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Pharmacokinetics of proline-rich tripeptides in the pig

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ABSTRACT

Tripeptides may possess bioactive properties. For instance, blood pressure lowering is attributed to the proline-rich tripeptides Ile-Pro-Pro (IPP), Leu-Pro-Pro (LPP), and Val-Pro-Pro (VPP). However, little is known about their absorption, distribution, and elimination characteristics. The aim of this study was to characterize the pharmacokinetic behavior of IPP, LPP, and VPP in a conscious pig model. Synthetic IPP, LPP, and VPP were administered intravenously or intragastrically (4.0 mg kg^{-1} BW in saline) to 10 piglets (approximately 25 kg body weight) in the postabsorptive state. After intravenous dosing, the elimination half-life for IPP was significantly higher ($P < 0.001$) than for LPP and VPP (2.5 ± 0.1, 1.9 ± 0.1, and 2.0 ± 0.1 min, respectively). After intragastric dosing, however, the elimination half-lives were not significantly different between the peptides (9 ± 1, 15 ± 4, and 12 ± 6 min, respectively). Maximum plasma concentrations were about 10 nmol l^{-1} for the three tripeptides. The fraction dose absorbed was 0.077 ± 0.010, 0.059 ± 0.009, and 0.073 ± 0.015%, for IPP, LPP, and VPP, respectively. Proline-rich tripeptides reach the blood circulation intact, with an absolute bioavailability of about 0.1% when administered via a saline solution. Because half-lives of absorption and elimination were maximally about 5 and 15 min, respectively, this suggests that under these conditions a bioactive effect of these tripeptides would be rather acute.

1. Introduction

In recent years, interest in the impact of functional foods to improve human health has grown [2,6,17,33]. Also, the knowledge about the beneficial role of bioactive peptides expanded drastically [24]. Bioactive properties of peptides that have been discussed include antimicrobial and antifungal effects, cholesterol lowering, and blood pressure lowering [24,27]. In many cases, bioactive peptides need to reach the blood circulation to exert their effect.

One of the beneficial effects that has been attributed to certain peptides, notably of the proline-rich tripeptides Ile-Pro-Pro (IPP), Val-Pro-Pro (VPP) and, to a lesser extend, Leu-Pro-Pro (LPP) (here collectively referred to as XPPs) is blood pressure lowering [18]. Blood pressure lowering after consumption of XPP-containing foods by hypertensive subjects was shown in several nutrition intervention trials [10,12,15,25,26]. The proposed mechanism of action is that these tripeptides are active angiotensin-converting enzyme (ACE) inhibitors [11,14]. It is well established that inhibition of

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ACE can effectively lower blood pressure [1], as ACE is one of the key enzymes controlling the rate of the renin–angiotensin–aldosterone system.

Efficacy studies usually present end point measurements (e.g. blood pressure), but hardly ever report plasma concentrations of the bioactive peptides involved. Of the XPPs, no data are available on absolute bioavailability or other pharmacokinetic/pharmacodynamic (PK/PD) properties. Because the required plasma concentration of XPPs known to exert ACE inhibition is about 1000-fold higher than reported plasma concentrations in animal or human trials [11], better knowledge of PK/PD properties may increase our understanding of the relationship between these bioactive peptides and the end point measurements. They could induce further investigations to other mechanisms of efficacy on blood pressure lowering than ACE inhibition that have not been studied yet [13,14].

In the current experiment, we used an interorgan pig model to study the pharmacokinetics of XPPs. This model allows the required intragastric (I.G.) and intravenous (I.V.) infusions of XPPs and sampling of arterial blood [16,30]. These combined measurements enable to measure a number of PK/PD characteristics of the XPPs, including the absolute bioavailability.

The aim of this study was to characterize the pharmacokinetic behavior of IPP, LPP, and VPP, administered intravenously or intragastrically as a single dose, in the conscious pig.

2. Materials and methods

2.1. Animals

Twelve, normotensive, pathogen-free, female piglets (Dutch Landrace × Yorkshire; 8–12 weeks of age; 25.2 ± 1.1 kg body weight (BW)) were used. Animals were allowed to adapt to individual housing in pens (2 m × 3 m) 1 week before surgery. Catheters were implanted into the stomach and the inferior caval vein, allowing infusion of peptides, and one into the abdominal aorta, enabling blood sampling [7]. After surgical placement of the catheters, animals were allowed to recover for 7 days before starting experiments. Surgical procedures were described previously [28]. Pigs were accustomed to a small movable cage (0.9 m × 0.5 m × 0.3 m), which allows the experiments to be performed in unrestrained, conscious animals. Piglets received 1 kg of a sow feed per day (Havens Voeders, Maashees, The Netherlands) and water was available ad libitum. After completing the experiment, the position of catheters was checked with X-ray under anesthesia. This study was approved by the animal experiment ethics committee of Maastricht University (2004-101).

2.2. Study design, treatments, and experimental procedures

Two experiments were conducted. The first aimed to determine the analytically feasible and potentially application-wise relevant dose of IPP, LPP, and VPP. In the second experiment, a single dose was used to assess the pharmacokinetic properties of each XPP in a larger number of pigs. All experiments were performed in overnight-fasted pigs. During the first 20 min of the experiment, a relatively large number of blood samples were taken. Because this could not be performed manually, a pump (Harvard 11 Plus, Harvard, USA) was used to collect blood continuously over short intervals, e.g. 0–2 min. In both experiments, synthetic IPP, LPP, and VPP (at least 98% pure) were used (Bachem, Weil am Rhein, Switzerland).

In both experiments, 1 ml blood was collected per time point in cooled heparinized tubes. Samples were centrifuged for 15 min at 3000 × g at 4 °C. Plasma was transferred into a tube containing 10 μl of 100 g trifluoroacetic acid l^{-1} (VWR, Amsterdam, The Netherlands). After mixing, the samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

2.3. Dose-finding study

The first experiment was performed in two pigs to establish the dose that allows reliable high performance liquid chromatography–mass spectrometry (LC–MS) determination of the half-lives of XPPs. Three tests were performed per pig. In a test, both pigs received 0.40, 4.0, or 10 mg of each XPP per kg BW. For IPP and LPP this is 1.2, 12, and 31 μmol kg^{-1} BW, and for VPP 1.3, 13, and 32 μmol kg^{-1} BW, respectively. The XPPs were dissolved in 40 ml iso-osmolar NaCl solution and after taking two baseline samples, XPPs were administered intravenously at t = 0 min. Subsequently, blood was collected at t = 0–2, 2–4, 4–6, 6–8, 8–10, 10–15, and 15–20 min and at 25, 30, 40, 50, 60, 90, 120, 150, and 180 min. There was a 24 h interval between tests, and it was verified whether XPP plasma concentrations in baseline samples had returned to baseline values.

2.4. Pharmacokinetic parameters for a single dose

In the second experiment, 10 pigs were given two randomized treatments: intravenous or intragastric infusion of a mixture of 4.0 mg IPP, 4.0 mg LPP, and 4.0 mg VPP per kg BW, dissolved in 40 ml iso-osmolar NaCl solution. After taking two baseline samples, at t = 0 min the XPPs were given and blood was subsequently collected at t = 0–2, 2–4, 4–6, 6–8, 8–10, 10–15, and 15–20 min, and at 25, 30, 40, 50, 60, and 90 min. There was a 24 h interval between tests, and it was verified whether XPP plasma concentrations in baseline samples had returned to baseline values.

2.5. Analytical procedures

Samples of infusates were analyzed for their XPP content using the following procedure. Firstly, 100 μl of the sample was mixed with 100 μl of a standard solution of universally ^{13}C labeled IPP [U-^{13}C-IPP] and VPP [U-^{13}C-VPP] (Biopeptide Co., San Diego, CA, USA). Secondly, the mixture was vortexed for 1 min, followed by centrifugation for 20 min at 16,000 × g at room temperature. Finally, 80 μl of the supernatant was pipetted into a 250 μl glass insert, and placed into an auto sampler vial. XPPs were quantified using LC–MS (Quattro II, Micromass, Milford, MA). This procedure allows determination of XPPs in the infusates covering a range of 1.9–123 μmol l^{-1}.

Plasma samples were analyzed for XPP content based on a previously described method [29]. Homogenized plasma (20 μl) was added to 50 μl internal standard solution, containing U-^{13}C-IPP, U-^{13}C-VPP, and U-^{13}C-LPP, and 480 μl water. After mixing, this aliquot was acidified with trifluoroacetic acid to pH < 3. Proteins were removed by heating the aliquot at 95 °C for 2 min, followed by centrifugation at 22,000 × *g* for 30 min at 15 °C. XPPs present in the supernatant were quantified by LC–MS (Quattro Ultima, Waters, Milford, MA). For I.V. measurements, the limit of quantification of this procedure was 18, 18, and 46 nmol l^{-1} for IPP, LPP, and VPP, respectively. For I.G. measurements, the limit of quantification of this procedure was 0.28, 0.28, and 0.71 nmol l^{-1} for IPP, LPP, and VPP, respectively.

2.6. Pharmacokinetic and statistical procedures

For I.V. infusions, plasma concentration–time curves were fitted using a 1- and 2-compartmental model, both with 1st order elimination. The compartment in the first model represents blood plasma; the two compartments in the second model may represent blood plasma and tissue (fluid). The latter model was introduced as there are indications that XPPs may be stored in a secondary compartment, like aorta tissue [19]. The following formulae were used to calculate the 1- and 2-compartment models, respectively:

$$c_t = \frac{D_{I.V.}}{V_d} e^{-k_{10}t} \quad (1)$$

$$c_t = \frac{D_{I.V.}}{V_d(\alpha-\beta)}\left[(k_{21}-\beta)\,e^{\beta t} - (k_{21}-\alpha\,e^{-\alpha t})\,e^{-\alpha t}\right] \quad (2)$$

where c_t is the plasma concentration of an XPP at time t (mol l^{-1}), $D_{I.V.}$ the amount of XPP dosed intravenously (mol), V_d the hypothetical distribution volume (l), k_{10} the elimination rate constant (min^{-1}), t the time (min), α the slope of mono exponential concentration line (min^{-1}), β the slope of mono exponential declining line (min^{-1}), and k_{21} is the distribution rate constant for transfer of an XPP from peripheral to central compartment (min^{-1}). No further lag time was assumed in these models. Baseline values for all XPPs were below the limit of detection, so no corrections for these values were needed.

Plasma concentration time curves observed after I.G. infusions were modeled using a 1-compartment model with 1st order absorption, lag time, and 1st order elimination:

$$c_t = \frac{D_{I.G.}k_{01}}{V_d(k_{01}-k_{10})}\left[e^{(-k_{10}(t-t_l))} - e^{(-k_{01}(t-t_l))}\right] \quad (3)$$

where c_t is the plasma concentration of an XPP at time t (mol l^{-1}), $D_{I.G.}$ the amount of XPP dosed in the stomach (mol), k_{01} the absorption rate constant (min^{-1}), V_d the hypothetical distribution volume (l), k_{10} the elimination rate constant (min^{-1}), t the time (min), and t_l is the lag time (min).

Uniform weighting and baseline correction was applied to all plasma concentration data. Baseline correction was calculated using plasma concentration of $t = -5$ min and $t = 0$ min. The Gauss–Newton method with Levenberg and Hartley modification [5] was used to estimate primary pharmacokinetic parameters V_d and k_{10} and, if applicable, k_{01}, k_{21}, and t_l. Furthermore, the following secondary pharmacokinetic parameters were calculated, if applicable: absorption half-life [$t_{1/2,a}$; min], elimination half-life [$t_{1/2,e}$; min], maximum XPP plasma concentration [C_{max}; mol l^{-1}], time of maximum XPP plasma concentration [t_{max}; min], clearance [Cl; l min^{-1}], and the area under the plasma concentration–time curve [AUC; mol l^{-1} min]. In case of pump-assisted sampling, the mean of the time window was taken.

The absolute bioavailability for an XPP was calculated using the following formula:

$$f = \frac{AUC_{I.G.}D_{I.V.}}{AUC_{I.V.}D_{I.G.}} \times 100\% \quad (4)$$

where f is the absolute bioavailability of an XPP (%), $AUC_{I.G.}$ the area under the plasma concentration–time curve obtained after I.G. infusion (mol l^{-1} min), $D_{I.V.}$ the I.V. infused XPP dose (mol), $AUC_{I.V.}$ the area under the plasma concentration–time curve obtained after I.V. infusion (mol l^{-1} min), $D_{I.G.}$ the I.G. infused XPP dose (mol).

Both models were compared to assess which described the observed plasma concentration best by using the Akaike information criterion [AIC] [3,31]. When comparing the two models, it was assumed that the lowest AIC would represent the best pharmacokinetic model. WinNonlin® version 5.0 (Pharsight, Mountain View, CA, USA) was used for all pharmacokinetic calculations.

Results are presented as mean ± S.E.M. Two-way ANOVA was used to test effects, and means were compared using Tukey's Multiple Comparison Test. These tests were performed using GraphPad Prism, version 4.03 (GraphPad Software, San Diego, CA, USA). Differences were considered significant if the P-value was < 0.05.

3. Results

3.1. Animals

After surgery and recovery, animals appeared healthy and had normal body temperature. Their growth (0.29 ± 0.02 kg day^{-1}) was normal given the restricted food intake of 1 kg day^{-1}. After experiments the catheters appeared to be in correct positions.

3.2. Dose-finding study

Plasma concentration–time curves from the dose-finding study were constructed. In Table 1 elimination half-life times are presented. This parameter appeared to be unrelated to the dose. Therefore, the dose in the second experiment was selected using following criteria: XPP plasma concentrations should enable calculation of pharmacokinetic parameters and the physiological range should be relevant. We used a dose of 4.0 mg kg^{-1} BW, since this dose gave a good response in the analyses. Very low plasma concentration were observed after administration of 0.4 mg kg^{-1} BW, also considering that the peptides would be given I.G. with an anticipated low bioavailability of XPPs. Finally,

Table 1 – Dose-finding study: elimination half-lives (min) for each XPP, obtained after dosing at $t = 0$ min

	Dose = 0.40 mg kg^{-1} BW[a]	Dose = 4.0 mg kg^{-1} BW[a]	Dose = 10 mg kg^{-1} BW[a]
IPP	5.0	2.7	6.7
	2.8	5.7	5.2
LPP	3.9	2.1	5.5
	2.2	4.6	4.1
VPP	4.5	2.2	5.5
	2.5	5.7	4.2

Individual data of the two pigs are presented.
[a] For IPP and LPP this equals to 1.2, 12, and 31 μmol kg^{-1} BW, for VPP to 1.3, 13, and 32 μmol kg^{-1} BW, respectively.

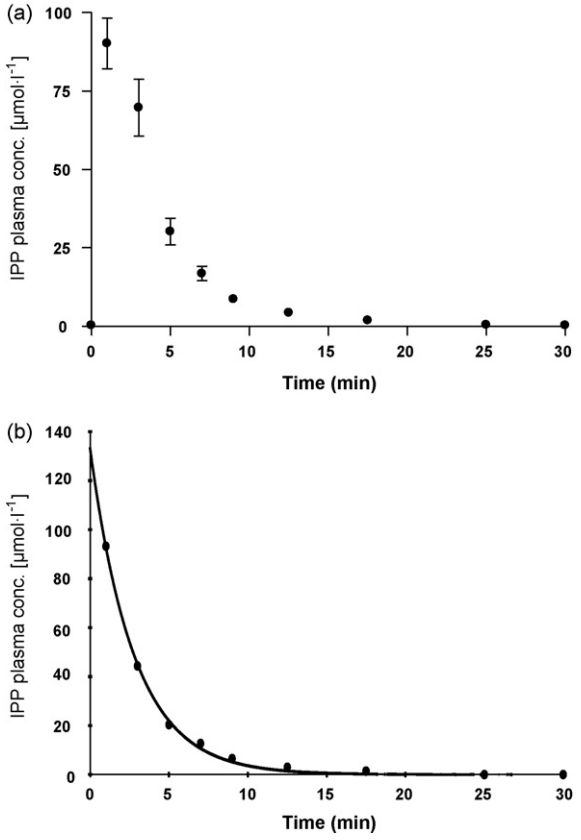

Fig. 1 – (a) *Top panel*: Observed plasma concentration–time curve for IPP in pigs after I.V. administration. Points are mean ± S.E.M. ($n = 10$) after baseline correction (0.31 μmol IPP l^{-1}). At $t = 0$ min, 4.0 mg IPP kg^{-1} BW (=12 μmol IPP kg^{-1} BW) was administered intravenously. Data points $t = 40$ min, 50 min, 60 min, and 90 min are not shown, because these concentrations were close to baseline concentrations. (b) *Lower panel*: Example of a modeled plasma concentration–time curve after I.V. administration after baseline correction. A 1-compartment model with no lag time was used. Points are observed values. Data from the mixing phase ($t < 1$ min) were excluded during modeling. The curve was back-extrapolated to $t = 0$ min. The limit of quantification was 0.018 μmol l^{-1}.

the 10 mg kg^{-1} BW dose was considered practically less relevant than the dose of 4.0 mg kg^{-1} BW.

3.3. *Pharmacokinetic parameters for a single dose*

As an example, the mean plasma concentration–time curve observed after I.V. infusion of IPP is presented in Fig. 1a, and of an individual modeled curve in Fig. 1b. As XPPs were already present at low concentrations in baseline conditions, corrections of 0.31, 0.31, and 0.32 μmol l^{-1} for IPP, LPP, and VPP, respectively, were made for I.V. infusions. From modeled curves, like presented in Fig. 1b, it was concluded that the mixing phase ended at about $t = 1$ min. On average, XPP plasma concentrations returned to baseline values at $t = 25$ min. Back-extrapolation predicted plasma concentrations during the mixing phase. In Table 2 calculated pharmacokinetic parameters are presented as obtained from plasma concentration–time curves after I.V. infusion using a 1-compartment model. There were significant differences between XPPs on half-life of elimination, on maximum XPP plasma concentration, and the AUC. Table 3 summarizes AIC values for the 1- and 2-compartment I.V. models. The average AIC value for the 2-compartment I.V. model was significantly lower than the one of the 1-compartment I.V. model. However, we were able to model the plasma concentration curves with a 2-compartment model only for 18 of the 30 observations, because pharmacokinetic parameter estimation did not converge in 12 cases.

As an example, the mean plasma concentration–time curve observed after I.G. infusion of IPP is presented in Fig. 2a, and of an individual modeled curve in Fig. 2b. The distribution phase after I.G. infusion ended at about $t = 3$ min. On average, plasma concentrations returned to baseline concentrations (1.0 nmol l^{-1} for all XPPs) at about $t = 60$ min. These concentrations were close to their limit of quantification. Modeled pharmacokinetic parameters are presented in Table 4. A significant difference between the maximum plasma concentrations of IPP and LPP vs. VPP was observed. After I.G. infusion of XPPs, half-lives of absorption were about 2 min for each XPP. Absorption and distribution of the XPPs took about 10 min and half-lives of elimination were about 10–15 min.

The fraction dose absorbed of IPP, LPP, and VPP were respectively 0.077 ± 0.010, 0.059 ± 0.009, and 0.073 ± 0.015% ($P < 0.06$).

4. Discussion

In this study pharmacokinetics of XPPs in the conscious pig was determined. XPPs appeared to have a low, but measurable absolute bioavailability. Half-lives of absorption and elimination after a single dose of IPP, LPP, or VPP, dissolved in a saline solution, are in the minute-range.

A human-like pig model was used. This model allows multiple dosing and sampling at locations that were, from an ethical point of view, not possible in humans. Also, the size of the pig allows multiple pharmacokinetic experiments with relative high frequency and volume of blood sampling, allowing accurate determination of key pharmacokinetic parameters. Very low XPP plasma concentrations (nmol l^{-1}-range) had to be measured, which is not possible using standard HPLC detectors. Therefore, a sophisticated, very

Table 2 – Pharmacokinetic parameters obtained after I.V. administration of 4.0 mg XPP kg^{-1} BW, in the pig[a,b,c]

XPP	V_d (l)	$t_{1/2,e}$ (min)	C_{max} (μmol l^{-1})	Cl (l min^{-1})	AUC (mmol l^{-1} min)
IPP	2.6 ± 0.2	2.5 ± 0.1	126 ± 10	0.74 ± 0.08	0.46 ± 0.05
LPP	2.5 ± 0.2	1.9 ± 0.1[c]	143 ± 8	0.94 ± 0.09[b]	0.40 ± 0.03[a]
VPP	3.1 ± 0.2	2.0 ± 0.1[c,d]	107 ± 7[a,d]	1.01 ± 0.08[b]	0.31 ± 0.03[c,e]

[a] V_d: hypothetical distribution volume; $t_{1/2,e}$: elimination half-life; C_{max}: maximum XPP plasma concentration; Cl: clearance; AUC: area under the plasma concentration–time curve. Values are mean ± S.E.M., $n = 10$.
[b] Significant differences vs. IPP: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$; or vs. LPP: [d]$P < 0.05$; [e]$P < 0.01$.
[c] Per kg BW, this equals to 12 μmol (IPP and LPP) or 13 μmol (VPP).

Table 3 – Values for the Akaike information criterion (AIC) for fitted XPP plasma concentrations using a 1- or 2-compartment model[a,b]

XPP	1-Compartment model		2-Compartment model	
	AIC	n	AIC	n
IPP	133 ± 7	10	99 ± 10	6
LPP	116 ± 9	10	87 ± 12	5
VPP	113 ± 8	10	79 ± 7	7

[a] Parameter estimated after intravenous infusion of 4.0 mg XPP kg^{-1} BW in the pig at $t = 0$ min. Values are mean ± S.E.M. $n < 10$ reflects unsuccessful modeling due to non-converging primary parameters.
[b] Differences of AICs between 1- and 2-compartment models are significant for all XPPs ($P < 0.05$).

selective and sensitive LC–MS technique was used, allowing quantification of XPPs at these low concentrations. We think that this combination of procedures is crucial to obtain reliable pharmacokinetic parameters.

Pharmacokinetic behavior of XPPs in the pig can be described with a 1- or 2-compartment model. Table 3 shows that a 2-compartment model yielded significantly lower values for AIC than a 1-compartment model, but estimates for primary parameters were obtained in a smaller number of cases when using a 2-compartment model. The low rates of transport of XPPs to and from the peripheral compartment (data not shown), indicate that a 1-compartment model is a good approximation of the 2-compartment model. With these results we cannot confirm or challenge suggestions in literature as if a second compartment would exist for XPPs, e.g. in aorta tissue [19].

In experiments with SHR rats [20,23,32] and normotensive or mildly hypertensive humans [21,22,25], usually 0.1–10 mg XPP or dipeptide per kg BW were supplied orally. In the dose-finding study 0.4–10 mg kg^{-1} BW was administered intravenously. The selected dose in the second experiment, 4.0 mg kg^{-1} BW, allowed accurate determination of pharmacokinetic parameters. The maximum XPP plasma concentration in this study, about 10 nmol l^{-1} for all XPPs (Table 4), is of the same order of magnitude as compared to a more applied study (20.4 mg IPP orally dosed resulted in a C_{max} of about 1 nmol l^{-1} in humans [11]).

Generally, after I.G. infusion, the fate of XPPs in vivo can be divided in an absorption, distribution, metabolism, and elimination phase. In this study metabolism was not studied, because only intact XPPs were measured in blood plasma. Short lag times obtained after I.G. infusion, about 2 min for all XPPs, show that transit of XPPs from the stomach, via the gut lumen into the blood circulation takes place remarkably quick. XPPs were administered in an iso-ionogenic NaCl solution. We hypothesize that this rapid transport is due to the low

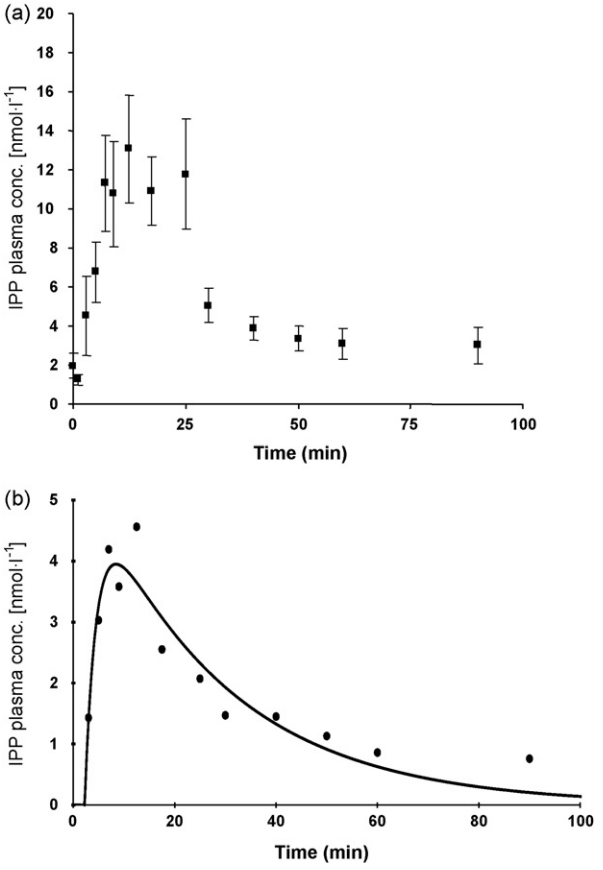

Fig. 2 – (a) *Top panel*: Observed plasma concentration–time curve for IPP in a pig after I.G. administration. Points are means before baseline correction ± S.E.M. ($n = 10$). At $t = 0$ min, 4.0 mg IPP kg^{-1} BW (=12 μmol IPP kg^{-1} BW) was administered intragastrically. (b) *Lower panel*: Example of an individual modeled plasma concentration–time curve after baseline correction. Points are observed values after baseline correction (1.0 nmol IPP l^{-1}). A 1-compartment model with no lag time was used to model the data. Data from the mixing phase ($t < 1$ min) were excluded from modeling. Note that the maximum IPP plasma concentrations after I.G. infusions were about a factor 1000 lower than after I.V. infusions (nmol l^{-1} vs. μmol l^{-1}-scale, respectively).

Table 4 – Pharmacokinetic parameters obtained after I.G. administration of 4.0 mg XPP kg^{-1} BW in the pig[a,b]

XPP	V_d (1000 l)	t_l (min)	$t_{1/2,a}$ (min)	t_{max} (min)	C_{max} (nmol l^{-1})	$t_{1/2,e}$ (min)	Cl (1000 l^{-1})	AUC (μmol l^{-1} min)
IPP	21 ± 3	1.8 ± 0.4	3.3 ± 0.5	8.6 ± 0.6	12 ± 3[c]	9.3 ± 1.1	1.7 ± 0.4	0.29 ± 0.06
LPP	34 ± 7	2.1 ± 0.5	2.0 ± 0.5	7.1 ± 0.7	11 ± 3[c]	15 ± 4	2.0 ± 0.5	0.26 ± 0.05
VPP	30 ± 6	2.0 ± 0.3	4.6 ± 1.1	8.9 ± 0.6	9 ± 2	12 ± 6	2.8 ± 0.8	0.22 ± 0.05

[a] V_d: hypothetical distribution volume; t_l: lag time; $t_{1/2,a}$: absorption half-life; t_{max}: time of maximum XPP plasma concentration; C_{max}: maximum XPP plasma concentration; $t_{1/2,e}$: elimination half-life life; Cl: clearance; AUC: area under the plasma concentration–time curve. A 1-compartment model was used. Values are mean ± S.E.M., $n = 10$.

[b] Per kg BW, this equals to 12 μmol (IPP and LPP) or 13 μmol (VPP).

[c] Difference significant vs. VPP ($P < 0.05$).

viscosity of the infusate in combination with the high capacity of peptide transporter 1 (PEPT1) [4,9]. The gut wall is a physical as well as a biological barrier for XPPs. The fraction dose absorbed for XPPs will be low, mainly due to peptidase activity in the lumen, the brush border membranes, and the cytosol. In the present study, absolute bioavailability was low and did not differ between IPP, LPP, and VPP. Absorption half-lives for the XPPs are about 3 min (Table 4). This is in agreement with results obtained by Foltz et al. [11], who observed increased IPP plasma concentrations 5–10 min after ingestion of a yoghurt drink containing IPP by humans.

Characteristics of the elimination phase clearly depend on the type of infusion (Tables 2 and 4). After I.G. administration, elimination half-lives of XPPs were about six times longer than after I.V. administration (about 12 and 2 min, respectively). This difference in half-life of elimination may be caused by a prolonged influx of XPPs during the elimination phase. We postulate that even longer half-lives for elimination can be achieved by administering XPPs in a different matrix. In the current experiment, they were infused in an iso-ionogenic NaCl solution. A (simulated) meal with higher viscosity may extend transport time from lumen to circulation, resulting in prolonged higher plasma XPP concentrations. Half-life of elimination for both I.V. and I.G. measurements are relatively short as compared to those observed by Foltz et al. [11]. In humans, they determined an half-life of elimination of 26 min. Possibly this is due to a species effect, but they used a more complicated food-matrix (yoghurt), which may result in the viscosity-related matrix effect described.

The data as presented in this study, show a fast pharmacokinetic profile with short elimination half-lives, but also low plasma concentrations reached. Kinetics of the breakdown-products (free amino acids and dipeptides) of the tripeptides tested were not measured in this study. We speculate, however, that the proline-rich tripeptides, despite that they are known to be relatively stable [8], are degraded in the enterocytes. Alternatively, they might be cleared from the plasma to organs or tissues. As we measure very low plasma response of the tripeptides this clearance must be very fast.

The pharmacokinetic properties of the tripeptides tested make it unlikely that physiological effects, like a reduction of blood pressure, are the result of prolonged high plasma concentrations. Therefore, such effects are induced either by very low concentrations, or there is build-up of an active concentration in certain tissues (e.g., aorta), or the effect is the result of an effect in the gut wall or gut lumen. For each bioactive peptide the exact mechanism needs to elucidated, however.

In summary, in conscious pigs proline-rich tripeptides reach the blood circulation intact. Infused via a iso-ionogenic solution of NaCl, their absolute bioavailability was about 0.1% and the half-life of elimination was maximally 15 min, suggesting that, under these conditions, a bioactive effect of these tripeptides would be rather acute. To investigate whether bioavailability or time of exposure to those tripeptides may be increased by supplementing them in a food or matrix with higher viscosity of digesta, further research is warranted.

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