	NOTE
RED BLOOD CELLS	Hematocrit (Hct) Hemoglobin Conc. (Hb) Total Erythrocyte Count (RBC) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Reticulocytes	Normal relative to control in rH- C1INH-treated groups, but lower in rH-C1INH-TIM3 group at day14 and return to normal at recovery Normal relative to control NC
WHITE BLOOD CELLS	lymphocyte count Neutrophil count Basophils, eosinophils count Monocyte/macrophage (day 24) Total leukocytes (WBC) Large unstained cells (LUC)	Normal relative to control NC Normal relative to control Normal relative to control in rH- C1INH-treated groups, but higher in rH-C1INH-TIM3 group at day14 and return to normal at recovery Normal relative to control
CLOTTING POTENTIAL	Fibrinogen Prothrombin time (PT) Activated partial thromboplastin time (APTT)	NC Normal relative to control Normal relative to control
OTHERS	Bone marrow cytology	NC

Table of Hematology Result Comments: No treatment-related changes in hematology parameters were found after treatment with rHC1INH. After 14 days, red blood cell count, hemoglobin, hematocrit (males and females), mean corpuscular volume, mean corpuscular hemoglobin. Platelet and monocyte count (all in females) were decreased and white blood cell count (males) was increased in the group treated with the C1-INHIBITOR TIM3.

Urine analysis: No clear treatment-related effects were seen after treatment with rH-C1INH or C1-INHIBITOR TIM3. After 14 days, urinary sodium concentration was decreased in males receiving 625 U/kg rHC1INH, and low potassium excretion was observed in males receiving the C1-INHIBITOR TIM3.

Systemic toxicity: There were no changes at necropsy which were considered to be associated with the test item at the end of both periods.

Gross Pathology: Animals of the rH-C1INH dose groups showed no treatment-related macroscopic changes. At necropsy, increased incidences of enlargement of the spleen (males and females) and foci on the lungs (females) were observed in group 5 (C1-INHIBITOR TIM3).

Group	Findings
1(Control)	No significant findings
2 (rH-C1IHN 25u/kg/day)	No significant findings
3 (rH-C1IHN 125U/kg/day)	No significant findings
4 (rH-C1IHN 6500U/kg/day)	No significant findings
5 (rH-C1IHN-TIM3 6500U/kg/day)	Enlarged spleen but return to normal at recovery

Microscopic examination:

Main group

RH-C1INH:

No findings were directly attributable to rH-C1INH, Procedure-induced findings.

Secondary effects of the infusion cannula and the method of tail restraint were generally comparable in control and rH-C1 NH treated groups.

C1-INHIBITOR TIM3:

No findings were directly attributable to C1-INHIBITOR TIM3, but there was a small treatment-related increase in incidence and/or severity of lesions in C1-INHIBITOR TIM3-treated animals compared to controls. A range of observations were related to the lesions provoked by the infusion cannula and the method of tail restraint. These were slightly more frequent/severe in C1-INHIBITOR TIM3 treated animals than in control.

Recovery group:

RH-C1INH - There were no treatment-related findings.

C1.INHIBITOR TIM3 - Heart: Minimal endothelial proliferation was present in 1/6 animals receiving C1-INHIBITOR TIM3.

Liver - Minimal histiocytosis was present in 1/6 control animals; slight histiocytosis was present in 1/6 animals receiving C1-INHIBITOR TIM3.

Study number (PCL-R-03-040.A01): rhGl INH Dose range finding study by the intravenous route in the cynomolgus monkey

Performing laboratory:

Test facility: -----(b)(4)-----

Test site: -----(b)(4)-----

Study initiation date: 26 May 2006

Final Report date: 02 February 2001

Test article batch/lot:

rH-C1INH (DP-CZ067): 144 U/mL

Control Vehicle(103203): Water for injection (----- (b)(4) -----)

Animal species and strain: Cynomolgus monkeys (*Macaca fascicularis*)

Breeder/supplier: -----(b)(4)-----.

Number of animal per group and sex: 2 (1 male and 1 female)

Age: 30 months

Body weight range: Male: 2.68 kg, Female: 2.33 kg.

Route and site of administration: Intravenous (short *b.i.d.* infusions). The animals were maintained in a restraining chair during infusion.

Volume of injection: Volume of 3.47, 6.94 and 13.89 mL/kg over 15 minutes at the dose levels 1000, 2000, 4000 and 6000 U/kg/day and volume of 20.83 mL/kg over 30 minutes at.

Frequency of administration and study duration: Twice daily with an interval of approximately 6 hours on days 0, 4, 7 and 11.

Dose: 1000, 2000, 4000, 6000 U/kg

Means of administration: Intravenous injection using an infusion pump attached to a microflex infusion set introduced into a vein after local disinfection with an aqueous solution of ethyl alcohol. The right cephalic and the right external saphenous vein used in rotation.

Report status: Final report

Summary

An escalating dose of RH-C1INH was administered to male and female cynomolgus monkey at dose levels of 1000, 2000, 4000 and 6000 U/kg/day given bid with a 6 hour interval and separated by 2 or 3 day of washout.

There was no mortality and no clinical signs considered related to treatment throughout the study. Hemorrhagic infiltration was noted at the injection sites as of the second day of treatment. The male tended to eat less food than pretest after the third treatment day (4000 U/kg) and after the fourth treatment day (6000 U/kg). There was a decrease in red blood cell count, hemoglobin concentration and packed cell volume, in both animals and a slight increase in reticulocyte count for the female, compared with pretest. Total bilirubin and serum aspartate aminotransferase activity were increased in both sexes at the end of the treatment period compared with pretest. At necropsy, the only finding possibly treatment related was a mottled aspect of the liver, for the male.

Experimental design

	Treatment days			
	0	4	7	11
Dose level (U/kg/day) ⁽¹⁾	1000 (2 x 500)	2000 (2 x 1000)	4000 (2 x 2000)	6000 (2 x 3000)
Dose concentrations (U/mL)	144	144	144	144
Flow rate (mL/kg/min)	0.231 (2)	0.463 (2)	0.926 (2)	0.694 (3)

(1) half of the dose given as a short infusion *b.i.d.* at 6 hour interval

(2) 15 minute infusion

(3) 30 minute infusion

Methods:

Endpoint	Methodology
Hematology	----- (b)(4) -----
Clinical chemistry	----- (b)(4) ----- -----
Coagulation	

Statistical analysis plan:

The following statistical methods were used to analyse the data:

- If the variables could be assumed to follow a normal distribution, the Dunnett-test (many-to-one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control group for each sex.
- The Steel-test (many-to-one rank test) was applied instead of the Dunnett-test if the data could not be assumed to follow a normal distribution.
- The Fisher Exact-test was applied to frequency data.

All tests were two-sided and in all cases $p \leq 0.05$ was accepted as the lowest level of significance.

Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables.

Test statistics were calculated on the basis of exact values for means and pooled variances. Individual values, means and standard deviations may have been rounded off before printing. Therefore, two groups may display the same printed means for a given parameter, yet display different test statistics

The following parameters were evaluated:

<i>Parameters</i>	<i>Frequency of Testing</i>
Cageside observation	Twice a day
Clinical observations	Daily, During the treatment period, animals were examined before and at least once after each dosing.
Body weight	days -7 and -3, on the first day of treatment (day 0) and at each dose change
Food consumption	daily, starting one week before initiation of treatment
Body temperature	NC
Blood pressure	NC
Clinical chemistry	once pretest (day -13) and at the end of the treatment period (day 12)
Hematology	once pretest (day -13) and at the end of the treatment period (day 12)
Coagulation	NC
Immunological response	NC
Evaluation of site of inoculation	NC
Necropsy	Two days after the last dose
Tissues for histopathology	Two days after the last dose
Urine	NC

(NC = not collected)

The following tissues and organs were collected from all animals at necropsy

system	ORGAN COLLECTED	ORGAN NOT COLLECTED
digestive	<i>liver, pancreas, stomach</i>	<i>esophagus, gall bladder, Large intestine (caecum, colon, rectum), small intestine (duodenum, jejunum, ileum) , salivary gland, mandibular, sublingual</i>
RESPIRATORY	<i>Lung (with main-stem bronchi), trachea,</i>	<i>nasal turbinate,s, trachea bifurcation</i>
CARDIOVASCULAR	<i>heart</i>	<i>aorta</i>
IMMUNOLOGIC/ HEMATOPOIETIC	<i>bone with marrow(sternum), thymus</i>	<i>bone with marrow (femur), lymph node (mandibular, mesenteric), spleen, lymph nodes(1 related to route of administration, and 1 from a distant location),</i>
UROGENITAL	<i>kidney, prostate, testes, ovaries</i>	<i>uterus, fallopian tubes, seminal vesicle, urinary bladder, uterus (with cervix), vagina, epididymis</i>
NEUROLOGIC		<i>Brain (cerebrum, cerebellum, medulla/pons), optic nerve, sciatic nerve, spinal cord (cervical, lumbar, mid-thoracic)</i>
HORMONAL	<i>adrenals</i>	<i>thyroid (with parathyroid glands), pituitary glands, mammary glands, Zymbal's Gland</i>
OTHER		<i>Harderian gland (if</i>

system	ORGAN COLLECTED	ORGAN NOT COLLECTED
		<i>present), eyes, , skin, tongue, skeletal muscle, brain</i>
GROSS LESIONS	All gross lesions	
INJECTION SITE OR SITE OF APPLICATION	Not taken	

Results:

Morbidity and mortality: No treatment related mortality occurred.

Clinical Signs: There were no clinical signs considered treatment-related. At detailed clinical examination, a slight hair loss on right hind limb was noted for the male and a sore on the tail was noted for the female at termination.

Body Weight

The male tended to eat less food than pretest after the third treatment day (4000 U/kg) and after the fourth treatment day (6000 U/kg). There was no effect of treatment on body weight and body weight gain at any time.

CLINICAL CHEMISTRY

	Male		Female	
	Day-13	Day 12	Day-13	Day 12
Gluc (mmol/L)	3.52	3.44	3.14	3.98
Urea (mmol/L)	5.17	5.64	5.23	5.23
Chol (mmol/L)	3.71	3.04	3.74	3.16
T. Bil (mcmol/L)	3.4	7.3	2.4	3.3
Prot (mcmol/L)	75	65	77	68
Creat (mcmol/L)	64	62	56	52
ALP (U/L)	1812	2167	1073	1238
ASAT (U/L)	30	188	32	153
ALAT (U/L)	31	41	39	38

Table of Clinical Chemistry Result Comments: ASAT was increased 6-fold increased for the male and 5-fold for the female. Bilirubin was doubled for the male and increased by 38 % for the female.

HEMATOLOGY

	Male		Female	
	Day-13	Day 12	Day-13	Day 12
RBC (T/L)	7.19	4.46	6.5	4.37
Hb (g/L)	132	84	115	78
PCV (%)	45.2	28.1	42.1	28.2
MCV (fL)	62.9	63.1	64.8	64.7
MCH (pg)	18.3	18.8	17.7	17.8
MCHC (g/L)	292	298	274	276
Reti (%)	0.1	0.2	0.2	2.2
Plat (G/L)	472	501	537	580
WBC (G/L)	8.99	6.35	6.68	7.14
N (G/L)	4.64	2.88	2.44	3.53
N (%)	51.6	45.3	36.6	49.4
L (G/L)	3.87	2.5	3.63	2.73
L (%)	43.1	39.4	54.4	38.3
M (G/L)	0.28	0.36	0.24	0.24
M (%)	3.1	5.7	3.6	3.4
E (G/L)	0.11	0.43	0.25	0.49
E (%)	1.2	6.7	3.7	6.8
B (G/L)	0.03	0.03	0.04	0.02
B (%)	0.3	0.5	0.6	0.3
Luc (G/L)	0.06	0.15	0.08	0.13
Luc (%)	0.7	2.4	1.2	1.8

Table of Hematology Result Comments: There was a decrease in red blood cell count, haemoglobin concentration and packed cell volume at the end of the treatment period compared with pretest. Reticulocyte count was increased for the female. Eosinophil count in both sexes and monocyte count in male no. 2001 were increased at the end of the treatment period compared with pretest. However the values remained within the normal range of background data for the species

Systemic toxicity: No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), clinical chemistry, gross anatomy or organ weight were found.

Gross Pathology: Liver pale/mottled was noted in the male.

Study number (PCL-R-03-043): rhC1-INH- 2-week intravenous (twice daily 15-minute infusion) toxicity study in the cynomolgus monkey followed by a 2-week treatment-free period.

Performing laboratory:

Test facility: -----(b)(4)-----

Test site: -----(b)(4)-----

Study initiation date: 05 June 2007

Final Report date: 23 April 2008

Test article batch/lot:

rhC1INH (DP-CZ193 and MA002): 144 U/mL

Control Vehicle: Water for injection (----- (b)(4) -----)

Control item: Sterile physiological saline (0.9 % NaCl) (----- (b)(4) -----
-----)

Animal species and strain: Cynomolgus monkeys (*Macaca fascicularis*), purpose bred animals

Breeder/supplier: ----- (b)(4) -----.

Number of animal per group and sex: 42 (21 males and 21 females)

Age: Males: 31 to 35 months, females: 32 to 35 months.

Body weight range: Males: 2.3 to 3.1 kg, females: 2.2 to 2.7 kg.

Route and site of administration: Intravenous (short infusion). A silastic catheter was implanted into the posterior vena cava via the right femoral vein. The remaining part of the catheter was tunneled subcutaneously to exit at the inter-scapular region. The catheter was attached to the delivery system via a tether system and a swivel joint. Infusions were performed with --- (b)(4) --- type 44 infusion pumps.

Volume of injection: 1.74 to 13.89 mL/kg over 15 minutes, for each administration

Frequency of administration and study duration: Twice daily with an interval of approximately 6 hours. Males and recovery animals: 14 days, Females: 15 days.

Dose: 0, 250, 500, 1000, 2000 U/kg/administration

Means of administration: Intravenous injection using an infusion pump attached to a microflex infusion set introduced into a vein after local disinfection with an aqueous solution of ethyl alcohol. The right cephalic and the right external saphenous vein used in rotation.

Report status: Final report

Summary

The toxicity of the test item (rhC1INH) was tested using the cynomolgus monkey. During the acclimatization period, a -(b)(4)- catheter was implanted into the posterior vena cava via the right femoral vein and the animals were used to determine the toxicity of the test item (rhC1INH) in the cynomolgus monkey following twice daily intravenous administration (15-minute infusion) for 2 weeks, to evaluate the regression of any toxic signs during a 2-week treatment-free period and to assess systemic exposure under the defined experimental conditions. No mortality occurred during the study. There were neither clinical signs nor any local reaction that could be related to treatment with the test item. The body weight and food consumption were not affected by treatment with the test item. There were no relevant ophthalmological findings. The cardiovascular functions were not affected by treatment with the test item. The test substance induced an increase in serum ALP and ASAT levels at 500 and 1000 U/kg/adm (up to 2 fold pretest values) and at 2000 U/kg/adm (up to 5 fold pretest values). There were no histopathological correlates at any dose. At the end of the recovery period, day 26, ALP and ASAT values for all treated groups were similar to pre-test values indicating the treatment-related effect on these parameters was reversible. Dose-related histopathological changes (microvacuoles in epithelial cells lining the renal tubules) were noted in the kidneys at 500 to 2000 U/kg/adm. The effects were minimal at 500 U/kg/adm but increased in severity and frequency at doses up to 2000 U/kg/adm. Following the treatment-free period, these changes were completely reversed at doses up to 1000 U/kg/adm. Given that only one high dose recovery animal (2000 U/kg/administration) had slight microvacuolation. The kidney is considered to be a target organ, and in view of the reversible, minor nature of the changes at doses up to 1000 U/kg/adm, possibly related to the accumulation of metabolically stable test substance in renal cells, only the high dose of 2000 U/kg/adm is considered to be an adverse effect level. Under the defined experimental conditions, the NOAEL (no observed adverse effect level) is considered to be 1000 U/kg/administration.

Experimental design

Group/Treatment	Dose (U/kg)	Volume (mL/kg)	Dose concentration (U/mL)	Number of animals			
				Week 3 ⁽¹⁾		Week 5 ⁽²⁾	
				Males	Females	Males	Females
1. Control	0	13.89	0	3	3	2	2
2. Low dose	250	1.74	144	3	3	/	/
3. Intermediate dose 1	500	3.47	144	3	3	/	/
4. Intermediate dose 2	1000	6.94	144	3	3	2	2
	2000	13.89	144	3	3	2	2
5. High dose							

(1): killed at the end of the treatment period.

(2): killed at the end of the treatment-free period.

adm.: administration.

/: not applicable.

Group 1 animals (control) received the control item (0.9 % NaCl).

Statistical analysis plan:

The following parameters were analyzed statistically for males and females separately:

- body weights on days -1, 6 and 13 and body weight gains over days -1 to 6, 6 to 13 and -1 to 13,
- heart rate, systolic and diastolic blood pressure on days -7, 2 and 12,
- hematology, coagulation, serum clinical chemistry and urinary volume, pH and specific gravity on days -9/-8 and 12/13,
- terminal body weight, absolute and relative organ weights at termination.

Groups with a sample size of less than three are excluded from the analysis for the parameter(s) concerned (where applicable).

For each parameter (except terminal body weights and organ weights), Levene's test was used to test the equality of variances across groups and Shapiro-Wilk's test was used to assess the normality of the data distribution in each group.

Data showing homogeneous variances across groups and normal distribution in all groups were analyzed using parametric procedures. Such analysis consisted of a one way analysis of variance (ANOVA) allowing for a group effect, followed, if the ANOVA test

was significant, by Student's t-test (pre-treatment data) or Dunnett's test to assess the significance of any differences between treated and control groups.

Data showing non-homogeneous variances across groups or a non-normal distribution in at least one group were analyzed using non-parametric methods. Such analysis consisted of the Kruskal-Wallis test, followed by the Wilcoxon's rank sum test if the Kruskal-Wallis test was significant.

These analyses were performed using a SAS software package.

For terminal body weights and organ weights, statistical analysis was performed by the data acquisition system (-----b(4)-----) as follows: Kolmogorov's test for normality of the data distribution in each group and Bartlett's test for homogeneity of variances across groups, followed by ANOVA and Dunnett's test for data showing equality of variances and normal distribution, or Kruskal-Wallis test and Dunn's test for data with non-homogeneous variances or non-normal distribution. Statistically significant results for pairwise comparisons are indicated on the tables next to the appropriate mean value, using the following abbreviations: *: p (probability) ≤ 0.05 or * or # (non-parametric): $p \leq 0.05$, **: $p \leq 0.01$ ** or ##: $p \leq 0.01$, ***: $p \leq 0.001$ for terminal body weights and organ weights. The ANOVA results are not reported.

The following parameters were evaluated:

<i>Parameters</i>	<i>Frequency of Testing</i>
Cageside observation	Twice a day
Clinical observations	Daily, During the treatment period, animals were examined before the first daily dosing, between each daily dosing and after the second daily dosing to detect any clinical signs or reaction to treatment. During the treatment-free period, animals were observed once daily.
Body weight	twice weekly pretest and once weekly during the treatment and treatment-free periods
Food consumption	daily
Ophthalmological examination	All animals: - once pretest, - once during week 2, - at the end of the treatment-free period (recovery period).
Body temperature	once before the initiation of treatment, once during week 2, once at the end of the treatment-free period
Cardiovascular examinations	All animals: - once pretest, after implantation (day -7), - on day 2 (third day of dosing) and on day 12: - before the first daily infusion, - 1 hour after the end of the second daily infusion. Recovery animals only: - on day 26.
Clinical chemistry	All animals:

<i>Parameters</i>	<i>Frequency of Testing</i>
	<ul style="list-style-type: none"> - once pretest, after implantation (day -9/-8 for males and females, respectively), - on day 12/13 (for males and females, respectively), Recovery animals only: <ul style="list-style-type: none"> - on day 26.
Hematology	All animals: <ul style="list-style-type: none"> - once pretest, after implantation (day -9/-8 for males and females, respectively), - on day 12/13 (for males and females, respectively), Recovery animals only: <ul style="list-style-type: none"> - on day 26.
Coagulation	All animals: <ul style="list-style-type: none"> - once pretest, after implantation (day -9/-8 for males and females, respectively), - on day 12/13 (for males and females, respectively), Recovery animals only: <ul style="list-style-type: none"> - on day 26.
Immunological response	All animals: <ul style="list-style-type: none"> - on days 0 and 13: - before the first daily infusion, - before the second daily infusion, - at the end of the second daily infusion, - 24 hours after the end of the first daily infusion (before the first infusion on that day). Recovery animals only: <ul style="list-style-type: none"> - at the end of the treatment-free period (day 26).
Evaluation of Implantation sites	daily during pretest and the treatment period
Necropsy	On the day after the last dose (day 14/15 for males and females, respectively) or at the end of the treatment-free period (day 28)
Tissues for histopathology	On the day after the last dose (day 14/15 for males and females, respectively) or at the end of the treatment-free period (day 28)
Urine	All animals: <ul style="list-style-type: none"> - once pretest, after implantation (day -9/-8 for males and females, respectively), - on day 12/13 (for males and females, respectively), Recovery animals only: <ul style="list-style-type: none"> - on day 26.

(NC = not collected)

The following tissues and organs were collected from all animals at necropsy

system	ORGAN COLLECTED	ORGAN NOT COLLECTED
digestive	<i>liver, pancreas, stomach, gall bladder, Large intestine (caecum, colon, rectum) , small intestine (duodenum, jejunum, ileum) , salivary gland, (mandibular, parotid, sublingual), esophagus</i>	
RESPIRATORY	<i>Lung (with main-stem bronchi), trachea,</i>	<i>nasal turbinate, trachea bifurcation</i>
CARDIOVASC ULAORTA	<i>Heart, aorta</i>	
IMMUNOLOGIC/ HEMATOPOIETIC	<i>bone with marrow(sternum), thymus, bone with marrow (femur)and articulation, lymph node (mandibular, mesenteric), spleen,</i>	<i>lymph nodes(1 related to route of administration, and 1 from a distant location),</i>
UROGENITAL	<i>kidney, prostate, testes, ovaries and oviducts, uterus, seminal vesicle, urinary bladder, uterus (with cervix) , vagina, epididymis</i>	<i>fallopian tubes</i>
NEUROLOGIC	<i>Brain (cerebrum, cerebellum, medulla/pons), optic nerve, sciatic nerve, spinal cord (cervical, lumbar, mid-thoracic)</i>	
HORMONAL	<i>adrenals thyroid (with parathyroid glands), pituitary glands, mammary glands</i>	<i>Zymbal's Gland</i>
OTHER	<i>eyes, skin, tongue, skeletal muscle</i>	<i>Harderian gland (if present)</i>
GROSS LESIONS	All gross lesions	
INJECTION SITE OR SITE OF APPLICATION	Implantation site. A sample was taken from the catheterized vein (corresponding to the end of the catheter) and another sample was taken 1.5 cm upstream of this point. One section was cut and examined from each block)	

Results:

Morbidity and mortality: No mortality occurred during the study.

Clinical Signs: The clinical signs including liquid faeces, rectal prolapse, vomit, swelling of a toe were noted but there were no dose-relationship. The sore on the neck and the scabs on the back seen in some animals were related to the administration procedure (damage produced by the jacket).

Implantation site observations: There was no local reaction that could be related to treatment with the test item. All the local reactions noted (including redness, induration or tumefaction) were related to the implantation procedure.

Ophthalmology: There were no ophthalmological findings which could be related to treatment with the test item.

Body Weight: When compared with the control group, a slightly lower body weight gain was noted over the whole treatment period in males given 2000 U/kg/administration (10 grams vs. 130 grams).

Food consumption: The food consumption was similar between control and treated animals.

Cardiovascular examinations: Arterial blood pressure : Systolic arterial pressures were increased 1 hour after transfusion in males given 2000 U/kg/administration but not in other group. The increases were not significant.

Heart rate: There were no relevant variations at the end of the treatment-free period in any animals.

Rhythm and cardiac conduction: No treatment-related effects occurred on PR or QT interval and QRS complex duration.

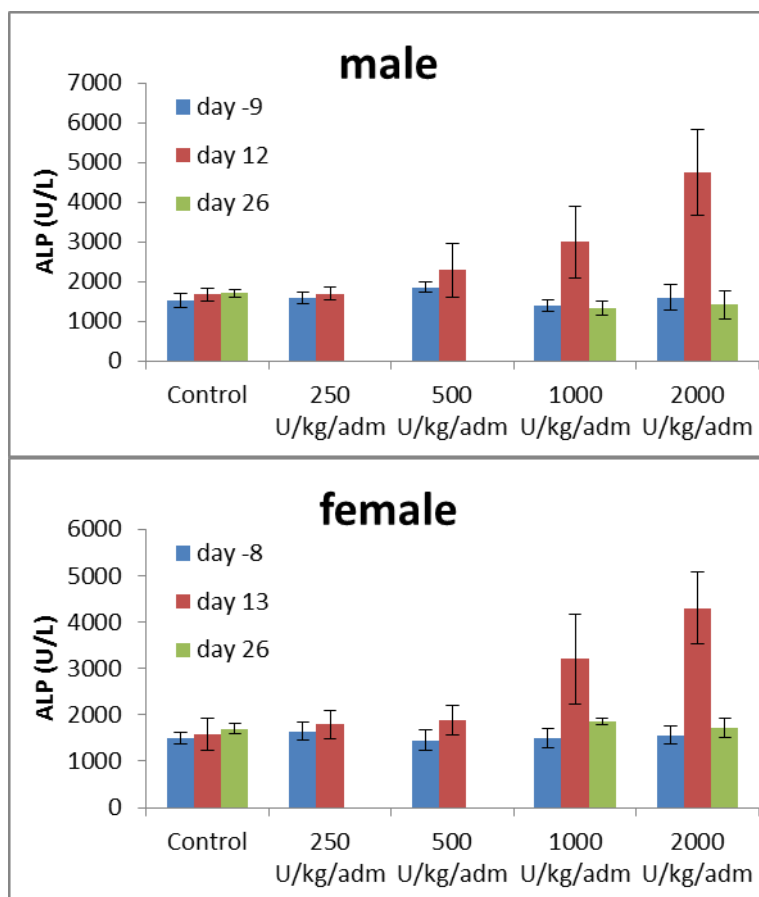
CLINICAL CHEMISTRY

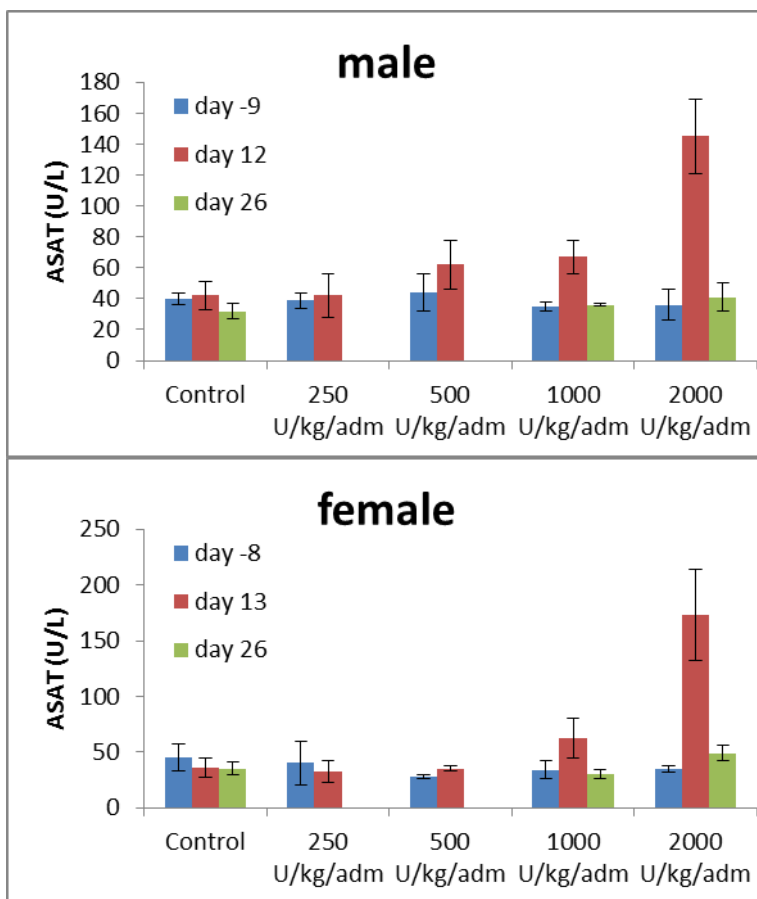
MEASUREMENT	PARAMETERS	NOTE
ELECTROLYTE BALANCE	Calcium, chloride, phosphorus potassium, sodium	Normal relative to control
CARBOHYDRATE METABOLISM	Glucose	Normal relative to control
A) HEPATOCELLULAR	Alanine aminotransferase (ALT or SGPT)	Normal relative to control
	Aspartate aminotransferase (AST or SGOT)	higher in 1000 and 2000 u/kg/ad treated groups

MEASUREMENT	PARAMETERS	NOTE
B) HEPATOBILIARY	Glutamate dehydrogenase Sorbitol dehydrogenase Total bile acids	NC
	Alkaline phosphatase (ALP)	higher on 1000 and 2000 u/kg/ad treated group
	Gamma-glutamyl transferase (GGT)	NC
	Total bile acids	NC
	Total bilirubin	Normal relative to control
ACUTE PHASE REACTANTS	C-reactive protein fibrinogen (also under coagulation),	NC NC
KIDNEY FUNCTION	Creatinine Blood urea nitrogen	Normal relative to control Normal relative to control
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Cholinesterase Total protein Fasting triglycerides	Normal relative to control Higher globulin in 1000 and 2000 u/kg/adm treated groups compare to control, but the individual values were similar to pretreated. Normal relative to control NC Normal relative to control NC
MUSCLE INJURY	CK-MB CK-MM CK-BB	NC

Table of Clinical Chemistry Result Comments: an increase in ALP activity was noted in males and females given 500, 1000 and 2000 U/kg/adm (statistically significant at 1000 and 2000 /kg/adm). An increase in ASAT activity was noted in males given 500,

1000 and 2000 U/kg/administration (statistically significant at 2000 U/kg/adm), and in females given 1000 and 2000 U/kg/adm (statistically significant at 1000 and 2000 U/kg/adm). These changes were no longer noted after a 2-week treatment-free period.





A significantly higher globulin serum concentration, associated with a significantly lower albumin/globulin ratio, was noted in females at 250, 1000 and 2000 U/kg/adm. This was no longer noted after a 2-week treatment-free period. However, this change was not dose-related and the individual values were similar to pretest values.

HEMATOLOGY

MEASURE MENT RELATED TO	PARAMETERS	NOTE
RED BLOOD CELLS	Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes	Normal relative to control Normal relative to control
WHITE BLOOD CELLS	lymphocyte count Neutrophil count Basophils, eosinophils count Monocyte/macrophage (day 24) Total leukocytes (WBC) Large unstained cells (LUC)	higher in female infused with 1000 and 2000 u/kg/ad treated groups at day 13, and return to normal at day 26 Normal relative to control Normal relative to control Normal relative to control Normal relative to control
CLOTTING POTENTIAL	Fibrinogen Prothrombin time (PT) Activated partial thromboplastin time (APTT)	NC Normal relative to control Normal relative to control
OTHERS	Bone marrow cytology	NC

Table of Hematology Result Comments: a statistically significantly lower absolute lymphocyte count was noted in group 4 and 5 females after 2 weeks of dosing. At the end of the 2-week treatment-free period, a decrease in total white blood cell count involving neutrophil count was noted in group 4 and 5 males. This was not noted in females and the values remained within the normal background control range.

Urine analysis: There were no changes in any parameters at any dose levels, which could be related to treatment with the test item.

Bioanalysis and antibody determination: The determination of rhC1INH and the IgG response against rhC1INH in plasma showed the exposure of the animals to the test item.

Systemic toxicity: No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), clinical chemistry, gross anatomy or organ weight were found.

Organ weight and terminal body weight: Thymus - mean absolute and relative thymus weights were lower than controls in all female treatment groups. For the absolute weight, this was dose-related. In males, mean absolute and relative thymus weights were higher than controls in all treatment groups and this was unrelated to the dose-level.

Spleen - All individual as well as the mean absolute and relative spleen weights were lower than controls in males at 2000 U/kg/adm. and females at 1000 or 2000 U/kg/adm. and the differences were dose-related.

Macroscopic findings: Spleen - The spleen appeared enlarged in male no. 2633 given 2000 U/kg/adm. The other macroscopic changes seen at the end of the 2-week treatment period were considered, from their incidence and nature, to be incidental.

Microscopic findings: Terminal sacrifice

Kidney - Dose-related minimal to severe, microvacuolation of the cytoplasm in epithelial cells lining the tubules was seen in males and females given 1000 or 2000 U/kg/adm. Male no. 2633 given 2000 U/kg/adm. had also slightly increased cellularity in the renal corpuscles with infiltration of the mesangium by vacuolated cells.

Thymus - A decrease in size of the cortical region of the thymus was seen in all groups. In controls, it was minimal, whereas, in treated animals it was minimal to moderate and dose-related. The severity was slightly higher in females.

Spleen - Minimal to slight increase in size of lymphoid follicles was seen in one control female and in treated animals at all dose levels and the incidence slightly increased toward 2000 U/kg/adm.

Lung - Inflammatory changes and perivascular mixed cell infiltration were seen in treated animals the severity being generally minimal and unrelated to the dose-level. Male no. 2633 given 2000 U/kg/adm. had a slight leucocytosis with intravascular accumulation of vacuolated cells consistent with macrophages.

Injection site and non-catheterized vein - Thrombus formation, changes in the vessel wall and inflammation in the perivascular tissue were seen at the injection site or in the vein sample collected downstream (non-catheterized vein) in both control and treated animals.

Liver - Male no. 2633 given 2000 U/kg/adm. had increase in sinusoid cellularity in the liver.

Study number (PCL-R-03-014): rhC1INH: Dose range finding study by the intravenous route in the pregnant rabbit

Performing laboratory:

Test facility: -----(b)(4)-----

Test site: -----(b)(4)-----

Study initiation date: 07 May 2003

Final Report date: 22 August 2005

Test article batch/lot:

rhC1-INH

- 04I01001: 144.8 units/ml (taken as 145 Units/ml for dose calculation),
- 04I01003: 146 units/ml (taken as 145 Units/ml for dose calculation),
- 04I02001: 142.3 units/ml,
- 04I03002: 150.8 units/ml,
- 04I03003: 145 units/ml.

Placebo - Sterile 20 mM citric acid buffer with 6.5 % sucrose, pH 6.8

Vehicle - Water for injection

Animal species and strain: -----(b)(4)----- rabbit, (b)(4)

Breeder/supplier: -----(b)(4)-----

Number of animal per group and sex: 44 primiparous females

Age: 17 to 19 weeks

Body weight range: 3 to 4.0 kg

Route and site of administration: intravenous into a margin ear vein

Volume of injection: approximately 4.5 ml/kg/day (the exact dose volume was adjusted to give the required dose level of 625 units/kg/day according to the concentration of the batch of test item in use).

Frequency of administration and study duration: Once daily, from day 6 (G6) to day 19 (G19) of gestation

Dose: 625 U/kg/day

Stability: at least 5 hours at room temperature

Means of administration: intravenous injection using a microflex infusion set introduced into an ear vein and a syringe mounted on an infusion pump.

Report status: Final report

Summary

Intravenous administration of rhC1INH caused slight maternal toxicity in the rabbit, as characterized by minor influences on maternal body weight gain and food consumption. There were, however, no adverse influences on the course and outcome of pregnancy under the conditions of this study, except for an equivocal slight delay in fetal ossification.

The detailed skeletal examinations revealed indications of delayed fetal ossification in the paws of the fetuses from the treated group. The severity of the observed abnormalities was minor, however, and was not considered to be indicative of any permanent deformity.

Experimental design

Group/ Treatment	Dose level (Units/kg/day	Dose volume*	Dose concentration
1. Control	0	4.5	0
2. Treated	625	4.5	140*

* Approximate 4.14 to 4.4 ml/kg/day (depending on concentration of batch of test item).

- Group 1 animals (control) received the placebo (see section 3.2.)
- Rationale for the dose selection: expected to result in a sufficient safety margin in terms of AUC with respect to the anticipated use in the human.

Methods:

Endpoint	Methodology
Hematology	
Clinical chemistry	
Coagulation	

Randomization procedure:***The following parameters were evaluated:***

Parameters	Frequency of Testing
Cageside observation	Twice a day
Clinical observations	observed once before and once after treatment
Body weight	days 0, 6,9, 13, 16,20,24 and 29 of gestation
Food consumption	the periods (days) of: 0 to 6,6 to 9,9 to 13, 13 to 16, 16 to 20,20 to 24 and 24 to 29 of gestation
Body temperature	NC
Blood pressure	NC
Ophthalmological examination	NC

Parameters	Frequency of Testing
Clinical chemistry	NC
Hematology	NC
Coagulation	NC
Immunological response	NC
Evaluation of site of inoculation	NC
Necropsy	day 29 of gestation
Tissues for histopathology	day 29 of gestation
Urine	NC

(NC = not collected)

The following tissues and organs were collected from all animals at necropsy

The ovaries and uterus were removed and examined, including examination of the placentae.

The following data were recorded:

- pregnancy status,
- number of corpora lutea,
- number and distribution of live fetuses,
- number and distribution of embryonic/fetal deaths,
- individual fetal weights,
- fetal sex (except for group 1 female no. 4324: one fetus not sexed in error)
- macroscopic evaluation of placentae.

Embryonic/fetal deaths were classified as:

- early: only placenta visible at termination,
- late: both placenta and embryonic tissue visible at termination.

Fetal examinations

All fetuses were examined visceraally and sexed at the time of cesarean section.

Results:

Morbidity: There was no mortality during the study.

Clinical Signs: There were no treatment-related changes in clinical condition during the study.

Body Weight: The rabbits given rhC1INH showed a slightly reduced mean body weight gain throughout the treatment period and the mean terminal body weight on day 29 of gestation was on average 5.7% lower in the treated group than in the control group.

Food consumption: Maternal food consumption was slightly reduced in the treated group throughout the treatment period.

Necropsy finding of adult females: Necropsy examination of the female rabbits did not reveal any treatment-related lesions.

Pregnancy incidence: There were 20 and 21 pregnant rabbits in the treated and control groups, respectively.

Pre-implantation data: The pre-implantation data, including numbers of corpora lutea, percentage pre-implantation loss and the resulting numbers of uterine implantation sites, were comparable in both groups.

Post-implantation data: The incidences of early and late resorptions and the resulting litter sizes were similar in the treated and control groups. There were no dead fetuses in either group.

Fetal data: The mean fetal weights and the sex ratio of the fetuses were not adversely influenced by treatment with rhC1INH.

Fetal examinations: External examination: The fresh external examinations of the fetuses did not reveal any obvious treatment-related abnormalities in either group. One control fetus (dam no. 4335) had multiple malformations of the head, spine, thorax and limbs.

Internal examination: In addition to the malformed control fetus identified at the external examination, which also had a gross disruption of the major thoracic blood vessels, two fetuses from separate dams given rhC1INH (nos. 4351 and 4359) were found to have major defects of the heart. The isolated nature of these malformations did not suggest an association with the test item.

The fresh internal examinations did not reveal any other soft tissue abnormalities in either group, except for one fetus from a treated dam (no. 4357) with slightly dilated renal pelvises.

Serial sectioning of the fixed fetal heads did not reveal any significant abnormalities in either group. One control fetus (dam no. 4324) had dilated IIIrd and IVth cerebral ventricles. Two fetuses from the treated group (dam nos. 4347 and 4356) had a porencephalic cyst and another treated fetus (dam no. 4350) had a vacuole in the midbrain. All of these findings in both groups are frequent observations in the rabbit and were not considered to be of any toxicological significance.

Skeletal examination

Two fetuses from each group had skeletal malformations. In addition to control fetus with multiple external abnormalities one fetus from each group (dam nos. 4325 and 4348) had defects of the thoracic vertebrae resulting in scoliosis. One fetus from a treated dam (no. 4357) had bilateral ectrodactily (missing digit).

The degree of ossification of the bones of the paws (phalanges in particular) tended to be less in the treated group than in the control group.

Study number (PCL-R-03-016): rhC1-INH - Embryo toxicity study by the intravenous route in the rat-segment II.

Performing laboratory:

Test facility: -----(b)(4)-----
-----.

Test site: -----(b)(4)-----

Study initiation date: 27 May 2003

Final Report date: 17 May 2004

Test article batch/lot:

rhC1-INH - (04I02002 then 04I01001)

Placebo: Sterile 20 mM citric acid buffer with 6.5 % sucrose, pH 6.8.

Vehicle: water for injection

Animal species and strain: -----(b)(4)----- rat s- -----(b)(4)-----

Breeder/supplier: -----(b)(4)-----

Number of animal per group and sex:

Main groups: 50 time-mated females

Satellite groups: 6 time-mated females.

Age: 10 to 12 weeks

Body weight range:

Main groups: 207 to 249 g at mating,

Satellite groups: 242 to 280 g on day 6 of gestation.

Route and site of administration: Intravenous injection via the tail vein.

Volume of injection: 4.2 or 4.4 mL/kg/day

Frequency of administration and study duration: Once daily, from day 6 to day 17 (G17) of gestation

Dose: 625 U/kg/day

Stability: Stability of the reconstituted test item: at least 5 hours at room temperature

Means of administration: Slow intravenous injection using a pump and a microflex infusion set introduced into a tail vein (0.5 mL/minute).

Report status: Final report

Summary

Intravenous administration of rhC1-INH caused a slight clinical reaction in the pregnant rat, characterized by transient swelling of the muzzle and limbs after dose administration. There were, however, no adverse influences on the course and outcome of pregnancy under the conditions of this study.

Experimental design

Group/Treatment	Dose Level units/kg/day	Dose Volume mL/kg/day	Dose Concentration (units/ml)
1. Control (placebo)	0	4.2 or 4.4	0
2. Rh-C1-INH	625	4.2 or 4.4	149 or 142.3

The following parameters were evaluated:

Parameters	Frequency of Testing
Cageside observation	Twice a day
Clinical observations	Daily, weekly full clinical examination
Body weight	days 0, 6, 11, 15, 18 and 20 of gestation
Food consumption	the periods (days) 0 to 6, 6 to 11, 11 to 15, 15 to 18 and 18 to 20 during gestation
Body temperature	NC
Blood pressure	NC
Ophthalmological examination	NC
Clinical chemistry	NC
Hematology	NC
Coagulation	NC
Immunological response	NC
Evaluation of site of inoculation	NC
Necropsy	on day 20 of gestation
Tissues for histopathology	on day 20 of gestation
Urine	NC

(NC = not collected)

The following tissues and organs were collected from all animals at necropsy

The ovaries and uterus were removed and examined, including examination of the placentae. The following data were recorded: pregnancy status, number of corpora lutea, number and distribution of live fetuses, number and distribution of embryonic/fetal deaths individual fetal weights, external fetal abnormalities, fetal sex.

Embryonic deaths were classified as: early: only placenta visible at termination, late: both placenta and embryonic tissue visible at termination.

Fetal examinations

Approximately one half of each litter was examined for visceral anomalies according to the Standard Operating Procedures of the testing facility then eviscerated. The eviscerated fetal carcasses were processed for skeletal examination. The skeletal examinations were performed following digestion of the soft tissues with aqueous potassium hydroxide, staining of the skeleton with alizarin red then passage into glycerol. The remaining fetuses were preserved in Harrisson's fluid for fixed visceral examination by the modified Wilson-Barrow technique. Fixed fetal examinations were performed under low power magnification.

Results:

Morbidity: There was no mortality during the study.

Clinical Signs: Most animal showed a dose-related effect, with a higher incidence of swelling of the muzzle and limbs in all rats given rh-C1INH. This sign persisted until the 4 hour observation in the majority of rats, but disappeared within one hour in some animals. Similar changes were observed in the majority of treated rats on the other days of treatment. The control rats were not affected. There were no other treatment-related changes in clinical condition.

Body Weight: The body weight profiles were similar in the treated and control groups throughout the study.

Food consumption: The treated and control groups consumed similar amounts of food throughout the study.

Necropsy finding of adult females: Necropsy examination of the adult female rats did not reveal any treatment-related lesions.

Pregnancy incidence: Twenty-three out of 25 females were pregnant in each of the treated and control groups.

Pre-implantation data: Percentage pre-implantation loss and the resulting numbers of uterine implantation sites - were similar in the treated and control groups.

Post-implantation data: The incidence of early resorptions and the percentage post-implantation loss were similar in the two groups. There were no late resorptions or dead fetuses in either group. All of the pregnant dams had viable fetuses at term.

Fetal data: The mean fetal weight was comparable in the treated and control groups. Fetal sex ratio was similar in the two groups.

Fetal examinations:

External observations: There were no fetuses with external abnormalities in either group.

Visceral observations: There were no fetuses with visceral malformations in either group.

Skeletal observations: There were no fetuses with skeletal malformations in either group.

Study number (PCL-R-03-007): Assessment of intravenous, intra-arterial and perivenous tolerance of rhC1INH in the rabbit after single administration

Performing laboratory:

Test facility: -----(b)(4)-----

Test article batch/lot:

rH-C1INH (04I01001).

vehicle: Sterile 20 mM citric acid buffer with 6.5 % sucrose, pH 6.8.

Sucrose Solution: Sterile 80% sucrose solution in water, pH 7.0

Saline: sterile isotonic saline

Animal species and strain: --(b)(4)-- rabbit, -----(b)(4)-----, (SPF-Quality).

Breeder/supplier: -----(b)(4)-----

Number of animal per group and sex: 4 males and 4 females

Age: 15 to 17 weeks

Body weight range: 2.6 -3.0kg

Route and site of administration: central ear artery, marginal vein

Volume of injection: 10 ml into the central ear artery, 5 ml into the marginal vein in the direction of the ear base, and 0.5 ml perivenously at a separate site.

Frequency of administration and study duration: Once, on day 1

Dose: 141 U/ml

Report status: Final report

Summary

Injection of rH-C1 NH intra-arterially, intravenously or perivenously into rabbit-ear caused only minor local effects (slight erythema), which were not significantly different from those caused by the control vehicle or the saline solution.

Experimental design

Group	Animal numbers		Treatment
	males	females	
1	1-2	5-6	saline (right ear) control vehicle (left
2	3-4	7-8	saline (right ear) rH-C11NH (left

Methods:

Endpoint	Methodology
Hematology	
Clinical chemistry	
Coagulation	

The following parameters were evaluated:

Parameters	Frequency of Testing
Cageside observation	Twice a day
Clinical observations	Once a day
Body weight	days 1 and 5
Skin reactions	Each injection site was observed once daily
Necropsy	On day 5

The following tissues and organs were collected from all animals at necropsy

The macroscopic appearance of the injection sites was recorded. The treated blood vessels and surrounding tissue of both ears were removed from each rabbit, clearly identified and fixed in 4% neutral buffered formaldehyde solution:

- (1) Intra-arterial treatment area (right ear)
- (2) Intravenous treatment area (right ear)
- (3) Perivenous treatment area (right ear)
- (4) Tip of the ear (right ear)
- (5) Intra-arterial treatment area (left ear)
- (6) Intravenous treatment area (left ear)
- (7) Perivenous treatment area (left ear)
- (8) Tip of the ear (left ear)

Results:

Morbidity: There was no mortality during the study.

Clinical Signs: There were no treatment-related changes in clinical condition during the study.

Body Weight: Body weights of animals on the day of treatment were between 2.55 and 3.01 kg. In all animals a small increase of weight was observed over the observation period.

Skin reactions: Slight erythema (grade 1) was noted in the majority of animals treated with any of the possible formulations (Saline vehicle control and rH-C1INH) and injected intravenously, perivenously or intra-arterially. In all cases, the erythema was no longer present on day 5.

Therefore, no significant difference was observed between the test substance, the control vehicle and the saline solution for the induction of local effects after any of the tested injection routes.