[Chem. Pharm. Bull. 36(3)1053—1062(1988)]

A Kinetic Study on Drug Distribution: Furosemide in Rats

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(Received June 25, 1987)

To rationalize the observed time courses of furosemide distribution in arbitrarily selected tissues of rats, a pharmacokinetic analysis based on the perfusion rate-limited model was carried out. Anatomical compartments include liver, kidneys, intestine, muscle and plasma. Required parameters were estimated on the basis of physiological principles for rats.

Keywords——furosemide; pharmacokinetics; perfusion model; tissue/plasma distribution coefficient; distribution; diffusion rate-limited distribution; perfusion rate-limited distribution

The work described here was undertaken to rationalize the observed time courses of furosemide distribution in arbitrarily selected tissues of rats, based on the model first proposed by Bischoff *et al.*[1] The aim was a better understanding of the disposition of furosemide, one of the most potent diuretics available.[2]

Experimental

Materials——Furosemide parenteral solution (Furosemide Injection "Mita" (20 mg/2 ml)) was supplied by Toyo Pharma Co., Ltd. The parenteral solution was diluted, if necessary, with a physiological saline solution to a concentration giving an injection volume of 1 ml. Other chemicals used were of reagent grade.

Animals——Male albino rats (Wistar strain), 6 to 7 weeks of age, weighing 200 ± 20 g after 20 h of abrosia, were restrained in a supine position. Furosemide (50 mg/kg) was injected *via* the jugular vein or administered orally using a peroral sonde.

Blood samples (1 ml each) were taken with a heparinized syringe, 10, 20, 30, 60, 120, 240, 300 and 480 min after furosemide dosing. Plasma was obtained by centrifugation (5 °C, 3000 rpm, 10 min) and stored frozen. Right after the blood sampling, the animal was sacrificed. Tissues such as brain, liver, intestine, kidney and muscle were excised, wiped lightly and blotted with a piece of gauze and stored frozen (−30 °C) until they were used for assay.

Urine samples were collected into a disposable centrifuge tube through a small funnel attached over the penis using an adhesive. Urine samples were taken before and 1, 2, 3, 4, 6, 8 and 25 h after furosemide i.v. injection (50 mg/kg). Collected urine samples were stored frozen until they were used for assay. Bile samples were collected through a bile duct cannula 1, 2, 4, 6 and 8 h after furosemide i.v. injection.

Plasma Protein Binding——After i.v. injection of furosemide (25 mg/kg) into rats, a blood sample (about 3 ml) was taken with a heparinized syringe, and centrifuged (3000 rpm, 10 min) at room temperature to obtain approximately 1 ml of plasma, of which 100 μl were reserved for the assay of "total" drug concentration. The rest of the plasma (0.5 to 1 ml) was placed in an ultrafiltration membrane cone (Amicon Co., Ltd.) in a Spitze tube. After exchange of the air inside the tube with oxygen mixture (95% O_2 + 5% CO_2), the tube was closed and centrifuged (2300 rpm, 15 min) at room temperature. The ultrafiltrate was used for the assay of unbound drug concentration.

Assay——Plasma, Blood and Tissue: Determination of furosemide concentration was carried out by high performance liquid chromatography (HPLC) following the procedure shown in Chart 1.

Bile and Urine: Determination of furosemide concentration (unchanged or unchanged + conjugated) was carried out spectrophotometrically, following the procedure shown in Chart 2.

Tissue Plasma Volume——Tissue plasma volume was determined with Evans blue,[3] according to the procedure shown in Chart 3.

Estimation of Model Parameters——Pharmacokinetic parameters other than the ones obtained from the literature were estimated by a model adaptation technique as follows (see Fig. 1-app of Appendix).

plasma sample 100 μl
+ H_2O 500 μl
+ phenacetin soln. (50 μg/ml) 200 μl
+ metaphosphoric acid soln. (100 mg/ml) 100 μl
+ diethyl ether 4.0 ml
↓ shake for 10 min
↓ centrifuge (3000 rpm, 5 min, room temperature)
↓ organic phase 3.0 ml
↓ evaporate to dryness at 40°C
+ mobile phase 100 μl
↓ HPLC UV

blood sample 100 μl
+ H_2O 1000 μl
+ phenacetin soln. (50 μg/ml) 200 μl
+ metaphosphoric acid soln. (100 mg/ml) 200 μl
+ diethyl ether 4.0 ml
↓ shake for 10 min
↓ centrifuge (3000 rpm, 5 min, room temperature)
↓ organic phase 3.0 ml
↓ evaporate to dryness at 40°C
+ methanol 50 μl + mobile phase 50 μl
↓ HPLC UV

tissue homogenate (20%) 500—2000 μl
+ H_2O 500 μl
+ phenacetin soln. (200 μg/ml) 100 μl (for kidney)
(10 μg/ml) 200 μl (for brain)
+ metaphosphoric acid soln. (100 mg/ml) 200—500 μl
+ diethyl ether 4.0 ml
↓ shake for 10 min
↓ centrifuge (3000 rpm, 5 min, room temperature)
↓ organic phase 3.0 ml
↓ evaporate to dryness at 40°C
+ methanol 50 μl + mobile phase 50 μl
↓ HPLC UV

measurement conditions

apparatus	:	Shimadzu LC-3A
column	:	4.0 mm × 15 cm stainless steel column packed with Merck Lichrosorb® RP-18 (5 μm)
detector	:	280 nm
mobile phase	:	methanol:H_2O:acetic acid = 200:280:4
flow rate	:	1.0 ml/min

Chart 1. Assay Flow Chart for Plasma, Blood and Tissue

Step 1: Parameters other than K_a, K_1, K_2 and r_{bi} were estimated from the tissue concentration data after i.v. administration (50 mg/kg), excluding intestine data.

Step 2: r_{bi} was estimated from biliary excretion data.

unchanged

bile 100 μl
+ buffer (pH 2) 1.0 ml
+ diethyl ether 3.0 ml
↓ shake for 10 min
↓ centrifuge at room temperature (3000 rpm, 5 min)
↓ organic phase 2.0 ml
+ buffer (pH 9) 5.0 ml
↓ shake for 10 min
↓ centrifuge at room temperature (3000 rpm, 5 min)
↓ aqueous phase 2.0 ml
+ 1 N HCl 2.0 ml
+ buffer (pH 9) 1.0 ml
↓ spectrofluorometry

unchanged + conjugate

bile 100 μl
+ 4 N HCl 0.5 ml
+ H_2O 0.5 ml
↓ heat (100°C, 1 h)
+ diethyl ether 3.0 ml
↓ shake for 10 min
↓ centrifuge at room temperature (3000 rpm, 5 min)
↓ organic phase 2.0 ml
+ buffer (pH 9) 5.0 ml
↓ shake for 10 min
↓ centrifuge at room temperature (3000 rpm, 5 min)
↓ aqueous phase 2.0 ml
+ 1 N HCl 2.0 ml
+ buffer (pH 9) 1.0 ml
↓ spectrofluorometry

apparatus: Shimadzu RF-503 wavelength: 340 nm (excitation) 415 nm (emission)

Chart 2. Assay Flow Chart for Bile

tissue (about 1 g)
+ conc. HCl 4.0 ml
↓ stand overnight at room temperature
+ benzalkonium chloride soln. (10%) 3.0 ml
↓ shake for 30 s
↓ left alone for 30 min
+ chloroform 7.0 ml for brain or muscle
12.0 ml for other tissue
↓ shake for 30 min
↓ after 3 to 4 agitations, the chloroform layer is withdrawn and filtered with filter paper
↓ filtrate
↓ spectrophotometry (620 nm)

plasma 50 μl
+ H_2O 10.0 ml
↓ mix well
↓ spectrophotometry (620 nm)

Chart 3. Analytical Method for Tissue Plasma Volume (Evans Blue)

Step 3: K_a and K_2 were estimated from plasma concentration data after oral administration (50 mg/kg).

Step 4: Intravenous administration data including intestine data were used to estimate K_1.

Step 5: Finally, all the parameters were determined simultaneously, using the parameter values obtained above as first estimates. For the model adaptation, a nonlinear least-squares regression program FKDM, which is based on the algorithm of Berman *et al.*,[4] was used on a digital computer (Digital Equipment Co., PDP-11/34).

Results and Discussion

Time Course of Plasma Concentration and Urinary Excretion

Plasma concentration of furosemide and cumulative amount excreted in urine after i.v. administration (50 mg/kg), and those after oral administration (50 mg/kg), are shown in Table I. In 8 h, 30 to 40% of the dose was excreted unchanged in urine after i.v. administration and 20 to 30% of the dose was excreted after oral dosing.

Non-linear least-squares fitting of a 2-exponential equation to the plasma data (i.v.) of Table I resulted in Eq. 1.

$$C_p = 284.37 \exp(-2.3059t) + 108.57 \exp(-0.2737t) \quad (1)$$

For a dose of 10 mg (body weight = 200 g), model-independent V_{dss}[5] and V_c were evaluated as 55.58 and 25.45 ml, respectively, using Eqs. 2 and 3.

$$V_{dss} = \text{dose} \int_0^\infty t C_p \, dt \Big/ \int_0^\infty C_p \, dt \quad (2)$$

$$V_c = \text{dose}/C_p(t=0) \quad (3)$$

Consequently, steady-state peripheral volume is estimated as 30.13 ml (= 55.58 ml − 25.45 ml).

Plasma Protein Binding

Unbound plasma concentration of furosemide was plotted against total concentration. The results are shown in Fig. 1. The fraction of unbound furosemide in plasma is small and less than 2.5% for total furosemide concentrations ranging from 10 to 250 g/ml. Unbound and total concentrations were correlated successfully with the following experimental equation.

$$C_{total} = (A+1)C_f + \frac{V_n C_f}{K_n + C_f} \quad (4)$$

where C_f and C_{total} are unbound and total concentrations, respectively. K_n, V_n and A are constants.

Nonlinear least-squares adaptation of the equation to the observed data of Fig. 1 gave parameter values of $A = 20.8 \pm 6.4$, $K_n = 1.26 \pm 0.54$ and $V_n = 157 \pm 51$ μg/ml. A solid curve shown in Fig. 1 represents the theoretical values calculated by means of Eq. 4.

TABLE I. Plasma Concentration and Urinary Excretion of Furosemide

Time (h)	Plasma conc. (μg/ml)	Cumulative amount excreted in urine (μg/200 g b.w.)	Time (h)	Plasma conc. (μg/ml)	Cumulative amount excreted in urine (μg/200 g b.w.)
Intravenous administration (50 mg/kg)			Oral administration (50 mg/kg)		
0.17	298. ± 38.7	—	0.17	23.9 ± 6.69	273. ± 26.7
0.33	228. ± 22.1	—	0.33	41.4 ± 7.25	813. ± 57.7
0.50	188. ± 18.6	—	0.50	53.0 ± 7.83	—
1.00	109. ± 7.19	1718. ± 85.3	1.00	65.7 ± 4.75	273. ± 26.7
2.00	66.5 ± 5.60	2292. ± 74.5	2.00	56.1 ± 5.63	813. ± 57.7
3.00	—	2749. ± 110.	3.00	—	1262. ± 61.4
4.00	36.3 ± 4.94	3040. ± 116.	4.00	27.4 ± 3.63	1614. ± 83.7
6.00	21.2 ± 6.44	3247. ± 120.	6.00	15.4 ± 1.43	2092. ± 126.5
8.00	11.7 ± 2.15	3380. ± 127.	8.00	13.0 ± 1.66	2360. ± 182.2
			24.00	—	2952. ± 177.5

Mean ± S.E., $n = 3$—6. b.w. = body weight.

TABLE II. Biliary Excretion of Furosemide after Intravenous Administration (50 mg/kg)

Time (h)	Cumulative amount excreted in bile (μg/200 g b.w.)
Unchanged	
1.0	199. ± 10.7
2.0	250. ± 13.4
3.0	279. ± 16.4
4.0	299. ± 17.8
6.0	321. ± 18.9
8.0	331. ± 16.9
Unchanged and conjugate	
1.0	391. ± 18.4
2.0	560. ± 2.75
4.0	669. ± 22.3
6.0	744. ± 11.5
8.0	782. ± 11.7

Mean ± S.E., $n=2$.

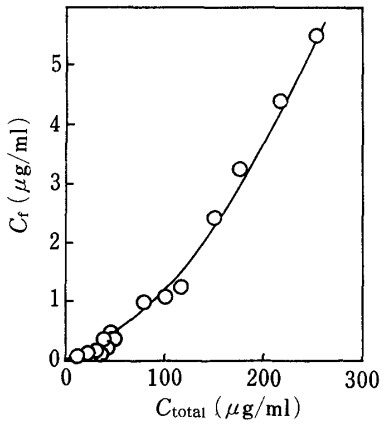

Fig. 1. Protein Binding of Furosemide in Rat Plasma

TABLE III. Tissue Concentration of Furosemide after Intravenous Administration (50 mg/kg)

Time (h)	Brain (μg/g)	Intestine (μg/g)	Liver (μg/g)	Kidney (μg/g)	Muscle (μg/g)	Lung (μg/g)	Plasma (μg/ml)
0.17	4.18 ± 1.36	54.0 ± 2.80	135. ± 40.7	674. ± 99.7	21.8 ± 0.668	51.6 ± 19.9	298. ± 38.7
0.33	2.91 ± 0.350	87.9 ± 18.0	88.4 ± 11.1	374. ± 55.9	23.8 ± 0.720	35.7 ± 3.18	228. ± 22.1
0.50	2.53 ± 0.457	82.0 ± 9.95	89.2 ± 5.53	339. ± 76.2	22.5 ± 3.89	30.9 ± 6.28	188. ± 18.6
1.00	1.30 ± 0.024	73.5 ± 18.2	28.5 ± 3.34	157. ± 12.6	16.3 ± 2.02	17.7 ± 5.60	109. ± 7.19
2.00	0.818 ± 0.210	93.1 ± 19.0	14.5 ± 1.66	82.1 ± 9.91	7.38 ± 1.63	10.2 ± 1.27	66.5 ± 5.60
4.00	0.488 ± 0.083	67.1 ± 24.4	8.78 ± 2.24	56.7 ± 17.1	3.70 ± 1.02	5.77 ± 1.79	36.3 ± 4.94
6.00	0.357 ± 0.010	95.7 ± 34.9	4.61 ± 0.401	26.8 ± 4.97	3.16 ± 1.52	2.87 ± 0.377	21.2 ± 6.44
8.00	—	118. ± 26.8	2.88 ± 0.025	19.0 ± 9.03	2.04 ± 1.03	2.38 ± 0.288	11.7 ± 2.15

Mean ± S.E., $n=2$—5.

Blood/Plasma Concentration Ratio

Blood and plasma concentrations were determined for the concentration range of 3 to 500 μg/ml. The blood/plasma concentration ratio was almost constant (B/P = 0.956 ± 0.263, $n=34$), suggesting a constant blood-cell/plasma concentration ratio.

Time Course of Biliary Excretion

The amount of unchanged furosemide excreted in bile and the total amount of unchanged and conjugated furosemide excreted in bile are shown in Table II. In 8 h after i.v. injection, 3% of the dose was recovered in the bile as unchanged form. The total amount of furosemide recovered in the bile in 8 h was 7 to 8% of the dose.

Time Course of Tissue Concentrations

Concentrations of furosemide in the brain, muscle, intestine, liver and kidneys after i.v. administration (50 mg/kg) are shown in Table III, Among the tissues examined, furosemide concentration was lowest in the brain and highest in the kidneys, the difference being two orders of magnitude. In the intestine, the time courses of furosemide concentration were different from those in other tissues. Enterohepatic circulation and dissociation of conjugates

were suspected, to be occurring.

Attainment of Tissue/Plasma Distribution Equilibrium

The logarithm of tissue furosemide concentration is plotted against the logarithm of plasma unbound furosemide concentration in Fig. 2. Discrepancies between the 45°-line and tissue concentration observed in muscle indicate that it takes some time for furosemide to achieve distribution equilibrium between muscle and plasma. On the basis of this fact, a diffusion rate-limited process was incorporated in the muscle compartment of the perfusion model described in Appendix A.

Tissue/Plasma Equilibrium Distribution Ratio

Bolus i.v. injection of furosemide (25 mg/kg) was followed by 3 h i.v. infusion (11.5 mg/kg/h) before a steady-state plasma concentration (100 μg/ml) was achieved. After the attainment of the steady state, rats were sacrificed and furosemide concentrations in plasma and in tissues were determined to calculate equilibrium distribution ratios. The results are shown in Table IV.

Observed tissue concentrations in the steady state were multiplied by the respective tissue weight values shown in Table V. The sum of the products was divided by the steady-state plasma concentration to get the observed steady-state tissue volume of 25.91 ml, which is about 86% of the steady-state peripheral volume evaluated above. The tissues in which

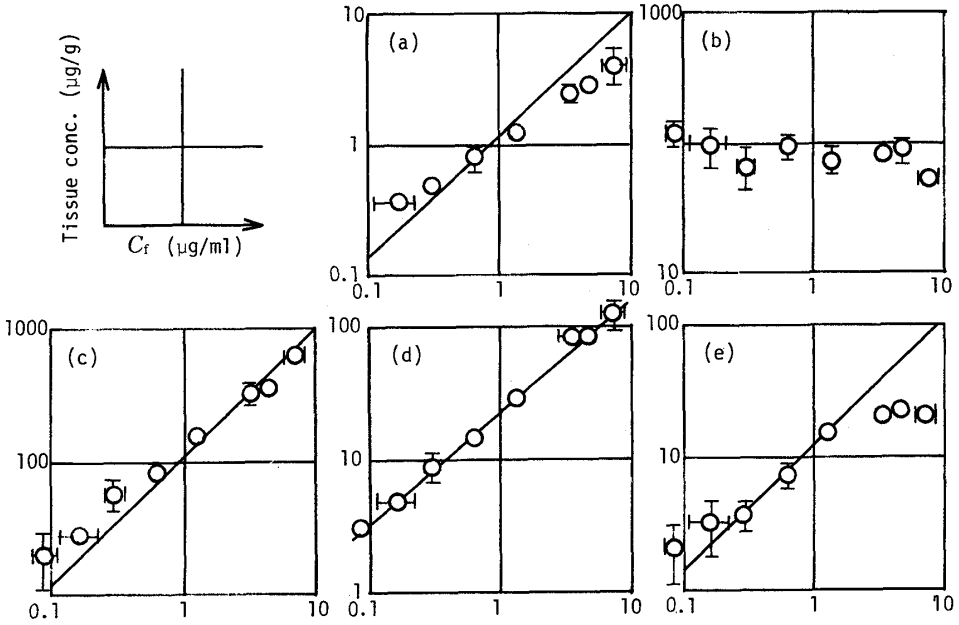

Fig. 2. Attainment of Tissue/Plasma Distribution Equilibrium (50 mg/kg i.v.)

a, brain; b, intestine; c, kidney; d, liver, e, muscle. Each point represents mean ± S.E. of 2—5 experiments.

TABLE IV. Tissue/Plasma Equilibrium Distribution Ratio

Tissue	Steady-state conc. (μg/g)	Ratio	Tissue	Steady-state conc. (μg/g)	Ratio
Brain	1.28 ± 0.332	0.0115	Muscle	18.2 ± 3.91	0.164
Liver	36.6 ± 4.38	0.330	Intestine	57.4 ± 18.1	0.517
Kidney	151. ± 9.46	1.36	Plasma	111. ± 3.91	1.00

Mean ± S.E., $n=3$ (25 mg/kg i.v. and 11.5 mg/kg/h infusion).

furosemide distribution was determined in this study account for more than 80% of the steady-state peripheral volume.

Physiological Parameters

Blood Flow Rate——In the present study, blood flow rates of the tissues were not determined, but the literature values were used.[6] Table V shows tissue plasma flow rates of the anesthetized rat.

Tissue Weight——For the weight of muscle, the literature value [6] was used, while for the other tissues, values measured with 38 rats were used (see Table V).

Tissue Plasma Volume——Tissue plasma volume data determined with Evans blue,[3] are shown in Table V. Tissue blood fractions were calculated from tissue plasma volume and hematocrit ($H_c = 0.435$).

Comparison of Predicted and Observed Values

Observed time courses of furosemide tissue distribution were compared with those predicted by the perfusion model, which is almost identical with the one first introduced by

TABLE V. Physiological Parameters

Tissue	Weight (g/200 g b.w.)	Plasma volume (ml/g)	Plasma flow (ml/h)
Brain	1.69±0.0728	0.0112±0.0005	24.3
Intestine	6.97±0.707	0.0860±0.0181	318.
Kidney	2.29±0.221	0.0849±0.003	300.
Liver	8.47±0.638	0.1270±0.0123	390.
Muscle	100.	0.0068±0.0004	180.

Mean±S.D., $n=38$ (tissue weight), 5—6 (plasma volume).

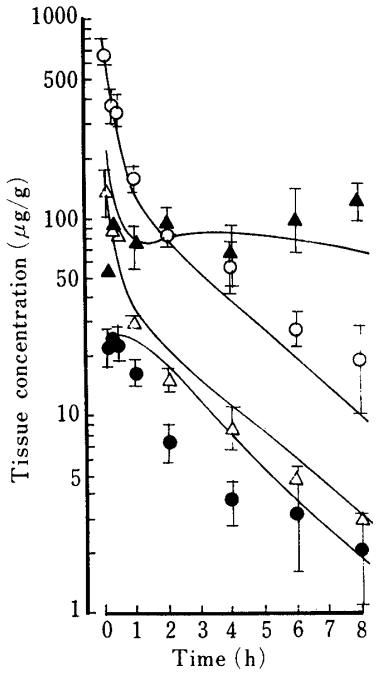

Fig. 3. Model Prediction *versus* Experimental Results (50 mg/kg i.v.)

▲, intestine; ○, kidney; △, liver; ●, muscle. Each point represents mean±S.E. of 2—5 experiments.

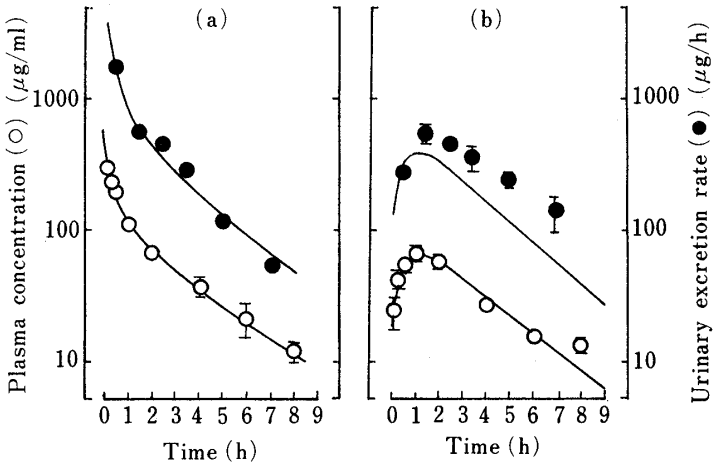

Fig. 4. Model Prediction *versus* Experimental Results

a, 50 mg/kg i.v.; b, 50 mg/kg *p.o.* Each point represents mean±S.E. of 3—6 experiments.

TABLE VI. Estimated Parameters

K_B:	$-0.6^{a)}$	K_1:	$0.103\,h^{-1}$	Cl_K:	5.70 ml/h
K_I:	35.8	K_2:	$0.466\,h^{-1}$	Cl_L:	62.8 ml/h
K_K:	120.	K_a:	$0.245\,h^{-1}$	r_{bi}:	0.203
K_L:	12.5				
K_M:	12.6	P_M:	$12.34\,h^{-1}$		

a) The distribution of furosemide to brain tissue being negligibly small, the negative value is an artifact.

Bischoff *et al.*[1] The model is described in Appendix A. Since the drug distributes to the blood cells and the blood/plasma concentration ratio is constant, perfusion model computation was performed on the basis of blood rather than plasma.

Calculation of the distribution coefficients of furosemide to the tissues (Appendix B) revealed that furosemide concentrations observed in the brain (Tables III and IV) were caused by furosemide in the blood space of the tissue and that distribution of furosemide to the brain itself was negligible Therefore, the brain was excluded from the model shown in Appendix A.

Figure 3 shows the model-predicted time courses of furosemide distribution in several body regions as well as observed data for 50 mg/kg furosemide in rats. Predicted and observed values are in reasonable agreement, except for muscle. In this study, muscle samples were taken exclusively from the abdominal region of the body and uneven drug distribution of muscle tissues of the other body regions may be a cause of the poor agreement. The relatively large P_M value ($12.34\,h^{-1}$) revealed that the delayed attainment of drug distribution to muscle was not necessarily due to a diffusion rate-limited process but could be due to the low perfusion rate (180 ml/h/100 g) to the tissue.

Figure 4 compares model predictions in plasma and urine with experimental data for (a) 50 mg/kg i.v. and (b) 50 mg/kg *p.o.* Parameters other than the ones shown in Table V were estimated by the model adaptation technique. Estimated parameter values are shown in Table VI.

The mathematical model used in this study reasonably predicted the detailed distribution of furosemide in tissues of rats. The perfusion model is based on anatomical compartments and inter-compartmental flow transport. The drug is cleared from the plasma by the kidneys, biotransformed by the liver and excreted in the bile, with subsequent partial intestinal reabsorption. The same model may be used for other animals with specific paramenters chosen for the species to be simulated.

Appendix

A. Description of the Model[1]

The model employs the lumped compartmental approach with the restriction that the volumes, flows and other properties should be physiologically meaningful and usually independently measured. The concept of flow-limited conditions is used for all body regions except muscle. The basic model, incorporating the enterohepatic circulation and hepatic elimination is shown in Fig. 1-app. The various symbols are defined in Table I-app.

The complete set of mass balance equations for the various anatomical body regions can be written as follows.

Plasma:

$$V_P\frac{dC_P}{dt}=Q_M C_M^{out}+Q_K C_K^{out}+(Q_L+Q_I)C_L^{out}-Q_P C_P \tag{1a}$$

Muscle:

$$V_M\frac{dC_M}{dt}=\frac{V_M f_M r_{B/P}}{1-H_c}\frac{dC_M^{out}}{dt}+V_M\left(1-\frac{f_M}{1-H_c}\right)\frac{dC_M^{*}}{dt}=Q_M(C_P-C_M^{out})\frac{r_{B/P}}{1-H_c} \tag{2a}$$

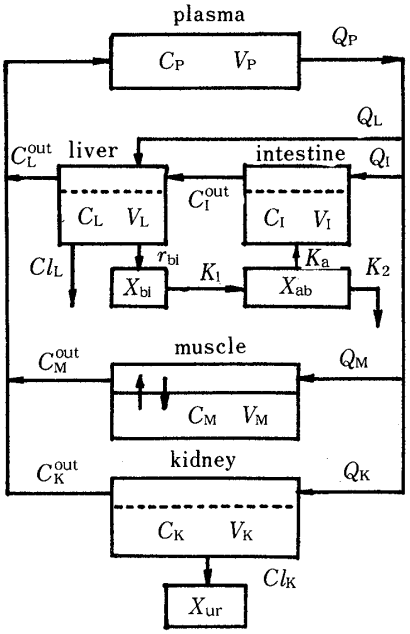

Fig. 1-app. Compartmental Model for Furosemide Disposition

TABLE I-app. List of Symbols and Definitions

C_X	Apparent drug concentration in the tissue specified by the subscript, μg/ml
C_X^*	Drug concentration in a substantial portion of the tissue, μg/ml
C^{in}	Drug concentration in the plasma entering the tissue, μg/ml
C_X^{out}	Drug concentration in the plasma returning from the tissue, μg/ml
$C_{X\,free}^{out}$	Unbound drug concentration in the plasma returning from the tissue, μg/ml
Cl_X	Clearance of the tissue specified by the subscript, ml/h
f_X	Plasma fraction of the tissue volume, dimensionless
H_c	Hematocrit, dimensionless
K_X	Tissue/plasma–water equilibrium distribution coefficient, dimensionless
K_1, K_2, K_a	Rate constant relative to biliary excretion and intestinal absorption, h^{-1}
P_X	Distribution rate constant of the tissue specified by the subscript, h^{-1}
Q_X	Plasma flow rate of the tissue specified by the subscript, ml/h
r_{bi}	Fraction of hepatic clearance responsible for biliary excretion, dimensionless
$r_{B/P}$	Blood/plasma concentration ratio, dimensionless
t	Time, h
V_X	Volume of the tissue specified by the subscript, ml
X_{ab}	Amount of drug in the absorption site, μg
X_{bi}	Amount of drug in the bile compartment, μg
X_{ur}	Cumulative amount of drug in urine, μg

Subscript (X)

I	Intestine
K	Kidney
L	Liver
M	Muscle
P	Plasma
T	Tissue (I, K, L or M)

$$\frac{dC_M}{dt}=P_M(C_{M\,free}^{out}-C_M^*/K_M) \tag{3a}$$

Kidney:

$$V_K\frac{dC_K}{dt}=\frac{V_K f_K r_{B/P}}{1-H_c}\frac{dC_K^{out}}{dt}+V_K\left(1-\frac{f_K}{1-H_c}\right)\frac{dC_K^*}{dt}$$

$$=\frac{Q_K r_{B/P}}{1-H_c}(C_P-C_K^{out})-Cl_K C_K^* \tag{4a}$$

Intestine:

$$V_I\frac{dC_I}{dt}=\frac{V_I f_I r_{B/P}}{1-H_c}\frac{dC_I^{out}}{dt}+V_I\left(1-\frac{f_I}{1-H_c}\right)\frac{dC_I^*}{dt}$$

$$=\frac{Q_I r_{B/P}}{1-H_c}(C_P-C_I^{out})+K_a X_{ab} \tag{5a}$$

Liver:

$$V_L\frac{dC_L}{dt}=\frac{V_L f_L r_{B/P}}{1-H_c}\frac{dC_L^{out}}{dt}+V_L\left(1-\frac{f_L}{1-H_c}\right)\frac{dC_L^*}{dt}$$

$$=\frac{r_{B/P}}{1-H_c}(Q_L C_P+Q_I C_I^{out}-(Q_L+Q_I)C_L^{out})-Cl_L C_L^* \tag{6a}$$

Absorption Site:

$$\frac{dX_{ab}}{dt}=K_1 X_{bi}-(K_a+K_2)X_{ab} \tag{7a}$$

Biliary Excretion:

$$\frac{dX_{bi}}{dt}=r_{bi}Cl_L C_L^*-K_1 X_{bi} \tag{8a}$$

Urinary Excretion:

$$\frac{dX_{ur}}{dt}=Cl_K C_K^* \tag{9a}$$

B. Distribution Coefficient of Drug in Tissue

Since $dC_T/dt=0$ at the steady state,

$$r_{B/P}Q_T\,(C^{in}-C^{out})/(1-H_c)-Cl_T C_T^*=0 \tag{10a}$$

$$r_{B/P}C^{out}\,f_T/(1-H_c)+(1-f_T/(1-H_c))\;C_T^*=C_T \tag{11a}$$

where subscript T stands for the respective tissue. Eliminating C_T^* from Eqs. 10a and 11a, Eq. 12a is obtained.

$$C^{out}=\frac{r_{B/P}Q_T C^{in}(1-f_T/(1-H_c))-Cl_T C_T(1-H_c)}{r_{B/P}(Q_T(1-f_T/(1-H_c))-Cl_T f_T} \tag{12a}$$

For tissues other than liver and kidneys, $C^{out}=C^{in}$ since $Cl_T=0$. Equation 13a is obtained from Eq. 11a.

$$C_T^*=\frac{r_{B/P}(C_T-C^{out}f_T/(1-H_c))}{1-f_T/(1-H_c)} \tag{13a}$$

And by definition,

$$K_T=\frac{C_T^*}{C_{T\,free}^{out}} \tag{14a}$$

At the steady state, $C^{in}=C_p$, therefore C^{in} and C_T are experimentally determinable. Q_T and f_T are known. Cl_T is estimated from the observed data by calculation, and $C_{T\,free}^{out}$ is calculated from the C^{out} value by using the mathematical expression for plasma protein binding of the drug. Consequently, the distribution coefficient K_T is calculated by using Eq. 14a.

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