



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

To: BLA STN 125350/0

Cross Reference: IND -(b)(4)-

From: Evi Struble, Ph.D.

Through: Dorothy E. Scott, M.D.

CC: Hon-Sum Ko, M.D., HFM-392
Pratibha Rana, RPM, HFM-370

Applicant: CSL Behring AG

Product: Hizentra™, Immune Globulin Subcutaneous (Human) (IGSC),
20% Liquid

Subject: Final Memo, Nonclinical Pharmacology/Toxicology

Table of Contents

Executive Summary	3
Animal Studies Performed with Hizentra™	3
Safety of Excipients	4
Safety of L-proline	4
References	4
BLA STN 125350/0 Complete Review.....	5
Conclusions	5
Brief Description of BLA Submission.....	5
Indication	5
Dose	5
Studies Performed with IgPro20, Main Findings	5
Studies Performed with L-proline, Main Findings	6
Safety of Excipients	8
Review of Studies Submitted.....	10
Studies Performed with IgPro20	10
Study No. -(b)(4)- 01/06; Effects of Sandoglobulin, IgPro10 and IgPro20 on blood pressure in rats	10

Study No. 143.143.552; Local Tolerance testing of Ready-to-Use Protein Solutions in the Rabbit (s.c., 96 h observation)	10
Study No. -(b)(4)- 01/05; A study on the bioavailability and pharmacokinetics of subcutaneously administered Vivaglobin, IgPro16 and IgPro20 in rabbits.....	11
Study No. -(b)(4)- 04/05; A study on the bioavailability and pharmacokinetics of subcutaneously administered IgPro16 and IgPro20 in rabbits.....	11
Study No. -(b)(4)- 02/06; A study on the pharmacokinetics of intravenously administered IgPro10 and subcutaneously administered IgPro20 after multiple dosing in rats.	12
Studies Performed with L-proline.....	12
Study 925/034; 5 Day Intermittent Intravenous Infusion Dose Range-finding Study in the Rat	12
Study 925/035; 4-week daily 7-hour intravenous infusion toxicity study in the rat.	13
Study No. 668316; L-proline– preliminary 7-day dose-range finding intravenous (7h) infusion study in --(b)(4)-- dogs.....	14
Study No. 668321; L-proline 28 day intravenous (7h) infusion toxicity study in the --(b)(4)-- dog with a 14 day recovery period.....	14
Study Number: -(b)(4)- 01/07; Effects of Early L-Proline or Glycine Administration on Morris Water Maze Performance in Rats (Treatment Phase).....	15
“--(b)(4)--” Study No.: 551; CSL Behring Study No.: ZLB 06_006.....	16
Study number 1657/ZLB/02; Effects of glycine and L-proline on behavior and physiological state as assessed by the Irwin test and on body temperature in rats... ..	17
Study -(b)(4)-30034; L-proline and glycine - Embryo toxicity study by daily 7-hour continuous intravenous infusion in the rat (Segment II).....	17
Study Number -(b)(4)- 08/06; Pharmacokinetics of L-proline in rats following single s.c. or i.p. injection.....	18
Study no. -(b)(4)- 03/06; Treatment of rat experimental allergic encephalomyelitis (EAE) with the subcutaneous immunoglobulin IgPro20	19

Executive Summary

Animal Studies Performed with Hizentra™

- 1) Local tolerance studies
 - a. Hizentra™ administered in rabbits s.c. up to volumes corresponding 7 times the maximum volumes applied to humans per infusion site and kg body weight caused erythemas and edemas.
 - i. Erythema development after Hizentra™ administration was comparable to that of the marketed subcutaneous immunoglobulin product Beriglobin P, a 16% IgG preparation licensed in EU.
 - ii. The frequency and intensity of edema was higher for Hizentra™ than the Beriglobin P control and it increased with the increasing IgG dosing. The frequency of edema was higher for proline than saline controls.
 1. Healing was observed during the three day observation period.
 - iii. No histological abnormalities were detected after s.c. infusion of Hizentra™.
 - b. Hizentra™ was applied to rabbits via intravenous (i.v.), intra-arterial (i.a.) and paravenous (p.v.) routes of administration to assess local reactions after correct and incorrect application.
 - i. Erythema and edema formation induced by test article and saline injection were slight and similar in incidence and severity after i.v. and i.a. application.
 - ii. Well defined erythemas and moderate to severe edemas were observed after p.v. administration of Hizentra™.
 1. Healing was observed during the three day observation period.
 - iii. There were no drug related macroscopical or histopathological findings after i.v., i.a. or p.v. application.
- 2) Pharmacokinetic studies
 - a. Pharmacokinetics of Hizentra™ in rats was investigated after repeated s.c. dosing on five consecutive days and relative bioavailability was assessed in comparison to Privigen administered intravenously. The overall bioavailability was 57%.
 - b. Single dose pharmacokinetic study in rabbits showed that the bioavailability of Hizentra™ is 80% of the bioavailability of Vivaglobin.
- 3) The pharmacodynamic effect of Hizentra™ was evaluated in hemodynamic safety pharmacology study after i.v. bolus administration in rats.
 - a. Hizentra™ caused moderate hypotension which was in the same range as for the marketed products Privigen and Sandoglobulin.

Safety of Excipients

The excipients used in Hizentra™ are L-proline and polysorbate 80 and the main impurity is octanoic (caprylic) acid. Safety of L-proline is summarized below. Other excipients and impurities present in Hizentra™ are also present in other approved Ig preparations and are considered to be safe when used in accordance with the label.

Safety of L-proline

L-proline is a naturally occurring amino acid and it is present in plasma at a concentration varying between 51–271 $\mu\text{mol/L}$ ¹. It passes the blood-brain barrier where may have neuroexcitatory activity. In adults L-proline is effluxed from the brain by several transporters, one or some of which may not be well developed in young children. In published research², juvenile rats subcutaneously injected with L-proline from birth to day 28 displayed short and long term memory loss in standard tests. The daily amounts of L-proline used in this study were more than 60 times higher than the exposure that would result from weekly administration of 400 mg/kg Hizentra. In tests performed by CSLB, L-proline at the same dose was not neurotoxic in juvenile rats when administered subcutaneously on five consecutive days, i.e. on days 9-13 of life, or once weekly for three weeks, i.e. on days 9, 16, and 23 of life.

Other studies performed by the sponsor showed safety of L-proline in animals.

References

1. Scriver's "The Online Metabolic and Molecular Bases of Inherited Diseases (OMMBID)" Chapter 81, Table 81.2.
2. Caren Serra Bavaresco, Emilio Luiz Streck, Carlos Alexandre Netto, and Angela Terezinha de Souza Wyse; Metabolic Brain Disease, Vol. 20, No.1, March 2005, pages 73-80.

BLA STN 125350/0 Complete Review

Conclusions

From the nonclinical prospective, this BLA may be approved.

Brief Description of BLA Submission

HizentraTM is a 20% protein liquid formulation of human immunoglobulin G (IgG) preparation for subcutaneous application. The 20% IgG solution is formulated with 250 mmol/L of L-proline at pH 4.8. IgPro20 also contains a nominal amount of 20 µg/mL polysorbate 80. In this review the name IgPro20 is used instead of HizentraTM because this was the name used in the application.

Indication

IgPro20 is indicated for the treatment of primary immunodeficiency (PI).

Dose

The dose will be individualized based on the patient's clinical response to IgPro20 therapy and serum IgG trough levels. The highest dose used in the clinical trials did not exceed 400 mg/kg.

Studies Performed with IgPro20, Main Findings

- 4) Local tolerance studies
 - a. IgPro20 administered in rabbits s.c. up to volumes corresponding 7 times the maximum volumes applied to humans per infusion site and kg body weight caused erythemas and edemas.
 - i. Erythema development after IgPro20 administration was comparable to that of the marketed SCIG product Beriglobin P, and to IgPro18.
 - ii. The frequency and intensity of edema was higher for IgPro20 than the Beriglobin P control and it increased with the increasing IgG dosing. The frequency of edema was higher for proline than saline controls.
 1. Healing was observed during the three day observation period.
 - iii. No histological abnormalities were detected after s.c. infusion of IgPro20.
 - b. IgPro20 applied to rabbits via intravenous (i.v.), intra-arterial (i.a.) and paravenous (p.v.) routes of administration to assess local reactions after correct and incorrect application.
 - i. Erythema and edema formation induced by test article and saline injection were slight and similar in incidence and severity after i.v. and i.a. application.
 - ii. Well defined erythemas and moderate to severe edemas were observed after p.v. administration of IgPro20.

1. Healing was observed during the three day observation period.
 - iii. There were no drug related macroscopical or histopathological findings after i.v., i.a. or p.v. application.
- 5) Pharmacokinetic studies
- a. Pharmacokinetics of IgPro20 in rats was investigated after repeated s.c. dosing on five consecutive days and relative bioavailability was assessed in comparison to IgPro10 (Privigen) administered intravenously. The overall bioavailability was 57% with 90% confidence interval of 49-67%.
 - b. Single dose pharmacokinetic study in rabbits showed that the bioavailability of IgPro20 is 80% of the bioavailability of Vivaglobin.
- 6) The pharmacodynamic effect of IgPro20 was evaluated in hemodynamic safety pharmacology study after i.v. bolus administration in rats.
- a. IgPro20 caused moderate hypotension which was in the same range as for the marketed products IgPro10 (Privigen) and Sandoglobulin.

Studies Performed with L-proline, Main Findings

Results from the animal studies performed with L-proline are summarized below.

- 1) Repeat-dose toxicity studies were conducted with L-proline in two species, rats and dogs.
 - a) In a 28 day study in rats, the No Observed Adverse Effect Level (NOAEL) was 1449 mg/kg/day. Slight reductions in body weight gain and reduced food consumption was observed in males at this dose in the 28-day study. There were no other treatment related changes in this or other dose groups.
 - b) In a 4-week study dogs the NOAEL was 4350 mg/kg/day, the highest dose used. Low level toxicities such as emesis for several animals, a reduction in food consumption, immature testes in 2/3 males were seen at this dose. These were not considered adverse and the weight gain was considered normal.
- 2) L-proline was not genotoxic when used in combination with nicotinamide and L-isoleucine using the Ames test, in vitro cytogenicity assay, a bacterial stress gene assay and a bone marrow micronucleus assay in mice.
- 3) L-proline was not teratogenic when administered i.v. at a dose of 1449 mg/kg/day during days 6 to 17 of gestation. At this dose, there was no indication of maternal or embryo-toxicity, and the dose tested was No Observed Effect Level (NOEL).
 - a) The impact of L-proline on fertility, early embryonic development and peri-natal development has not been assessed. L-Proline is a naturally occurring amino acid and this is acceptable.
- 4) L-proline was not neurotoxic in a neurotoxicity studies in rats.
 - a) L-proline did not significantly affect the behavior of rats during or after infusion for 5-days whereas minor to moderate effects were seen with glycine. Cumulated doses of L-proline and glycine induced slight statistically significant increases in body temperature after 5 days of infusion when compared to control groups.

- b) L-proline caused no neurological effects in rats when administered s.c. at a dose of 2 g L-proline/kg bw.
- 5) L-proline was not neurotoxic when investigated in juvenile rats.
 - a) In an acute neurotoxicity and a Morris water maze task study in juvenile rats, L-proline administered at doses 1500-2000 mg L-proline/kg on days 9-13 or on days 9, 16, and 23 was shown not to have any negative effects in short and long term memory and in the nervous system development in rats.
- 6) In published research, juvenile rats injected s.c. with high doses of L-proline from birth to day 28 displayed short and long term memory loss in standard tests.
- 7) Pharmacokinetic studies with L-proline were performed using intravenous infusion to rats and dogs, as well as s.c. and i.p. injection in rats.
 - a) In rats L-proline administered s.c. and i.p. at a dose of 2.0 or 4.0 g /kg bw respectively was eliminated quickly from the serum with a half-life of L-proline s.c. 1.5 h and baseline levels reached at 8 h after injection for most animals.
 - b) Plasma levels after a single s.c. administration of 1.9 g/kg bw L-proline to juvenile rats were about 15 mmol/L L-proline 15 minutes after application and declined to about 16% of this concentration 4 hours after injection. Thus, elimination from plasma in juvenile animals was slower compared to adult rats.
 - c) In rats L-proline administered i.v. up to a dose of 1449 mg/kg/day for 5 or 28 consecutive days showed no accumulation of L-proline in the serum. The total daily excretion of L-proline in urine on Day 28 was less than 2% of the administered L-proline dose.
 - d) In dogs, L-proline administered i.v. at a dose 2170 and 4350 mg/kg for 7 or 28 consecutive days showed no accumulation of L-proline in the serum; less than 5% of the infused L-proline dose was excreted with urine.
- 8) Sponsor has performed no distribution study and only limited excretion studies with L-proline. The following has been derived from the studies performed by the sponsor and the published literature.
 - a) L-proline is a naturally occurring amino acid and present in plasma at a concentration of 266–35 $\mu\text{mol/L}$. It is tubularly reabsorbed in kidneys uses the same transporter as glycine and hydroxyproline and thus not secreted in the urine of healthy individuals.
 - i) In studies performed by the sponsor, L-proline administered i.v. in dogs and rats was not accumulated in plasma and only a limited amount is excreted in urine, namely 5 and 2% respectively.
 - b) L-proline injected into muscle tissue is rapidly transported to the liver and to a lesser degree the intestine where it is used for protein synthesis and also converted to L-glutamine.
 - c) L-proline passes the blood-brain barrier (BBB) where it acts as a low potency member of the class of excitatory amino acids. In adults it is effluxed by the amino acid transporter ATA2, the orphan transporter v7-3 (solute carrier 6a15, or B0AT2), and the PROT transporter. One or some of these transporters may not be well developed in young children.

Safety of Excipients

The excipients are L-proline and polysorbate 80 and the main impurity is octanoic (caprylic) acid.

Octanoic acid is a naturally occurring medium chain fatty acid and has been used in similar amounts in other Ig products such as Gamunex (IGIV 10%). In albumin solutions is used up to concentration of 4 mmol/L (5% albumin) and 25 mmol/L (25% albumin).

Table 1: Exposure to the Excipients/Impurities

	IgPro20		Flebogamma 10%		Gamunex 10%	
	Conc.	Dose#	Conc.	Dose^	Conc.	Dose^
Polysorbate 80	30 µg/mL	60 µg/kg	200 µg/mL	1600 µg/kg		
Octanoic (Caprylic) Acid	-(b)(4)- -----	-(b)(4)- -----			1.3 mmol/L	10.4 µmol/kg

#Calculated using a dose of 400 mg/kg

^Calculated using a dose of 800 mg/kg

L-proline is used as a stabilizer at a concentration of 250 mmol/L IgPro20 (= 28.75 g/L). L-proline is present as an excipient in the licensed biologic Privigen (human IGIV preparation) and aminoacid mixtures intended for parenteral nutrition such as 8.5% FreAmine® III, B. Braun Medical Inc.

The acute exposure to L-proline from IgPro20 use in the clinic will result to approximately 58 mg/kg bw L-proline once weekly. This amount is lower than with the “parent” IVIG IgPro10 (i.v. doses of 287.5 mg/kg L-proline with IgG doses of 1g/kg) and lower than the doses for parenteral nutrition intended for sub-chronic administration. For example up to 255 mg/kg/day L-proline are given to healthy and 348 mg/kg to malnourished or traumatized infants with 8.5% FreAmine® III, B. Braun Medical Inc.

Table 2: Maximum human doses of IgG and L-proline administered with IgPro20 and approved products formulated with L-proline

Product	Route of Administration	Maximum daily dose: IgG (mg/kg bw)	Maximum daily dose: L-proline (mg/kg bw)
IgPro20	s.c.	400*	58
IgPro20	s.c.	1000^	145
IgPro10 (Privigen)	i.v.	1000	288
8.5% FreeAmine® III [#]	i.v.	n.a.	255-348

*The highest dose used in clinical trials

^Theoretical maximal dose, not likely to be used in the clinic

[#]Labeled “For short-term use in adult patients”; also used in infant and pediatric populations.

Reviewer Conclusions and Labeling Comments

Based on the limited toxicity seen in animal studies performed with L-proline, the exposure to this excipient from IgPro20 use in PI adult patients is judged to be safe. In contrast, the animal data and their relevance to the safe use of L-proline in pediatric populations are not conclusive. In published research (Bavaresco et al., 2005), juvenile rats injected s.c. with high doses of L-proline from birth to day 28 displayed short and long term memory loss in standard tests. This effect was not observed in a study performed by the sponsor where L-proline was administered during five consecutive days, namely days 9 through 13 of life or once weekly on days 9, 16, and 23 of life. The clearance of L-proline was shown to be less effective in young rats when compared to adult rats.

In conclusion, due to lack of pediatric data on the safety and PK properties of L-proline in infant and very young children and the inconclusive results from animal studies, it is recommended that the conclusions from the above mentioned animal study(ies) be included in the label, Section 8.4 “Pediatric Use”.

Other excipients present in HizentraTM are judged to be safe when the proposed product is used according to the label.

Review of Studies Submitted

Studies Performed with IgPro20

Study No. -(b)(4)- 01/06; Effects of Sandoglobulin, IgPro10 and IgPro20 on blood pressure in rats

Aim: to assess the hypotensive effects of two batches of IgPro20 in comparison to one batch of IgPro10 (Privigen), Sandoglobulin (a marketed IVIG) and saline. The primary study

Model: -(b)(4)- rats

Design: N= 24 F -(b)(4)- rats, with a body weight of 216 to 303 g and an age of 6 to 8 weeks, were assigned to 5 groups of 5 animals each, receiving 250 mg/kg of either IgPro10 (2.5 mL/kg) or Sandoglobulin (2.08 mL/kg) or two different batches of IgPro20 (1.25 mL/kg) intravenous bolus injection. A group of 4 rats receiving single injections of 2.0 mL/kg isotonic saline served as controls.

Outcome measurements: the minimal, mean systolic and diastolic arterial blood pressure during the observation period of 3 hours.

The blood pressures were recorded invasively in the carotid artery.

Results

Sandoglobulin caused the expected moderate drop of mean arterial blood pressure to about 70% of saline controls. IgPro10 as well as IgPro20, batch 43109-00002 caused a similar hypotension and appeared not different from Sandoglobulin. The second batch of IgPro20 (number 43109-00001) appeared to cause a slightly more pronounced hypotension. This batch also showed this effect faster than the first batch.

Conclusion

IgPro20 was well tolerated with regard to effects on blood pressure. The hypotension caused was only moderate and in the same range as IgPro10 (Privigen) and Sandoglobulin.

Study No. 143.143.552; Local Tolerance testing of Ready-to-Use Protein Solutions in the Rabbit (s.c., 96 h observation)

Performing Laboratory: -----(b)(4)-----

GLP: Yes

Aim: To determine the local tolerance of IgPro and compare it to the formulation buffer, a control IgG (Beriglobin P) and saline in rabbits.

Model: ----(b)(4)----- Rabbits -(b)(4)-

Design: Randomly assigned 3M and 3F -(b)(4)- rabbits per group for a total of 6 treatment groups, four IgPro formulations (10, 16, 18 and 20% IgG), and two controls (one L-Proline and one Beriglobin P). The test article formulations, buffer and control protein were administered as a s.c. infusion on the back of animals, caudally from the scapula, at a volume of 2.5 mL/kg and at an infusion rate of 5 mL/kg/h using an infusion pump. As a control, s.c. infusion of saline was administered in the same animals in a different site 24 hrs later given at the same volume and infusion rate.

Outcome measurements: Severity of pain after a bolus injection, as well as erythema and edema after bolus and infusion administration were traced and graded according to a grading system

from 0 to 4. Clinical observations were performed twice daily on the day of application and daily until study day 4; necropsy, macroscopic examination, histopathology of the application sites and the surrounding tissue (e.g. muscle and lymph node) were performed on study day 4.

Results

Erythema formation:

Well defined to moderate test article dependent appearance of erythema was observed after the s.c. infusion of the third higher concentration of IgPro (IgPro18) and after s.c. bolus and infusion of the fourth higher concentration of IgPro (IgPro20). The degree of erythema development was comparable to that of Beriglobin P.

Edema formation:

The appearance of edema was dependent of the administration of test article or control protein and was significantly different from L-Proline and saline. The increase of frequency and intensity of edema formation with the increasing immune globulin dosing suggests a dose dependency effect.

Pain reaction:

No major difference could be observed between test article formulations, formulation buffer, control protein or saline solution.

Macroscopic and histological findings such as discoloration and hematomas were not different in test and control animals and in saline controls.

Very slight to slight inflammation was seen in all control protein and saline solution treated groups but their incidence was higher in test article groups than in the negative controls.

No abnormality was observed in animals treated with the formulation buffer.

Study No. -(b)(4)- 01/05; A study on the bioavailability and pharmacokinetics of subcutaneously administered Vivaglobin, IgPro16 and IgPro20 in rabbits

Design: A total of 3 groups of 10 -(b)(4)- rabbits (5 males and 5 females) were injected subcutaneously with IgPro20, Vivaglobin, IgPro16 at a dose of 400 mg product/kg bw. Blood samples were drawn for the determination of human IgG at baseline and at 1 h, 4 h, and 1, 2, 3, 4, 8, 10, 14 and 21 days following the s.c. injection.

Results

All three immunoglobulins were absorbed from the subcutaneous space in a similar way. The IgG plasma levels increased rapidly and C_{max} levels were reached after 2 to 3 days, followed by a monoexponential decrease.

The AUC values showed a high variability between animals. The bioavailability as measured by the three AUC-variables was very similar for IgPro16 and Vivaglobin P. The bioavailability of IgPro20 was 80% to 84% of that of the 16% formulations; these differences were not statistically significant at the 5% level.

Conclusion

IgPro20 showed lower AUC values than IgPro16 and Vivaglobin P, however, the high variability of single values might have influenced these results.

Study No. -(b)(4)- 04/05; A study on the bioavailability and pharmacokinetics of subcutaneously administered IgPro16 and IgPro20 in rabbits

Aim: to compare IgPro20 with IgPro16 after one subcutaneous injection in (b)(4) rabbits.

Design: A total of 2 groups of 20 animals (10 males and 10 females) were used. Group 1 received IgPro16 and group 2 IgPro20. All animals were injected s.c. with 400 mg product/kg bw.

Blood samples were drawn for the determination of human IgG at baseline and at 1 h, 4 h, and 1, 2, 3, 4, 8, 10, 14 and 21 days following the s.c. injection.

Results

The IgG plasma levels in both treatment groups increased rapidly with a peak after 2 to 3 days, followed by a monoexponential decrease.

The areas under the curve were similar for both products showing bioequivalence of the two products in rabbits at a significance level of 5%.

Conclusion

The two immune globulin preparations show bioequivalence.

Study No. -(b)(4)- 02/06; A study on the pharmacokinetics of intravenously administered IgPro10 and subcutaneously administered IgPro20 after multiple dosing in rats.

Aim: to compare the bioavailability of IgPro20 after repeated subcutaneous injection with the bioavailability of IgPro10 after repeated intravenous administration.

The test articles were:

A total of 4 groups of 10 female rats each were used:

Group 1: IgPro20 100mg/kg bw/day (0.5 mL/kg) s.c.

Group 2: IgPro20 400mg/kg bw/day (2.0 mL/kg) s.c.

Group 3: IgPro10 100mg/kg bw/day (1.0 mL/kg) i.v.

Group 4 : IgPro10 400mg/kg bw/day (4.0 mL/kg) i.v.

All animals were injected once daily on five consecutive days.

Results

The plasma concentration – time curves after s.c. administration of IgPro20 and i.v. application of IgPro10 in doses of 100 or 400 mg/kg bw show similar rates of elimination. However, absorption after s.c. administration was four times faster in the higher dose group.

The bioavailability of the 100 mg/kg dose of s.c. versus i.v. administration was 61% and the bioavailability of the 400 mg/kg dose was 53%; not statistically significantly different.

Studies Performed with L-proline

Study 925/034; 5 Day Intermittent Intravenous Infusion Dose Range-finding Study in the Rat

Design: A total of 5 groups of 5 male -(b)(4)- rats were used, a control group (physiological saline) and low dose and high dose L-proline and glycine groups, respectively. In addition, satellite animals (3 males/group) were used for toxicokinetics.

The following doses were applied:

L-proline low dose: 579 mg/kg bw/day

L-proline high dose: 1449 mg/kg bw/day

Glycine low dose: 378 mg/kg bw/day

Glycine high dose: 945 mg/kg bw/day

The test articles were infused for 5 consecutive days. Serum samples were taken from satellite animals on Day 1 before (pre-infusion), 3 hours after start of the infusion and just

before termination of the infusion; on Day 2 before infusion; on Day 5 before infusion, 3 hours after start of the infusion and just before termination of the infusion.

Conclusion

There was no evidence of toxicity in either dose group with glycine or L-proline. Therefore the high dose level, i.e. 1449 mg/kg bw/day for L-proline and 945 mg/kg bw/day for glycine is NOAEL.

Study 925/035; 4-week daily 7-hour intravenous infusion toxicity study in the rat

Design: GLP Study

A total of 5 groups of 20 -(b)(4)- rats (10 females and 10 males) were used, a control group (physiological saline) and low dose and high dose L-proline and glycine groups, respectively.

The following doses were applied:

L-proline low dose: 579 mg/kg bw/day

L-proline high dose: 1449 mg/kg bw/day

Glycine low dose: 378 mg/kg bw/day

Glycine high dose: 945 mg/kg bw/day

The test articles were infused for 28 consecutive days. Additional animals, 10 animals per group (5 females and 5 males) were kept for a further two weeks without treatment after the 28-days infusion period to evaluate the regression of any toxic signs. In addition, satellite animals (3 animals/sex/group) were used for toxicokinetics.

Outcome measurements: Morbidity, mortality checks and clinical examinations, ophthalmology, clinical laboratory (hematology, serum clinical chemistry, coagulation, urine analysis).

All animals were sacrificed at termination (Day 29) or at the end of the 2-week treatment-free period (Day 43). Full necropsy was performed on each animal with histopathology at the high dose and control animals.

Results

Daily intravenous administration (7-hour infusion) of L-proline (579 and 1449 mg/kg/day) to the rat for four weeks was not associated with any marked changes indicative of toxicity. The only treatment-related changes were slight (not statistically significant) reductions on body weight gain and food consumption, especially in males.

Toxicokinetics

There was a dose-dependent increase in the peak serum concentrations of L-proline, up to 13 and 9 times baseline levels for males and females, respectively.

No accumulation of L-proline in serum occurred at both doses.

Excretion

Total daily excretion of L-proline in urine was low: 4.4 and 2.2 (control), 6.0 and 3.1 (low dose L-proline), 45.6 and 62.5 (high dose L-proline) in males and females, respectively. This corresponds to means of 0.3% and 0.2% (low dose L-proline) and 0.8% and 1.9% (high dose L-proline) of total daily doses of L-proline administered.

Conclusions

1449 mg/kg/day for L-proline could be identified as a NOAEL under the defined experimental conditions.

Study No. 668316; L-proline– preliminary 7-day dose-range finding intravenous (7h) infusion study in --(b)(4)-- dogs

Aim: to determine the toxicity of L-proline following 7 h daily continuous i.v. administrations on 7 consecutive days and to set suitable levels of L-proline for a subsequent 28-day study in dogs.

Design: GLP study

A total of 6 animals, in 3 groups of one male and one female dog each were used: L-proline low dose: 2170 mg/kg bw/day; infusion rate 9 mL/kg bw/h L-proline high dose: 4350 mg/kg bw/day; infusion rate 18 mL/kg bw/h

Control group (physiological saline); infusion rate 18 mL/kg bw/h

All animals were infused for 7 h on 7 consecutive days using surgically implanted catheters.

Outcome measurements: Morbidity and mortality, body weights, food consumption, hematology and clinical chemistry and blood samples for toxicokinetics, urine samples Necropsy was performed on each animal and organ weighing; no histological examinations were performed.

Results

TK

Highest L-proline concentrations measured were 16.5 mmol/L (male) and 14.5 mmol/L (female) at the end of the high dose infusion (4350 mg/kg) on Day 6, corresponding to an approximately 100 fold increase in L-proline serum concentration. In all animals serum concentrations reached baseline values (pre-treatment) 24 h after the start of the last infusion.

Excretion

In the high dose group male dogs excreted 3.25 – 4.27% of the infused dose. Female dogs excreted 0.66 – 4.65% of the administered dose. In the low dose group, all animals excreted less than 1% of the infused dose.

Conclusions

The intravenous infusion for 7 hours daily of L-proline at dose levels of 2170 and 4350 mg/kg bw (infusion rates of 9 and 18 mL/kg/h) on 7 consecutive days was well tolerated by --(b)(4)-- dogs.

Study No. 668321; L-proline 28 day intravenous (7h) infusion toxicity study in the --(b)(4)-- dog with a 14 day recovery period

Aim: to determine the toxicity of L-proline and the reversibility of any effects following 7h daily continuous i.v. administration on 28 consecutive days followed by a 14 day treatment-free recovery period.

Design: GLP study

A total of 26 --(b)(4)-- dogs (13 females and 13 males) were used; they were assigned to 3 main study groups of 3 males and females each:

Group 1: control group (physiological saline); infusion rate 18 mL/kg/h

Group 2: 2170 mg L-proline/kg bw/day; infusion rate 9 mL/kg/h

Group 3: 4350 mg L-proline /kg bw/day; infusion rate 18 mL/kg/h

An additional 2 male and 2 female dogs were assigned to Groups 1 and 3 and were kept for a further two weeks without treatment after the 28-days infusion period to evaluate

the regression of any toxic signs. All animals were infused for 7 h on 28 consecutive days *via* surgically implanted catheters.

Outcome measurements: Clinical observations, body weights and food consumption, ophthalmoscopy investigations, electrocardiography investigations, blood samples for hematology and clinical chemistry investigations, urine samples, detailed necropsy upon sacrifice, with organ weight analysis and histological evaluation of > 40 kinds of tissue including brain.

Results

There were no unscheduled deaths.

A reduction in food consumption was generally noted for animals in the high dose group; not seen during the recovery period.

The urinary electrolytes (UNa, UCl) were reduced for Groups 2 and 3 when compared with Group 1. Urinary potassium (UK) and urinary creatinine (UCr) levels were much lower than the pretrial values for all three groups. Excretion of urinary creatinine (exCrea) was not affected by the large volumes that were administered.

Immature testes were noted for the 2/3 males in the high dose group.

TK

Administration of 2170 mg L-proline/kg was followed by a 35-fold transient increase in L-proline serum concentrations from baseline, infusions of doses of 4350 mg/kg led to an approximate 75- fold increase in serum concentrations of L-proline with male animals showing higher peak serum concentrations than females. No accumulation of L-proline in the serum occurred over the whole treatment period of 28 days.

Conclusions

The intravenous (7 h) infusion of L-proline at dose levels of 2170 and 4350 mg/kg and at infusion rates of 9 and 18 mL/kg/h, respectively, for 28 consecutive days was considered to be well tolerated by --(b)(4)-- dogs. Histologically, there were no changes that were indicative of any treatment related effects. The No Observed Adverse Effect Level (NOAEL) was considered to be 4350 mg/kg.

Study Number: -(b)(4)- 01/07; Effects of Early L-Proline or Glycine Administration on Morris Water Maze Performance in Rats (Treatment Phase)

L-proline, glycine and vehicle were administered s.c. to n=24/group male -(b)(4)- rats divided into 3 groups using two dosing schedules:

Schedule “1” was 1.5 g/kg s.c. L-proline or 1.0 g/kg s.c. glycine twice daily for 5 consecutive days from day of life 9-13 (simulating the maximum duration for IVIG application to Idiopathic thrombocytopenic purpura patients)

Schedule “2” was a non-consecutive dosing scheme, i.e. 1.5 g/kg s.c. L-proline or 1.0 g/kg s.c. glycine twice on day of life 9, 1.8 g/kg s.c. L-proline or 1.2 g/kg s.c. glycine twice (15.6 mMol/kg) on day of life 16 and 2.0 g/kg s.c. L-proline or 1.3 g/kg s.c. glycine (17.4 mMol/kg) twice on day of life 23, simulating IVIG application to primary immunodeficiency patients.

16 randomly selected rats / group were transferred to -----(b)(4)-----
---- on the 37th day of life to perform the learning and memory tests.

Results

Local intolerance to the subcutaneous injection of the 2 Mol/L L-proline solution. 6 out of 16 animals of schedule “1” and 10 out of 16 animals of schedule “2” displayed an uni-

or bilateral loss of fur at the injection site, with a diameter of about 2-4 mm. Also red skin and local swelling were observed. Complete recovery was seen at the end of the observation period.

“(b)(4)” Study No.: 551; CSL Behring Study No.: ZLB 06_006

n=16 male (b)(4)- rats per test group. After pre-treatment, the animals were transferred from CSL Behring to (b)(4)- between the 40th and 45th day of life where the learning tests were performed. Final test sessions were: for reference memory at the 60th day of life and for working memory from 68th to 71st day of life.

Outcome Measures

Body weight and neurological, working and reference memory parameters, such as

- Latency to find the position of the removed platform (s)
- Distance travelled to reach the position of the removed platform (cm)
- Distance travelled relative to the linear (shortest) distance between starting point and platform (%)

Results

The animals of all test groups showed a successful acquisition of reference memory and a good performance in the testing of reference and working memory. There were no indications of any negative effects on acquisition of reference memory, reference or working memory by any of the different pre-treatments.

Differences found in supplementary parameters of the tests were attributed to chance.

There were no differences in the health state of the animals of the different pre-treatment groups. Differences in body weight appeared to be unrelated to pre-treatment and be due to the normal variability.

PK

Proline concentration was measured 15 and 240 minutes after administration. Plasma concentration of proline 10 times higher than background was found at the 240 minutes time point (16% of administered proline remaining) confirming previous published data that proline clearance in young rats is less efficient than adult rats (9% remaining at 4 hours in female adult rats).

L-proline concentration (mmol/L) (mean ±SD)		
Timepoint (min)	Saline	L-proline
Baseline	0.25 ±0.004	0.27 ±0.04
15	0.28 ±0.04	14.6 ±1.4
240	0.24 ±0.006	2.6 ±0.16

Conclusions

Proline does not cause acute neurotoxicity in newborn rats.

Study number 1657/ZLB/02; Effects of glycine and L-proline on behavior and physiological state as assessed by the Irwin test and on body temperature in rats.

Design: GLP 5-day neurotoxicity study (Irwin test) in male -----(b)(4)----- rats weighing 195 – 340 g after 7 h daily continuous i.v. administration of L-proline or glycine for the first 4 days and approximately 3 h on day 5.

A total of 6 groups of 10 male animals/group were used: no treatment, control vehicle (physiological saline), and one of each groups L-proline 579 mg/kg/day, L-proline 1449 mg/kg/day, Glycine 378 mg/kg/day and Glycine 945 mg/kg/day. The test articles were infused for 5 consecutive days. On Day 5 all animals were necropsied.

Outcome measures: standardized observations and tests to assess the neurobiological state of rodents including measures of autonomic, sensorimotor functions, convulsive behavior and excitability.

Rectal temperature was recorded before the beginning of the administration and just after each Irwin test.

Results

Animals treated with L-proline showed some small modifications of typical behavior, such as decreased reactivity on days 1 and 5 post-infusion, decrease in the number of rears on day 1 of infusion, CNS excitability including some clonic convulsions in one animal on day 5 of infusion. However, all these modifications were not statistically significant when compared to those observed after vehicle treatment and were smaller than changes after glycine administration.

Cumulated doses of L-proline induced statistically significant increases in body temperature after 5 days of infusion when compared to vehicle and/or naive group. This hyperthermia was higher after L-proline treatment than after glycine administration; however the differences in body temperature increases between the two amino acids tested were not statistically significantly different.

Conclusions

L-proline might have an advantage over glycine which is frequently used as excipient in IVIGs.

Study -(b)(4)-30034; L-proline and glycine - Embryo toxicity study by daily 7-hour continuous intravenous infusion in the rat (Segment II)

Aim: This Segment II teratogenicity study was conducted to evaluate the effects of the test items L-proline and glycine on embryonic and fetal development of the rat when administered by intravenous infusion during the period of organogenesis Day 6 – Day 17 of gestation.

Design: GLP Study

The test articles were administered by continuous i.v. infusion daily for 7h from Day 6 – Day 17 of gestation.

A total of 3 groups of 25 mated females were used. In addition, satellite animals (4 animals/ treated group, 2 animals in the control group) were used for toxicokinetics. The first group was the 0.9% physiological saline control group, the second group received glycine and the third group was administered with L-proline at a dose of 945 mg/kg bw/day glycine and 1449 mg/kg bw/day L-proline respectively.

Outcome measures:

The main parameters were morbidity/mortality, clinical signs, body weight, and food consumption. At Day 20, the animals were sacrificed. At necropsy, pregnancy status, number of corpora lutea, number and distribution of intra-uterine implantations, individual fetal weight and sex of fetuses were recorded. Each fetus was examined for external defects. Approximately half of each litter was examined for visceral abnormalities then eviscerated. The eviscerated carcasses were processed for skeletal examination. The remaining fetuses were preserved for fixed soft tissue examination.

Results

TK:

There was an increase in the peak serum concentrations up to about 9 fold baseline levels for L-proline and up to about 7 fold baseline levels for glycine with mean peak serum concentrations being higher at Day 6 than at Day 17 of gestation for both amino acids. No accumulation of L-proline or glycine in serum occurred.

Conclusions

There were no indications of maternal toxicity or of embryo-toxicity or teratogenicity in the groups given either glycine (300 mmol/L) or L-proline (300 mmol/L) by 7-hour daily intravenous infusion at a dose volume of 42 mL/kg/day. A NOEL for maternal toxicity, fetotoxicity and teratogenicity can therefore be defined at a daily dose of 1449 mg L-proline/kg body weight and 945 mg glycine/kg body weight, respectively.

Study Number -(b)(4)- 08/06; Pharmacokinetics of L-proline in rats following single s.c. or i.p. injection

Aim: to assess acute neurotoxicity of L-proline at high serum concentration of the amino acid.

Design:

A single dose pharmacokinetic study in female -(b)(4)- rats weighing approximately 250 g with scoring for clinical signs for acute neurotoxicity was conducted with s.c. or i.p. administration of L-proline.

A total of 5 groups of 6 female animals were used: 2 g L-proline/kg bw s.c., 2 and 4 g L-proline/kg bw i.p., control solution (saline) s.c. and i.p. administration.

Outcome measurements:

Assessment of clinical signs including signs of neurotoxicity including acute, seizures and cramps as well as blood samples

Results

There were no unscheduled deaths.

Subcutaneous administration of L-proline: There were no clinical signs including signs of acute neurotoxicity in the group treated with L-proline.

Intraperitoneal administration of L-proline: Major clinical signs observed in animals receiving low and high dose proline and the sodium chloride control solution included a transient high muscle tonus in the hind limbs accompanied by vocalization in some animals immediately upon dosing with vocalization also at later time points. In the proline treated groups increased defecation was observed with some dose relationship.

Toxicokinetics of L-proline after s.c. injection:

There was a rapid 70 fold increase in L-proline mean serum concentration from 170 $\mu\text{mol/L}$ to a maximum mean peak concentration of 11990 $\mu\text{mol/L}$ 15 min after injection

of the L-proline solution. Elimination of L-proline from serum was fast (half-life:1.5 h), baseline L-proline levels were reached 8 h after L-proline injection.

Toxicokinetics of L-proline after i.p. injection:

A dose-dependent increase of L-proline serum concentrations was observed with maximum concentrations obtained 15 min after dosing. At 15 min L-proline serum concentrations were in a range between 433 - 17517 $\mu\text{mol/L}$ in the low-dose group and in a range between 1265 – 43752 $\mu\text{mol/L}$ in the high-dose group. Elimination of L-proline from serum was fast, 8 h after dosing baseline serum levels were reached in all animals except for one which still showed slightly elevated levels at this time point.

Conclusion

Subcutaneous injection of high doses of L-proline results in transient high L-proline serum concentrations early after administration thus allowing assessment of acute toxic effects. No clinical signs of acute neurotoxicity were found after subcutaneous administration of L-proline.

Study no. -(b)(4)- 03/06; Treatment of rat experimental allergic encephalomyelitis (EAE) with the subcutaneous immunoglobulin IgPro20

Model: Experimental allergic encephalomyelitis (EAE) in -(b)(4)- rats.

Design: N= 70 F -(b)(4)- rats, assigned to 7 groups of 10 rats each.

Dose: IgPro20, Privigen (IgPro 10) were injected s.c. or i.v respectively at doses 100; 200 and 400 mg/kg on 5 consecutive days. Isotonic saline served as a placebo control.

Outcome measurement: The sum of performance scores served as the primary study variable; secondary variables were: incidence of EAE, time to onset of symptoms, time to death and mortality.

Results

In all IgG treatment groups, a lower sum of scores was observed than in the placebo group. The differences are not significant for the 100 mg/kg group but are significant for the 200 or 400 mg/kg groups. Scores from the s.c. group were higher than scores from the i.v. group. 1/10 animals died after s.c. administration in each of the 200 and 400 mg/kg IgPro20 groups and no animals died after i.v. application of 200 and 400 mg/kg IgPro10 compared to 5/10 in the placebo group.

Reviewer Conclusion

In this model i.v. application of immunoglobulins appears more effective than the s.c. application in slowing down the EAE and preventing mortality.