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Bioequivalence Study of Ranitidine Tablet

Chang-Koo Shim§, Jae-Sun Hong, Chang-Ki Lee*, Ik-Soo Han* and Kwang-Sik Choi*

*College of Pharmacy, Seoul National University, Seoul 151-742, and *National Institute of Safety Research, Seoul 122-020, Korea*

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Abstract□A bioequivalence study of ranitidine tablets was conducted according to the Korean Guidline for the Bioequivalence Test using twelve healthy male subjects. The plasma concentration-time curves of ranitidine from the test and reference tablets showed profound multiple peak phenomenon in each subject as reported earlier. However, the area under the plasma concentration-time curve (AUC) and the maximum plasma concentration at the first peak (C_{max1}) of the two preparations was proven to be equal when analyzed satistically according to the criteria of the guidline; *i.e.*, statistical power ($1-\beta$) was calculated to be over 0.8 under the condition of $\alpha = 5\%$ and Δ (minimum detectable difference) = 20%, and the confidence interval of the difference in AUC at 95% confidence level was in the range of $\pm 20\%$, which statisfied the criteria of bioequivalence. Equivalence of the peak concentration of ranitidine at the second peak (C_{max2}), and the time to reach the first (T_{max1}) and second (T_{max2}) peaks were not statistically guaranteed in this study. More subjects were needed to verify the bioequivalence of C_{max2}, T_{max1} and T_{max2} between the two tablets. However, we conclude that the test and reference tablets are bioequivalent taking the therapeutic characteristics of the ranitidine preparations into consideration.

Keywords□Ranitidine tablet, bioequivalence test, cross-over design, AUC, multiple plasma peak.

Ranitidine has been used as a potent H_2-receptor antagonist. It was reported to show unusual pharmacokinetic behavior by producing a significant secondary peak in the plasma concentration-time curve after oral administration[1-4]. The first peak occurred at 0.5 to 2.5 h and the second peak at 3 to 6 h after dosing ranitidine tablet to Korean male subjects[5]. There were great variations in the plasma concentration-time profiles among subjects. However, the intrasubject variation of ranitidine pharmacokinetics was small over one week under the controlled conditions in spite of its great intersubject variation[5].

The great intersubject variation in ranitidine pharmacokinetics might evoke the bioequivalence problem of the ranitidine preparations. In this study, bioequivalence of the ranitidine tablet was tested using twelve healthy Korean male subjects.

EXPERIMENTS

Drugs

Curan tablet (Ildong Pharm. Co. Korea) was used as a test drug and Zantac (Wyeth) was used as a reference drug. Each tablet contained ranitidine chloride equivalent to 150 mg of its base.

Experimental design and protocol

The study was carried out employing a two-way cross-over design of twelve healthy male subjects (Table I) using a 2×2 Latin square design (Table II). The tablets (Test and Reference tablets) were administered over a period of one week. All subjects were fasted from 9 p.m. after standard meal on the night before the experiment to avoid the effect of food[6,7]. Tablets were administered between 8 and 9 a.m. on the next day with 200 m*l* of water. No food or drink other than water was permitted until 4 h after dosing, and no sleeping was allowed during the experiment. Bread (200g), milk (180 m*l*) and water (*ad libitum*) were allowed 4 h after drug administration. Venous blood samples (5 m*l*) were withdrawn from a forearm on heparin (25-unit vacuum tube) through an indwelling butterfly needle (18 or 21 G), immediately before and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 h after dosing. After centrifugation, the plasma samples were transferred to sterilized vials and frozen at

§To whom correspondence should be addressed.

Table I. Profile of the subjects

Subject No.	Age (year)	Height (cm)	Weight (kg)
1	34	168	67
2	40	168	70
3	39	180	71
4	27	170	60
5	27	173	77
6	31	177	72
7	29	170	67
8	34	176	67
9	34	169	70
10	29	175	77
11	27	170	61
12	23	159	50
Mean	31.2	171.3	67.4
SD	4.9	5.2	7.3

Table II. Dosing schedule to 12 volunteers

Group	Subject	Period	
		I	II
1	1 – 6	Test	Reference
2	7 – 12	Reference	Test

– 20°C until analysis.

High-performance liquid chromatographic assay of ranitidine in plasma

Ranitidine hydrochloride was measured using a liquid chromatographic method described by Carey and Martin[8] after minor modification. To 0.5 m*l* plasma were added 0.5 m*l* of 0.1 M disodium hydrogen phosphate (pH 9.30) and 3 m*l* of *n*-octanol. After vortexing for 3 min and centrifusing for 10 min (6000 × g), 2.6 m*l* of the upper octanol layer was transferred to another tube and 250 μl of 0.0004% (w/v) procaine hydrochloride, an internal standard, in 0.1 M phosphate buffer (pH 6.0) was added. After vortexing for another 3 min and centrifusing for 10 min (6000 × g), the octanol layer was discarded by aspiration. Of the aqueous phase, 100 μl was injected onto the HPLC column (30 × 0.39 cm i.d., stainless steel; Waters, P/N 27324), containing 10μ μ-Bondapak C18 reversed-phase material. All tubes were used after silanization with dichlorodemethylsilane. The pump and variable detector were also from Waters (model 510 and 481, respectively). The peak area ratio of ranitidine against internal standard at 313 nm was calculated by a Spectra-Physics automatic intergrator (model 4290). The mobile phase was a 60:40 mixture of 0.05 M phosphate buffer (pH 7.0) and methanol. The flow rate was 1.0 m*l*/min and the mean operating pressure was 1800 psi. A standard curve was run with each set of determinations and prepared by adding known amounts of ranitidine hydrochloride to plasma. Linearity of the standard curves was found in the range from 5 to 800 ng/m*l*.

Bioavailability parameters

The area under the plasma ranitidine concentration-time curve from time zero to 12 h ($AUC_{0\text{-}12}$) was calculated by the trapezoidal rule. The area from time 12 h to infinity was estimated by C_{12}/k, where C_{12} is the ranitidine concentration at 12 h and k is the apparent elimination rate constant of ranitidine obtained from the slope of log-linear portion of the curve by least square regression analysis. Moreover, the area from time to infinity, $AUC_{0\text{-}\infty}$ was calculated by Eq. 1[9];

$$AUC_{0-\infty} = AUC_{0-12} + C_{12}/k \qquad \text{(Eq. 1)}$$

Maximum plasma concentration (C_{max}) and time maximum concentration (T_{max}) were directly read from the plasma concentration-time curve.

Statistical analysis

Analysis of variance (ANOVA) for a cross-over design was carried out for the pharmacokinetic parameters in order to determine the sources of variation[10, 11]. For hypothesis testing, statistical power (1- β), minimum detectable difference (Δ) and the confidence intervals were estimated to evaluate the accuracy of this bioequivalence study[10-13].

RESULTS

Plasma concentration of ranitidine

Plasma concentrations of ranitidine after oral administration of the test and reference tablets to twelve subjectsd are shown in Fig. 1. There was a great variation in the plasma concentration-time profile among subjects as previously reported[1-5]. There were two or more distinct peaks in all the curves of the subjects and distinct double peaks were shown in the average plasma concentration-time cruves. This phenomenon is much more profound than ever reported[1, 4], and not consistent with some previous reports[7, 14-17]. Inter- and intrasubject variations of ranitidine pharmacokinetics were recently reported by Shim and Hong[5].

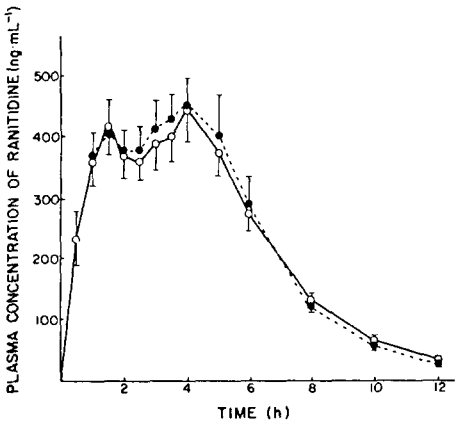

Fig. 1. Average plasma concentration-time profiles of ranitidine from 12 subjects.
Each data point represents the mean (±SE) plasma concentration of the test (○) and reference (●) tablets.

Table III. *AUC* (ng·hr/ml) data for the test and reference tablets in twelve subjects

Group	Subject	Period I	Period II	Sum
I	1	1947	1968	3915
	2	3189	3217	6406
	3	2565	2982	5547
	4	2242	2727	4969
	5	2807	2186	4993
	6	5672	4465	10137
	Total	18422	17545	35967
II	7	3717	3485	7198
	8	4030	3172	7202
	9	2374	2913	5287
	10	2016	1905	3921
	11	2053	2632	4685
	12	2197	2491	4688
	Total	16383	16598	32981
Sum	Period	34805	34143	68948
	Drug	35020	33928	

Mean *AUC* of reference tablet: 2918.3, Mean *AUC* of the test tablet: 2827.3. Difference in *AUC* between the two tablets against the reference tablet: 3.1%.

The concentration curves of the two tablets were very similar in pattern and they were almost superimposable. The Student's *t*-test did not show any significant differences between concentrations of the two tablets at each sampling time-points. No significant differences were found between the two tablets for the maximum plasma concentrations at the first (C_{max1}) and the second (C_{max2}) peaks, and the times to reach the first (T_{max1}) and second (T_{max2}) peaks.

Table IV. ANOVA table for AUC

Source of variation	*DF*	*SS*	*MS*	*F*
Between Subjects	11	17146990	1558818	8.589
Group or Sequence	1	371504	371504	0.221
Subject/Group	10	16775490	1677549	9.244
Time Periods	1	18272	18272	0.101
Drugs	1	49664	49664	0.273
Residual	10	1814816	181481.6	
Total	23	19029740		

$F(1, 10) = 4.96$, $F(10, 10) = 2.98$. *DF*: degree of freedom, *SS*: sum of squares, *MS*: mean squares, *F*: variance.

Table V. Symmetrical confidence intervals for *AUC* data

α (%)	1-β (%)	Δ (%)	n	Confidence intervals of δ
5	85	20	12	
	80	18.6	12	$-10.2\% < \delta < 16.4\%$
	80	20	10.9	
10	95	20	12	
	80	13.5	12	$-7.6\% < \delta < 13.9\%$
	80	20	8.5	

Calculated according to Westlake[11]. α : probability of Type-I error, β: probability of Type-II error, Δ: minimum detectable difference, n: total number of subjects, δ: difference of bioavailability between two tablets.

Analysis of variance (ANOVA) of the data

Table III shows *AUC* data of the twelve subjects. There were no significant differences between the two tablets. Table IV shows the result of ANOVA for *AUC* data in Table III. Significant differences ($p < 0.05$) in the *F* ratio of *Between Subjects* and *Subject/Group* were observed by ANOVA for a cross-over design. It shows that there was a significant intersubject variation in *AUS* values. However, there were no significnat differences in the *F* ratios of *Group or Sequence*, *Time Periods* and *Drugs*. No significant difference in *Group or Sequence* confirms the cross-over study was properly done.

Table V shows the accuracy of the experiment for the *AUC* data. Under the condition of $\alpha = 5\%$, 1-$\beta = 80\%$ and $n = 12$, Δ for *AUC* was calculated to be 18.5%. Under the condition of $\alpha = 5\%$, $\Delta = 20\%$

Table VI. C_{max1} (μg/ml) **data of the test and reference tablets in twelve subjects**

Group	Subject	Period I	Period II	Sum
I	1	527.6	489.0	1016.6
	2	584.6	634.9	1219.5
	3	445.3	397.0	842.3
	4	446.6	470.3	916.9
	5	293.1	260.1	553.2
	6	758.6	620.8	1379.4
	Total	3055.8	2872.1	5927.9
II	7	448.1	402.2	850.3
	8	651.0	447.2	1098.0
	9	472.9	543.5	1016.4
	10	380.4	364.9	745.3
	11	492.4	650.0	1142.4
	12	331.8	286.0	617.8
	Total	2776.6	2693.6	5470.2
Sum	Period	5832.4	5565.7	11398.1
	Drug	5749.4	5648.7	

Mean C_{max1} for the reference tablet: 2874.7, Mean C_{max2} for the test tablet: 2824.4, Difference in C_{max1} between the two tablets against the reference tablet: 1.8%.

Table VII. ANOVA table for C_{max1}

Source of variation	*DF*	*SS*	*MS*	*F*
Between Subjects	11	328563.0	29869.4	6.21
Group or Sequence	1	8729.5	8729.5	0.27
Subject/Group	10	319833.5	31983.4	6.66
Time Periods	1	2964.0	2964.0	0.61
Drugs	1	423.5	423.5	0.08
Residual	10	48050.0	4805.0	
Total	23	380000.5		

Table VIII. C_{max2} (μg/ml) **data of the test and reference tablets in twelve subjects**

Group	Subject	Period I	Period II	Sum
I	1	237.7	246.7	484.4
	2	679.4	617.5	1296.9
	3	485.9	329.2	815.1
	4	305.2	596.6	901.8
	5	356.4	322.2	678.6
	6	1003.6	840.7	1844.3
	Total	3068.2	2952.9	6021.1
II	7	668.4	759.2	1427.6
	8	629.9	678.0	1307.9
	9	365.8	434.3	800.1
	10	285.3	323.1	608.4
	11	360.6	471.6	832.2
	12	385.8	563.5	949.3
	Total	2695.8	3229.7	5925.5
Sum	Period	5764.0	6182.6	11946.6
	Drug	6297.9	5648.7	

Mean C_{max2} of the reference tablet: 3149.0, Mean C_{max2} of the test tablet: 2824.4, Difference in C_{max2} between the two tablets against the reference tablet: 10.3%.

Table IX. ANOVA table for C_{max2}

Source of variation	*DF*	*SS*	*MS*	*F*
Between Subjects	11	857704.5	77973.1	10.3
Group or Sequence	1	380.5	380.5	0.0
Subject/Group	10	857324.0	85732.4	11.3
Time Periods	1	7300.5	7300.5	1.0
Drugs	1	17560.6	17550.5	2.3
Residual	10	75971.0	7597.1	
Total	23	958536.5		

and n = 12, 1- β for *AUC* was calculated to be 0.85. With 95% confidence, the confidence interval of the difference in bioavailability between the two tablets was −10.2% to 16.4%, which satisfied the criteria (±20%) for bioequivalence[18]. From these results, *AUC*, which indicates the total amount absorbed, was virtually proven to be equal for the two tablets.

Table IV and VIII show the maximum concentration data of the first (C_{max1}) and second (C_{max2}) plasma peaks, the Table VII and IX show the ANOVA tables for them. Table X-XIII show the data of the time to reach the first (T_{max1}) and the second (T_{max2}) peaks and their ANOVA tables.

From Table VI and VII, it was found that crossover study was properly done for C_{max1} since *F* ratios *Group or Sequence, Time Periods* and *Drugs* were smaller than *F* values from *F*-table. However, *F* ratios of *Between Subjects* and *Subject/Group* were larger than those from *F*-table. It may be due to significant intersubject variations in C_{max1} values. Similar conclusions can be extracted from Table VIII and IX for C_{max2}, and Table X and XI for T_{max1}. However,

Table X. T_{max1} (hr) data for the test and reference tablets in twelve subjects

Group	Subject	Period I	Period II	Sum
I	1	1.5	1.5	3.0
	2	1.5	1.5	3.0
	3	1.5	1.5	3.0
	4	0.5	0.5	1.0
	5	0.5	0.5	1.0
	6	2.5	2.0	4.5
	Total	8.0	7.5	15.5
II	7	1.0	1.5	2.5
	8	1.5	1.0	2.5
	9	1.0	1.0	2.0
	10	2.0	2.0	4.0
	11	0.5	1.0	1.5
	12	2.5	1.5	4.0
	Total	8.5	8.0	16.5
Sum	Period	16.5	15.5	32.0
	Drug	16.0	16.0	

Mean T_{max1} of the reference tablet: 16.0, Mean T_{max1} of the reference tablet: 16.0, Difference in T_{max1} between the two tablets against the reference tablet: 0%.

Table XI. ANOVA table for T_{max1}

Source of variation	*DF*	*SS*	*MS*	*F*
Between Subjects	11	7.33	0.67	7.0
Group or Sequence	1	0.04	0.04	0.1
Subject/Group	10	7.29	0.73	7.6
Time Periods	1	0.04	0.04	0.4
Drugs	1	0.00	0.00	0.0
Residual	10	0.96	0.10	
Total	23	8.33		

Table XII. T_{max2} (hr) data for the test and reference tablets in twelve subjects

Group	Subject	Period I	Period II	Sum
I	1	5.0	4.0	9.0
	2	3.0	3.0	6.0
	3	3.0	3.0	6.0
	4	2.5	3.0	5.5
	5	3.0	4.0	7.0
	6	5.0	4.0	9.0
	Total	21.5	21.0	42.5
II	7	4.0	4.0	8.0
	8	4.0	3.5	7.5
	9	3.5	6.0	9.5
	10	3.0	3.5	6.5
	11	4.0	4.0	8.0
	12	4.0	3.0	7.0
	Total	22.5	24.0	46.5
Sum	Period	44.0	45.0	89.0
	Drug	45.5	43.5	

Mean T_{max2} of the reference tablet: 43.5, Mean T_{max2} of the test tablet: 45.5, Difference in T_{max2} between the two tablets against the reference tablet: 4.4%.

Table XIII. ANOVA table for T_{max2}

Source of Variation	*DF*	*SS*	*MS*	*F*
Between Subjects	11	9.46	0.86	1.6
Group or Sequence	1	0.67	0.67	0.8
Subject/Group	10	8.79	0.88	1.7
Time Periods	1	0.04	0.04	0.1
Drugs	1	0.17	0.17	0.3
Residual	10	5.29	0.53	
Total	23	14.96		

Table XII and XIII for T_{max2} show that all the F ratios in ANOVA table (Table XIII) are smaller than those values in F-table. It implies that there was no intersubject variation in T_{max2} and cross-over design was properly done for T_{max2}. No intersubject variation in T_{max2} might be due to long-term scale sampling of the plasma in part.

Table XIV shows the symmetrical confidence intervals for C_{max1}, C_{max2}, T_{max1} and T_{max2}. The number of subjects (n) needed to confirm the bioequivalence was calculated from the following equation.

Table XIV. Symmetrical confidence intervals for C_{max1}, C_{max2}, T_{max1} and T_{max2}

Parameters	Δ(%)	Confidence intervals of δ	n
C_{max1}	18.1	$-11.4\% < \delta < 14.9\%$	12
C_{max2}	23.0	$-4.8\% < \delta < 25.4\%$	14
T_{max1}	28.6	$-21.1\% < \delta < 21.1\%$	24
T_{max2}	25.2	$-13.1\% < \delta < 21.8\%$	18

Δ and δ were calculated at $\alpha = 5\%$, $1-\beta = 80\%$ and confidence level = 95%.

$$\lambda\{\alpha,\ 1-\beta,\ 2(n-1)\} = \sqrt{n}\delta^*/S \qquad \text{(Eq. 2)}$$

where λ means the noncentrality of the data obtained from the Noncentrality table. S means the route values of the residuals. α , $1-\beta$, n and δ * are probability of Type-I error (5%), power to detect 20% difference of the means between two tablets at α , number of total subjects needed to confirm the bioequivalence, and the difference values of each parameters to be detected (20% of the mean value of the reference tablet) respectively.

From the relation in Eq. 2, it was calculated that 12 subjects are needed to prove the bioequivalence of the tablets for AUC and C_{max1}. However 14, 24 and 18 subjects were needed for C_{max2}, T_{max1} and T_{max2} (Table XIV).

Considering the very small difference of C_{max1} (1.8%) between test and reference tablets (Table VI) together with Δ and δ (Table XIV), it was concluded that C_{max1} of the two tablets is bioequivalent when tested with twelve subjects. There were very small differences between test and reference tablets in C_{max2} (10.3%), T_{max1} (0.0%) and T_{max2} (4.4%) which were in the range of bioequivalence criteria of Korea[18]. However, Δ and δ of them were a little larger than the criteria (20% for Δ and $\pm 20\%$ for δ) for the bioequivalence with the twelve subjects used in this study (Table XIV).

Equivalence of C_{max2}, T_{max1} and T_{max2} were not statistically guaranteed in this study. However, we conclude that test tablet and reference tablet are bioequivalent, taking the following characteristics of ranitidine into consideration; (1) rapid onset of the effect is not required, (2) C_{max2}, T_{max1} and T_{max2} do not seem to influence the effectiveness of the drug during a long-term treatment by the usual administration of twice a day.

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