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Information for the package leaflet regarding proline used as an excipient in medicinal products for human use

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Information for the package leaflet regarding proline used as an excipient in medicinal products for human use

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Executive summary

Proline is an amphiphillic, naturally occuring non-essential amino acid which is present in human plasma and a major component of human proteins. The daily intake with food is about 5200 mg. The normal proline plasma level is in the range of 266 ± 35 µmol/l.

Proline is not commonly used in pharmaceuticals as an excipient. It is approved for use in Privigen (IVIg) and Hizentra (SCIg) solution for injection as a stabiliser for IgG. Proline is also contained in low amounts in several vaccines, e.g. Havrix 720 Kinder (Hepatitis A).

Proline is not currently listed in the Annex of the guideline on 'Excipients in the Label and Package Leaflet of Medicinal Products for Human Use' [1]. However, two major safety concerns have been identified regarding an excessive exposure to proline. Both concerns are related to neurotoxicity following excessive exposure to proline.

In patients suffering from hyperprolinaemia, a rare autosomal recessive inherited disorder leading to a dysfunction of proline metabolism resulting in elevated plasma (normal 51–271 µM; HP type I < HP type II (up to 500–3700 µM), it is reported that this may lead to seizures or other neurological abnormalities and specific forms of schizophrenia. Consequently, additional exposure of these patients to proline should be limited as much as possible and a warning has been added to the package leaflet accordingly.

As for the second concern, some published pharmacological and behavioural research studies in rats indicate that long-term administration of high doses of proline have effects on brain development in juvenile rats (Wyse/Bavaresco). However, these animal models are not considered to be relevant for non-deficient adults or children who would not be exposed for a prolonged period to high levels of proline. Therefore no related warning has been included in the package leaflet.

62 **Proposal for new information in the package leaflet**

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Proline	All routes of administration	Zero	This medicine contains x mg of proline in each <dosage unit/unit volume> <which is equivalent to x mg/<weight><volume>>. Proline may be harmful for patients with hyperprolinaemia, a rare genetic disorder in which proline builds up in the body. If you (or your child) have hyperprolinaemia, do not use this medicine unless your doctor has recommended it.	In patients suffering from hyperprolinaemia, exposure to proline should be minimised. Use in patients suffering from hyperprolinaemia only if strictly necessary (e.g. if no alternative treatment is available).

Scientific background

1. Characteristics

1.1. Category (function)

Proline [(2-S)-Pyrrolidine-2-carboxylic acid] is used in pharmaceuticals as an excipient and as an active substance in nutritional supplements. In IVIg the use of proline as a stabiliser has been demonstrated to improve stability by inhibition of IgG aggregation, dimer formation and solution discolouration. This is postulated to result from the interaction of hydrophobic groups on proline with hydrophobic domains of IgG molecules, inhibiting hydrophobic interactions of two IgG molecules preventing excessive dimerisation (Bolli et al., 2010 [3]).

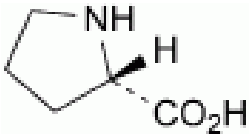
1.2. Physico-chemical Properties

Category

Proline (Ph. Eur. terminology standard term for L-proline) is an amphiphillic, naturally occurring non-essential amino acid which is present in human plasma and a major component of human proteins (Maeder et al., 2011 [24]). Currently, proline is mainly manufactured by fermentation.

Properties

$C_5H_9NO_2$



The substance is a white or almost white, crystalline powder or colourless crystals, which are very soluble in water and freely soluble in ethanol (Ph. Eur. 01/2008:0785).

European Pharmacopeia specifications include the analysis for ninhydrin-positive substances by thin-layer chromatography, in order to exclude the presence of other amino-acids over 0.5%. The relative molecular mass of proline is 115.1.

1.3. Use in medicinal products

Proline is quite rarely used in pharmaceuticals as an excipient.

For example, its use as an excipient is approved for Privigen (IVIg) EMEA/H/C/000831 - formulated with 250 mmol/L proline at pH 4.8 and has stability at room temperature for 3 years (Wasserman et al., 2009 [31]).

Hizentra (20% human normal immunoglobulin SCIg) solution for injection has also been approved by the EMA for subcutaneous administration (EMEA/H/C/002127). The product is stable at 25ºC for 30 months and has been formulated with 250 mmol/L proline (Jolles et al., 2011 [20]). Proline can bind to the hydrophobic regions of the IgG molecules and prevent interactions/breakdown and oxidation, thereby stabilizing the molecule (Hagan et al., 2012 [16]).

In lower amounts proline is also contained in Repatha (evolocumab, mAB) and several vaccines, e.g. Havrix 720 Kinder (Hepatitis A).

As an active substance proline is used as an oral nutritional supplement in OTC supplements by a number of companies in doses ranging from 500 mg/d to 1000 mg/d and in numerous amino acid mixtures intended for parenteral nutrition such as 10% FreAmine® III, B. Braun Medical Inc.

Proline may also be used as food additive, but exposure to humans through food is in orders of magnitude higher than the anticipated level of exposure from the use as flavouring agents (WHO/JEFCA [32]).

Of note, under certain exceptional physiological conditions (severe burn, preterm neonate), i.e. when endogenous synthesis cannot meet metabolic need of proline, additional intake via dietary source is needed (conditionally indispensable) (Dietary Reference Intakes: The Essential Guide to Nutrient Requirements [10]; Wu, 2009 [10]); Wu et al., 2011 [34]).

2. Pharmacy-toxicological data

2.1. Toxicology

Published toxicological data for proline is limited. Conventional non-clinical studies for proline were predominantly generated in support of the development and approval of Hizentra and Privigen and are published in condensed form in the EPARs of those approved products. Further toxicological data has been summarised by the WHO (WHO FOOD ADDITIVES SERIES: 54 [32]). Only few additional data were collected from other published sources. Results from animal studies performed with proline are summarised below.

There is no clear statement on the formal GLP status of the reviewed non-clinical data. At least the studies conducted for regulatory purposes, i.e. those supporting marketing approval of Hizentra and Privigen and the subchronic toxicological study performed under the Japanese food regulation (Tada et al., 2010 [30]) should have been performed in line with GLP guidance or at least under comparable regulatory acceptable standards.

Safety pharmacology

Published pharmacological studies from the literature indicate that proline is an amino acid with potential CNS toxicity. A general overview on behavioural and neurochemical effects of proline is reviewed by Wyse and Netto (2011 [37]).

Several published pharmacological and behavioral research studies in rats (i.e. watermaze test, open field test) in which proline was given SC (twice daily) at high doses for several weeks to juvenile rats (generally from post-natal day (PND) 6 to day 28, but later timeframes up to PND 39 have also been investigated) indicate that long-term administration of high doses of proline have effects on brain development in juvenile rats (Moreira et al., 1989 [23]; Delwing et al., 2003 [6]; Shanti et al., 2004 [29], Bavaresco et al., 2005 [2]; Delwing, 2006 [8]; Ferreira et al., 2011 [14]). A similar “experimental model of hyperprolinemia” was used in these studies. This model was designed to achieve sustained levels of proline in blood and brain comparable to those of human type II hyperprolinemia patients. Using PK studies, administered doses (12800 to 16400 µmol of Pro/g bw) were adjusted to achieve plasma proline levels between 1000 and 2000 µM, similar to those found in hyperprolinemic type ll patients (Moreira et al., 1989 [23]). Please refer also to section Pharmacokinetic in juvenile animals below.

The findings from the behavioral studies indicate that treatment with proline impairs spatial memory in rats and that this effect established in early life. Study reports further indicate that locomotor activity was not adversely affected. Beside this, these and other studies found that high proline levels inhibit

enzyme activities as acetylcholinesterase, Na+, K+-ATPase, and aminotransferases, induce degenerative changes in the brain (Pontes et al., 1999 [26]; Shanti et al., 2004 [29]; Delwing et al., 2005 [7]) energy deficit (Delwing et al., 2007 [9]; Ferreira et al., 2010 [14]) and oxidative stress (Delwing et al., 2003 [6]).

Overall, there is evidence that high proline concentrations in the CNS can induce neurotoxicity when administered repeatedly at high doses leading to overload/exhausting of defence mechanisms and enzymatic capacity can effect brain development particularly in juvenile animals.

Privigen contains high amounts of proline and is indicated in children of all age groups. In order to address the literature data on putative effects of high doses of proline on brain development several safety pharmacological studies were conducted in support marketing authorisation of Privigen.

Standard safety pharmacology studies with proline revealed no clinical signs of neurotoxicity, and no relevant modifications of behaviour were seen with proline in rats (Study 1657/ZLB/02 Irwin Test with proline, Study PSR 08/06 Pharmacokinetics of proline in Rats following a single s.c. or i.p. Injection) (EPAR Hizentra [17]).

A safety pharmacology study (Irwin test, study no. 1657/ZLB/02) in rats was conducted under conditions relevant for the clinical situation of Privigen, i.e. a 7 hrs/day infusion. In this study proline was compared to glycine, a broadly used excipient in IVIG solutions that is neurologically active. Groups of ten male rats were intravenously infused with two doses of proline (579 and 1449 mg/kg bw/day) or glycine (378 and 945 mg/kg bw/day) for 7 hours during the first 4 days and during approximately 3 hours on day 5. It was concluded that proline did not significantly affect the behaviour of animals during and after infusion at both doses tested (EPAR Privigen [27]).

A Morris water maze task study in newborn rats (exact age not known) with subcutaneous administration of proline with daily proline doses up to 4000 mg/kg administered daily for 2-5 consecutive days or once every 2–4 weeks was performed. Morris water maze performance was tested at postnatal days 54 to 71, and the treated rats showed no change in spatial memory acquisition or retention as compared to the control rats, which themselves showed good learning and reproducible performance (EPAR Privigen [27]).

Another study in rats (Study PSR 08/06) that was designed to assess acute neurotoxicity of proline at high proline serum concentrations, measuring clinical signs and proline serum concentrations after subcutaneous or intraperitoneal bolus injections. Groups of six female rats were subcutaneously injected with 2 g proline/kg bw or intraperitoneally with 2000 mg and 4000 mg proline/kg bw, respectively. Animals of two control groups were subcutaneously and intraperitoneally injected with sodium chloride solution of the same osmolarity as the high-dose proline solution used. Clinical signs with special reference to neurotoxicity were assessed during 24 hours. Blood samples for measurement of proline serum concentrations were also collected. After subcutaneous injection of 2000 mg proline/kg bw which resulted in maximum serum concentrations of 12000 µmol/L, no clinical signs were found. All other groups of animals including control group animals showed comparable clinical signs primarily shortly after test article administration.

Following intraperitoneal injection, a high variation in proline serum concentrations between animals within the same test article group was observed. Conclusions as to the toxicity of proline were therefore only drawn from results obtained after subcutaneous administration of proline. No significant effects on behaviour (Irwin test) at doses of proline up to 5 times the maximum dose administered with Privigen in clinical studies were found except for a slight rise of body temperature after 5 days of treatment. After single subcutaneous application of 2000 mg proline/kg bw/day to female rats, leading

to much higher serum concentrations than achieved with an intravenous infusion scheme, no clinical signs of neurotoxicity, especially no signs of seizures, were observed (EPAR Privigen [27]).

Pharmacokinetic in juvenile animals

The efficiency of clearance of proline by young as compared to adult rats was less after a single subcutaneous administration (Study PSR0307 Pharmacokinetics of proline or glycine following a single subcutaneous administration in young rats). This pharmacokinetic observation was expected from literature (EPAR Hizentra [17]).

In some published pharmacological and behavioral research studies in rats, proline was given SC (twice daily) at high doses for several weeks to juvenile rats (generally from post-natal day (PND) 6 to day 28. The model was designed to achieve sustained levels of proline in blood and brain comparable to those of human type II hyperprolinemia patients. PK data for this model were determined in the study of Moreira (Moreira et al., 1989 [23]). In order to obtain PK data, neonatal rats were sacrificed either at PND 7, 14, 21 or 28 and Proline concentrations were determined in blood and cerebrum. Using PK data, administered doses were adjusted to achieve plasma proline levels between 1000 and 2000 µM, similar to those found in hyperprolinemic type II patients.

Of note is that subcutaneous administration produced a non-steady elevation in proline levels, with high peak plasma concentrations of 12000–14000 µmol/l one hour post-injection, dropping to 1300–2600 µmol/L six hours post injection (saline control 70–100 µmol/L proline). PK data further indicate that permeability of blood-brain barrier to proline and proline metabolism changes with the age of the rats. While proline metabolism and excretion increase as age advances, proline permeability through the blood-brain barrier decreased with age. It seems that blood-brain barrier of young adult 28-day-old rats is practically impermeable to exogenous proline, whereas permeability was higher the younger the rat (PND 7 > 14 > 28).

Toxicity after single administration

LD50 (oral, rat) of > 5110 mg/kg has been published (Merck Sicherheitsdatenblatt, Stand 17.08.2006). No clinical signs were found in a safety pharmacological study with single subcutaneous injection of 2000 mg proline/kg bw (c_{max} 12000 µmol/L). Since proline is also substantial part of human proteins (Maeder et al., 2011 [24]) and diet with normal daily intake of approximately 5200 mg (WHO FOOD ADDITIVES SERIES: 54, original literature: Institute of Medicine (2002) [32]) a low acute toxic potential can be assumed.

Toxicity after repeated administration

<u>Rat</u>

In a 5-day repeat-dose intravenous dose finding study (Study o. 25/034), groups of male rats were administered daily for 7 hours with low and high dose proline (579 and 1449 mg/kg bw/day). Other groups of rats were infused with low and high dose glycine (378 and 945 mg glycine/kg bw/day) or with physiological saline. Serum concentrations of proline and glycine were assessed on Day 1 (before infusion, 3 hrs after the start of the infusion and at the end of infusion), on Day 2 (before infusion) and on Day 5 before infusion, 3 hrs after the start of the infusion and at the end of infusion). The high dose level represented the maximal daily dose that could be infused in the animals. There were no signs of toxicity in either dose group with glycine or proline. The No Observed Adverse Effect Level (NOAEL) was therefore the high dose level, i.e. 1449 mg proline/kg bw/day and 945 mg glycine/kg bw/day, respectively. Maximum serum levels of 4000 µmol/L were found at the NOAEL. The high dose was considered appropriate as upper dose in the final 28-day toxicity study (EPAR Privigen and Hizentra [27, 17]).

In a subsequent 28-day repeated dose intravenous study (Study No. 925/035), groups of female and male rats were administered daily for 7 hours with low and high dose proline (579 and 1449 mg proline/kg bw/day) as in the study before. Other groups of rats were infused with low and high doses of glycine (378 and 945 mg glycine/kg bw/day) or with physiological saline. Serum concentrations of proline and glycine were assessed on Days 1, 7, 14 and 28 before and at the end of the infusion. Urine was collected at termination during 24 hours. The high dose group represented the maximal daily dose that could be infused in the animals and was well tolerated and not associated with any marked changes indicative of toxicity. There were no unscheduled deaths throughout the study. No treatment-related clinical signs were observed in any group and there were no treatment-related eye lesions. There was no obvious influence of treatment on the haematology and serum clinical chemistry parameters. There were no treatment-related effects on the urine parameters. No obvious effects of treatment with proline and glycine were observed in organ weights, or after macroscopic and microscopic examinations of the tissues. The only treatment-related changes were slight (not statistically significant) reductions in body weight gain and food consumption during the first two weeks of treatment, especially in males. These affected principally the animals treated with both doses proline, and glycine at 945 mg/kg/day, whereas glycine at 378 mg/kg/day was not affected. NOAELs of 1449 mg/kg/day for proline and 945 mg/kg/day for glycine could be established under the defined experimental conditions. Maximum serum levels of 4100 µmol/L were found at the NOAEL (EPAR Privigen and Hizentra [27, 17]).

In a 30-day study, a group of seven white female Sprague-Dawley rats were given proline at a dose of 50 mg/kg bw per day in water. A group of 10 rats served as the control group. After 30 days, all animals were weighed, necropsied, and subjected to a complete gross examination. Histopathology and microscopic examinations of the liver and kidneys were conducted. Samples of serum were obtained for determination of enzyme activities, and concentrations of creatinine and total protein. There were no treatment-related effects in rats given proline at 50 mg/kg bw/day compared with the controls (Published in WHO/JEFCA report, original literature Kampel et al., 1989 [21]). Toxicokinetic parameters were not determined.

In order to assess possible toxicity of proline when used as an ingredient of supplements, health foods and cosmetics in japan, a subchronic oral toxicity study of proline was conducted with groups of 10 male and 10 female Fischer 344 rats fed a powder diet containing 0%, 0.625%, 1.25%, 2.5% and 5.0% of proline for 90 days. No treatment-related clinical signs and mortality were noted. There were no clear treatment-related effects with regard to body weight, food intake or urinalysis data. The average daily water intakes of the treated female groups were significantly increased compared to the controls. The haematology (red blood cell parameter) and serum biochemistry (glucose, blood urea nitrogen, creatinine or uric acid) of the treated male and/or female groups were lower than those of the control groups. However, these changes were lacked dose-dependence, and no abnormalities were found in corresponding pathological findings. The NOAEL was determined to be a dietary dose of 5.0% (2772 mg/kg body weight/day for males and 3009 mg/kg body weight/day for females) (Tada et al., 2010 [30]). Toxicokinetics parameters were not determined.

Dog

In 7-day and 28-day repeat-dose toxicity studies in dogs, daily intravenous proline doses of up to 4350 mg/kg bw showed no overt toxicity (Study ZLB 06_009, 668316 proline preliminary 7-day dose range finding intravenous (7h) infusion study in beagle dogs and Study CSL 07_002, 668321 28 Day Intravenous (7h) Infusion Toxicity Study in the Beagle Dog with a 14 Day Recovery Period). The NOAEL for proline was considered to be 4350 mg/kg bw (EPAR Hizentra [17]). Toxicokinetic

parameters were not determined. High safety margins (of 75 relative to the maximum clinical exposure) on mg/kg basis could be established.

There is no data from repeated-dose toxicity studies with long term administration of proline.

Genotoxicity

In an assay for reverse mutation in Salmonella typhimurium, negative results were reported for proline at concentrations of up to 57550 µg/plate (500 µmol/l) with and without metabolic activation, in S. typhimurium strains TA1530, TA1531, TA1532 and TA1964 (Published in WHO/JEFCA report, original literature Green & Savage, 1978 [32]).

Proline did not produce a clinically relevant genotoxic response in a sister chromatid exchanges assay (SCEs) in human lymphocytes at concentrations of up to 115 mg/ml (Cited in WHO/JEFCA report, original literature Xing & Na, 1996 [32]).

For proline no genotoxic effects were shown in results from in vitro and in vivo genotoxicity/clastogenicity assays in combination with L-isoleucine and nicotinamide (Study 22196 Salmonella thyphimurium/ mammalian microsome plate incorporation assay (Ames Test), Study 49196 Bacterial reverse mutation test (Ames Test) using the pre-incubation method, Study CLE 1554-3-D5140 Induction of chromosome aberration in cultured Chinese Hamster Ovary (CHO) cells, Study Zen-0995 Pro-Tox (C): Bacterial Stress Gene Assay (16 constructs) with solutions containing nicotinamide, proline, leucine, and isoleucine). Considering the known metabolism of the three excipients, which suggest a low interaction between the compounds; these studies were considered relevant for proline as a single excipient as well (EPAR Hizentra [17]).

Overall, there is no evidence of a genotoxic potential.

Carcinogenicity

Proline is an endogenously present substance lacking genotoxic potential. There is no evidence of a cancer causing potential.

Reproductive and developmental toxicity

In an Embryo Toxicity study in rats (Study No. AA30034), proline was not teratogenic or embryotoxic when administered intravenously (7-hour daily infusion) at doses of 1449 mg/kg/day during days 6 to 17 of gestation. There was also no indication for maternal toxicity. 1449 mg/kg/day are considered to be the NOAEL (EPAR Hizentra [17]).

Juvenile toxicity study

Conventional juvenile toxicity studies are not available.

Some published pharmacological and behavioural research studies as well as some conventional safety pharmacology studies are described in section ‘other toxicity studies’.

The efficiency of clearance of proline by young as compared to adult rats was less after a single s.c. administration (please refer to section “Pharmacokinetics in juvenile animals above) (EPAR Hizentra [17]).

Other studies

Evidence of retrograde amnesia has been shown in both chick and mice (Davis and Cherkin, 1977, 1979 [4, 5]).

2.2. Toxicokinetics

In a 5-day repeat-dose intravenous dose finding study (Study No. 925/034), groups of male rats were administered daily for 7 hours with low and high dose proline (579 and 1449 mg/kg bw/day). There was a dose-dependent increase in the peak serum concentration of proline up to 13–14 times baseline levels, equivalent to approximately 4000 µmol/l for the high dose group. Three hours after the start of the infusion 83 and 71% of peak concentrations were reached at low and high dose proline, respectively. There was no indication of accumulation of proline in serum in both the doses tested (EPAR Privigen [27]).

In a 28-day repeated dose intravenous study (Study No. 925/035), groups of female and male rats were administered daily for 7 hours with low and high dose of proline (579 and 1449 mg proline/kg bw/day) as in the study before. There was a dose-dependent increase in the peak serum concentrations of proline, up to 14 and 9 times baseline levels for males and females, respectively. Maximal serum levels of 3100–4100 µmol/l and 2200–2800 µmol/l proline were found at termination of the infusion on days of measurements at the high dose level for males and females, respectively, without a trend to an increase or decrease during the 4 weeks of the study. No accumulation of occurred at both doses as serum concentrations of proline were similar to baseline levels during the treatment period before daily infusions. As expected total daily excretion of proline in urine was low (EPAR Privigen).

In a single dose pharmacokinetic study (Study No. PSR 08/06) groups of female rats were injected s.c. with 2000 mg proline/kg bw, or i.p. with 2000 mg proline/kg bw or 4000 mg proline/kg bw. Control groups received i.p. or s.c. injections of sodium chloride solution of the same osmolarity as the high-dose proline solution. Peak serum concentrations of 12000 µmol/l (mean; 70 fold baseline) for s.c. treated animals and up to 18000 µmol/l and 43000 µmol/l after i.p. administration were reached 15 min after injection. Proline was eliminated quickly from the serum with baseline levels reached at 8 hours after injection for all animals except one high-dose i.p administered animal. Following i.p. injection, a high variation in proline serum concentrations between animals within the same test article group was observed (EPAR Privigen [27]).

In an intravenous teratogenicity study (Study No. AA30034) proline and glycine were compared. Groups of pregnant rats were administered from Days 6–17 of gestation over 7 h/day with 300000 µmol/l proline (42 ml/kg bw/day equivalent to 1449 mg/kg bw/day) or 300000 µmol/l glycine (42 ml/kg bw/day equivalent to 945 mg/kg bw/day) or, as a control, physiological saline (42 ml/kg bw/day). Serum concentrations of proline and glycine were assessed on Days 6 and 17 of gestation before and at the end of the infusion. Mean maximal serum levels on Days G6 and G17 were 3530 µmol/l and 2570 µmol/l proline in the animals infused with proline, and 1670 µmol/l and 1230 µmol/l glycine in the animals infused with glycine, respectively. No accumulation of proline and glycine occurred. Serum concentrations of glycine were only marginally influenced by proline infusions and vice versa (EPAR Privigen [27]).

In an “experimental model of hyperprolinemia” (Moreira et al., 1989), juvenile rats received subcutaneous doses of proline of 12800 to 18200 µmol/kg bw (1500 to 2100 mg/kg bw) twice daily from PD 6 till PD 28. This model was used in the numerous publications (Delwing et al., 2003 [6]; Bavaresco et al., 2005 [2]; Delwing, 2006 [8]) which indicate vulnerability of the immature, developing brain to high doses of proline. This model was designed to achieve sustained levels of proline in blood and brain comparable to those of human type II hyperprolinemia patients with proline levels between 1000 and 2000 µM.

356 **Table 1.** Extract from the EPAR for Privigen

Rat Study duration	Proline: mean C_{max} at highest dose tested
Safety Pharmacology and Pharmacokinetics; exploratory study – Single dose, s.c. (Study No. PSR 08/06)	12000 µmol/l
Repeat dose toxicity – 5 days (Study No. 925/034)	4000 µmol/l
Repeat dose toxicity – 28 days (Study No. 925/035)	3100–4100 µmol/l (males) 2200–2800 µmol/l (females)
Reproduction Toxicity (segment II) (Study No. AA30034)	2600–3500 µmol/l

357 # 3. Pharmacokinetics (in humans)

358 Proline is a nonessential neutral amino acid and a component of nutritional proteins; the daily intake
359 with food is about 5200 mg. The normal proline plasma level is in the range of 266 ± 35 µmol/l.
360 Proline is formed from and metabolized to glutamate. However, under certain physiological conditions
361 (severe burn, preterm neonate), i.e. when endogenous synthesis cannot meet metabolic need of
362 proline, additional intake via dietary source is needed (conditionally indispensable) (Dietary Reference
363 Intakes: The Essential Guide to Nutrient Requirements [11]; Wu, 2009 [35]; Wu, 2011 [35]). Proline is
364 part of diet with normal daily intake of approximately 5.2 g (www.nap.edu). The normal proline plasma
365 level is in the range of 266 ± 35 µmol/L. Proline is formed from and metabolised to glutamate. Normal
366 serum proline levels vary by age: 3–10 years 68–148 µmol/L; 6–18 years 58–324 µmol/L and > 18
367 years 102–336 µmol/L (Wu, 2006 [33]).

368 The half-life (t1/2) of proline is approx. 5 hours.

369 It is tubularly reabsorbed in kidneys uses the same transporter as glycine and hydroxyproline and thus
370 not secreted in the urine of healthy individuals.

371 Proline passes the blood-brain barrier (BBB) where it acts as a low potency member of the class of
372 excitatory amino acids. In adults it is effluxed by the amino acid transporter ATA2, the orphan
373 transporter v7-3 (solute carrier 6a15, or B0AT2), and the PROT transporter (FDA Memo). One or some
374 of these transporters may not be well developed in young children.

375 **Privigen (IVIG)**

376 Privigen is stabilized with 250000 µmol/l proline (proline has a molecular weight of 115.13 g/mol→
377 28.75 g/l).

378 Proline physiological serum range: 266 ± 35 µmol/l.

379 The serum concentrations of proline were measured in both clinical studies of Privigen (one study in
380 primary immunodeficiency patients (PID) and one in immune thrombocytopenia (ITP).

381 The pre-infusion levels in all subjects were within the expected physiological range.

382 In the PID study, all patients (n= 80, ages 3–69 years) received doses between 0.20 to 0.88 g/kg
383 Privigen per infusion given at 3 to 4 week intervals. The median pre-infusion proline levels prior to
384 the first and second infusion and also prior to the infusion at week 12 were between 207 and

236 µmol/l. The median post-infusion proline serum concentrations were 1927 µmol/l after the first infusion and 1793 µmol/l after the second infusion. As expected, median proline values after infusion increased with increasing median dose of Privigen.

In the ITP study (n=57; ages: 15–69 years), a dose of 1 g/kg was administered for two consecutive days. The median pre-infusion levels of proline prior to the first and second infusions were 199 µmol/l and 446 µmol/l, respectively. Proline serum concentration increased from pre-infusion levels to median post-infusion levels of 2606 µmol/l after the first and 2951 µmol/l after the second infusion. There was no cumulative effect of proline seen in the ITP study.

In an analysis of the ITP study the applicant showed that the rate of all temporally associated AEs as well as that of the most frequent temporally associated AE, namely headache, did not increase with higher proline serum concentrations.

The serum concentrations of L-glutamate were measured by HPLC in conjunction with the proline assessment, but were only analysed retrospectively. Post-infusion levels of L-glutamate did not increase to the same degree as proline concentrations. The median concentration of L-glutamate after the first infusion was about 1.4 times above baseline. The transiently increased L-glutamate serum concentrations after Privigen administration were lower than the glutamate serum concentrations measured after a protein rich meal, where increases of > 2 fold have been reported.

Based on literature data, excretion of proline into the milk of lactating women and proline exposure of nursing infants were assessed. The baby's proline dose derived from IgPro10 treatment of the mother is considerably lower than the proline amounts naturally taken in with milk proteins or than would be administered with amino acid solutions for parenteral nutrition. Proline uptake from milk therefore does not pose a risk for the baby. This available information in humans, in conjunction with the animal reproduction toxicity data and the data in juvenile animals obtained with proline, supports the safety of proline applied to lactating women with Privigen. This information is considered sufficient to justify not including any precautionary statements regarding proline uptake with milk after IgPro10 administration to mothers in sections 4.6 and 5.3 of the Summary of Product Characteristics [28].

Hizentra

Two PID studies were performed, one in the EU and one in the USA.

In the EU study in 51 PID patients (ages: 3–60 y) the mean dose per week was 120 mg/kg (range 59–243 mg/kg)

Proline was measured during 1 dosing interval at Week 28 ± 1. Mean pre-infusion levels were 259 µmol/l, the level peaked at ~10 minutes after the infusion (370 µmol/l; max 662 µmol/l). One day after infusion, the serum proline concentration had returned to approximately the same concentration as before the infusion. There were no signs of proline accumulation. In 3/23 subjects individual measurements showed proline concentrations were above the upper limit of the normal range (450 µmol/). No relevant differences were seen in children.

In the US study in 49 PID patients (ages: 5–72 y) the mean dose per week was 181.5 mg/kg. The maximum proline level was 789 µmol/l. In comparison in the studies with Privigen median levels of 1927 µmol/l (in PID study) and 2951 µmol/l (in ITP study) were reached.

No additional concerns with regard to proline are raised by these studies with the s.c product.

Table 2. Partially adapted from Hagan et al. [16]

	Route **Proline content**	**Studies**	**Dose IgG**	**Maximum proline dose**	**Proline serum level post-infusion** µmol/L
IgPro 10 Privigen	i.v. 250 mmol/L	PID (3-69 y) N= 80	Range: 0.20 – 0.88 g/kg/month (long-term dosing)	253 mg/kg/month	Post day 1 Median: 1927
		ITP (15-69 y) N= 57	2 g/kg (short-term dosing) Maximum daily dose: 1 g/kg/d	288 mg/kg/d	Post day 1 Median: 2606 Max 7963* Post dose day 2 Median 2951 Max: 9661 **
IgPro 20 Hizentra	s.c. 250 mmol/L	PID (3-60 y) N= 51	Mean: 0.120 g/kg/week Range: 0.059-0.243 (long-term dosing)	34 mg/kg/week	Mean: 370 Max: 662
		PID (5 -72y) N= 49 N =18 PK	Mean: 0.213 g/kg/week Range: 0.072-0.379 (long-term dosing)	55 mg/kg/week	Post dose day 1 Median: Max: 789
10% FreAmine® III	i.v	n.a	n.a (short-term dosing)	255–348 mg/kg	n.a.

*In the ITP study the patient with the highest proline level post-dose on the first Day (7963 µmol/l)experienced mild AEs of which 2 were considered "possibly" related to the treatment (+ Coomb's test and bilirubin increase) but not to proline. This subject had a baseline of 180 µmol/L and returned to baseline by Day 4 post-dose.

**The patient with the highest proline level post-dose on the second Day (9661µmol/l) experienced mild –severe AEs (headaches, fever, dizziness sleepiness, - the severe headache was considered "probably" related to the treatment but as this is a typical side-effect of IVIG it is difficult to say what role proline played in this AE. This subject had a baseline of 125 µmol/l and returned to baseline by Day 8 post-dose.

4. Clinical safety data

4.1. Safety studies

Two immunoglobulins authorised in the EU are currently in use and contain proline as an excipient: Privigen given intravenously and Hizentra given subcutaneously. Depending on the indication, the IV product is generally administered at doses of 200 mg/kg to 800 mg/kg per infusion every 3 to 4 weeks in the replacement setting of primary immunodeficiency patients (PID) and at doses of 1000 mg/kg was for two consecutive days in the immunomodulatory setting (e.g. ITP, GBS, Kawasaki). The second product is administered weekly or biweekly at doses of 100 mg/kg to 200 mg/kg in replacement therapy.

As animal studies in rats suggest that proline induces oxidative stress which may be involved in the brain dysfunction observed hyperprolinemic patients, further investigations were undertaken during the MAA of Privigen to address this issue (see above).

4.1.1. Patients with hyperprolinaemia

From patients suffering from hyperprolinaemia, a rare autosomal recessive inherited disorder leading to a dysfunction of proline metabolism resulting in elevated plasma (normal 51–271 µM; HP type I < HP type II (up to 500–3700 µM) it is reported that this may lead to seizures or other neurological 1* forms of schizophrenia.

Consequently, additional exposure of these patients to proline should be limited as much as possible.

This is in line with Privigen's and Hizentra's current labellings which contraindicate their use for patients suffering from hyperprolinaemia.

Privigen

SmPC Section 4.3 Contraindications: Patients with hyperprolinaemia.

PIL section 2. Do NOT take Privigen if you suffer from hyperprolinaemia (a genetic disorder causing high levels of the amino acid proline in the blood). This is an extremely rare disorder. Only a few families with this disease are known worldwide.

Hizentra

SmPC Section 4.3 Contraindications: Patients with hyperprolinaemia type I or II.

PIL section 2 Do NOT inject Hizentra if you suffer from hyperprolinaemia (a genetic disorder causing high levels of the amino acid proline in the blood).

4.1.2. Patients with normal proline metabolism

Some published pharmacological and behavioral research studies in rats (i.e. watermaze test, open field test) in which proline was given SC (twice daily) at high doses for several weeks to juvenile rats (generally from post-natal day *(*PND) 6 to day 28, but later timeframes up to PND 39 have also been investigated) indicate that long-term administration of high doses of proline have effects on brain development in juvenile rats (Wyse/Bavaresco). The model used in these studies claims to be adjusted to achieve plasma proline levels between 1000 µM and 2000 µM, comparable to those found in type ll hyperprolinemic patients (Moreira et al., 1989 [23]).

From other studies using a similar animal model it is reported that proline provokes several neurotoxic effects in rats, such as memory impairment (Delwing et al., 2006 [8]) inhibition of enzymes activities as acetylcholinesterase, Na+, K+-ATPase, and aminotransferases (Pontes et al., 1999 [25], 2001 [26]; Shanti et al., 2004 [29]; Delwing et al., 2005 [7]) energy deficit (Delwing et al., 2007 [9]; Ferreira et al., 2010 [14]) and induction of oxidative stress (Delwing et al., 2003 [6]). This data indicate that high proline concentrations in the CNS can induce some neurotoxicity when administered repeatedly at high doses and may effect brain development in juvenile animals.

Of note Privigen and Hizentra (approved in the European Union via the centralised procedure) contain high levels of proline and are indicated for the use in children of all age groups. In order to address the neurotoxic potential of proline, specific non-clinical safety studies, with the particular intention to mimic the clinical dose, application route and schema of Privigen and Hizentra were conducted. On the basis of these studies, non-clinical data for proline reveal no special risk for humans based on safety

pharmacology and toxicity studies. This situation is reflected in the current labelling in sections 5.3 of the SmPCs of Privigen and Hizentra [28, 18].

In sum, some published pharmacological and behavioural research studies in juvenile rats indicate that long-term administration of high doses of proline may have effects on brain development in juvenile rats. However, the results from Privigen in newborn rats were reassuring and suggested a non-neurotoxic potential of proline, administered as an excipient with Privigen, on the developing brain. Furthermore, no effects on brain development or any other special risk for humans (with an intact proline metabolism) were observed in the conventional non-clinical studies conducted with proline for approval of Privigen and Hizentra. These somewhat contradictory results may possibly be explained by the different administration schedules and pharmacokinetics, i.e. high subcutaneous doses of proline twice daily versus intermittent dosing mimicking clinical administration schedule of Privigen and Hizentra and indicate a threshold for neurotoxicity in-between the exposition mimicking the clinical scheme for Privigen/Hizentra and the exposition as described in the animal model causing proline levels comparable to those of hyperprolinaemia type II patients depending on dose, administration schedule (route, duration and frequency of exposure) and probably on the age of the animals.

A certain risk for neurotoxicity along with elevated plasma levels of proline is known from patients suffering from hyperprolinaemia (up to 500–3700 µM, normal range 266 ± 35 µmol/L).

Considering that

a) the development of gray and white matter, myelination, synaptogenesis, pruning, and synaptic modification continues in early childhood and synaptogenesis further continues to adolescence [15];

b) the blood-brain-barrier is not fully developed until approx. 6 months;

c) an exact threshold for children cannot be deduced from the pre-clinical studies the relative safety limit extrapolated from proline doses administered in the Privigen and Hizentra studies and parenteral amino acid mixtures could serve as a guidance for intravenous and subcutaneous administration levels of proline;

there might be a potential risk for neurotoxicity following long-term administration of high doses of proline to very young individuals.

However, that standard toxicity studies did not identify any toxic effects and the signal for neurotoxicity has been observed experimentally in a model specifically designed to study hyperprolinaemia using very specific conditions. Taken this into account and because proline is only rarely used as an excipient in adequately labeled medicinal products, it was felt that a common labelling recommendation in the excipient guideline concerning neurotoxicity was not reasonable.

4.2. Safety post marketing

From the marketing authorisation data for both Privigen and Hizentra no clear safety signals were detected with regard to proline. To broaden the safety data base on the paediatric population, an intensified Pharmacovigilance protocol was proposed to uncover signals on relevant neurological effects. This dedicated Signal Detection focuses on spontaneous ADR reports, including cases from the scientific literature, concerning Kawasaki disease, the paediatric population and neurologic disorders after Privigen administration.

In the last PRAC PSUR 07 Oct 2013 (on all IVIGs and SCIGs) the main focus was on haemolysis, allergic/hypersensitivity reactions/anaphylactic reactions, thromboembolic events, an acute renal

failure. For Hizentra the MAH was asked to comment, on whether there had been any suspected cases of hyperprolinaemia in the post-marketing observation spontaneously reported since launch.

5. Risk assessment

5.1. Pre-clinical data

Proline is a physiological, conditional-essential amino acid and a component of nutritional proteins with the daily intake with food of about 5200 mg.

Results from a 5-day Irwin test in rats receiving daily intravenous 7 h infusion of proline at doses of up to 4350 mg/kg (resulting in a plasma exposure of 4000 µmol/L) did not show any significant neurobehavioral finding. In 5- and 28-day repeat-dose toxicity studies with subcutaneous administration to rats there were no signs of toxicity (NOAEL 4350 mg/kg bw). There was also no toxicological finding in a 3 months oral toxicity study in rats (NOAEL 2772 mg/kg bw).

In 7-day and 28-day repeat-dose toxicity studies in dogs, daily intravenous (7h infusion) proline doses of up to 4350 mg/kg bw (NOAEL) showed no overt toxicity. There are no data from repeated-dose toxicity studies with long term administration of proline.

Available in vitro and in vivo data provide sufficient assurance of the absence of genotoxic activity.

There was no evidence for maternal or reproductive toxicity in a segment II study in rats treated intravenously (7-hour daily infusion) at doses of up to 1449 mg/kg/day during days 6 to 17 of gestation (NOAEL).

The only notable evidence for potential toxicity of proline came from some published studies pertaining to hyperprolinaemia. These studies have shown that repeated administration of, high doses of proline have effects on brain development in very young rats. In an “experimental model of hyperprolinemia” (Moreira et al., 1989 [23]), juvenile rats received subcutaneous doses of proline of 12800 to 18200 µmol/kg bw (1500 mg/kg bw to 2100 mg/kg bw) twice daily from PND 6 till PND 28 or PND 15 to PND 39. The finding that particularly very young individuals are vulnerable to high doses of proline is supported by PK data that indicate that permeability of blood-brain barrier to proline and proline metabolism changes with the age of the rats. While proline metabolism and excretion increase as age advances, proline permeability through the blood-brain barrier decreased with age. It seems that blood-brain barrier of young adult 28-day-old rats is practically impermeable to exogenous proline, whereas permeability was higher the younger the rat (PND 7 > 14 > 28).

Further evidence supporting the vulnerability of particular young individuals as the brain rapidly develops in this phase and since protective mechanisms like the blood brain barrier or systemic clearance are immature in rats aged < PND 35 and human equivalent age groups; i.e. immature development of blood-brain barrier (BBB) and amino acid efflux transporters (see above). Furthermore, pharmacokinetic studies in juvenile animals have shown that efficiency of systemic clearance of proline by young was less when compared to adult rats.

There are no data for adult or older juvenile animals with repeated or longer term administration of proline addressing specific neurotoxic endpoints (e.g. dosing started later than PND 15). On the basis of the available pharmacokinetic and toxicological data it might however be speculated that animal > day 28 to 35 of age are less vulnerable to proline and may tolerate higher doses or a more often dosing schedule without adverse neurological effects (see below).

Of note is that specific behavioural studies have been conducted in the context of the MAAs for Privigen and Hizentra with the aim to qualify the excipient proline and to address concerns from the published

experimental studies indicating that proline may have neurological effects on brain development in young rats. No effects on brain development or any other special risk for humans (with an intact proline metabolism) were observed in these studies mimicking the clinical dosing schedule of Privigen and Hizentra. Particularly in a Morris water maze task study in newborn rats with subcutaneous administration of proline with daily proline doses up to 4 g/kg administered daily for 2–5 consecutive days or once every 2–4 weeks there was no finding in water maze performance at postnatal days 54 to 71, and the treated rats showed no change in spatial memory acquisition or retention.

These apparently contradictory results may be explained by the different administration schedules used, i.e. daily application of high subcutaneous doses for several weeks in the “experimental model of hyperprolinemia” versus high single doses as given in standard safety pharmacology studies or doses administered in a intermitted schedule, i.e. daily of up to 4000 mg/kg for 2–5 consecutive days or once every 2–4 weeks and indicate a threshold for neurotoxicity in-between the exposition mimicking the clinical scheme for Privigen/Hizentra and the exposition as described in the animal model causing proline levels comparable to those of hyperprolinaemia type II patients - depending on dose, administration schedule (route, duration and frequency of exposure) and probably on the age of the animals. It might be speculated that the repeated twice daily subcutaneous administration inducing high peak plasma concentrations of 12000 –14000 µmol/l induce overload/exhausting of defence mechanisms and enzymatic capacity of brain tissues, resulting in CNS toxicity.

Overall, since results from animal studies are inconclusive, it is difficult to draw firm conclusions from the current somewhat contradictory data, but data indicate that high repeated doses of proline may pose a possible risk for the developing brain particularly in young individuals. A NOAEL/NOEL for neurotoxicity in healthy young animals for proline might be established in-between the exposition mimicking the clinical scheme for Privigen/Hizentra and the exposition as described in the animal model causing proline levels comparable to those of hyperprolinaemia type II patients - depending on dose, administration schedule (route, duration and frequency of exposure) and probably on the age of the animals.

There were no other notable non-clinical findings from conventional studies of safety pharmacology, acute and repeated dose toxicity, genotoxicity and reproductive toxicity. Repeated dose toxicity studies have been conducted with rats (SC) and dogs (IV) of up to 28 days (NOAEL 4350 mg/kg) and in dogs (oral) of up to 3 months (NOAEL 2772 mg/kg bw).

5.2. Clinical aspects

The effects of consistently high proline plasma levels can be seen in hereditary hyperprolinemia type I (HPI) and type II (HPII). Each type is caused by an autosomal recessive inborn error of the proline metabolic pathway.

- HPI is caused by an abnormality in the proline-oxidizing enzyme (POX). The POX gene (PRODH) is located on chromosome 22 (22q11.21). This region is deleted in congenital malformation syndromes including velo-cardio-facial syndrome, DiGeorge Syndrome and conotruncal anomaly face syndrome. This gene locus is also related to susceptibility to schizophrenia. The incidence of chromosome 22q11.2 deletion syndrome is ~27:100 000 live births. Approx. 50% of patients with 22q11.2 deletion have hyperprolinemia and approx. 77% of patients with 22q11.2 deletion are immunodeficient (mainly thymic hypoplasia); antibody defects are only present in 15%. Data from European and US registries for immunodeficiencies showed that 3% of patients with DiGeorge syndrome require IgG therapy. An estimated 1675–2400 people with 22q11.2 deletion could be treated with IgG therapy in the USA. Half of these may be hyperprolinemic.

- HPII is caused by a deficiency of Δ-1-pyrroline-5-carboxylate (P5C) dehydrogenase (P5CDh). The P5CDh gene (ALDH4A1) is located on chromosome1 (1p36.13).

In patients with type I and II hyperprolinemia their proline serum levels are 10–15 times higher than the normal level (proline physiological serum range: 266 ± 35 µmol/L). If not diagnosed early in life some individuals with these genetic disorders will exhibit neurological and psychiatric disorders, including seizures, mental retardation and schizophrenia (Mitsubuchi, 2014 [22]).

Thus, the developing brain seems to be sensitive to consistently high proline levels.

In products containing proline as an excipient one of the concerns is that proline passes the blood-brain barrier (BBB) (The PDCO accepts 6 months as the age at which the BBB is developed). Proline is effluxed by various amino acid transporters (see above) that may not be fully developed in very young children.

As the brain development continues in early childhood into adolescence and as an exact threshold for proline serum levels in children cannot be deduced from the pre-clinical studies, it would be advisable for long-term treatment to avoid plasma proline levels seen in symptomatic hyperprolinemic patients; this would approx. be 10x physiological serum range, i.e. ~2500 µmol/L.

However, the following aspects require further differentiation in assessing a possible danger resulting from increased proline levels:

a. Will the treatment be given short-term as a single dose as for Privigen in ITP or Kawasaki or long-term treatment as in PID (Privigen/Hizentra)?

b. Will the dosing interval in long-term treatment allow for a sufficient and rapid normalisation of proline levels?

As the half-life of proline is approx. 5 hours and the dosing intervals of Hizentra (low dose; weekly) and Privigen (higher dose, 3–4 weekly) should not lead to any accumulation, given an intact proline metabolism. Proline is eliminated from the plasma by about 50% within 2 hours after end of infusion, and more than 90% within 24 hours.

Thus, a relative safety limit may be extrapolated from Proline doses administered in the Privigen and Hizentra studies and parenteral amino acid mixtures could serve as guidance for intravenous and subcutaneous administration levels of proline.

Conclusion

In children and adolescents with an intact proline metabolism, parenteral short-term single doses of a treatment containing proline as an excipient can be given at an amount of ≤ 350 mg/kg/daily.

In children and adolescents with an intact proline metabolism, parenteral long-term repeated doses of a treatment containing proline as an excipient can be given at an amount of ~0.060 g/kg/weekly or ~250 mg/kg/monthly.

6. Safety information relevant for the package leaflet

Proline is not listed in the current Annex to the European Commission 'Guideline on Excipients in the Label and Package Leaflet of Medicinal Products for Human Use [1, 12].

A certain risk for neurotoxicity along with elevated plasma levels of proline is know from patients suffering from hyperprolinaemia (up to 500–3700 µM, 266 ± 35 µmol/L). This is in line with the labelling of the approved proline containing medical products is contraindicated in patients with

hyperprolinaemia, i.e. the use of Privigen and Hizentra (proline containing IgG medical products) is contraindicated in Patients suffering from hyperprolinaemia type I and II).

Therefore, the risk of the **use of proline in hyperprolinaemia** has been included in the Annex.

Evidence for potential toxicity of proline in very young individuals came from some published studies pertaining to hyperprolinaemia. Using a similar “experimental model of hyperprolinemia”, these studies have shown that repeated administration of high doses of proline have effects on brain development in very young rats (subcutaneous doses of proline of 12800 to 18200 µmol/kg bw (1500 mg/kg bw to 2100 mg/kg bw) twice daily from PND 6 till PND 28 or PND 15 to PND 39).

There is also some evidence for a potential risk for neurotoxicity following long term administration of high doses of proline to very young individuals (see section 4.1.2), but there is no proline specific labelling concerning the use of Privigen and Hizentra in children (approved for children (0–18 years)) and adults (with an intact proline metabolism). This is justified on basis of animal behavioural tests reflecting clinical schedules of Privigen and Hizentra without relevant findings.

However, the current use of proline is restricted to a few products only (e.g. in Privigen (IVIg), Hizentra (ICIg), Repatha (evolocumab, mAB) and probably some vaccines (single dose)), there were no noteworthy findings in standard toxicology studies and since the data that indicate a neurotoxic potential of proline came from a model to study hyperprolinemia with very specific experimental conditions, a specific general warning for the use of proline in young children and/or adolescents (not suffering from hyperprolinemia) will not be meaningful.

Qualification of unusual proline amounts in upcoming MAAs/CTAs may be addressed individually by a product specific discussion either way.

A specific general warning for the use of proline in young children and/or adolescents (not suffering from hyperproloinemia) is not meaningful.

There is also no proline specific labelling in nutritional supplements. The use of such products is generally contraindicated in “patients suffering from amino acid metabolism disorders”. In nutritional supplements, proline is added as “active compound” and in consequence is out of the scope of this document.

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Annex 1. Causes for concerns identified for approved Biological medicinal products (Privigen and Hizentra)

	Privigen (solution for infusion) IVIg	**Hizentra** 200 mg/ml solution for subcutaneous injection
SmPC 5.3 Preclinical safety data	"Immunoglobulins are a normal constituent of the human body. L-proline is a physiological, non-essential amino acid. The safety of Privigen has been assessed in several preclinical studies, with particular reference to the excipient L-proline. Some published studies pertaining to hyperprolinaemia have shown that long-term, high doses of L-proline have effects on brain development in very young rats. However, in studies where the dosing was designed to reflect the clinical indications for Privigen, no effects on brain development were observed. Non-clinical data reveal no special risk for humans based on safety pharmacology and toxicity studies."	"Immunoglobulins are a normal constituent of the human body. L-proline is a physiological, non-essential amino acid. The safety of Hizentra has been assessed in several preclinical studies, with particular reference to the excipient L-proline. Non-clinical data reveal no special risk for humans based on safety pharmacology and toxicity studies."
PIL 2. What you need to know before you are given XXX	**Do NOT take Privigen** - if you suffer from hyperprolinaemia (a genetic disorder causing high levels of the amino acid proline in the blood). This is an extremely rare disorder. Only a few families with this disease are known worldwide. **Privigen contains proline** You must not take it if you suffer from hyperprolinaemia (see also section 2 "What you need to know before you are given Privigen"). → Tell your doctor prior to treatment.	**Do NOT inject Hizentra** - if you suffer from hyperprolinaemia (a genetic disorder causing high levels of the amino acid proline in the blood). **Hizentra contains proline** You must not take it if you suffer from hyperprolinaemia (see also section 2 "What you need to know before you use Hizentra"). Please tell your doctor prior to treatment.
Comments	→ Proline is used as an excipient for parenteral Ig (IV or SC) immunoglobulins Privigen and Hizentra. Considering the wording in	

	5.3 of SPC that there is no special risk of the use of L-proline as an excipient in relation to hyperprolinaemia, is it relevant to include this information about proline and hyperprolinaemia in the PIL. → Please note that the information in the PIL related to hyperprolinaemia is not consistent with the SPC.

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