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BIOAVAILABILITY OF SEVEN FUROSEMIDE TABLETS IN MAN

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ABSTRACT

A seven-way crossover study was conducted in 14 healthy male volunteers to evaluate the relative bioavailability of seven different marketed 40 mg furosemide tablets. Each dose was administered as a single tablet after an overnight fast, and blood samples were obtained for 16 hours. Plasma was assayed by HPLC. There were no statistically significant differences among the seven products for the mean peak concentration (1·01–1·29 μg/ml), mean time of peak (1·2–2·1 h) or mean area under the plasma concentration–time curves, which differed by less than 14 per cent. However, one product exhibited greater intersubject variability, and on this basis was considered inequivalent to the other six products.

Furosemide is a potent and widely used diuretic. Currently the United States Food and Drug Administration (USFDA) has granted approval to at least twelve manufacturers of 40 mg furosemide tablets, based in part on bioavailability data obtained in human subjects. In addition, the USFDA has granted an 'AB' therapeutic equivalence evaluation to each of these products,[1] which is understood by many to indicate the therapeutic equivalence and interchangeability of these products.

The objective of the present investigation was to directly compare the bioavailability of seven 40 mg furosemide tablet products which had previously been approved by the Food and Drug Administration.

KEY WORDS Furosemide tablets Human bioavailability HPLC assay

EXPERIMENTAL

Dosage forms

The seven furosemide tablet products† selected for study were obtained

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†Product No. 1: Hoechst-Roussel Pharmaceuticals, Lot 606103; No. 2: Chelsea Laboratories, Lot 304013; No. 3: Cord Laboratories, Lot 53532; No. 4: Lederle Laboratories, Lot 727-1731; No. 5: Mylan Pharmaceuticals, Lot K032C; No 6: Parke Davis Laboratories, Lot MC1GRA; No. 7: Superpharm Corp., Lot 83E053.

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from local suppliers, and represented the seven manufacturers which had received USFDA approval at the time of the study. At least one year remained until expiration for all products.

Clinical protocol

Fourteen male volunteers (23–28 years, 61–93 kg) underwent a physical examination, urinalysis, and hematological and blood chemistry (SMA 18/90) determinations, to ensure that they were in good health. All subjects provided written informed consent. Subjects' body weights were within 10 per cent of normal as defined by common actuarial tables. The subjects were instructed to refrain from all medications for the week prior to the study and during the study period. No alcohol was allowed for 24 h prior to each treatment period or for 16 h after the dose. In order to reduce any risk of dehydration, the subjects were required to drink 360 ml of a fluid-electrolyte replenisher (Squincher ®) the night before each study day, 4 h after the dose, and following the collection of the last blood sample. Additionally, the subjects were required to drink 360 ml of water upon awakening each study day.

Using a randomized crossover design described by Williams,[2] each subject received each of the seven products at 3-day intervals, with no two subjects receiving the same dosing sequence. All doses were administered after an overnight fast with 240 ml of water. No food was allowed until 4 h after the dose, when a standard meal (131 g carbohydrate, 61 g fat, and 30 g protein: content available on request) was given. The meals were identical for each of the seven dosing days. Immediately prior to each 40 mg dose and at 0·5, 1, 2, 3, 4, 6, 8, 12, and 16 h after each dose, 7 ml blood samples were obtained from each subject via a 19 gauge intermittent intravenous infusion set (Deseret Medical Inc.) or venipuncture. The blood was placed into 7 ml sterile, evacuated test tubes containing 100 units of heparin (Venoject®, Terumo Medical) and centrifuged at 3000 rev min^{-1} for 15 min. The plasma was removed and stored at $-10°$ until assayed.

Analytical method

Plasma furosemide concentrations were determined using an HPLC assay similar to that reported by Rapaka *et al.*[3] Standard curves were prepared each day that subject samples were assayed, using an acetonitrile precipitation method. A 200 µl aliquot of plasma was combined with 50 µl of an aqueous solution of furosemide (Sigma Chemical) (0·20–4·0 µg ml^{-1}); 50 µl of a methanol solution of warfarin (Endo Labs) (1 mg ml^{-1}), used as the internal standard; and 400 µl of acetonitrile. Subject plasma samples and blank samples were prepared with aliquots of water and methanol instead of the furosemide and internal standard solutions, as appropriate. The sample mixtures were vortexed for 10 s and centrifuged for 15 min at 3000 rev min^{-1} and $-10°$. The supernatant was transferred to a silanized conical tube and

evaporated to dryness at 50° under a stream of nitrogen. The residue was reconstituted with 100 µl of the mobile phase, and centrifuged for 15 min at 3000 rev min^{-1} and −10°. The clear supernatant was transferred to autosampler vials, and 10–20 µl was injected (Model WISP 710B, Waters Associates) into the HPLC system. The HPLC column (µ-Bondapak C_{18}, Waters Associates) mobile phase (37·5 per cent acetonitrile: 62·5 per cent 0·08 M phosphoric acid) and fluorescence detector (Schoeffel Model FS 970) were identical to those utilized by Rapaka *et al.*[3] A guard column (µ-Bondapak C_{18} Corasil, Waters Associates) was employed between the injector and HPLC column.

Data analysis

The time of maximum plasma concentration (t_{max}) and maximum plasma concentration (C_{max}) were determined by inspection of individual subject data. The elimination rate constant (k) for each dose was determined by least-squares fitting of the post-absorption concentration–time data. The area under the plasma concentration–time curve from 0 to 16 h (AUC_{0-16}) was calculated using the trapezoidal method. The $AUC_{0-\infty}$ was calculated by addition of the AUC to the last log-linear plasma concentration employed for the determination of k, and the plasma concentration at that time divided by k.

Analysis of variance was used to evaluate statistically significant differences ($p < 0{\cdot}05$) at each sampling time, as well as values for t_{max}, C_{max}, and AUCs. In cases where significant differences occurred, the Newman-Keuls *a posteriori* test was used to evaluate which subjects, treatment sequence or dosage forms were different. A power analysis[4] was used to evaluate the potential for statistical errors based on $\alpha = 0{\cdot}05$ and $\beta = 0{\cdot}2$.

A series of AUC ratios were also computed for each subject, comparing the $AUC_{0-\infty}$ for each product to the $AUC_{0-\infty}$ for the other six products. This type of analysis has been termed the 75:75 Rule[5,6] and provides information regarding the equivalence of different dosage forms in a given subject.

RESULTS AND DISCUSSION

Analytical method

The HPLC assay demonstrated good linearity ($r \geq 0{\cdot}999$) over a furosemide plasma concentration range of 0·05–1·0 µg ml^{-1}, with a lower limit of sensitivity of 0·01 µg ml^{-1}. The precision of the assay, as determined from the relative standard deviations of eight replicates at each concentration assayed over a four-day period, were 2·0, 3·7, 4·3, 7·2, and 15·0 per cent for the 1·0, 0·5, 0·25, 0·10, and 0·05 µg ml^{-1} standards, respectively. No interfering peaks were noted in the HPLC chromatograms in the vicinity of either the furosemide or internal standard for blank plasma samples, or plasma samples obtained during the course of the bioavailability study.

Table 1. Mean values*

Parameter	Products 1	2	3	4	5	6	7
Conc 0·5 h	0·16	0·42	0·50	0·58	0·14	0·27	0·41
($\mu g\ ml^{-1}$)	(156)†	(80)	(91)	(80)	(162)	(107)	(79)
Conc 1 h	0·55	1·04	0·92	1·07	0·53	0·70	0·87
($\mu g\ ml^{-1}$)	(89)	(36)	(46)	(45)	(94)	(70)	(62)
Conc 2 h	0·70	0·77	0·74	0·85	0·54	0·81	0·75
($\mu g\ ml^{-1}$)	(62)	(30)	(43)	(44)	(66)	(46)	(43)
Conc 3 h	0·56	0·62	0·50	0·53	0·52	0·67	0·53
($\mu g\ ml^{-1}$)	(52)	(43)	(39)	(57)	(56)	(44)	(61)
Conc 4 h	0·47	0·40	0·39	0·37	0·52	0·44	0·34
($\mu g\ ml^{-1}$)	(74)	(68)	(86)	(75)	(70)	(59)	(70)
Conc 6 h	0·15	0·14	0·15	0·13	0·17	0·15	0·12
($\mu g\ ml^{-1}$)	(51)	(45)	(55)	(67)	(45)	(51)	(44)
Conc 8 h	0·06	0·06	0·06	0·05	0·08	0·06	0·05
($\mu g\ ml^{-1}$)	(55)	(61)	(66)	(76)	(52)	(52)	(54)
Conc 12 h	0·01	0·01	0·01	0·01	0·02	0·01	0·01
($\mu g\ ml^{-1}$)	(121)	(122)	(145)	(217)	(86)	(134)	(149)
Conc 16 h	<0·01	<0·01	<0·01	<0·01	0·01	<0·01	<0·01
($\mu g\ ml^{-1}$)	(374)	(260)	(374)	(374)	(188)	(374)	(374)
Peak conc	1·07	1·16	1·13	1·29	1·01	1·14	1·04
($\mu g\ ml^{-1}$)	(27)	(23)	(29)	(23)	(21)	(24)	(41)
Time of	1·89	1·38	1·31	1·29	2·14	1·94	1·21
peak (h)	(58)	(56)	(67)	(69)	(58)	(52)	(46)
AUC_{0-16}	3·00	3·49	3·32	3·48	2·99	3·34	3·10
($\mu g\ h\ ml^{-1}$)	(22)	(24)	(28)	(30)	(22)	(19)	(42)
k	0·40	0·42	0·39	0·48	0·35	0·42	0·42
(h^{-1})	(32)	(36)	(25)	(35)	(44)	(20)	(37)
$AUC_{0-\infty}$	3·04	3·51	3·33	3·50	3·04	3·35	3·12
($\mu g\ h\ ml^{-1}$)	(22)	(24)	(28)	(30)	(21)	(20)	(39)

*Each value represents the mean of the 14 subjects.
†Relative standard deviation given in parentheses (S.D. × 100/mean).

Plasma concentrations at each sampling time

The mean furosemide plasma concentrations for each of the seven products are given in Table 1. There were no statistically significant differences ($p > 0{\cdot}05$) noted among the products with the Newman-Keuls analysis, with the following exceptions: at 0·5 h Product 4 was greater than Products 1 and 5; at 1 h Products 2 and 4 were greater than Products 1 and 5; and at 8 h Product 5 was greater than Product 4.

Peak concentration, time of peak concentration, k *and AUC values*

The C_{max} for the product with the lowest value was 95 per cent that of Product 1, and was 78 per cent that of the tablet with the highest C_{max}. The

differences among t_{max} values for the seven products was 0·9 h. The $AUC_{0-\infty}$ for the product with the lowest value was identical to the $AUC_{0-\infty}$ for Product 1, and was 87 per cent of that seen for the tablet with the highest $AUC_{0-\infty}$. None of these differences were statistically significant ($p > 0{\cdot}05$). The harmonic mean half-life for the subjects ranged from 1·3 to 2·8 h, which is consistent with ranges reported by others after intravenous and oral administration of furosemide to healthy human subjects.[7–9]

Phase and subject differences

Except for the 0·5 h sample concentration, there were significant differences noted among subjects for all values given in Table 1 ($p<0{\cdot}05$). There were no significant differences ($p > 0{\cdot}05$) observed among the seven dosing phases.

Power analysis

Because of the variability in the values given in Table 1, 28 or more subjects would have been required for a 20 per cent difference to be statistically significant. For the 14 subjects utilized in the study a 29 per cent difference among products for C_{max} and $AUC_{0-\infty}$ would have been significant. The degree of variability observed in this study is likely to be the result of both inter- and intrasubject variability in the absorption of furosemide. This conclusion is supported by a recent report[10] in which a two-fold range in AUC was found within a subject after oral administration of 40 mg furosemide tablets on two occasions, and the intersubject range in AUC varied over a four-fold range. In contrast, when the same subjects were given two doses of furosemide intravenously, the inter- and intrasubject variability in AUC was much less.

Product interchangeability

It seemed logical to assume that dosage forms which are considered 'therapeutically equivalent'[1] should permit such products to be used interchangeably in any given patient. Table 2 summarizes the results of a subject-by-subject comparison of the $AUC_{0-\infty}$ ratios for Products 2–7, using Product 1 as the denominator (reference). Strict adherence to the 75:75 rule indicates that only for Product 5 do at least 75 per cent of the subjects have $AUC_{0-\infty}$ values which are within 75–125 per cent of those found for Product 1. If the limits of the analysis are expanded, requiring that 70 per cent of the subjects must have $AUC_{0-\infty}$ ratios within the range of 70–130 per cent, then only Product 7 fails. One criticism of the 75:75 rule is that a variable reference product will decrease the probability for the acceptance of a second product, when the two AUC values are compared in each subject. Further, in the clinical setting it is possible that a patient may be receiving any one of the seven products included in this study, and may subsequently receive any of the other six products. Thus, it is useful to consider the degree of variability to

Table 2 75:75 Comparison of $AUC_{0-\infty}$ using Product 1 as reference

Subject	Per cent (relative to Product 1) Prod 2	Prod 3	Prod 4	Prod 5	Prod 6	Prod 7
1	130	144	114	120	66	128
2	93	126	103	80	120	108
3	112	109	120	85	113	86
4	112	97	103	89	95	62
5	121	135	92	125	90	119
6	104	104	126	107	127	85
7	115	88	130	102	145	93
8	115	74	118	108	101	88
9	94	90	85	78	81	67
10	158	79	107	94	170	77
11	154	173	154	176	205	205
12	113	137	126	118	104	69
13	81	109	176	90	104	167
14	148	89	80	73	100	108
Subjects Within 75:125	10/14	8/14	9/14	12/14	9/14	8/14
Per cent	71%	57%	64%	86%	64%	57%
Subjects Within 70:130	11/14	10/14	12/14	13/14	10/14	9/14
Per cent	79%	71%	86%	93%	71%	64%

be anticipated if free interchange is permitted among any of the seven products. To evaluate this type of variability, six additional tabulations of $AUC_{0-\infty}$ ratios were prepared, using the same approach illustrated in Table 2, with each of the other six products used as the reference product. A summary of these results is shown in Table 3. These data are expressed in terms of the total percentage of subjects within the stipulated range for the six products compared to the given reference. For example, when Product 1 was used as the reference, as shown in Table 2, 56 of the 84 AUC ratios (67 per cent) were within the range of 75–125 per cent, and 65 of the 84 AUC ratios (77 per cent) were within the range of 70–130 per cent. Since Product 7 appeared to exhibit the greatest intersubject variability, the data were also analysed deleting Product 7. As shown in Table 3, between 66 and 71 per cent of the subjects exhibited AUC ratios between 75 and 125 per cent when Product 7 was deleted, regardless which of the other six products was employed as the reference. Similarly 73–80 per cent of the subjects had AUC ratios between

Table 3. Summary of individual subject AUC ratios, using each of the seven furosemide tablet products as a reference dosage form

Reference product no.	Per cent of subjects within the indicated AUC per cent range			
	75:125 per cent range		70:130 per cent range	
	Including Product 7	Without Product 7	Including Product 7	Without Product 7
1	67	69	77	80
2	69	73	75	77
3	68	66	74	73
4	65	71	75	77
5	68	69	77	79
6	64	69	68	73
7	50	–	58	–

70 and 130 per cent, using any of the first six products as the reference. This analysis suggests that if all seven products are considered interchangeable, including Product 7, the potential exists that only 58 per cent of the subjects will exhibit a change in AUC of $\leq$ 30 per cent if they receive Product 7 and are then changed to the other six products. The actual number of subjects outside the range of 70–130 per cent for each of the six products, using Product 7 as the reference is (6/14), (6/14), (4/14), (7/14), (4/14), and (8/14) for Products 1–6, respectively, for a total of 35/84 subjects (42 per cent). In contrast, as shown in Table 3, if Product 7 is deleted from consideration, only 20–27 per cent of the subjects will exhibit AUC ratios outside the range of 70 to 130 per cent, regardless which of the remaining six products is employed as the reference. The greater intersubject variability demonstrated by Product 7 could be anticipated from the greater relative standard deviation for $AUC_{0-\infty}$ shown in Table 1. However, a quantitation of the potential significance of this observation is somewhat difficult without the type of analysis shown in Tables 2 and 3.

It was concluded that even though each of the seven products performed satisfactorily in terms of an analysis of conventional bioequivalence parameters, because of the variability demonstrated by Product 7, only six of the products could be considered interchangeable.

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