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Report

Influence of Tablet Dissolution on Furosemide Bioavailability: A Bioequivalence Study

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In order to evaluate the *in vitro* dissolution and *in vivo* bioavailability relationship for furosemide, a bioequivalence study was carried out. Furosemide (40 mg) was administered orally to 12 normal volunteers in a 6 × 6 crossover design using six products (five tablets and one solution) obtained from three pharmaceutical companies. Plasma and urine concentrations of furosemide were quantitated by high-performance liquid chromatography (HPLC). Plasma furosemide profiles were analyzed by noncompartmental methods. Compared to the oral solution, all of the formulations exhibited lower peak furosemide concentrations, longer mean residence times, and, in some cases, diminished bioavailability (range, 66–96%). Similar results were obtained when the reference product (a rapidly dissolving tablet) was used as the standard. All of the products failed the 75/75 rule when compared to either reference standard, apparently because of large intersubject variability. The total amount of furosemide excreted in urine could be associated with the percentage drug dissolved (*in vitro*) at 30 min. The pH 5.6 dissolution medium (compared to pH 4.6) appears to be an appropriate test medium for assuring batch uniformity and bioequivalence of furosemide products.

KEY WORDS: furosemide; bioavailability/bioequivalence; dissolution; intersubject variability.

INTRODUCTION

Dissolution characteristics of a drug product have been determined to be an important parameter to assure the bioavailability of a drug product (1,2). However, only a limited number of examples are in the literature that have successfully related *in vitro* dissolution properties with the *in vivo* bioavailability of a drug (3,4), while many others have failed to establish this relationship (5–8).

Furosemide, an anthranilic acid derivative with potent diuretic properties, is rapidly but incompletely absorbed following oral administration. Moreover, the dissolution characteristics of furosemide tablets have been found to be widely variable and to be influenced by the dissolution media (9). Several furosemide products with varying dissolution characteristics have been marketed. In order to examine the *in vitro* dissolution and *in vivo* bioavailability relationship, and also to establish *in vitro* dissolution requirements for furosemide, a bioequivalence study was carried out.

EXPERIMENTAL

Bioavailability Study Design. Twelve normal, healthy male volunteers participated in the bioavailability trial, which was a 6 × 6 crossover design. Two subjects received each of six treatments (A, B, C, D, E, and F) on each of 6 successive weeks. Doses were separated by a 1-week washout period. Furosemide was administered as a 40-mg oral dose in the following treatments:

A—Hoechst-Roussel (Lasix) tablet, lot number 601160;
B—Mylan tablet, lot number E018D;
C—Pharmadyne tablet, lot number 18359;
D—Hoechst-Roussel (Lasix) tablet, lot number 601329;
E—Pharmadyne tablet, lot number 18360; and
F—Hoechst-Roussel (Lasix) solution, lot numbers 619535 and 619529.

Lot numbers of these products are different from those studied by Prasad *et al.* (9).

Blood samples were collected at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 9.0, and 12.0 hr following drug administration. The samples were centrifuged at room temperature and the plasma was collected. Urine was collected for 24 hr following drug administration. Plasma

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and urine samples were frozen (−20°C) until assayed for furosemide.

Dissolution Study. Five lots of furosemide tablets (see above) were tested for dissolution in acetate buffer at both pH 4.6 and pH 5.6 using a USP Apparatus II at 50 rpm (9). Samples were collected at 15, 30, 45, and 60 min, filtered, and assayed for furosemide. Dissolution properties of each lot were determined using six replicates.

Chemical Analysis. Analysis of plasma and urine samples for furosemide was carried out using an established high-performance liquid chromatographic (HPLC) separation and fluorescence detection method (10). The concentrations of conjugated metabolites of furosemide in urine were also quantitated following β-glucuronidase treatment (10).

Pharmacokinetic analysis. The time course of furosemide concentration in plasma was analyzed using noncompartmental methods (11,12). The area under the concentration versus time profile (AUC) and the area under the first moment of the concentration versus time profile (AUMC) were evaluated by trapezoidal rule with extrapolation to infinity (11). The slope of the terminal elimination phase was estimated by log-linear regression of the terminal-phase concentration–time points. The mean residence time in the body following administration of the various furosemide treatments was determined by

$$\text{MRT} = \text{AUMC/AUC} \quad (1)$$

The mean dissolution time *in vivo* (MDT *in vivo*) (12) was then estimated for each product in each subject by

$$\text{MDT } \textit{in vivo} = \text{MRT,tablet} - \text{MRT,solution} \quad (2)$$

The relative bioavailability factor (F,rel) for each product in each subject was calculated as

$$F,\text{rel} = \text{AUC(product)/AUC(solution)} \quad (3)$$

whereas a relative bioavailability term with respect to the reference product D (F,rel-ref) for each product was calculated as

$$F,\text{rel-ref} = \text{AUC(product)/AUC(reference)} \quad (4)$$

Observed peak furosemide concentrations (C_{max}) and time to reach maximum concentration (T_{max}) were also noted.

In vitro dissolution data were analyzed using moment theory (13,14). The mean *in vitro* dissolution time (MDT *in vitro*) describing the first moment of the dissolution rate–time curve was calculated according to

$$\text{MDT } \textit{in vitro} = \text{AUMC}'/\text{AUC}' \quad (5)$$

where AUMC′ and AUC′ are the area under the first moment and the area under the curve, respectively, for a plot of the dissolution rate as a function of time (13,14).

Statistical Analysis. Pharmacokinetic data were subjected to analysis using the General Linear Model of the Statistical Analysis System (SAS). Type I SS (sequential sums of squares) were used to test the effects of treatment, sequence, treatment week, prior treatment, and subject on each of the derived pharmacokinetic parameters. Only a priori tests were undertaken. Contrast statements were used to compare products to the reference standard solution (product F) and to the reference tablet (product D). Statistical significance was defined as a P level < 0.05. In addition, the FDS 75/75 rule was applied to AUC and C_{max} data (15). The 75/75 rule used as a measure of inter- and intrasubject variability states that, using each subject as his/her own control, 75% of the subjects taking the test product should exhibit a relative bioavailability (AUC and C_{max}) within 75–125%.

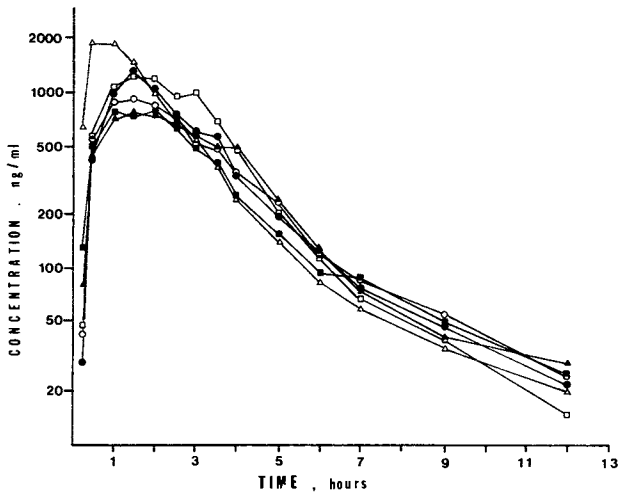

Fig. 1. Mean furosemide plasma concentrations as a function of time for six treatments (Treatment A, ●; Treatment B, ○; Treatment C, ■; Treatment D, □; Treatment E, ▲; Treatment F, △).

RESULTS AND DISCUSSION

The effects of dosing sequence, week of administration, and prior treatments were not significant for any parameter. Therefore, the following discussion is based upon SAS model statements in which analyses were not adjusted for these effects.

The mean furosemide plasma concentration versus time profiles for all six treatments are shown in Fig. 1, and cumulative urinary excretion–time profiles for furosemide are depicted in Fig. 2. The results of the pharmacokinetic analysis

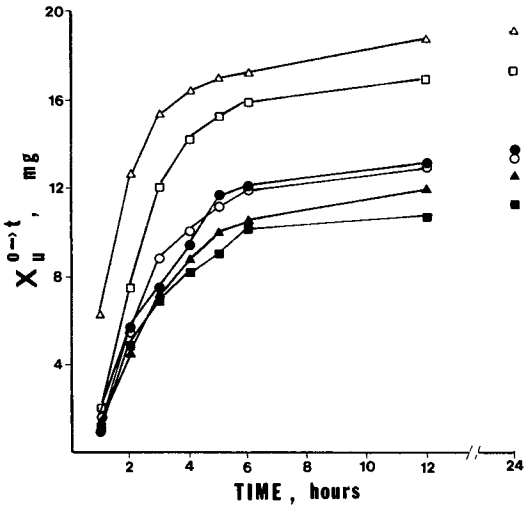

Fig. 2. Mean cumulative furosemide urinary excretion as a function of time for six treatments. See the legend to Fig. 1 for symbols.

Table I. Mean (±SD) Parameters for Six Oral Furosemide Products Following Random Crossover Treatment of Twelve Healthy Subjects

Parameter	A	B	C	D	E	F
AUC (μg/ml/hr)	3745 ± 1196	3379 ± 981	3047 ± 1388	4291 ± 1630	3367 ± 1314	4768 ± 1559
C_{max} (μg/ml)	1404 ± 681	1200 ± 503	1160 ± 785	1938 ± 939	1193 ± 579	2421 ± 865
T_{max} (hr)	1.79 ± 0.75	1.75 ± 0.78	1.08 ± 0.47	1.71 ± 0.69	1.71 ± 1.05	0.708 ± 0.33
Beta (l/hr)	0.306 ± 0.140	0.271 ± 0.097	0.252 ± 0.118	0.286 ± 0.128	0.283 ± 0.234	0.245 ± 0.11
X_u, free (mg)	12.9 ± 4.0	13.3 ± 3.0	13.1 ± 5.1	14.5 ± 4.5	13.1 ± 8.3	20.9 ± 5.9
X_u, total (mg)	18.3 ± 4.9	17.9 ± 5.1	18.7 ± 6.5	20.5 ± 4.2	16.7 ± 8.5	24.0 ± 10.5

are summarized in Table I. All of the tablets were absorbed to a lesser extent (as assessed by AUC, X_u, and C_{max}) and at a slower rate (as assessed by C_{max}, T_{max}, and MDT) than the orally administered solution of furosemide (Treatment F). In fact, with the exception of beta, all of the parameters differed from those for Treatment F.

When the other tablet treatments (A, B, C, and E) were compared to the reference tablet formulation (Treatment D), all parameters except beta were again significantly different. This was true not only for Treatments B and C, but also for Treatment A.

When Treatments A, B, C, and E were compared among themselves, the only significant difference concerned Treatment C, which had a significantly shorter T_{max} than the other three treatments. For all practical purposes, therefore, Treatments A, B, C, and E are considered to be bioequivalent. Treatment C had peak concentrations which occurred earlier, but the resultant AUCs and peak concentrations did not differ.

Tablet formulations A, B, C, and E were absorbed between 70 and 91% compared to the reference tablet formulation (Treatment D). All tablet formulations had relative bioavailabilities which ranged between 66 and 96% compared to the solution (Table II). The FDA has subsequently removed the two unapproved products, C and E (Pharmadyne products), from the market. These products had mean relative AUC values of 66 and 75% compared to the oral solution. The total amounts of furosemide excreted in the urine (X_u, total, in Table I), in general, support the plasma data with regard to bioavailability estimates. Considerable variability was also noted for the urinary excretion data.

Previous furosemide bioavailability studies suggest a wide intersubject variability in the absorption and excretion of furosemide from oral dosage forms (10,16). The results of the present study support this observation. A large intersubject variability was demonstrated in that all tablet dosage forms failed the prescribed FDA 75/75 rule when comparing the AUC and C_{max} for the tablets versus the solution and the test tablets versus the reference tablet (Treatment D).

Mean dissolution profiles in acetate buffer for the five tablet formulations are presented in Figs. 3a and 3b. Moment analysis of the dissolution data is summarized in Table II. Although the dissolution profiles varied, no statistically significant correlations were found between the percentage dissolved at 30 or 60 min and the AUC for the various tablets (Table II). The correlation between the mean *in vitro* and the

Table II. Mean (±SD) Drug Absorption Parameters for Furosemide Following Six Oral Treatments in Twelve Healthy Subjects and Dissolution Characteristics of the Five Tablet Formulations

Parameter	A	B	C	D	E	F
In vivo						
F,rel-ref (%)	91.3 ± 28.4	83.7 ± 26.1	70.1 ± 13.5	—	81.5 ± 27.7	—
F,rel (%)	83.6 ± 31.3	74.6 ± 23.1	66.4 ± 29.3	96.0 ± 38.7	75.4 ± 38.3	—
MRT (hr)	3.57 ± 1.18	3.61 ± 0.90	3.61 ± 0.81	3.01 ± 0.44	3.95 ± 1.26	2.46 ± 0.52
MDT (hr)	1.11 ± 1.45	1.15 ± 0.83	1.15 ± 1.00	0.55 ± 0.65	1.49 ± 1.06	—
In vitro						
pH 4.6						
% dissolved in 30 min	43.1	69.0	47.4	65.8	32.0	—
% dissolved in 60 min	58.5	84.9	66.8	77.6	55.9	—
MDT (hr)	0.48	0.35	0.33	0.29	1.06	—
pH 5.6						
% dissolved in 30 min	63.4	94.6	74.6	78.8	18.5	—
% dissolved in 60 min	85.7	98.9	83.2	90.6	31.7	—
MDT (hr)	0.45	0.13	0.22	0.24	1.14	—

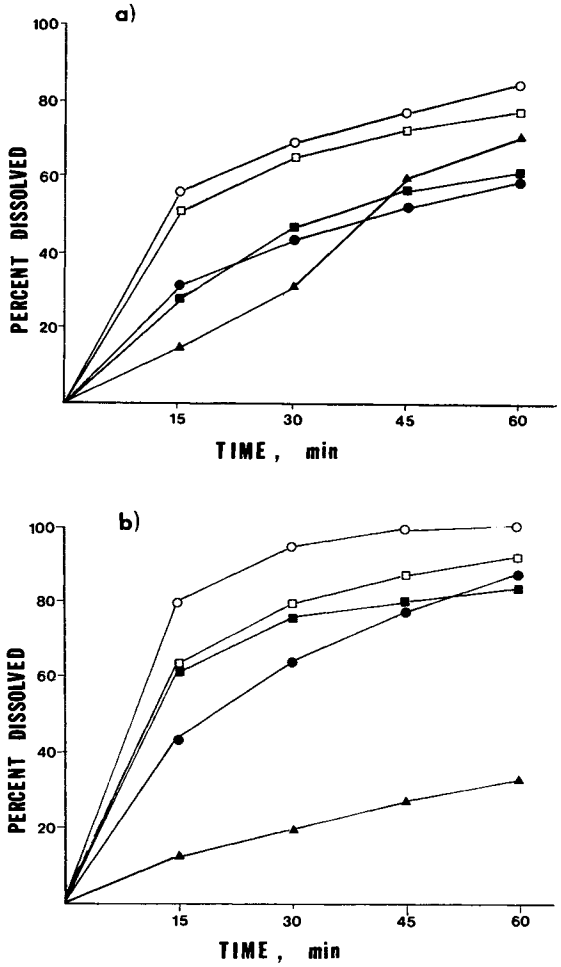

Fig. 3. Mean *in vitro* dissolution profiles of five furosemide products (see the legend to Fig. 1 for symbols) at pH 4.6 (a) and pH 5.6 (b).

mean *in vivo* MDT parameters approached statistical significance ($r = 0.640$ and $r = 0.733$ for the pH 5.6 and 4.6 dissolution media, respectively). However, the analysis of individual data revealed that the product MDT *in vitro* was a poor predictor of the individual subjects' MDT *in vivo* parameters ($r = 0.051$ for the pH 4.6 dissolution medium). This, again, is probably due to the large intersubject variability.

Although individual plasma levels could not be correlated with dissolution data, better correlations were observed between the mean values for total furosemide excreted in the urine and the *in vitro* dissolution parameters, such as the percentage dissolved at 30 min at pH 4.6 ($r = 0.625$), the percentage dissolved at 30 min at pH 5.6 ($r = 0.604$), the *in vitro* MDT at pH 4.6 ($r = -0.773$), and the *in vitro* MDT at 5.6 ($r = -0.670$). However, these correlations were not statistically significant ($P > 0.1$). In agreement with previous work (9), the pH 5.6 dissolution studies did not discriminate among the products. A comparison of the dissolution profiles for all five products at pH values of 4.6 and 5.6 shows that the products dissolve faster and more completely in pH 5.6 (Fig. 3). Since product A and product B are essentially bioequivalent yet exhibit marked differences in their dissolution at pH 4.6, it appears that the pH 4.6 dissolution medium is overly discriminative. Therefore, pH 5.6 dissolution medium appears to be an appropriate test medium for assuring batch uniformity and bioequivalence of furosemide products.

Previous furosemide bioavailability studies have attempted to correlate *in vitro* dissolution properties with *in vivo* measurements of bioavailability. Kingsford *et al.* (19) reported a good correlation between the percentage of several different furosemide tablets dissolved in 30 min and the percentage of furosemide recovered in the urine. In contrast, Crismon *et al.* (20) found no difference in plasma AUCs and urine output for two tablets that exhibited significantly different dissolution rates. Our own results are more consistent with the latter report. Some of the differences in the conclusions of these studies may arise from differences in dissolution methodology (9,19) or in time scaling. However, we believe that the most troublesome aspect of furosemide bioavailability testing arises from the large inter- and intrasubject variability in furosemide kinetics (10,16). Grahnen *et al.* (16) have stated that "intraindividual variation in absorption is a confounding factor in bioavailability studies of furosemide." We are currently conducting an additional bioavailability study in which normal volunteers receive each of three different furosemide dosage forms twice, according to a randomized six-way crossover design. The results of this study should help identify the relative contribution of the intra- and intersubject variability in estimating furosemide bioavailability.

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