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A controlled-release matrix tablet of furosemide: design, in vitro evaluation, pharmacological and pharmacodynamic evaluation

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Summary

Microporous polypropylene powder (Accurel) was used for the formulation of a matrix tablet of furosemide. The formulation of the matrix tablet was based on known absorption data of furosemide in the literature. Six male volunteers participated in an in-vivo study, in which they received a 60 mg matrix tablet, a 60 mg oral solution and an i.v. bolus injection of 40 mg furosemide. The bioavailability of furosemide calculated from the AUCs and from numerical deconvolution was resp. 76.6 ± 14.9 and $73.8 \pm 17.5\%$ ($P < 0.05$) for the oral solution and 40.0 ± 17.8 and $37.9 \pm 19.4\%$ ($P < 0.05$) for the matrix tablet. Peak diuretic effects were similar to the effects of a regular furosemide tablet. The high diuretic efficacy of the matrix tablet is remarkable (compared to the oral solution and the i.v. injection) although the bioavailability is relatively low. The possibility of controlled-release dosing for furosemide has been discussed from a pharmacological point of view. The pharmacodynamic response was studied in relation to the furosemide concentrations in the urine.

Introduction

The design of a slow release dosage form for furosemide

In previous publications (Verhoeven and Junginger, 1985; Verhoeven et al., 1987) we presented data of controlled-release tablets based on microporous polymeric powders. The microporous powders, like e.g. polypropylene (PP), show excellent tabletting properties. A great advantage of their use is the possibility of direct tabletting: drug, additives and polymeric powder(s) are simply blended and compressed either by hand or machine. Both matrix tablets and coated tablets of microporous polymers are under investigation (Pat. pend.). The design of a slow-release dosage form has to be based on known pharmacokinetic and biopharmaceutical aspects of the drug that will be incorporated in such a system. As for furosemide, many investigations have been published over the years, but still many questions about its absorption kinetics remain unanswered. Although the bioavailability of furosemide in humans has an average of 60–65% (Benet, 1979; Waller et al., 1982), furosemide shows a strong inter- and intra-individual variability in the absorption (Waller et al., 1982; Grahnén, 1984). Several suggestions for

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possible causes have been made. First, the stability of furosemide in gastric and duodenal fluids was investigated. Although unstable in acidic solutions, hardly any degradation was found in these fluids (Beermann et al., 1975; Andreasen et al., 1982; Lee, 1983). A second cause might be site-limited absorption. In vivo data from rats, published by Chungi et al. (1979), show that the absorption from the stomach is more rapid than from the small intestine. Lee and Chiou (1983) confirmed these data in rats; no absorption could be detected from the large intestine. They also studied the first-pass metabolism as a third possible cause. Approximately 30% of the oral dose, again in rats, was lost due to first-pass metabolism in the wall of the stomach and gut. This first-pass effect seems to be strongly site-dependent: the stomach scores highest in the rate of metabolism. These authors suggest that parallel phenomena might occur in humans. So far, only Loew et al. (1984) presented data on the absorption of furosemide from different intestinal sites in humans. Using a remotely controlled-release capsule they found an absorption of about 20% in the ileocecal region and only 3% in the ascending colon. Although one might question the necessity of sustained-release furosemide dosing, efforts have been made to develop a retard formulation (Lasix retard, Eutensine). These efforts were based on the clinical need to prevent the high peak diuresis, normally seen after dosing a regular tablet of furosemide. Especially elderly patients suffer from side-effects like weakness and tiredness (Morgan et al., 1979; Pothuizen and Chadha, 1982; Beermann, 1982). A retard formulation might furthermore be advantageous over thiazide diuretics, particularly in hypertensive patients suffering from some degree of cardiac insufficiency or who have an impaired renal function (Leary and Asmal, 1980; Ebihara et al., 1983). These arguments seem to justify the development of such a formulation for furosemide.

If one decides to formulate a retard formulation of furosemide, the design should meet the following demands: the site-limited absorption compels to develop a dosage form that will release all drug in 4–6 h, which is the average passage time after fasting, for a tablet in the stomach and small intestines (Davis et al., 1984a). Furthermore, the stomach might be the most important site of absorption, so dosing has to start here and no lag-times should be allowed for the release of drug from the dosage form.

The rate of metabolism in rats is highest in the stomach, so, if one assumes similar effects in humans, it might be necessary to dose here at a relatively higher rate than in the small intestines.

Wilson et al. (1975) concluded from their study that more than 20 mg oral furosemide is necessary to provoke a sufficient diuretic effect. So, to be effective, the dosage form should release at least 20 mg within a relatively short time interval.

This led us to the choice of a 60 mg matrix tablet formulation with an in vitro release of 90% in 6 h (see Fig. 1A), which we considered optimal. Interesting from a technical point of view is the low solubility of furosemide in acidic solutions. Especially dosing in the stomach is then challenging.

Stüber et al. (1982) and Ebihara et al. (1983) described the use of a retard formulation of furosemide (Lasix retard, Eutensine) in humans. These dosage forms consist of a gelatin capsule filled with furosemide pellets that are coated with shellac. This acid-resistant coating shows an increasing solubility at a pH above 7. The in vitro dissolution testing of this capsule shows a lag time in drug release at pH 7.8 (Stuber et al., 1982). Therefore, similar effects can be expected in the in vivo absorption of furosemide from this dosage form. The in vivo data of this capsule vary largely: Stüber et al. (1982) found a relative bio-availability of 81% compared to a normal tablet, Ebihara et al. (1983) established only 41% and Beermann (1982) found 73%.

Davis et al. (1984b) have shown that pellets spread to some degree within the intestines but do not distribute particularly widely. The $t_{50\%}$ of release for pellets from the stomach was 79 ± 20 min whereas for tablets the $t_{50\%}$ was 164 ± 92 ($n = 6$).

For furosemide the stomach is probably the major absorption organ. So, a matrix tablet design with a sufficient release of drug in the stomach might offer a more balanced approach for retard formulation.

In summary, our initial objectives were two-fold: firstly, we wished to evaluate our dosage form concept, in which we use microporous polymers, and secondly, we wished to check if the postulated design for furosemide dosing could prevent the peak diuresis without the loss of efficacy.

Materials and Methods

The microporous polypropylene (void space 70% v/v) and microporous polylactic acid (void space 60% v/v) were gifts from ENKA AG Obernburg. The furosemide was provided by Hoechst (W994). The additives in the tablet were all of Dutch Pharmacopoeia quality. Other chemicals were all of analytical grade. The HPLC equipment consisted of a Waters Associates pump M45, an auto-injector WISP 710 B and a Z-module, with a μBondapack C18 insert (8 × 100 mm), both of Waters Associates. The fluorimeter was a Shimadzu RF-530. The flame photometer was a Perkin Elmer 460 (emission 589.6 nm, slit 0.7 nm). The dissolution equipment consisted of a water-bath with 6 vessels, paddles, etc., designed according to the specifications of the USP XX, a pneumatic Rheodyne 6 position valve, an 8 channel Ismatec tubing pump, a Shimadzu UV190 spectrophotometer (330 nm) with a 3 mm quartz flow-through cell, an Apple PC IIe and a controlling interface.

Tablet formulation

Drug and additives (see Table 1) were all sieved to obtain particle sizes smaller than 200 μm. They were then blended and compressed into tablets on an excenter press. Technical data are given in Table 1.

HPLC assay

Sample preparation and HPLC assay were performed according to Kerremanns et al. (1982). The flowrate was set at 2.0 ml per min.

In vitro dissolution testing

The in vitro dissolution testing was performed according to the USP XX paddle method at 37.0 ± 0.1 °C and 50 rpm. As dissolution media we used: 0.1 N HCl, 0.05 M phosphate buffer pH 6.8 and demineralized water. In acidic medium furosemide is prone to degradation giving erratic results for the amount of furosemide released, but, as we found that the extinction coefficients of furosemide and its major degradation product, 4-chloro-5-sulfamoyl anthranilic acid (CSA), are approximately equal at 330 nm (0.0113 $l \cdot cm^{-1} \cdot mg^{-1}$ for furosemide and 0.0121 $l \cdot cm^{-1} \cdot mg^{-1}$ for CSA) errors will be small, especially because the residence time in the acidic solution was only 1 h. A better way of testing in acidic milieu is, of course, using the described HPLC assay. The amount of Tris buffer released in time, which should be associated with the release of furosemide, was calculated by a combined titration of the two components and by simultaneous registration of furosemide release by UV analysis. The principles of the experiment are discussed in the appendix (see Fig. 9).

In vivo study design

The protocol was approved by the Ethical Committee of the University Hospital, Leiden.

Six informed male volunteers (age 20–28 years) participated in the study after clinical and physical examination. After both oral and written information they volunteered by giving their written informed consent. No alcoholic or caffeinated beverages were allowed from 12 h preceding and during the experiment. In a cross-over design they received, after a 12 h fast, a matrix tablet of 60 mg furosemide with 100 ml of water, an oral solution of 60 mg furosemide in 50 ml of water, washed down with 50 ml water, and an i.v. bolus injection of 40 mg. Blood and urine samples were collected according to the following scheme. *Plasma*: i.v. bolus 0, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 min; solution 0, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min; matrix tablet 0, 15, 30, 60, 90, 120, 150, 180, 240, 360 and 480 min. *Urine*: 0–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–12 and 12–24 h. To compensate for the loss of volume they took 250 ml of lemonade at resp. 1, 2, 3 and 4 h after the start of the experiment. Lunch was allowed 4 h after the start. Plasma and urine samples were stored at −20 °C until further anal-

ysis by HPLC (within two weeks). Urinary sodium concentrations were measured by flame spectrophotometry.

Pharmacokinetic and statistical analysis

The plasma data were evaluated using two-compartmental analysis. The areas under the curve (AUC) were calculated by the log–trapezoidal rule.

The bioavailability (F_{AUC}) was calculated by:

$$F_{AUC} = \frac{D_{iv}}{D_{or}} \times \frac{AUC_{or}}{AUC_{iv}}$$

c_{max} and t_{max} are experimental data points. The absorption profiles were calculated by numerical deconvolution, according to Vaughan and Dennis (1978), using the i.v. bolus injection as a reference. By making Weibull fits of these curves we estimated the plateau values for absorption. These were used to calculate the bioavailability (F_{Deconv})

$$F_{Deconv} = \frac{\text{plateau value}}{D_{or}}$$

Statistical moment analysis according to Riegelman and Collier, (1980) was used to calculate the MRT, the MDT from the tablet and the MAT from the oral solution.

$$MRT = AUMC/AUC$$

$$MDT = MRT_{tab} - MRT_{sol}$$

$$MAT = MRT_{sol} - MRT_{iv}$$

The intra-individual data were compared with a Student t-test for paired observations, assuming a Student distribution. The intra-individual response to the different dosage forms has been compared by analysis of covariance (Table 5). The pharmacokinetic parameters were calculated by two-compartmental analysis (Waller et al., 1982).

Results and Discussion

The formulation of a matrix tablet for furosemide

The composition of the tablet was optimized with respect to the porosity and to the solubility of furosemide from this tablet.

For matrix tablets it is known that the drug-induced porosity caused by dissolution of the drug, is a rate-determining step for release of drug; the nature of the material used for the matrix is of less importance. This is only true for matrix tablets, as coated tablets show a large dependence on the properties of the polymeric coating compound (Verhoeven et al., 1987). Fig. 1B shows the release of furosemide at increasing amounts of furosemide and Tris buffer (see below) in the matrix tablet. The influence of the microporous polypropylene was checked by changing it for increasing amounts of microporous polylactic acid. Although being a completely different (i.e. hydrophilic) polymer, polylactic acid showed no significant changes in the rate of release. The next step was the optimization of the amount of Tris that was used in the tablet. Furosemide shows only very low solubility in acidic and neutral media, which results in only very little release of drug. The knowledge of the importance of the gastric absorption requires to improve the solubility in acidic environment. We did this by creating an appropriate microclimate around the furosemide crystals in the tablet with Tris. This addition might also improve the stability of furosemide in the dosage form. Fig. 1C shows the release of drug at an increasing amount of Tris in the matrix tablet. We found that at least equimolar amounts of Tris buffer were necessary to create a maximal effect on the rate of release of furosemide (see Fig. 1D). Table 1 gives the optimal formulation data of the matrix tablet, including several additives that were used for optimization of the technical behaviour of the blend. No adverse effects are expected from these compounds. Shibab et al. (1979) showed that PEG 6000 has a positive effect on the solubility of furosemide even in water, probably due to the better cosolvency of the carbowax-water mixture. This all resulted in the dissolution profiles presented in Fig. 1A.

In vivo absorption data

The plasma-data of the in vivo study, are summarized in Table 2. One of the objectives of this study was to prevent the peak effects of fur-

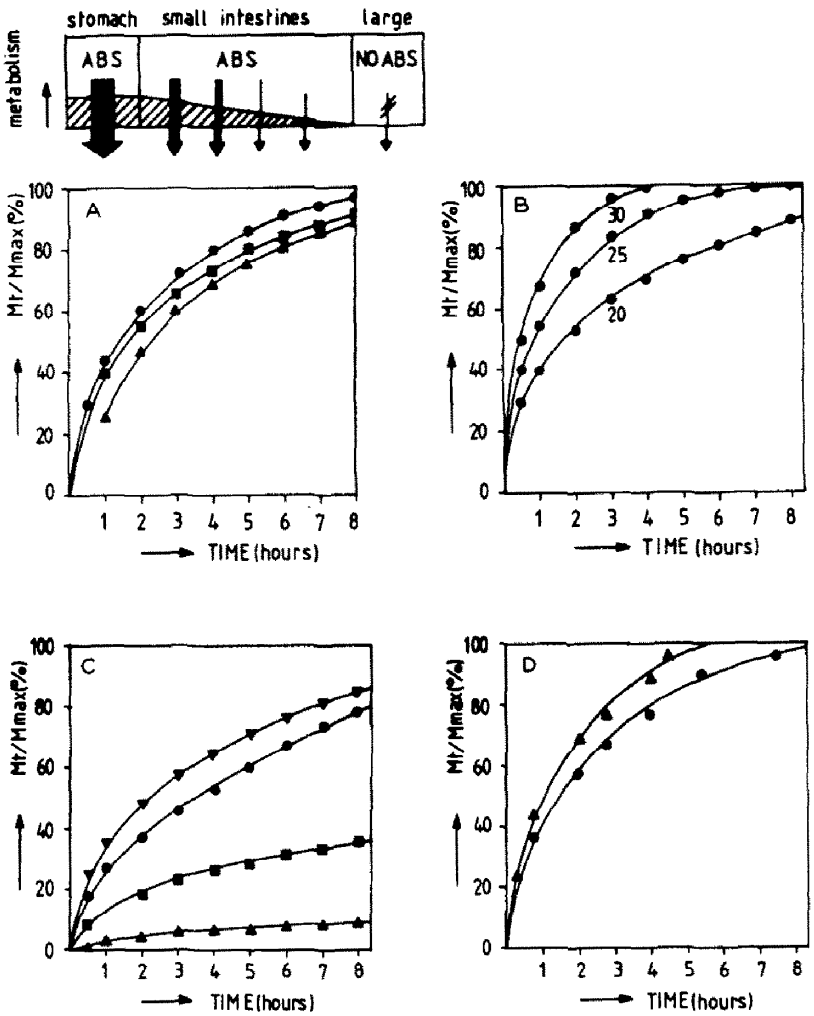

Fig. 1. A: schematic representation of the possible absorption (ABS) and metabolism profile of furosemide in the g.i.tract. Experimental in-vitro dissolution data from the designed matrix-tablet: ●, demineralized water; ■, phosphate buffer pH 6.8; ▲, 1 h 0.1 N HCl, then an increase to pH 6.8 phosphate buffer. B: effect of the amount of soluble material (furosemide and tris) on the in vitro release of furosemide from a matrix tablet in demineralized water, 20: 20% furosemide, 20% tris; 25: 25% furosemide, 25% tris; 30: 30% furosemide, 30% tris; 5% PEG 6000, 0.2% Aerosil 200, 0.5% Mg stearate and up to 100% microporous PP. C: influence of Tris on the release of furosemide from a matrix tablet in demineralized water. ▲, 0% Tris, 20% NaCl; ■ 5% Tris, 15% NaCl; ● 10% Tris, 10% NaCl; ▼ 15% Tris, 5% NaCl and 20% Tris, 0% NaCl fall together. 25% furosemide, 5% PEG 6000, 0.2% Aerosil 200, 0.5% Mg stearate and up to 100% microporous PP. D: Simultaneous registration of the release of tris buffer ▲ and furosemide ● from a matrix tablet in demineralized water (see Appendix). The S.D. for all dissolution curves is below 2.0%.

osemide. Therefore a comparison was made of c_{max} and t_{max} for both the oral dosage forms. The c_{max} of the matrix tablet is significantly smaller than the c_{max} of the oral solution ($P < 0.01$). The difference in t_{max} is also statistically significant ($P < 0.002$). As we did not study a regular tablet of furosemide, we took the in vivo data of Martin et al. (1984) as a reference, to compare the matrix tablet with the conventional tablet (Lasix). They found a t_{max} of 66 ± 24 min and a c_{max} of 1.66 ± 0.59 mg/l ($n = 12$). The comparison between this tablet and our matrix tablet results in a significant difference between the maximum plasma concentrations, but no significant difference for the

TABLE 1

The formulation of the matrix tablet of furosemide

Composition	Particle size (μm)	% ($g/_g$)
Furosemide	≦ 200	25.0
Tris	≦ 200	25.0
PEG 6000	≦ 200	5.0
Magnesium stearate	≦ 200	0.5
Aerosil 200		0.2
PP (void space 70%)	≦ 200	44.3
Diameter	12 mm	
Shape	convex	
Weight	244.6 ± 3.6 mg ($n = 15$)	
Crushing strength	105 ± 4N ($n = 6$)	
In vitro drug release	$93.6 \pm 1.7\%$ ($n = 7$) in 8 h at pH 6.8	
Stability	no significant weight loss at 25 rpm during 5 min.	

Variation is given in S.E.M.

matching times. This means that the peak plasma level is reached at the same time but the plasma concentrations after the matrix tablet are significantly smaller. After 240 min the plasma data of both dosage forms are not significantly different. The results of the bioavailability calculations are also given in Table 2. The data sets for the F_{AUC} and the F_{DECONV} show similarity, though these last data have the tendency to give lower calculated bioavailabilities. This difference might be explained by the fact that the log-trapezoidal rule gives a fairly accurate estimate of the last part under the plasma curve, but the accuracy of the plateau level of the absorption curve is strongly dependent on the last data point(s), giving an underestimation of the bio-availability when not enough plasma data are available for the plateau level.

The calculated absorption curves are given in Fig. 2. The data from the Statistical Moment Analysis are given in the lower part of Table 2. A paired Student's *t*-test shows that the *MRT* of the tablet is significantly longer ($P < 0.02$) than the *MRT* of the solution. This means that the dissolution from the matrix tablet is a rate-limiting step in the absorption process. But the *MDT*, which is independent from the extent of bioavailability of the product is relatively short (66 ± 44 min) implying a short effective period of drug release. This seems to confirm the data of the in-vivo animal experiments (Chungi et al., 1979; Lee and Chiou, 1983), which give evidence for the stomach as the most important absorption site. Dosage forms that release their drug only after passage of the stomach might show a low bioavailability as indeed can be

TABLE 2

Plasma data and calculated parameters for the 6 volunteers

Parameter	units	A	B	C	D	E	F	Mean ± S.E.M. *
t_{max}^{sol}	min	61	45	44	29	21	60	43 ±17
c_{max}^{sol}	mg/l	2.14	3.05	3.03	2.84	2.76	1.70	2.59 ± 0.58
t_{max}^{tab}	min	104	60	90	61	73	90	80 ±19
c_{max}^{tab}	mg/l	0.86	1.10	0.52	0.73	0.86	1.40	0.93 ± 0.26
AUC_{0-24}^{iv}	mg min/l	193	245	315	280	241	171	240 ±56
AUC_{0-24}^{sol}	mg min/l	189	327	326	309	253	264	278 ±57
AUC_{0-24}^{tab}	mg min/l	94	148	109	136	160	189	139 ±37
F_{AUC}^{sol}	%	65.3	89.0	66.8	71.2	67.7	99.6	76.6 ±14.9
F_{AUC}^{tab}	%	31.4	38.4	25.2	30.4	42.2	72.5	40.0 ±17.8
F_{DECONV}^{sol}	%	56.0	80.1	62.3	63.9	64.3	107.5	73.8 ±17.5
F_{DECONV}^{tab}	%	30.2	34.3	21.0	25.0	40.3	77.2	37.9 ±19.4
MRT_{iv}	min	41	66	67	65	56	115	68 ±26
MRT_{sol}	min	136	129	113	133	94	169	129 ±26
MRT_{tab}	min	149	198	202	242	177	177	191 ±31
MAT	min	95	63	46	68	38	54	61 ±21
MDT	min	13	69	89	109	83	8	62 ±44

* $P < 0.05$.

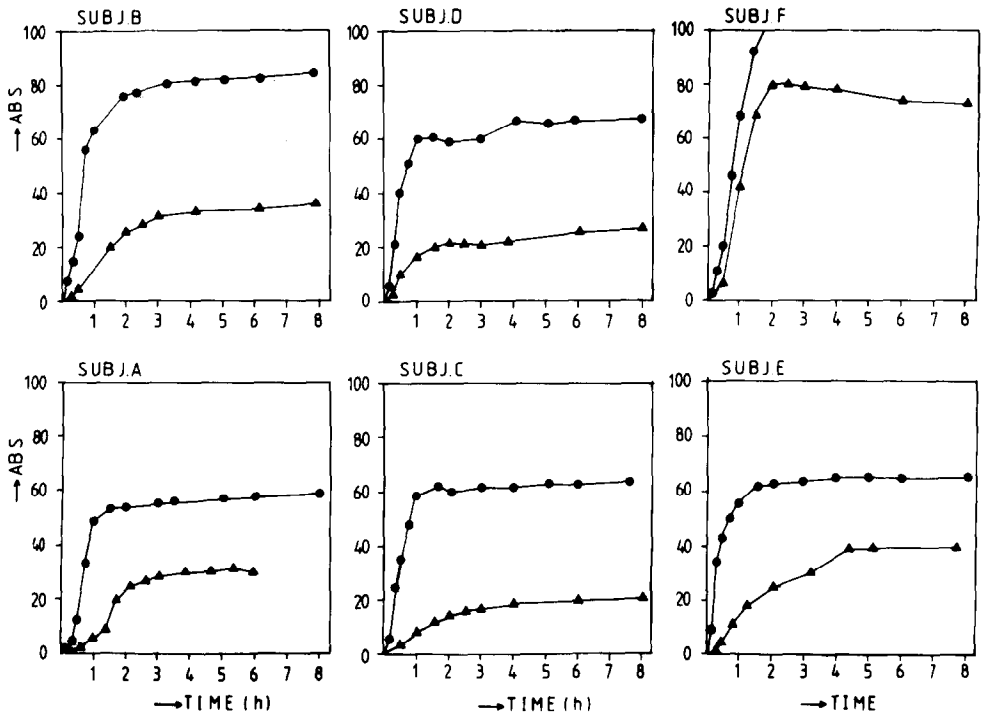

Fig. 2. In vivo absorption profiles (%) of furosemide from the oral solution (●) and the matrix tablet (▲) for 6 subjects.

seen from studies of Stüber et al. (1982) and Ebihara et al. (1983). We fitted the in vitro drug release curve with a Weibull function (Riegelman and Collier, 1980). This then gives the *MDT* in vitro, which can be compared with the calculated mean dissolution time in vivo. The calculated in-vitro *MDT* is 252 min for the phosphate buffer, which is approximately 4 times longer than the calculated in-vivo *MDT* (see Table 3). Calculation of the MDT_{ave}, by Weibull fits of the absorption curves, results in 41 ± 12 min for the oral solution and 91 ± 26 min for the matrix tablet. From these the MDT_{ave} can be deduced giving 50 ± 33 min. This is in good agreement with the results of the Statistical Moment Analysis.

One might try to increase the bioavailability of furosemide of the matrix tablet by dosing the tablet after breakfast, thus enhancing the residence time in the stomach. Davis et al. (1984) showed that a tablet might be released within the hour on an empty stomach, which in our case means a reduced absorption. Furosemide itself shows reduced plasma levels after a breakfast (Kelly et al., 1974; Welling, 1977) although the saluretic response seems equal to the response after dosing on an empty stomach. The influence of food on the dosage form itself has been studied in our laboratory (Verhoeven and Junginger, 1985). Though these effects are minor for our matrix tablets, some reduction in the rate of release has been observed.

Pharmacologic and pharmacodynamic evaluation

Next, we would like to show a representative data set from our experiments (subject B). Fig. 3C, E seem to show some hysteresis. Average pharmacokinetic data of the intravenous experiments are given in Table 4. These data show a good similarity with other experimental data sets for furosemide (Waller et al., 1982; Kelly et al., 1974). Ogata et al. (1983) showed that the best approach for evaluating the pharmacodynamic effect of furosemide is based on the urine data and not on the plasma data. Also Brater (1985) stresses this relation (see Fig. 5).

As mentioned before, the scope of this study was the prevention of peak diuretic effects. Fig. 6 gives the urine production during the first four 60-min intervals; the in vivo data of Martin et al. (1984) were taken as a reference (Lasix 40 mg).

As presented, the differences are small for the different dosage forms. The only observation one can make is the high diuretic effect of both the i.v. injection and the oral solution (see Fig. 4), indicating the immediate and efficient bioavailability of furosemide from the oral solution.

So far, no advantage has been demonstrated for the matrix tablet as peak diuretic effects are similar to the regular tablet of furosemide (Martin et al., 1984). Therefore, we would like to discuss in more detail the diuretic effect in relation to the excretion of furosemide in urine. As we have demonstrated the input profiles are strongly different for the 3 dosage forms. This is schematically presented in Fig. 7. Still, the diuretic response over the first 4 h is similar and the same accounts for the sodium excretion.

Thus it seems more relevant to study the amount of furosemide at the active site in relation to the diuretic effects. But concentrations in this compartment can only be measured indirectly in the excreted urine, so only overall effects can be established. Fig. 8 gives the excretion profiles for furosemide in urine for subject B. When we combine these with the data of Table 6 the superiority of the matrix tablet is clearly demonstrated.

Kelly et al. (1977) demonstrated that after i.v. and oral administration (tablet) of the same dose of furosemide (80 mg), the diuretic response was the same, although the bioavailability of the oral dose was only 60%. Branch et al. (1977) found similar results, though they did not specify the oral dosage form. Our data show comparable results and now it is possible to differentiate between the two oral dosage forms. It seems that oral retard dosing is more effective than an oral solution: one can reach the same diuretic effects with a lower dose ($F \times D$) of furosemide.

I.v. administration scores unfavourably suggesting that even for clinical application oral administration might be favourable. But one has to realize that the oral absorption might be altered in pa-

TABLE 3

Weibull fits of the absorption curves from the oral solution and matrix tablet of furosemide, compared to the in-vitro data of the matrix tablet

	MAT (min)	Shape factor	Lag time (min)	Plateau (%)
Solution				
subject				
A	46.2	2.76	1.2	56.0
B	49.3	2.19	−3.5	80.1
C	31.6	1.47	1.9	62.3
D	30.0	1.56	2.1	63.9
E	29.4	1.19	1.3	64.3
F	58.6	2.02	1.7	107.5
$\bar{x} \pm$ S.E.M. *	40.9 ± 10.1			72.4 ± 15.7
Matrix tablet				
subject				
A	111.8	2.65	−2.3	30.2
B	84.1	1.15	5.1	34.3
C	103.6	1.11	1.4	21.0
D	63.4	0.93	1.4	25.0
E	120.4	1.19	5.3	40.3
F	59.7	2.59	7.1	77.2
$\bar{x} \pm$ S.E.M. *	90.5 ± 21.0			38.0 ± 16.8
In vitro (matrix tablet)				
$\bar{x}$, phosphate buffer pH 6.8 ± S.E.M. *	252 ± 60	0.61	0.00	117.6 ± 9.0
$\bar{x}$, H_2O ± S.E.M. *	193 ± 31	0.65	−0.00	116.5 ± 6.3

* $P < 0.1$.

TABLE 4

Pharmacokinetic data of intravenous experiments calculated by two-compartmental analysis, $P < 0.05$ ($n = 6$)

Parameter	Mean ± S.E.M.	Unit
α	0.044 ± 0.012	min^{-1}
β	0.011 ± 0.005	min^{-1}
A	4.39 ± 1.36	mg/l
B	1.58 ± 0.79	mg/l
K_{21}	0.020 ± 0.009	min^{-1}
Kc	0.025 ± 0.006	min^{-1}
K_{12}	0.011 ± 0.003	min^{-1}
Vc	0.102 ± 0.054	l/kg
Vd_{ss}	0.183 ± 0.157	l/kg
Cl_T	0.169 ± 0.035	l/min
Cl_R	0.121 ± 0.024	l/min

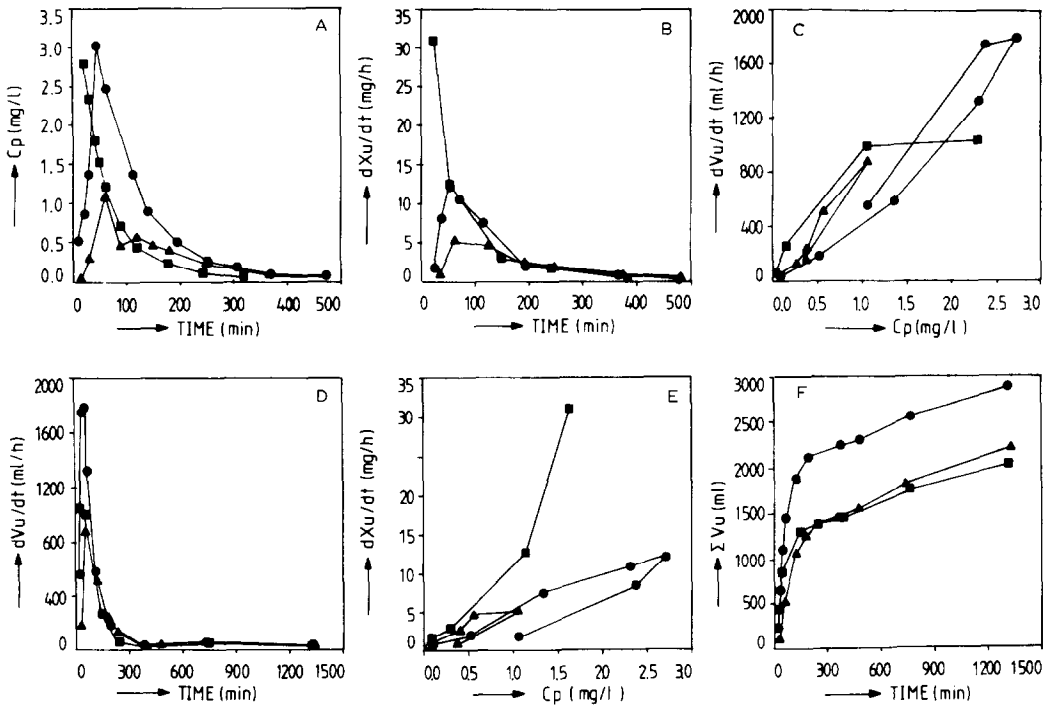

Fig. 3. Data set of subject B. The data points are connected chronologically. (■), Bolus i.v. injection 40 mg; (●), oral solution 60 mg; (▲), matrix tablet 60 mg. A: plasma concentration of furosemide vs time. D: urine flow (dVu/dt) vs time. B: furosemide excretion rate in the urine (dXu/dt) vs time. E: furosemide excretion rate in the urine (dXu/dt) vs the plasma concentration. C: urine flow (dVu/dt) vs the plasma concentration. F: cumulative urine production vs time.

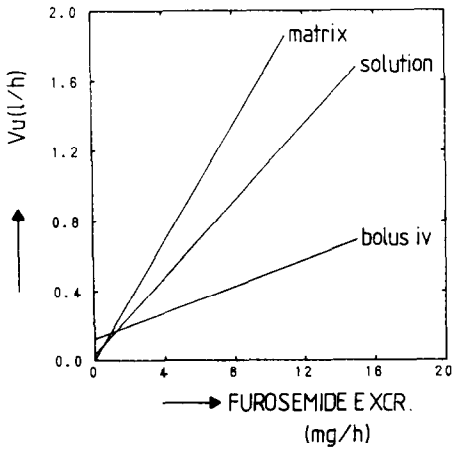

Fig. 4. The average urine flow rate vs the furosemide excretion in urine. The S.E.M.s are 65% for the matrix tablet, 16% for the solution and 13% for the bolus i.v.

tients. Intraindividual comparison of the matrix tablet and the i.v. bolus injection shows a significantly ($P < 0.05$) higher urine flow rate. The effects between both oral dosage forms are not significantly different.

Hammarlund and Paalzow (1982) studied dose-dependent kinetics in rats. They gave increasing i.v. doses of furosemide and established an approximately linear response curve up to 40 mg/kg. As they used bolus injections, the input profiles were similar for all experiments. It would have been interesting though if they had also studied the response after the same i.v. dose, but at different input profiles, using an infusion. Experiments like these might succeed in giving a basis for plasma–response relationships and could

then offer details on the necessary oral input profile. But, as has been discussed oral bioavailability of furosemide is a very complex problem.

Grahnén (1984) demonstrated that, even using the same dosage form, large variations in bioavailability can be expected for furosemide. So, the possibility of controlled release dosing seems questionable, but retard dosing might still offer advantages, especially in elderly patients. In this view, also the results of Stüber et al. (1982) and Ebihara et al. (1983) should be considered, as they established a sufficient diuretic response.

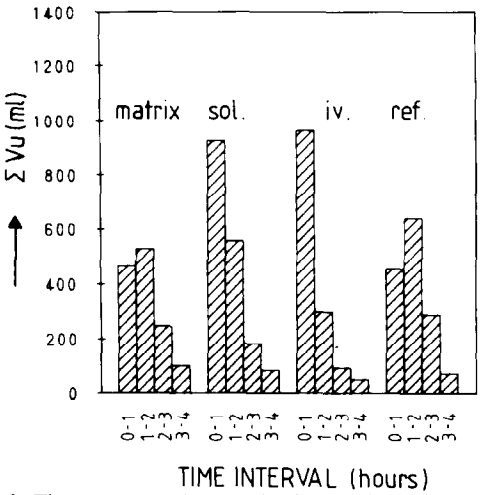

Fig. 6. The average urine production during the first four hourly intervals. (See Martin et al., 1984.)

Conclusions

The present study demonstrates that furosemide is no hit-and-run drug. On the contrary, adequate urine levels during a relatively long time are necessary to provoke the wanted diuretic effect. It seems that the efficiency of the drug can be increased when the drug concentrations in urine are kept at a constant level; low concentrations of drug may then be adequate.

Beermann (1982) and Brater (1985) suggested that fast dosing induced urinary concentrations of furosemide that transiently exceed those that induce maximal effect, which leads to some waste of drug. This agrees with data of Bhise et al. (1984) that a threshold concentration is necessary to provoke the liquid membrane effect; higher concentrations might show less increment in the diuretic effect than expected. This might well be

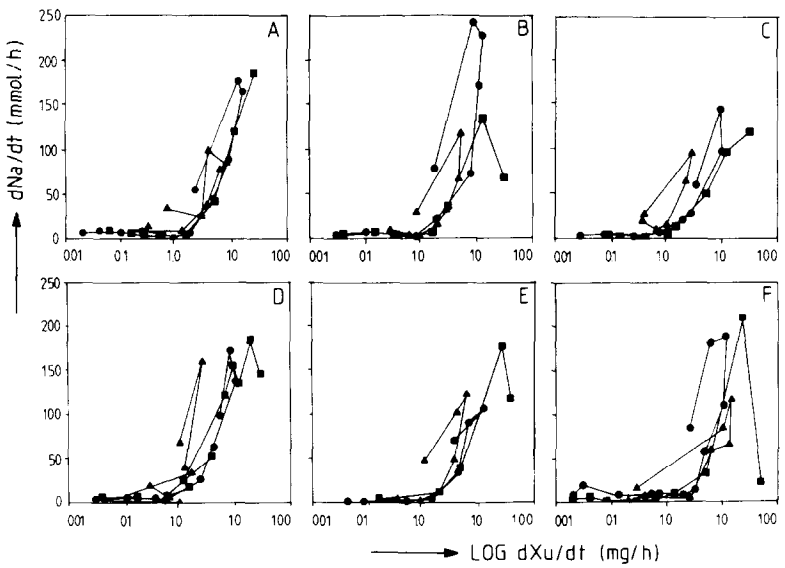

Fig. 5. The log dose–response curves (dXu/dt vs dNa/dt) for all subjects. The data points are connected chronologically. (■), Bolus i.v. injection 40 mg; (●), Oral solution 60 mg; (▲), matrix tablet 60 mg. No saturation of the effect can be observed (Brater, 1985).

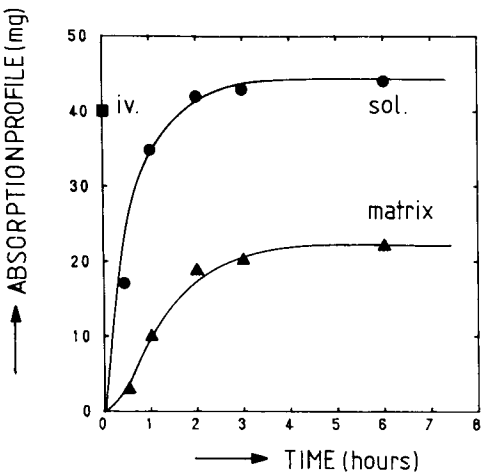

Fig. 7. The average drug input profiles for the different dosage forms.

connected with the sigmoidal adsorption kinetics which are often seen for surfactants; above the critical micellar concentration (CMC), no extra adsorption occurs.

The value for the CMC is 8.3×10^{-5} M and this is expected to be even less at the high salt concentrations in urine. Our data seem to confirm this suggestion although we did not observe a saturation of the diuretic effect (Fig. 8): a fast increment after i.v. dosing (29 ± 3 mg furosemide excreted in urine) gives similar diuretic effects as after oral (matrix) dosing (19 ± 11 mg furosemide

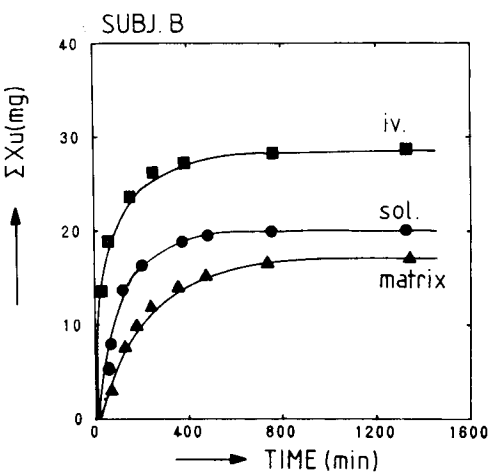

Fig. 8. Furosemide excretion in urine vs time for the different dosage forms (subject B).

TABLE 5

The urine flow Vu (y) versus the furosemide excretion rate in urine Xu (x) for the matrix tablet, oral solution and the i.v. bolus injection

	Equation	Correlation	P (1)	P (2)
Matrix				
subject				
A	$y = 92x + 67$	0.8958	n.s.	n.s.
B	$y = 143x - 54$	0.9265	0.01	n.s.
C	$Y = 220x + 16$	0.9660	0.01	0.05
D	$y = 359x - 48$	0.9061	0.05	0.01
E	$y = 150x - 5$	0.8778	0.05	n.s.
F	$y = 47x + 22$	0.9637	n.s.	0.01
Solution				
subject				
A	$y = 90x + 33$	0.9777	0.01	
B	$y = 136x + 31$	0.9212	0.01	
C	$y = 107x + 13$	0.9520	0.01	
D	$y = 126x + 7$	0.9544	0.01	
E	$y = 92x + 14$	0.9804	0.05	
F	$y = 111x + 83$	0.8749	0.01	
Bolus i.v.				
subject				
A	$y = 40x + 150$	0.9483		
B	$y = 36x + 98$	0.8970		
C	$y = 31x + 99$	0.9105		
D	$y = 44x + 200$	0.8044		
E	$y = 33x + 113$	0.8499		
F	$y = 41x + 78$	0.9528		

The response to the matrix tablet and solution are compared to the response of the bolus injection by analysis of covariance. Also both oral dosage forms have been compared. The level of significance is given in the last column: (1) oral vs i.v., (2) matrix vs solution; n.s., non-significant.

excreted in urine) with a much slower excretion profile (see Table 5). This shows that the diuretic effect is not related to c_{max}.

TABLE 6

Urinary data (mean ± S.E.M.) for the different dosage forms ($P < 0.05$)

Parameter	I.v. bolus	Solution	Matrix tablet
ΣVu_{0-4} (ml)	1391 ± 278	1752 ± 509	1345 ± 110
ΣVu_{0-24} (ml)	2164 ± 428	2642 ± 698	2327 ± 405
ΣNa^{+}_{0-4} (mmol)	168 ± 39	206 ± 69	179 ± 22
ΣNa^{+}_{0-24} (mmol)	247 ± 93	297 ± 119	287 ± 47
$F \times D$ (mg)	40.0 ± 1.0	44.5 ± 7.4	23.0 ± 10.9
ΣXu_{0-24} (mg)	29.0 ± 2.9	18.4 ± 3.6	19.4 ± 11.1

To be able to establish more or less steady-state urinary conditions, more detailed studies are needed on the relation between steady-state plasma levels and urinary levels and their effects. Only then, a controlled effect of furosemide might be reached.

Nevertheless, absorption problems of furosemide hinder an easy approach for formulating oral controlled-release dosage forms. Rectal controlled delivery might offer perspectives here. In conclusion, peak diuretic effects could not be abolished by the matrix tablet concept, probably due to a relative increase in efficiency of the drug from this tablet.

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Abbreviations

AUC	area under the curve
AUMC	area under the first moment curve
MAT	mean absorption time
MDT	mean dissolution time
MRT	mean residence time
F	bioavailability
PP	(microporous) polypropylene
t_{max}	time of maximum in plasma curve
c_{max}	maximum concentration in plasma curve
ΣVu	cumulative urine production
Vu	urine flow
Xu	furosemide excreted in urine
ΣNa	cumulative sodium excretion in the urine
ave	average
M_t	amount of drug released at time t
M_{max}	amount of drug in the tablet
D	dose

Appendix

A rather time consuming, but simple acid–base titrimetric analysis was performed to determine both furosemide and Tris buffer concentrations.

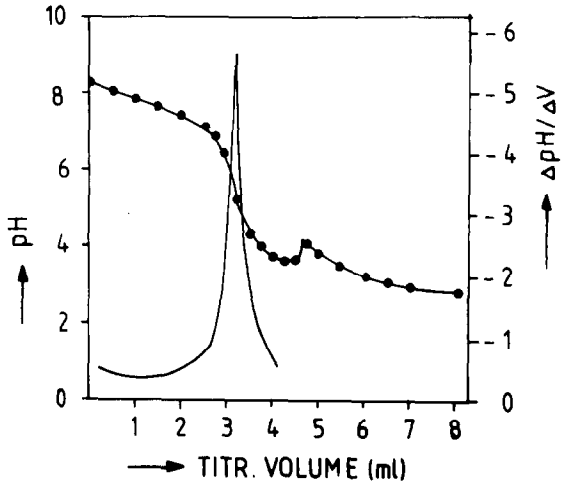

Fig. 9. Titration curve of the mixture of Tris and furosemide: 5.0 mmol Tris and 2.0 mmol furosemide in 10 ml water, titrated with HCl 0.1 N (see Appendix). The small peak at pH 4–5 is caused by the precipitation of furosemide from the solution.

Furosemide is a bifunctional acid ($pKa_1 = 3.8$, $pKa_2 = 7.5$) and Tris is a monofunctional base ($pK_b = 5.9$). Furosemide appears in solution partly as the monovalent H-Fur$^-$ and partly as the Fur^{2-} ion, in presence of an excess of Tris. Titration of the mixture with hydrochloric acid gives two equivalence points. In the first part of the titration the excess Tris is titrated and furosemide is completely converted to the monovalent ion (equivalence point at pH = 5.5); in the second part, the monovalent H-Fur$^-$ is converted to the H_2-Fur molecule. The second equivalence point does not give a clear change of pH and is therefore not useful. At known furosemide concentrations the first equivalence point can be applied to determine the Tris concentration (see Fig. 9). The furosemide concentrations were determined by UV analysis. The titrimetric equivalence point was detected by potentiometric pH measurements. The $\Delta pH/\Delta V$ curves were derived. The accuracy was about 1%.

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