

MEMORANDUM



**Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research**

Pharmacology / Toxicology Review Memorandum

Date: September 10, 2009
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To: Felice D'Agnillo Ph.D. (BLA Chairperson) and Nannette Cagungan
(BLA regulatory project manager)
Subject: STN 125287/0 – Non-clinical review of (Berinert[®] P - C1 Esterase
Inhibitor, Pasteurized) for the treatment of acute attacks in patients with
hereditary angioedema
Sponsor: CSL Behring, Marburg, Germany

Recommendation: STN 125287/0 can be approved from a pharmacology and toxicology perspective when the sponsor adequately addresses non-clinical toxicology in the product labeling.

Proposed Indication:

C1 Esterase inhibitor is indicated as a C1 Inhibitor replacement therapy for use in patients with Hereditary Angioedema (HAE), also known as C1 inhibitor deficiency. C1 Esterase inhibitor increases antigenic and functional serum levels of C1 inhibitor, thereby replacing the deficient C1 inhibitor activity in pediatric and adult HAE patients to control HAE attacks.

Conclusions of the review:

Berinert[®] P is supplied as single-use vials that contain 500 Units (U) of lyophilized human C1 inhibitor and is reconstituted with 10 mL of Sterile Water for Injection. The two vials are combined to provide a single dose of C1 esterase inhibitor at a concentration of 50 U/mL (40.0-62.5 U/mL) to be administered by the intravenous route.

When reconstituted with the appropriate volume of diluent, contains the following excipients per 10 mL reconstituted vial:

Sodium chloride 7.0-10 mg/ml (70-100 mg/vial)

Sodium citrate 2.5-3.5 mg/mL (25-35 mg/vial)

Glycine (amino acetic acid) 8.5-11.5 mg/mL (85-115 mg/vial)

General clinical pharmacology: Berinert[®] P dosing is proposed at a level of 20 units per kg body weight administered via the intravenous route. The human pharmacokinetics of Berinert[®] P displays a V_{dss} nearly identical to the plasma volume, Cl_{total} = ---b(4)----- and plasma elimination half-life of --b(4)--

The product demonstrates a reasonable risk to patients with hereditary angioedema at 10 fold the proposed clinical dose as defined primarily by 14 day repeat dosing in the rat.

Roster of Non-clinical Studies

All study reports have been translated from German to English.

Pharmacology/animal pharmacokinetics:

COM 01/04 (GLP – No) – *In vitro* complement activation (Berinert[®] P batch ---b(4)----)

57-23 (GLP – Yes) – Safety pharmacology in dogs (Berinert[®] P batch ---b(4)-----)

V-626 (1-3) (GLP – No) – Pharmacokinetics in rats (Berinert[®] P batch ---b(4)----)

PSK 02/05 (GLP – No) – Pharmacokinetics in rabbits (Berinert[®] P batch ---b(4)----)

Toxicology/non-clinical safety:

57-1HS – Part 1 (GLP – Yes) – Acute dose toxicity in mice (Berinert[®] P batch ---b(4)--)

57-1HS – Part 2 (GLP – Yes) - Acute dose toxicity in rats (Berinert[®] P batch ---b(4)----)

856351 (GLP – Yes) – Repeat dose toxicity in rats (Berinert[®] P batch ---b(4)----)

57-23 (GLP – Yes) – Intravenous tolerance rabbit (Berinert[®] P batch ---b(4)-----)

265.143.905 (GLP – Yes) – Subcutaneous administration in rabbits (Berinert[®] P ---b(4)----)

V-211d (GLP – Yes) – Neoantigenicity in rabbits and guinea pigs (Berinert[®] P ---b(4)---)

Overview of Safety:

COM 01/04 (GLP – No) – Influence of Berinert® P batch ---b(4)---- on complement activity in human or rat plasma.

Objective: To compare the effect of Berinert® P batch ---b(4)---- on complement activity in human and rat plasma with the end point being the IC50 for inhibition of complement components C1 r and C1 s. This study serves as a justification for the use of rats as a species which is pharmacologically similar to humans.

Methods:

Normal rat plasma and human plasma were used as the complement source. C1-INH (Berinert® P batch ---b(4)----) was diluted in each plasma type over a range of concentrations from 0.0095 U/mL to 1.9 U/mL. See treatment group table below.

Materials:

Rat plasma – From -b(4)- rats (female), weight range 400-500 g from ---b(4)----- . Blood collected in citrate.

Human plasma – Standard human plasma, ---b(4)----

Berinert® P – Batch # ---b(4)----

Treatment #	Test group	Final concentration
1	Berinert® P in human plasma	1.9 U/mL
2	Berinert® P in human plasma	1.4 U/mL
3	Berinert® P in human plasma	1.19 U/mL
4	Berinert® P in human plasma	0.59 U/mL
5	Berinert® P in human plasma	0.29 U/mL
6	Berinert® P in human plasma	0.15 U/mL
7	Berinert® P in human plasma	0.095 U/mL
8	Berinert® P in human plasma	0.0095 U/mL
9	Berinert® P in rat plasma	1.9 U/mL
10	Berinert® P in rat plasma	1.4 U/mL
11	Berinert® P in rat plasma	1.19 U/mL
12	Berinert® P in rat plasma	0.59 U/mL
13	Berinert® P in rat plasma	0.29 U/mL
14	Berinert® P in rat plasma	0.15 U/mL
15	Berinert® P in rat plasma	0.095 U/mL
16	Berinert® P in rat plasma	0.0095 U/mL

Results:

C1-INH concentration	Complement activity (percent of normal)			
	Human plasma		Rat Plasma	
1.9 U/mL	11.2	12.9	9.9	10.2
1.4 U/mL	34.3	32.9	30.6	29.1
1.19 U/mL	45.4	45.0	37.8	38.5
0.59 U/mL	75.0	71.8	73.9	73.9
0.29 U/mL	84.3	94.0	88.7	85.8
0.15 U/mL	98.1	116.9	107.7	85.4
0.095 U/mL	106.6	84.3	97.0	86.8
0.00950 U/mL	103.3	116.4	93.5	93.9

Plotted averaged data (n=2 per time point) fit to a four parameter regression model indicate a C1 inhibitory concentration at 50% (IC50) = 1.0529 in human plasma and an IC50 = 1.0108 in rat plasma.

Reviewer conclusions **COM 01/04**: The data indicate a similar pharmacologic activity of Berinert® P in rat and human plasma. While, it is likely that other species would show similar C1-inhibition compared to humans, the data presented are acceptable to demonstrate that the rat is a reasonable species for demonstration of non-clinical safety.

57-23 (GLP – Yes) – Safety pharmacology study in dogs and a local intravenous tolerance study in rabbits after introduction of a further step in processing (Berinert® P batch ---b(4)-----).

Objective: To evaluate the general safety pharmacology of hepatitis C virally inactivated C1-INH batches ---b(4)-----in beagle dogs (n=2 male and n=1 female) and local intravenous tolerance in rabbits (n=3 male and n=2 female).

Methods:

Test substance – hepatitis safe C1 inactivator HS batch -----b(4)----- (3,000 U/10 mL)
Vehicle control - 0.9% NaCl

Animals:

Safety pharmacology – Male and female beagle dogs from CSL's in house breeding colony (mean body weight = 10.5 kg)

Local intravenous tolerance testing – Male and female rabbits from CSL's in house breeding colony (mean body weight = 2.5 kg)

Study design:

Dosing safety pharmacology- Dogs received an escalating dose regimen of 500 U, 1000 U and 2000 U for a total of 3500 U (333 U/kg). All doses were administered into the lateral marginal ear vein.

Parameters measured:

- Arterial blood flow
- Heart, pulse and respiratory rates
- Cutaneous oxygen partial pressure
- Cardiac output
- Systemic vascular resistance
- Stroke volume
- Respiratory flow
- Central body temperature
- Blood pressure
- Pneumatogram
- dp/dt
- ECG
- Hematology (erythrocytes, leucocytes and thrombocytes)

Dosing local tolerance – Each rabbit received a single intravenous dose of 500 U (200 U/kg) (injection time 1 minute) into the lateral marginal ear vein.

Parameters measured:

- The local reaction to single injections was evaluated after administration and 24 hours post administration.

Results:

Safety Pharmacology – The cardiovascular and respiratory parameters measured at a cumulative dose of 3500U (333 U/kg) showed no abnormalities. Hematological evaluation demonstrated a slight decrease in coagulation time and thrombocyte aggregation.

Local intravenous tolerance – No abnormal histopathology was observed in tissue excised from the venous injection site area.

Reviewer's conclusions [57-23](#) – At approximately 15 fold the proposed clinical replacement dose; Berinert[®] P does not induce any discernible adverse cardio-respiratory effects in beagle dogs. Minimal effects on thrombogenic potential could be observed at this dosing level. It is known that doses in children and neonates administered Berinert[®] P do exhibit thrombogenicity at 90 U/kg (4.5 fold the recommended clinical dose).

[V-626 \(1-3\)](#) (GLP – No) – Pharmacokinetics of C1-inactivator in rats (Berinert[®] P batch -b(4)-) following intravenous injection. (An additional study was performed in rabbits to determine subcutaneous bioavailability).

The determination of and validation of Berinert[®] P in rabbit plasma is detailed in study report MEV-29r (Determination of the functional C1-Inhibitor activity in rabbit plasma)

Objective: To evaluate the pharmacokinetics of Berinert[®] P in the rat, one of the primary species chosen for toxicological evaluation.

Methods:

Test substance - Berinert[®] P lot # --b(4)-- (50 IU/mL)

Animals:

Rat - -b(4)- CSL colony raised 200g male and female

Study design:

PK was evaluated in 1 male and 1 female animal per dosing level in anesthetized and conscious rats as follows:

Experiment 626-1 (anesthetized)

Rat 1, 3 - Berinert[®] P 61.5 IU/kg one dose intravenous

Rat 2, 4 - Berinert[®] P 123 IU/kg one dose intravenous

Blood sampling (0, 5 min, 10, min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr post injection)

Experiment 626-2 (conscious)

Rat 1, 3 - Berinert[®] P 61.5 IU/kg one dose intravenous

Rat 2, 4 - Berinert[®] P 123 IU/kg one dose intravenous

Blood sampling (0, 1 hr, 5 hr, 8 hr, 24 hr, 30 hr post injection)

Experiment 626-3 (conscious)

Rat 1, 3 - Berinert[®] P 61.5 IU/kg one dose intravenous

Rat 2, 4 - Berinert[®] P 123 IU/kg one dose intravenous

Blood sampling (0, 6 hr, 24 hr, 48 hr, 72 hr and 96 hours post injection)

Results:

In the three groups of rats the initial C1-INH levels of 24 +/- 10.8% increased approximately 8-fold within the initial hour after dosing. C1-INH activity began to decline after 24 hours and by 48 hours plasma C1-INH levels returned to baseline (approximately 25%).

PTS-4r – A study on the bioavailability (F) and pharmacokinetics of subcutaneously administered Berinert P in rabbits.

Objective: To evaluate the bioavailability of Berinert[®] P after subcutaneous administration (200 U/kg) compared to the intravenous dosing (200 U/kg) route of administration. A secondary objective was to determine all PK parameters for each route of administration at the 200 U/kg dosing level.

Methods:

Test substance - Berinert[®] P (lot # ---b(4)----)

Animals: -b(4)- rabbits n=10M and 10F (2.8 kg)

Study design:

Group #	Treatment	Dose/Vol./route	N (m/f)
1	Berinert [®] P	200 U/kg/4.0 mL/kg/intravenous	10 (5/5)
2	Berinert [®] P	200 U/kg/4.0 mL/kg/subcutaneous	10 (5/5)

Blood sampling: 0, 0.5 h, 1 h, 2 h, 3h, 16 h, 20 h, 1, 2, 3, 4, 14 and 21 days after a single dose.

Results:

The initial intravenous dose demonstrated a rapid decline in plasma concentration from 0 to 16 hours. Such that the end of the initial elimination phase ending at 24 hours mated the C_{max} after subcutaneous dosing (2 U/mL).

Pharmacokinetic parameters PTS-4r

		Intravenous (200 U/kg)	Subcutaneous (200 U/kg)
	N	10	10
AUC _{0-tlast} (d*U/mL)	Mean	6.18	4.68
	SD	0.79	0.51
AUC _{0-inf} (d*U/mL)	Mean	6.29	4.80
	SD	0.82	0.51
t _{max} (days)	Mean	0.03	1.05
	SD	0.02	0.34
C _{max} (U/mL)	Mean	7.53	1.70
	SD	0.87	0.15
α elimination t _{1/2} (days)	Mean	0.16	---
	SD	0.03	---
β elimination t _{1/2} (days)	Mean	1.41	0.90
	SD	0.22	0.15
Clearance	Mean	38.9	54.1
	SD	4.3	5.6
Volume of distribution	Mean	61.0	69.8
	SD	10.3	12.7

<i>F</i>	<i>Parameter</i>	<i>Ratio</i>	<i>90% CI</i>
s.c./i.v.	AUC _{0-inf}	0.75	69.3-83.0

Reviewer conclusions **V-626(1-3) and PTS-4r** – These data suggest that comparable PK following intravenous dosing in the rabbit and human exist. The sponsor suggests that subcutaneous dosing in the rabbit shows 75% bioavailability and as a result this dosing route may be an option. The bioavailability in humans following subcutaneous dosing would require evaluation in order to accurately make this conclusion.

57-1HS – Part 1 (GLP – Yes) – A single-dose intravenous toxicity study of C1-inactivator HS in mice and rats

Objective: To determine the acute dose toxicity of C1-inactivator HS in mice and rats following human equivalent maximum doses (120 U and 300 U) of C1-inactivator. These doses represent the equivalent maximum clinical dose per unit body weight (20 U/kg).

Methods:

Test substance:

C1-inhibitor HS batch # ---b(4)----

Control:

0.9% NaCl

Animals:

Mice - -b(4)- mice CSL breeding colony (21-25 g), 10 male and 10 female

Rats - -b(4)-- (110 g – 5 weeks old), 10 male and 10 female

Dosing:

Mice – 30, 60, 120 U/animal via the tail vein

Rats – 100, 200, 300 U/animal via the tail vein

Study Design:

Mice and rats were dosed as stated above on day one, histology was performed on day 14 while clinical parameters (e.g. food/water intake, body weight and body temperature) were measured from day 1-14.

Results:

Body weight development – Normal

Clinical observations – Normal

Terminal observations (Histology) – Normal

Reviewer's conclusions – The study shows that mice and rats demonstrate a reasonable toxicity profile at the maximum clinical intended doses. However, the study does demonstrate points of weakness in its design (e.g. multitudes of the maximum clinical

dose (10x) were not studied and an early evaluation group (2 days post dosing) was not included).

856351 (GLP – Yes) – 14-Day intravenous toxicity (bolus) study in the --b(4)--- rat.

Objective: To assess the cumulative toxicity of Berinert[®] P when administered daily to rats intravenously as a bolus for 14 days. Additionally the time dependency of neutralizing antibody formation to Berinert[®] P or endogenous C1-inhibitor in rats was studied.

Methods:

Test substance: Berinert[®] P (batch # --b(4)-----)

Control: Sterile water for injection

Animals: --b(4)-- outbred -b(4)- rats (N=60 male 135-162 g and N=60 female 112-134 g)

Study Design:

	Group 1 (0 U/kg/day)	Group 2 (20 U/kg/day)	Group 3 (60 U/kg/day)	Group3 (200 U/kg/day)
Males A	1-5	16-20	31-35	46-50
Males B	6-10	21-25	36-40	51-55
Males C	11-15	26-30	41-45	56-60
Females A	61-65	76-80	91-95	106-110
Females B	66-70	81-85	96-100	111-115
Females C	71-75	86-90	101-105	116-120
A – Sacrifice at 24 hours after 6 th treatment B – Sacrifice at 24 hours after 10 th treatment C – Sacrifice at 24 hours after 14 th treatment Dose volume = 4 mL/kg Group 1 – control Group 2 – represents the clinical dose Group 3 – represents 3x the clinical dose Group 4 – represents 10x the clinical dose				

Observations:

Viability/mortality – Twice daily

Clinical signs – Once prior to first administration; twice daily on days 1-3; once daily thereafter during treatment period

Food consumption – Twice weekly during acclimatization and treatment

Body weight – Twice weekly during acclimatization and treatment

Ophthalmoscopic examination - During acclimatization and at the end of treatment

Clinical/laboratory investigation – days 7, 11 and 15

Hematology – days 7, 11 and 15

- erythrocyte count
- Hb
- MCV
- Red cell volume distribution width
- MCH
- MCHC
- Hemoglobin concentration distribution width
- Platelet count
- Reticulocyte count
- Reticulocyte maturity index
- Total leukocyte count
- Differential leukocyte count
- Thromboplastin time
- Activated partial thromboplastin time

Clinical Biochemistry – days 7, 11 and 15

- glucose
- urea
- creatinine
- total bilirubin
- total cholesterol
- triglycerides
- phospholipids
- AST
- ALT
- lactate dehydrogenase
- glutamate dehydrogenase
- CK
- alkaline phosphatase
- gamma-glutamyl-transferase
- sodium
- potassium
- chloride
- calcium
- phosphorous
- total protein
- albumin
- globulin

Urinalysis - days 7, 11 and 15

- volume
- specific gravity
- color

- appearance
- pH
- glucose
- ketone
- urobilinogen
- bilirubin
- erythrocytes

Antibody and C1 plasma level determination – days 7, 11, 15

Pathology - days 7, 11, 15

Results (summary):

Viability/mortality: No pre-mature mortalities

Clinical signs: No clinically relevant differences were noted in any of the animals in any group prior to administration or on any days 1-3 during the treatment period

Food consumption – No relevant changes in food intake were noted in Berinert[®] P treated animals at any of the dosing levels evaluated.

Body weight –No differences were found in groups treated with increasing doses of Berinert[®] P.

Ophthalmoscopic examination – No Berinert[®] P related ophthalmology changes were detected amongst groups.

Clinical/laboratory investigation – days 7, 11 and 15

Hematology – days 7, 11 and 15

Most notable was a trend toward an increase in platelets observed in female rats at the 60 and 200 U/kg dosing levels. Inconsistent changes were seen in hematological parameters (shortened PTT, elevated neutrophils, decreased MCH) in both male and female rats. However, these observations were not treatment or doses related and therefore are not likely to be attributable to Berinert[®] P.

Clinical Biochemistry – days 7, 11 and 15

There were no consistent treatment or dose related changes in clinical biochemistry parameters discernible at 7, 11 or 15 days. Inconsistent changes were seen in potassium, triglyceride, chloride, fatty acids and globulins. However, these observations were not treatment or doses related and therefore are not likely to be attributable to Berinert[®] P.

Urinalysis - days 7, 11 and 15

There were no consistent treatment or dose related changes in urinary parameters discernible at 7, 11 or 15 days. Inconsistent changes were seen in urine density and erythrocyte count. However, these observations were not treatment or doses related and therefore are not likely to be attributable to Berinert® P.

C1 plasma level determination – days 7, 11, 15

	Mean C1 Esterase Inhibitor Plasma Levels (% increase over T0)					
	24 h after 6 th treatment		24 h after 10 th treatment		24 h after 14 th treatment	
	Female	Male	Female	Male	Female	Male
Group1 (saline)	23.85	11.61	18.47	3.75	13.93	9.59
Group 2 (20 U/kg)	23.23	16.04	24.6	21.33	30.59	17.95
Group 3 (60 U/kg)	48.78	33.58	28.54	32.1	42.63	38.51
Group 4 (200 U/kg)	112.51	157.05	115.43	169.92	141.83	172.25

Antibody formation against Berinert® P

	Mean antibody against C1 Inhibitor (antibody titer – ---b(4)-----)					
	24 h after 6 th treatment		24 h after 10 th treatment		24 h after 14 th treatment	
	Female	Male	Female	Male	Female	Male
Group1 (saline)	0.86	0.43	0.54	0.76	76.81	112.37
Group 2 (20 U/kg)	1.79	0.53	0.90	0.73	1.98	5.48
Group 3 (60 U/kg)	0.24	0.56	0.60	1.39	0.27	14.43
Group 4 (200 U/kg)	0.40	0.67	0.17	0.39	2.86	2.79

* Relative to control with the exception of the day 15 sampling in the control animals, neutralizing antibody production are not an influence on the overall toxicological findings for 14 day dosing.

Pathology - days 7, 11, 15

Organ weights - There were no consistent treatment or dose related changes in organ weights or organ/body weight ratios discernible at 7, 11 or 15 days.

Macroscopic findings - There were no consistent treatment or dose related changes in macroscopic lesions discernible at 7, 11 or 15 days in treatment groups compared to control animals.

Microscopic findings – Phlebitis, periphlebitis, thrombophlebitis and perivascular hemorrhages were observed at the injection sites of all animals (treatment and control) and are the common result of physical rather than chemically induced local trauma.

The other various microscopic findings noted do not appear to be treatment or dose related.

265.143.905 (GLP – Yes) – Local tolerance testing of Berinert[®] P versus saline 0.9% In the rabbit.

Objective: Determine the local tolerance of Berinert[®] P to physiological saline in male and female -----b(4)----- Rabbits following subcutaneous administration.

Methods:

Group	#/sex	Substance	Side	Route	Dose	Volume
1	3 male	Berinert [®] P/Saline	L/R	s.c.	25 U/kg	0.44
	3 female	Berinert [®] P/Saline		s.c.	25 U/kg	0.44
2	3 male	Berinert [®] P/Saline		s.c.	75 U/kg	1.32
	1 female	Berinert [®] P/Saline		s.c	75 U/kg	1.32
	2 female	Berinert [®] P/Saline		s.c.	75 U/kg	1.32

After s.c. injection, clinical observations were performed three times on day 0, twice daily on days 1, 2, and 3.

Results:

Erythema formation – One instance of grade 2 erythema occurred in the 25 U/kg group none in the 75 U/kg group and none in the saline treated group.

Edema formation – Edema was not observed in males or females of the control, 25 U/kg or 75 U/kg groups.

Pain reaction – One instance of a grade 1 pain reaction was noted in one male dosed with 25 U/kg. No pain reactions were noted in males or females dosed s.c. with control or 75 U/kg.

Histopathology- No product or dose related histopathology changes were noted in the tissue surrounding the s.c. injection site.

V-211d (GLP – Yes) – Testing for possible formation of antigenic components through out the modification of product processing

Through out the different phases of processing applied to CSLs C1-Inhibitor (Berinert[®] P) there appears to be little evidence of processes related increases in neutralizing and or non-neutralizing antibody production.