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EFFICACY OF URINE SAMPLES IN BIOAVAILABILITY STUDY OF RANITIDINE

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ABSTRACT

Urinary excretion of ranitidine is known to be almost 70% of the intact drug , therefore this drug would be a good candidate for bioavailability studies using urine samples. In this study the bioequivalency of two marketed formulations using both urine and plasma samples were investigated. 'Ranitidine' 150 mg tablets (generic) and 'Zantac' 150 mg tablets were compared in a double blind crossover study using eight healthy male volunteers. A simple and rapid HPLC method was also developed to analyze the drug concentration in both urine and plasma. Double peak phenomenon, observed in plasma samples, was omitted when the urine samples were used. Bioavailability of the two formulations calculated from urinary data were not significantly different, whereas the plasma data were considerably different (based on C_{max} & T_{max} but not AUC). Pharmacokinetic parameters resulted from urine regarding the rate of the absorption ($T_{max\text{-}ud}$, $(dD_u/dt)_{max}$, $K_{a\text{-}ud}$) did not correlate well with their respective plasma parameters (T_{max}, C_{max}, K_a), whereas those of absorption extent and elimination rates (plasma AUC, K and urinary D_u^{∞}, K) were well correlated. It is concluded that the urine sampling which has advantages of easy sample collection and extraction could be used for determination of the extent of absorption and rate of the elimination of ranitidine, since similar parameters can be obtained with easier sample collection and extraction, whereas for determination of absorption rate, C_{max} & T_{max} plasma data are preferred.

Key words: Ranitidine, Bioavailability, HPLC, Plasma, Urine.

INTRODUCTION

Ranitidine is a specific H_2-receptor antagonist used to treat peptic and duodenal ulcers (1). There are many reports studying the bioavailability of ranitidine using serum or plasma data (2-6), but using urine samples in bioavailability studies is not reported. As urinary excretion of ranitidine is around 70% of the intact drug (1,7), it could be a good candidate for bioavailability studies by urine samples. For this purpose, a rapid, and sensitive HPLC method to evaluate the bioavailability of this drug in healthy subjects using both plasma and urine samples was developed. Correlation between urine and plasma samples and thereafter the efficacy of urinary data in bioequivalency studies were investigated.

MATERIALS AND METHODS

Chemicals

Zantac 150 mg tablets (Glaxo Canada Inc.) and Ranitidine 150 mg tablets (Arya Pharmaceutical Co., Iran) were obtained from the regular commercial sources. Ranitidine reference standard was donated by Arya Pharmaceutical Co. (Tehran, Iran). [5-Nitro-(2-amino-1,3,4 thiadiazole) imidazole] (8) was used as internal standard. HPLC grade methanol and acetonitrile (Merck, Germany) were used for high-pressure liquid chromatography analyses. Other chemi-cals used in the study were of analytical grades.

Subjects and Sampling

The study was approved by the local ethics committee. Eight healthy male volunteers were selected for participation in the study (age: 26-30 years., weight: 57-80 kg). Subjects with pyloric or abnormalities of the renal, hepatic, cardiovascular, endocrine, respiratory or hematopoietic systems and also subjects requiring regular medication during the week prior to the trial and those taking any prescription or non-prescription drugs within 24 hours preceding the beginning of the study were excluded from the study. Absence of abnormalities was determined by medical history, physical examination, and the proper clinical laboratory tests. All study volunteers provided written informed consent.

The experiment was designed as a double blind crossover study, in which each subject received a single oral dose of each medication with an interval of at least 3 weeks. All subjects were required to fast overnight before administration to

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each study treatment, and food and drink which offered on each study day were identical for all subjects and dosing sessions. Prior to the drug administration a 10 mL urine sample was taken for use as a control sample. The urine samples were collected during the periods 0-2, 2-4, 4-6, 6-8, 8-10 and 10-24 hours after dosing.

Blood samples were collected in heparinized tubes just prior to dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 5, 6.5, 8, 10 and 12 hours after drug administration. Plasma was immediately separated from the blood samples. All samples were kept frozen (-20 °C) until analysis.

Preparation of standard solutions

A stock solution containing either 1 mg/mL ranitidine or internal standard was prepared in methanol. The working standard solutions of ranitidine were prepared by dilution of the stock solutions up to 10 µg/mL and 250 µg/mL with deionized water for plasma and urine samples, respectively.

Sample extraction procedure and chromatographic conditions were according to Ahmadiani et al (9) with few modifications as follows;

Sample extraction procedure

Plasma Samples: To 500 µL plasma in a 1.5 mL plastic tube (Eppendorf, Germany) were added 10 µL internal standard (40 ng/mL) and 100 µL trichloroacetic acid. The tube was mixed for 1 minute on a vortex mixer and centrifuged for 5 minutes (8000 rpm). The supernatant was filtered through a 0.22 µm membrane filter (Millipore Assoc., USA) and 25 µL of the solution was injected to the column.

Urine samples: To 20 µL of urine were added 40 µL of the internal standard (40 ng/mL) and 940 µL deionized water. The tube was mixed on a vortex mixer for one minute and filtered through a 0.22 µm filter (Millipore Assoc., USA). Twenty five µL of the filtrate was injected to the column.

Apparatus and chromatographic conditions

The HPLC column was NovaPak C_{18} (4 µm particle size, 15 cm x 3.9 mm I.D.). Mobile phase consisted of the phosphate buffer (0.1 M, pH=5.5), sodium lauryl ether sulphate (2 mM), methanol, acetonitrile (30:30:20:19.6). A flow rate of 0.6 mL/min was maintained by the use of a Waters-501 solvent delivery system (Waters Assoc., USA). The column effluent was monitored by a Lambda-Max-481 UV variable wavelength detector (Waters Assoc., USA) set at 320nm to a minimum of 20 ng/mL. The retention times were found 4 and 5 minutes for internal standard and ranitidine respectively.

Pharmacokinetic analysis

Plasma data: K (elimination rate constant) was calculated from the slope of elimination points on straight line obtained from terminal phase of the log plasma concentration versus time curve (last four points). K_a (absorption rate constant) was calculated from the plasma data using residual method. Half life ($t_{1/2}$) was calculated from Eq. (1):

$$t_{1/2} = \ln(2) / K \qquad (1)$$

T_{max1}, T_{max2} (observed first and second peak T_{max}), C_{max1} and C_{max2} (observed first and second peak C_{max}) were obtained from the experimental data.The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule to 12h and then extrapolated to infinity using the terminal rate constant value (10, 11)

Urine data: K_{-ud} was defined as the elimination rate constant calculated from the slope of elimination straight line which was obtained from terminal phase of $\log(D_u^{\infty}-D_u)$ vs. time curve (last four points of urine data). K_{a-ud} was defined as the absorption rate constant calculated from urinary data (Eq. 2):

$$D_u^{\infty}-D_u = D_u^{\infty} / (K_{a-ud}-K_u).(K_a.e^{-K.t} - K.e^{-Ka.t}) \qquad (2)$$

The intercept of the above equation was equal to $D_u^{\infty}/K_{a-ud}-K_{-ud}$ by means of which K_{a-ud} was calculated. $(dD_u/dt)_{max-o}$ was defined as the observed maximum urinary excretion rate or the urinary excretion rate (dD_u/dt) versus mid point time. $(dD_u/dt)_{max-c}$ was defined as the calculated maximum urinary excretion rate (Eq. 3):

1: $D_u^{\infty} = F.K_e D_o / K$

2: $dD_u/dt = K_e K_a F D_o / K_a - K(e^{-K.t} - e^{-Ka.t})$

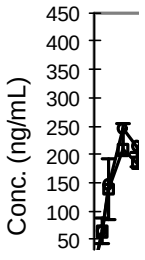
1&2 =>

$$(dD_u/dt)_{max-c} = D_u^{\infty}.K_{a-ud}.K_{-ud} / (K_{a-ud}-K_{-ud}) . (e^{-\mathbf{K}\text{-ud}.\mathbf{tmax}\text{-ud-c}} - e^{-\mathbf{K}a\text{-ud}.\mathbf{tmax}\text{-ud-c}}) \qquad (3)$$

T_{max-ud} was defined as time of peak urinary excretion observed on the dD_u/dt versus mid point time (urinary data). D_u^{∞} was defined as the cumulative urinary excretion up to 24 hours. $t_{1/2-ud}$ was defined as half-life of the drug calculated from urinary data (Eq. 4):

$$t_{1/2-ud} = \ln(2) / K_{-ud} \qquad (4)$$

$Cl_{(renal)}$ was defined as renal clearance of the drug calculated from the slope of dD_u/dt versus plasma



concentration. (12)

The F ratios defined as the pharmacokinetic parameter of the test (Ranitidine) were calculated by dividing to the same parameter in the reference (Zantac).The parameters included: $(dD_u/dt)_{max\text{-}o}$, D_u^{∞}, $K_{a\text{-}ud}$, $K_{\text{-}ud}$, $T_{max\text{-}ud}$ of urine (F_{1U} to F_{5U}) and C_{max}, AUC, K_a, K, T_{max} of plasma (F_{1P} to F_{5P}).

The derived parameters were subjected to paired *t*-test and 90% confidence interval to evaluate the significance of the differences. p-value of less than 0.05 and *F* values of less than 80% and/or more than 120% of the relative means were considered significant (13).

RESULTS AND DISCUSSION

Bioequivalency study

Plasma levels of ranitidine at each time point achieved with ranitidine and zantac tablets are plotted in figure 1. Urinary excretion rate of ranitidine at each mid point ($(t_n+t_{n+1})/2$) achieved with 150 mg ranitidine tablets and 150 mg zantac tablets are plotted in figure 2. Pharmacokinetic parameters were calculated using Drug-knt software (14). The examined urinary and plasma parameters are summarized in tables 2 and 3. The *F* values are illustrated in table 4.

The resulting pharmacokinetic parameters were in a good agreement with the previous reports (1-7). A double peak phenomenon that could be due to enterohepatic recycling (6, 15) was ob-served in both formulations. The first and the second peaks were observed around 1 h and 4 h after drug administration respectively. The first peak had a plasma concentration of about 60% that of the second one. Therefore the second peak was considered as the overall T_{max} and C_{max} in the bioequivalency study.

There were no significant differences in AUC's for both formulations. Although C_{max} and T_{max} values in two groups based on *t*-test were not significantly different, the plasma 90% confidence intervals obtained for C_{max} and T_{max} (test to reference) ratios ($F1_P$ & $F5_P$) did not lie within the 80-120% of their respective mean values. The results of plasma data showed that the two formulations were not bioequivalent (based on C_{max} & T_{max}).

Figure 1- Ranitidine plasma concentration-time profile after administration of one single oral dose of test and reference products to eight healthy subjects. (mean±SD)

It is known that urinary D_u^{∞}, $(dD_u/dt)_{max\text{-}o}$, $T_{max\text{-}ud}$, $K_{a\text{-}ud}$, $K_{\text{-}ud}$ correspond to AUC, C_{max}, T_{max}, K_a, K of plasma, respectively (11). No significant difference in two formulations were observed based on urinary parameters (D_u^{∞}, $(dD_u/dt)_{max\text{-}o}$ & $T_{max\text{-}ud}$), corresponding to plasma data. These observations may be indicative of inadequacy of the urine samples in the absorption phase.

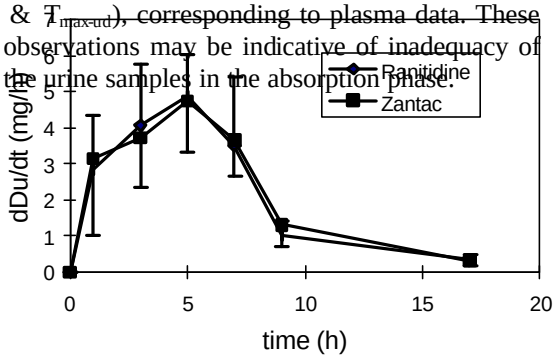


Figure 2 Urinary excretion rate profile after administration of one single oral dose of test and reference products to eight healthy subjects. (mean±SD)

Plasma and urine relationship

For further investigation of adequacy of urinary samples in bioavailability of ranitidine, *F* value were also calculated for the following parameters: $K_{a\text{-}ud}$, $K_{\text{-}ud}$ of urine (F_{3U}, F_{4U}) and K_a, K of plasma (F_{3P}, F_{4P}). Although $K_{a\text{-}ud}$, $K_{\text{-}ud}$, K_a and K of the two formulations based on *t*-test were not statistically different. *F* values,

Table 1. Analysis reproducibility of plasma and urinary samples (n=4).

	Concn. (ng/mL)	*Within day CV%*	*Day to day CV%*
Plasma	100	2.27	1.70
	200	1.50	3.00
	750	0.75	3.40

	Mean	1.51	2.70
	SD	0.76	0.89
Urine	400	0.95	1.90
	800	0.52	2.12
	1500	1.70	0.93
	Mean	1.06	1.65
	SD	0.60	0.63

Table 2. Pharmacokinetic parameters obtained from plasma data after administration of two formulations. (mean±SD, n=8)

	K_a (/h)	K (/h)	$T_{1/2}$ (h)	$AUC_{(0-12)}$ (ng.h/mL)	$AUC_{(0-\infty)}$ (ng.h/mL)	T_{max1} (h)	T_{max2} (h)	C_{max1} (ng/mL)	C_{max2} (ng/mL)
Test	1.7 ±0.5	0.2 ±0.02	4.7 ±0.1	1837.7 ±185.1	1864.0 ±187.0	1.0 ±0.0	3.0 ±0.8	244.9 ±45.1	392.2 ±75.1
Ref	2.7 ±1.1	0.2 ±0.03	4.7 ±0.3	1841.6 ±216.9	1868.4 ±217.0	1.1 ±0.3	3.8 ±0.5	212.2 ±42.2	326.2 ±82.6
p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S. Non-significant p value (in paired t-test)
T_{max1} Time to reach first peak plasma concentration (observed)
T_{max2} Time to reach second peak plasma concentration (observed)
C_{max1} Maximum plasma concentration of the first peak (observed)
C_{max2} Maximum plasma concentration of the second peak (observed)

Table 3. Pharmacokinetic parameters obtained from urinary data after administration of the two formulations. (mean±SD, n=8)

	$(dD_u/dt)_{max-o}$ (mg/h)	$(dD_u/dt)_{max-c}$ (mg/h)	D_u^{∞} (mg)	K_{a-ud} (/h)	K_{-ud} (/h)	$T_{1/2-ud}$ (h)	$Cl_{(renal)}$ (mL/min/Kg)	T_{max-ud} (h)
Test	5.8 ±0.8	5.6 ±1.2	36.9 ±3.7	0.7 ±0.1	0.3 ±0.1	2.5 ±0.5	6.6 ±1.2	4.0 ±1.9
Ref	5.6 ±1.1	5.6 ±1.2	39.2 ±5.7	0.6 ±0.1	0.3 ±0.1	2.4 ±0.4	6.0 ±0.7	3.8 ±2.1
p-val.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S. Non significant p value (in paired t-test)
$(dD_u/dt)_{max-o}$ Maximum urinary excretion rate (observed)
$(dD_u/dt)_{max-c}$ Maximum urinary excretion rate (calculated)
D_u^{∞} Cumulative urinary excretion
K_{a-ud} Absorption rate constant obtained from urinary data
K_{-ud} Elimination rate constant obtained from urinary data
$T_{1/2-ud}$ Elimination half-life obtained from urinary data
$Cl_{(renal)}$ Renal clearance
T_{max-ud} Time to reach maximum urinary excretion rate (observed)

Table 4. Ratio of different parameters (F values) calculated from pharmacokinetic parameters. (mean±SD)

Factor:	$F1_U$	$F2_U$	$F3_U$	$F4_U$	$F5_U$	$F1_P$	$F2_P$	$F3_P$	$F4_P$	$F5_P$
Mean	1.08	1.00	1.06	0.97	1.00	1.25	1.01	0.71	1.04	0.79
SD	0.24	0.13	0.62	0.20	0.26	0.32	0.17	0.36	0.25	0.14
95% Confidence Interval	0.94 - 1.18	0.93 - 1.07	0.69 - 1.42	0.86 - 1.09	0.85 - 1.16	1.06 - 1.43	0.91 - 1.11	0.50 - 0.92	0.90 - 1.19	0.71 - 0.88

$F1_U$: $(dDu/dt)_{max\text{-}o\text{-}R}/(dDu/dt)_{max\text{-}o\text{-}Z}$ $F1_P$: $C_{max\text{-}R}/C_{max\text{-}Z}$ $F1_U$:$(dDu/dt)_{max\text{-}o\text{-}R}/(dDu/dt)_{max\text{-}o\text{-}Z}$
$F2_U$:$D_u^{\infty}{}_{\text{-}R}/D_u^{\infty}{}_{\text{-}Z}$ $F2_P$: $AUC_{(0\text{-}12)\text{-}R}/AUC_{(0\text{-}12)\text{-}Z}$ $F2_U$: $D_u^{\infty}{}_{\text{-}R}/D_u^{\infty}{}_{\text{-}Z}$
$F3_U$: $K_{a\text{-}ud\text{-}R}/K_{a\text{-}ud\text{-}Z}$ $F3_P$: $K_{a\text{-}R}/K_{a\text{-}Z}$ $F3_U$: $K_{a\text{-}ud\text{-}R}/K_{a\text{-}ud\text{-}Z}$
$F4_U$: $K_{\text{-}ud\text{-}R}/K_{\text{-}ud\text{-}Z}$ $F4_P$: $K_{\text{-}R}/K_{\text{-}Z}$ $F4_U$: $K_{\text{-}ud\text{-}R}/K_{\text{-}ud\text{-}Z}$
$F5_U$: $T_{max\text{-}ud\text{-}R}/T_{max\text{-}ud\text{-}Z}$ $F5_P$: $T_{max\text{-}R}/T_{max\text{-}Z}$ $F5_U$: $T_{max\text{-}ud\text{-}R}/T_{max\text{-}ud\text{-}Z}$

calculated for $K_{a\text{-}ud}$ and K_a (F_{3U} & F_{3P}) did not lie within 80-120% of their respective mean values based on both 90% confidence interval. It was therefore concluded that $K_{a\text{-}ud}$ and K_a are different in two formulations.

The K values of two formulations were not statistically different, which might be as a result of the calculation of their values from the terminal plasma profile points, where it is not affected by the absorption differences in different formulations and is in accord with the above suggestion on invalidity of urinary data for determination of absorption phase para-meters. (*3.2*) One reason might be that urinary sampling times being far apart in the absorption phase as opposed to plasma sampling.

In summary, the two formulations on the basis of urinary excretion parameters; D_u^{∞}, $T_{max\text{-}ud}$, $(dD_u/dt)_{max\text{-}o}$, $K_{a\text{-}ud}$, K_{ud} were non-different, but on the basis of C_{max}, T_{max}, K_a of plasma, which corresponds to urinary $(dD_u/dt)_{max\text{-}o}$, $T_{max\text{-}ud}$, $K_{a\text{-}ud}$ respectively, at 90% confidence intervals of *F* values (though none were statistically different based on *t*-test) there were not bioeqivalent. The two formulations had also no considerable difference in terms of plasma AUC and K.

It is concluded that urinary data could be used instead of the plasma data in determination of absorption extent and elimination rate of ranitidine due to its comparability in results and non-invasive procedure as well as easier sample collection, extraction procedure, assay and non existence of the double peak phenomenon in urinary data, which in turn can ease bioavailability evaluations (16). However, for determination of the absorption rate constant (K_a), T_{max} and C_{max}, plasma data are preferred. This phenomenon may be attributed to less frequent sampling in the first hours of urine sampling, since repetitive urine samplings with short time intervals is not practically possible (as opposed to plasma).

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