The influence of drug input rate on the development of tolerance to frusemide

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Aims Understanding the impact of drug input rate on its pharmacokinetic-pharmacodynamic relationship may lead to a more optimal drug therapy. The aim of the present study was to investigate the influence of the rate of administration on tolerance development to frusemide, by giving the drug at four different infusion rates.

Methods Eight healthy volunteers were given 10 mg of frusemide on four different occasions, as a constant-rate intravenous infusion during 10, 30, 100 and 300 min, respectively. Urinary volume and contents of frusemide and sodium were measured in samples collected over 8 h.

Results The four different infusion rates systematically influenced the frusemide excretion rate versus diuretic and natriuretic response relationship. Counter-clockwise hysteresis occurred for the most rapid infusion rate, whereas a progressive clockwise hysteresis was observed for the slower infusions, indicating development of tolerance. For each subject, diuresis and natriuresis were modeled for all four treatments simultaneously, using a feedback tolerance model. It was not possible to describe the data using a model without tolerance. The time course of tolerance development showed remarkable differences between the infusion rates. The intensity of maximum tolerance development was significantly less for the slowest infusion rate compared with the more rapid infusions and it appeared significantly later. However, no differences in diuretic or natriuretic response were found between the treatments.

Conclusions The direction of the hysteresis loop is dependent on the input rate of frusemide. After the administration of a single low dose of frusemide, the time course of tolerance, rather than the integrated time course of tolerance, is influenced by the drug input rate.

Keywords: frusemide, drug input rate, pharmacodynamics, tolerance

Introduction

In the past 20 years pharmaceutical dosage forms have become more complex in their design. Novel drug delivery systems have been developed that achieve pharmacological effects not solely based on chemical structure, but also on the basis of controlling the rate of administration of the drug [1]. Clinically important changes in drug effects may result from changes in drug delivery [2]. For the loop diuretic frusemide, it has been shown that if identical total doses are given, a slow and constant input of the drug into the body induces a higher total effect compared with a rapid administration. This has been explained by investigating the efficiency of the drug [3–9]. However, the pharmacokinetic-pharmacodynamic relationship of frusemide may be altered by development of tolerance [6, 9, 10]. It has been suggested that for drugs that elicit compensatory homeostatic mechanisms,

a slow input of the drug will trigger fewer homeostatic reactions, leading to a higher cumulated pharmacological response. On the other hand, for drugs that elicit 'true tolerance', a slow input over a prolonged period of time will enhance the development of tolerance, reducing the total response [11].

The rapid intravenous administration of multiple frusemide doses activates counter-regulatory mechanisms leading to a profound decrease in diuretic and natriuretic effects [12]. However, clockwise hysteresis, (a sign of tolerance development) has been observed during slow input of the drug, for example after the administration of oral doses of frusemide in combination with food or of controlled release formulations [5, 7, 10]. Understanding the consequences of changing drug input rate for its pharmacokinetic-pharmacodynamic profile may lead to more adequate drug therapy. In the present study, frusemide was administered four times as a single dose using different infusion rates, with the aim to investigate and model the influence of the rate of administration on tolerance development to the drug.

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Methods

Subjects

Eight white male subjects participated in the study after giving written informed consent and after approval had been obtained from the Huddinge University Hospital Ethics Committee. Their ages ranged from 26 to 41 years and their body weights from 66 to 84 kg. All subjects were considered healthy according to detailed medical history and physical examination, which included an ECG and laboratory investigations. Smokers or heavy consumers of caffeine (more than four cups of coffee daily) were excluded from participating in the study. None of the subjects regularly used any medications.

Study design

The study had a randomized cross-over design using two replicate 4×4 latin squares balanced for possible carry-over effects. The four frusemide infusions were administered on separate study days with intervals of at least 1 week. To standardize experimental circumstances, no medications were allowed within 3 days before each study day. Also, the subjects were asked to refrain from alcohol and extreme physical activity within this period. Standardized meals were provided the day before and during each study day with a total content of 159 mmol sodium and 81 mmol potassium day⁻¹ and caffeinated drinks were not allowed during this time. Urine was collected for 24 h on the day before each study day to assess adherence to the diet and to estimate basal diuresis. The subjects fasted overnight and the study started in the morning at the closure of the 24 h urine collection. Before the start of the experiment, a cannula was inserted into an antecubital vein of each arm and the subjects assumed the supine position for 30 min. Blood samples were taken to measure plasma active renin, angiotensin II, aldosterone, antidiuretic hormone (ADH), atrial natriuretic peptide (ANP) and catecholamines (adrenaline, noradrenaline and dopamine). After the blood samples, each subject emptied his bladder and was weighed, after which the administration of frusemide was started (at 0 h). Frusemide 10 mg ml⁻¹ (Furix®, Benzon Pharma, Copenhagen, Denmark) was diluted with saline solution to a concentration of 1 mg ml⁻¹. A 10 mg dose of frusemide was given intravenously of four different infusion rates, 60 ml h⁻¹, 20 ml h⁻¹, 6 ml h⁻¹ and 2 ml h⁻¹ using a Syringe-Minder 90 infusion pump (CRITIKON, UK). The total infusion times were 10, 30, 100 and 300 min, respectively. The infusion fluid was protected from light. The subjects provided urine samples by voiding at 15 min intervals for the first 5 h and at 30 min intervals for the last 3 h after every dose. Blood samples were taken to measure plasma active renin, angiotensin II, aldosterone, ADH, ANP and catecholamines at 15, 30, 105 and 300 min and at 6 and 8 h after dosing. Blood samples were always taken before voiding and the subjects remained in the supine position, except when voiding. Every 30 min, the subjects drank 50 ml water. Lunch was served 5 h after dosing. Plasma samples were stored at -70°C. The urine volumes were weighed

and aliquots were carefully protected from light and stored at -70°C until analyzed for frusemide and sodium.

Analytical methods

Frusemide concentrations in urine were determined in duplicate by h.p.l.c. [13]. The lower limit of quantitation (LLQ) was 0.125 µg ml⁻¹. The intra-assay coefficient of variation at 1 µg ml⁻¹ was 6.4% and the inter-assay coefficient of variation was 7.6%. Sodium, plasma active renin, angiotensin II, aldosterone, ADH, ANP and catecholamines were analyzed elsewhere according to standard methods.

Data analysis

For frusemide, the pharmacological response may be monitored by measuring changes in volume diuresis or in the excretion rate of sodium. As loop diuretics exert their diuretic effects mainly from the luminal surface of the renal tubule [14], the pharmacological effects of frusemide are adequately described as a function of the urinary excretion rate of the drug [15, 16]. An apparent delay in the onset of diuretic response in relation to the urinary excretion rate of frusemide is commonly observed. Indirect-response models may be applied when a time lag exists between the concentration of a drug in plasma or biophase and the pharmacodynamic response [17, 18]. In the present study, a modified indirect-response model was used for analysis of the pharmacokinetic-pharmacodynamic relationship [12, 19]. The frusemide excretion rate (pharmacokinetics) and diuresis and natriuresis (pharmacodynamics) were modeled in separate steps and all subjects were analyzed individually. A multiexponential model was applied to describe the time course of the frusemide excretion rate (ER in µg min⁻¹) from 0 to 8 h:

$$ER = \sum_{i=1}^n A_i \times e^{-\alpha_i \times t} \quad (1)$$

The most appropriate pharmacokinetic model was selected by residual analysis. Each infusion rate was modelled separately in order to describe the observed frusemide excretion rates as closely as possible. The pharmacokinetic parameters were then fixed and ER served as input to the pharmacodynamic model. For each subject, the pharmacodynamic model was regressed to the diuresis and natriuresis data for all four frusemide infusion rates simultaneously (PCNONLIN version 4.2, Scientific Consulting Inc., Cary, N.C., USA). Uniform weights (a constant variance) were applied.

The basic premise of an indirect-response model is that a measured response (R) to a drug is produced by indirect mechanisms. Factors controlling the production (k_{in}) or the loss (k_{out}) of a response variable may be stimulated or inhibited by the drug [17, 18]. In our study, the response variables measured are diuresis (in ml min⁻¹) and natriuresis (in mmol min⁻¹). The rate of change of the diuretic (natriuretic) response (R) over time with no frusemide present can then be described as follows:

$$\frac{dR}{dt} = k_{in} - k_{out} \times R \quad (2)$$

where k_{in} represents the zero-order constant for production of the diuretic response and k_{out} defines the first-order rate constant for loss of response.

Because frusemide inhibits the reabsorption of chloride, sodium and water [14], an indirect-response model was used assuming frusemide to increase the diuretic (natriuretic) response by inhibiting k_{out} according to an inhibition function $I(ER)$:

$$I(ER) = 1 - \frac{I_{max} \times ER^\gamma}{IC_{50}^\gamma + ER^\gamma} \quad (3)$$

where I_{max} represents the maximum effect attributed to the drug, IC_{50} represents the frusemide excretion rate producing 50% of the maximum drug-induced inhibition potency and γ is the sigmoidicity factor. The rate of change of diuretic (natriuretic) response over time with frusemide [12, 13, 18] can then be described as follows:

$$\frac{dR}{dt} = k_{in} - k_{out} \times I(ER) \times R \quad (4)$$

Maximum inhibition is obtained when $ER \gg IC_{50}$ and $I(ER)$ then approaches $1 - I_{max}$. When ER approaches zero, the net effect approaches the baseline effect, i.e. $I(ER) \rightarrow 1$.

To account for tolerance, an endogenous regulator (or modifier) M was implemented into the model as a feedback mechanism [12, 19]. An increase in response R causes an increase in modifier M . For simplicity, M is assumed to be solely governed by R and a single rate constant k_{tol} as follows:

$$\frac{dM}{dt} = k_{tol} \times R - k_{tol} \times M \quad (5)$$

When M increases, it counter-balances R , as M is assumed to stimulate k_{out} according to a stimulation function $S(M)$:

$$S(M) = \left(1 + \frac{M}{M_{50}} \right) \quad (6)$$

M_{50} was assumed to be equal to unity. The full model can now be described as follows:

$$\frac{dR}{dt} = k_{in} - k_{out} \times \left(1 - \frac{I_{max} \times ER^\gamma}{IC_{50}^\gamma + ER^\gamma} \right) \times R \times (1 + M) \quad (7)$$

M is defined according to equation 5. A value for maximum response including tolerance development ' R_{max} ' (basal + drug-induced) can be calculated from the parameter values obtained [12] according to

$$R_{max} = -\frac{1}{2} + \sqrt{\frac{1}{4} + \frac{k_{in}}{k_{out} \times (1 - I_{max})}} \quad (8)$$

At equilibrium, the steady-state values of R and M are equal.

For an overall evaluation, the same tolerance model was also applied to pooled data of all eight subjects (784 observations), for both diuresis and natriuresis. Here, the pharmacokinetic parameters obtained from simultaneous modeling of all subjects for each infusion rate were used as input.

Calculations

The rate and extent of tolerance development was further investigated by calculating the time course of the modifier M and the time and size of maximum tolerance development ($t_{max,M}$ and M_{max}) from the estimated parameters for each frusemide treatment. Also, the AUC of M was calculated from the predicted M values using the linear trapezoidal rule, extrapolated to infinity. Differences in M_{max} and AUC of M between the four treatments for diuresis and natriuresis were analyzed by repeated measures ANOVA. For pairwise comparisons, P values were adjusted using the Bonferroni multiple comparisons test. Differences in $t_{max,M}$ between the treatments were analyzed by Friedman's ANOVA for repeated measures, followed by Dunn's test for multiple comparisons. Differences in total recovery of frusemide in urine, total diuresis and total natriuresis between the four treatments were analyzed by repeated measures ANOVA. For pairwise comparisons, P values were adjusted using the Bonferroni multiple comparisons test.

Results

The administration of frusemide at different infusion rates had a profound effect on the pharmacokinetic-pharmacodynamic relationship of the drug. Figures 1–4 display the individual data of subject 2, which are representative of all subjects. The most rapid (10 min) infusion gave rise to distinct counter-clockwise hysteresis of the frusemide excretion rate *vs* response curve, indicating a delay between the excretion rate and the diuretic effect. In this case, the

Figure 1 Diuresis (a) and natriuresis (b) *vs* frusemide excretion rate following the 10 min (●), 30 min (▲), 100 min (○) and 300 min (△) infusion in subject 2.

Figure 2 Observed (symbols) and predicted (solid lines) frusemide excretion rate *vs* time following the 10 min (●), 30 min (▲), 100 min (○) and 300 min (△) infusion in subject 2.

Figure 3 Observed (symbols) and predicted (solid lines) diuresis (a) and natriuresis (b) *vs* time following the 10 min (●), 30 min (▲), 100 min (○) and 300 min (△) infusion in subject 2.

input rate of the drug was greater than the rate of equilibrium between the pharmacokinetics (ER) and the pharmacodynamics (R), as well as the rate of equilibrium between the pharmacodynamics (R) and the development of tolerance (M). However, a clockwise hysteresis loop was observed after the 30 min infusion, which increased in size after the slower infusion times of 100 and 300 min (Figure 1). With these three infusion rates, the rate of input was slower than the PK-PD equilibrium rate, as well as the rate of tolerance development. It was not possible to describe the data using a model without tolerance. The clockwise hysteresis indicated acute within-dose tolerance development to the diuretic effect of frusemide for the slower infusion rates. However, there was no difference in total diuresis or

Figure 4 Simulation of tolerance development (M) for diuresis (a) and natriuresis (b) *vs* time following the 10 min (solid line), 30 min (broken line), 100 min (line with large dots) and 300 min (line with small dots) infusion in subject 2. The horizontal bars indicate the respective infusion times.

total natriuresis between the four treatments. There was also no difference in total urinary recovery of frusemide between the treatments. The mean $\pm$ s.d. values and the 95% confidence intervals for the recovery of frusemide (mg) were: 6.7 ± 0.9 ; (5.9–7.5) for the 10 min infusion, 6.4 ± 0.5 ; (6.0–6.8) for the 30 min, 6.5 ± 1.2 ; (5.5–7.5) for the 100 min and 6.2 ± 0.4 ; (5.8–6.5) for the 300 min infusion. The total diuretic and natriuretic response from 0 to 8 h obtained from all subjects is shown in Table 1.

A good fit for the frusemide excretion rate was obtained for all subjects. A tri-exponential model with first-order input and first-order output, or a model with constant intravenous input and first-order output gave the best description of the data, depending on the infusion rate and the shape of the frusemide excretion rate *vs* time curve (Figure 2). The pharmacokinetic parameters obtained were introduced into the pharmacodynamic tolerance model. Tables 2 and 3 present the pharmacodynamic parameter estimates obtained from the fit of the tolerance model for diuresis and natriuresis, respectively. Interestingly, the rate constant for tolerance development k_{tol} was consistently higher for natriuresis than for diuresis. The mean half-life for tolerance development was 42 min for natriuresis *vs* 139 min for diuresis. Figure 3 shows the observed and calculated values of diuretic and natriuretic response of subject 2. High consistency was obtained between the predicted and observed data for all subjects, except for the diuresis data of subject 8. The subset of the diuresis and natriuresis data after the 100 min infusion was left out from

Table 1 Total diuresis and natriuresis after an infusion dose of 10 mg frusemide given over 10, 30, 100 and 300 min. Basal diuresis and natriuresis were subtracted. Mean, s.d. and 95% confidence intervals, $n = 8$.

Subject	Diuresis (ml)				Subject	Natriuresis (mmol)			
	10	30	100	300		10	30	100	300
1	622	830	994	1342	1	95	108	129	120
2	631	779	1178	1413	2	106	107	129	121
3	1263	1039	466	1438	3	134	104	27	128
4	512	990	1356	1411	4	83	116	147	149
5	1511	1424	1532	1718	5	127	114	102	135
6	1366	1097	1427	1234	6	187	130	139	108
7	1143	1247	1488	1090	7	107	126	141	88
8	2092	1374	2329	1716	8	169	108	183	160
Mean	1142	1097	1346	1420	Mean	126	114	125	126
s.d.	539	237	528	216	s.d.	36	10	45	23
95% CI	692–1593	899–1296	904–1788	1240–1601	95% CI	96–156	106–122	87–162	107–145

the analysis of subject 3. This was needed because of the extreme deviation of this subset from the remaining data of subject 3 and also in comparison to similar subsets of the other subjects. For subject 6 imprecise parameter values were obtained for k_{in} and k_{out} , but not for the other parameters. This may be due to the fact that the first datapoint of this subject already was the highest in the dataset, causing the estimates of k_{in} and k_{out} to be uncertain. Otherwise, a good parameter precision was obtained for all subjects.

The different infusion rates caused striking differences in the time course of tolerance development for both diuresis and natriuresis. This is illustrated by Figure 4, showing the calculated values of the modifier M as a function of time. It can be observed that the time course of tolerance development was very similar for the 10 min and 30 min infusions as shown by similar shapes of M . However, the time of maximum tolerance development occurred later for the slower infusions of 100 min and 300 min, respectively. Interestingly, the time course of M clearly lagged behind with respect to the infusion rate for the 10, 30 and 100 min infusions, but not for the 300 min infusion. Here, the development of tolerance closely followed the pattern of induced diuresis and natriuresis, returning back to baseline after the infusion was stopped.

The time ($t_{max,M}$) and size (M_{max}) of maximum tolerance development are shown in Tables 4 and 5, respectively. The baseline value for M was subtracted from the peak height to yield the net increase caused by the frusemide treatment. The time of maximum tolerance development appeared significantly later for the 300 min infusion compared to the 10 min and 30 min infusions for both diuresis and natriuresis. The mean difference between the $t_{max,M}$ of the 300 min infusion and the 10 min infusion was 3.9 h for diuresis and 4.1 h for natriuresis, respectively. The mean difference between the 300 min and the 30 min infusion was 3.8 h for diuresis and 4.0 h for natriuresis, respectively (Table 4). Also, the peak value of M (M_{max}) was significantly lower for the slowest infusion compared to all other infusion rates for both diuresis and natriuresis (Table 5). In spite of these notable differences in the appearance of tolerance development as a function of the infusion rate, the AUC values of M were rather similar. There was only a significant difference between the total AUC of the most rapid and the slowest infusion for diuresis ($P < 0.05$), but not for the other infusion rates. There was no difference in total AUC of M for natriuresis. The mean $\pm$ s.d. values of total AUC for diuresis ($\text{ml min}^{-1} \text{h}$) were: 14 ± 1.8 for the 10 min, 15 ± 1.1 for the 30 min, 17 ± 1.8 for the 100 min and 18 ± 3.9 for the 300 min infusion. The mean $\pm$ s.d. values of total AUC for natriuresis ($\text{mmol min}^{-1} \text{h}$) were 2.1 ± 0.5 , 2.2 ± 0.3 , 2.7 ± 1.1 and 2.1 ± 0.7 , respectively.

Similar results were obtained when diuresis and natriuresis data were modeled for all subjects pooled together (the diuresis data of subject 8 were included in this analysis). Also in this case, there was no difference in AUC of M for natriuresis. For diuresis, the AUC showed a tendency towards slight increase with infusion rate.

In spite of the acute development of tolerance for all four infusion rates, no hormonal counter-regulatory actions could be observed. Levels of plasma active renin, angiotensin II,

Table 2 PD modeling of diuresis. k_{in} is the zero-order rate constant for production of diuretic response, k_{out} is the first-order rate constant for loss of diuretic response, I_{max} is a parameter representing the maximum effect of the drug on the inhibition function, IC_{50} represents the frusemide excretion rate producing 50% of I_{max} , γ is the sigmoidicity factor and k_{tol} is the first-order rate constant for production and loss of M. R_0 and R_{max} are secondary parameters representing basal diuresis and maximum diuresis including tolerance development (basal + drug-induced). Mean (s.d.) $n=7$.

Subject	Parameter							
	k_{in} ($ml (min h)^{-1}$)	k_{out} (h^{-1})	I_{max}	IC_{50} ($\mu g min^{-1}$)	γ	k_{tol} (h^{-1})	R_0 ($ml min^{-1}$)	R_{max} ($ml min^{-1}$)
1	233	41	0.94	8	2.0	0.3	1.9	9
2	600	177	0.97	8	2.5	0.5	1.4	10
3	240	53	0.96	6	1.7	0.6	1.7	10
4	177	42	0.97	6	1.6	0.4	1.6	12
5	335	69	0.94	4	1.1	0.2	1.8	9
6	2768	451	0.97	7	1.6	0.3	2.0	13
7	709	96	0.95	11	1.9	0.2	2.3	12
8	—	—	—	—	—	—	—	—
Mean	723	133	0.96	7	1.8	0.3	1.8	11
s.d.	924	148	0.01	2	0.4	0.1	0.3	2

Table 3 PD modeling of natriuresis. k_{in} is the zero-order rate constant for production of natriuretic response, k_{out} is the first-order rate constant for loss of natriuretic response, I_{max} is a parameter representing the maximum effect of the drug on the inhibition function, IC_{50} represents the frusemide excretion rate producing 50% of I_{max} , γ is the sigmoidicity factor and k_{tol} is the first-order rate constant for production and loss of M. R_0 and R_{max} are secondary parameters representing basal natriuresis and maximum natriuresis including tolerance development (basal + drug-induced). Mean (s.d.) $n=8$.

Subject	Parameter							
	k_{in} ($mmol (min h)^{-1}$)	k_{out} (h^{-1})	I_{max}	IC_{50} ($\mu g min^{-1}$)	γ	k_{tol} (h^{-1})	R_0 ($mmol min^{-1}$)	R_{max} ($mmol min^{-1}$)
1	18	134	0.96	10	2.5	0.8	0.12	1.5
2	111	910	0.97	9	2.3	0.8	0.11	1.7
3	44	467	0.98	5	1.7	0.9	0.09	1.7
4	33	369	0.99	5	1.7	0.8	0.08	2.2
5	45	365	0.97	5	1.6	0.5	0.11	1.5
6	263	1589	0.99	13	2.6	3.7	0.14	2.9
7	66	447	0.97	11	2.3	0.4	0.13	1.8
8	47	456	0.98	3	1.3	0.4	0.09	1.9
Mean	78	592	0.98	8	2.0	1.0	0.11	1.9
s.d.	80	457	0.01	3	0.5	1.1	0.02	0.5

Table 4 The time of maximum tolerance development (t_{maxM}) after an infusion dose of 10 mg frusemide given over 10, 30, 100 and 300 min.

Subject	t_{maxM} for Diuresis (h) Infusion time (min)				Subject	t_{maxM} for Natriuresis (h) Infusion time (min)			
	10	30	100	300		10	30	100	300
1	1.15	1.55	2.03	5.17	1	0.90	1.19	1.91	5.06
2	0.90	0.97	1.95	5.05	2	0.78	0.71	1.87	5.03
3	1.24	1.15	—	5.05	3	1.01	1.01	—	5.03
4	1.00	1.18	1.91	5.07	4	0.85	0.98	1.85	5.04
5	2.17	2.16	2.66	5.28	5	1.26	1.46	2.11	5.06
6	1.15	1.27	2.12	5.26	6	0.43	0.65	1.77	5.18
7	1.05	1.23	1.99	5.13	7	0.90	1.07	1.90	5.07
8	—	—	—	—	8	1.47	1.23	2.07	5.10
Mean	1.24***	1.36*	2.11	5.14	Mean	0.95***	1.04**	1.93	5.07
s.d.	0.43	0.39	0.28	0.10	s.d.	0.31	0.27	0.12	0.05

Differences in treatments were analyzed by Friedman's ANOVA for repeated measures, followed by Dunn's test for pairwise comparisons. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ when compared with the 300 min infusion.

Table 5 The size of maximum tolerance development ($M_{\max}$) after an infusion dose of 10 mg frusemide given over 10, 30, 100 and 300 min.

$M_{\max}$ for diuresis (ml min^{-1})					$M_{\max}$ for natriuresis (mmol min^{-1})				
Infusion time (min)					Infusion time (min)				
Subject	10	30	100	300	Subject	10	30	100	300
1	3.32	3.66	3.63	2.80	1	0.82	0.87	0.82	0.41
2	5.01	4.79	5.57	3.89	2	0.85	0.81	0.82	0.45
3	5.36	4.87	—	3.29	3	0.94	0.83	—	0.42
4	4.15	4.88	5.27	3.74	4	0.85	1.03	1.04	0.58
5	1.93	2.07	2.25	2.02	5	0.54	0.59	0.62	0.36
6	3.84	3.95	3.92	2.82	6	2.35	2.29	1.48	0.37
7	3.25	3.52	3.89	1.90	7	0.65	0.71	0.76	0.25
8	—	—	—	—	8	0.75	0.67	0.86	0.51
Mean	3.84**	3.96***	4.09***	2.92	Mean	0.97*	0.97*	0.91*	0.42
s.d.	1.16	1.02	1.20	0.78	s.d.	0.57	0.55	0.28	0.10

Differences in treatments were analyzed by repeated measures ANOVA, followed by the Bonferroni multiple comparisons test for pairwise comparisons.

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ when compared with the 300 min infusion.

aldosterone and ANP did not show any systematic changes during the day. The catecholamine and ADH values could not be evaluated, due to technical problems with the analyses.

Discussion

Much is unknown about the factors that cause variability in pharmacodynamics and the mechanisms involved [20]. In the present study, there was a striking difference in the time course and appearance of tolerance development to frusemide, depending on the rate of input of the drug. Distinct clockwise hysteresis loops indicating tolerance development were observed for the slower infusions, in contradistinction to the most rapid infusion rate of frusemide. Instead, a counter-clockwise hysteresis indicated the delay between the frusemide excretion rate and the diuretic effect. In this case, the drug input rate exceeded the rate of loss of response and tolerance development throughout the duration of the infusion.

In this study, the AUC of the modifier M, used to quantify the development of tolerance after each infusion, was rather similar for all treatments. However, the similarity in AUC values may be a consequence of the characteristics of the present feedback model rather than a demonstration that total tolerance development was the same. In this model, the AUC value of M is not independent from the AUC of R (total response), which was the same for all infusions. Nevertheless, the similarity in total diuretic and natriuretic response and total frusemide recovery may indicate that there was no or very little difference in total tolerance development to the same dose. The onset of tolerance development was much more rapid for the 10 and 30 min infusions, compared to the slower input rates. For the slowest infusion, tolerance had reached its maximum only after more than 5 h for both diuresis and natriuresis. Also, the peak value of tolerance development was much lower for the 300 min infusion compared to the other treatments.

In our study, the kinetics of the response variable R was found to be very fast in comparison to the kinetics of the

regulator M. That is, the value of k_{out} was high in comparison to k_{tol} . For diuresis, the mean half-life for tolerance development was 139 min, whereas the mean half-life for the first-order loss of response (k_{out}) was only 0.3 min. For natriuresis, the mean half-lives were 42 and 0.1 min, respectively. The relatively low value of k_{tol} rate-limited the development of tolerance, causing this to clearly lag behind the infusion rate and the diuretic and natriuretic response. For the most rapid infusion, the clockwise hysteresis was even entirely obscured by the counter-clockwise hysteresis loop caused by the indirect response of the drug [12, 17]. However, the level of response after the slowest 300 min infusion was low enough to enable M to exert a more direct feedback, leading to a large clockwise hysteresis loop.

It has been suggested that the influence of drug input rate on tolerance development is not only dependent on the rate of administration itself, but also on the mechanism responsible for tolerance [11]. Castañeda *et al.* approached this issue by considering tolerance as either being caused by compensatory homeostatic (counter-regulatory) mechanisms or by more intrinsic processes ('true tolerance'). For drugs that elicit compensatory homeostatic mechanisms, such as nifedipine, a slow input of the drug would trigger fewer homeostatic reactions, leading to an increased pharmacological response. For drugs that elicit 'true tolerance', such as nitrates, a slow input would enhance the development of tolerance, reducing the response [11].

With nifedipine [21], the concentration-effect relationships for two infusion rates were profoundly different. This was suggested to be caused by stronger counter-regulation in baroreceptor reflex activation of the higher infusion rate. Similar observations have been reported for other calcium antagonists such as nisoldipine [22] and other vasodilator agents such as prazosin [23]. On the other hand, a slow and constant drug input rate has been found disadvantageous for the anti-anginal effect of nitrates, because tolerance occurs after several doses [24]. Psychomotor and subjective adverse effects of diazepam were found to be less after the administration of controlled release capsules compared to

plain tablets in healthy volunteers [25] despite similar plasma concentrations. This was attributed to stronger tolerance development for the controlled release formulation. Administration of an intravenous bolus dose of morphine to rats causes less tolerance than two different constant rate infusions [26]. However, tolerance development was stronger for the higher than for the lower infusion rate. For many drugs, it may prove difficult to separate counter-regulation from 'true tolerance'. In many cases, the mechanisms for the development of tolerance are not known. Tolerance may develop differently for different drug effects and several factors may be involved concomitantly, with different rates of onset.

For frusemide, acute development of tolerance may be due to the activation of counter-acting mechanisms. The profound decrease in diuretic response after the repetitive administration of frusemide as rapid intravenous infusions was associated with a significant rise in plasma active renin and a decrease in atrial natriuretic peptide levels [12]. Activation of the renin-angiotensin-aldosterone system and the sympathetic nervous system have been proposed to mediate the acute development of tolerance to the drug [27]. Suppression of ANP may be involved in the postdiuretic phase of renal sodium retention [28]. However, in the present study, no changes in these hormone levels could be observed. The total frusemide dose of 10 mg may have been too low to render any detectable changes in the hormones analyzed. Also, the extensive urine collection protocol causing the subjects to frequently change from the supine position may have been a complicating factor, although the blood sampling and sample handling procedures were highly standardized. It seems likely that local intrarenal mechanisms contributed to the acute development of tolerance. Homeostatic regulations such as resetting of the tubuloglomerular feedback or changes in the tubulointerstitial hydrostatic and osmotic pressure gradients may be mechanistically involved in causing tolerance phenomena, although this has not been studied in humans [29].

In the present study, as in our previous study [12], tolerance development was stronger for natriuresis than for diuresis, as shown by higher values for k_{tol} . This is likely to be related to the type of fluid replacement used [12] and also indicates that the renal response pattern and tolerance development for diuresis and natriuresis are partly independent. Prediction of the total diuretic response to frusemide is complicated and determined by the total amount of drug delivered to the site of action, the time course of that delivery and the pharmacokinetic-pharmacodynamic relationship between the frusemide excretion rate and the diuretic effect [3]. This relationship is nonlinear and subject to both counter-clockwise and clockwise hysteresis. In patients, a combination of disease-dependent pharmacokinetic and pharmacodynamic factors may be involved in causing diuretic resistance [30, 31]. Our findings suggest that with the present dose, the time course of tolerance development to frusemide, rather than total tolerance, is influenced by the drug input rate. A slow and constant administration of the drug may lead to a higher diuretic efficiency and delay and blunt the development of tolerance. Clinically, a relatively slow input may also prevent the compensatory renal sodium retention that may occur, not

only during the time of diuretic activity, but also well after the drug effect has subsided and which may antagonize the net diuretic effect [8, 29, 32, 33]. This study showed the influence of drug input rate on the shape of the concentration-effect curve. Care should be taken not to interpret the size of the clockwise hysteresis loop as a measurement of tolerance development or to assume its absence if such a hysteresis is not observed, since this is not necessarily the case. The influence of the frusemide input rate on the diuretic response stresses the importance of finding the optimal dosage regimen to overcome diuretic resistance in different patient populations.

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References

- 1 Banerjee PS, Robinson JR. Novel drug delivery systems: an overview of their impact on clinical pharmacokinetic studies. *Clin Pharmacokinet* 1991; **20**: 1–14.
- 2 Goldman P. Rate-controlled drug delivery. *N Engl J Med* 1982; **307**: 286–290.
- 3 Kaojarern S, Day B, Brater DC. The time course of delivery of furosemide into urine: an independent determinant of overall response. *Kidney Int* 1982; **22**: 69–74.
- 4 Alván G, Helleday L, Lindholm A, Sanz E, Villén T. Diuretic effect and diuretic efficiency after intravenous dosage of frusemide. *Br J Clin Pharmacol* 1990; **29**: 215–219.
- 5 Alván G, Paintaud G, Eckernäs S-Å, Grahnén A. Discrepancy between bioavailability as estimated from urinary recovery of frusemide and total diuretic effect. *Br J Clin Pharmacol* 1992; **34**: 47–52.
- 6 van Meyel JJ, Smits P, Russel FG, Gerlag PG, Tan Y, Gribnau FW. Diuretic efficiency of furosemide during continuous administration versus bolus injection in healthy volunteers. *Clin Pharmacol Ther* 1992; **51**: 440–444.
- 7 Paintaud G, Alván G, Eckernäs S-Å, Wakelkamp M, Grahnén A. The influence of food intake on the effect of two controlled release formulations of furosemide. *Biopharm Drug Dispos* 1995; **16**: 221–232.
- 8 Dormans TPJ, van Meyel JJM, Gerlag PGG, Tan Y, Russel FGM, Smits P. Diuretic efficiency of high dose furosemide in severe heart failure: bolus injection versus continuous infusion. *J Am Coll Cardiol* 1996; **28**: 376–382.
- 9 Wakelkamp M, Alván G, Paintaud G. Natriuretic efficiency of frusemide as a consequence of drug input rate. *Br J Clin Pharmacol* 1997; **43**: 481–491.
- 10 Hammarlund MM, Odling B, Paalzow LK. Acute tolerance to furosemide diuresis in humans. Pharmacokinetic-pharmacodynamic modeling. *J Pharmacol Exp Ther* 1985; **233**: 447–453.
- 11 Castañeda-Hernández G, Caillé G, du Souich P. Influence of drug formulation on drug concentration-effect relationships. *Clin Pharmacokinet* 1994; **26**: 135–143.
- 12 Wakelkamp M, Alván G, Gabrielsson J, Paintaud G. Pharmacodynamic modeling of furosemide tolerance after multiple intravenous administration. *Clin Pharmacol Ther* 1996; **60**: 75–88.
- 13 Wakelkamp M, Alván G, Paintaud G. The time of maximum effect for model selection in pharmacokinetic-

- pharmacodynamic analysis applied to frusemide. *Br J Clin Pharmacol* 1998; **45**: 63–70.
- 14 Burg MB. Tubular chloride transport and the mode of action of some diuretics. *Kidney Int* 1976; **9**: 189–197.
 - 15 Odland B, Beermann B. Renal tubular secretion and effects of furosemide. *Clin Pharmacol Ther* 1980; **27**: 784–790.
 - 16 Holford NHG. Parametric models of the time course of drug action. In *The in vivo study of drug action*. Eds van Boxtel CJ, Holford NHG, Danhof M. Amsterdam: Elsevier, 1992: 61–70.
 - 17 Dayneka NL, Garg V, Jusko WJ. Comparison of four basic models of indirect pharmacodynamic responses. *J Pharmacokinet Biopharm* 1993; **21**: 457–478.
 - 18 Jusko WJ, Ko HC. Physiologic indirect response models characterize diverse types of pharmacodynamic effects. *Clin Pharmacol Ther* 1994; **56**: 406–419.
 - 19 Gabrielsson J, Weiner D. *Pharmacokinetic and pharmacodynamic data analysis: Concepts and applications*. (1st and 2nd ed.) Stockholm: Swedish Pharmaceutical Press, 1994, 1997.
 - 20 Danhof M. Does variability in drug disposition explain (all) variability in drug effects? In *Topics in pharmaceutical sciences* Eds Breimer DD, Crommelin DJA, Midha KK, 1989. The Hague: International Pharmaceutical Federation, 1989: 573–586.
 - 21 Kleinbloesem CH, van Brummelen P, Danhof M, Faber H, Urquhart J, Breimer DD. Rate of increase in the plasma concentration of nifedipine as a major determinant of its hemodynamic effects in humans. *Clin Pharmacol Ther* 1987; **41**: 26–30.
 - 22 van Harten J, van Brummelen P, Zeegers RRECM, Danhof M, Breimer DD. The influence of infusion rate on the pharmacokinetics and haemodynamic effects of nisoldipine in man. *Br J Clin Pharmacol* 1988; **25**: 709–717.
 - 23 Elliott HL, Vincent J, Meredith PA, Reid JL. Relationship between plasma prazosin concentrations and α -antagonism in humans: comparison of conventional and rate-controlled (Oros) formulations. *Clin Pharmacol Ther* 1988; **43**: 582–587.
 - 24 Fung H-L. Pharmacokinetic determinants of nitrate action. *Am J Med* 1984; **76**(suppl. 6A): 22–26.
 - 25 Mattila M. Acute and subacute effects of diazepam on human performance: comparison of plain tablet and controlled release capsule. *Pharmacol Toxicol* 1988; **63**: 369–374.
 - 26 Ekblom M, Hammarlund-Udenaes M, Paalzow L. Modeling of tolerance development and rebound effect during different intravenous administrations of morphine to rats. *J Pharmacol Exp Ther* 1993; **266**: 244–252.
 - 27 Sjöström PA, Odland BG, Beermann BA, Hammarlund-Udenaes M. On the mechanism of acute tolerance to furosemide diuresis. *Scand J Urol Nephrol* 1988; **22**: 133–140.
 - 28 de Wardener HE. Idiopathic edema: Role of diuretic abuse. *Kidney Int* 1981; **19**: 881–892.
 - 29 Sjöström PA, Beermann BA, Odland BG. Delayed tolerance to furosemide diuresis. Influence of angiotensin converting enzyme inhibition by lisinopril. *Scand J Urol Nephrol* 1988; **22**: 317–325.
 - 30 Wilcox CS, Mitch WE, Kelly RA, *et al.* Response of the kidney to furosemide I. Effects of salt intake and renal compensation. *J Lab Clin Med* 1983; **102**: 450–458.
 - 31 Brater DC. Resistance to diuretics: mechanisms and clinical implications. *Adv Nephrol* 1993; **22**: 349–369.
 - 32 Brater DC. Resistance to loop diuretics. Why it happens and what to do about it. *Drugs* 1985; **30**: 427–443.
 - 33 Ferguson JA, Sundblad KJ, Becker PK, Gorski JC, Rudy DW, Brater DC. Role of duration of diuretic effect in preventing sodium retention. *Clin Pharmacol Ther* 1997; **63**: 203–208.

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