1 TITLE PAGE	CLINICAL STUDY REPORT	Version: 1.0

Title: Pharmacokinetic Berinert® P study of subcutaneous (s.c.) versus intravenous (i.v.) administration in subjects with moderate hereditary angioedema – the PASSION study.	Version date: 23-Mar-2011
	INN: C1 esterase inhibitor
	Study/Protocol No.: CE1145_1001
	Substance No.: CE1145
Study Phase: I	Batch No.: 311 617 11E 206 617 11F
First subject in: 22-SEP-2008	Indication studied: PK investigation of s.c. versus i.v. administration of Berinert® P.
Last subject out: 01-NOV-2010	Date of early termination: Not applicable
Principal investigator:	PD Dr. Wolfhart Kreuz, Dr. Inmaculada Martinez-Saguer Centre of Paediatrics III, Department of Haematology, Haemostaseology and Oncology, Comprehensive Care Centre for Thrombosis and Haemostasis, Johann-Wolfgang-Goethe-University Hospital Theodor-Stern-Kai 7 60596 Frankfurt am Main, Germany
Person responsible for the study report:	PD Dr. Wolfhart Kreuz Dr. Inmaculada Martinez-Saguer
Sponsor:	Dean of the Department for Medicine of Johann-Wolfgang-Goethe-University Frankfurt am Main Theodor-Stern-Kai 7 60590 Frankfurt am Main, Germany

This study was performed in accordance with Good Clinical Practice, including the archiving of essential documents. This report has been prepared in accordance with the ICH Harmonized Tripartite Guideline on the Structure and Content of Clinical Study Reports, dated July 1996 (CPMP/ICH/135/95), the Declaration of Helsinki (revised version Somerset West, 1996), German Drug Law (AMG), and applicable national regulations and laws of the country in which the study was carried out.

2 SYNOPSIS

Title of the study:	Pharmacokinetic Berinert® P study of subcutaneous (s.c.) versus intravenous (i.v.) administration in subjects with moderate hereditary angioedema (PASSION study)
Investigator:	PD Dr. Wolfhart Kreuz, Dr. Inmaculada Martinez-Saguer Centre of Paediatrics III, Department of Haematology, Haemostaseology and Oncology, Comprehensive Care Centre for Thrombosis and Haemostasis, Johann-Wolfgang-Goethe-University Hospital Theodor-Stern-Kai 7 60596 Frankfurt am Main Germany
Study center(s):	Centre of Paediatrics III, Department of Haematology, Haemostaseology and Oncology, Comprehensive Care Centre for Thrombosis and Haemostasis, Johann-Wolfgang-Goethe-University Hospital Theodor Stern Kai 7 60596 Frankfurt am Main Germany. Phase-I-Unit Clinical Trial Centre Rhine-Main (KSRM), Schleusenweg 22 60528 Frankfurt am Main Germany
Indication:	Pharmacokinetic (PK) investigation of s.c. versus i.v. administration of Berinert® P.
Study period:	Total Study duration: 26 months Start of Study: 22-SEP-2008. End of Study: 01-NOV-2010. Treatment duration: 1 day First subject first visit: 22-SEP-2008 Last subject last visit: 01-NOV-2010

Period per subject: approximately 6 months

Study phase: I

Objectives:

Primary objective:

To investigate and compare pharmacokinetics of study medication (Berinert® P) in hereditary angioedema (HAE) subjects after subcutaneous and intravenous administration.

Secondary objectives:

- Documentation of adverse events (AEs).
- Screening for α-C1-inhibitor (α-C1-INH) antibodies.
- Investigation of virus markers.

Methodology: Single centre, randomized, open-label, cross-over pharmacokinetic study.

Number of subjects:

Screened:	24
Randomized:	24
Full analysis set (FAS):	24
Safety set (SAF)	24
Per-protocol set (PPS):	23

Study population: 24 subjects of both genders with an established diagnosis of HAE.

Inclusion criteria:

- Subjects with an established diagnosis of HAE type I (C1-INH activity <50% and C1-INH antigen <15.4 mg/dl) or HAE type II (C1-INH activity <50% and C1-INH antigen in normal or elevated concentration of dysfunctional protein).
- Male and female subjects with an age of ≥18 years.
- Subjects providing an informed consent.

Exclusion criteria:

- Subjects without an established diagnosis of HAE.
- Last C1-INH administration less than 7 days ago and/or acute attack.
- Subjects with acquired angioedema (AAE).

	• All other types of angioedema not associated with C1-INH deficiency. • Treatment with any investigational drug (exclusive drugs appropriate for the treatment of acute angioedema) 30 days before study treatment. • Treatment with any other drug appropriate for the treatment of acute angioedema within 7 days before start of study treatment at each phase. • Danazol prophylaxis. • Prophylaxis with antifibrinolytics, ε-aminocaproic acid, tranexamic acid. • Subjects with a known hypersensitivity to study medication (Berinert® P). • Pregnant women (pregnancy rapid assay required for women with childbearing potential), women currently breast-feeding, or with the intention to breast-feed (3 female subjects were not tested due to their high age) • Subjects with malignant diseases. • Subjects with immunodeficiencies such as established acquired immunodeficiency syndrome. • Subjects with concurrent serious or acute illness or infection as per investigators judgment. • Subjects with mental conditions which render the subject or its legally acceptable representative unable to understand the nature, scope and possible consequences of the study.
Sample size	Samples of 12 subjects per each starting group, i.e. samples of 24 subjects in total per first study arm and again of 24 subjects after cross-over.
Investigational Medicinal Product:	C1-INH concentrate, pasteurized (Berinert® P).
Reference therapy/placebo:	Not applicable.
Dose schedule and mode of administration:	• Subjects with HAE randomized to investigation of s.c. administration first received 1,000 U of study medication (Berinert® P; volume: 20 ml) s.c. at two different locations via 2 pumps over the period of 15 min.

- Infusion pumps: OMT Microjet Crono Super PID.
- Subjects with HAE randomized to investigation of i.v. administration first received 1,000 U of study medication (Berinert® P; volume: 20 ml) i.v. over the period of 3 min.
- After the observation phase, subjects crossed-over to the alternative administration type; at least 7 days wash out.
- In total: administration of twice 1,000 U of Berinert® P to 24 subjects, one time i.v., the other time s.c., 48,000 units (U) needed.

Criteria for evaluation:

Study endpoints:

- Bio-availability of s.c. relative to i.v. administration of study medication.
- Safety of s.c. and i.v. administration of study medication.

Pharmacokinetics/ pharmaco-dynamics:

Individual courses of C1-INH levels, from these the following pharmacokinetic parameters were derived:

- Area under the curve (AUC) (for dose of 1,000 U per subject ([U x hours (h)] /ml).
- Time to maximum concentration (T_{max}; h).
- Maximum concentration (C_{max}).
- Terminal elimination half-life ($t_{1/2}$).
- Mean residence time (MRT; h).
- Total clearance (Cl; ml/[kg x h]).
- Volume of distribution at steady state (V_{ss}; ml/kg).
- In-vivo recovery (IVR).
- Classical IVR (% rise/U/ml).
- Incremental IVR (response) (% rise/U/kg body weight).

Safety:

- Serological virus markers at baseline and 3 months after each application of investigational medicinal product (antibodies against Hepatitis A virus, Hepatitis B virus surface antigen, antibodies against Hepatitis B virus surface antigen, antibodies against core protein of Hepatitis B virus if not vaccinated or non responding; antibodies against Hepatitis C virus (anti-HCV), antibodies against human immunodeficiency virus (anti-HIV) type 1 and 2, for all, to be done in laboratory III).
- Polymerase chain reaction (PCR) at baseline for: HCV, HIV

(laboratory III).

- PCR after 3 months for HCV, HIV after each infusion of study medication (laboratory III).
- Investigation for α-C1-INH antibodies at baseline and 3 months after each administration of study medication (laboratory IV).
- Adverse events (AEs) over the study period.
- Physical examination at baseline and 3 months after each administration.
- Vital signs (blood pressure, heart rate, temperature) before and after each application of study medication and during s.c. application.

Pharmacokinetic sampling times:

i.v. and s.c. samples: 0, 0.25, 0.5, 0.75 h and 1, 2, 4, 6, 8, 12, 16, 20, 24, 36, 48, 60, 72, 120,168, 336 (±48) and 504 (±48) h.

Statistical methods:

PK analysis:

- PK was assessed by means of non-linear regression, taking into account individual endogenous baseline concentrations of C1-INH. Individual profiles were described by an open one-compartment model. Standard formula was used to obtain individual PK variables. The incremental in-vivo recovery (IVR) for C1-INH was obtained from the maximum plasma level within 4 h after administration of the study drug.
- The distribution of PK parameters and IVR per treatment was analyzed descriptively, including 90% confidence intervals (based on log-transformed data) and graphs (boxplots).
- Exploratory analysis of variance and graphs checked PK parameters for period, sequence (group) and carry-over effects.

Bioavailability analysis:

The bioavailability of Berinert® P s.c. treatment relative to Berinert® P i.v. treatment was determined by the geometric mean quotient of individual areas under the curves (AUCs, not extrapolated, dose-adapted, with negative values set to 0; no interpolation of values). Ninety percent confidence intervals were indicated.

Safety analysis:

Safety analysis was descriptive. Laboratory investigations and AEs were documented during study period. Later investigations and AEs, if any, were displayed in total.

Summary:

Pharmacokinetic and pharmacodynamic results:

The mean relative bioavailability of Berinert® P s.c. relative to Berinert® P i.v. was 39.7345%, with the 90%-CI ranging from 27.3442% to 57.7390%. ANOVA of C1-INH-activity revealed a significant influence for 'treatment' ($p = 0.0001$), whereas 'period' and 'treatment*period' had no influence on C1-INH activity.

Further pharmacokinetic measurements on C1-INH activity showed that after s.c. administration compared to i.v. administration, overall activity was lower, time to maximum concentration was longer, maximum concentration was lower, elimination half-life and MRT were longer, and mean volume of distribution was lower. The results for C1 antigen and C4 antigen were similar, but individual subjects treated with s.c. medication reached the AUC values achieved with i.v. treatment (AUC_{st} minimum and maximum values for both pathways: 16.02 – 102.34 h x mg/ml for i.v. treatment versus 0 – 104.37 h x mg/ml for s.c. treatment).

Under s.c. administration, the mean C1-INH activity reached its maximum of approximately 26% after 48 h. The maximum level after i.v. administration was approximately 50%, measured directly after infusion. In some subjects, the activity level after s.c. administration remained stable until at least 120 h after administration.

The results for C1-antigen levels were similar: after s.c. administration, the maximum was reached after 48 h (mean: 0.07 mg/ml), overall, the C1-antigen levels after reaching the maximum were more stable with s.c. than with i.v. administration.

Mean C4 antigen levels rose quicker and to a greater extent when Berinert® P was given intravenously. The mean maximum was 10 mg/dl, which was reached after 72 h; after this point, the mean value decreased rapidly. With s.c. administration, the peak level was reached after 120 h (mean:

7.5 mg/dl) and was more stable than after i.v. infusion.

Analysis of variance showed that for most PK parameters only 'treatment' had a significant influence, but not 'period' or 'treatment x period'. Exceptions were $t_{1/2}$ and clearance for both C1-INH activity and C4 antigen, where no factor was found to be significant, and V_z for C4 antigen, where both 'treatment' and 'period' influenced the PK value significantly.

The pharmacodynamic analysis concentrated on the IVR within 4 h, 24 h and without time limit. For the classical IVR there was a rise of 95.17 versus 5.46 %/(U/ml) within 4 h and 96.34 versus 18.50 %/(U/ml) within 24 h. For incremental IVR, it was a rise of 2.63 versus 0.15 %/(U/ml) within 4 h and 2.66 versus a 0.51 %/(U/ml) within 24 h. Without time constraint the mean classical IVR after s.c. treatment was less than one third of the IVR after i.v. treatment. That is, maximum increase in plasma of s.c. Berinert® could not be reached within 24 h, which was the case after i.v. administration.

In both the i.v. and the s.c. phase the percentages of cleaved high-molecular weight kininogen (cl HK) decreased between t = 0 h and t = 36 h. This decrease was more marked in the i.v. phase, where the percentage of cl HK dropped from 46.02% at baseline to 37.14% after 36 h. Under s.c. treatment, the lowest value was 41.96%. After this time point, the values rose again and approximately reached baseline values.

Safety results:

Overall, 16 subjects (66.7%) of the SAF experienced 46 treatment-emergent AEs. Fourteen AEs could be seen in the i.v. group, 32 AEs in the s.c. group. Most treatment-emergent AEs (N = 42, [91.3%]) were of mild intensity, the remaining four AEs were of moderate intensity. No AEs were of severe intensity.

Causality assessment revealed none (N=10), probable, possible, unclassified (N=0) or unlikely (N=4) causal assessment to study medication in patients in the i.v. group in contrast to patients in the s.c. group, where none (N=9), probable (N=5), possible (N=2), unlikely (N=0) and unclassified (N=16) causality assessments took place.

One serious adverse event occurred in the i.v. group, none in the s.c. group. The event, a case of 'Pneumonia', resolved without sequelae, had no relationship to the study medication,

and was of moderate intensity.

The five AEs judged to be probable - as well as the two AEs judged to be possible related to the study medication - occurred all in the s.c. group and were all of mild intensity. All these AEs included 'Application site irritation' (4 probable and 2 possible AEs) and 'Application site haematoma' (1 probable AE).

Sixteen AEs were unclassified with regard to causality assessment to study medication in the s.c. group and the MedDRA preferred term included 'Application site irritation' (N=5), 'Application site swelling' (N=6), 'Application site haematoma' (N=3) and 'Application site pain' (N=1).

In the s.c. group however, all AEs (N=32, [100%]) were of mild nature, where the only 4 moderate AEs all occurred in the i.v. group (one rhinitis, one oropharyngeal pain, one sinusitis, one pneumonia). The number of treatment-emergent AEs was more than twice as high in the s.c. phases (Berinert® P i.v.: 14 vs. Berinert® P s.c.: 32). The majority of treatment-emergent AEs (N=23) were administration site conditions as 'Application site irritation', 'Application site haematoma', 'Application site swelling', and 'Application site pain'. They were all experienced by subjects during s.c. treatment.

However, another dysbalance between groups was seen for infections and infestations (17.4% of all AEs) with the majority in the i.v. group (13% of all AEs), comprising 6 AEs in the i.v. group and 2 AEs in the s.c. group. This is also true for respiratory, thoracic and mediastinal disorders with the majority of cases (11% of all AEs) in the i.v. group with 5 AEs in the i.v. group in contrast to the s.c. group with 1 AE.

No subject died in the course of the study. One treatment-emergent SAE was documented, which was already described before.

No clinically relevant changes in laboratory parameters over time were found. Additionally, there were no significant differences between the treatment groups. None of the laboratory values found to be abnormal was assessed to have clinical relevance by the investigator.

Two subjects under i.v. treatment developed a positive result for anti-HAV 3 months after baseline, after being negative before, which was explained by previous vaccination. For anti-

HBc, 1 subject in the i.v. phase was found positive at baseline and negative 3 months later. In the s.c. phase, 1 subject changed from borderline at baseline to positive 3 months later. This subject had a hepatitis B infection that was present already in the past. So, in summary, no virus transmissions due to the administration of Berinert® P during the study period were identified. No positive polymerase chain reactions (PCR) for either hepatitis C (HCV) or human immunodeficiency virus (HIV) were observed.

For vital signs, neither abnormal changes over time nor significant differences between the treatment groups were found.

In the s.c. phase, the anti-C1-INH antibody titre of one subject changed from below cut off point at baseline to above cut off point 3 months later. This antibody, however, proved to be not an inhibitory anti-C1-INH antibody. Otherwise, no changes of antibodies from below cut off to above cut off point were observed.

Conclusions:

The results of the pharmacokinetic analyses revealed that Berinert® P s.c. has a bioavailability of 39.7% compared to i.v. administration. There was, however, a difference between subjects treated before the substantial amendment no. 1 came into effect, and subjects treated afterwards. The relative bioavailability for the first group was 45.35%, and for the latter group of subjects 27.31%. This difference can probably be explained with the conservative nature of the data analysis (including negative values set to 0, no interpolation and baseline-correction). The area under the curve for the 6 subjects treated after amendment 1 was larger than for the other subjects, resulting from the prolongation of measurement time to 504 h, which revealed more negative values for i.v. AUCs than for s.c. AUCs. As it was felt that internal C1-Inhibitor generation could not be ruled out at this point to interfere and due to the fact that s.c. C1-INH activity values in principle were more in the lower range, it was decided to set all negative values to "0" as the risk for creating false high bioavailabilities was considered to be too high otherwise.

As expected for s.c. treatment, the maximum activity of C1-INH, as well as maximum C1 antigen levels were reached only

after a certain accumulation time, in this case 48 h. For both parameters, and C4 antigen as well, it was shown that C1-INH activity and antigen levels after s.c. treatment almost never reached the maximum values achieved after i.v. administration. However, peak values were more stable after s.c. administration than after i.v. administration.

The comparison of elimination half-lives revealed that Berinert® P is eliminated much slower (mean $t_{1/2}$ was about half as long as after i.v. administration) when given subcutaneously. The reason for this is probably the formation of depots under the skin after s.c. injection, resulting in a slow release of Berinert® P into surrounding tissues and the vascular system. It should be kept in mind, however that due to s.c. administration, the mean $t_{1/2}$ obtained here should be interpreted with care due to the challenges with regard to resorption in this compartment and that the reference half-life value of C1-INH referred to here is derived from the i.v. administration.

In both treatment groups a drop in the percentage of cl HK was observed after injection. This was more marked after i.v. administration of the study medication. From the low number of observations (n = 6 in each group) no conclusion on the effect on either treatment on cl HK can be drawn, but it is apparent that Berinert® P is able to lower the amount of cl HK, which is known to rise during HAE attacks.

In only one subject an increase in anti-C1-INH antibodies was identified from baseline to 3 months after, but this antibody was found to provide no inhibitory potential.

The safety evaluation revealed no unexpected risks of s.c. Berinert® P administration. Most treatment-emergent AEs were administration site conditions, which can be expected after drug injection. Laboratory profiles and vital signs evaluations did not show any relevant findings. For 4 subjects, presence of virus antibodies from negative or borderline at baseline to positive at the end of the study were found, but these conversions could be explained by vaccinations and hepatitis infection earlier in life. No positive PCR for either hepatitis B or hepatitis C were identified over the study period.

Date of report: 23-MAR-2011