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Original Article

Comparative Bioavailability of Ranitidine Tablets in Healthy Volunteers

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Abstract

The pharmacokinetic parameters of domestic and imported ranitidine hydrochloride tablets (Ranitidine, formulated and manufactured by Kharazmi Pharmaceuticals, Iran, and Zantac ® manufactured by Glaxo, UK) were measured in 14 healthy subjects following oral administration of a single 300-mg dose of each brand and compared for bioequivalence evaluation.

The pharmaceutical equivalency of both formulations was shown by in vitro characterization and dissolution testing. The comparative bioavailability of the two products was then determined in a single-blind, single dose, randomized, cross-over study in 14 healthy volunteers. A sensitive, rapid and precise high performance liquid chromatography (HPLC) method was used to measure concentrations of ranitidine in plasma samples collected up to 12 hours following each dose. Pharmacokinetic parameters, including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, elimination rate constant (*k*) and half life were determined for both formulations.

Analysis of the data revealed that the variations in all pharmacokinetic parameters were not statistically significant ($p > 0.05$), and the 90% confidence intervals for the test/reference mean ratios of the plasma pharmacokinetic variables lie within the conventional bioequivalence range of 80-125%. Therefore, both formulations were comparable based on the in vitro characterization and were bioequivalent in terms of Cmax and AUC.

The two formulations were considered to be bioequivalent.

Keywords: Ranitidine; Zantac®; Comparative Bioavailability; Bioequivalent; Pharmacokinetic Parameters; HPLC Detection.

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Introduction

Ranitidine, as a specifically histamine H_2-receptor antagonist, heals gastric and duodenal ulcers by reducing gastric acid output as a result of H_2-receptor blockade. This drug is also indicated for the management of hypersecretory conditions, such as Zollinger – Ellison syndrome and systemic mastocytosis.

The effects of factors, such as food intake, formulation, age and hepatic diseases, on blood concentrations of ranitidine have been described by several researchers (1-3). High performance liquid chromatography (HPLC) method has been extensively used for the analysis of ranitidine from biological fluids (plasma or urine) due to high sensitivity and selectivity (4-9).

The main purpose of this work was to compare the pharmacokinetic parameters of a commercially available preparation (Ranitidine, formulated and manufactured by Kharazmi Pharmaceuticals, Iran) with those of an imported product (Zantac®, manufactured by Glaxo, UK). In the first step, a simple, rapid and sensitive HPLC procedure was developed for the quantification of the drug present in the

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plasma. In the second step, the pharmacokinetic parameters of ranitidine (i.e., C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, elimination rate constant, *k*, and half life) were compared following the administration of 300-mg single oral doses of both brands to each of 14 healthy male volunteers.

Experimental

Materials

Ranitidine tablets and standard powder were supplied by Kharazmi Pharmaceuticals (Iran). Zantac ® tablets and standard metronidazole powder (as internal standard) were kindly donated by Glaxo (UK) and Farchemia (Italy), respectively. HPLC-grade methanol and acetonitrile, and all other analytical grade reagents (ammonium acetate, monobasic potassium phosphate, phosphoric acid and zinc sulfate) were obtained from Merck Company (Germany). Double-distilled, purified and filtered water was used throughout the study.

Methods

Invitro studies

Weight variation, content uniformity, assay and dissolution time tests were all carried out according to USP XXIII procedures (10).

Instruments and chromatographic conditions

A Shimadzu Model LC-6A HPLC system (Japan) equipped with a Rheodyne Model 7125 manual injector valve (with a 20-µl loop), a Shimadzu Model LC-6A solvent delivery pump, and a Shimadzu SPD-6AV, UV-visible detector operating at 320 nm connected to a Shimadzu Model CR6A data integrator were employed. Chromatographic separation was performed on a reversed 3.9 mm× 300 mm i.d. C_{18} - µbondapak (5 µ particle size) column.

The mobile phase, composed of acetonitrile-monobasic potassium phosphate buffer solution with the ratio of 1:9 (pH 3.6, adjusted with phosphoric acid), was prepared and degassed by ultrasonication before use and pumped at a flow rate of 1.1 $ml.min^{-1}$. All experiments were carried out at 37 °C.

Extraction from plasma

Heparinized blood samples from various volunteers were centrifuged and plasma was then collected and frozen at –20 °C. 900 µl of thawed plasma, 50 µl of internal standard (metronidazole) solution (20 $\mu g.ml^{-1}$) and 50 µl deionized, double distilled water were mixed. After the addition of 300 µl HPLC grade methanol and 200 µl zinc sulfate solution (0.7M), the tube was vortex-mixed for 30 seconds and then centrifuged at 3000 rpm for 5 minutes. A 20-µl aliquot of the supernatant was injected into HPLC system and quantification was done by comparing the peak area ratios of ranitidine and internal standard.

Calibration curve for plasma

Thawed and drug-free plasma (900 µl) was pipetted into a disposable test tube and spiked with 50 µl of standard stock solution of ranitidine (with increasing concentration of 2, 6, 10, 15, 20, 30, 40 and 80 $\mu g.ml^{-1}$) and 50 µl of the internal standard solution (20$\mu g.ml^{-1}$). The mixture was assayed as described above. Peak area ratios (ranitidine/internal standard) were measured and plotted versus plasma concentrations, in order to construct the calibration curve for plasma.

In vivo bioequivalence study

A single dose, randomized, two period, two treatment, two sequence, crossover study with equal doses of the test and reference products and a 2-week washout period between the phase I and phase II dosing was designed in this study.

14 healthy male volunteers aged from 23 to 37 years (mean 31 years) and weighed from 50 to 85 kg (mean 64 kg) were selected based on acceptable physical examination, medical history and clinical laboratory test results. They were asked to avoid taking any medication for at least two weeks before the study and until after the study was completed. After an overnight fast, 300 mg ranitidine and Zantac ® (2 ×150-mg tablets) were given orally with 250 ml of tap water to each of the subjects. A standardized meal was served to each of the participating subjects at 3 hrs after dosing.

Venous blood samples (5 ml) were collected pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, and 10 hours post-dose. Blood samples were

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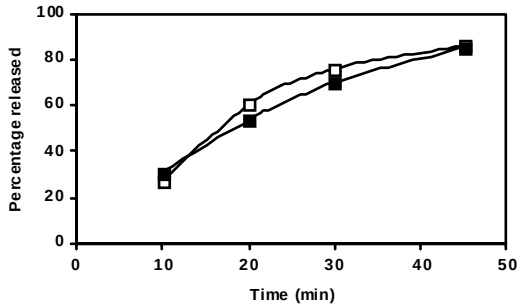

Figure 1. Dissolution rate profile for Zantac® (■) and ranitidine (□) tablets

centrifuged and plasma samples were separated promptly and immediately frozen until assayed. The second phase of the study began following a 2-week washout period. The plasma samples were thawed and then analyzed by a developed HPLC method, as described.

Pharmacokinetic analysis

The maximum plasma concentration and the time to reach the maximum concentration for ranitidine were determined directly from the raw data. The area under the plasma concentration–time curve up to the last available sampling time (AUC_{0-t}) was calculated by the trapezoidal rule. The elimination rate constant (k) was then estimated by the regression analysis of the terminal points on the plasma concentration curve for each subject. Areas under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) were calculated by adding the ratio of the last measurable drug concentration in the decay phase of the curve and the appropriate rate constant to the corresponding AUC_{0-t}. The apparent half-life for the drug in plasma was determined , using $t½ = 0.693/k$ equation. The two one-sided hypotheses at the $\alpha = 0.05$ level of significance was tested for all pharmacokinetic parameters by constructing the 90% , 95% and 99% confidence intervals for the ratio between the test and reference averages (10).

Results and Discussion

Invitro studies

Two tablet formulations were evaluated from the pharmaceutical point of view and the results obtained from content uniformity, weight variation and assay studies indicated

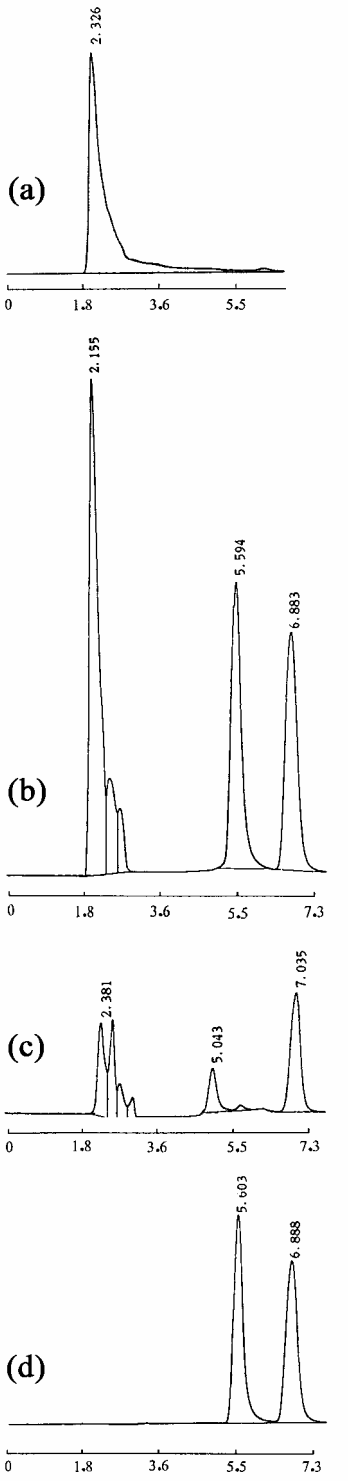

Figure 2. Chromatograms from human plasma: (a) blank plasma; (b) blank plasma spiked with 1.5 $\mu g.ml^{-1}$ ranitidine and 1$\mu g.ml^{-1}$ metronidazole (internal standard); (c) plasma sample taken 2.5 hr after oral administration of 300 Zantac® tablet with 1$\mu g.ml^{-1}$ metronidazole ; (d) ranitidine solution containing 1.5 $\mu g.ml^{-1}$ ranitidine and 1$\mu g.ml^{-1}$ metronidazole.

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Table 1. Inter- and intra-day precisions of ranitidine assay in plasma

Concentration added ($\mu g.ml^{-1}$)	Concentration measured (Mean ± SD)	
	Intra-day (n=3)	Inter-day (n=3)
0.50	0.58 ± 0.021 (5.55)	0.57 ± 0.018 (4.83)
1.00	0.89 ± 0.017 (3.08)	1.01 ± 0.011 (1.80)
2.00	2.18 ± 0.065 (4.82)	2.28 ± 0.025 (1.61)

Values in parentheses are coefficient of variations

that the test and reference products comply with the relevant standards in official compendium. Comparison of the dissolution rates of ranitidine and Zantac ® tablets revealed that the release rate was a little faster for ranitidine, while the percentage release from the two brands was equivalent after 45 min (Figure 1).

Chromatography

Typical chromatograms are illustrated in Figure 2. Under the chromatographic conditions described, ranitidine and internal standard peaks were well resolved and no interfering peak at ranitidine peak position was observed. Chromatographic elution was undertaken for 15 minutes, and the average retention times of ranitidine and internal standard were 5.5 and 6.8 minutes, respectively.

Calibration curve, quantification and detection limits

The calibration curve for the determination of ranitidine in human plasma, obtained by plotting the peak area ratios of ranitidine and internal standard against their corresponding known concentrations, was found to be linear over the range of 0.5 - 4 $\mu g.ml^{-1}$. The corresponding regression equation was $y = 0.5617\ x + 0.0525$ ($r = 0.9904$), where y is the peak area ratio and x is the ranitidine concentration ($\mu g.ml^{-1}$) in plasma. The limit of detection (LOD) and limit of quantification (LOQ) (signal-to-noise ratio greater than 3) were 4 $ng.ml^{-1}$ and 40 $ng.ml^{-1}$, respectively.

Calculation of recovery and precision

Recovery was defined as the peak area of the extracted samples divided by the peak area produced for the equivalent amount of drug injected directly onto the column from aqueous solution. The average recovery was found to be about 109% at different plasma concentrations. The precision of the method was verified by evaluating intra- and inter-day variations (i.e., replicate analysis of plasma-based controls spiked with three known concentrations). The intra- and inter-day precisions of ranitidine assay are shown in Table 1.

Selection of internal standard

The choice of internal standard was based on its separation from ranitidine and endogenous blank plasma peaks and its consistent recovery during extraction procedure.

Table 2. Mean pharmacokinetic parameters for ranitidine and Zantac® tablets in healthy volunteers (n=14) as determined from the plasma concentration data after administration of 300-mg oral dose.

Drug	C_{max} ($\mu g.ml^{-1}$)	T_{max} (hr)	AUC_{0-t} ($\mu g.hr.ml^{-1}$)	$AUC_{0-\infty}$ ($\mu g.hr.ml^{-1}$)	k_{elim} (hr^{-1})	$t_{½}$ (hr)
Ranitidine						
Mean	1.34	3.21	17.81	18.74	0.27	2.70
± SD	0.38	0.91	2.04	2.27	0.01	0.47
± SEM	0.10	0.24	0.55	0.61	0.01	0.13
CV (%)	28.72	28.43	11.45	12.10	19.09	17.44
Zantac®						
Mean	1.21	3.21	17.66	18.45	0.28	2.56
± SD	0.37	1.01	2.13	2.20	0.05	0.44
± SEM	0.10	0.27	0.57	0.59	0.01	0.12
CV (%)	30.18	31.54	12.04	11.92	19.38	17.22

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Stability

Ranitidine was stable in human plasma samples for more than one month, stored at freezer. Spiked plasma samples, extracted following the described procedure and then stored at 4°C, remained stable at least 24 hrs without significant degradation.

Pharmacokinetic data and statistical analysis

Table 2 lists the mean pharmacokinetic parameters for both ranitidine and Zantac ® tablets, determined from the plasma concentration-time data. All subjects showed double peaks following administration of both tablets, except one for which a single peak was observed. However, when the mean concentrations were plotted as a function of time, double peaks disappeared and the curve exhibited one maximum. Figure 3 shows average plasma concentration versus time for ranitidine and Zantac ® tablets in 14 subjects.

Miller (1984) has showed that ranitidine produced a blood concentration curve with a pronounced secondary peak in the drug concentration profile after oral dosing on a fasting stomach (9). He proposed a model to describe this unusual pharmacokinetic behavior. Based on this model, ranitidine accumulates mainly from the systemic circulation into a depot compartment and in response to food intake, a reabsorption phenomenon takes place and the drug accumulated in depot is spontaneously released into the gut (absorption compartment). Alkasi and his coworkers (1989) also reported, in their bioequivalency studies, the appearance of double peaks following administration of ranitidine (1).

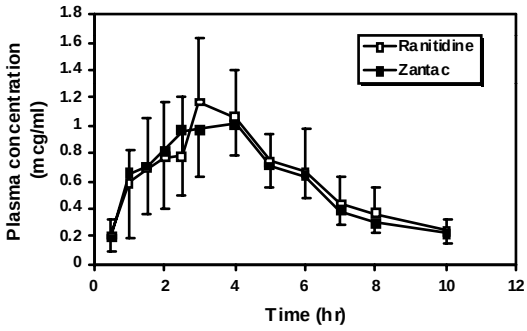

Figure 3. Average plasma concentration versus time plot for Zantac ® (■) and ranitidine (□) tablets in 14 healthy subjects.

The profile of concentration-time curves obtained in this study is in good agreement with those observed by Alkasi and Miller (1,9). It should be noticed that due to the difference between the time of appearance of C_{max} in the subjects, the coefficients of variation have relatively increased, while lower coefficients of variation were calculated for AUC.

The pharmacokinetic parameters utilized for the comparison between the two formulations were C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, elimination rate constant (k) and half life. The AUC_{0-t} after oral administration was calculated by the linear trapezoidal method and the $AUC_{0-\infty}$ was determined using the terminal elimination rate constant calculated by the regression analysis of the last six measurable drug concentrations.

Comparison of the data obtained for the pharmacokinetic parameters between the two formulations revealed that the differences were not statistically significant ($p > 0.05$). It should be mentioned that the highest concentration attained was considered to be the C_{max} for each subject individually, irrespective of the fact that double peak phenomenon was observed for ranitidine.

Table 3. The ratio of product averages at different confidence intervals

Pharmacokinetic parameter	Confidence interval		
	90%	95%	99%
C_{max}	101.12 – 119.49	99.28 – 121.71	95.38 – 125.69
T_{max}	85.11 – 121.93	81.81 – 126.84	75.04 – 138.29
AUC_{0-t}	98.23 – 103.52	97.66 – 104.11	96.44 – 105.44
$AUC_{0-\infty}$	98.40 – 104.68	97.74 – 105.40	96.29 – 106.28
k	87.56 – 102.74	86.03 – 104.57	82.78 – 108.67
$t_{½}$	97.33 – 114.21	95.63 – 116.24	92.02 – 120.80

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In this study, log transformation was employed in the analysis of bioequivalence data in order to achieve the general comparison based on the ratio of the two averages rather than the difference. When the data are analyzed on the log transformed basis, FDA Division of Bioequivalence uses a range of 80% to 125% for the ratio of product averages as the standard equivalence criterion. In this regard, the 90%, 95% and 99% confidence intervals were then calculated for the pharmacokinetic parameters. Table 3 indicates the calculated ranges for the ratios of the product averages at different confidence intervals. As seen, the ratio of averages exists between the standard equivalence criterion of 80% to 125% at 99% (except fot T_{max}) confidence interval. In conclusion, the study indicated that both formulations are comparable, based on the in vitro characterizations, and bioequivalent in terms of C_{max} and AUC. It was also found that any difference between the two formulations was not statistically significant.

Acknowledgement

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