4 March 2024
EMA/CHMP/332530/2015
Committee for Medicinal Products for Human Use (CHMP)

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

Draft agreed by Excipients Drafting group	1 August 2018
Adopted by CHMP for release for consultation	20 September 2018
Start of public consultation	19 November 2018
End of consultation (deadline for comments)	31 May 2019
Agreed by Non-clinical Working Party (NcWP)	18 January 2023
Adopted by CHMP	20 March 2024
Date of publication	4 March 2024

Keywords	*Excipients, Package leaflet, Proline, Hyperprolinaemia*

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Executive summary

Proline is an amphiphilic, naturally occurring non-essential amino acid which is present in human plasma and a major component of human proteins. The daily intake with food is about 5 200 mg. The normal proline plasma levels are in the range of 266 ± 35 micromol/L (µM).

Proline is not commonly used in pharmaceuticals as an excipient. It is approved for use in Privigen, immunoglobulin G (IgG) for intravenous (IV) administration (IVIgG) and Hizentra, immunoglobulin G for subcutaneous (SC) administration (SCIgG) as a stabiliser. 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' [2]. However, two major safety concerns related to neurotoxicity have been identified regarding excessive exposure to proline.

In patients suffering from hyperprolinaemia (HP) Type I and II, two rare autosomal recessive inherited disorder leading to a dysfunction of proline metabolism resulting in elevated plasma proline levels (normal 51–271 µM; HP type I < HP type II (up to 500–3,700 µM), it is reported that this may lead to seizures or other neurological abnormalities and specific forms of schizophrenia. Despite the absence of evidence that these neurological manifestations are caused by proline, the disorder is characterised by high levels of proline in the blood, and additional exposure of these patients to proline should be limited as much as possible. Therefore, 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 [40]; Bavaresco [3]). 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.

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 immunoglobulin G (IgG) for intravenous (IV) administration (IVIgG), the use of proline as a stabiliser has been demonstrated to improve stability by inhibiting 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 [4]).

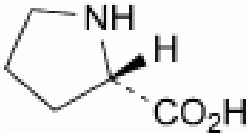
1.2. Physico-chemical properties

Category

Proline (Ph. Eur. terminology standard term for L-proline) is an amphiphilic, naturally occurring non-essential amino acid which is present in human plasma and a major component of human proteins (Maeder et al., 2011 [25]). 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 specification includes 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 g/mol.

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 10% immunoglobulin G for IV administration (IVIgG) formulated with 250 mmol/L (mM) proline at pH 4.8 and has stability at room temperature for 3 years (Wasserman et al., 2009 [35]).

Hizentra 20% immunoglobulin G for subcutaneous (SC) administration (SCIgG) has been formulated with 250 mM proline and is stable at 25 ºC for 30 months (Jolles et al., 2011 [23]), has also been approved by EMA. Proline can bind to the hydrophobic regions of the IgG molecules and prevent interactions/breakdown and oxidation, thereby stabilising the molecule (Hagan et al., 2012 [18]).

In lower amounts, proline is also contained in a human IgG2 monoclonal antibody (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 over the counter (OTC) supplements and in numerous amino acid mixtures intended for parenteral nutrition.

Proline may also be used as food additive, but exposure of humans to proline through food is in orders of magnitude higher than the anticipated level of exposure from its use as a flavouring agent (WHO/JEFCA [36]).

Of note, under certain exceptional physiological conditions, i.e. when endogenous synthesis cannot meet metabolic need of proline (severe burn, preterm neonate), additional intake via a dietary source is needed (conditionally indispensable) (Dietary reference intakes: The essential guide to nutrient requirements [12]; Wu, 2009 [39]); Wu et al., 2011 [38]).

2. Pharmacological and toxicological data

2.1. Toxicology

Published toxicological data for proline are 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 European public assessment reports (EPARs) of these approved products. Further toxicological data have been summarised by the World Health Organization (WHO) (WHO Food additives series: 54 [36]). Only few additional data were collected from other published sources. Results from animal studies performed with proline are summarised below.

With the exception of the regulatory studies conducted under good laboratory practice (GLP) to support the marketing authorisation of IVIgG and SCIgG, there is no information on the formal GLP status of the available non-clinical data. GLP compliance or at least comparable regulatory acceptable standards can also be assumed for the subchronic toxicological study conducted under the Japanese food regulation for regulatory purposes (Tada et al., 2010 [33]).

Safety pharmacology

Published pharmacological studies from the literature indicate that proline is an amino acid with potential central nervous system (CNS) toxicity. A general overview on behavioural and neurochemical effects of proline is reviewed by Wyse and Netto, 2011 [40].

Several published pharmacological and behavioural research studies in rats (i.e. water maze test, open field test) in which proline was given subcutaneously (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 had effects on brain development in juvenile rats (Moreira et al., 1989 [27]; Delwing et al., 2003 [8]; Shanti et al., 2004 [32], Bavaresco et al., 2005 [3]; Delwing, 2006 [10]; Ferreira et al., 2011 [16]). A similar experimental model of hyperprolinaemia 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 hyperprolinemic patients (normal 51–271 µM; HP type I < HP type II (up to 500–3,700 µM).

Using pharmacokinetic (PK) studies, proline was administered at doses of 12.8 to 18.2 µmol per gram of body weight (µmol/g bw) to achieve plasma proline levels between 1 and 2 mM (ten times over normal proline serum levels), similar to those found in hyperprolinemic (HP) type ll patients (Moreira et al., 1989 [27]) (see section 'Pharmacokinetic in juvenile animals' below).

The findings from the behavioural studies indicate that treatment with proline impairs spatial memory in rats and that this effect establishes in early life. Study reports further indicate that locomotor activity was not adversely affected. Besides, 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 [28]; Shanti et al., 2004 [32]; Delwing et al., 2005 [9]) energy deficit (Delwing et al., 2007 [11]; Ferreira et al., 2010 [16]) and oxidative stress (Delwing et al., 2003 [8]).

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 and can affect brain development particularly in juvenile animals.

IVIgG contains high amounts of proline and is indicated in children of all age groups. To address the literature data on putative effects of high doses of proline on brain development, several safety pharmacology studies were conducted in support of the marketing authorisation of IVIgG. 19

Irwin test, study No. 1657/ZLB/02: A safety pharmacology study in rats was conducted under conditions relevant for the clinical situation of IVIgG, i.e. a 7 h/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 1,449 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 [30]).

A Morris water maze task study in newborn rats (exact age not known) with SC administration of proline with daily proline doses up to 4 g/kg administered daily for 5 consecutive days, or once every 2–4 weeks was performed. Morris water maze performance was tested at PND 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 [30]).

Study PSR 08/06: Another study in rats investigated the acute neurotoxicity of proline at high proline serum concentrations after SC or IP bolus injections. Groups of six female rats were injected proline SC 2 g/kg bw or IP 2 g and 4 g/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 SC injection of 2 g/kg bw, which resulted in maximum serum concentrations of 12 mM, no clinical signs were found. All other groups of animals, including control group animals, showed comparable clinical signs primarily shortly after test article administration (EPAR Privigen [30]). Following IP 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 SC administration of proline. After single SC application of 2 g/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 [30]).

Overall, standard safety pharmacology studies with proline revealed no clinical signs of neurotoxicity and no relevant modifications of behaviour were seen with proline in rats.

Pharmacokinetic in juvenile animals

The efficiency of clearance of proline in young as compared to adult rats was lower after a single SC administration (Study PSR0307 pharmacokinetics of proline or glycine following a single SC administration in young rats). This PK observation was expected from literature (EPAR Hizentra [19]).

In some published pharmacological and behavioural research studies in rats, proline was given SC (twice daily) at high doses for several weeks to juvenile rats, generally from 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 HP patients. PK data for this model were determined in the study of Moreira (Moreira et al., 1989 [27]). 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 1 and 2 mM, similar to those found in hyperprolinemic type II patients.

Of note is that SC administration produced a non-steady elevation in proline levels, with high peak plasma concentrations of 12–14 mM one-hour post-injection, dropping to 1.3–2.6 mM six hours post injection. PK data further indicate that permeability of the blood-brain barrier (BBB) 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 BBB decreased with age. In this study, it seems that BBB 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

No clinical signs were found in a safety pharmacology study with single proline SC injection of 2 g /kg bw (C_{max} 12 mM) in rats. Since proline is also substantial part of human proteins (Maeder et al., 2011 [25]) and diet with normal daily intake of approximately 5.2 g (WHO Food additives series: 54, [36]), a low acute toxic potential can be assumed.

Toxicity after repeated administration

Rat

In a 5-day repeated dose IV dose finding study (Study No. 925/034), groups of male rats were administered daily for 7 hours with low and high doses of proline (579 and 1,449 mg/kg bw/day). 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 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 administered with glycine or proline. The No Observed Adverse Effect Level (NOAEL) was therefore the high dose level, i.e. 1,449 mg/kg bw/day for proline and 945 mg/kg bw/day for glycine. Maximum serum levels of 4 mM 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 [30, 19]).

In a subsequent 28-day repeated dose IV study (Study No. 925/035), groups of female and male rats were administered daily for 7 hours with low and high doses of proline (579 and 1,449 mg/kg bw/day) as in the study mentioned above. Other groups of rats were infused with low and high doses of glycine (378 and 945 mg/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 for 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 of proline and glycine at 945 mg/kg/day, whereas animals treated with glycine at 378 mg/kg/day were not affected. NOAELs of 1,449 mg/kg/day for proline and 945 mg/kg/day for glycine could be established under the defined experimental conditions. Maximum serum levels of 4.1 mM proline were found at the NOAEL (EPAR Privigen and Hizentra [30, 19]).

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/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 [36], original literature Kampel et al., 1989 [24]). Toxicokinetic parameters were not determined.

To assess potential toxicity of proline when used as an ingredient of supplements, health foods and cosmetics in Japan, a subchronic oral toxicity study with proline was conducted in 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 parameters) and serum biochemistry (glucose, blood urea nitrogen, creatinine or uric acid) parameters of the treated male and/or female groups were lower than those of the control groups. However, these changes were not dose-dependent, and no abnormalities were found in corresponding pathological findings. The NOAEL was determined to be a dietary dose of 5.0% (2,772 mg/kg bw/day for males and 3,009 mg/kg bw/day for females) (Tada et al., 2010 [33]). Toxicokinetic parameters were not determined.

Dog

In 7-day and 28-day repeat-dose toxicity studies in dogs, IV proline doses of up to 4,350 mg/kg bw/day showed no overt toxicity (Study ZLB 06_009, 668316 proline preliminary 7-day dose range finding IV (7 h) infusion study in beagle dogs and Study CSL 07_002, 668321 28-day IV (7 h) infusion toxicity study in the Beagle Dog with a 14-day recovery period). The NOAEL for proline was considered to be 4,350 mg/kg bw (EPAR Hizentra [19]).

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

Genotoxicity

In a reverse mutation assay in *Salmonella typhimurium*, negative results were reported for proline at concentrations of up to 57 550 µg/plate (500 µM) with and without metabolic activation, in strains TA1530, TA1531, TA1532 and TA1964 (published in WHO/JEFCA report [36], original literature Green & Savage, 1978 [36]).

Proline did not produce a clinically relevant genotoxic response in a sister chromatid exchanges assay (SCE) in human lymphocytes at concentrations of up to 115 mg/mL (cited in WHO/JEFCA report, original literature Xing & Na, 1996 [36]).

No genotoxic effects were shown in in vitro and in vivo genotoxicity/clastogenicity assays conducted with proline in combination with L-isoleucine and nicotinamide (Study 22196 Salmonella typhimurium/ 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 suggests a low interaction between the compounds, these studies were considered relevant for proline as a single excipient as well (EPAR Hizentra [19]).

Overall, there is no evidence of a genotoxic potential of proline.

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 embryotoxicity study in rats (Study No. AA30034), proline was not teratogenic or embryotoxic when administered intravenously (7-hour daily infusion) at doses of 1,449 mg/kg/day during days 6 to 17 of gestation. There was also no indication for maternal toxicity. The NOAEL was defined at 1,449 mg/kg/day (EPAR Hizentra [19]).

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 'Safety pharmacology'.

The efficiency of clearance of proline in young as compared to adult rats was lower after a single SC administration (see section 'Pharmacokinetic in juvenile animals' above)19.

Evidence of retrograde amnesia has been shown in both chick and mice (Davis and Cherkin, 1977, 1979 [6, 7]).

2.2. Toxicokinetics

In a 5-day repeated dose IV, dose finding study (Study No. 925/034), groups of male rats were administered daily for 7 hours with low and high doses of proline (579 and 1,449 mg/kg bw/day). There was a dose-dependent increase in the peak serum concentration of proline up to 13–14 times the baseline levels, equivalent to approximately 4 mM for the high dose group. Three hours after the start of the infusion 83 and 71% of peak concentrations were reached at the low and high proline dose group, respectively. There was no indication of accumulation of proline in serum at both doses tested (EPAR Privigen [30]).

In a 28-day repeated dose IV study (Study No. 925/035), groups of female and male rats were administered daily for 7 hours with low and high doses of proline (579 and 1,449 mg proline/kg bw/day) as in the study above. There was a dose-dependent increase in the peak serum concentrations of proline, up to 14 and 9 times the baseline levels for males and females, respectively. Maximal serum levels of 3.1–4.1 mM and 2.2–2.8 mM proline were found at termination of the infusion on the 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 proline 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 [30]).

In a single dose PK study (Study No. PSR 08/06) groups of female rats were injected SC proline 2 g/kg bw, or IP proline 2 g /kg bw or 4g /kg bw. Control groups received IP or SC injections of sodium chloride solution of the same osmolarity as the high-dose proline solution. Peak serum concentrations of 12 mM (mean; 70-fold baseline) for SC treated animals and up to 18 mM and 43 mM after IP 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 IP administered animal. Following IP injection, a high variation in proline serum concentrations between animals within the same test article group was observed (EPAR Privigen [30]).

In an IV 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 proline 300 mM (42 mL/kg bw/day equivalent to 1,449 mg/kg bw/day) or glycine 300 mM (42 mL/kg bw/day equivalent to 945 mg/kg bw/day) or physiological saline (42 mL/kg bw/day) as a control. Serum concentrations of proline and glycine were assessed on gestation days 6 and 17 of gestation before and at the end of the infusion. Mean maximal serum levels on days G6 and G17 were 3.53 mM and 2.57 mM in the animals infused with proline, and 1.67 mM and 1.23 mM 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 [30]).

In an "experimental model of hyperprolinaemia" (Moreira et al., 1989 [27]), juvenile rats received SC doses of proline of 12.8 to 18.2 mmol/kg bw (1.5 to 2.1 g/kg bw) twice daily from PND 6 till PND 28. This model was used in the numerous publications (Delwing et al., 2003 [8]; Bavaresco et al., 2005 [3]; Delwing, 2006 [10]) 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 HP patients with proline levels between 1 and 2 mM.

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, SC (Study No. PSR 08/06)	12 mM
Repeat dose toxicity – 5 days (Study No. 925/034)	4 mM
Repeat dose toxicity – 28 days (Study No. 925/035)	3.1–4.1 mM (males) 2.2–2.8 mM (females)
Reproduction Toxicity (segment II) (Study No. AA30034)	2.6–3.5 mM

3. Pharmacokinetics (in humans)

Proline is a nonessential neutral amino acid and a component of nutritional proteins; the daily intake from food is about 5.2 g of which 75–80% are absorbed to the blood (Adibi et al., 1967 [1]). Up to 15.8 mmol/kg/h are synthesised under fasting conditions (Jaksic et al., 1987 [22]), thus more than 3 g/day in an adult. Synthesis is completely inhibited after parenteral administration of high doses of L-proline (Jaksic et al., 1987 [22]), indicating a feedback mechanism. Proline is formed from and metabolised to glutamate. Under certain 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 [13]; Wu, 2009 [38]; Wu, 2011 [4]). Proline is part of diet with normal daily intake of

approximately 5.2 g (Dietary reference intakes [11]). Normal systemic weekly uptake of L-proline from the diet is 364 mg/kg for adults and about 1200 mg/kg for children (1 to 5 years old) reflecting the higher protein need of children in growth (calculated from Adibi et al., 1967 [1]). Normal serum proline levels vary by age: 3–10 years 68–148 µM; 6–18 years 58–324 µM and > 18 years 102–336 µM (Wu, 2006 [37]) and are reported to be in the range of 266 ± 35 µM in adults by Druml et al., 2001 [14].

The half-life ($t_{1/2}$) of proline is approximately 5 hours.

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

Proline passes the 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 (Zaragozá, R., 2020 [41]), the orphan transporter v7-3 (solute carrier 6a15, or B0AT2) (Broer A. et al., 2006 [5]), and the PROT transporter (Fremeau R.T. et al., 1992 [17]). One or some of these transporters may not be well developed in young children.

Privigen (IVIgG)

Privigen is stabilised with 250 mM proline (proline has a molecular weight of 115.13 g/mol→ 28.75 g/L).

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

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

In the PID study, all patients (n= 80, ages 3–69 years) received doses between 0.20 to 0.88 g/kg IVIgG per infusion given at 3–4-week intervals. The median proline levels before the first and second infusion, and before the infusion at week 12 were between 207 and 236 µM. The median post-infusion serum proline levels were 1,927 µM after the first infusion and 1,793 µM after the second infusion. As expected, median post-infusion proline levels increased with increasing median doses of IVIgG.

In the ITP study (n=57, ages 15–69 years), a dose of 1 g/kg of IVIgG was administered daily for two consecutive days. The median pre-infusion levels of proline prior to the first and second infusions were 199 µM and 446 µM, respectively. Proline serum concentrations increased from pre-infusion levels to median post-infusion levels of 2,606 µM after the first, and 2,951 µM after the second infusion. There was no cumulative effect of proline seen in the ITP study.

In an analysis of the ITP study the data showed that the rate of all temporally associated adverse events (AEs) as well as that of the most frequent temporally associated AEs, 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 the baseline. The transiently increased L-glutamate serum concentrations after IVIgG 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 breast-feeding infants were assessed. The proline dose in infants derived from IVIgG treatment of the mother is considerably lower than the proline amounts naturally occurring in milk proteins or

than would be administered with amino acid solutions for parenteral nutrition. Proline intake from milk therefore does not pose a risk for the baby. This available information in humans, in conjunction with the animal reproductive toxicity data and the data in juvenile animal studies obtained with proline, support the safety of proline in breast-feeding mothers treated with IVIgG. This information is considered sufficient to justify not including any precautionary statement regarding proline intake from milk after IVIgG administration to mothers in sections 4.6 and 5.3 of the summary of product characteristics (SmPC) [31].

Hizentra (SCIgG)

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

In the EU study (n=51, ages 3–60 years) the mean dose per week was 120 mg/kg (range 59–243 mg/kg).

Proline was measured during one dosing interval at Week 28 ± 1. Mean pre-infusion levels were 259 µM, and peak levels were observed at approximately 10 minutes after the infusion (370 µM; max 662 µM). One day after the infusion, the serum proline concentration had returned to approximately pre-infusion levels. 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, at 450 µM. No relevant differences were seen in children.

In the US study (n=49, ages: 5–72 years) the mean dose per week was 181.5 mg/kg, i.e. approximately 50% higher than in the EU study. The maximum proline level observed was 789 µM, lower than in the studies conducted with IVIgG where median levels of 1,927 µM (in the PID study) and 2,951 µM (in the ITP study) were reached.

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

Table 2. Partially adapted from Hagan et al. [18], data based on clinical studies

	Route **Proline content**	**Studies**	**Dose IgG**	**Maximum proline dose**	**Proline serum level post-infusion (µM)**
IVIgG Privigen	IV 250 mM	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: 1,927
		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: 2,606 Max 7,963* Post dose day 2 Median 2,951 Max: 9,661 **
SCIgG Hizentra	SC 250 mM	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	Mean: 0.213 g/kg/week Range: 0.072–0.379	55 mg/kg/week	Post dose day 1 Median:

		N=18 PK	(long-term dosing)		Max: 789
10% FreAmine III	IV	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 (7,963 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 (9,661 µmol/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: IVIgG and SCIgG. 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 PID and at doses of 1g/kg for two consecutive days in the immunomodulatory setting (e.g. ITP, GBS, Kawasaki) or at a dose of 2 g/kg bw in a single infusion for Kawasaki disease, corresponding to between 57.6 mg and 576 mg/kg proline (see also table 2). The SCIgG is administered weekly or biweekly at doses of 100 mg/kg to 200 mg/kg in replacement therapy and at doses of 200 to 400 mg/kg weekly in immunomodulatory therapy for chronic inflammatory demyelinating polyneuropathy (CIDP), corresponding to between 14.4 mg/kg and 57.6 mg/kg proline.

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

4.1.1. Patients with hyperprolinaemia

In patients suffering from hyperprolinaemia (HP), 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 500–3,700 µM) it is reported that seizures or other clinical symptoms may occur.

The causal relationship is not established (e.g. the clinical signs could be due to other metabolites affected by these enzyme deficiencies). In the absence of data establishing the safety of exposure of subjects with HP I and II to additional proline, this exposure 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 type I or II".*

Product information leaflet (PIL) section 2: *"Do NOT take Privigen if you suffer from hyperprolinaemia type I or II (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 behavioural research studies in rats (i.e. water maze 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, 2005 [40]; Bavaresco, 2011 [3]). The model used in these studies claims to be adjusted to achieve plasma proline levels between 1mM and 2mM, comparable to those found in type ll hyperprolinemic patients (Moreira et al., 1989 [27]).

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 [10]), inhibition of enzymes activities as acetylcholinesterase, Na^+, K^+-ATPase, and aminotransferases (Pontes et al., 1999 [28], 2001 [29]; Shanti et al., 2004 [32]; Delwing et al., 2005 [9]) energy deficit (Delwing et al., 2007 [11]; Ferreira et al., 2010 [16]) and induction of oxidative stress (Delwing et al., 2003 [8]). These data indicate that high proline concentrations in the CNS can induce some neurotoxicity when administered repeatedly at high doses and may affect brain development in juvenile animals.

Of note, IVIgG and SCIgG contain high levels of proline and are indicated for use in children of all age groups. To address the neurotoxic potential of proline, specific non-clinical safety studies, were conducted with the particular intention to mimic the clinical dose, application route and schema of IVIgG and SCIgG. Based on these studies, non-clinical data obtained for proline safety pharmacology and toxicity studies reveal no special hazard for humans. This is reflected in the current labelling in sections 5.3 of the SmPCs of IVIgG and SCIgG [31, 20].

In summary, 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 IVIgG in newborn rats were reassuring and suggested a non-neurotoxic potential of proline, administered as an excipient with IVIgG, 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 the marketing authorisation of IVIgG and SCIgG. These results, which at first may seem contradictory, can be explained by the differences in administration regimens and pharmacokinetics, (i.e. high SC doses of proline twice daily versus intermittent dosing mimicking the clinical administration schedule of IVIgG and SCIgG) and indicate a threshold for neurotoxicity in-between the exposition mimicking the clinical scheme for IVIgG/SCIgG 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 500–3,700 µM, normal range 266 ± 35 µM).

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 (Tau, 2010 [34]);

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

an exact threshold for children cannot be deduced from the pre-clinical studies. The relative safety limit extrapolated from proline doses administered in the IVIgG and SCIgG studies and parenteral amino acid mixtures could serve as a guidance for IV and SC 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, standard toxicity studies did not identify any toxic effects and the signal for neurotoxicity has been observed in an experimental model specifically designed to study hyperprolinaemia using very specific conditions. Taking this into account, the amount of daily systemic synthesis of the nonessential amino acid proline in humans, the feedback inhibition of its synthesis under conditions of administration of proline through the diet or as excipient of medicinal products, the lower or comparable doses of proline taken up after infusion of such products as compared to dietary intake, and because proline is rarely used as excipient in such adequately labelled medicinal products, it was felt that a common labelling recommendation in the excipient guideline concerning neurotoxicity was not necessary.

4.2. Safety post marketing

Data submitted post-marketing for IVIgG and SCIgG did not identify safety signals regarding 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 adverse drug reaction (ADR) reports, including cases from the scientific literature, concerning Kawasaki disease, the paediatric population and neurologic disorders after IVIgG administration.

Current safety concerns for both products include among others haemolysis, hypersensitivity reactions/anaphylactic reactions, thromboembolic events, aseptic meningitis syndrome (AMS) and transmission of infectious agents.

Exacerbation of existing hyperprolinaemia is identified as an important potential risk for SCIgG. The marketing authorisation holder (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. Non-clinical data

Proline is a physiological, conditional-essential amino acid and a component of nutritional proteins with the daily intake from food of about 5.2 g.

Results from a 5-day Irwin test in rats receiving daily IV 7-hour infusion of proline at doses of up to 1,149 mg/kg (resulting in a plasma exposure of 4 mM) did not show any significant neuro-behavioural findings. In 5- and 28-day repeat-dose toxicity studies with SC administration to rats there were no signs of toxicity (NOAEL 1,149 mg/kg bw). There were also no toxicological findings in a 3-month oral toxicity study in rats (NOAEL 2,772 mg/kg bw).

In 7-day and 28-day repeat-dose toxicity studies in dogs, daily IV (7-hour infusion) proline doses of up to 4,350 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 of maternal or reproductive toxicity in a study in rats treated intravenously (7-hour daily infusion) with proline at doses of up to 1,449 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 hyperprolinaemia (Moreira et al., 1989 [27]), juvenile rats received SC doses of proline of 12.8 to 18.2 mmol/kg bw (1,500 mg/kg bw to 2,100 mg/kg bw) twice daily from PND 6 till PND 28 or PND 15 to PND 39. This finding is showing that particularly very young individuals are vulnerable to high doses of proline is supported by PK data that indicate that permeability of BBB 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 BBB decreased with age. In this study, it seems that BBB 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 supports the vulnerability of particular young individuals as the brain rapidly develops in this phase and since protective mechanisms like the BBB or systemic clearance are immature in rats aged < PND 35 and human equivalent age groups, i.e. immature development of BBB and amino acid efflux transporters (see above). Furthermore, PK studies in juvenile rats have shown that efficiency of systemic clearance of proline in young was lower 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). Based on the available PK 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 IVIgG and SCIgG 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 IVIgG and SCIgG. Particularly in a Morris water maze task study in newborn rats with SC administration of proline at 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. twice daily application of high SC doses for several weeks in the "experimental model of hyperprolinaemia" versus high single doses as given in standard safety pharmacology studies or doses administered in a intermitted schedule, i.e. of up to 4 g/kg daily 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 HP 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 SC administration inducing high peak plasma concentrations of 12–14 mM 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 HP 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 in rats (SC and IV, NOAEL 1,449 mg/kg) and dogs (IV, NOAEL 4,350 mg/kg) of up to 28 days (NOAEL 4,350 mg/kg) and in dogs of up to 3 months (Oral, NOAEL 2,772 mg/kg bw).

5.2. Clinical aspects

Consistently high proline plasma levels are observed in hereditary HP type I (HPI) and type II (HPII). Each type is caused by an autosomal recessive inborn error of the proline metabolic pathway. 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).

HPI is caused by an abnormality in the proline-oxidizing enzyme (POX). The POX gene (PRODH) is located on chromosome 22 (22q11.21). There is a heterozygous deletion of this region 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 hyperprolinaemia (less severe than in HPI, with homozygous deletion of the POX gene) and approx. 77% of patients with 22q11.2 deletion are immunodeficient (mainly thymic hypoplasia); antibody defects are only present in 15% of patients. Data from European and US registries for immunodeficiencies showed that 3% of patients with DiGeorge syndrome require IgG therapy. An estimated 1,675–2,400 people with 22q11.2 deletion could be treated with IgG therapy in the USA. Half of these may be hyperprolinemic. There is no evidence to suggest that patients with chromosome 22q11.2 deletion syndrome, including those with DiGeorge syndrome, would be affected by transient elevations in plasma L-proline following treatment with currently registered drugs within the EU (Hagan, 2012 [18]).

In patients with HPI and HPII their proline serum levels are 10–15 times higher than the normal level (proline physiological serum levels range: 266 ± 35 μM). 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 [25]).

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 BBB (The Paediatric committee of the European medicines agency (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 approximately be 10x physiological serum range, i.e. ~2.5 mM.

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 IVIgG in ITP or Kawasaki or long-term treatment as in PID (IVIgG/SCIgG)?

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 approximately 5 hours and the dosing intervals of SCIgG (low dose; weekly) and IVIgG (higher dose, 3–4 weekly) should not lead to any accumulation, given an intact proline metabolism. No accumulation of proline was seen in clinical studies. Proline is eliminated from the plasma by approximately 50% within 2 hours after the end of infusion, and more than 90% within 24 hours.

Conclusion

A relative safety limit may be extrapolated from proline doses administered in the IVIgG and SCIgG studies and parenteral amino acid mixtures could serve as guidance for IV and SC administration levels of proline (see table 2).

In children, adolescents and adults with an intact proline metabolism, parenteral short-term single doses of a treatment containing proline as an excipient can be given at an amount up to 576 mg/kg of proline.

In clinical practice, proline doses up to 57.6 mg/kg are given at weekly intervals without any AE related to proline reported. However, the highest tolerable proline dose in humans with an intact proline metabolism is not known.

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" [2, 15].

A certain risk for neurotoxicity along with elevated plasma levels of proline is known from patients suffering from hyperprolinaemia (up to 500–3,700 µM, normal 266 ± 35 µM). This is in line with the labelling of the approved proline containing medical products which are contraindicated in patients with HP, i.e. the use of IVIgG and SCIgG is contraindicated in patients suffering from HPI and HPII.

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 was obtained from some published studies pertaining to hyperprolinaemia. Using an experimental model of hyperprolinaemia, these studies have shown that repeated administration of high doses of proline have effects on brain development in very young rats (SC doses of proline of 12.8 to 18.2 mmol/kg bw (1500 mg/kg bw to 2100 mg/kg bw) twice daily from PND 6 to 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 IVIgG and SCIgG 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 IVIgG and SCIgG without relevant findings.

However, as the current use of proline as an excipient is restricted to a few products only (e.g. in Privigen (IVIgG), Hizentra (SCIgG), Repatha (human IgG2 monoclonal antibody, evolocumab) and probably some vaccines (single dose)), there were no noteworthy findings in standard toxicology studies. Since the data that indicate a neurotoxic potential of proline came from a model to study HP (with very specific experimental conditions), a specific general warning for the use of proline in young children and/or adolescents not suffering from HP is not considered meaningful.

Qualification of unusual proline amounts in upcoming MAAs/clinical trial applications (CTAs) may be addressed individually by a product specific discussion.

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.

References – Bibliography

1. Adibi, SA., Seymour, JG., and Menden, E., 'The Kinetics of Amino Acid Absorption and Alteration of Plasma Composition of Free Amino Acids After Intestinal Perfusion of Amino Acid Mixtures', The American Journal of Clinical Nutrition. January 1967, 20(1): p. 24–33.
2. Annex of the European Commission guideline 'Excipients in the labelling and package leaflet of medicinal products for human use' (EMA/CHMP/302620/2017).
3. Bavaresco, C.S., Streck, E.L., Netto C.A., Wyse A.T., 'Chronic hyperprolinemia provokes a memory deficit in the Morris water maze task', Metab Brain Dis. 2005 Mar; 20(1), p. 73–80.
4. Bolli, R., Woodtli, K., Bärtschi, M., Höfferer, L., Lerch, P., 'L-proline reduces IgG dimer content and enhances the stability of intravenous immunoglobulin (IVIg) solutions', Biologicals 2010, vol. 38, p. 150–157.
5. Broer A., Tietze N., Kowalczuk S., Chubb S., Munzinger M., Bak L.K. and Broer S., The orphan transporter v7-3 (slc6a15) is a Na+-dependent neutral amino acid transporter (B0 AT2)´, Biochem. J. (2006) 393, 421–430.
6. Davis, J. L. and Cherkin, A., 'Intraventricular L-proline induces retrograde amnesia in mice', IRCS Med. Sci. 5, 1977, p. 88–94.
7. Davis, J. L. and Cherkin, A., 'Retrograde amnesia in chicks and mice induced by 3,4,dehydro-D-L-proline, a proline analog', Pharmacol. Biochem. Behav, 10, 1979, p. 643–645.
8. Delwing, D., Bavaresco, C., Chiarani, F., Wannmacher, C., Wajner, M., Dutra-Filho, C., Wyse, A., 'In vivo and in vitro effects of proline on some parameters of oxidative stress in rat brain', Brain Research 2003, vol. 991, p. 180–186.
9. Delwing, D., Chiarani, F., Bavaresco, C., Wannmacher, C., Wajner, M., Dutra-Filho, C., Wyse, A.,' Protective effect of antioxidants on brain oxidative damage caused by proline administration', Neurosci Res. May 2005; 52(1): p. 69–74.
10. Delwing, D., Sarkis, J., Wyse, A., 'Proline induces alterations in nucleotide hydrolysis in rat blood serum', Mol Cell Biochem, Nov. 2006 Nov; 292 (1-2): p. 139–44.
11. Delwing, D., Delwing, D., Sarkis, J., Wyse, A., 'Proline induces alterations on nucleotide hydrolysis in synaptosomes from cerebral cortex of rats', Brain Research 2007, vol. 1149, p. 210–215.
12. Dietary Reference Intakes: 'The Essential Guide to Nutrient Requirements', http://www.nap.edu/catalog/11537.html
13. 'Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids', Macronutrients 2005.
14. Druml W, Heinzel G, Kleinberger G., 'Amino acid kinetics in patients with sepsis' Am J Clin Nutr 2001, 73:908–13.
15. European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use', March 2018.
16. Ferreira, A.G., Scherer, E.B., da Cunha, M.J., Machado, F.R., Cunha, A.A., Graeff, J.S., Netto, C.A., Wyse, A.T., 'Physical exercise reverses cognitive impairment in rats subjected to experimental hyperprolinemia', Neurochem Res. Dec , 36(12), p. 2306–15. doi: 10.1007/s11064-011-0555-6. Epub Jul 2011.

17. Fremeau R.T., Caron M.G., Blakely R.D., ´Molecular cloning and expression of a high-affinity l-proline transporter expressed in putative glutamatergic pathways of rat brain´, Neuron. 1992; 8:915–926.

18. Hagan J.B. et al., 'Safety of L-proline as a stabilizer for immunoglobulin products', Expert Rev Clin Immunol, Feb. 2012, 8(2), p. 169-78. doi: 10.1586/eci.11.97.

19. HIZENTRA (EPAR): https://www.ema.europa.eu/documents/overview/hizentra-epar-summary-public_en.pdf

20. HIZENTRA (SUMMARY OF PRODUCT CHARACTERISTICS): https://www.ema.europa.eu/documents/product-information/hizentra-epar-product-information_en.pdf

21. Institute of Medicine (2002) 'Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol', Protein and Amino Acids, Washington, DC, USA: The National Academies Press (available at http://www.nap.edu/books/0309085373/html/).

22. Jaksic, T., Wanger, DA., Burke, JF., and Young, VR., 'Plasma Proline Kinetics and the Regulation of Proline Synthesis in Man' Metabolism. November 1987, 36(11): p. 1040–1046.

23. Jolles, S., Bernatowska, E., De Gracia, J., Borte, M., Cristea, V., Peter, H., Belohradsky, B., Wahn, V., Neufang-Hüber, J., Zenker, O, Grimbacher, B., 'Efficacy and safety of Hizentra in patients with primary immunodeficiency after a dose-equivalent switch from intravenous or subcutaneous replacement therapy', Clinical Immunology 2011, vol. 141. p. 90–102.

24. Kampel, D., Kupferschmidt, R. and Lubec, G. (1989) 'Toxicity of d-proline', In: Lubec, G. & Rosenthal, G.A., eds, Amino Acids: Chemistry, Biology and Medicine. Amsterdam: ESCOM Science Publishers BV, pp. 1164–1171.

25. Maeder, W., Lieby, P., Sebald, A., Spycher, M., Pedrussio, R., Bolli, R., 'Local tolerance and stability up to 24 months of a new 20% proline-stabilised polyclonal immunoglobulin for subcutaneous administration', Biologicals 2011, vol. 39, p. 43–49.

26. Mitsubuchi, H. et al., 'Biochemical and clinical features of hereditary hyperprolinemia', Pediatrics International, Volume 56, Issue 4, August 2014, p. 492–496.

27. Moreira, J.C., Wannmacher, C.M., Costa, S.M., Wajner, M., 'Effect of proline administration on rat behavior in aversive and nonaversive tasks', Pharmacol Biochem Behav. April 1989, 32(4), p. 885–90.

28. Pontes, Z.E., Oliveira, L.S., Baveresco, C.S., Streck, E.L., Dutra-Filho, C.S., Wajner, M., Wannmacher, C.M., Wyse, A.T., 'Proline administration decreases Na+,K+-ATPase activity in the synaptic plasma membrane from cerebral cortex of rats', Metab Brain Dis. Dec 1999, 14(4), p. 265–72.

29. Pontes, Z.L., Oliveira, L.S., Franzon, R., Wajner, M., Wannmacher, C.M., Wyse, A.T., 'Inhibition of Na+,K+-ATPase activity from rat hippocampus by proline', Neurochem Res. 2001 Dec, 26(12), p. 1321–6.

30. PRIVIGEN (EPAR): https://www.ema.europa.eu/documents/overview/privigen-epar-summary-public_en.pdf

31. PRIVIGEN (SUMMARY OF PRODUCT CHARACTERISTICS): https://www.ema.europa.eu/documents/product-information/privigen-epar-product-information_en.pdf

32. Shanti N.D., Shashikumar K.C., Desai P.V., 'Influence of proline on rat brain activities of alanine aminotransferase, aspartate aminotransferase and acid phosphatase', Neurochem Res. 2004 Dec. 29(12), p. 2197–206.

33. Tada Y.N., Yano, H., Takahashi, K., Yuzawa, H., Ando, Y., Kubo, A., Nagasawa, N., Ohashi, A., Ogata, D., Nakae, 'Toxicological evaluation of L-proline in a 90-day feeding study with Fischer 344 rats', Regulatory Toxicology and Pharmacology, 2010, 58, p. 114–120.

34. Tau G.Z., Peterson, B.S., 'Normal Development of Brain Circuits', Neuropsychopharmacology, Jan 2010; 35(1), p. 147–168.

35. Wasserman, R., Church, J., Peter, H., Sleasman, J., Melamed, I., Stein, M., Bichler, J., 'Pharmacokinetics of a new 10% intravenous immunoglobulin in patients receiving replacement therapy for primary immunodeficiency', European Journal of Pharmaceutical Sciences 2009, vol. 37 p. 272–278.

36. WHO FOOD ADDITIVES SERIES: 54, 'Safety evaluation of certain food additives', Prepared by the Sixty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), p. 435-480. http://whqlibdoc.who.int/publications/2006/9241660546_eng.pdf

37. Wu, A. 'Clinical Guide to Laboratory Tests (4th Edition)', Saunders (2006).

38. Wu, G., Bazer F.W., Burghardt R.C., Johnson G.A., Kim S.W., Knabe D.A., 'Proline and hydroxyproline metabolism: implications for animal and human nutrition'. Amino Acids (2011) 40:1053–1063, DOI 10.1007/s00726-010-0715-z.

39. Wu, G., 'Amino acids: metabolism, functions, and nutrition', Amino Acids 37, p. 1–17 (2009).

40. Wyse A.T. and Netto, C.A. 'Behavioral and neurochemical effects of proline, Biochemical and clinical features of hereditary hyperprolinemia', Metab Brain Dis 2011, 26, p. 159–172.

41. Zaragozá, R.,´Transport of Amino Acids Across the Blood-Brain Barrier´, Front Physiol. 2020; 11: 973.