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DETERMINANTS OF RESPONSE TO FRUSEMIDE IN NORMAL SUBJECTS

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1 The pharmacokinetic and diuretic response of frusemide have been investigated in six normal subjects. Frusemide (80 mg) was administered (a) intravenously to unstressed subjects, (b) orally to unstressed subjects, (c) orally to sodium depleted subjects who had received 80 mg oral frusemide 36 h previously followed by a 20 mmol sodium, 160 mmol potassium diet.

2 After i.v. administration, the logarithmic plasma concentration-time curve was biexponential. There was a linear relationship between the frusemide plasma concentration in the β-phase of elimination and the rate of sodium excretion. Urinary clearance of frusemide was 60% of total plasma clearance, similarly recovery of frusemide in the urine over 36 h was 65% of the dose administered. These observations suggested an extrarenal route of elimination.

3 After oral administration there was also a linear relationship between frusemide plasma concentration and rate of sodium excretion. Oral bioavailability estimated from the ratio of the areas under the plasma concentration-time curve (AUC) and urine recovery over 36 h after i.v. and oral administration was approximately 50%, yet the diuretic response was similar.

4 The AUC of the β-phase after i.v. administration was similar to the total AUC after oral administration suggesting that response was related to drug present in a tissue pool rather than in plasma. After sodium depletion, there was no change in frusemide kinetics, however the diuretic response decreased. Once again, there was a significant relationship between plasma concentration and rate of sodium excretion. This relationship during the elimination phase after oral administration to sodium depleted subjects was significantly shifted to the right compared to the elimination phase after oral administration to unstressed subjects, suggesting a change in plasma concentration response.

5 In conclusion, the response to frusemide is determined by the concentration of drug in the tissue compartment. This response is modified by factors controlling sodium homeostasis.

Introduction

Frusemide is an anthranilic acid diuretic which acts on the ascending limb of Henle to induce a brief but potent diuretic response. Pharmacokinetic studies suggest that after oral administration approximately 50% of the dose is available to the systemic circulation in comparison to the intravenous route of administration (Calesnick, Christensen & Richter, 1966; Kelly, Cutler, Forrey & Kimpel, 1974; Beermann, Dalen, Lindstrom & Rosen, 1975). A relationship between plasma concentration of frusemide and diuretic response (Rupp, 1974) would suggest that oral administration should be associated with a smaller total diuretic response. However Kelly *et al.* (1974) observed an equal diuretic response when normal subjects received the same dose of frusemide orally and intravenously. This observation has been investigated in an attempt to understand factors which influence the relationship between the plasma concentration of frusemide and the magnitude of the diuretic response.

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Methods

Six normal healthy male volunteers (ages 20-25 years) gave informed consent for the protocol which was approved by the hospital ethical committee. Each subject received frusemide (80 mg) (Hoechst) by intravenous and oral administration on separate occasions. On a third occasion sodium deprivation was induced by frusemide (80 mg) orally, followed by a 20 mmol sodium, 160 mmol potassium diet for 36 h after which frusemide (80 mg) was again administered orally. After i.v. administration, urine and blood were obtained at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 240, 300 min and the urine excreted over the subsequent 31 h was collected. After both

oral administrations, blood and urine were obtained at 30, 45, 60, 90, 120, 150, 180, 240 and 300 min, together with subsequent urine passed over 36 h. Urine samples were spontaneously voided and blood samples were drawn from an indwelling needle in a peripheral brachial vein.

Plasma frusemide was measured by a modification of the method of Hadju & Haussler (1964). Plasma (1 ml) was acidified with 1N HCL (1 ml) and extracted into methylene chloride (10 ml). Methylene chloride (8 ml) was extracted with 0.05 M sodium borate buffer (3 ml), then the buffer (2 ml) was acidified by 1N HCL (1 ml) and the fluorescence determined using an Aminco Bowman spectrofluorimeter set at 350 nm excitation, and 416 nm emission. Standard curves were obtained by adding 0.5, 1, 2.5 an; 10 μg/ml to the blank plasma of each subject.

Urinary frusemide was measured by the method of Hadju & Haussler (1964). Thin layer chromatography of frusemide in urine was by the method of Haussler & Wicha (1965). Urine sodium and potassium were measured by flame photometry.

Calculations

The plasma decay curve of frusemide fits a biexponential function:

$$C_{p(t)} = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{Equation 1}$$

where $C_{p(t)}$ is the plasma concentration at time t, A and B are constants, α and β are the rapid and slow exponential constants with appropriate half-lives $T\frac{1}{2}(\alpha)$ and $T\frac{1}{2}(\beta)$.

$T\frac{1}{2}(\alpha)$ and $T\frac{1}{2}(\beta)$ have been calculated from least squares regression analysis of log plasma concentration v time relationship. Clearance was estimated from:

$$\text{Clearance} = \frac{\text{Dose}}{\frac{A}{\alpha} + \frac{B}{\beta}} \qquad \text{Equation 2}$$

The area under the plasma concentration curve (AUC) had been estimated using the trapezoidal method, and the volume of distribution of the slow phase of elimination ($V_{d(\beta)}$) from

$$V_{d(\beta)} = \frac{\text{Dose}}{B} \qquad \text{Equation 3}$$

Kinetic and dynamic parameters after each administration of frusemide have been compared by Student's paired *t*-test.

Results

The major metabolite of frusemide, 4-chloro-5-sulphamoyl-anthranilic acid did not cross react with the fluorimetric assay used for plasma estimations, but was quantitatively measured in the spectrophotometric urine assay. However, thin layer chromatography of urine from subjects after both intravenous or oral administration of frusemide failed to reveal significant amounts of this metabolite.

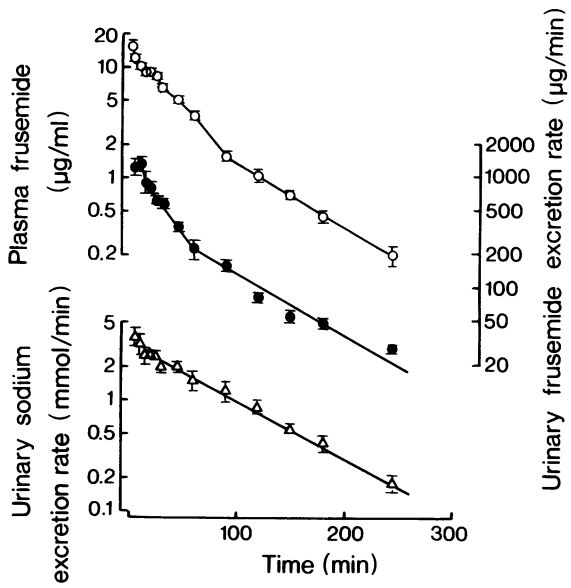

Figure 1 Mean ± s.e. mean changes in plasma frusemide concentration (○), urinary rate of frusemide excretion (●) and the rate of sodium excretion (▲) after intravenous administration of frusemide (80 mg) to six normal male subjects.

Following intravenous administration of frusemide to six normal subjects, the mean plasma concentration declined rapidly with a bi-exponential decay (Figure 1). The half-life of the late phase of elimination was 50 min with a volume of distribution of 11.9 litres. The mean total clearance was 125 ml/min, while mean urine clearance was 75 ml/min (Figure 2). The rate of excretion of frusemide in the urine was parallel to the plasma concentration decay curve. Recovery of the dose of frusemide administered from the urine over 36 h was 65% (Table 1). The diuretic response to frusemide, measured by the rate of sodium excretion, was brisk with a maximal response within 5 min; this then declined exponentially with a similar half-life to the plasma concentration curve. Thus there was a linear relationship between the plasma concentration of the drug after equilibration and the rate of sodium excretion (Figure 3). During the early phase of drug distribution and elimination there was less of a response for any given plasma concentration. The urine concentra-

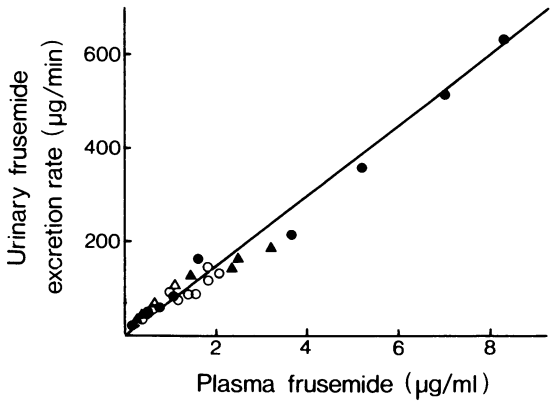

Figure 2 The relationship of the mean urinary rate of frusemide excretion and plasma frusemide concentration after administration of frusemide (80 mg) intravenously (●), orally to unstressed subjects (○) and orally to subjects who had been acutely sodium deprived (▲). The y intercept of slope of the regression is not significantly different from zero. The mean slope through zero provides a measure of urine clearance of 75 ml/min.

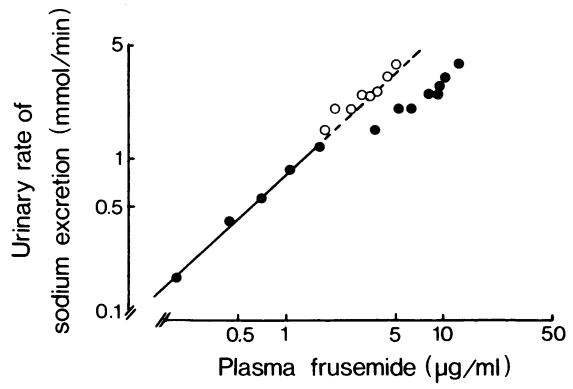

Figure 3 The relationship of the plasma concentration and urinary rate of sodium excretion after intravenous administration of frusemide (80 mg) to six normal subjects, (●) actual data points (confidence limits can be seen in Figure 1), (○) extrapolated plasma concentrations from the β phase. The regression line was calculated from the data during the terminal exponential of the plasma concentration-time curve (i.e. the last five points).

tion did not have any consistent relationship with diuretic response.

Following oral administration, the time of onset of absorption to time of peak plasma concentration was variable between subjects, however once absorption started the plasma concentration rapidly achieved a maximum and then declined exponentially with a mean half-life of 79 min. The variability in onset accounts for the broad based response of the mean results with a peak plasma concentration 90 min after drug administration (Figure 4). Bioavailability was assessed by comparison of the area under the plasma concentration-time curve (AUC) and the recovery of frusemide in urine. After oral and i.v. administration the ratio of the AUC was 0.49 and the ratio of the total recovery of frusemide in the urine over 36 h was 0.52 (Table 1). The urinary rate of frusemide excretion and the urinary rate of sodium excretion were parallel to the plasma concentration curve throughout the period under investigation (Figure 4), while the urine concentrations of frusemide had no consistent relationship with the diuretic response.

Table 1 The pharmacokinetics and diuretic responses (mean ± s.e. mean) over 5 h in six normal male subjects after administration of frusemide (80 mg) intravenously, orally and orally after acute sodium deprivation had been induced by previous frusemide administration 36 h prior to the study and maintained by a low sodium diet.

	Pharmacodynamics			*Pharmacokinetics*	
	Urine volume	*Sodium*	*Potassium*	*Area under plasma concentration-time curve*	*Frusemide recovery in urine over 36 h*
	(ml)	*(mmol)*	*(mmol)*	*(μg/ml h)*	*(mg)*
Intravenous	1979 ± 153	238 ± 16	45 ± 5	636 ± 19	52 ± 3.8
Oral	2023 ± 142	203 ± 10	48 ± 8	322 ± 49*	25 ± 2.9*
Oral after sodium deprivation	1312 ± 66*†	128 ± 10*†	50 ± 5	387 ± 38*	27 ± 1.7*

* $P < 0.05$ paired Student's t-test in comparison to intravenous administration
† $P < 0.05$ paired Student's t-test comparing oral administration to oral administration after sodium deprivation

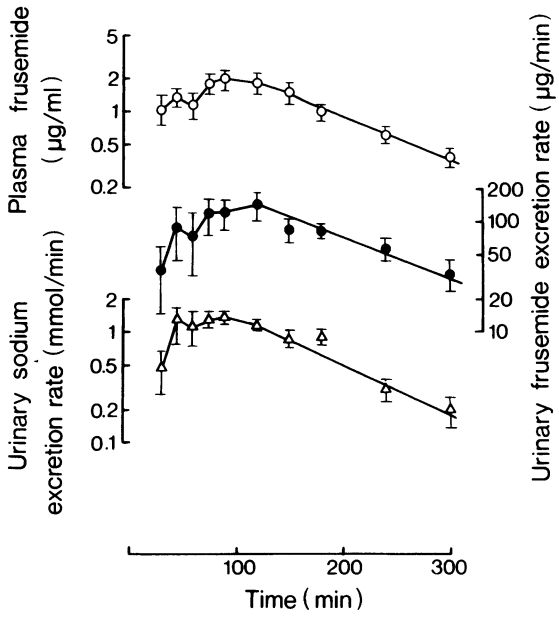

Figure 4 Mean ± s.e. mean changes in plasma frusemide concentration (○), urinary rate of frusemide excretion (●) and the rate of sodium excretion (△) after oral administration of frusemide (80 mg) to six normal unstressed male subjects.

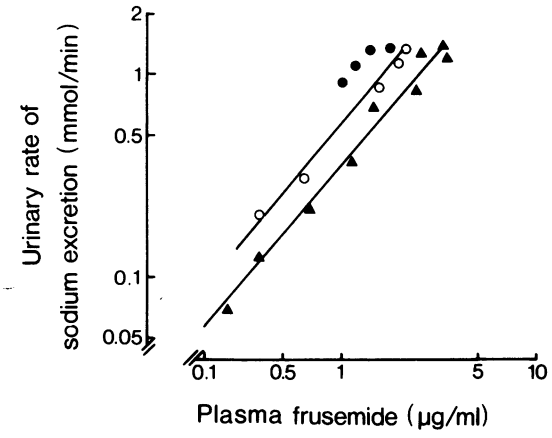

Figure 5 The relationship of the plasma frusemide concentration and urinary rate of sodium excretion after oral administration of frusemide (80 mg) to six normal male subjects.
(●) absorption phase in subjects not previously stressed
(○) decay phase in subjects not previously stressed
(▲) decay phase in subjects who had received frusemide (80 mg) 36 h earlier to induce acute sodium deprivation. The sodium deficit was maintained by a 20 mmol sodium, 160 mmol potassium diet during the intervening 36 h.

Administration of frusemide (80 mg) orally followed by a low sodium diet for 36 h induced a sodium deficit of 233 ± 12.7 mmol (mean ± s.e. mean). After the second administration of frusemide orally, the onset of diuretic response was early in all subjects. The mean peak plasma concentration was achieved 45 min after drug administration, it then declined exponentially with a mean half-life of 68 min. There were no significant differences in AUC or in recovery of frusemide in the urine between the two oral doses of frusemide (Table 1).

In contrast to the pharmacokinetic parameters, the diuretic response of urine volume, sodium excretion and potassium excretion were similar after oral and i.v. administration to unstressed subjects. However there was a significant one third decrease in urine volume and sodium excretion

Table 2 Regression analysis of log transformed relationship between frusemide plasma concentration and rate of sodium excretion in six normal subjects after administration of frusemide (80 mg) (a) intravenously, (b & c) orally, (d) orally after acute sodium depletion.

	Intravenous	*Oral*		
		Unstressed		*Sodium depleted*
	β phase (a)	*Absorption (b)*	*Decay (c)*	*Decay (d)*
Number of data points	5	3	5	7
r value	0.999	0.996	0.997	0.990
y Intercept	1.819*	−0.993	−0.908†	−1.987†‡
Slope	0.91*	1.40	1.21	1.20

* $P < 0.05$ in comparison to c and d
† $P < 0.05$ in comparison to b
‡ $P < 0.05$ in comparison to c

after oral administration of frusemide to sodium deprived subjects (Table 1).

After both oral doses of frusemide the relationship between plasma concentration and rate of sodium excretion was similar to that observed after i.v. administration. There was a highly significant correlation between the plasma concentration during the elimination phase after oral administration to unstressed subjects and the rate of sodium excretion (Table 2). Although there were only few data points during the absorption phase, these demonstrated hysteresis with a greater response for individual plasma concentrations than during the elimination phase (Figure 5). After oral administration of frusemide to sodium deprived subjects, the linear regression between plasma concentration and rate of sodium excretion was parallel but significantly shifted to the right in comparison to the elimination phase after oral administration to unstressed subjects (Table 2, Figure 5).

Discussion

The intensity of response following administration of many drugs is directly related to drug concentration at the site of action, with the duration of effect being dependent on the rate of elimination. Thus a study of the relationship between the pharmacokinetics and pharmacodynamics of frusemide after administration by different routes and under different conditions might allow an understanding of factors which influence variation in response. The results of the present study suggest that the response to frusemide is related to the concentration of drug in a tissue compartment, and that this response can be influenced by additional factors which are involved in determining sodium homeostasis.

Using a spectro-fluorimetric assay to estimate plasma concentration of frusemide in normal subjects; half-life, volume of distribution and total plasma clearance were similar to previously reported studies which have utilized both fluorimetric and ^{35}S labelling techniques (Kelly *et al.*, 1974; Rupp & Zapf, 1973; Beermann *et al.*, 1975). The spectrophotometric assay used to measure urinary frusemide estimated total sulphonamide nucleus, it therefore measured both parent drug and metabolites. However, thin layer chromatography failed to demonstrate significant quantities of 2-amino-4-chloro-5-sulphamoyl anthranilic acid, after either oral or intravenous administration. Although this compound is thought to be the major metabolite of frusemide (Haussler & Wicha, 1965), other attempts to find it in man have also been unsuccessful (Beermann *et al.*, 1975). It is possible that other metabolites might have been present but the majority of the drug estimated was probably parent compound.

Following intravenous administration, the plasma concentrations were initially high and rapidly fell due to both distribution and elimination. After approximately 100 min the drug appeared to be in equilibrium between plasma and tissues, with the terminal plasma decay curve representing elimination only. The ratio of recovery of frusemide in the urine to the dose administered, and the urine and plasma clearances suggested a significant extrarenal route of elimination of approximately 40%. This might be by metabolism, direct biliary excretion which is less likely as only small quantities of [^{35}S]-frusemide were detected in jejunal juice aspirates (Beermann *et al.*, 1975) or it might be secreted into the colon. Whatever the mechanism, the total extrarenal route of elimination assumes importance in patients with renal disease as it is able to increase and compensate to some extent for the decline in renal elimination (Cutler *et al.*, 1974).

The initial high plasma concentration was matched by a high rate of excretion of sodium. This was maximal within a few minutes and then declined exponentially in parallel to the β-phase of the plasma concentration curve. Thus during the early distribution phase, the rate of sodium excretion was more closely related to the concentration extrapolated from the β-phase of elimination curve rather than to the actual plasma concentration itself. During the late phase of elimination the rate of sodium excretion was linearly related to the plasma concentration (Figure 3). If the disposition of frusemide can be described by a two compartment system, these observations would imply that the response was related to the amount of drug in the tissue compartment (Wagner, Aghajanian & Bing, 1968), however in physiological terms there are probably numerous compartments with the rapidity of onset of action suggesting that the site of action is a compartment receiving a high rate of drug delivery. A similar linear relationship has also been observed between the urinary excretion rate of ^{203}Hg mercaptomerin sodium and urine flow rate (Levy, Calsnick & Wase, 1964).

The rate of excretion of frusemide in the urine was parallel to the plasma concentration during both the early and late phases of elimination. In contrast, the initially high urine concentration fell and then as the diuresis diminished rose again. So that neither the rate of urinary excretion nor the urinary concentration of frusemide related to the response throughout the whole period of the diuresis.

After oral administration, a systemic bioavailability of approximately 50% was suggested by the

ratio of the recovery of frusemide in the urine and the AUC after oral and intravenous administration (Table 1). This is rather lower than 65% reported by Beermann *et al.* (1975), or 60% reported by Kelly *et al.* (1974).

With a low bioavailability and a similar relationship between plasma concentration and rate of sodium excretion after both oral and i.v. administration (Figure 4), oral administration would be expected to induce a smaller diuretic response. However, the diuretic response over 5 h was not significantly different when the same dose of frusemide was given by the two routes of administration (Table 1). This is in contrast to when frusemide is administered orally as different formulations with or without meals. When the drug is given by the same route of administration, similar AUCs are associated with equal diuretic responses even when there are different shapes of the plasma concentration-time curves (Kelly *et al.*, 1974).

If the response to frusemide is dependent on drug in a tissue compartment rather than the concentration of drug in the plasma, then from the viewpoint of response, bioavailability will depend on the amount of drug distributed into that tissue. Thus although the ratio of the AUC after oral and intravenous administration defines the pharmacokinetic systemic bioavailability of a drug (Koch Weser, 1974), for frusemide the ratio of the AUC of the β-phase after i.v. administration (402 μg/ml min) and the AUC after oral administration (322 μg/ml min) define bioavailability in terms of the pharmacological response.

The range of response to an individual dose of frusemide is wide, (Stason, Cannon, Heinman & Laragh 1966), and there is also a wide range in response when one subject is treated with the same dose over a period of time. These variations have been attributed to variations in activity of the homeostatic mechanisms for sodium (Nickolls, Espiner, Donald & Hughes, 1974). In the present study, acute sodium deprivation prior to drug administration was able to influence the plasma concentration-response relationship. The changes in this relationship were rapid enough to detect an hysteresis effect when comparing the absorption phase to the decay phase after the first oral dose of frusemide. In the decay phase after frusemide administration to sodium depleted subjects the regression line of the concentration response relationship was further shifted to the right (Figure 5), thus with increasing sodium deprivation there was a decrease in response to any given plasma concentration of frusemide without requiring any change in the pharmacokinetics of frusemide. These changes in the plasma concentration-response curves are suggestive of competitive inhibition of an apparent renal receptor. It does not, however, provide information on the mechanism of inhibition.

In conclusion, this study had demonstrated a close relationship between the concentration of frusemide in a tissue compartment and the rate of sodium excretion in the urine. Furthermore, this relationship can be influenced by acute sodium depletion.

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