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Discrepancy between bioavailability as estimated from urinary recovery of frusemide and total diuretic effect

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1 Frusemide was given at a dose of 60 mg as two oral controlled release (CR) formulations and as plain tablets in a randomised, balanced, three way cross over design to 26 healthy volunteers. Urinary volume, and contents of frusemide, sodium, chloride and potassium were measured in samples taken over 24 h.

2 There was a marked difference between the CR formulations on one hand and the plain tablets on the other, in excretion of frusemide and diuresis *vs* time. The total diuretic/saluretic effect was only marginally lower (19 and 28% respectively, $P < 0.05$) after CR compared with plain tablets although the fraction absorbed was markedly decreased (39 and 51% lower, respectively, $P < 0.05$), estimated as urinary recovery of frusemide. The total diuresis of the two CR formulations did not differ although the urinary recovery was significantly different ($P < 0.05$).

3 The diuretic effect *vs* frusemide excretion rate showed minimal counter-clockwise hysteresis after plain tablets while the CR formulations produced clockwise hysteresis indicating tolerance.

4 In agreement with the concept of efficiency, the higher diuretic/saluretic effect per amount of excreted frusemide may be a consequence of the slower output of frusemide in urine with the CR formulations compared with plain tablets. The major part of the pharmacological effect was produced with a higher efficiency after CR compared with plain tablets. It should be noted that the pharmacokinetics of a drug and its pharmacodynamic potency independently determine the total response.

5 This study shows that bioavailability assessments might give different results depending on whether parameters like area under the curve or excreted amounts of drugs are evaluated in contrast to results obtained when total effect is measured. This discrepancy is of profound importance for the bioavailability/bioequivalence concept.

Keywords frusemide pharmacokinetics pharmacodynamics

Introduction

Traditionally, pharmacological effects have been modelled as a function of drug concentrations (Holford & Sheiner, 1981). Another way of expressing pharmacodynamic relationships is to define the dependent variable as effect per concentration and study it as a function of concentration. The new variable is named efficiency (Kaojarern *et al.*, 1982), to be different from effect, and gives an estimate of how much effect is obtained per unit of stimulus through the concentration range. According to the principle of diminishing returns with increasing concentrations (Holford & Sheiner, 1981) and the existence of a maximum effect, efficiency will obviously decrease at the high end of the effect-concentration range. A succinct expression for efficiency through the whole course of drug action can be derived from a theoretical effect-concentration relationship. It can be used to explain that the pharmacokinetics of a drug is an important factor for the cumulated pharmacological effect (Alván *et al.*, 1990; Hammarlund *et al.*, 1985; Kaojarern *et al.*, 1982).

Frusemide is a drug that shows absorption limited kinetics which means that the absorption is limiting the rate of the excretion of drug in urine (Hammarlund *et al.*, 1984). Frusemide excretion rate has been shown to determine the pharmacological response (Hammarlund & Benet, 1989) but influences the total diuretic effect as

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well, as diuretic efficiency, which is understood as effect per unit of excreted drug, will vary with drug excretion rate (Alván *et al.*, 1990; Hammarlund *et al.*, 1985; Kaojarern *et al.*, 1982).

The primary aim of this study was to assess the relative bioavailability of a new controlled release (CR) frusemide formulation with regard to renal excretion of unchanged drug and total diuretic effect. However, it could also be used to illustrate the ambiguity in the present definition of bioavailability as being 'rate and extent of delivery to the site of action . . .' (Balant *et al.*, 1991). As clearly stated by this source, extent and rate are properties of the dosage form and may not be directly related to pharmacological or therapeutic effects.

Methods

Twenty-seven healthy Caucasian subjects participated in the study after giving informed consent and after approval had been obtained from an Ethics Committee. There were 18 males and 9 females. Their ages ranged between 19 and 29 years and their body weights from 54 to 94 kg. They fasted overnight and had the tablets given with 300 ml water in the morning. Food and fluid intake was strictly standardised during the 24 h study periods. Lunch was served 4 h after dose, an afternoon snack after 7 h, dinner after 10 h and finally an evening snack was given after 12 h. The total intake of fluid during meals was 1200 ml (water, decaffeinated coffee and fruit juice). Isovolumetric oral replacement of voided volumes was done using a balanced solution of carbohydrates and electrolytes. The study was randomised, balanced and doses were given at least at 1 week intervals. The study formulation was Furix Retard® (FR) and the CR reference was Lasix Retard® (LR). The plain tablet was Furix® (F). All doses were 60 mg but an assay of batch content was performed and deviations (0.2, 6 and 2.5%, respectively) were adjusted for. Urine samples were obtained by voiding at 15 min intervals for the first 1.5 h, 30 min intervals from 1.5 to 3 h and then hourly up to 10 h. Urine from 10 to 16 h was collected every other hour and the last portion was from 16 to 24 h after dosage. The volumes were weighted and aliquots frozen at −20° C. Frusemide concentrations were determined by h.p.l.c. (Hammarlund & Paalzow, 1982). Detector problems occurred during analysis of samples from one subject, leaving 26 evaluable subjects. Electrolytes were assayed by ion selective electrodes.

Calculations

The sigmoid E_{max} model, also known as the Hill equation, is expressed by:

$$E = \frac{E_{max} \cdot C^S}{C_{50\%}^S + C^S} + E_0 \qquad (1)$$

There is a close agreement between the pharmacological effect of frusemide on the excretion of volume, sodium and chloride (Alván *et al.*, 1990). If we select to study volume diuresis, E expresses the diuretic effect in ml min^{-1}, E_0 is basal diuresis, E_{max} is the maximum drug induced diuresis, $C_{50\%}$ is the frusemide excretion rate associated with half maximum induced diuresis and S is a fitting parameter known as slope factor (Hill, 1910; Holford & Sheiner, 1981). In effect studies C usually denotes the independent variable concentration, but in the case of frusemide, C represents urinary drug excretion rate. This exchange can be done because loop diuretics are considered to act from the endotubular side (Burg, 1976; Odlind, 1979; Odlind & Beermann, 1980). The excretion rate during a sufficiently short time can then be approximated with the amount or concentration of drug available at the site of action. Equation 1, which is an expression of pharmacological effect (E), can be transformed to express efficiency (Eff) by dividing both sides by C (Kaojarern *et al.*, 1982):

$$Eff = \frac{E - E_0}{C} = \frac{E_{max} \cdot C^{S-1}}{C_{50\%}^S + C^S} \qquad (2)$$

If E is the diuretic effect on urine volume, Eff is expressed in ml μg^{-1}. Efficiency will tell how much effect is obtained per unit of stimulus as a function of stimulus (C). Effect and efficiency will have different shapes when expressed *vs* C as previously shown (Alván *et al.*, 1990; Kaojarern *et al.*, 1982). The excretion rate associated with maximum efficiency (C_{effmax}) is a function of S and $C_{50\%}$ (Kaojarern *et al.*, 1982):

$$C_{effmax} = [C_{50\%}^S (S - 1)]^{1/S} \qquad (3)$$

It can be deduced that C_{effmax} is less than $C_{50\%}$ for $1 < S < 2$ and greater than $C_{50\%}$ for $S > 2$. For $S < 1$ the efficiency is ever increasing with decreasing C. A total or time averaged efficiency can be calculated for the whole effect event and is equal to

$$\text{Total Eff} = \frac{\int_0^{24} (E - E_0)\, dt}{\int_0^{24} C dt} = \frac{\text{Total induced diuresis}}{Ae} \qquad (4)$$

in which Ae is the total frusemide excretion in 24 h. In all the calculations and in Figures 2–4 basal diuresis (E_0) was subtracted. For urine volume, the values were 0.6, 0.7 and 0.7 ml min^{-1} which represented mean diuresis 16–24 h after FR, LR and F, respectively. Mean residence times (MRT) were calculated from the urinary excretion data (Rowland & Tozer, 1989).

Statistical analysis

Treatment differences were evaluated by analysis of variance (ANOVA). The statistical analyses were based on log-transformed data and on the ordinary linear model (Jones & Kenward, 1989) utilising the SAS GLM procedure corresponding to the three-treatment, three-period cross-over design used. Due to the nature of the

Table 1 Urinary excretion data. *Ae* is amount excreted, Max $\Delta Ae/\Delta t$ is maximum excretion rate, MRT is mean residence time. Total efficiency is adjusted for basal diuresis. Mean ± s.d. ($n = 26$)

	Furix Retard® *(FR)*	*Formulation* *Lasix Retard®* *(LR)*	*Furix®* *(F)*
Frusemide			
Ae (mg)	10·9 ± 3.4[ab]	8.7 ± 3.3[a]	17.8 ± 3.5
Max $\Delta Ae/\Delta t$ ($\mu g\ min^{-1}$)	39 ± 18[ab]	30 ± 20[a]	140 ± 51
Mean residence time (h)	6.6 ± 1.2[ab]	7.7 ± 2.1[a]	4.7 ± 1.8
Volume			
Diuresis (ml)	4270 ± 153[a]	3800 ± 130[a]	5260 ± 140
Total efficiency ($ml\ \mu g^{-1}$)	0.337 ± 0.149[a]	0.362 ± 0.224[a]	0.242 ± 0.082
Sodium			
Ae (mmol)	244 ± 66[a]	237 ± 69[a]	303 ± 70
Max $\Delta Ae/\Delta t$ ($mmol\ min^{-1}$)	1.01 ± 0.41[a]	0.92 ± 0.61[a]	2.71 ± 0.71
Total efficiency ($mmol\ \mu g^{-1}$)	0.017 ± 0.006[a]	0.020 ± 0.011[a]	0.013 ± 0.004
Chloride			
Ae (mmol)	249 ± 73[a]	235 ± 73[a]	297 ± 66
Max $\Delta Ae/\Delta t$ ($mmol\ min^{-1}$)	1.17 ± 0.42[a]	1.04 ± 0.65[a]	2.90 ± 0.70
Total efficiency ($mmol\ \mu g^{-1}$)	0.018 ± 0.006[a]	0.018 ± 0.007[a]	0.014 ± 0.004

$P < 0.05$ when compared with [a]the plain tablet, [b]the other CR formulation.

study, carry-over effects were not included in the model. Ninety percent confidence intervals were calculated according to Schuirmann (1987). This is a standard procedure in bioequivalence studies and relates to the fact that two or more formulations are compared. Thus, a two sided test is applied at a 5% α level with the hypothesis that either of the formulations may have the highest bioavailability in a pairwise comparison.

Table 2 Treatment ratios as % and 90% confidence intervals for the different formulations. FR is Furix Retard®, LR is Lasix Retard® and F is Furix®. *Ae* is amount excreted, Max $\Delta Ae/\Delta t$ is maximum excretion rate. Total efficiency is adjusted for basal diuresis

	FR/LR	*FR/F*	*LR/F*
Frusemide			
Ae	121(106–138)	56(49–64)	46(41–53)
Max $\Delta Ae/\Delta t$	140(109–179)	26(20–33)	18(14–23)
Volume			
Diuresis	107(93–124)	74(64–85)	69(59–79)
Total efficiency	98(81–118)	128(106–154)	131(108–159)
Sodium			
Ae	99(90–109)	75(68–83)	76(69–84)
Max $\Delta Ae/\Delta t$	118(97–143)	34(28–41)	29(24–35)
Total efficiency	89(75–107)	121(101–145)	138(114–162)
Chloride			
Ae	100(91–110)	77(70–85)	77(70–85)
Max $\Delta Ae/\Delta t$	119(99–143)	36(30–44)	31(26–37)
Total efficiency	99(88–111)	131(116–146)	132(117–148)

Results

Frusemide excretion rate and diuresis are shown in Figure 1. Tables 1 and 2 display kinetic and diuretic data obtained. The ratios between recovered amounts of frusemide for the formulations were all significantly ($P < 0.05$) different from unity. The total diuresis was significantly higher after Furix® than after the two CR formulations. However, despite the fact that FR and LR had a urinary recovery that was 39 and 51% lower than that of Furix®, total urinary volume was only 19 and 28% lower, respectively. The total diuresis of the two CR formulations did not differ statistically although the urinary recovery was significantly different ($P < 0.05$). The relationship between diuresis (adjusted for E_0) and frusemide excretion rate is shown in Figure 2. This relation appears as hysteresis loops. Hysteresis was minimal and counter-clockwise after the plain tablet indicating a barely visible delay of the effect in relation to the frusemide excretion rate. There was clockwise hysteresis after the two CR formulations which shows that tolerance developed during the course of drug action. A secondary increase in diuresis was apparent between 8 and 10 h, for all the formulations (Figures 1 and 2). Figure 3 shows the relation between diuretic efficiency and frusemide excretion rate. The data from 4 to 24 h are displayed with smaller symbols for visual clarity. These late points reflect the development of tolerance and an increase in diuresis probably not directly caused by the drug. Urinary samples containing less frusemide than corresponding to an excretion rate of 2 $\mu g\ min^{-1}$ are not included in Figures 2 and 3 because of the error associated with the estimates when frusemide excretion is close to 0. Figure 4 displays the data as cumulative diuresis *vs* cumulative excretion of frusemide. This figure shows that both cumulative drug induced diuresis and cumulative frusemide excretion start at the origin and develop differently for the two dosage forms.

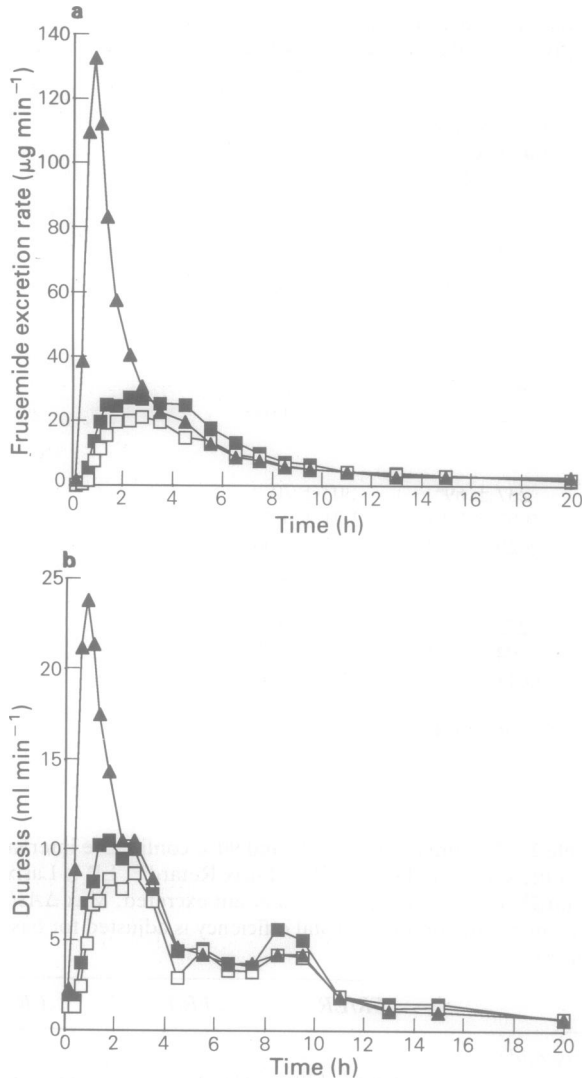

Figure 1 Frusemide excretion rate (a) and diuresis (b) following three 60 mg oral dosage forms (▲ Furix® plain tablets, ■ Furix Retard®, □ Lasix Retard®). The values are plotted against the midpoint of each sampling interval. Mean data from 26 subjects.

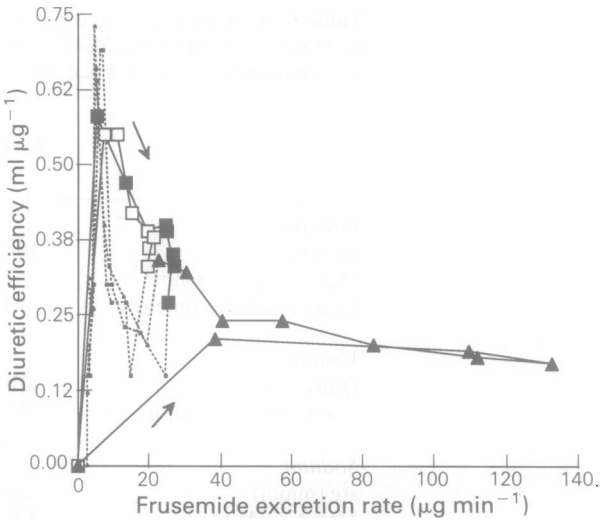

Figure 3 Diuretic efficiency (urine volume adjusted for basal diuresis per amount excreted frusemide) *vs* frusemide excretion rate following three 60 mg oral dosage forms during the first 4 h after dose (▲ Furix® plain tablets, ■ Furix Retard®, □ Lasix Retard®) and from 4 to 24 h (– – –). Mean data from 26 subjects. Arrows indicate direction of hysteresis.

Discussion

This study confirms the observations by Beermann (1982) that CR formulations of frusemide produce almost as much diuresis as plain tablets although the bioavailability is markedly lower. Efficiency is higher for low values of frusemide excretion rate (Figure 3). Thus, the excretion rate will be in the range close to maximum efficiency for a longer time if the drug gets into the body by slow oral absorption, compared with the cases when a dosage form with rapid absorption is given or a bolus dose is administered. This explains why plain tablets produce approximately the same total response as a similar i.v. bolus dose although only about half of it is absorbed (Hammarlund *et al.*, 1985) and why, in our study, CR

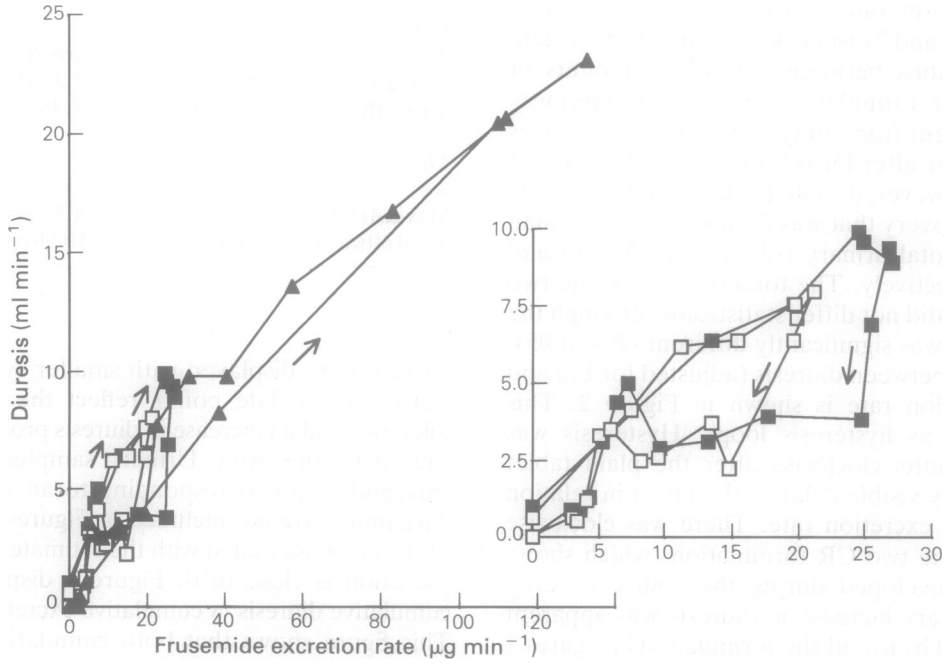

Figure 2 Diuresis *vs* frusemide urinary excretion rate following three 60 mg oral dosage forms (▲ Furix® plain tablets, ■ Furix Retard®, □ Lasix Retard®). Mean data from 26 subjects, basal diuresis has been subtracted. Insert shows data from the CR formulations and arrows indicate direction of hysteresis.

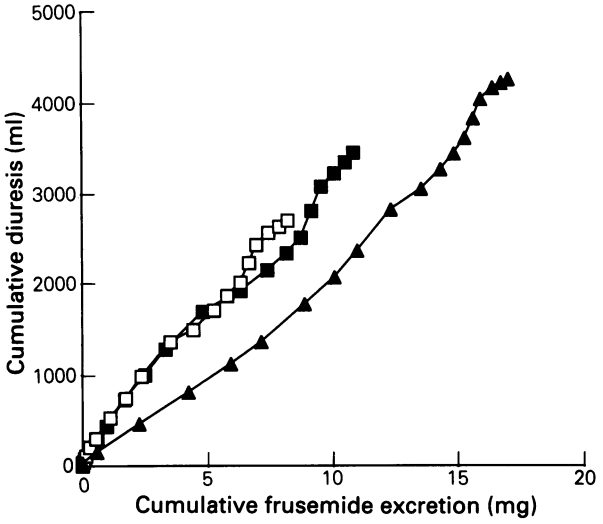

Figure 4 Cumulative mean total diuresis *vs* cumulative mean frusemide excretion following three 60 mg oral dosage forms to 26 subjects (▲ Furix® plain tablets, ■ Furix Retard®, □ Lasix Retard®). Basal diuresis is subtracted.

formulations still give not much less response although the fraction absorbed is about 50% lower.

The efficiency concept was recently criticised by Noormohamed (1990) and Noormohamed & Lant (1991). Given that the Hill equation adequately models the diuretic effect, the efficiency is a simple mathematical consequence (Alván *et al.*, 1991). In a recent paper, Noormohamed & Lant (1991) have chosen to display their data only as cumulative excretion of sodium *vs* cumulative excretion of the loop diuretic piretanide. Displaying the data in this way will however disregard the pharmacodynamic relationship expressed e.g. as the Hill equation. Figure 4 shows that there is no intercept of excreted drug that would be 'wasted' as suggested to occur for intravenous piretanide by Noormohamed & Lant (1991).

Sommers *et al.* (1991) discuss the effect of probenecid on the diuretic response to i.v. frusemide. According to the parameters presented for the Hill equation, there was a quite different pharmacodynamic-pharmacokinetic relationship after probenecid was given. We think that the marked increase in $C_{50\%}$ and decrease in S reflect tolerance or counter regulation. These changes are very much in line with preliminary calculations on our present data and results from others (Hammarlund *et al.*, 1985). The observed changes in Hill parameters are in our view not consistent with an increase in effects of frusemide caused by probenecid (Sommers *et al.*, 1991) nor is it likely that probenecid leads to 'normalisation of the responsiveness' (Noormohamed & Lant, 1991). We recommend that the simple theory of efficiency is applied and exhausted before more complicated assumptions about pharmacodynamic interactions are made. Probenecid will attenuate the disposition of the diuretic into urine (Homeida *et al.*, 1977; Honari *et al.*, 1977) and thus enable the drug to work in the excretion rate range of high efficiency for a comparatively longer time than when it is given alone.

The effect-concentration and efficiency-concentration relationships are shown in the papers by Kaojarern *et al.* (1982) and Alván *et al.* (1990). In accordance with the efficiency principle, Rudy *et al.* (1991) reported a significantly increased 12 h sodium excretion when bumetanide was given as a continuous infusion compared with the case when a similar total dose was administered as two i.v. bolus infusions. Very consistent results were obtained in a study where frusemide was infused at different rates to dogs (Lee *et al.*, 1986).

The present investigation confirms that 'the time course of delivery of a loop diuretic into urine is an independent [from intrinsic activity and maximum response] determinant of overall diuretic response' (Koajarern *et al.*, 1982). The ultimate goal of bioavailability and bioequivalence studies is to assess and evaluate differences in clinical effects related to the dosage form and this should be more emphasized than the comparison of drug concentrations in future bioavailability investigations (Levy, 1991).

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