BIOPHARMACEUTICS & DRUG DISPOSITION, VOL. 3, 345–359 (1982)

DOSE-DEPENDENT PHARMACOKINETICS OF FUROSEMIDE IN THE RAT

M. M. HAMMARLUND* AND L. K. PAALZOW

Department of Biopharmaceutics and Pharmacokinetics, University of Uppsala, Biomedical Center, Box 580, S-751 23 Uppsala, Sweden

ABSTRACT

The pharmacokinetics of furosemide were investigated in the rat at doses of 10 and 40 mg kg^{-1} corresponding to doses of 80 and 320 mg given to humans based on body surface area. A three-compartment open model with renal excretion taking place from the shallow peripheral compartment gave the best fit to the data. The terminal half-life of furosemide was found to change from 29 min for the 10 mg kg^{-1} dose to 49 min for the 40 mg kg^{-1} dose. This change could be detected as a change in the apparent volume of distribution caused by decreased protein binding at increasing plasma concentrations of furosemide. The total plasma clearance did not change significantly although metabolic and renal clearances both changed. The renal clearance was found to be dependent on the free fraction of furosemide in plasma and thus increased with increasing plasma concentrations. The metabolic clearance decreased with increasing dose indicating a saturable metabolism of furosemide.

KEY WORDS Furosemide Pharmacokinetics Rat Plasma Urine i.v.

INTRODUCTION

Furosemide, *N*-furfuryl-4-chloro-5-sulfamoylanthranilic acid, is a potent diuretic agent with interesting kinetic properties in humans. It is widely used in clinical practice and many studies have been performed on the pharmacokinetics of this drug in humans both in normal and diseased states (for review see Benet[1] and Cutler and Blair[2]). The pharmacokinetic data from animal studies are, however, sparse.[3–5]

Among the characteristic kinetic properties of furosemide are a high protein binding[2] and an active renal secretion via the organic acid secretory mechanism.[6–8] The excretion of unchanged drug into the urine is about 50–70 per cent of the intravenous dose[1] and the metabolism is reported to consist of a glucuronidation step with subsequent biliary elimination of part of the glucuronide. After an intravenous dose, from 6–18 per cent is found in the

*Addressee for reprints: Dr. Margareta Hammarlund, Department of Biopharmaceutics and Pharmacokinetics, University of Uppsala, BMC, Box 580, S-751 23 Uppsala, Sweden.

0142–2782/82/040345–15$01.50 *Received 5 April 1982*

 Revised 7 June 1982

faeces of healthy subjects.[9] The oral availability is incomplete and about 60–70 per cent in healthy subjects.[1, 2]

Furosemide exerts its effect by inhibition of the active chloride transport in the thick ascending limb of the loop of Henle.[6] It has been proposed that the site of action is located at the luminal side of the nephron,[7] which indicates that furosemide has to be excreted into the tubule to be able to exert its effect. This finding also indicates that a correlation between the diuretic effect and the renal excretion rate of furosemide can be expected rather than a relation to the plasma concentration. This has also been reported.[3, 10–13]

Consequently, processes that might decrease or increase furosemide excretion to its site of action will also affect the obtained response. The possibilities of kinetic influences on the effect of furosemide are obvious. Furosemide is therefore an interesting model drug for basic studies on the interrelationships between kinetic parameters and physiological variables.

The rat was chosen as model species for this study. In this investigation the basic pharmacokinetic properties of furosemide will be presented.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats, weighing 200 ± 28 g (S.D.) were used throughout the study. The rats had free access to food and water until the start of the experiment.

During the experiment, the rats had free access to water. No other attempt was made in this study to compensate for urinary fluid losses during the experiment.

The rats were divided into the following experimental groups.

Intravenous bolus injection—Group I

Two hours before the experiment, the rats were implanted with two silicon rubber cannulae (Silastic, 0·02 in i.d.; 0·037 in o.d.) in the jugular veins under light ether anaesthesia, allowing an exact amount of intravenous furosemide to be administered and serial sampling of blood to be done (0·4 ml). Each sample was replaced by an equal volume of saline. The maximum cumulative blood volume withdrawn from any one animal never exceeded 10 per cent of the total blood volume. During the experiment the rats were conscious and unrestrained.

Furosemide 10 or 40 mg kg^{-1} was given as a bolus dose, and blood samples were taken at 1·0, 2·5, 5·0, 7·5, 10.0, 12·5, 17·5, 22·5, 30·0, 42·5, 60·0, 85·0, 140·0, and 210·0 (only 40 mg kg^{-1}) min.

The samples were immediately protected from light and centrifuged and the plasma was frozen ($-20°$) until analysed.

The renal excretion of furosemide was not measured in this group of rats.

Renal excretion—Group II

Two days before the administration of the furosemide dose, the rats were implanted under light ether anaesthesia with silicon rubber cannulae in the jugular veins as described for Group I. Via an incision in the abdomen, the urinary bladder was opened and cannulated with a catheter (PE 205). It was tied so that the dead space in the bladder became as small as possible and another thread was tied to close the urethra. The venous and urinary catheters were exteriorized through the abdomen via a metal fistula extending about 6 cm outside the skin.

The rat was placed in a cage with a track through which the fistula was protruded. The urine could then be collected directly into a small plastic vial which was attached to the fistula under the cage. The rat was freely movable along the track. Two rats were placed in each cage. This arrangement made it possible to take blood and urine samples at exact points of time without stressing the rat.

The day before the experiment, the rat was allowed to rest and normal urinary output was measured. On the day of the experiment 1·0, 2·5, 10, 20, 40, or 100 mg kg^{-1} of furosemide were given as i.v. bolus doses through the venous cannula. Urinary samples were collected at 5, 10, 15, 20, 25, 35, 50, 70, 100, 180, and 240 (only 40 mg kg^{-1}) min. During the collection, the vial was protected from light. The samples were weighed and frozen until analysed. Control blood samples were taken in order to compare the plasma concentrations of this group of rats with those of the Group I rats.

Protein binding

The binding of furosemide to rat plasma albumin was studied by equilibrium dialysis at 37° for 7 h[14] within the concentration range 11–425 μg ml^{-1} of furosemide. Fresh rat serum was used. No addition of heparin was made to the blood during the collection as this was shown to dramatically influence the binding of furosemide to albumin.

Chemical assay

Furosemide was determined in plasma and urine by a high-performance liquid chromatographic method with u.v.-detection according to Lindström.[15] Some modifications were made. The procedure was adapted to small plasma or urine samples (0·1–0·3 ml). The mobile phase consisted of 40 per cent acetonitrile in phosphate buffer, pH 2·5, 0·02 *M*. A column (250 × 4·6 mm i.d.) packed with ODS-Hypersil C_{18} 5 μm, Shandon, was used. This gave a retention time for furosemide of 7 min with a flow of 1·0 ml min^{-1}. The minimum detectable quantity (MDQ) was 1·2 ng (injected amount) or $3{\cdot}6.10^{-12}$ moles, which, according to this method, corresponds to a concentration in the sample of 0·15 μg ml^{-1} with a sample volume of 0·1 ml.

Effort was made to keep the samples out of light and to standardize the extraction procedure in order to minimize the hydrolysis of furosemide to the

hydrolytic product 4-chloro-5-sulfamoylanthranilic acid in acidic milieu[16] or in the light.

Furosemide was kindly supplied by Hoechst, Ltd, Sweden.

Pharmacokinetic analysis

Exponential or differential equations were fitted to the plasma and urine concentration–time data by the non-linear least squares regression program, NONLIN, and run on an IBM 370 computer.

The data points were given different weights (equal, $1/y$, $1/y^2$). Several runs with different initial estimates were performed to avoid local minima in the sum of square surfaces. Significance for and between data was obtained by using conventional statistical methods such as linear regression, analysis of variance, and *t*-tests. The goodness of fit of computed data to observed data was based on coefficient of correlation (r), coefficient of determination (r^2), and standard deviation of parameters.

Pharmacokinetic symbols and their calculations

Plasma concentration data:

$$C_p = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} + C \cdot e^{-\gamma \cdot t} \tag{1}$$

Area under the plasma concentration–time curve:

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma} \tag{2}$$

Apparent volume of distribution:

$$V_{d,area} = \frac{Dose}{\gamma \cdot AUC} \tag{3}$$

Apparent volume of distribution of the central compartment:

$$V_c = \frac{Dose}{A+B+C} \tag{4}$$

Total plasma clearance:

$$Cl = \frac{Dose}{AUC} = \text{renal clearance} + \text{non-renal clearance} \tag{5}$$

Elimination half-life:

$$t_{\frac{1}{2},\gamma} = \frac{0{\cdot}693 \cdot V_{d,area}}{Cl} \tag{6}$$

Rate of renal excretion at pseudo-equilibrium:

$$\frac{dA_e}{dt} = \text{renal clearance} \cdot C_p \tag{7}$$

Unbound plasma clearance:

$$Cl_u = \frac{Cl}{f_u} \quad (f_u = \text{fraction unbound in plasma}) \tag{8}$$

Unbound volume of distribution:

$$V_{d,u} = \frac{V_{d,area}}{f_u} \tag{9}$$

RESULTS

Selection of proper dose to the rat

In the experiments on rats in Group II, the cumulative volume of urine found up to 180 min after the administration of furosemide, was plotted against the logarithm of the dose (Figure 1). A sigmoid dose–response curve was obtained with a maximum urine volume of 14 ml obtained at doses of 40 mg kg^{-1} or more.

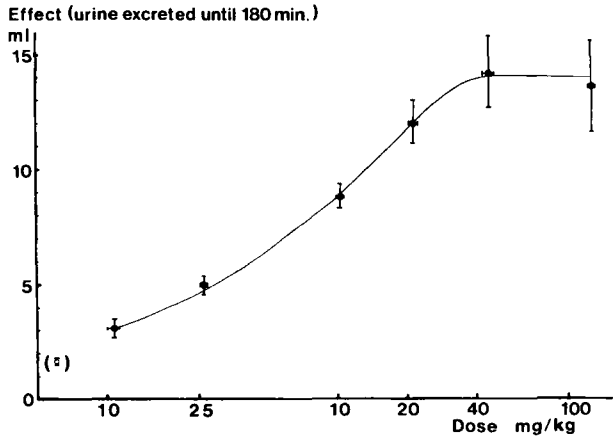

Figure 1. Dose–response curve of furosemide. Volume of urine (ml) excreted for 180 min plotted against log dose. Mean values ±S.E.M. (Φ represents the normal urine volume excreted for the same period)

From this dose–effect curve 10 and 40 mg kg^{-1}, representing half-maximal and maximal effect, were chosen for the plasma concentration studies. The lower dose corresponds to a human dose of 80 mg based on body surface area. The magnitude of the greatest possible response is, of course, influenced by our choice not to compensate for urinary fluid losses.

Calculations based upon total plasma concentration data

Plasma levels of furosemide and pharmacokinetic interpretation. The time course of furosemide concentrations in plasma (C_p) after i.v. administration of

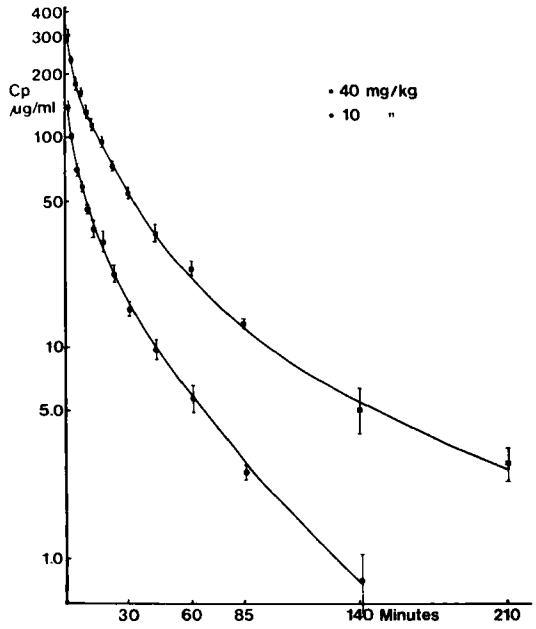

Figure 2. Plasma concentration of furosemide after intravenous adminstration. Each point represents the mean ±S.E.M., $n = 5$ (10 mg kg^{-1}), $n = 6$ (40 mg kg^{-1}). The solid line represents the curve fit to a tri-exponential equation (1)

10 and 40 mg kg^{-1} to the rats in Group I is shown in Figure 2. A triexponential equation (1) gave the best fit to the plasma data. The obtained coefficients and exponents of this equation are shown in Table 1. The computer fit of the data gave coefficients of determination (r^2) of 1·000 as shown in Table 1. The three rate constants of furosemide disposition (α, β, γ), all decreased significantly

Table 1. Pharmacokinetic data of furosemide given intravenously to rats. Mean values ±S.D.

Dose mg kg^{-1}	Constants A (µg ml^{-1})	α (min^{-1})	B (µg ml^{-1})	β (min^{-1})	C (µg ml^{-1})	γ (min^{-1})	$t_{\frac{1}{2}\gamma}$ (min)	r^2
10	106·0 ±28·9	0·640 ±0·149	70·8 ±8·6	0·0863 ±0·0097	21·5 ±5·2	0·0240 ±0·0033	28·9	1·000
40	139·0 ±27·8	0·312 ±0·098*	165·0 ±27·6	0·0557 ±0·0043†	37·1 ±15·4	0·0141 ±0·0042*	49·2	1·000

*$p < 0·01$.
†$p < 0·001$.

Table 2. Apparent volumes of distribution and total plasma clearance of furosemide based on plasma data from i.v. bolus doses. Mean values ± S.D.

Dose (mg kg^{-1})	V_c (ml)	$V_{d,area}$ (ml)	Cl (ml min^{-1})
10	11·7 ± 2·69	49·1 ± 12·3	1·18 ± 0·212
40	26·6 ± 6·92†	106 ± 28·7*	1·50 ± 0·345

*$p < 0{\cdot}01$.
†$p < 0{\cdot}001$.

when the dose was increased from 10 to 40 mg kg^{-1}. The volume of distribution ($V_{d,area}$, V_c) was significantly increased while the total plasma clearance was slightly increased, although not statistically significant (Table 2).

Consequently, the significant increase in the apparent volume of distribution was directly reflected in an increase of the biological half-live ($t_{\frac{1}{2},\gamma}$, (6)).

Renal excretion of furosemide. The renal excretion of unchanged furosemide was evaluated at the six dose levels for the animals of Group II. Despite the intravenous mode of administration, maximum excretion rate was not obtained until the interval 5–10 min following injection.

In Figure 3 the cumulative percentage of the dose that was eliminated unchanged into the urine up to 180 min was plotted against the dose. The part of the dose that was eliminated via the kidneys was increased in a linear manner with the logarithm of the dose, and rose from 27 per cent at 1·0 mg kg^{-1} to 38 per cent at 100 mg kg^{-1} ($F = 8{\cdot}67$, $p < 0{\cdot}01$). The increase was not significant between 10 and 40 mg kg^{-1} ($p > 0{\cdot}05$).

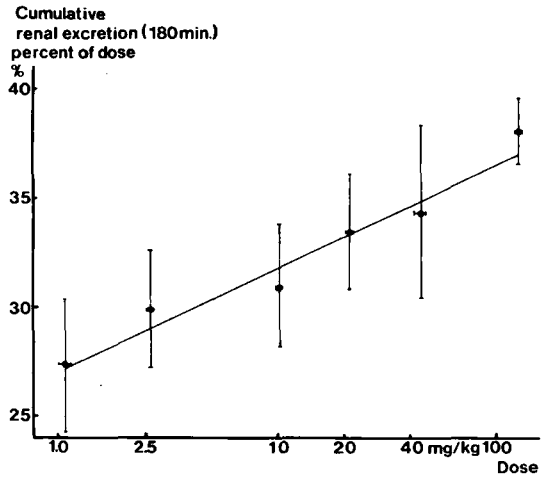

Figure 3. The cumulative excretion of unchanged furosemide into the urine up to 180 min expressed as a percentage of the dose. Mean values ± S.E.M.

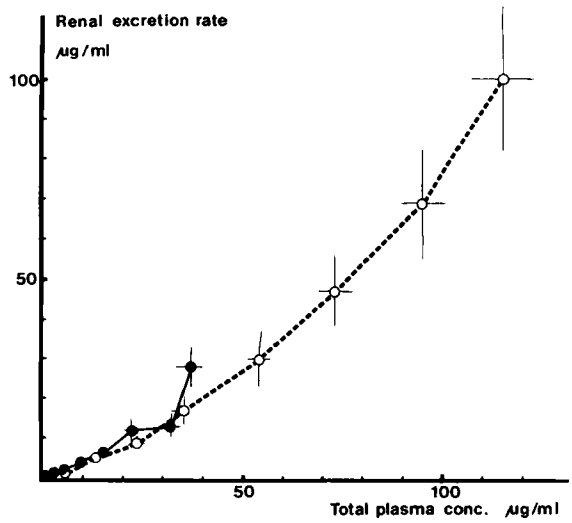

Figure 4. Plot of the renal excretion rate of furosemide against the total plasma concentration at pseudo-equilibrium. Mean values ±S.E.M.

For 10 and 40 mg kg^{-1}, the plot of furosemide excretion rate against the plasma concentration during pseudo-equilibrium (from 12·5 min) is shown in Figure 4 (equation (7)). This figure indicates that the renal clearance increases with the plasma concentration. This finding is in good agreement with the increase in the percentage of the dose that is excreted unchanged via the kidneys with increasing dose as shown in Figure 3.

Overall pharmacokinetic modelling of furosemide kinetics in the rat. An overall picture of the behaviour of total furosemide concentrations in the rat was accomplished by simultaneously using both plasma levels and values of cumulative urinary excretion in the calculation of the possible kinetic model. The best fit to the data was accomplished by a three-compartment open model with the renal excretion taking place from the shallow peripheral compartment. The model is depicted in Figure 5. The results obtained from the final computer fit are listed in Table 3.

The renal excretion rate constant k_{20} was found to be significantly increased ($p<0·01$) when the dose was increased from 10–40 mg kg^{-1} which is in

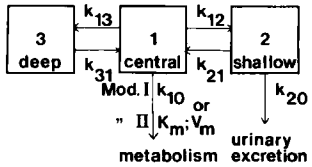

Figure 5. Three-compartment open model of furosemide. k_{20} represents the renal excretion rate constant. In Model I, linear kinetics is applied for the elimination from the central compartment (non-renal elimination) while in Model II Michaelis–Menten kinetics is used for this step

Table 3. Rate constants of the three-compartment open model I depicted in Figure 5. Mean values +S.D.

Dose (mg kg^{-1})	Rate constants (min^{-1}) k_{12}	k_{21}	k_{13}	k_{31}	k_{10}	k_{20}	r^2
10	0·203 ±0·017	0·178 ±0·026	0·0188 ±0·0079	0·0239 ±0·0106	0·0701 ±0·0028	0·0311 ±0·0034	1·000
40	0·110 ±0·012**	0·216 ±0·054	0·228 ±0·0052	0·0282 ±0·0079	0·0359 ±0·0026†	0·0505 ±0·0104*	1·000

*$p<0·01$.
†$p<0·001$.

accordance with the increased renal clearance (Figure 4). The rate constant k_{12} and k_{10} (non-renal elimination rate constant) were both found to be significantly decreased ($p<0·001$) whereas all the other rate constants remained unchanged with the dose.

These results thus give an indication of a saturable metabolism of furosemide. In order to clarify this finding, Michaelis–Menten kinetics was tried for the metabolic step in the calculations. A similar value for V_m and K_m, repectively, was obtained for the two doses as can be seen in Table 4. However, due to the large number of parameters, the standard deviation of the parameter estimates of V_m and K_m was large. The other rate constants of the model remained unchanged compared to the data obtained using only first order rate constants (Table 3).

This Michaelis–Menten type of the three-compartment model provided an excellent fit to both plasma and cumulative renal excretion data (Figure 6).

Data treatment based upon unbound drug levels in plasma

Protein binding. The equilibrium dialysis at serum concentrations of 11–425 μg ml^{-1} of furosemide showed that the free fraction increased linearly with increasing total drug concentration according to the relationship $f_u\% = 0·020 \cdot C_p + 0·67$ ($r^2 = 0·985$), valid between 11 and 425 μg ml^{-1}, i.e. an increase in the free fraction from 0·92 to 9·1 per cent within this concentration range (Figure 7).

Table 4. Rate constants, maximum rate of metabolism V_m, and the Michaelis–Menten constant K_m according to Model II depicted in Figure 5. Mean values +S.D.

Dose (mg kg^{-1})	Rate constants (min^{-1}) k_{12}	k_{21}	k_{13}	k_{31}	k_{20}	V_m (μg min^{-1})	K_m (μg ml^{-1})	r^2
10	0·229 ±0·022	0·173 ±0·037	0·0377 ±0·0244	0·0264 ±0·0148	0·0267 ±0·0062	66·8 ±69·2	45·8 ±74·6	1·000
40	0·114 ±0·013	0·207 ±0·060	0·0451 ±0·0391	0·0254 ±0·0290	0·0471 ±0·0136	92·8 ±297·0	37·7 ±215·0	1·000

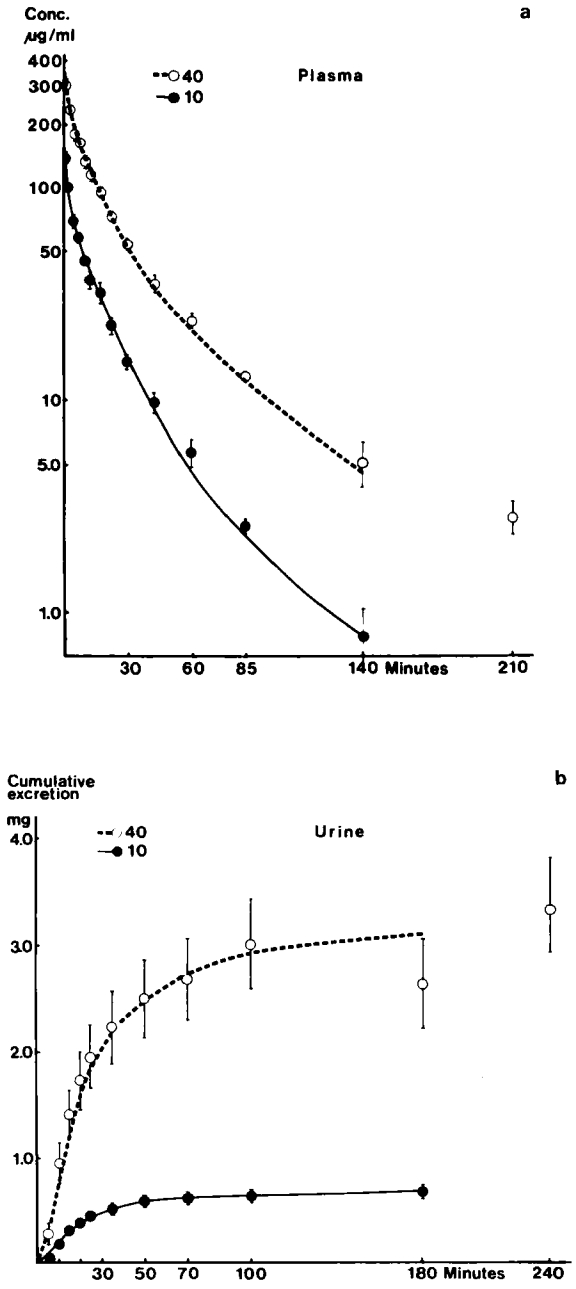

Figure 6. Plasma concentrations (a) and cumulative renal excretion (b) of unchanged furosemide. Each point represents the mean ±S.E.M. The lines represent the curve fit according to Model II depicted in Figure 5

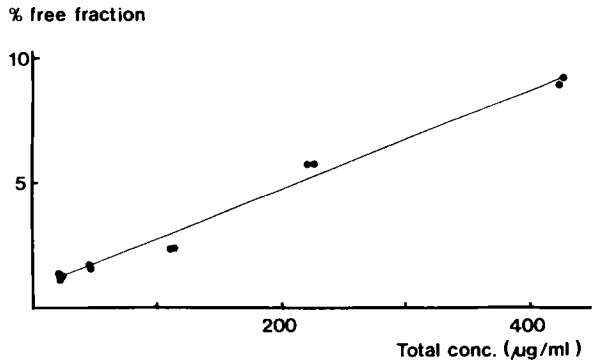

Figure 7. Protein binding of furosemide. Percentage free fraction (f_u%) plotted against total plasma concentration. Each point represents individual dialysis experiments

Renal clearance. Taking into consideration the saturation of the protein binding of furosemide, the increased free fraction in plasma could explain the above-mentioned concentration–dependent increase of the renal clearance of total drug. This was also the case. When calculating the free renal clearance of furosemide this concentration–dependent property disappeared (Figure 8). In Figure 8 there is also an indication of a saturable process with reference to the active transport mechanism when renal clearance is divided into glomerular filtration and active renal secretion. (A rough estimation of the maximum transport capacity (T_m) is $4{\cdot}9\,\mu g\,min^{-1}$ and a K_m of $0{\cdot}11\,\mu g\,ml^{-1}$ (free concentration), corresponding to a total concentration of $12\,\mu g\,ml^{-1}$ at half-maximal transport rate.) Glomerular filtration of free drug is $26\,ml\,min^{-1}$.

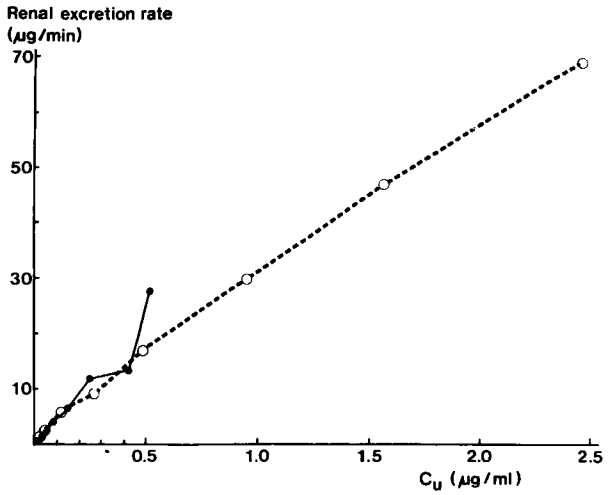

Figure 8. Renal excretion rate of furosemide plotted against the free concentration of furosemide in plasma (C_u) at pseudo-equilibrium

Volume of distribution. Recalculating the plasma concentration data using free drug concentration similar volumes of distribution ($V_{d,u}$) were obtained for the two doses ($V_{d,u}$ = 1014 ml and 1096 ml for the 10 and 40 mg kg^{-1} dose, respectively).

The unbound plasma clearance, however, was somewhat decreased between the two doses (66·5 ml min^{-1} and 53·2 ml min^{-1}, respectively), which indicates a tendency towards saturation of some elimination step.

DISCUSSION

After i.v. bolus doses of furosemide, the total plasma concentration declined in a tri-exponential manner. The rate constant of the terminal phase decreased with increasing dose and thus, the half-life increased from 29–49 min indicating a non-linear pharmacokinetic behaviour. This non-linearity could be detected as an independent increase in both the total plasma clearance and the volume of distribution. As plasma clearance was increased but less compared to the volume of distribution, the increase in the biological half-life is a reflection of the change in the latter. An increase in the volume of distribution is considered to be a consequence of a decrease in plasma protein binding or an increase in tissue binding. Furosemide is a highly protein-bound drug[17–19] and can therefore be suspected of showing changes in protein binding e.g. in diseased states and during drug interactions.

Our results indicate a very high protein binding (99·1 per cent at 11 μg ml^{-1}) in the rat. Other authors have reported protein binding of 91–99 per cent (for review see Reference 2) in humans. As they do not clearly mention whether heparin has been used when sampling the blood, this possible influence on the degree of binding cannot be ruled out. Our experience indicates a strong displacement capacity of heparin. The studies made by Prandota and Pruitt[17] with pure human plasma albumin and buffer can be ruled out in this respect. In our study, the free fraction of furosemide was found to be linearly related to the total plasma concentration with an increase of about ten times for the free fraction within the concentration range used (Figure 7). A concentration-independent binding has been reported in man at serum levels of 1–100 μg ml^{-1} (Reference 19) and 1–30 μg ml^{-1} (Reference 17). Using the ultrafiltration technique Andreasen *et al.*[18] found a change in the free fraction from 1–5 per cent within the concentration range 10–400 μg ml^{-1}. By means of the equilibrium dialysis technique Prandota and Pruitt[17] also found a linear increase in the free fraction from 2–9 per cent within the range 30–500 μg ml^{-1}, which is consistent with our results.

Some authors have reported an increase in the volume of distribution of furosemide in patophysiological conditions in humans, such as nephrotic syndrome[19] and hepatic cirrhosis[20] characterized by decreased serum albumin concentrations. This pronounced influence of the serum albumin levels on the degree of furosemide binding is well documented.[17,19,20] In patients with

normal albumin concentrations the protein binding of furosemide has not been shown to change appreciably within the therapeutic plasma concentration range as this seldom exceeds 20 $\mu g\,ml^{-1}$. The equi-effective diuretic concentration in the rat is, however, much higher and, consequently, in this species the importance of changes in protein binding is obvious. When the obtained values of the apparent volume of distribution were corrected for the protein binding, the unbound volume of distribution was found to be independent of the dose.

Furosemide is a low-clearance drug. Consequently, its clearance will be proportional to the free fraction in plasma. The renal clearance of furosemide is composed of a passive filtration and an active secretion. Only the free fraction can be filtered whereas the influence of the free fraction on the secretion has been questioned. For a long time, the secretion has been considered to be independent of the protein binding of drugs, since this binding is reversible and a rapid equilibration takes place. Bowman[8] has, however, found that the renal secretion of furosemide is dependent on the free fraction. This controversy can be discussed parallel to the dependence of hepatic metabolism on the free fraction of a drug. Wilkinson and Shand[21] discuss the possible 'stripping' of drug molecules from the binding sites during the passage through the liver. Two types of extraction are possible, one restricted or limited to the circulating free drug and the other non-restricted as both free and bound drug can be removed by the liver. Drugs with low clearance are said to show mainly restricted behaviour. It is plausible, although not yet confirmed, that this model is also valid for the active renal secretion of drugs. In that case, according to Bowman,[8] the secretion of furosemide would be restricted, i.e. in part dependent on the free fraction of the drug and thus an increased free fraction of furosemide would also increase not only the filtration, but also the secretion of the drug in the kidneys.

Our results show an increase in the renal excretion of unchanged drug with increasing dose (Figure 3) as well as an increased renal clearance with increasing total plasma concentration (Figure 4). When the unbound concentration was plotted against renal excretion rate (Figure 8), the renal clearance was concentration-independent. Even a tendency towards a saturation of the active transport mechanism can be seen. The pharmacokinetic modelling approach also shows a significant change in the renal excretion rate constant k_{20} (Table 3). Together, these data clearly show the strong influence of the free fraction in plasma on the renal clearance of furosemide. Although our compartmental analyses show that the renal elimination of furosemide takes place from a peripheral compartment, we have chosen to plot the plasma concentration against the renal excretion rate of furosemide in order to calculate the clearance (Figures 4 and 8). However, this was performed in the post-distributive phase when the change in concentration in the shallow compartment parallelled that in the plasma compartment.

An interesting and unexpected observation in the fit of the plasma and urine data to the three-compartment open model was the decrease in the rate constant

of elimination from the central compartment k_{10} with increasing dose. This constant represents the fractional rate of non-renal elimination. This might be a consequence of a saturable metabolism which might be expected for a drug like furosemide metabolized by conjugation with glucuronic acid.

Furthermore, glucuronidation is known to be a more dominant pathway for drug metabolism in the rat compared to humans. This might indicate that humans reach saturation at lower concentration compared to rats. By using Michaelis–Menten kinetics similar values for V_m and K_m, respectively, were obtained for the two doses (Table 4) without any change in the other rate constants. Due to the uncertain estimates of the parameters V_m and K_m, detailed studies on the mechanism and capacity of hepatic metabolism is called for.

The compartmental analyses of plasma and urine concentration of furosemide yielded a best fit to a three-compartment open model. However, a non-linear binding in plasma can produce concave plasma vs time curves and thus extra exponential terms.[22] This possibility was investigated by fitting the free drug concentrations of furosemide to multi-exponential equation. However, the free drug levels also declined in a tri-exponential manner. Obviously, in the rat, furosemide not only shows a variable renal clearance dependent upon the free fraction in plasma, but also possesses a saturable hepatic metabolism.

ACKNOWLEDGEMENTS

For skilful technical assistance during the experiments, we want to thank Ms. A. Ohlsson and Ms. L. Alari as well as Mr. P. Westberg.

Financial support was received from the I. F. Foundation for Pharmaceutical Sciences and the C. D. Carlsson Foundation which is gratefully acknowledged.

REFERENCES

1. L. Z. Benet, *J. Pharmacokin. Biopharm.*, **7**, 1 (1979).
2. R. E. Cutler and A. D. Blair, *Clin. Pharmacokin.*, **4**, 279 (1979).
3. D. E. Smith and L. Z. Benet, *Pharmacol.*, **19**, 301 (1979).
4. S. K. Chakrabarti and J. Brodeur, *Can. J. Physiol. Pharmacol.*, **58**, 205 (1979).
5. J. Prandota and M. Wilimowski, *Polski Tyg. Lek.*, **31**, 9 (1976).
6. M. B. Burg, *Kidney Int.*, **9**, 189 (1976).
7. M. Burg, L. Stoner, J. Cardinal and N. Green, *Am. J. Physiol.*, **225**, 119 (1973).
8. R. H. Bowman, *Am. J. Physiol.*, **229**, 93 (1975).
9. W. Rupp, *Scot. Med. J.*, **19**, 5 (1974).
10. B. Odlind and B. Beermann, *Clin. Pharmacol. Ther.*, **27**, 784 (1980).
11. J. B. Hook and H. E. Williamson, *J. Pharmacol. Exp. Ther.*, **149**, 404 (1965).
12. M. Homeida, C. Roberts and R. A. Branch, *Clin. Pharmacol. Ther.*, **22**, 402 (1977).
13. J. Honari, A. D. Blair and R. E. Cutler, *Clin. Pharmacol. Ther.*, **22**, 395 (1977).
14. M. Ehrnebo, S. Agurell, B. Jalling and L. O. Boréus, *Eur. J. Clin. Pharmacol.*, **3**, 189 (1971).
15. B. Lindström *J. Chromatogr.*, **100**, 189 (1974).
16. J. E. Cruz, D. D. Maness and G. J. Yakatan, *Int. J. Pharm.*, **2**, 275 (1979).
17. J. Prandota and A. W. Pruitt, *Clin. Pharmacol. Ther.*, **17**, 159 (1974).
18. F. Andreasen and P. Jakobsen, *Acta Pharmacol. et Toxicol.*, **35**, 49 (1974).

19. A. Rane, J. P. Villeneuve, W. J. Stone, A. S. Nies, G. R. Wilkinson and R. A. Branch, *Clin. Pharmacol. Ther.*, **24**, 199 (1978).
20. E. Keller, G. Hoppe-Seyler, R. Mumm and P. Schollmeyer, *Eur. J. Clin. Pharmacol.*, **20**, 27 (1981).
21. G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).
22. P. J. McNamara, J. T. Slattery, M. Gibaldi and G. Levy, *J. Pharmacokin. Biopharm.*, **7**, 397 (1979).