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Comparative Bioavailability of Two Oral Formulations of Ranitidine

Francisco J. Flores-Murrieta[a,b,*], Alejandra Toledo[c], Miriam del Carmen Carrasco-Portugal[a,c], Gerardo Reyes-García[a], Juan Rodríguez-Silverio[a,b], Roberto Medina-Santillán[a] and Jorge E. Herrera[a]

[a] *Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina del Instituto Politécnico Nacional, México, D.F., México*

[b] *Unidad de Investigación, Instituto Nacional de Enfermedades Respiratorias, Secretaría de Salud, México, D.F., México*

[c] *Instituto Nacional de Pediatría, Secretaría de Salud, México, D.F., México*

ABSTRACT: The current requirement of the Mexican Authorities to demonstrate the interchangeability of ranitidine formulations is to establish that the dissolution profile of the drug shows similarity. In order to establish if this requirement is adequate, the bioavailability of two formulations that did not meet this similarity were compared. Twenty-five female volunteers received 150 mg ranitidine (Azantac® or Midaven®) under fasting conditions in two separate sessions using a cross-over design. Plasma samples were obtained at selected times for a period of 12 h and stored frozen at −80°C until analysed. Ranitidine plasma levels were determined and pharmacokinetic parameters were obtained. Values (mean ± SEM) were: C_{max} 528.85 ± 25.34 and 563.03 ± 33.25 ng/ml, t_{max} 2.76 ± 0.19 and 2.79 ± 0.18 h, and $AUC_{12\,h}$ 2694.94 ± 99.50 and 2648.51 ± 133.38 ng.h/ml, for Azantac® or Midaven®, respectively. No statistically significant difference was obtained in the parameters evaluated. Moreover, 90% confidence limits were 96.6%–116.2% and 90.7%–105.1% for C_{max} and $AUC_{12\,h}$ ratios, respectively, indicating that the formulations tested are bioequivalent, despite the dissimilarity in the dissolution profile of the formulations. These results suggest that the comparative dissolution profile is not an adequate test to demonstrate the interchangeability of ranitidine formulations.

Key words: ranitidine; Azantac; Midaven; dissolution profile

Introduction

Ranitidine is an H_2 antagonist that is widely used in the treatment of peptic ulcer and in the control of acid secretion [1,2]. It has been reported that the effect of ranitidine on acid secretion does not show any significant variability, however, inter-individual variability in bioavailability is wide. In addition, it has been reported that a long antisecretory activity of ranitidine is observed, although the short half-life of the drug varies between 2 and 8 h [3]. Additionally, it has been reported that ranitidine concentrations that produce 50% of the maximal effect are about 100 ng/ml and the maximal effect is reached at concentrations of about 600 ng/ml [4].

So far, several generic formulations of ranitidine are sold in Mexico, and the current requirement to demonstrate interchangeability of ranitidine formulations is to compare the dissolution profile of the formulations according to the Mexican Pharmacopeia [5], with a f_2 value of at least 50 [6]. *In vitro* studies on ranitidine dissolution have been reported, and several strategies for detecting differences between formulations have been suggested [7,8]. According

*Correspondence to: Unidad de Investigación, Instituto Nacional de Enfermedades Respiratorias, SSA, Calz. De Tlalpan 4502, Col. Sección XVI, 14080 México, D.F., México.
E-mail: fjfloresmurrieta@prodigy.net.mx; fjfloresmurrieta@yahoo.com.mx

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to the classification of drugs based on permeability and solubility, ranitidine has been classified as a class III drug (highly soluble, low permeability), and, as mentioned above, the current requirement for demonstrating the interchangeability of ranitidine formulations in Mexico is to obtain a similarity factor (f_2) of at least 50 in the comparative dissolution profiles of the reference and test formulations, using the USP apparatus 2 [6]. In order to establish if this approach is adequate, it was decided to compare the bioavailability of two formulations that did not meet the criteria of *in vitro* interchangeability (f_2 higher than 50).

SUBJECTS, MATERIAL AND METHODS

Drugs and reagents

The ranitidine standard was obtained from the United States Pharmacopeia, the nizatidine standard was purchased from Sigma. Midaven® tablets of 150 mg (test formulation) were provided by Merck Mexico, S.A. de C.V. (Mexico City, Mexico). Commercially available Azantac® tablets of 150 mg (reference formulation) were manufactured by Glaxo Smith Kline, S.A. de C.V. (Mexico City, Mexico). Deionized water was prepared using an EasyPure system (Waters Inc., Milford, MA, USA). Acetonitrile of chromatographic grade was obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

Dissolution profile

The ranitidine dissolution profile was evaluated according to the Mexican Pharmacopeia [5], using the USP apparatus 2 (paddle) at 50 rpm using water as a dissolution media. Samples were obtained at 10, 15, 20, 45 and 60 min and the ranitidine concentration was measured using a validated spectrophotometric method at 340 nm.

Subjects

Twenty-five young female subjects weighing (mean $\pm$ SEM) 58.60 $\pm$ 1.22 kg, of 158.96 $\pm$ 0.92 *cm* height and 23.88 $\pm$ 0.66 years old volunteered for this study. Individual demographic data are shown in Table 1. All subjects were fit according to medical history, medical examination and suitable laboratory tests. None of the subjects was an alcohol or drug abuser or was taking any concomitant medication at the time of the study. All volunteers read the protocol that was approved by the research and ethics committees and was carried out following the recommendations of the Declaration of Helsinki, and gave written informed consent before entering the study.

Table 1. Demographic data and sequence of administration of volunteers that were enrolled in the comparison of the bioavailability of two formulations of ranitidine. Formulation A corresponds to Azantac® and formulation B corresponds to Midaven®

				Sequence of administration	
Subject	Age (years)	Weight (kg)	Height (cm)	Session 1	Session 2
1	22	52.5	151	A	B
2	22	52	160	B	A
3	23	59	162	A	B
4	22	63	156	A	B
5	21	66	162	B	A
6	23	70	162	B	A
7	23	54	162	A	B
8	21	54.5	155	A	B
9	27	56	160	B	A
10	24	70	171	B	A
11	20	53	156	B	A
12	32	59	161	A	B
13	21	55	157	A	B
14	26	58	155	A	B
15	23	64.5	158	B	A
16	21	64	157	B	A
17	22	59.5	168	B	A
18	27	66	164	A	B
19	21	48.5	160	A	B
20	33	62	158	A	B
21	26	50	153	B	A
22	24	55	160	A	B
23	24	64	156	B	A
24	27	56	157	A	B
25	22	53.5	153	B	A

Study design

The study was carried out according to a randomized cross-over design. Volunteers arrived at the hospital the night before the drug administration. After an overnight fast, a cannula

was placed in a suitable forearm vein and a blood sample was obtained. Then, subjects received an administration of 150 mg ranitidine (formulation A or B, according to the sequence shown in Table 1), and blood samples were obtained at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after drug administration. Samples were centrifuged to obtain plasma that was stored frozen at −80°C until analysed by a high-performance liquid chromatographic (HPLC) method. After a 1 week washout period, the subjects went back to the hospital for the administration of the second formulation which proceeded in the same form as the first session.

Determination of plasma levels of ranitidine

Plasma ranitidine concentrations were determined by an HPLC method based on the report by Castañeda-Hernández *et al*. [3]. Briefly, 0.5 ml plasma samples were placed in conical glass tubes and 0.1 ml of 2 μg/ml of nizatidine, used as internal standard, was added. The samples were alkalinized by the addition of 0.1 ml of 2.5 M sodium hydroxide and extracted with 3 ml dichloromethane by agitation in a vortex mixer for 1 min. The organic layer was transferred to another conical glass tube and evaporated to dryness in a water bath under a stream of nitrogen. The dry residue was redissolved in 0.2 ml of mobile phase (see below) and 50 μl aliquots were injected into the chromatographic system. Separation of compounds was carried out using a Symmetry C18 column (15 cm length × 3.9 mm i.d.) of 5 μm particle size eluted with a mixture of 0.05 M potassium dihydrogen phosphate solution adjusted to pH 6.5 with sodium hydroxide and acetonitrile (88:12, v/v). The flow rate was kept constant at 1 ml/min and detection of compounds was carried out by absorbance at 313 nm. Under these conditions, the method was linear in the range 20–1000 ng/ml, accuracy was between 95.5% to 109.9% and the coefficient of variation was always lower than 3.5%, indicating that this method is suitable for pharmacokinetic studies of ranitidine.

Pharmacokinetic and statistical analysis

Individual plasma level-time curves were constructed and the maximal concentration (C_{max}) and the time to reach this maximum (t_{max}) were obtained direct from these curves. The area under the plasma levels against the time curve ($AUC_{12\,h}$) was obtained by the trapezoidal rule [9] extrapolation to infinity (AUC_{∞}) was determined by dividing the last concentration by the elimination rate constant [9]. The half-life was obtained by log-linear regression of the terminal decay phase.

Pharmacokinetic parameters (C_{max} and $AUC_{12\,h}$) were log transformed and compared by analysis of variance for a cross-over design. The ratios and 90% confidence limits for both, C_{max} and $AUC_{12\,h}$ with both formulations were calculated and two one-sided *t*-tests [10] were employed to evaluate if the confidence limits were within the acceptance criteria (80%–125%) [11,12]. All pharmacokinetic and statistical analyses were carried out using the WinNonlin Professional software v. 2.1 (Pharsight, Palo Alto, CA, USA).

Results

Figure 1 shows the dissolution profile of the two formulations of ranitidine evaluated. It can be seen that dissolution of Midaven® was faster than that observed with the reference formulation (Azantac®). However, this difference was not reflected in significant differences in the bioavailability of ranitidine, as shown in Figure 2. In fact, this figure shows mean plasma-levels time curves after administration of both formula-

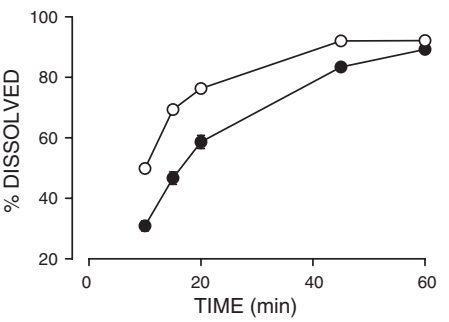

Figure 1. Comparative dissolution profile of ranitidine formulations, Azantac® (black circles) and Midaven® (white circles), using the USP apparatus 2. Each point corresponds to 12 tablets ± SEM

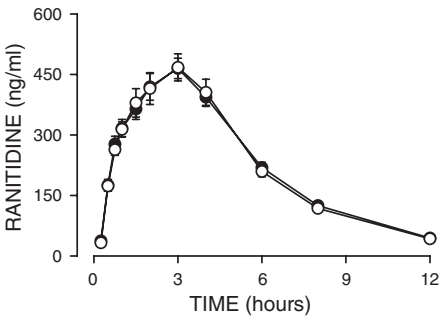

Figure 2. Mean (± SEM) ranitidine plasma levels after administration of 150 mg ranitidine in two oral formulations (Azantac®, black circles, or Midaven®, white circles) to 25 female healthy volunteers

tions. It can be seen that the ranitidine levels increased reaching a maximum of about 550 ng/ml in about 2.5 h. Then, the concentrations decayed with a half-life of about 2.6 h. The pharmacokinetic parameters are shown in Table 2. No statistically significant difference was observed in any parameter. In order to establish whether the formulations tested were bioequivalent, the ratios of C_{max} and of $AUC_{12\,h}$ as well as the 90% confidence limits of these ratios were calculated (Table 3). Then, two one-sided *t*-tests were performed for each parameter and the probability of exceeding the limits of acceptance (80%–125%) are shown in Table 3. It can be seen that the probabilities of exceeding the limits of acceptance were always lower than 0.05, indicating that the formulations tested are bioequivalent.

Table 2. Pharmacokinetic parameters obtained after administration of 150 mg ranitidine in two oral formulations to 25 healthy volunteers. Data are expressed as mean ± SEM. Formulation A corresponds to Azantac® and formulation B corresponds to Midaven®

Parameter	Formulation A	Formulation B
C_{max} (ng/ml)	528.85 ± 25.34	563.03 ± 2.79
t_{max} (h)	2.76 ± 0.194	2.79 ± 0.176
$AUC_{12\,h}$ (ng.h/ml)	2694.94 ± 99.50	2648.51 ± 133.38
AUC_{∞}	2867.31 ± 102.40	2816.20 ± 136.41
$t_{1/2}$ (h)	2.62 ± 0.086	2.66 ± 0.079

Table 3. Ratio and 90% confidence limits of C_{max} and $AUC_{12\,h}$ of the formulations tested, as well as, probability of exceeding the limits of acceptance for bioequivalence

Parameter	Obtained value (confidence limits)	Probability of having outside values
C_{max}	1.05945	<0.80 p = 0.0000
	(0.9657 – 1.1623)	>1.25 p = 0.0152
$AUC_{12\,h}$	0.97589	<0.80 p = 0.0000
	(0.9066 – 1.0505)	>1.25 p = 0.0000

Discussion

A comparison of the bioavailability of two oral formulations of ranitidine was carried out in this study. The formulations showed a dissimilarity in the dissolution profile, since f_2 was lower than 50, the criteria accepted in Mexico for demonstrating interchangeability [5,6], notwithstanding, both meet the Mexican Pharmacopeia criteria for dissolution (80% in 45 min) [5]. However, despite the dissimilarity in the dissolution profile, the formulations tested were bioequivalent, indicating that the dissolution profile seems not to be an adequate method to demonstrate the interchangeability of ranitidine formulations. A waiver has been established for *in vivo* bioavailability and bioequivalence studies for Class I drugs (highly soluble, highly permeable), since according to those characteristics, the rate and extent of drug absorption is unlikely to depend on drug dissolution and/or the gastrointestinal transit time. Recently, it has been suggested that a waiver should also be applied for Class III drugs (highly soluble, low permeability), such as ranitidine [13], since the absorption of Class III drugs is essentially controlled by the gut wall permeability of the drug and not by the drug's solubility. However, some excipients may modify the absorption of this class of drugs [13]. On the other hand, the use of the USP apparatus 2 has been questioned for demonstrating the interchangeability of ranitidine generic formulations, since some formulations that failed to pass the f_2 test are bioequivalent [7]. To solve this problem, it has been suggested that USP apparatus 1 [8] or 3 [7] may be adequate, however, no information on *in vivo–in vitro* correlation studies have been reported.

This study reports that a dissolution profile using the USP apparatus 2 seems not to be

adequate for demonstrating the interchangeability of ranitidine, since formulations that did not meet similarity in dissolution profile are bioequivalent. Based on the results observed in this study, it seems that the dissolution profile for Class III drugs is not an adequate test to demonstrate the interchangeability of generic formulations, since differences in this profile may not be reflected in differences in the bioavailability of the drug. A complete evaluation of *in vitro–in vivo* correlation studies using a different USP dissolution apparatus should provide a basis for an adequate selection of a technique that best predicts the bioavailability of ranitidine.

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References

1. Strum WB. Ranitidine. *JAMA* 1983; **250**: 1894–1896.
2. Zeldis JB, Friedman LS, Isselbacher KJ. Ranitidine: a new H2-receptor antagonist. *N Engl J Med* 1983; **309**: 1368–1373.
3. Castañeda-Hernández G, Flores-Murrieta FJ, Granados-Soto V, *et al*. Pharmacokinetics of oral ranitidine in Mexicans. *Arch Med Res* 1996; **27**: 349–352.
4. Franco C, Flores-Murrieta FJ, Castañeda-Hernández G. Pharmacokinetic-pharmacodynamic modeling of oral ranitidine: implications in efficacy and safety. *Proc West Pharmacol Soc* 1997; **40**: 107–109.
5. *Farmacopea de los Estados Unidos Mexicanos*, 7a Edición, Tomo II. México, 2000; 1743.
6. Norma Oficial Mexicana NOM-177-SSA1-1998. *Diario Oficial de la Federación*. Tomo DXLVIII No. 4. Mayo 7 de 1999; 44–67.
7. Yu LX, Wang JT, Hussain AS. Evaluation of USP apparatus 3 for dissolution testing of immediate-release products. *AAPS Pharm Sci* 2002; **4**: 1–5.
8. Cappola ML. A better dissolution method for ranitidine tablets USP. *Pharm Dev Technol* 2001; **6**: 11–17.
9. Rowland M, Tozer TN. *Clinical Pharmacokinetics. Concepts and Applications*, 3rd edn. Lea & Febiger: Philadelphia, London, 1995.
10. Schuirmann DJ. A comparison of the two one-sided tests procedure, the power approach for assessing the equivalence of average bioavailability. *J Pharmacokinet Biopharm* 1987; **15**: 657–680.
11. Pabst G, Jaeger H. Review of methods and criteria for the evaluation of bioequivalence studies. *Eur J Clin Pharmacol* 1990; **39**: 5–10.
12. Steinijans VW, Hauschke D, Jonkman JHG. Controversies in bioequivalence studies. *Clin Pharmacokinet* 1992; **22**: 247–253.
13. Blume HH, Schug BS. The biopharmaceutics classification system (BCS): class III drugs—better candidates for BA/BE waiver? *Eur J Pharm Sci* 1999; **9**: 117–121.

