DETERMINATION OF BIOEQUIVALENCE OF TWO OMEPRAZOLE PREPARATIONS: OMERAN (EUROPHARM) AND LOSEC (ASTRA)

Leucuta Sorin-Emilian, Iuga Cristina*, Capalneanu Radu**, Popa Adina*, Preda Manuela**

REZUMAT

Determinarea bioechivalenţei a două preparate cu omeprazol: OMERAN (Europharm) şi LOSEC (Astra)

In această lucrare s-a determinat bioechivalenţa a două produse de uz oral cu omeprazol, produsul de testat Omeran (Europharm, Brasov) şi produsul de referinţă Losec (Astra), ambele cu un conţinut de 20 mg omeprazol, în granule enterosolvente condiţionate, în capsule gelatinoase. Studiul s-a efectuat pe 12 voluntari sănătoşi, care au primit fiecare preparat, într-o ordine întamplătoare, la interval de o săptămână. Concentraţiile plasmatice ale omeprazolului s-au determinat cantitativ cu o metodă validată de cromatografie de lichide de înaltă performanţă (aparat HP 1100 Series). Parametrii farmacocinetici caracteristici ai omeprazolului s-au determinat din valorile individuale ale concentraţiilor plasmatice în funcţie de timp (Kinetica 3, Inna Phase. S-a demonstrat că atât mărimea absorbţiei, cât şi viteza de absorbţie din cele două preparate studiate a fost identică; cele două produse medicamentoase pot fi considerate interschimbabile sub aspectul biodisponibilităţii.

Cuvinte cheie: omeprazol; biodisponibilitate/ bioechivalenţă

ABSTRACT

The bioequivalence of two oral preparations of the omeprazole, the test preparation Omeran (Europharm, Brasov) and the reference product, Losec (Astra), both in a dose of 20 mg omeprazole, was assessed in an open, cross-over, randomized trial in 12 healthy male volunteers. A HPLC method with U.V. detection was used for the assessment of plasma omeprazole concentrations. The pharmacokinetic characteristics for omeprazole were determined from the plasma concentration-time data (Kinetica 3, Inna Phase). The tested product was bioequivalent with the reference product both as amount and rate of absorption; these products are interchangeable.

Key words: omeprazole; bioavailability; bioequivalence

Omeprazole is a substituted benzimidazole which is activated in the acidic compartment of the parietal cell, inhibits gastric acid secretion by binding to active proton pumps (H^+-, K^+-ATPase) in the secretory membrane of the parietal cell. Omeprazole is available as a capsule containing enteric-coated (acid –protected) granules. The granules' polymer coating dissolves only at pH of more than 6, allowing release of omeprazole in the duodenum.

Absorption is rapid with peak plasma levels of omeprazole occurring within 0.5 to 3.5 hours. Absolute bioavailability, compared to intravenous administration, is about 30-40% at a dose of 20 mg, due in large part to presystemic metabolism. In healthy subjects the plasma half-life is 0.5 to 1 hour, and the total body clearance is 500-600ml/min. The drug is metabolized by hepatic cytochrome P-450 isoenzyme CYP2C19, resulting in a very short plasma half-life of 40-60 minutes, although it is as long as 2 hours in 3-5% of whites who do not have the specific isoenzyme. The majority of the dose was eliminated in urine as at least six metabolites. The metabolites have very little or no antisecretory activity (1-3).

Methods

Subjects

A number of 12 healthy male adult volunteers participated in the study.

Their mean age ±S.D. was 22.83 (±1.11) years, body weight of 73.91 (±8.05) kg and height of 180 (±6.6) cm. On the basis of medical history, physical examination and clinical laboratory test results no subject had a history evidence of hepatic, renal, gastrointestinal or hematologic deviations. The volunteers were asked to abstain from taking any drug or alchohol for at least 2 participants before they accepted to participate the study. The study protocol was approved by the University Ethical Committee.

Study design

The study design was a single dose, two treatments, two periods, and two-sequence crossover with a one-week washout period between treatments. Each subject received a single dose of 20 mg omeprazole capsules of either brand, with 150 ml of water, after an overnight fast for at least 10

University of Medicine and Pharmacy „Iuliu Hatieganu" Cluj-Napoca
**Prof.dr.Leucuta S.E.; Sef lucrari dr. C.Iuga; Sef lucr.dr. A.Popa; Sef lucr.dr. M.Preda: Faculty of Pharmacy, Bioequivalence Laboratory;*
***Prof.dr. R.Capalneanu: Institute of Heart „N.Stancioiu" Cluj-Napoca*

hours. Subjects were allowed to eat a standard meal after 4 hours and after 8 hours after drug administration. Multiple blood samples were collected before and at 0.5;1;1.5;2;2.5,3;4;6; and 8 hours after the dose. The plasma was then separated after centrifugation and stored frozen at -20°C pending analysis.

Standard in vitro tests

Both the test formulation, Omeran (Europharm) capsules, and the reference product, Losec (Astra), capsules, were examined for conformation to compendial standards and the manufacturer specifications for weight variation, disintegration, dissolution and assay. Both the brands met the specifications with USP 23 and the manufacturer specifications.

Analysis of plasma samples

For quantification of omeprazole a high-pressure liquid chromatographic methods was developed. A HP 1100 Series liquid chromatograph system composed of pump, autosampler, thermostat and U.V. detector were used. A Kromasil C 18 150 mm x 4.6 mm (5μm) column and a Zorbax SB-C18 4.6 x 12.5 mm (5μm) precolumn were used. The mobile phase was containing CH_3OH:H_2O (55:45, v/v), triethanolamine 1% at pH=7 with H_3PO_4 85%. The flow rate: 1ml/min, temperature: 28°C. The injection volume: 30 ml. Detection: 302 nm.

Standard solutions and sample preparation

Standard solutions were prepared by dissolving omperazole and internal standard phenacetine (0.1 mg/ml) in methanol. One ml of the plasma samples was mixed with internal standard (phenacetin), NaOH 0.02 M and $CHCl_2$: ethylether (2:2.5), vortex and centrifuged. Organic phase was evaporated and the extract was reconstituted in mobile phase and injected. Calibration curves in plasma were linear over the range of 25-500 ng/ml. The limit of quantification: 15 ng/ml. Intra-day and inter-day validation: 0.3-7.3 (C.V.%) for precision and accuracy. Recovery: 81-99%.

Pharmacokinetic analysis

The parmacokinetic characteristics for omeparzole were determined from the plasma concentration-time data. The maximum plasma concentrations (Cmax)and time to reach maximum plasma concentrations (Tmax) were obtained directly by inspection of the individual drug plasma concentration-time data. The area under the plasma concentration-time curve (AUCt) up to the last time showing a measurable concentration (Ct) was determined by using the linear trapezoidal rule (4,5). The apparent elimination rate constant Kel was calculated by a computer programm (Kinetica 3, Inna Phase). The AUCi from zero to infinite values (express the magnitude of absorbtion) were determined by adding the quotient of Ct and Kel to the calculated by using the equation: T1/2=ln2/Kel. The sampling period covered more than 90% of total area under the curves for both brands.

Statistical analysis

The two-way analysis of variance (ANOVA) for crossover design was used to assess the efect of formulations, periods, sequences and subjects within sequence on the bioequivalence parameters studied: area under the curve (AUC), Cmax and Tmax. AUC and Cmax of the two formulations were assessed by Schuirmann's two one-sided t test. Non parametric Friedman test for the Tmax was performed under the assumption of additive model (6-11). All analyses of the data were performed with the statistical software package (Kinetica 3, Inna Phase).

Results and discussion

Table 1 shows the results of the mean plasma concentrations of omeprazole following oral administration of 20 mg omeprazole of the two brands to 12 healthy volunteers.

Time (hours)	OMERAN (ng/ml)	S.D.	LOSEC (ng/ml)	S.D.
0.5	<L.Q.		<L.Q.	
1	35.36	68.5	50.4	101.2
1.5	153.9	166.8	159.6	161
2	196	128	184.6	139
2.5	151	86.5	171.8	98.3
3	105.4	69.2	140.8	93.1
4	41.5	30.8	60.5	59.3
6	11.6	11.4	10.7	9.96
8	2	4.2	5.94	8.2

L.Q.=limit of quantification

Table 2 shows the parametric 90% confidence intervals of the mean values of the AUC and Cmax as well as the point estimates for test/reference ratio assuming multiplicative model, and nonparametric (Friedman) confidence interval for Tmax.

Pharmacokinetic parameters	Tested formulation Omeran (Europharm) Mean ± S.D.	Reference formulation Losec (Astra) Mean ± S.D.	Confidence interval 90%
ASCt (ng.h/ml) (ln)	2.578 (0.042)	2.616 (0.033)	0.81-1.04
Cmax (ng/ml) (ln)	2.433 (0.144)	2.445 (0.045)	0.80-1.18
Tmax (hours)	2.1167 (0.537)	2.208 (0.582)	CHI^2=0.083 (N.S.) (Friedman)

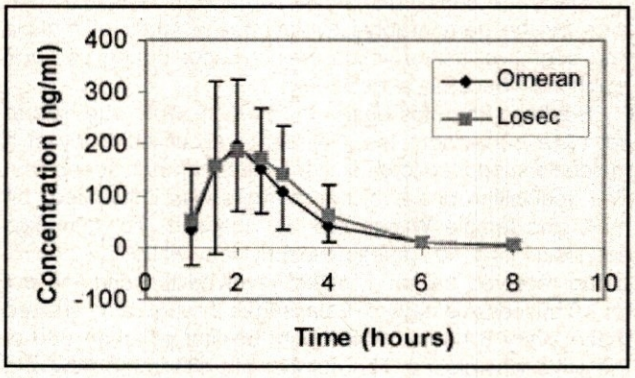

Fig.1 shows the mean plasma concentration time curves of omeprazole for the two brands of omeprazole.

It can be seen that the mean plasma concentration time profile from tested and reference brands are almost superimposed.

The parameters of both Omeran (Europharm) and Losec (Astra) are similar and consistent with reported values in literature.

The relative bioavailability (F) of omeprazole from test product versus reference product was F%=(ASC test/ASC reference). 100=(380/426).100=89%. The difference is without clinical significance.

The confidence limits for the mean AUCt and Cmax indicated thet these values are entirely within the bioequivalence acceptable range of 80-125% (Schuirman't test). With regard to the characteristic of Tmax, untransformed data were used and the bioequivalence range was expressed in absolute difference instead of proportions. The difference between the two formulations is non significant (Friedman test).

In conclusion, based on the pharmacokinetic and statistical results of this study, the bioequivalence of the test product and the reference product was demonstrated as the amount and rate of absorption, and we can assume interchangeability of both preparations in clinical practice.

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