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Bioavailability and Kinetics of the Antihypertensive Casein-Derived Peptide HLPLP in Rats

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ABSTRACT: The aim of this study was to investigate the oral bioavailability and kinetics of the milk casein-derived peptide HLPLP, which had previously demonstrated antihypertensive effect in spontaneously hypertensive rats. HLPLP disposition after single intravenous (4 mg/kg body weight) and oral (40 mg/kg body weight) doses was studied in rats. Plasma concentrations of HLPLP [β-casein fragment f(134−138)], and two derived fragments found after HLPLP administration, LPLP [β-casein fragment f(135−138)] and HLPL [β-casein fragment f(134−137)], were determined by ultrahigh performance liquid chromatography (UPLC) coupled on line to a Q-TOF instrument. For HLPLP, the elimination half-lives ($T_{1/2\beta}$) were 7.95 min after intravenous and 11.7 min after oral administration. The volume of distribution at steady state (V_{ss} = 30.8 L/kg) suggests a considerable uptake of HLPLP into tissues. HLPLP was converted to the peptides LPLP and HLPL. After HLPLP intravenous administration, the elimination half-lives ($T_{1/2\beta}$) for these biotransformed peptides, LPLP and HLPL, were 8.38 and 10.9 min, respectively. After oral administration, HLPLP was rapidly absorbed with an absorption half-life ($T_{1/2a}$) of 2.79 min. The oral bioavailability of HLPLP was found to be 5.18%. Our study suggested that HLPLP was rapidly absorbed and eliminated after oral administration, biotransformed into smaller fragments LPLP and HLPL, and distributed throughout the body by the circulation blood. The present pharmacokinetic information from a preclinical kinetic study in rats can also play an important role in designing future kinetic studies in humans for assessing HLPLP dose−response relationship.

KEYWORDS: *antihypertensive peptide HLPLP, oral bioavailability, plasma disposition, rats, tandem MS*

INTRODUCTION

Research on bioactive peptides has led to the discovery of particular protein sequences that modulate physiological functions with hormone- or druglike activity.[1] There are several peptides derived from food proteins that interact with the renin−angiotensin system and reduce arterial blood pressure. Much work has been performed to assess the in vitro activity of food peptides on the angiotensin-I-converting enzyme (ACE). However, only in some cases has the correspondence between in vitro and in vivo effects been demonstrated.[2] Discrepancy between ACE-inhibitory and antihypertensive activity of peptides can be due to their degradation during gastrointestinal digestion or the impaired access to the target organ in a sufficient amount, or a mechanism different from ACE inhibition may be involved.[3]

Milk fermentation represents one of the strategies most commonly employed to produce peptides with biological activity. For instance, recently *Kluyveromyces marxianus* has been demonstrated to produce antihypertensive lactoferrin-derived peptides, with ACE-inhibitory activity in vivo.[4] Milk fermented using selected strains of *Enterococcus faecalis* showed acute antihypertensive effect in spontaneously hypertensive rats (SHR) after a single oral administration.[5] In following experiments, the decrease of the arterial blood pressure was demonstrated after long-term intake of fermented milk.[6] The β-casein fragment (f) (133−138), with the sequence LHLPLP, was identified as one of the major peptides responsible for the biological activity of the fermented product. In fact, it is a potent in vitro ACE inhibitor (IC_{50} of 5.5 μM) and antihypertensive peptide which causes a decrease in the systolic blood pressure in SHR of 25.3 mmHg at a dose of 2 mg/kg when orally administered.[7] Simulated gastrointestinal digestion has shown that the sequence LHLPLP is resistant to digestive enzymes,[8] but upon incubation using Caco-2 cells, it was partly hydrolyzed by brush border peptidases that cleaved the peptide bond between Leu and His, releasing the pentapeptide HLPLP. The peptide HLPLP is also resistant to in vitro gastrointestinal digestion and brush border peptidases of Caco-2 cells, being rapidly transported through the cell monolayer.[9] This peptide HLPLP has been also demonstrated to exert antihypertensive activity in SHR.[10] Later on, the production of the active pentapeptide HLPLP by enzymatic hydrolysis from caseinate was also optimized[11] as an alternative to the fermentation with *E. faecalis* strains because its use in the food industry is controversial due to pathogenic potential.[12] Because the peptide HLPLP might be commercially attractive as an active compound, it is important to investigate its capacity to reach blood circulation.

Previous studies on oral bioavailability of selected food-derived peptides have reported their presence in plasma at pico- and nanomolar concentrations using very selective and sensitive

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analytical techniques.[13] van Platerink et al.[14] developed a sensitive method to detect peptides in human plasma samples with detection limits from 0.01 to 0.001 ng/mL. Although the research on the absorption of antihypertensive peptides has revealed that certain short peptides, di- and tripeptides such as VY and IPP, can reach bloodstream undegraded after oral administration, either in fasted or fed states, very low absorption extents were reported.[15−17] To the best of our knowledge, kinetic studies with longer peptides than tripeptides from dietary sources have not been reported.

Because the information regarding the kinetic profile is needed to establish a scientific basis for efficacy in the use of any food supplement, the objective of this study was to investigate the oral bioavailability and the kinetic behavior of the antihypertensive peptide HLPLP after its administration to rats, determining HLPLP plasma concentrations using a sensitive and specific analytical method. In addition, this technique allowed us to monitor the plasma concentrations of two derived peptide fragments, LPLP [β-casein fragment f(135−138)] and HLPL [β-casein fragment f(134−137)].

MATERIALS AND METHODS

Chemicals. The peptides HLPLP, β-casein f(134−138), HLPL, β-casein f(134−137), HLP, β-casein f(134−136), LPLP, β-casein f(135−138), LPL, β-casein f(135−137), PLP, and β-casein f(136−138) were used in this study. These peptides were prepared in-house following the solid-phase synthesis method with fluorenyl-methoxy-carbonyl (FMOC), using a 431A peptide synthesizer (Applied Biosystems Inc. Überlingen, Germany). Their purities, verified by reversed phase high performance liquid chromatography−ultraviolet−mass spectrometry (RP-HPLC-UV-MS) as previously described,[18] were 98.0%, 97.68%, 96.20%, 93.43%, and 99.36%, respectively. Peptide PLP, with a purity of 92.94%, was purchased from SynPeptide CO, LDT (Shanghai, China). All other chemicals were of the highest quality grade and obtained from commercial sources.

Animals and Experimental Design. The study was undertaken in accordance with the ethics requirements and authorized by the official ethical committee of University Complutense of Madrid. Adult male Wistar rats (Charles River Inc., Margate, Kent, UK) each weighing 220−230 g at 9 weeks of age were used. The animals were individually housed in polycarbonate cages with sawdust bedding and maintained in environmentally controlled rooms (22 ± 2 °C and 50 ± 10% relative humidity) with a 12 h light/dark cycle (light from 08.00 to 20.00 h). Food (A04 rodent diet, Panlab SL, Barcelona, Spain) and water were available ad libitum. The rats were divided into two groups, one group of 60 animals (group 1) received the synthetic peptide HLPLP intravenously and the other group of 84 animals (group 2) orally. Group 2 rats were deprived of food for 12 h before the single oral administration of 40 mg/kg body weight, but they had ad libitum access to water. The peptide was dissolved in distilled water (10 mg/mL) and was administered by oral gavage in a volume of 1 mL peptide solution/rat of 250 g of body weight. Group 1 rats were given a single iv injection of 4 mg/kg body weight into the lateral tail vein (in this case a peptide solution of 4 mg/mL was prepared, and 0.25 mL peptide solution/rat of 250 g of body weight was injected). The iv 4 mg/kg and oral 40 mg/kg doses of peptide HLPLP were selected on the basis of preliminary experiments where both doses and routes of administration did not show any adverse effects, abnormal clinical signs, behavioral changes, body weight changes, or change in food and water consumption in the animals treated (data not shown). The oral HLPLP dose was higher than the iv dose due to the low absolute oral bioavailability described for other food-derived peptides in pigs, approximately 0.1%.[17] The administered oral dose is higher than that reported to cause a significant decrease in the systolic blood pressure in spontaneously hypertensive rats (SHR).[10] Five additional animals were used as control to collect blank blood samples and to prepare calibration curves from plasma control fortified with the synthetic peptides HLPLP, LPLP, and HLPL.

Animals in groups 1 and 2 were killed by cervical dislocation (six animals at each time) and then exsanguination at 0, 1.5, 2, 3, 5, 8, 10, 15, 20, and 30 min after iv and at 0, 3, 5, 10, 15, 20, 30, 40, 45, 50, 55, 60, 65, and 70 min after oral administration of peptide HLPLP, respectively. Time points were selected based on preliminary experiments, and the limit of quantification (LOQ) of the analytical method was taken into account. Blood samples (1 mL) were withdrawn from the jugular vein and collected in heparinized tubes. Plasma was separated by centrifugation and stored frozen at −80 °C until analyzed. Blood samples at times greater than 70 or 30 min after oral or iv HLPLP doses, respectively, were not collected, because preliminary experiments (data not shown) showed that at these times no levels of the synthetic peptides HLPLP, LPLP, and HLPL were detected.

Analysis of Plasma Samples. *Plasma Sample Preparation.* Plasma samples from rats were prepared by adding 40 μL of an aqueous solution of 10% of trifluoroacetic acid (TFA) to 1 mL of plasma and homogenized in a vortex, and the mixture was heated at 99 °C for 2 min to precipitate proteins, as previously described.[14] Then the samples were centrifuged (MiniSpin, Eppendorf, Hamburg, Germany) at 8500*g* for 30 min. The supernatant was purified using mixed mode cation exchange cartridges (Bond Elut Plexa PCX, 60 mg, 1 mL, Agilent, Santa Clara, CA). Cartridges were preconditioned with 1500 μL of methanol and 2500 μL of Milli-Q water (Millipore, Bedford, MA). The plasma sample, previously mixed (1:3 v/v) with an aqueous solution containing 2% orthophosphoric acid, was loaded. The cleaning step consisted of the addition of 1500 μL of 2% formic acid in Milli-Q water and 2500 μL of a mixture composed of 50% methanol and 50% acetonitrile (v/v). The elution step was carried out by adding 1 mL of ammonia at 5% (35% purity) in methanol:acetonitrile (50:50, v/v). The elution volume was afterward totally dried by vacuum centrifugation (SpeedVac SC 200 Savant, Irving, TX). For the injection, the dried samples were reconstituted with 50 μL of 0.1% formic acid.

To prepare the standard curves, the synthetic peptides HLPLP, LPLP, and HLPL were diluted in Milli-Q water to final concentrations that ranged from 0.08 to 300 ng/mL, corresponding to 0.13 to 521 nmol/L, 0.18 to 682.9 nmol/L, and 0.17 to 625.9 nmol/L, respectively, in rat control plasma. Immediately after addition of the peptide solution to the plasma, they were treated as described previously for the preparation of plasma samples from rat assays. The calibration curves were injected on the same day as the rat samples.

Peptide Analysis by UPLC-Q-TOF. The analyses of samples were performed on an Acquity ultrahigh performance liquid chromatography (UPLC) instrument from Waters (Milford, MA), coupled to a Microtof-QII (Bruker Daltonik, Bremen, Germany). The LC-MS system was controlled by HyStar 3.2 software (Bruker). The column employed for the analyses was an Acquity UPLC BEH 130 C_{18} of 2.1 mm × 100 mm (Waters), with a particle size of 1.7 μm. The analyses were run at 30 °C, the flow rate was 0.2 mL/min, and the injection volume was 25 μL. A linear gradient was set from 0 to 45% of solvent B (acetonitrile/formic acid 0.1%) and 55% of solvent A (water/formic acid 0.1%) in 16 min. The nebulizer pressure was set at 3 bar, the temperature of the source at 180 °C, and the capillary voltage at 4.5 kV. MS full-scan acquisitions were first performed during UPLC runs, using a 50−1200 mass-to-charge ratio (m/z) range. The method developed was pseudoselected reaction monitoring (SRM) in which the parent ion with m/z 576.343 was selected for fragmentation. The peptide HLPLP was identified by its retention time and also by its fragmentation profile. The identification of the possible derived peptide fragments, i.e., LPLP and HLPL, HLP, LPL, and PLP, was performed using the retention time and the extraction of the characteristic molecular ions m/z 439.284, 479.290, 366.206, 342.231, and 326.200, respectively. The Q-TOF analyzer was calibrated on a daily basis in MS mode, using the m/z ratios of adduct ions arising from sodium formate as reference.

Data Analysis. The mean plasma concentration versus time after iv and oral administration was sequentially fitted to one-, two-, and

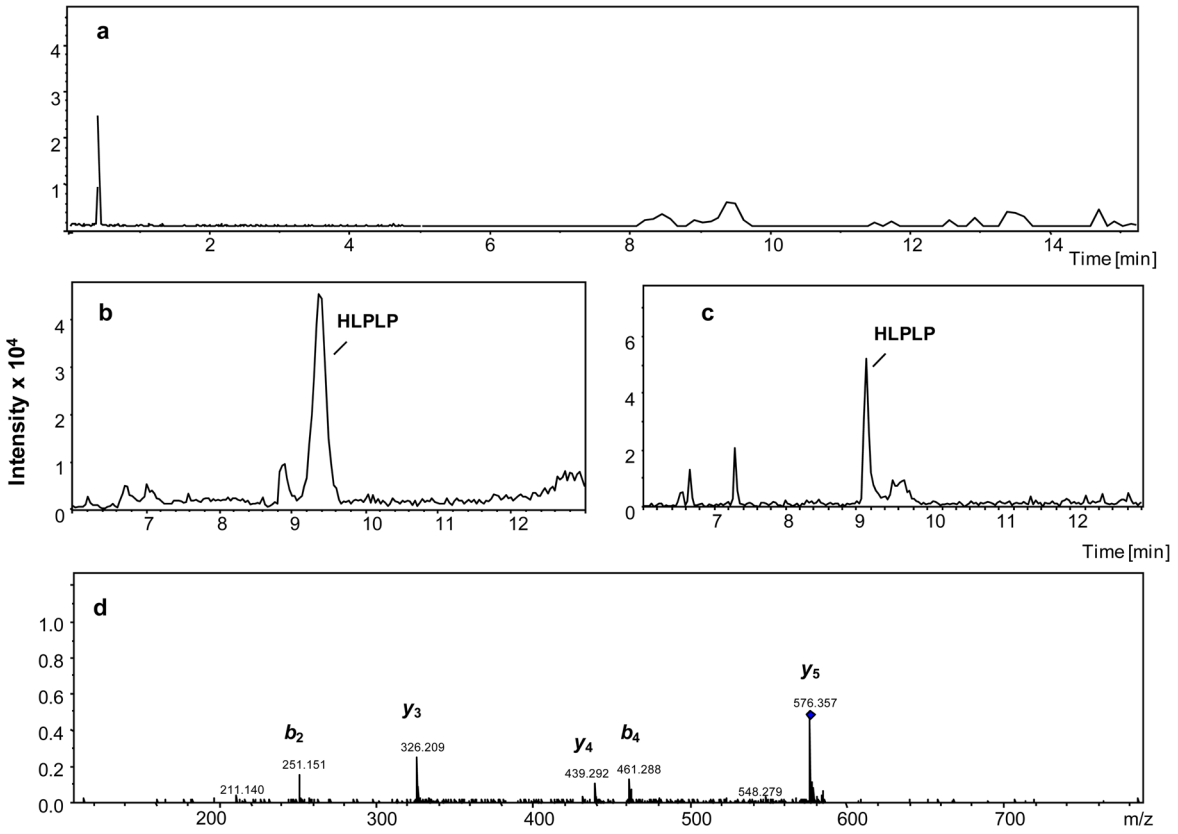

Figure 1. (a) Extracted ion chromatogram (EIC) of HLPLP (m/z 576.343; t_R 9.4), LPLP (m/z 439.284; t_R 10.5), and HLPL (m/z 479.290; t_R 8.4) in blank plasma. EIC of the peptide HLPLP (m/z: 576.343; t_R 9.4 min) (b) at 15 after oral administration and (c) at 20 min after intravenous administration. (d) Fragmentation pattern of HLPLP at 15 min after oral administration.

multiple-compartment models, using the computer program WinNonlin (Version 6.3; Pharsight Corporation, Mountain View, CA). The model was determined for best fit on the basis of a smaller value for the Akaike's Information Criterion (AIC).[19] The two-compartment model was the best fit for the two animal groups. This model was used to establish pharmacokinetic characteristics. Mean plasma concentration curves of peptide HLPLP after a single iv and oral administration and those of the peptides LPLP and HLPL (metabolites in plasma) after a single iv administration of HLPLP were fitted to the following exponential equations:

$$C = A_1 \times e^{-\alpha t} + A_2 \times e^{-\beta t} \text{ (iv)}$$

$$C = A_1 \times e^{-\alpha t} + A_2 \times e^{-\beta t} - A_3 \times e^{-K_a t} \text{ (oral)}$$

where C is the plasma concentration of the compound, A_1, A_2, and A_3 are mathematical coefficients (i.e., A_1 and A_2 are the plasma concentrations extrapolated to time zero of the first and second elimination phases of the compound and A_3 for the absorption phase), α is the hybrid rate constant for the distribution phase, β is the hybrid rate constant for the elimination terminal phase (i.e., α and β are the slopes of the first and second elimination phases of the compound disposition), and t is the time. Absorption half-life ($T_{1/2a}$), half-life of α phase ($T_{1/2\alpha}$), the half-life of β phase ($T_{1/2\beta}$), distribution rate constants for transfer of the compound from the central to the peripheral compartment (K_{12}) and from the peripheral to the central compartment (K_{21}), and the elimination rate constant (K_{10}) were calculated using standard equations as described.[20,21] After iv and oral administration, the area under the concentration−time curves (AUC) was calculated as follows:

$$\text{AUC} = (A_1/\alpha) + (A_2/\beta)$$

or

$$\text{AUC} = (A_1/\alpha) + (A_2/\beta) - (A_3/K_a)$$

where bioavailability (F) is $(\text{dose}_{iv} \times \text{AUC}_{oral})/(\text{dose}_{oral} \times \text{AUC}_{iv})$.

Total plasma clearance (CL) was calculated, using the following formula:

$$\text{CL} = [\text{dose (mg/kg)}]/\text{AUC}$$

or

$$\text{CL} = [\text{dose (mg/kg)}](F)/\text{AUC}$$

Mean residence time (MRT) (only for HLPLP iv administration) was calculated as follows:

$$\text{MRT} = [(A_1/\alpha^2) + (A_2/\beta^2) \times (1/\text{AUC})]$$

Volume of distribution in the central compartment (V_1) (only for HLPLP iv administration) was determined as follows:

$$V_1 = [\text{dose (mg/kg)}]/A_1 + A_2$$

Apparent volume of distribution in the second compartment (V_2) (only for HLPLP iv administration) was determined as follows:

$$V_2 = (V_1) \times (K_{12}/K_{21})$$

Volume of distribution at steady state (V_{ss}) (only for HLPLP iv administration) was determined as follows:

$$V_{ss} = \text{MRT} \times \text{CL}$$

Maximum drug plasma concentration (C_{max}) after oral administration and the time at which C_{max} was achieved (T_{max}) was determined directly from the concentration versus time curve.

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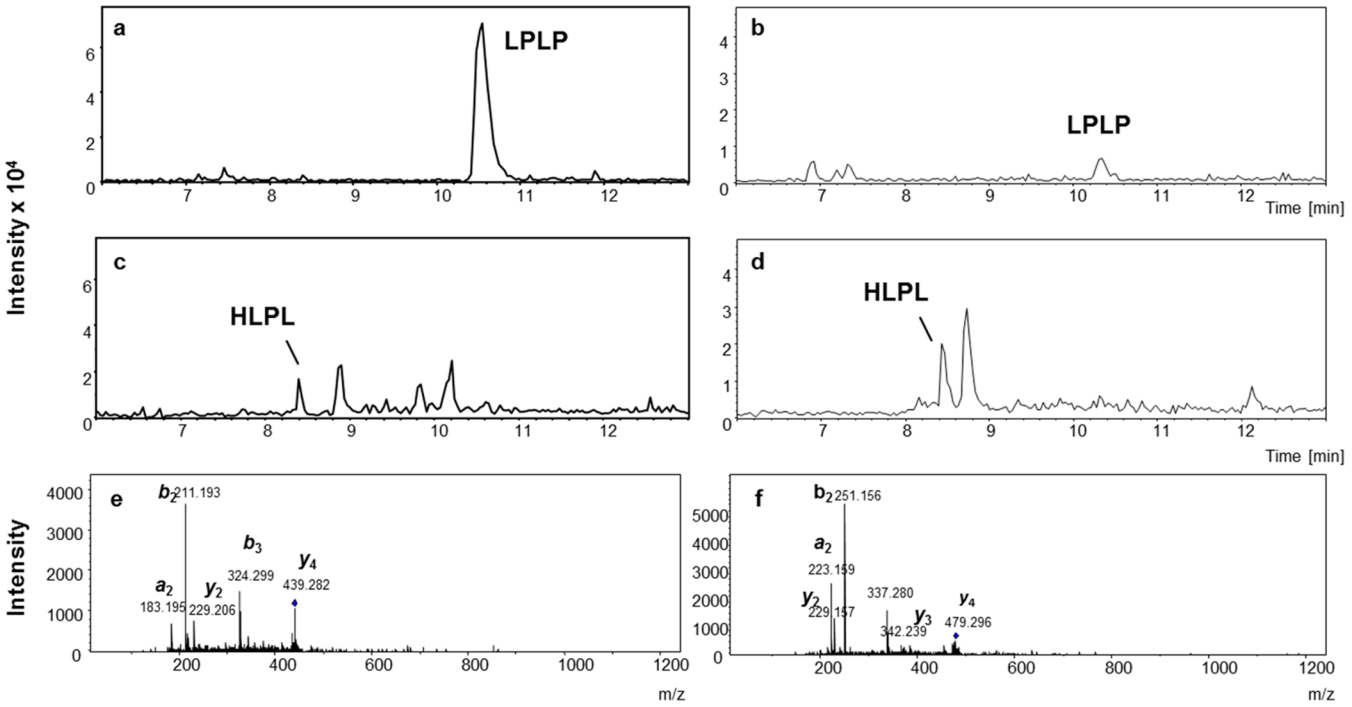

Figure 2. (a) Extracted ion chromatogram (EIC) of LPLP (t_R 10.5 min) at 2 min after intravenous administration and (b) at 40 min after oral administration of the peptide HLPLP; (c) EIC of HLPL (t_R 8.4 min) at 2 min after intravenous administration and (d) at 60 min after oral administration of HLPLP; fragmentation pattern of (e) LPLP and (f) HLPL.

RESULTS

Quantitative Determination of Peptides by UPLC-Q-TOF. In preliminary experiments, the quantification of the HLPLP peptide was attempted with a sample preparation protocol consisting of the addition of 10% TFA to 1 mL of plasma followed by heating at 99 °C for 2 min to precipitate the proteins and further centrifugation. However, this protocol did not allow determining plasma peptide concentrations below 12.5 ng/mL. Therefore, peptide purification and concentration was investigated by using different solid-phase extraction (SPE) cartridges, such as reversed phase C_{18} cartridges, mixed-mode cation, anion, and weak cation exchangers, and several solvent mixtures for the elution step. The best recovery values were obtained with a cation exchanger with mixed sorbent characteristics suitable for polar and nonpolar bases. The elution solvent selected was ammonia at 5% (35% purity) in methanol:acetonitrile (50:50, v/v).

The calibration curves provided linear results for HLPLP concentration values from 0.13 to 521 nmol/L in plasma (R^2 = 0.99). Figure 1a shows the extracted ion chromatogram of a blank plasma sample indicating the masses of the pentapeptide HLPLP and both tetrapeptides, HLPL and LPLP, where no interferences with endogenous compounds are expected. The extracted ion chromatogram of the pentapeptide after oral and iv administration and its fragmentation pattern are shown in Figure 1b, 1c, and 1d, respectively. One of the most intense fragment ions was observed at m/z 326.207, corresponding to y-type ion y_3. Other major fragment ions were detected at m/z 251.149 and 461.290, corresponding to b-type ions b_2 and b_4, respectively. These fragments (y_3, b_2, and b_4) resulted from the cleavage at N-terminal proline, which often generates highly intense b- and y-type fragment ions.[22] The recovery was calculated as the ratio between the concentration determined in a blank plasma sample spiked with HLPLP and further subjected to SPE purification and the concentration determined in a spiked sample after SPE purification. This was performed in triplicate using two concentrations of the peptide. The recovery values ranged from 96.9% to 98.7%. The reproducibility was estimated by the analyses of five spiked plasma extracts purified by SPE on three different days separated by 10 days, and repeatability by performing these determinations in consecutive analyses. Relative standard deviations (RSD) of the peak areas were 6% and 5% for reproducibility and repeatability, respectively. These values reflect the high performance achieved in the present study in terms of accuracy and precision. The limit of detection (LOD) was estimated to be 0.03 nmol/L, equivalent to 0.02 ng/mL, and was calculated as the concentration of peptide that gave a signal equal to 3 times the standard deviation of the blank. The LOQ was estimated at 0.17 nmol/L, equivalent to 0.10 ng/mL, by using 10 times the standard deviation of blank signal. With respect to the peptides LPLP and HLPL, the calibration curves provided linear results from 0.18 to 682.9 nmol/L (R^2 = 0.98) and 0.17 to 625.9 nmol/L (R^2 = 0.99), respectively. The LOD values for LPLP and HLPL were estimated at 1.13 ng/mL (0.50 nmol/L) and 0.11 ng/mL (0.22 nmol/L), respectively. The LOQ values for these peptides resulted in 1.67 ng/mL (3.71 nmol/L) and 0.36 ng/mL (0.75 nmol/L), respectively.

Pharmacokinetics of Peptide HLPLP and Its Biotransformed Peptides LPLP and HLPL. In the plasma samples collected at time 0, i.e., before HLPLP iv and oral administration, peptides HLPLP, HLPL, and LPLP were not detected. The two peptide fragments (m/z 479.290, 439.284) corresponding to HLPL and LPLP respectively, were found in plasma from 1.5 min until 30 min after iv administration of HLPLP. However, after oral administration, peptide fragments were detected between 5 and 60 min although they could not be quantified at all time points. Figure 2 shows the extracted

ion chromatogram of the derived peptides, LPLP and HLPL, at different times after oral and iv administration, and their corresponding fragmentation patterns.

Mean plasma concentrations of peptide HLPLP and its biotransformed peptides LPLP and HLPL after single HLPLP iv administration and mean plasma concentrations of peptide HLPLP after single oral administration are shown in Figure 3.

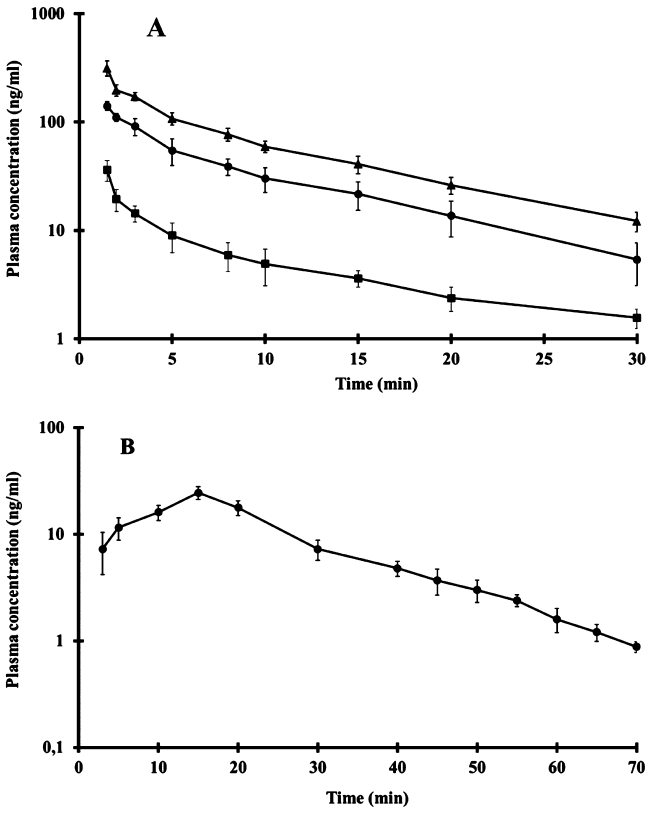

Figure 3. (A) Mean plasma concentrations of the peptides HLPLP (●), LPLP (▲), and HLPL (■) after single HLPLP intravenous dose of 4 mg/kg of body weight. (B) Mean plasma concentrations of the peptide HLPLP (●) after single oral dose of 40 mg/kg of body weight. Data represent mean ± SD values for six rats (i.e., six rats/time) (one-way ANOVA). Symbols without bars indicate that SD is within the symbols.

Table 1. Kinetic Parameters for Peptide HLPLP and Its Biotransformed Peptides LPLP and HLPL after a Single HLPLP iv Dose of 4 mg/kg Body Weight in Rats

parameters[a]	HLPLP	LPLP	HLPL
A_1 (ng/mL)	189	537	91.8
A_2 (ng/mL)	75.7	141	9.60
α (1/min)	0.64	0.77	0.90
β (1/min)	0.09	0.08	0.06
$T_{1/2\alpha}$ (min)	1.08	0.90	0.77
$T_{1/2\beta}$ (min)	7.95	8.38	10.9
V_1 (L/kg)	15.1	–	–
V_2 (L/kg)	15.7	–	–
V_{ss} (L/kg)	30.8	–	–
K_{12} (1/min)	0.25	0.35	0.42
K_{21} (1/min)	0.24	0.23	0.14
K_{10} (1/min)	0.23	0.28	0.40
AUC (μg min/L)	1163	2404	253
MRT (min)	8.95	8.97	9.87
CL (L/min/kg)	3.41	–	–

[a]Kinetic parameters were calculated from the mean plasma concentration−time curves.

Table 2. Kinetic Parameters for Peptide HLPLP after Oral Administration in Rats

parameters[a]	oral dose (40 mg/kg body weight)
B_1 (ng/mL)	242
B_2 (ng/mL)	56.9
B_3 (ng/mL)	299
α (1/min)	0.29
β (1/min)	0.06
K_a (1/min)	0.25
$T_{1/2\alpha}$ (min)	2.38
$T_{1/2\beta}$ (min)	11.7
$T_{1/2a}$ (min)	2.79
AUC (μg min/L)	603
F (%)	5.18
CL (L/min/kg)	3.41
C_{max} (ng/mL)	20.1
T_{max} (min)	11.8

[a]Kinetic parameters were calculated from the mean plasma concentration−time curve.

Analysis of plasma concentration-versus-time curves indicated a biphasic decrease after iv and oral administration. Good fit of the observed data for a two-compartment open model was obtained. The values of the kinetic parameters of HLPLP and its derived peptides LPLP and HLPL after iv administration of HLPLP are presented in Table 1. Values of the relevant kinetic variables that described absorption and disposition kinetics of HLPLP after oral administration are presented in Table 2.

■ DISCUSSION

The limited information available on the absorption of antihypertensive peptides derived from food proteins makes it difficult to interpret the functional properties of these bioactive peptides and to make an efficacy assessment as a new food supplement. These topics are commonly under debate for most functional ingredients.

Efficacy and safety assessment of many substances are normally extrapolated from animal data to humans. Laboratory animals, particularly rodents, are widely used for these evaluations. When several factors such as differences in species sensitivity are considered, kinetic variables are of major importance and should be taken into account in extrapolation of laboratory data to humans, because changes in various kinetic parameters are either easily determined experimentally or can be predicted with a reasonable accuracy. It is apparent that the principles of pharmacokinetics are indispensable in the overall assessment of human health risk.

Kinetic characteristics combined with dynamic patterns should be considered in the efficacy evaluation of peptide HLPLP. Preclinical pharmacokinetic information in experimental animals, such as the rat, is essential in designing further pharmacokinetic studies in humans which should be applied to the safe and effective use of this bioactive peptide.[23]

To the best of our knowledge, the present paper is the first report of plasma disposition of HLPLP peptide in rats using a selective analytical method to evaluate its pharmacokinetic profile. The LC-MS determination of the peptide was carefully optimized by selecting the parameters and analysis mode

providing the highest signal-to-noise ratio. The validation parameters used show that the method is reliable and sensitive and allow an adequate characterization of the disposition of peptide HLPLP in rat plasma. In addition, this analytical method also allowed the identification and the quantification of two derived fragments, LPLP and HLPL, from the hydrolysis of HLPLP by the action of plasma peptidases. However, the presence of shorter fragments derived from the action of plasma peptidases on HLPLP cannot be excluded. The inherent limitations of the mass spectrometry techniques to identify small peptides and the complexity of the matrix may have impaired detection of shorter fragments.

This study reports the kinetics of the peptide HLPLP after a single iv (4 mg/kg body weight) and oral (40 mg/kg body weight) administration in rats. Plasma disposition of HLPLP after iv and oral administration in rats as well as the appearance of the peptide fragments, LPLP and HLPL, after iv administration of HLPLP were best described by use of a two-compartment open model. Disappearance of the peptide from plasma was characterized by an initial rapid distribution phase followed by a rapid elimination phase. van der Pijl et al.[17] reported in pigs that elimination from plasma of the proline-rich tripeptides IPP, VPP, and LPP followed a two-compartment decay process only for 18 of the 30 observations. Therefore, it was concluded that the pharmacokinetic behavior of these peptides in pigs can be described with both one- and two-compartment models, although values for AIC for fitted peptide plasma concentrations after iv administration were lower using a two-compartment model.[17]

In the present study, after iv administration of HLPLP at 4 mg/kg, the distribution phase was rapid ($T_{1/2\alpha}$ = 1.08 min) and with a high value of V_{ss} (30.79 L/kg) which suggests that the peptide HLPLP is distributed into extravascular tissues. The elimination half-life ($T_{1/2\beta}$) calculated after iv administration was 7.95 min, much longer than that reported in pigs for peptides IPP, LPP or VPP (range $T_{1/2el}$ = 2.5−3.1 min)[17] The $T_{1/2\beta}$ of peptide HLPLP increased from 7.95 min after iv to 11.7 min after oral administration. This may indicate that the plasma disposition of HLPLP after oral administration could be conditioned by the absorption process.

Peptide HLPLP was extensively biotransformed to peptides LPLP and HLPL in rats and, after iv administration, represented 206% and 21.8% of the HLPLP plasma concentrations, respectively, as calculated by the ratio between the AUC for LPLP or HLPL and AUC for HLPLP. This suggests that these biotransformed peptides might contribute to the physiological activity of the peptide. In this respect, further experiments aiming at the evaluation of the activity of these derived peptides are already in progress.

When orally administered, HLPLP was rapidly absorbed through the gastrointestinal tract in rats, as reflected in a $T_{1/2a}$ of 2.79 min. This $T_{1/2a}$ is comparable to that previously obtained in pigs for the tripeptides IPP, LPP, and VPP, where after a single oral dose of 4 mg/kg body weight, the absorption half-life ranged from 2.0 to 4.6 min.[17] The absorption rate of the peptide HLPLP was low with a C_{max} of 20.1 ng/mL at 11.8 min (T_{max}) after an oral dose of 40 mg/kg body weight. However, it has to be highlighted that, as shown after iv administration, HLPLP is rapidly hydrolyzed by plasma peptidases into smaller fragments but were not considered in the calculation of absorption rate. In addition, it is hypothesized that other smaller fragments, tri- and dipeptides, might be released by the action of the peptidases, and due to the rapid degradation of the peptide, the effective absorption is underestimated. Nevertheless, the oral bioavailability found for HLPLP (5.18%) in rats was higher than that estimated for peptides IPP, LPP, and VPP in pigs, for which approximately 0.059−0.077% of the dose was absorbed.[17] Probably, the low bioavailability of HLPLP observed in the present study could be due to first-pass effect (metabolism in gastrointestinal tract or liver before reaching systemic circulation).[24,25] However, the absolute bioavailability of 5.18% after oral administration of the peptide HLPLP, which is not high for a small organic molecule, is valuable data for a peptide, given that they are subjected to extensive metabolism by peptidases before, during, and after the absorption process. The bioavailability of a chemical compound is a prime consideration in formulation approaches, especially when attempting to increase bioavailability through physico-chemical and technical manipulations in regard to the dosage form. Further studies in this concern should be conducted.

In summary, the present study indicates that when orally given, peptide HLPLP is absorbed and distributed throughout the body by the bloodstream. The kinetic characteristics of peptide HLPLP identified in this study warrant further research on possible new ways of supplementation in food to increase its absorption and time of exposure. The present pharmacokinetic information in rats can play an important role designing future kinetic studies in humans for assessing HLPLP dose−response relationship. In addition, this work reports an accurate analytical method for the determination of HLPLP, which also allowed the identification and quantification of some derived peptide fragments. Although the pentapeptide reaches the bloodstream, its rapid degradation in plasma affects the estimation of the kinetic parameters. This study raises, as well, the question about the final active form of dietary compounds, and specifically of dietary peptides, in the organism.

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Notes

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