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PHARMACOKINETICS OF FUROSEMIDE AFTER THREE DIFFERENT SINGLE ORAL DOSES

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ABSTRACT

Furosemide solution was orally administered to 21 healthy adult males to determine dose proportionality over the dose range used and the reproducibility of disposition following a repeated dose. Furosemide solution was given in doses of 20, 40, and 80 mg, with the 40 mg dose repeated once. Blood was collected for 12 hours post-dose and urine for 24 hours. The maximum plasma concentrations resulting from 20, 40, and 80 mg doses were significantly different ($p < 0.05$). Dose normalized maximum concentrations for the 20 and 80 mg doses were significantly different ($p < 0.05$). Mean time to Cp_{max} was 50 minutes, with no differences observed among doses. Plasma AUCs were significantly different ($p < 0.05$) for 20, 40, and 80 mg. Dose normalized AUCs were not significantly different. Mean amounts of furosemide in urine (X_u) were 9·62, 16·7, and 32·0 mg for the 20, 40, and 80 mg doses, respectively. These amounts were significantly different ($p < 0.05$); dose normalized amounts were not significantly different. Renal clearances of furosemide following the three doses were not significantly different. Regressions of Cp_{max}, AUC and X_u on dose were significant. There were no significant differences in Cp_{max}, t_{max}, AUC or X_u for 40 mg given on two separate days. Renal clearance of furosemide was statistically different for 40 mg given on two separate days, but the difference was not clinically significant. The pharmacokinetics of furosemide are linear over the dosage range studied. Furosemide 40 mg given on two separate days results in similar disposition parameters.

KEY WORDS Furosemide Pharmacokinetics Dose proportionality

INTRODUCTION

Furosemide is one of the most commonly prescribed diuretics, being used clinically to treat volume and electrolyte imbalances. The site of furosemide activity is the luminal surface of the ascending loop of Henle.[1] The drug reaches this site of activity via active secretion into the proximal tubule through the

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organic acid pathway.[2] Because this pathway is capacity limited, it is conceivable that non-linearity of furosemide kinetics might be observed if the drug were given in high enough doses to saturate the secretory pathway.

Although the pharmacokinetics of furosemide in normal volunteers and diseased patients have been reported by a number of investigators, there is little published literature on the linearity of furosemide kinetics. Cutler *et al.*[3] administered furosemide intravenously in doses of 40, 80, and 120 mg to four normal volunteers. Regressions of elimination rate constant, apparent volume of distribution and serum furosemide clearance on dose revealed no significant correlations. Regression of area under the serum concentration time curves versus administered dose resulted in a correlation coefficient of 0·84. The authors concluded there were no significant changes in furosemide pharmacokinetics in normal subjects receiving doses of 40–120 mg.

The present study was conducted to determine dose proportionality of furosemide administered as an oral solution over the dosage range of 20–80 mg when given to healthy adult males. In addition, the reproducibility of disposition of a repeated furosemide 40 mg dose was studied. This was included in the study design because of large intrasubject variability observed in previous work on furosemide pharmacokinetics.[4]

Analyses of the data demonstrate the pharmacokinetics of furosemide to be linear over the dosage range studied. In addition, pharmacokinetic parameters resulting from two separate 40 mg oral doses are similar.

MATERIALS AND METHODS

Study design

Twenty-one healthy males, 21 to 34 years of age (mean 24), weighing between 65 and 89 kg (mean 74), who were in good physical condition as determined by physical examination and clinical laboratory tests, volunteered to participate in the study. Informed consent was obtained from each subject. The protocol had approval of the The University of Texas at Austin Institutional Review Board.

An open Latin-square design was used to study 21 subjects, divided into three groups of seven. Subjects were randomly assigned to each group. Lasix® injection solution* (10 mg furosemide ml^{-1}) was administered orally as a 2 ml (20 mg), 4 ml (40 mg), or 8 ml (80 mg) diluted to a final volume of 50 ml with tap water. The container was rinsed with another 50 ml of water, and this was also administered to the subject. Lasix® solution for injection was used in preference to Lasix® Oral Solution because the latter contains sorbitol and glycerin. These inclusions delay furosemide absorption and would have added an unnecessary variable in the present study. Subjects received 20, 40, and 80 mg of furosemide solution in randomized order on three different days. All subjects received a

* Lasix® 10 ml ampules for injection, Lot No. 613080, supplied by Hoechst-Roussel Pharmaceuticals Inc.

repeat 40 mg dose on the last study day. Seven-day washout periods separated the study days.

All subjects abstained from medications, smoking, and alcohol for one week prior to and throughout the study. Subjects fasted for 12 hours before each drug administration, and three hours thereafter.

Following drug administration, blood samples (10 ml) were collected through an indwelling intravenous catheter placed in a forearm vein, using a plastic syringe with immediate transfer to heparinized tubes. Blood was collected immediately before and at 0·25, 0·5, 0·75, 1, 1·5, 2, 2·5, 3, 4, 5, 6, 8, 10, and 12 hours after drug administration. Plasma was separated and frozen at $-20°$ until assayed. During each study day, urine was collected immediately before drug administration and for the following periods: 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–8, 8–12 and 12–24 hours. Urine volumes were recorded and an aliquot frozen at $-20°$ until assayed.

Furosemide assay

An HPLC method for furosemide analysis in plasma and urine, similar to one published previously[4] was used. Furosemide was supplied by Hoechst-Roussel Pharmaceuticals Inc. (Somerville, N. J.) and hydroflumethiazide by Bristol Laboratories (Syracuse, N.Y.). All other chemicals used were reagent grade or better.

To 1 ml of plasma was added 20 µl of a stock solution of hydroflumethiazide (2 mg ml^{-1} prepared in methanol; final concentration of internal standard hydroflumethiazide was approximately 20 µg ml^{-1}) and, in the case of standard curve samples, 100 µl of a stock solution of furosemide (prepared in methanol and protected from light[6]) to yield final furosemide concentrations of 0·05–2·5 µg ml^{-1}. The sample was acidified with 100 µl of 6M HCl and immediately extracted with 5 ml of anhydrous diethyl ether. Following a brief centrifugation to separate phases, 4 of 5 ml of the ether phase was transferred to another tube, evaporated under a gentle stream of nitrogen, and reconstituted in 0·25 ml of 0·02 M glycine buffer, pH 11. A portion (100 µl) of the glycine buffer phase was chromatographed using a Berkman HPLC system including a 4·6 mm I.D. × 25 cm, 10 micron Pell ODS column (Whatman) and fluorescence detection (Gilson Spectra/Glo filter fluorometer, excitation filter 330–400 nm, emission filter 460–600 nm). The mobile phase was methanol–water–acetic acid (35 : 65 : 3) used at a flow rate of 1·0 ml min^{-1}. Furosemide typically eluted with a retention time of approximately 8·8 min, while the internal standard hydroflumethiazide typically eluted with a retention time of approximately 4·0 min.

The sensitivity limit for furosemide in plasma was estimated to be 0·05 µg ml^{-1} with a S/N > 5. Calibration curves (furosemide/hydroflumethiazide peak area ratio versus concentration (in µg ml^{-1} of furosemide in plasma)) were prepared daily with a recording integrator, and these curves had an average $r = 0·999$ with negligible Y-intercept values. HPLC determinations on seven spiked plasma samples (10·0, 5·00, 2·50, 1·00, 0·50, 0·25, and 0·05 µg ml^{-1})

indicated an average accuracy of 103 per cent and a precision of ±10·2 per cent (S.D.).

Urine samples were spiked and analysed the same as plasma samples except that a 4 ml portion of the extract was back extracted with 1 ml of pH 11·0 glycine buffer prior to injection into the HPLC column. Urine samples extracted showing $> 10\,\mu g\,ml^{-1}$ furosemide were diluted appropriately with glycine buffer before injection. The sensitivity limit for furosemide in urine was estimated to be $0{\cdot}5\,\mu g\,ml^{-1}$ with a S/N > 5. Calibration curves (furosemide/hydroflumethiazide peak area ratios versus concentration in $\mu g\,ml^{-1}$ of furosemide in urine) were prepared daily and had an average $r = 0{\cdot}999$ with negligible Y-intercept values. HPLC determinations on a series of five spiked urine samples (10·0, 5·00, 2·50, 1·00, and $0{\cdot}50\,\mu g\,ml^{-1}$) indicated an average accuracy of 98·4 per cent and a precision of ±2·4 per cent (S.D.).

Data analysis

Area under the plasma concentration–time curve (AUC) was calculated for zero to 12 hours using the trapezoidal rule. The AUC was truncated at 12 hours because secondary maxima in plasma concentrations obscured the terminal log-linear phase in many subjects, making corrections to AUC_{0-inf} unreliable. Because most subjects had plasma concentrations below assay sensitivity or near the sensitivity limit at the time of the last blood sample, a significant portion of the AUC was not lost by not calculating the AUC to infinity. Renal clearance (Cl_R) was calculated with the equation: $Cl_R = X_u^{0-12}/AUC$ where X_u^{0-12} is the total amount of unchanged drug excreted in the urine from 0–12 hours. The maximum plasma concentration achieved (Cp_{max}) and time to maximum plasma concentration (t_{max}) were observed from the measured plasma concentrations following drug administration. AUC, Cp_{max}, and X_u^{0-24} (total amount of unchanged drug excreted in the urine from 0–24 hours) of each subject were normalized for dose by dividing the individual values by two for the 40 mg doses and by four for the 80 mg dose.

Two-way analysis of variance, with the Least Significant Difference test[7] utilized for *a posteriori* comparison and Student's *t*-test for paired data, were used to make statistical evaluations of the data. Regression analysis was used to evaluate linearity of the pharmacokinetic parameters in relation to dose. An α level of less than 0·05 was accepted as evidence of statistical significance.

RESULTS

Mean plasma concentrations of furosemide resulting from the administration of 20 mg, 40 mg (repeated once), and 80 mg are depicted graphically in Figure 1. Pharmacokinetic parameters resulting from the four administered doses are given in Table 1. Maximum plasma concentrations from the three different doses were significantly different. Dose normalized maximum plasma concentrations were significantly different for the 20 mg and 80 mg doses. Comparison of Cp_{max}

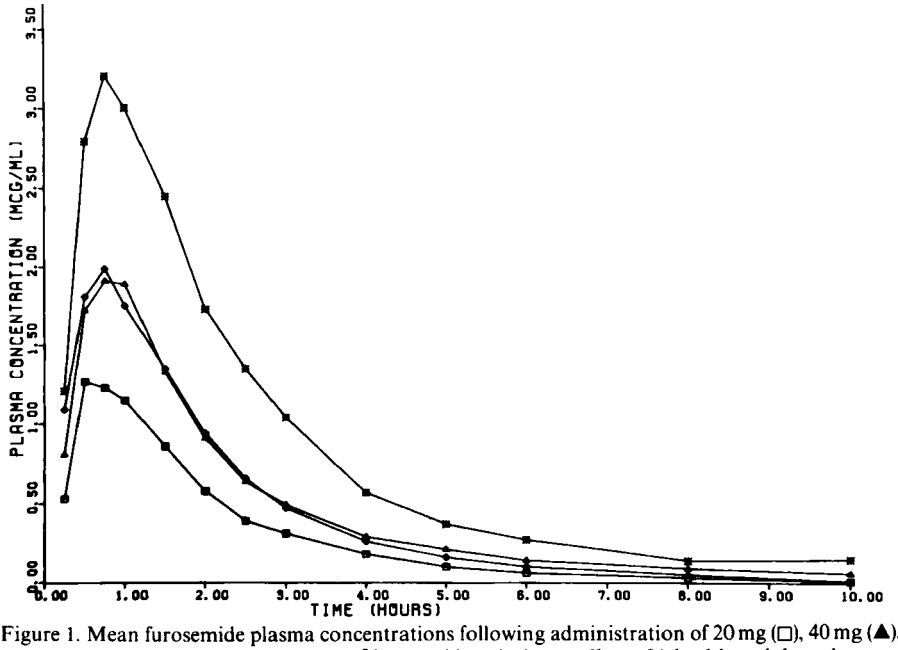

Figure 1. Mean furosemide plasma concentrations following administration of 20 mg (□), 40 mg (▲), repeat 40 mg (◆), and 80 mg (*) of furosemide solution orally to 21 healthy adult males

Table 1. Mean (±S.D.) pharmacokinetic parameters resulting from administration of furosemide 20 mg, 40 mg, and 80 mg, with a repeat dose of 40 mg, to 21 healthy adult men

	20 mg	40 mg	Repeat 40 mg	80 mg	Dose-normalized 40 mg	Dose-normalized 80 mg
Cp_{max} ($\mu g\,ml^{-1}$)	1·34±0·49	2·21±0·71	2·27±0·59	3·54±1·33	1·10±0·36	0·89±0·33
t_{max} (h)	0·89±0·37	0·82±0·25	0·79±0·40	0·81±0·29	—	—
AUC ($\mu g\,ml^{-1}\,h$)	2·69±1·29	4·62±1·47	4·41±1·16	8·28±2·80	2·31±0·73	2·07±0·70
X_u (mg)	9·62±2·07	16·7±4·22	19·2±4·54	32·0±9·72	8·34±2·11	8·00±2·43
Cl_R ($ml\,min^{-1}$)	65·4±24·2	61·7±19·8	72·4±16·5	63·7±14·5	—	—

resulting from the two 40 mg doses revealed no significant difference when furosemide 40 mg was administered on two separate days.

Mean t_{max} was 50 minutes, with no differences observed among doses. Comparison of t_{max} resulting from the two 40 mg doses showed no significant difference when the same dose was administered on two separate days.

Areas under the plasma concentration–time curves were significantly different for the 20 mg, 40 mg, and 80 mg doses. Dose normalized AUCs were not

significantly different. The AUCs resulting from separate administration of furosemide 40 mg were not significantly different.

Mean amounts of furosemide appearing in urine in 24 hours were 9·62 mg, 16·7 mg, and 32·0 mg for the 20 mg, 40 mg, and 80 mg doses, respectively. Cumulative urine recovery of furosemide is illustrated in Figure 2. Amounts of drug in the urine from the three different doses were significantly different. When the amounts were normalized for dose, there were no significant differences. Comparison of X_u resulting from the two 40 mg doses revealed no significant difference.

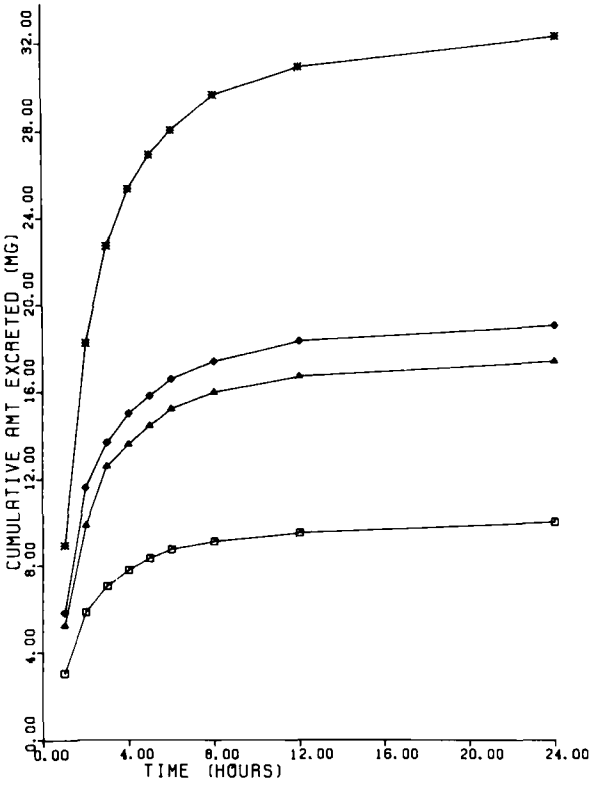

Figure 2. Mean cumulative amounts of furosemide in urine following administration of 20 mg (□) 40 mg (▲), repeat 40 mg (◇), and 80 mg (*) of furosemide solution orally to 21 healthy adult males

Renal clearance of furosemide did not change significantly with dose. Renal clearances of the two 40 mg doses were statistically significantly different; the difference is not clinically significant.

Regression analysis of Cp_{max}, t_{max}, AUC, X_u and Cl_R versus dose were performed. Linear relationships were determined between dose and Cp_{max}, AUC and X_u, with correlation coefficients of 0·62, 0·72, and 0·51, respectively. These correlations are illustrated in Figures 3, 4, and 5. Time to maximum plasma concentration and Cl_R were not significantly correlated to dose.

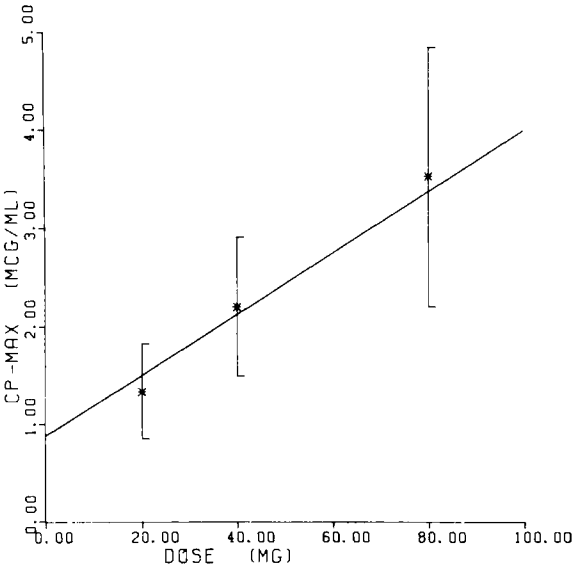

Figure 3. Mean (±S.D.) CP_{max} versus orally administered doses of furosemide in 21 healthy adult males ($r = 0{\cdot}62$). Regression lines calculated from all individual data points

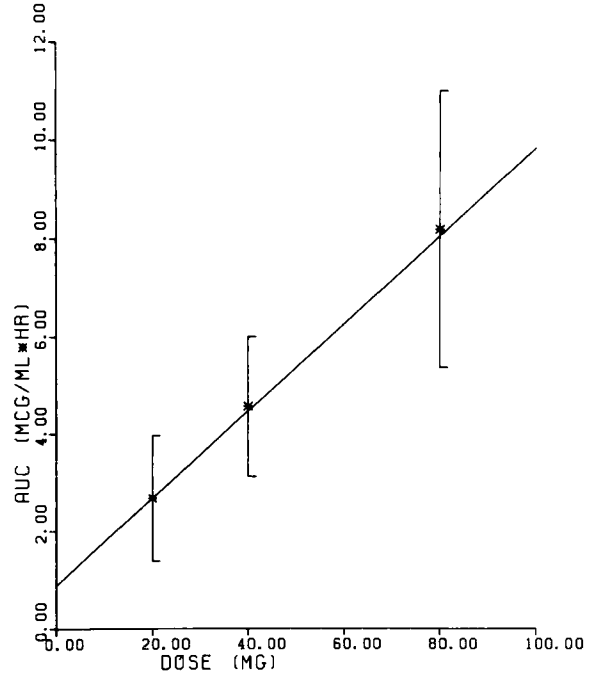

Figure 4. Mean (±S.D.) AUC versus orally administered doses of furosemide in 21 healthy adult males ($r = 0{\cdot}72$). Regression lines calculated from all individual data points

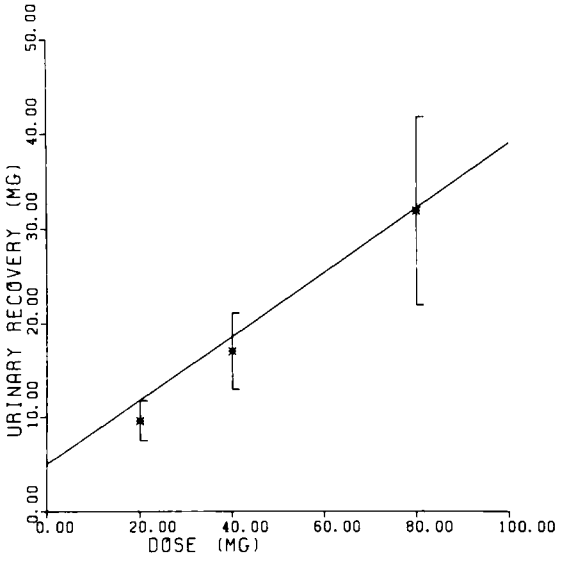

Figure 5. Mean (±S.D.) X_u versus orally administered doses of furosemide in 21 healthy adult males (r = 0·51). Regression lines calculated from all individual data points

DISCUSSION

Furosemide is primarily eliminated renally by secretion.[8] Theoretically, elimination of the drug may change with dose, as the secretion process is saturated. However, in this study the pharmacokinetics were linear over the dosage range of 20–80 mg. This agrees with earlier data by Cutler *et al.*[3] who studied the dosage range of 40–120 mg in four volunteers. While the disposition of furosemide may change when very large doses are given, its disposition is clearly not changed with the most commonly used doses as utilized in this study.

A large intra- and intersubject variability in the disposition of furosemide was demonstrated in this investigation. This variability has been previously observed in our laboratories and prompted the study of the reproducibility of disposition with a repeat 40 mg dose.[4] The reason for such variability has not been identified in our present work. However, several reasonable explanations can be offered. If furosemide is absorbed only in a very short segment of the upper gastrointestinal tract, variability in absorption kinetics may be influenced by gastric emptying time and/or transit time. Biliary recycling of furosemide has been previously suggested[4] as contributing to variability in disposition. The presence of secondary maxima in the plasma concentration–time curves of many subjects in this study resembles our previous observations.[4] Despite the intrasubject variability noted in the present data, there were not clinically significant differences in all pharmacokinetic parameters when two 40 mg doses were given. This demonstrates reproducibility of results regardless of possible variability in disposition due to reasons discussed above.

In summary, the disposition of furosemide is linear over the dosage range of 20–80 mg in healthy adult men. The administration of furosemide 40 mg on two separate days results in similar disposition parameters.

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REFERENCES

1. M. B. Burg, L. Stoner, J. Cardinal and N. Green, *Am. J. Physiol.*, **225**, 119 (1973).
2. J. B. Hook and H. E. Williamson, *J. Pharmacol. Exp. Ther.*, **149**, 404 (1965).
3. R. E. Cutler, A. W. Forrey, G. Christopher and B. M. Kimpel, *Clin. Pharmacol. Ther.*, **15**, 588 (1974).
4. E. S. Waller, S. F. Hamilton, J. W. Massarella, M. A. Sharanevych, R. V. Smith, G. J. Yakatan and J. T. Doluisio, *J. Pharm. Sci.*, **71**, 1105 (1982).
5. K. Carr, A. Rane and J. C. Frolich, *J. Chromatogr.*, **145**, 421 (1978).
6. A. L. Kerremans, Y. Tan, C. A. M. Van Ginneken and F. W. J. Gribman, *J. Chromatogr.*, **229**, 129 (1982).
7. R. E. Kirk, *Experimental Design: Procedures for the Behavioral Sciences*, Brooks Cole Publishing Co., Belmont, California, 1968, pp. 87–88.
8. R. E. Cutler and A. D. Blair, *Clin. Pharmacokinet.*, **4**, 279 (1979).