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Pharmacokinetics of Furosemide in Man After Intravenous and Oral Administration. Application of Moment Analysis

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Summary. Furosemide 40 mg was administered to 8 healthy subjects as an i.v. bolus dose, as 1 tablet in the fasting state, and as 1 tablet and a solution after food intake. The i.v. data gave a total body clearance of 162 ± 10.8 ml/min and a renal clearance of 117 ± 11.3 ml/min; the volume of distribution at steady state was 8.3 ± 0.6 l. Oral administration gave a bioavailability of the tablet (fasting) of 51%. Food intake slightly reduced the bioavailability, but not to a significant extent. There was no significant difference in availability between the tablet and the solution. Moment analysis gave a mean residence time after the i.v. dose, $MRT_{i.v.}$, of 51 ± 1.5 min. The mean absorption times (MAT) for all oral doses were significantly longer than the $MRT_{i.v.}$, indicating absorption rate-limited kinetics of furosemide. On average, food delayed the absorption by 60 min. The MAT for the tablet in the postprandial state was significantly longer than for the solution, indicating dissolution rate-limited absorption of the tablet.

Key words: furosemide; bioavailability, pharmacokinetics, oral administration, i.v. administration, drug absorption, moment analysis, food effect, dissolution effect

Furosemide is a weak acid (pKa 3.9), which is absorbed incompletely from the gastro-intestinal tract. There have been reports of considerable intra- and interindividual variability in its bioavailability [1, 2], and speculations about the cause of its low and variable bioavailability have mentioned site-specific absorption or dissolution problems [3].

As a weak acid, furosemide could be absorbed both from the stomach and from the intestine. In spite of an unfavourable pH for absorption, most weak acids are absorbed mainly from the intestine, because of its greater absorptive area. In the rat, however, Chungi et al. [3] have shown a greater absorptive capacity for furosemide from the stomach than from the intestine despite the much smaller absorptive area of the former. Whether this might also be the case in humans has not been confirmed.

The presence of food delays stomach emptying and the absorption of drugs that are mainly taken up from the intestine. Drugs absorbed from the stomach are influenced by food to a much smaller extent. In the present study furosemide was given both fasting and postprandially to investigate whether the stomach was an important absorption site, and also to compare the common experimental situation of giving the drug in the fasting state with a clinically more relevant situation, i.e. when taken together with food.

The rate of absorption is frequently described by the peak plasma concentration (C_{max}) and the time to reach this concentration (t_{max}). These measurements are rather rough estimates and provide meagre information about the absorption process itself. The use of statistical moments for this type of calculation has many advantages, as it clearly defines and separates the rates of absorption and dissolution from the rate of disposition. This important information cannot be obtained from C_{max} and t_{max} calculations alone.

Inter- and intraindividual differences in absorption after an oral dose could be due to physiological differences in gastrointestinal motility and/or to differences in the kinetic behaviour of the drug. It was decided, therefore, to present individual data in order to give a more realistic picture of the kinetics of furosemide in humans.

Materials and Methods

Subjects

8 healthy subjects (5 women, 3 men) volunteered for the study. They were between 22 and 32 years old and weighed 55 to 82 kg. The study was approved by the Ethics Committee at the University Hospital of Uppsala. The volunteers all signed written consent for participation in the study.

Study Design

The volunteers participated on four different experimental days, at least one week apart, according to the following scheme. All experiments started at 8 a.m.

Furosemide 40 mg was given in a randomized cross-over design as

1. i.v. bolus dose to fasting subjects (Lasix® injection solution 10 mg/ml).
2. Orally (Lasix 40 mg tablet) to fasting subjects.
3. Orally (Lasix 40 mg tablet) after breakfast.
4. Orally as a solution (Lasix injection solution 10 mg/ml) after breakfast.

The fasting subjects had had no food for 10 h preceding the experiment. In them the oral dose was taken with 200 ml of water. The breakfast consisted of tea, two rolls with butter and cheese and one egg. The oral doses after breakfast were given with 200 ml orange juice.

As standardized lunch (one portion of meat balls or chicken) was given after 4 h. The total Na^+ and K^+-intakes were 48 and 30 mmol, respectively. 200 ml water was given every hour for 8 h.

Venous blood samples were taken through an indwelling catheter at 0, 2.5, 5, 7.5, 10, 12.5, 22.5, 37.5, 52.5, 67.5, 82.5, 105, 135, 165, 210, 270, 330, 390 and 450 min and 24 h after administration of the i.v. bolus dose. Urine was collected by spontaneous voiding in fractions at 0–15, 15–30, 30–45, 45–60, 60–75, 75–90, 90–120, 120–150, 150–180, 180–240, 240–300, 300–360, 360–420, 420–480 min, 480 min–24 h and 24–32 h.

After the oral doses, venous blood samples were taken at 0, 15, 30, 45, 67.5, 82.5, 97.5, 112.5, 127.5, 142.5, 165, 195, 225, 255, 285, 315, 345, 390 and 450 min and 24 h. The corresponding urine samples were collected in fractions at 0–30, 30–60, 60–75, 75–90, 90–105, 105–120, 120–135, 135–150, 150–180, 180–210, 210–240, 240–270, 270–300, 300–330, 330–360, 360–420, 420–480 min, 480 min–24 h and 24–32 h. Thus, a blood sample was always taken at the mid-point of a urine collection interval.

Analysis of Plasma and Urine

The concentrations of furosemide in plasma and urine were measured by HPLC with fluorimetric (excit. 345 nm, emiss. 418 nm) and UV (280 nm) detection, respectively.

The extraction procedure and the sensitivity of the UV detection are described elsewhere [4]. 0.2 ml plasma and 0.5 ml urine were used in the assay.

The fluorimetric assay had a lower sensitivity limit of 10 ng/ml for a sample volume of 0.2 ml plasma.

Pharmacokinetic Symbols and Calculations

Plasma concentrations:

$$C = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} + C \cdot e^{-\gamma \cdot t} \quad \text{Eq. (1)}$$

The area under plasma concentration-time curves (AUC) was calculated using the trapezoidal rule. The residual area ($t = 450$ to ∞) was calculated as C_{450}/γ.

Total plasma clearance is composed of renal plus non-renal clearances:

$$Cl = \frac{Dose}{AUC_\infty} = Cl_r + Cl_{nr} \quad \text{Eq. (2)}$$

Renal clearance:

$$Cl_r = \text{Amount excreted unchanged}/AUC_\infty \quad \text{Eq. (3)}$$

Apparent volume of distribution:

$$V_{d,\,area_\beta} = Cl/\beta \quad \text{Eq. (4a)}$$

$$V_{d,\,area_\gamma} = Cl/\gamma \quad \text{Eq. (4b)}$$

Apparent volume of distribution at steady state:

$$V_{d,\,ss} = \frac{Dose \cdot \int_0^\infty t \cdot Cdt}{(AUC_\infty)^2} \quad \text{Eq. (5)}$$

Differential equations describe furosemide kinetics after the i.v. dose according to Fig. 2:

$$C = \frac{X_c}{V_c} \quad \text{Eq. (6)}$$

$$\frac{dX_c}{dt} = -(k_{12} + k_{13} + k_m + k_e) \cdot X_c + k_{21} \cdot X_S + k_{31} \cdot X_D \quad \text{Eq. (7)}$$

$$\frac{dX_S}{dt} = k_{12} \cdot X_c - k_{21} \cdot X_S \quad \text{Eq. (8)}$$

$$\frac{dX_D}{dt} = k_{13} \cdot X_c - k_{31} \cdot X_D \quad \text{Eq. (9)}$$

$$\frac{dX_u}{dt} = k_e \cdot X_c \quad \text{Eq. (10)}$$

X = amount; C = concentration in plasma; V_c = volume of the central compartment

Bioavailability:

$$BA\% = \frac{AUC_{p.o.,\infty} \cdot 100}{AUC_{i.v.,\infty}} \quad \text{Eq. (11)}$$

Model-independent Treatment of Data

To interpret the data on oral administration the method of statistical moments was used [5, 6], which has the advantage of being independent of a specific pharmacokinetic model. It gives valuable information about the overall properties of the time courses of absorption and disposition processes in the body.

The mean residence time (MRT) is defined as the mean time for the intact drug molecules to transit through the body and includes a composite of all kinetic processes:

$$MRT = \frac{\int_0^\infty t \cdot Cdt}{\int_0^\infty Cdt} = \frac{AUMC_\infty}{AUC_\infty} \quad \text{Eq. (12)}$$

The mean absorption time (MAT) is a measure of the rate of absorption and can be calculated as the difference between the MRT for extravascular administration and the MRT for intravenous administration:

$$MAT = MRT_{extravasc.} - MRT_{i.v.} \quad \text{Eq. (13)}$$

The mean dissolution time (MDT) can be calculated in the same manner, as the difference between the MAT for a solid dosage form and the MAT for a solution:

$$MDT = MAT_{solid} - MAT_{soln.} \quad \text{Eq. (14)}$$

It was decided to calculate the difference in absorption time between the fasting and postprandial states and to call it the mean gastrointestinal transit time (MGT), as a representation of the delay in absorption caused by food intake:

$$MGT = MAT_{postprandially} - MAT_{fasting} \quad \text{Eq. (15)}$$

If these processes were to behave like log-normally distributed curves, the mean times (MRT, MAT, MDT and MGT) by definition [6] would be the times for 63.2% of the amount of drug present to be eliminated, absorbed, dissolved or transported by the processes involved.

Data Analysis

The experimental data were fitted to the model by the non-linear least squares programs DARE-MINUIT [7] and NONLIN.

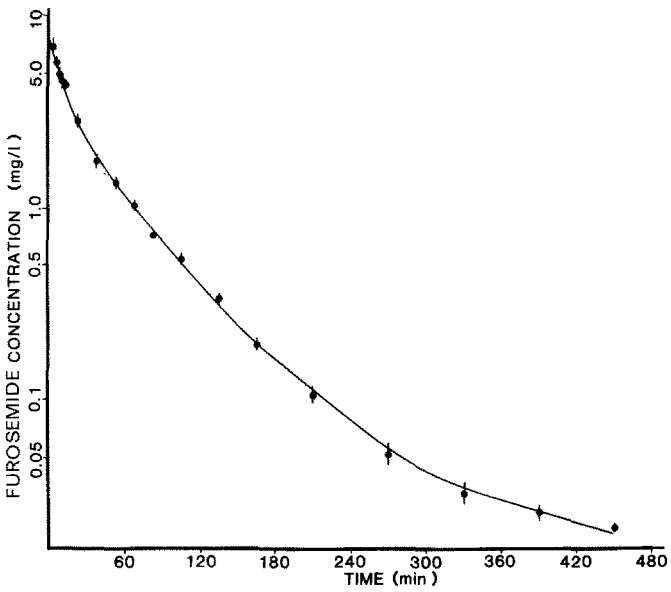

Fig. 1. Plasma furosemide concentrations following intravenous administration of 40 mg to healthy subjects. Mean ± SEM. The solid line represents the fit according to a triexponential equation

Exponential or differential equations were used. Plasma and urine data were fitted simultaneously to obtain a more reliable estimate of the parameters of the differential equations.

Several runs were performed with different initial estimates to avoid local minima in the sum of squared surfaces; the data were given different weights (1.0, 1/y, $1/y^2$, 1/SD) to obtain the best fit. The significance of differences of and between data was determined using conventional statistical methods, such as linear regression, analysis of variance and *t*-tests. The goodness of fit of computed to observed data was assessed by determination of the coefficient of correlation (r), coefficient of determination (r^2) and standard error of estimate (SEE) of the parameters or the coefficients of variation (C.V.%).

Results

I. V. Bolus Dose

A triexponential function Eq. (1) gave the best fit to the individual data after intravenous injection. The mean data are shown in Fig. 1 and the resulting pharmacokinetic parameters in Table 1. The terminal phase of the plasma-concentration-time curve is usually called the biological half-life of the drug. In this case, the terminal phase (γ) contributed to the total AUC by only 10%, whereas the β-phase contributed to the AUC by 72%. Thus, the β-phase is

Table 1. Pharmacokinetic data of furosemide 40 mg given intravenously

Constant/ coefficient	Parameter estimate ± SEE	Half-life	% of AUC
A [mg/l]	4.33 ±0.32		
α [min^{-1}]	0.0954 ±0.0079	7.3 min	18
B [mg/l]	3.39 ±0.18		
β [min^{-1}]	0.0190 ±0.00049	36.4 min	72
C [mg/l]	0.0882 ±0.00074		
γ [min^{-1}]	0.00341 ±0.00046	3.39 hrs	10

Table 2. Rate constants (min^{-1}) for i.v. bolus dose of furosemide according to the model depicted in Fig. 2. Mean values: weight 1/SD

Rate constant	Parameter estimate ± SEE
k_{12} [min^{-1}]	0.0232 ±0.00203
k_{21} [min^{-1}]	0.0409 ±0.00244
k_{13} [min^{-1}]	0.00334 ±0.000068
k_{31} [min^{-1}]	0.00131 ±0.000022
k_m [min^{-1}]	0.00713 ±0.000169
k_e [min^{-1}]	0.0231 ±0.000168
V_c [l]	5.45 ±0.19

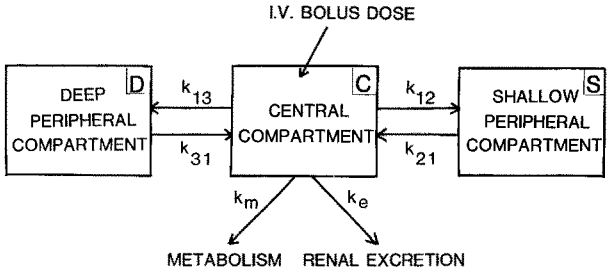

Fig. 2. Compartmental model for furosemide following intravenous administration with renal and non-renal elimination taking place from the central compartment

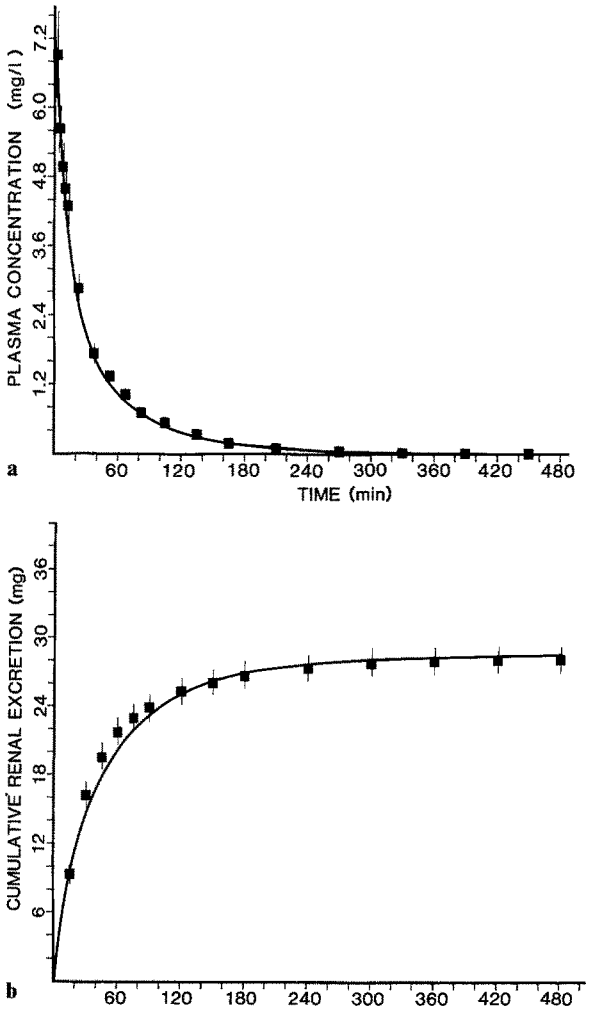

Fig. 3a, b. Furosemide plasma concentrations and cumulative renal excretion following intravenous administration of 40 mg to healthy subjects. Mean ± SEM. The solid line represents simultaneous fit of the model in Fig. 2 to both plasma and urine data

here considered to represent the biological half-life of furosemide and was calculated to be 36 min.

The total plasma clearance of furosemide was 162 ± 10.8 ml/min. Of this, the renal clearance was 117 ± 11.3 ml/min (72%) and the extrarenal clearance (metabolism) was 45 ml/min (Table 3). The volume of distribution, $V_{d,ss}$; Eq. (5) was found to be 8.3 ± 0.6 l while $V_{d,\beta}$ was 8.5 l and $V_{d,\gamma}$ was 47.5 l.

The simultaneous fitting of the differential equations; Eqs. (7–10) to plasma and urine data enabled us to conclude that renal excretion took place from the central compartment; the model is depicted in Fig. 2 and the resulting fit to the data is shown in Fig. 3 a and b. The simultaneous fitting to the two datasets gave a coefficient of determination of 0.989. The rate constants are shown in Table 2 together with the standard errors of the parameters estimated.

Oral Doses

The mean plasma concentration-time curves and the cumulative renal excretion curves for the three oral doses can be seen in Fig. 4 a and b, and the individual plasma concentration-time curves are depicted in Fig. 5. As can be seen in Fig. 5, there was considerable interindividual variation in the shapes of the curves, especially for the tablet given to fasting subjects. The maximal concentration after this dose (C_{max}) showed a threefold range of 0.52 to 1.7 mg/l, and the time for maximal concentration (t_{max}) varied fivefold between 30 and 142.5 min. Many of the curves had two distinct peaks.

The presence of food dramatically changed the shapes of the curves for most individuals. The maximal plasma concentration was reduced and t_{max} was

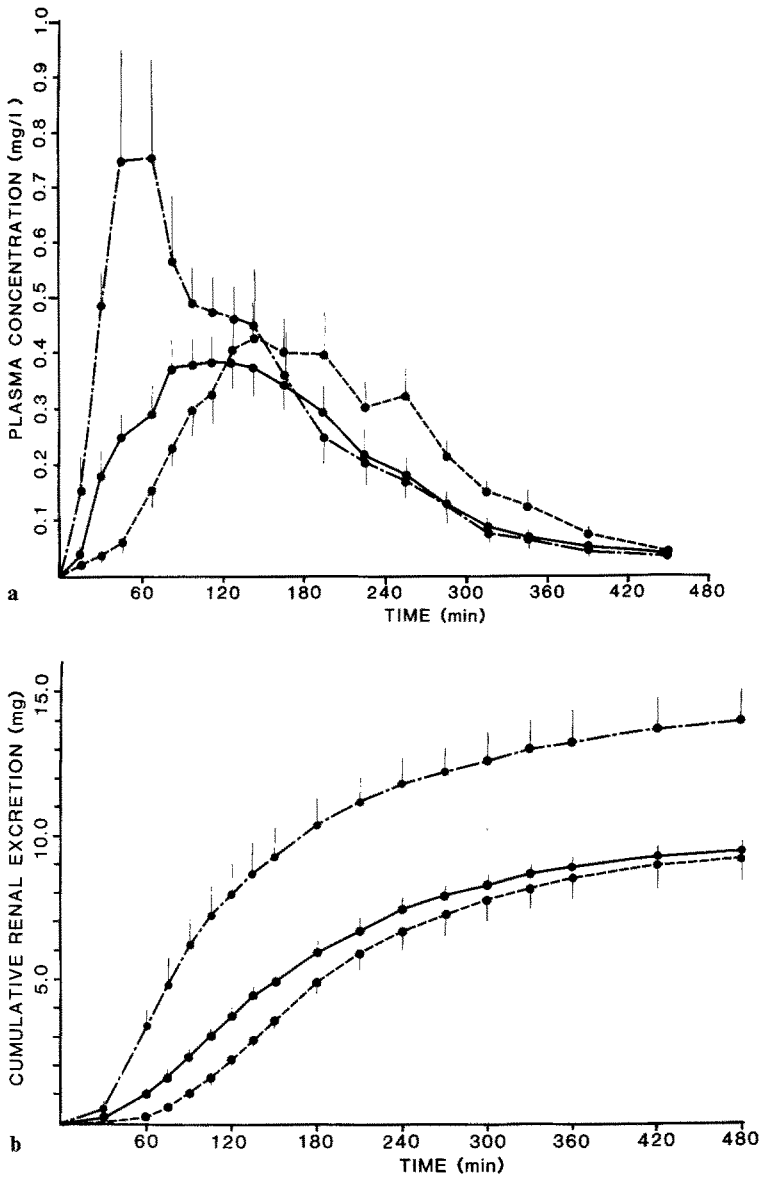

Fig. 4a, b. Furosemide plasma concentrations and cumulative renal excretion following oral doses of 40 mg to healthy subjects. Mean ± SEM. - - - - - - tablet, fasting; — — — tablet postprandially; ———— solution postprandially

delayed. For the tablet given postprandially C_{max} varied between 0.23 and 0.77 mg/l, and t_{max} varied between 112.5 and 195 min. For the postprandial solution C_{max} varied between 0.32 and 0.64 mg/l and t_{max} varied between 45 and 225 min.

Extent of Absorption

The oral availability was calculated according to Eq. (11). Due to the pronounced interindividual differences in the AUCs, a significant difference was not found between the oral doses, although there was a tendency towards lower values after food intake (Table 4; Fig. 6), as the bioavailability was 51.3, 43.3 and 37.4%, for tablet fasting, postprandial tablet and postprandial solution, respectively.

Two-way analysis of variance of the AUCs did not show any significant difference between fasting and non-fasting subjects nor between tablet and solution. When comparing all three types of administration, there was a small significant difference between the subjects ($F=3.18$, $p<0.05$). There was no significant difference when paired doses were compared.

Rate of Absorption

I. Model-independent Approach by Moment Analysis. The calculated model-independent parameters MRT, MAT, MDT and MGT, Eqs. (12–15) are listed in Table 5. The mean residence time after intrave-

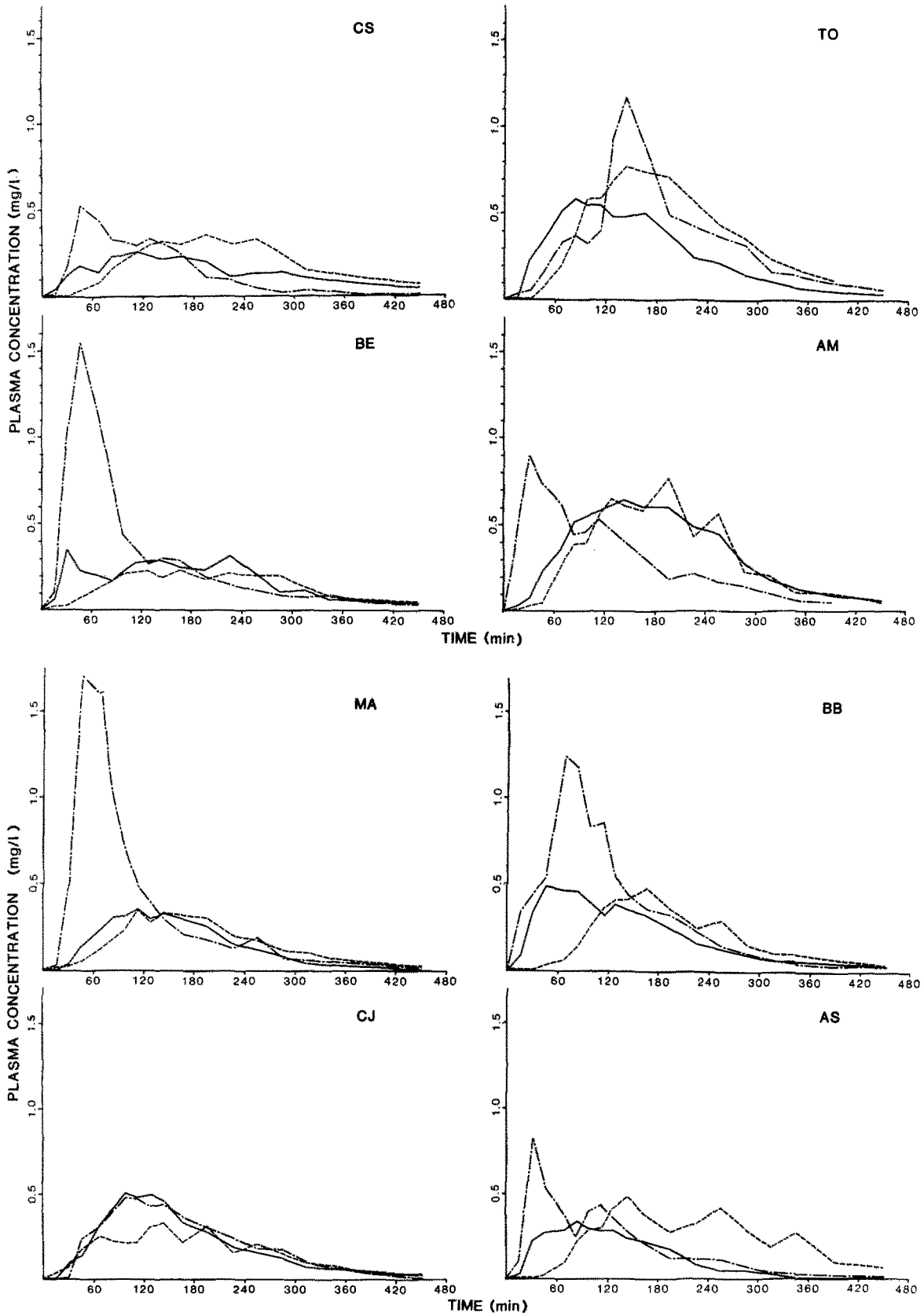

Fig. 5. Individual plasma concentrations of furosemide after oral doses of 40 mg to healthy subjects: - - - - - - tablet, fasting; — — — tablet postprandially; ———— solution postprandially

Table 3. Total plasma clearance, renal clearance and steady-state volume of distribution for furosemide after intravenous and oral administration of 40 mg

Subject	Cl [ml/min]	Renal clearance, Cl_r [ml/min]				$V_{d,ss}$ i.v. dose	
	i.v. dose	i.v. dose	tablet fasting	tablet postprand.	solution postprand.	[l]	[l/kg]
CS	140.0	95.7	207.0	107.0	136.0	6.91	0.125
BE	218.0	186.0	116.0	98.4	125.0	10.7	0.173
TO	174.0	125.0	106.0	78.4	104.0	10.1	0.123
AM	127.0	81.6	121.0	83.7	62.3	6.54	0.122
MA	185.0	128.0	121.0	127.0	160.0	9.26	0.127
CJ	146.0	116.0	149.0	91.5	150.0	7.28	0.112
BB	136.0	96.4	112.0	112.0	121.0	6.20	0.101
AS	171.0	105.0	155.0	78.2	166.0	9.48	0.153
Mean	162.0	117.0	136.0	97.1	128.0	8.31	0.129
±SEM	10.8	11.3	11.9[a]	6.19[a,b]	12.0[a,c]	0.62	0.008

[a] N.S. compared with i.v. dose; [b] $p<0.02$ compared with tablet, fasting; [c] $p<0.05$ compared with tablet, postprandially

Table 4. Bioavailability and renal excretion of furosemide after intravenous and oral administration of 40 mg

Subject	AUC [mg·min/l] i.v.	Bioavailability [%]			Amount eliminated unchanged in urine [mg] in 32 h			
		tablet fasting	tablet postprand.	solution postprand.	i.v.	tablet fasting	tablet postprand.	solution postprand.
CS	286.0	32.0	26.7	32.0	27.4	16.9	7.53	10.6
BE	183.0	80.5	40.5	48.3	34.1	14.7	6.74	9.85
TO	230.0	73.0	79.7	52.7	28.8	17.0	13.6	11.0
AM	315.0	41.3	47.8	50.5	25.7	13.2	11.3	8.48
MA	217.0	69.2	33.6	30.7	27.6	15.8	8.33	8.91
CJ	273.0	27.2	36.1	26.5	31.7	8.52	8.08	8.94
BB	295.0	51.5	31.4	33.6	28.4	15.2	9.72	9.89
AS	233.0	35.8	50.9	24.8	24.5	10.4	8.56	8.07
Mean	254.0	51.3	43.3	37.4	28.5	16.1	10.1	11.2
±SEM	±15.9	±7.24	±5.94[a]	±3.99[a]	1.09	±0.97	±0.83[b]	0.40[b]

[a] N.S. compared with tablet, fasting; [b] $p<0.001$ compared with tablet, fasting

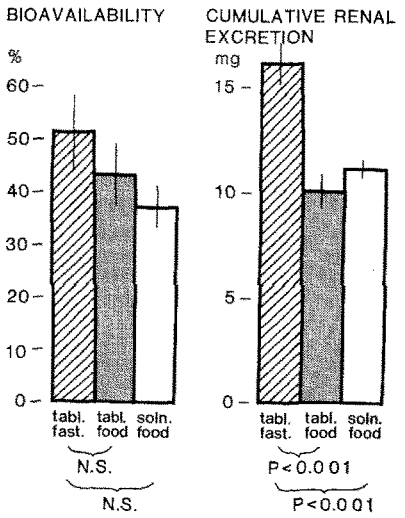

Fig. 6. Bioavailability and cumulative renal excretion over 32 h of furosemide given orally

nous administration ($MRT_{i.v.}$) showed very low interindividual variability and averaged 51 ± 1.5 min.

The MATs for all the oral doses are significantly longer than the MRT for the intravenous dose. This is evidence that furosemide kinetics after oral doses are rate-limited by absorption, so-called flip-flop behaviour.

The greatest interindividual variability in MAT was found for tablets given to fasting subjects (C.V. 28%), whereas the presence of food seemed to diminish the interindividual differences in the rate of absorption, even though the solution was more variable than the tablet (C.V. 19% vs 8%).

After giving tablets to fasting subjects, the mean absorption time was 84 ± 8.4 min, which was 60 min shorter than after food intake (144 ± 3.9 min). This shows that food intake significantly delayed the absorption process of furosemide ($p<0.001$), the delay being represented by the mean gastrointestinal transit time, MGT.

Table 5. Statistical moment analysis of furosemide after intravenous and oral doses (minutes)

Subject	$MRT_{i.v.}$	MAT tablet, fasting	MAT tablet, postprand.	MAT solution postprand.	MDT postprand.	MGT
CS	51.2	118.0	126.0	111.0	15.3	8.7
BE	48.4	68.2	150.0	126.0	24.8	82.2
TO	59.5	124.0	138.0	98.2	40.1	14.6
AM	51.9	73.9	140.0	131.0	8.9	65.8
MA	49.7	60.5	136.0	109.0	26.6	75.1
CJ	51.2	79.8	158.0	130.0	28.1	78.2
BB	44.9	79.4	150.0	99.8	50.4	70.8
AS	54.3	67.7	157.0	69.6	87.2	89.1
Average	51.4	83.9	144.0	109.0	35.2	60.6
±SEM	1.50	8.37[a]	3.93[a, b]	7.25[a, c, d]	8.74	11.0
±C.V.	8.25%	28.2%	7.69%	18.8%	70.3%	51.2%

[a] $p<0.001$ compared to MRT i.v.; [b] $p<0.001$ compared to MAT, tablet fasting; [c] $p<0.001$ compared to MAT, tablet postprand.; [d] $p<0.05$ compared to MAT, tablet fasting

Table 6. Comparison of compartmental analysis (one-compartment model) and statistical moment approach for oral administration of furosemide 40 mg. For a flip-flop model comparison should be made between $MRT_{i.v.}$ and $1/k_a$ and MAT and $1/K_E$, respectively

Subject	$MRT_{i.v.}$	Tablet, fasting			Tablet, postprandially			Solution, postprandially		
		$1/k_a$	MAT	$1/K_E$	$1/k_a$	MAT	$1/K_E$	$1/k_a$	MAT	$1/K_E$
CS	51.2	66.2	118.0	102.0	86.2	126.0	105.0	46.3	111.0	133.0
BE	48.9	–	68.2	–	100.0	150.0	130.0	90.1	126.0	107.0
TO	59.5	66.7	124.0	169.0	80.0	138.0	172.0	51.8	98.2	113.0
AM	51.9	7.19	73.9	131.0	57.5	140.0	202.0	65.4	131.0	169.0
MA	49.7	–	60.5	–	79.4	136.0	133.0	47.4	109.0	114.0
CJ	51.2	20.9	79.8	81.3	104.0	158.0	158.0	79.4	130.0	106.0
BB	44.9	–	79.4	–	51.3	150.0	193.0	20.3	99.8	125.0
AS	54.3	–	67.7	–	97.1	157.0	175.0	45.9	69.6	83.3

The mean absorption time (MAT) of the solution was significantly shorter than of the tablet ($p<0.001$), indicating that, in the postprandial state, the solution was absorbed more rapidly than the tablet. The difference between these two MATs gives the mean in vivo dissolution time (MDT) for the tablet when food is present, and was calculated to be 35 ± 8.7 min. It indicates that absorption is rate-limited by transport across the gastrointestinal membranes or stomach emptying, as the MDT was shorter than the MAT for the solution.

II. Comparison Between Conventional Compartmental Analysis and Moment Analysis. For all subjects and modes of administration, except BE, MA, BB and AS given tablets fasting, a one-compartment model could be fitted to the oral data (the fasting curves for BE, MA, BB and AS exhibited multiexponential characteristics). This was done to compare these results with those obtained by moment analysis. According to the definition [6] for linear models, the $MRT_{i.v.}$ is usually equal to $1/K_E$ (K_E 'elimination rate' constant) and the MAT should be equal to $1/k_a$ (k_a 'absorption rate' constant).

The results (Table 6) show that for furosemide in most cases there was a good correlation between MAT and $1/K_E$ and $MRT_{i.v.}$ and $1/k_a$, respectively, proving that the elimination kinetics of furosemide after oral administration is absorption rate-limited (flip-flop).

Simulating curves from the individual values of $MRT_{i.v.}$ and MAT gave as good a fit to the data as the model approach, but was considerably less time-consuming than the compartmental analysis, and also gave a more reliable answer about which process was rate-limiting.

Renal Elimination

The renal elimination of unchanged furosemide in the 32 h following its administrations is shown in Table 4 and Fig. 6. A significant decrease ($p<0.001$) in the amount of furosemide eliminated in the urine can be seen on comparing the fasting and non-fasting states. No significant difference could be found

between the tablet and solution after food intake. The renal clearance (Table 3) was not significantly lower for any of the oral doses as compared to the intravenous bolus dose. Food, therefore, had no obvious influence on the renal clearance of furosemide. In some cases the differences in renal clearance between the oral doses reached significance (Table 3).

Discussion

Intravenous Administration

The pharmacokinetics of furosemide in healthy volunteers has previously been studied by several authors [1, 2, 8, 9, 10, 11, 14, 15]. The present results, with an average total plasma clearance of 162 ml/min, renal clearance of 117 ml/min and a volume of distribution of 8.3 l are in good agreement with those reports. However, probably due to the frequent and prolonged blood sampling in our schedule, it was found that the furosemide concentration in plasma declined in a triexponential manner, yielding half-lives for the β- and γ-phases of 36 min and 3.3 h, respectively, in contrast to the biexponential decline with a terminal half-life of 50–80 min (β-phase) previously reported [2, 8, 9, 11]. Rupp [10] also found a triexponential decline in the plasma concentrations. The γ-phase identified here contributed only 10% to the AUC. We believe that the longer half-life for the terminal phase reported by others is probably a consequence of mixing the true β-phase and the unidentified γ-phase.

By simultaneously fitting the model to plasma and urine data (Fig. 3 a, b), furosemide elimination was found to occur exclusively from the central compartment. In such a case, the volume of distribution at steady state is a kinetic parameter which is independent of the elimination rate constant [12]. Theoretically, the calculated apparent volume of distribution ($V_{d,\,area}$) for a two-compartment model should not differ by more than 10% from the $V_{d,\,ss}$ [13]. When $V_{d,\,area}$ was calculated using the final slope γ, the volume of distribution was 47.5 l, whereas the second slope, β, yielded an apparent volume of distribution of 8.5 l. It can thus be concluded that $V_{d,\,area,\,\beta}$ was a more appropriate estimate of the apparent volume of distribution than $V_{d,\,area,\,\gamma}$, since it had about the same magnitude as $V_{d,\,ss}$, although it was not calculated from the terminal slope.

The simultaneous fit of the three-compartment model to plasma and urine data indicated that the rate of renal excretion was directly proportional to the plasma concentration. This is of importance when discussing the relationship between the diuretic effect and drug level at the site of action. A subsequent paper will deal with the findings about the relationship between the plasma and urine concentrations of furosemide and its diuretic effect.

Oral Administration

A mean oral availability in healthy volunteers of between 37 to 51% was found depending on the mode of administration. It was slightly, but not significantly decreased by food intake. Smith et al. [1] found an absolute availability of 43% on administering tablets to fasting subjects, whereas Waller et al. [2] calculated it to be 64% for the solution and 71% for the tablet. Other authors have found a bioavailability of furosemide of 50 to 75% [8, 9, 14, 15]. In the present study the bioavailability for tablets both with and without food varied about threefold between individuals, whereas the postprandial solution showed a twofold variability (Table 4).

There was a pronounced difference between subjects in the shapes of the curves for tablets given in the fasting state (Fig. 5). This was also observed by Waller et al. [2]. The maximal concentration varied threefold and t_{max} varied fivefold. The variation might be a consequence of differences in stomach emptying between individuals, with a consequent influence on the absorption of furosemide from the intestine. In that case the overall absorption rate would be determined by the rate of transfer of the drug from the stomach to the duodenum. Gastric emptying is influenced by the volume in the stomach, type of content (solid or liquid) and by humoral control. Waller et al. [2] and Smith et al. [1] have suggested that the second peak phenomenon might be a consequence of enterohepatic cycling of furosemide. Clements et al. [16], in a paper describing acetaminophen absorption, have evaluated gastric emptying patterns. The occurrence of multiple peaks in the plasma concentration curves was explained by the interruption of gastric emptying. In our study double peaks were seen especially after giving furosemide to fasting subjects. The presence of food seemed to diminish the interindividual differences and the irregularities in gastric emptying, as the plasma concentration curves were more uniform and smoother. Furthermore, the differences in t_{max} between individuals after food intake were not so pronounced as after tablets given to fasting subjects. The plasma concentrations produced by the doses given postprandially remained close to the maximal concentration for a considerable time, making the estimation of t_{max} uncertain.

Chungi et al. [3] have described furosemide absorption in the rat. They concluded that there was greater absorptive capacity for furosemide from the

stomach than the small intestine despite its smaller surface area. Their explanation for this finding was pH-dependence of the absorption of furosemide. The assumption that absorption occurs at different rates from two or more sites in the gastrointestinal tract could be adapted to our mean data for the tablet taken fasting (Fig. 4a), with a fast rise in the curve together with a smaller second peak. The large inter-individual differences in t_{max}, however, contradict the theory that absorption preferentially takes place from the stomach. The fast rise in the curve for some of the subjects might instead be explained as 'bolus' emptying of a fraction of the dose into the duodenum [16].

Treating the data for oral administration by moment analysis has many advantages in evaluating the rate of absorption of drugs. The approach is independent of any assumption about which model is most accurate for the drug investigated. This is important for a drug like furosemide, which shows multicompartmental disposition and irregular shapes of the oral curves. By this approach it proved possible to distinguish between the influence of food and solid dosage form on the absorption rates of furosemide in a manner not feasible by any other means.

The results show that the mean residence time in the body after the intravenous dose was 51 min (Table 5). The mean absorption time when giving tablets to fasting subjects was 84 min. Furosemide thus remains longer in the gastrointestinal tract than in the rest of the body ($p < 0.001$), which is a criterion of flip-flop kinetics, i.e. after oral administration furosemide kinetics is rate-limited by absorption.

The MAT for the tablet given after food intake was significantly longer than the corresponding MAT in the fasting state (144 vs. 84 min; $p < 0.001$). This difference is called the mean gastrointestinal transit time. Thus, on average, the presence of food delayed absorption of a tablet by 60 min. The mechanisms for this might be delayed gastric emptying with subsequent slower transfer of the drug from the stomach to the duodenum. Food might also influence the dissolution rate of the tablet, although this is less probable.

The MAT for the solution was significantly shorter than that for the tablet in the postprandial state (109 vs. 144 min; $p < 0.001$). However, as this MAT is longer than the MDT, transmucosal transfer rather than dissolution of the tablet is the rate-limiting step. The value of the MDT (35 min), is an in vivo measurement. It is the difference between the behaviour of the solution and the tablet and does not indicate anything about how the solution behaves in the stomach. Furosemide might be precipitated, as its solubility is low. If this were the case, the 'true' MDT would be longer. It should also be pointed out that this comparison was made in the postprandial state.

The compartmental analysis does not give any definite clue to which is the rate-limiting process in the overall kinetics of furosemide. The reason for this is that the absorption and elimination rate constants (Table 6) are quite close to each other and that the existence of the γ-phase for the i.v. data complicates interpretation of the oral doses, i.e. which phase is elimination and which absorption. However, from the model-independent approach with moment analysis, it was concluded that the kinetics of furosemide was rate-limited by absorption. Another draw-back of the compartmental analysis is that the data are smoothed when calculating k_a, the absorption rate constant. This constant represents a first-order rate process, which is seldom in accordance with reality considering the physiology of the absorption process with stomach emptying and dissolution processes as possible determining factors.

It is possible to speculate why the oral bioavailability is only about 50%. The moment analysis showed that absorption from the lumen in the gastrointestinal tract was the determining factor in overall absorption. Some authors have theorised that furosemide absorption might be site-specific [3]. This theory is neither contradicted nor confirmed by the present study.

Furosemide is known to be conjugated in the liver to a glucuronide [2, 8, 17], which is in part excreted via the bile into the duodenum. Rupp [10] has shown that within 5 days after intravenous administration of 35-S-furosemide, 12% of the activity was found in the faeces as unchanged drug. This figure seems reasonable, as in our study 28% of the clearance was non-renal. Part of the glucuronide is also eliminated in the urine (14% of the dose according to refs. [2 and 17]). The furosemide glucuronide is probably deconjugated in the intestine by the bacterial flora. This part of the drug could subsequently be reabsorbed, as suggested by Waller et al. [2], but contradicting this possibility is the finding that for oral doses as much as 50% of a dose has not been absorbed when it has reached the more distal parts of the intestine. What is excreted via the bile and deconjugated would then be only a minor part of the total drug present. Thus, biliary or some other type of excretion of furosemide into the intestine seems primarily to be an elimination pathway.

From a clinical point of view this investigation has indicated that the presence of food makes the bioavailability and rate of absorption of furosemide less variable between individuals. Food also diminishes the sharp peak in plasma concentration which is of importance for the diuretic effect.

By using statistical moment analysis it was possible to determine that absorption is the rate-limiting step for furosemide kinetics after oral administration, and also that dissolution is not the rate-limiting step for absorption of the tablet. By this approach, the delaying influence of food intake on furosemide absorption could also be defined. It is concluded from these data that in man furosemide is mainly absorbed from the intestine and that transmucosal transport problems may be the principal cause of its low bioavailability.

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