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Short communication

Pharmacokinetics and bioavailability study of L-ornithine–L-aspartate in healthy volunteers—A comparative study of two oral formulations

P. Kowalski *, M. Bieniecki

Medical University of Gdańsk, Faculty of Pharmacy, Hallera 107, PL-80-416 Gdańsk, Poland

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Abstract

The aim of the evaluation was to establish bioequivalence between two oral 3.0 g sachet forms of L-ornithine–L-aspartate (LOLA). It was designed as randomised, two-way crossover study with a 1-week washout interval. Blood samples were collected throughout a 12 h period after administration of reference and test product to 12 fasting healthy male volunteers. Plasma were analyzed by sensitive, reproducible, accurate and rapid capillary electrophoresis (CE) method with UV detection. Many pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$ and K_{el} were determined from plasma concentration. First three of them after log-transformation of data were examined for bioequivalence. Based on ANOVA with 90% confidence level no significant difference was found. All of tested parameters were found to be within the bioequivalence acceptance range of 80–125%. Based on these and other statistical tests it was concluded that Hepatil® is bioequivalent to Hepa-Merz granulate®.

Keywords: L-ornithine–L-aspartate (LOLA); Capillary electrophoresis; Bioequivalence study

1. Introduction

Bioequivalence and bioavailability trials play a key role in the drug development period for both new drug products and their generic equivalents. With the increasing availability and use of generic products, the new products need testing for their bioequivalency before they are marketed. In the present study, the bioequivalence of two brands of L-ornithine–L-aspartate (LOLA) sachet formulations was evaluated in healthy male volunteers. In clinical practice, tablets are one of the most convenient and acceptable solid dosing forms. However, for some geriatric patients who have difficulty swallowing capsules, a suspension or liquid form is preferred. Furthermore, solution forms generally result in faster and more complete absorption of drug, since a dissolution step is not required. This formulations should overcome genuine swallowing problems and prevent cover refusal to swallow in uncooperative individuals, potentially reducing confrontations with medical staff and improving medication compliance. Differences in the quality of the granules coating are a potential limiting factor for a vivo performance of the drug and various product may cause different bioavailability parameters. Thus we developed and established the bioequivalence of the sachet forms.

Both ornithine and aspartate are endogenic amino acids, which are required by the body for a large number of metabolic processes. L-ornithine is a non-protein, basic amino acid and in the natural way, get into the urea cycle, which produce urea from ammonia in the liver. The elevation of L-ornithine level produces acceleration detoxification processes in the liver and brain. Moreover, there is known a influence to carbohydrates, lipids and amino acids metabolism.

Recently, several controlled clinical trials have been undertaken using LOLA, and in all cases, it was found to be effective in lowering blood ammonia concentrations by increasing urea synthesis. Thus it has been widely used in hepatic encephalopathy and to improve neuropsychiatric status in patients with chronic liver failure [1–6].

L-ornithine is used also as a nutritional supplement principally for its putative anabolic activity, along with L-arginine, used in very high amounts, may promote muscle building activity by increasing levels of growth promoting (anabolic) hormones [7]. Likewise, accumulating evidence suggests that LOLA restores muscle protein synthesis, an effect which can be exploited in patients suffering from cancer or HIV infections.

* Corresponding author. Tel.: +48 58 3493130; fax: +48 58 3493130.
E-mail address: piotrpl@wp.pl (P. Kowalski).

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Moreover, LOLA increased the tolerance towards cytostatic drugs in patients receiving chemotherapy [8,9]. Preliminary studies have shown an LOLA given intravenously to patients hospitalized with burns, trauma and severe infection may help improve protein balance, wound healing and decrease recovery time. Other studies showed significant improvements of clinical values and liver function in humans undergoing radiation therapy receiving LOLA as well as during long lasting tuberculostatic and neoplastic therapy [10].

This paper, based on recently described CE method for assaying LOLA in human plasma [11], presents pharmacokinetic study, carried out on 12 male volunteers. For the first time are reported also the results of a bioequivalence trials with the test and the reference in granulate formulation, in both the cases with 3 g of the active ingredient. Although L-ornithine–L-aspartate is the stable salt of two amino acids, in this work both L-ornithine and L-aspartic acid were determined separately.

2. Materials and methods

2.1. Study products

The purpose of this study was to determine the pharmacokinetic parameters of two brands of L-ornithine–L-aspartate 3 g sachets and then to compare these parameters statistically to evaluate the bioequivalence between the two products. Hepatil® (Polfa, Krakow, Poland) was used as test product while Hepa-Merz granulate® (Merz Pharma GmbH and Co. KGaA, Germany) was used as reference product.

2.2. Study subjects

Twelve healthy, non-smoking, male volunteers, aged with a range 21–28 years (mean ± S.D., 24.1 ± 2.5 years), weight 65–85 kg (75.6 ± 6.1 kg), height 173–190 cm (181.2 ± 6.6 cm) took part in this study. The clinical protocol was approved by the local Ethics Committee and all the volunteers gave written informed consent after they had receive detailed instructions about the aims, restrictions and possible adverse effect which could be experienced as a result of taking the drug. Before entering the study, volunteers had a routine physical examination and were subjected to a set of laboratory tests (blood biochemistry, hematology, and urine analysis), which were found to be normal. Alcoholics were excluded from this study. Subjects did not receive any medication during the 2 weeks period prior to the start and during the study period.

2.3. Drug administration, sample collection and preparation

Administration of the two products (reference and test) to the subjects was carried out by means of two-way crossover design with a 1-week washout period. Volunteers were randomly divided into two equal groups and assigned to one of the two sequences of administration. Each subject was fasted overnight prior to the experiment, and food was withheld for 3 h after dosing. Each subject received a single dose of 3 g sachet of either brand dissolved in 250 ml of water. Approximately 5 ml venous blood samples for LOLA assay were collected into heparinized tubes at the following times: immediately before drug administration (O), and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 8.0, and 12.0 h after dosing. The plasma was then separated after centrifugation and stored frozen at −20 °C until quantitative analysis. After a period of 7 days the study was repeated in the same manner to complete the crossover design. The frozen plasma samples form each of volunteers were thawed in an ice water bath and subsequently were prepared to CE injection according the procedure described early [11].

2.4. Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was performed by use of WinNonlin® version 4.0.1. computer program. Data obtained from individual volunteers was subjected to non-compartmental pharmacokinetic analysis. Various pharmacokinetic parameters such as area under curve (AUC), peak plasma concentration (C_{max}), time to reach the peak (T_{max}), elimination rate constant (K_{el}), elimination half-life ($T_{1/2}$), and absorption efficiency were determined for each volunteer. The elimination rate constant (K_{el}) was obtained as the slope of the linear regression of the log-transformed concentration versus time data in the terminal portion of the curve. AUC_{0-t} was determined by linear trapezoidal rule. $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_{last}/K_{el}$, where C_{last} is the last measurable concentration. Pharmacokinetic parameters as C_{max} and T_{max} were determined by inspection of the individual plasma-concentration time profiles.

To assess the final bioequivalence decision pharmacokinetic parameters $AUC_{0-\infty}$, AUC_{0-t} and C_{max}, obtained from plasma analysis, were evaluated with tests: two-way ANOVA as classical and Westlake way, two one-sided *t*-test procedure and Anderson–Hauck procedure. The products were considered bioequivalent when there were no statistically significant differences between two compared parameters [12]. In the extrapolation procedure not more than 20% was added to AUC_{0-t} to calculate $AUC_{0-\infty}$, which means that the blood sampling period selected was appropriate.

3. Results and discussion

3.1. Endogenous amino acids

It is difficult to become human plasma devoid of ornithine and aspartate, because the both amino acids are manufactured by the body, and naturally occur in human organism. Therefore, their levels in human plasma before administration of LOLA is higher than zero (Figs. 1 and 2). The examination of endogenous substation always appear a question if the estimate level is produce by organism or it is effect of our procedure or else drug. To eliminate this factor of uncertainty some made a baseline. In this case it wasn't necessary because the main purpose was to examined a average bioequivalence between drugs and not to analyze each level in one probant. The second condition: in the bioequivalence study participate the same subjects, so probably

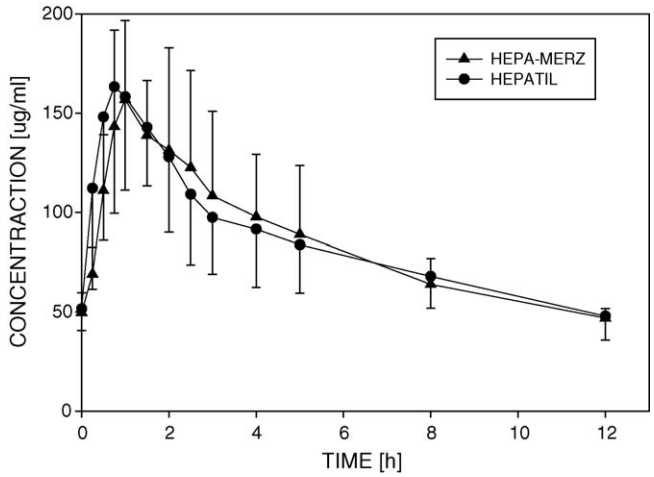

Fig. 1. Mean plasma concentration–time profiles of L-ornithine after single oral administration of LOLA (granulate formulation) of test (Hepatil) and reference (Hepa-Merz) drugs.

the naturally base level of LOLA was the same in both amino acids case.

3.2. CE method

The assay was found to be selective, accurate and precise with a linear range of 10–280 μg/ml for L-aspartic acid and 20–280 μg/ml for L-ornithine. These concentrations correspond well with therapeutic ranges of both amino acids. The validation data of the electrophoretic method of LOLA plasma samples have been investigated previously [11]. The main advantages of the application of CE for the determination of LOLA in plasma are short analysis time (8 min), low cost of the reagents used as the background electrolyte and a simple procedure for sample preparation. Moreover, because aqueous buffer is applied, it is possible to use low UV wavelength detection and to determinate the amino acids without derivatisation. The high degree of clean up from the plasma matrix resulted in samples that did not cause any capillary adsorption problems into the capillary. The same capillary could be used for weeks without problems using the washing steps described above. The electrophoretic method used in this study yielded satisfactory results for determination of LOLA in human plasma, and has been successfully used for pharmacokinetic studies after oral administration in sachet forms.

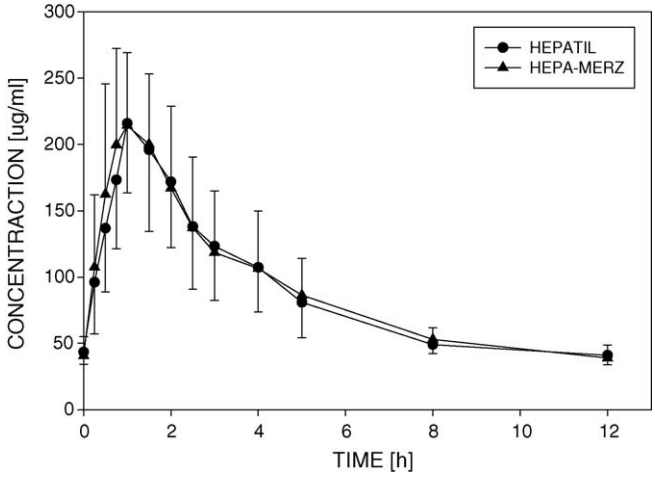

Fig. 2. Mean plasma concentration–time profiles of L-aspartic acid after single oral administration of LOLA (granulate formulation) of test (Hepatil) and reference (Hepa-Merz) drugs.

Table 1
Mean pharmacokinetic parameters of L-ornithine in two products

Pharmacokinetic parameter	Hepatil	Hepa-Merz
$AUC_{0-\infty}$ (μg h/ml)	1113.2 ± 228.4	1141.7 ± 329.9
AUC_{0-t} (μg h/ml)	953.2 ± 244.8	954.4 ± 243.7
C_{max} (μg/ml)	182.2 ± 40.7	188.9 ± 61.0
T_{max} (h)	1.2 ± 0.5	1.0 ± 0.5
K_{el} (h^{-1})	0.17 ± 0.05	0.16 ± 0.04
$T_{1/2}$ (h)	4.4 ± 1.3	4.5 ± 1.3
V_d (l)	9.4 ± 4.5	9.1 ± 3.7
MRT (h)	6.7 ± 1.2	6.7 ± 1.7
% Extrapolated	15.8 ± 6.4	16.3 ± 7.7

$AUC_{0-\infty}$: area under the curve extrapolated to infinity; AUC_{0-t}: area under the curve up to the last time (t) in which drug was measured; C_{max}: the maximum plasma concentration; T_{max}: the time to reach peak concentration; K_{el}: the apparent elimination rate constant; $T_{1/2}$: the apparent elimination half-life; V_d: volume of distribution; MRT: mean residence time.

3.3. Pharmacokinetic study

This paper describes for the first time the pharmacokinetic studies of L-ornithine and L-aspartate granulate formulations in dose 3 g on a 12 volunteer group by using electrophoretic method. We have examined the single dose pharmacokinetics of LOLA as a sachet formulations, in crossover study. The mean L-ornithine and L-aspartic acid concentration–time profiles after administration of the test and reference formulations in 12 subjects are depicted in Figs. 1 and 2, respectively. Intake of either formulation produced similar plasma concentration–time profiles for both amino acids. All basic pharmacokinetic parameters calculated for the test formulation were close to those of the reference formulation and there were no statistically significant differences between the two products (Tables 1 and 2). Results

Table 2
Mean pharmacokinetic parameters of L-aspartate in two products

Pharmacokinetic parameter	Hepatil	Hepa-Merz
$AUC_{0-\infty}$ (μg h/ml)	1264.6 ± 162.7	1317.8 ± 336.9
AUC_{0-t} (μg h/ml)	1031.0 ± 231.2	1067.4 ± 243.4
C_{max} (μg/ml)	225.4 ± 49.6	229.9 ± 55.6
T_{max} (h)	1.1 ± 0.4	1.1 ± 0.5
K_{el} (h^{-1})	0.15 ± 0.08	0.16 ± 0.06
$T_{1/2}$ (h)	5.6 ± 2.5	5.3 ± 2.8
V_d (l)	10.2 ± 5.9	8.8 ± 3.7
MRT (h)	7.5 ± 2.7	7.3 ± 3.1
% Extrapolated	19.3 ± 9.9	17.8 ± 11.2

$AUC_{0-\infty}$: area under the curve extrapolated to infinity; AUC_{0-t}: area under the curve up to the last time (t) in which drug was measured; C_{max}: the maximum plasma concentration; T_{max}: the time to reach peak concentration; K_{el}: the apparent elimination rate constant; $T_{1/2}$: the apparent elimination half-life; V_d: volume of distribution; MRT: mean residence time.

and estimated parameters are reported as mean ± S.D. Comparative pharmacokinetic study of the two LOLA products, test formulations (Hepatil) and reference formulation (Hepa-Merz) after single dose regimen by administration of 3.0 g LOLA in volunteers revealed that the parameters which indicate the amount of drug absorbed into the body (AUC_{0-t}) and the relative rate of drug absorption (C_{max}) obtained from these two brands were very similar.

Maximum plasma concentration (C_{max}) for L-aspartic acid after administration of the tested and reference preparation occurred at a similar time (T_{max}) 1.1 ± 0.4 h and 1.1 ± 0.5 h, respectively; and amounted to 225.4 ± 49.6 ug/ml for tested and 229.9 ± 55.6 ug/ml for reference preparation (Table 2). Whereas, for L-ornithine of tested and reference products C_{max} amounted 182.2 ± 40.7 ug/ml and 188.9 ± 61.0 ug/ml, respectively (Table 1). It should be noted that the C_{max} values are slightly more favorable for reference product, nonetheless none of all investigated parameters obtained from 12 subjects were outside the range of 0.8–1.25. A consequence of a higher C_{max} value for reference product is a considerably larger area under curve ($AUC_{0-\infty}$) for L-ornithine (1141.7 ± 329.9 μg h/ml) as well as for L-aspartic acid (1317.8 ± 336.9 μg h/ml).

Due to small differences in the values of C_{max} and $AUC_{0-\infty}$ the tested formulation no large differ also with reference to the apparent elimination rate constant (K_{el}), volume of distribution (V_d), and mean residence time (MRT) for analyzed compounds. ANOVA for $AUC_{0-\infty}$, AUC_{0-t} and C_{max} parameters, after log-transformation of the data, showed no statistically significant difference between the two products. The present study demonstrates also that both L-ornithine as well as L-aspartic acid are well absorbed after oral administration of a 3 g dose of LOLA. Moreover, both LOLA formulations were well tolerated by the enrolled volunteers, with no adverse effect being reported.

4. Summary and conclusion

In this study of two commercial brands (test and reference granulate formulations) of LOLA in healthy volunteers, a similar rate and extent of absorption for products were found to be bioequivalent. The means and standard deviations of pharmacokinetic parameters for the two products are very similar, indicating that the pharmacokinetics of LOLA in the two granulate formulations are also similar. The applied CE method for the determination of LOLA displayed good precision, accuracy and specificity, required only a small amount of buffer solutions, was fast, with reasonable limit of quantification for both amino acids, thereby enabling its use in bioequivalence trials. To determinate concentration of LOLA in clinical trials, the use of CE would be ideal due to the availability of autosamplers and the speed at which the data is obtained. No significant sequence effect was found for all of the bioavailability parameters, indicating that the crossover design was properly performed. In conclusion, above results indicate that the two medications of LOLA (Hepa-Merz and Hepatil) are bioequivalent, and thus may be prescribed interchangeably.

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