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Pharmacodynamic analysis of the furosemide-probenecid interaction in man

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Pharmacodynamic analysis of the furosemide-probenecid interaction in man. Probenecid pretreatment in man increased the overall response to furosemide in contrast to animal studies in which probenecid decreased response by inhibiting proximal renal tubular secretion of furosemide to its active site. We administered i.v. 40 mg of furosemide to eight normal volunteers with and without probenecid pretreatment and measured serum and urinary furosemide concentrations by high performance liquid chromatography to determine the mechanism of probenecid's effect. Probenecid pretreatment significantly increased serum furosemide concentration. Urinary furosemide excretion rate paralleled urinary sodium excretion rate; both were initially decreased but were later increased by probenecid pretreatment. Probenecid pretreatment decreased renal and nonrenal clearance of furosemide (1.04 ± 0.31 vs. 0.29 ± 0.06 ml/kg/min, $P < 0.05$; and 1.00 ± 0.18 vs. 0.27 ± 0.03 ml/kg/min, $P < 0.004$, respectively). Although probenecid inhibited renal clearance for the duration of the study, accumulation of furosemide in serum from concomitant effects on nonrenal clearance allowed more furosemide to appear in the urine at later times, increasing response. This analysis demonstrated the importance of probenecid's effects on nonrenal elimination of furosemide in determining the overall response to furosemide. The relationship between furosemide concentrations and response depicted a sigmoid dose-response curve. Probenecid shifted the serum dose-response relationship to the right but did not affect the relationship between urinary furosemide excretion rate and response, demonstrating the importance of the urinary (as opposed to serum) concentration-response relationship of furosemide in normal man. This relationship will provide a valuable tool for assessing response to diuretics in various disease states where resistance to diuretics occurs.

Analyse pharmacodynamique de l'interaction furosémide-probénécide chez l'homme. Le pré-traitement par le probénécide chez l'homme augmente la réponse globale au furosémide par opposition aux études chez l'animal où le probénécide diminue cette réponse en inhibant la sécrétion tubulaire proximale de furosémide. Nous avons administré i.v. 40 mg de furosémide par voie à huit volontaires normaux, avec ou sans pré-traitement par le probénécide, et mesuré les concentrations de furosémide sériques et urinaires par chromatographie liquide à haute résolution afin d'étudier le mécanisme de l'effet du probénécide. Le pré-traitement par le probénécide augmente significativement la concentration sérique de furosémide. L'excrétion urinaire de furosémide est parallèle à l'excrétion urinaire de sodium. Ces deux dernières sont initialement diminuées mais ultérieurement augmentées par le pré-traitement au moyen de probénécide. Le pré-traitement par le probénécide diminue les clearances rénale et non-rénale du furosémide (1,04 ± 0,31 vs. 0,29 ± 0,06 ml/kg/min, $P < 0,05$; et 1,00 ± 0,18 vs. 0,27 ± 0,03 ml/kg/min, $P < 0,004$, respectivement). Bien que le probénécide diminue la clearance rénale pendant la durée de l'étude, l'accumulation sérique de furosémide due aux effets sur la clearance non rénale permet l'apparition dans l'urine de quantités plus importantes à des temps ultérieurs, ce qui augmente la réponse. Cette analyse démontre l'importance des effets du probénécide sur l'élimination non rénale de furosémide dans le déterminisme de la réponse globale au furosémide. La relation entre les concentrations de furosémide et la réponse décrit une courbe dose-réponse sigmoïde. Le probénécide déplace la courbe dose-réponse sérique vers la droite mais n'affecte pas la relation entre l'excrétion urinaire de furosémide et la réponse, ce qui démontre l'importance de la relation entre la concentration urinaire (à la différence de la concentration sérique) et la réponse. Cette relation fournit un instrument utile pour évaluer la réponse aux diurétiques dans divers états où la résistance à ces drogues est observée.

Furosemide is one of the most potent and frequently used diuretics [1–3]. Although its clinical effectiveness has been well established from prior studies, pharmacokinetic and dynamic correlates have only recently been investigated [4–7]. The new development of a specific and sensitive assay for furosemide has made it possible to probe the determinants of the response to furosemide and to better understand the pharmacology of the drug, which, in turn, may allow use of measures of the drug as a probe of the pathophysiology of renal salt and water metabolism.

Furosemide inhibits active chloride transport throughout the thick ascending limb of the loop of Henle, also preventing the reabsorption of sodium which passively follows chloride [8–10]. Studies with microperfusion of isolated segments of renal tubules [11] showed that furosemide's site of action is at the luminal side of the nephron. Because fu-

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rosemide is an organic acid [2] and is highly bound to serum proteins [12, 13], it gains access to its site of action by secretion at the proximal tubule at the nonspecific transport pathway for organic acids [14, 15]. Prior studies in animals have shown that coadministration of probenecid, an organic acid which competes for secretion, decreased furosemide secretion by the proximal tubule of the nephron and decreased the amount of furosemide available at its site of action. Intravenous administration to dogs of 50 mg/kg of probenecid over 5 min completely attenuated the response to intraarterial infusion of 1 μg/kg/min of furosemide [16]. In contrast, our laboratory recently showed in normal volunteers that pretreatment with probenecid caused an increased response to 40 mg of furosemide administered intravenously [17]. Sodium excretion over 8 hours increased from 262 ± 16 to 358 ± 11 mEq ($P < 0.005$). The time course of this effect showed that the initial response to furosemide was decreased by probenecid, but after 1 to 2 hours, probenecid caused an increased response to the diuretic; the overall effect was an increased total amount of sodium excreted over 8 hours. These data suggested a more complex interaction in man between probenecid and furosemide than had been described in experimental animals.

In the present study, we determined the mechanism by which probenecid increased the natriuretic effect of furosemide, and in so doing, established a relationship between urinary concentrations of furosemide and natriuretic effect for this population of normal volunteers.

Methods

Study protocol. We studied eight subjects, three women and five men, who were 22 to 27 years old. Subjects had normal medical histories, physical examinations, and screening blood chemistries, blood counts, and urinalyses. Each subject was fully informed of the nature of the study and signed an informed consent approved by the Committee on Human Research at the University of Texas Health Science Center at Dallas.

This paper reports results of those studies assessing the mechanism and the pharmacodynamics of the probenecid-furosemide interaction. These subjects were studied a total of ten times each with several different diuretic regimens, all of which were conducted in random order and with an interval of at least 1 week between each phase of the study. The effect of probenecid on the response to furosemide has been published [17].

In this report, we analyzed the results of studies after intravenous administration over 2 to 3 min of 40 mg of furosemide with and without pretreatment with probenecid, and control studies with administration of no drug and with probenecid alone. Subjects ingested 1 g of probenecid at bedtime the night before and on arising the morning of the study (30 to 60 min before administration of furosemide).

Subjects had ingested a diet containing 150 mEq of sodium and 60 to 80 mEq of potassium per day for 3 days as outpatients on the General Clinical Research Center. They collected a 24-hour urine sample beginning the morning of the third day to assess adherence to the diet. The sodium content of this 24-hour urine specimen was not significantly different before any of the phases of the study (0.09 ± 0.015 and 0.10 ± 0.017 mEq/min before furosemide alone and with probenecid, respectively). The study began on the morning of the fourth day at the close of the 24-hour urine collection. The day of the study, patients skipped breakfast (including caffeine containing beverages) but ate lunch (at least 3 hours after the start of the study).

Heparinized scalp-vein needles were placed in each forearm, one for administration of furosemide and replacement fluids and one for obtaining blood samples. Blood samples were drawn at 0, 5, 10, 20, 30, 45, 60, 80, 100, 120, 180, 240, 300, 360, 480 min, and 24 hours after administration of furosemide. All urine was collected by spontaneous voiding at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 24 hours. During the first 2 to 3 hours of the study, urine output was replaced by isovolumic amounts of lactated Ringer's solution administered by vein over the time interval of the subsequent collection period. When urine output had decreased to approximately 250 ml/hr, fluids were administered orally. In the control studies, all urine losses were replaced orally. No changes occurred in serum sodium, potassium, chloride, or creatinine in any study. Creatinine clearance did not change throughout the study. Consequently, we elected to express response as sodium excretion rate in milliequivalents per minute.

Assays. The assay for concentrations of furosemide in urine and in serum samples was performed with a Waters model ALC-GPC-204 high-performance liquid chromatograph equipped with a Waters model 440 dual wavelength detector and a dual channel 10-mV Omniscribe recorder (Waters Associates, Inc., Milford, Massachusetts 07157). The separation was effected with a 30 cm × 4 mm reverse-phase "μ Bondapak C_{18}" column (Waters Assoc.) which was eluted at 2.0 ml/min with an

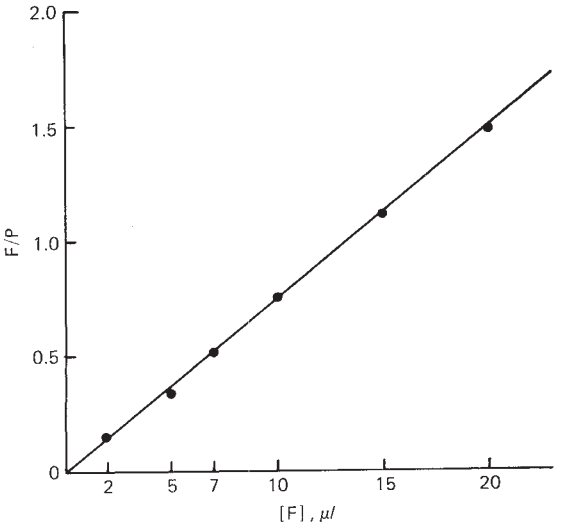
acetonitrile/water (23/77 by volume) solution buffered to pH 3.8 with 0.01 M acetate. The assay for furosemide in studies where probenecid was administered was changed from the "normal" furosemide assay (25/75 by volume acetonitrile/water at pH 4.0 to 4.5) to separate the furosemide and phenobarbital (the internal standard) peaks from interfering peaks due to probenecid and its metabolites. The column effluent was monitored simultaneously at 280 nm for furosemide absorption and at 254 nm for phenobarbital.

Urine samples were forced through a 0.5-μ syringe filter, and 50 μl of urine was then pipetted into a test tube containing 0.2 ml of water and 50 μl of a standard phenobarbital solution (0.40 mg/ml). The solution was mixed well, and 10 to 20 μl were injected onto the column.

Serum samples were prepared by pipetting 0.2 ml of serum and 10 μl of phenobarbital standard into a test tube; 0.4 ml of acetonitrile was added to precipitate serum proteins, and the mixture was centrifuged for 10 min. The supernate was poured off, and the acetonitrile was evaporated with a gentle stream of nitrogen. The remaining residue from this evaporated supernate was reconstituted with 50 μl of the mobile phase, and 10 to 20 μl were injected onto the column.

Prior to each series of patient samples, known solutions containing given amounts of phenobarbital standard and varying amounts of furosemide standard were injected onto the column, and a standard curve was constructed by plotting the furosemide/phenobarbital peak height ratios (F/P) against the amount of furosemide in each standard sample. The amount of furosemide in patient samples was obtained by using the measured F/P ratio in a linear regression program to calculate the actual amount of furosemide in that specific patient sample by comparison to the standard curve. A standard curve is depicted in Fig. 1, and a typical trace of the chromatographic separation of a representative urine specimen is shown in Fig. 2. Trace A occurred with furosemide administration alone, and trace B occurred with coadministration of probenecid and furosemide. The additional peaks in trace B presumably represent probenecid and its metabolites. Ten determinations of standard serum samples at three different concentrations of furosemide were 0.0572 ± 0.0015, 0.2913 ± 0.002, and 0.896 ± 0.006 for concentrations of approximately 0.06, 0.3, and 0.9 μg/ml, respectively.

Creatinine was measured in serum and urine with a Technicon autoanalyzer. Sodium and potassium



[F], μl	F, mm	P, mm	F/P
2	0.90	5.50	0.16
5	1.95	5.35	0.36
7	2.80	5.50	0.51
10	4.00	5.30	0.75
15	5.50	4.95	1.11
20	7.10	4.80	1.48

r = 0.9998
m = 0.074
b = 0.005

Fig. 1. *Typical standard curve for assay of furosemide relating the ratio of heights of peaks of furosemide (F) to heights of peaks of phenobarbital (P) (the internal standard) versus concentration of furosemide ([F]).* r denotes the correlation; m, the slope; and b, the y-intercept which should not differ from zero.

were measured with an Instrument Laboratories model 143 flame photometer. Chloride was measured with a Buchler chloridometer, model 4-2500.

Data analyses. Following intravenous administration, serum furosemide concentration vs. time curves appeared to be described by a two-compartment pharmacokinetic open model of the format:

$$CP = Ae^{-\alpha t} + Be^{-\beta t}$$

where Cp is serum concentration at time t, A and B are constant coefficients or "intercepts," and α and β are fast and slow disposition rate constants. Data were analyzed by computer fitting using a nonlinear least squares program (NONLIN) [18]. The degree of fit of the data to the model was evaluated by examining r^2, the proportion of the variance in the data which is explained by the model [19]. The plasma clearance was defined as the dose administered divided by the total area under the plasma concentrations vs. time curve. Renal clearance was obtained for each study by plotting the plasma fu-

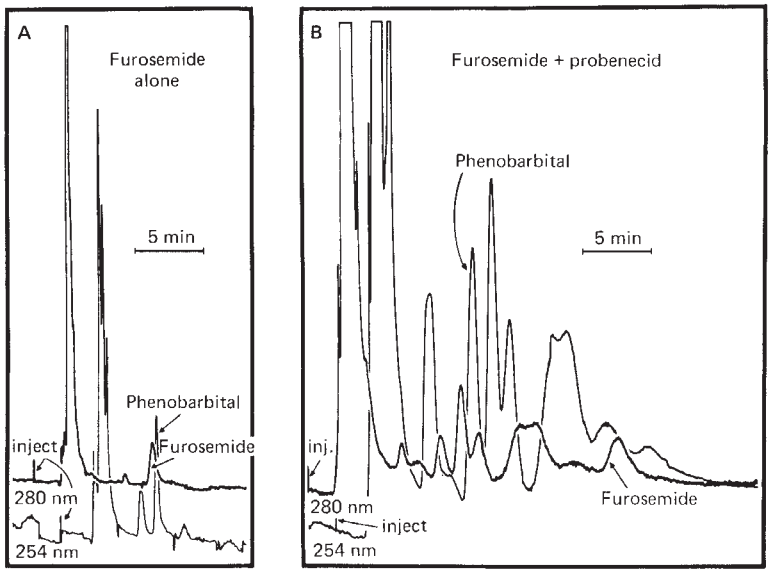


Fig. 2. *Representative chromatographic tracing of a urine specimen after furosemide alone* (**A**) *and after furosemide plus probenecid* (**B**). The additional peaks in trace B represent probenecid and its metabolites (unlabeled).

rosemide concentration at the midpoint of a urine collection period vs. urinary furosemide excretion rate. This plasma furosemide concentration was determined from the computer-calculated curve fitting the plasma furosemide data. Renal clearance is the slope of this plot derived as the least squares line regressed through the origin [20]. The intercepts of least squares linear regressions not forced through the origin were tested against zero [21] and were not significantly different from zero. Nonrenal clearance was defined as the difference between plasma clearance and renal clearance. These parameters were analyzed as the mean obtained from fitting of data from individual studies. When the parameters were obtained from fitting the meaned data, results did not differ significantly.

Significance of differences was tested with paired *t* tests. All significant differences in this report have *P* values of <0.05. Group data are expressed in standard renal physiologic and pharmacokinetic terms as mean ± SEM.

Results

Figure 3 shows the effect of probenecid on the serum concentration of furosemide for all subjects after administration of 40 mg of furosemide intravenously. Fitting these curves to a two-compartment pharmacokinetic model resulted in an r^2 of 0.995 and 0.993 (with and without probenecid pretreatment, respectively). It is apparent from Fig. 3 that pretreatment with probenecid significantly increased serum furosemide concentrations. The elimination half-life of furosemide, determined as the mean of analyses of each study, increased from 105.4 ± 16.4 to 168.9 ± 15.1 min ($P < 0.033$). Deriving these parameters from curves fit to mean data gave similar results. These elimination half-lives are longer than those previously reported [4, 5, 7, 22]. We followed concentrations of furosemide for longer periods of time. By so doing, the elimination phase was more clearly defined and resulted in a longer elimination half-life. Examination of the time course of probenecid's effect showed that initial furosemide concentrations were not significantly different, but after 30 min probenecid caused persistently higher serum concentrations of furosemide. The plasma clearance of furosemide decreased after probenecid pretreatment from 2.04 ± 0.42 to 0.56 ± 0.05 ml/kg/min ($P < 0.011$). The volume of distribution at steady state was not changed by probenecid, 0.110 ± 0.015 and 0.161 ± 0.051 liter/kg ($P \approx 0.346$) with and without probenecid pretreatment, respectively. Consequently, the effect of probenecid occurred by decreasing the elimination of the drug rather than by affecting the volume in which it distributes.

Probenecid could affect the pharmacokinetics and dynamics of furosemide by changing its binding to serum proteins. Binding of furosemide to serum proteins, however, remains the same over the con-

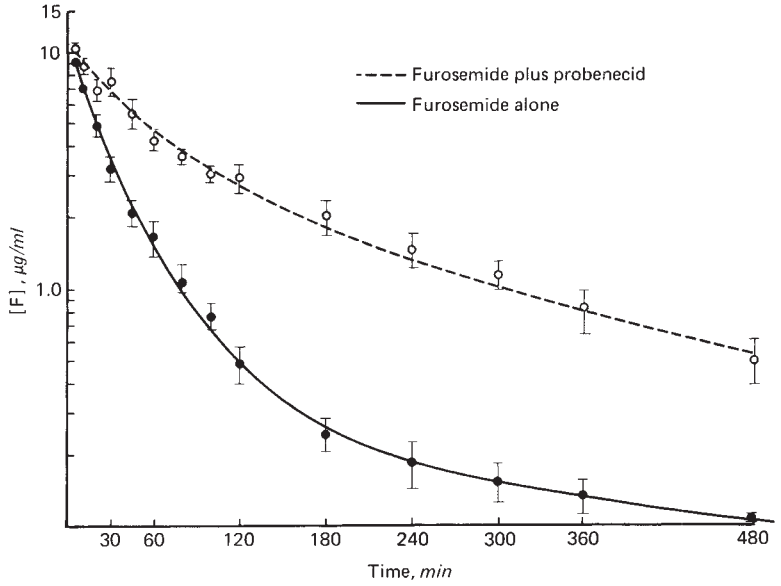


Fig. 3. *Effect of probenecid pretreatment on the serum concentration ([F]) over time after administration i.v. of 40 mg of furosemide. Closed symbols* represent furosemide alone. *Open symbols* represent furosemide plus probenecid. The *lines* represent curves computed by iterative least squares fitting of the data. *Brackets* represent SEM.

centrations of furosemide attained in this study, and probenecid does not affect extent of binding [13, 23].

The effects of probenecid pretreatment on urinary furosemide excretion rate (upper curves) and on response to furosemide expressed as urinary sodium excretion rate (lower curves) are shown in Fig. 4. After probenecid pretreatment, the initial furosemide excretion rate was significantly decreased ($P < 0.026$). After approximately 60 min, however, probenecid pretreatment caused an increased urinary excretion rate of furosemide. The effect of probenecid on the natriuretic response to furosemide shown in the lower part of Fig. 4 parallels the urinary excretion rate of furosemide. The initial urinary excretion rate of sodium after pretreatment with probenecid was less than that with furosemide alone, but after approximately 60 min the response with probenecid pretreatment became significantly higher. Although the initial decrement in urinary sodium excretion rate does not reach statistical significance, the P value is equal to 0.053.

Fig. 5 shows the relationship between serum furosemide concentration and urinary furosemide excretion rate. Each point in the figure represents the mean of observations in one collection period for all subjects. Probenecid pretreatment decreased renal clearance of furosemide determined as the slopes of these plots from 63.8 ± 15.6 to 20.6 ± 5.2 ml/min ($P < 0.036$). The renal clearance of furosemide, factored by body weight, decreased from 1.04 ± 0.31

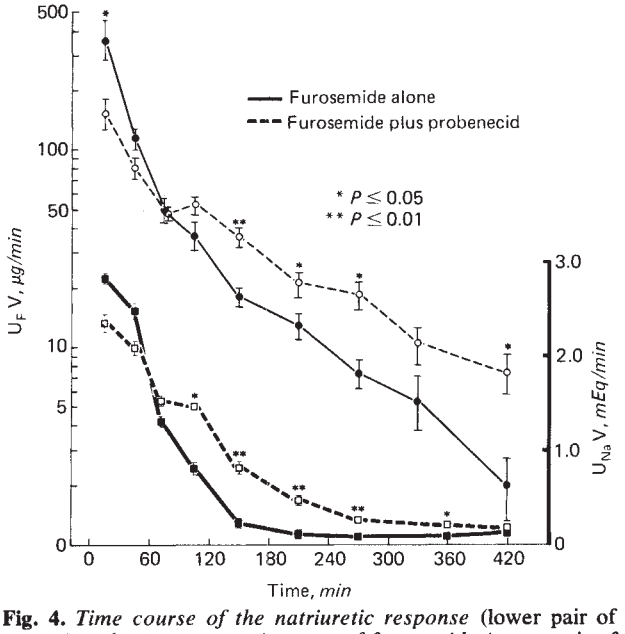


Fig. 4. *Time course of the natriuretic response* (lower pair of curves) *and urinary excretion rate of furosemide* (upper pair of curves) *with and without probenecid pretreatment*. Symbols are as in Fig. 3. Absence of brackets implies a SEM within the area of the symbol.

to 0.29 ± 0.06 ml/kg/min after probenecid pretreatment ($P < 0.05$). Probenecid also significantly decreased nonrenal clearance of furosemide from 1.00 ± 0.18 to 0.27 ± 0.03 ml/kg/min ($P < 0.004$). The effect of probenecid on renal clearance was prolonged and persisted throughout the study, as indicated in Fig. 6.

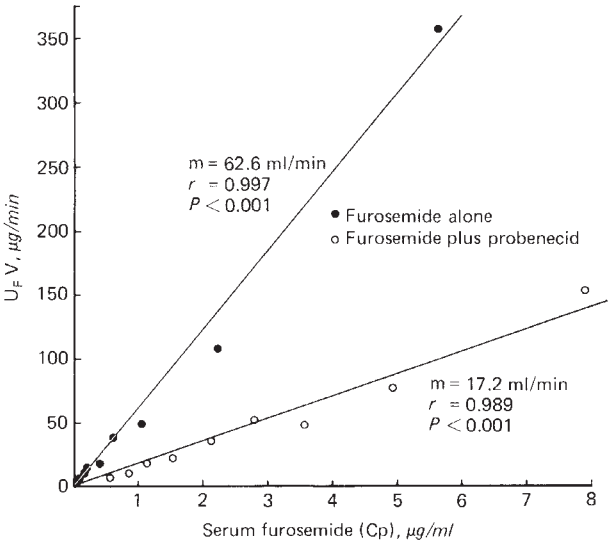


Fig. 5. *Relationship between urinary excretion rate and serum concentration after intravenous administration of 40 mg of furosemide. Symbols* are as in previous figures. The *lines* represent linear least squares regressions of the data. Their slopes (m) represent renal clearance of furosemide.

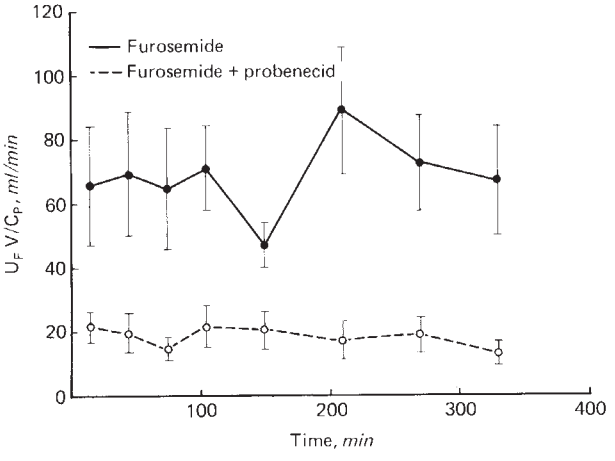


Fig. 6. *Effect of probenecid on renal clearance* ($U_F V/C_P$) *of furosemide over time*. Symbols are as in previous figures.

Figure 7 depicts "dose-response" curves for concentrations of furosemide in serum and urine related to natriuretic effect. Probenecid caused a significant shift to the right of the relationship between serum concentration of furosemide and sodium excretion rate; in contrast, the relationship between urinary concentration of furosemide and sodium excretion rate was not affected by probenecid pretreatment. This finding verifies the previously assumed hypothesis that urinary concentrations of furosemide rather than concentrations in serum best reflect concentrations of diuretic reaching its site of action.

Discussion

The results of this study clarify the mechanism by which pretreatment with probenecid increased the response to furosemide as described in an earlier report from our laboratory [17]. In addition, this study verified the heretofore assumed hypothesis derived from studies in animals that furosemide inhibits chloride transport from the luminal rather than the peritubular surface of the nephron [11], and that secretion of furosemide into the lumen at the proximal tubule is a prerequisite for its diuretic action [10].

These same mechanisms also apply to other organic acid diuretics such as thiazides [24] and ethacrynic acid [25]. Several studies in animals showed that probenecid attenuated the natriuretic effect of chlorothiazide [24, 26-28] and of furosemide [16, 29], presumably by competing with and decreasing diuretic transport at the organic acid secretory pathway [30]. Similar studies in man, however, did not support the findings from in vitro and in vivo animal studies [7, 17, 31-33]. Several possible mechanisms might explain the difference in response between man and experimental animals.

The present study clarified the mechanism of the complex interaction between probenecid and furosemide in man in which probenecid caused an initial decrease followed by a later increase in response [17]. This effect could have occurred by a changing effect of probenecid on delivery of furosemide to its site of action in which an initial inhibition of transport of furosemide dissipated as probenecid itself was eliminated, allowing more furosemide to be transported to its active site at later times. Probenecid, however, caused a persistent decrease in furosemide secretion and in renal clearance (Fig. 6). Importantly, probenecid suppressed nonrenal as well as renal clearance of furosemide. This long lasting, dual effect of probenecid on nonrenal and renal elimination of furosemide, rather than a changing effect of probenecid on renal clearance over time, caused amounts of furosemide appearing at the active site to change through the time course of the study. Probenecid's concomitant inhibition of nonrenal clearance (probably hepatic) allowed accumulation of sufficient amounts of furosemide in serum at later times to cause greater amounts of furosemide to be delivered into the urine despite continued suppression of renal clearance. This phenomenon had not been observed in animal studies, because furosemide was administered into the renal artery and no ramifications of probenecid's effect on nonrenal clearance of furosemide could be observed. Consequently, pharmacodynamic analysis of the interaction between furosemide and proben-

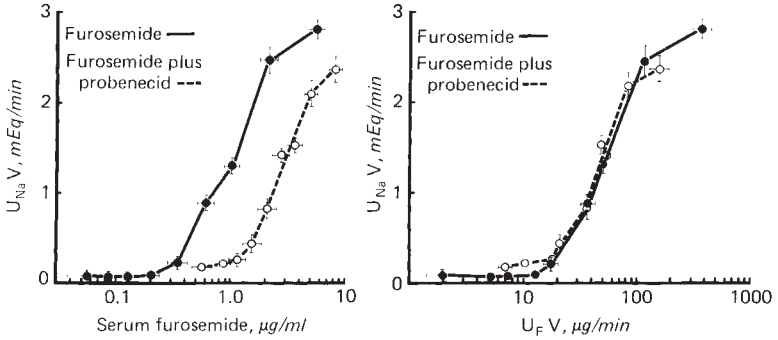


Fig. 7. *"Dose-response" curves for furosemide after a 40 mg intravenous dose.* The *left panel* depicts the relationship between concentrations of furosemide in serum and response expressed as sodium excretion rate. The *right panel* depicts the relationship between the urinary excretion rate of furosemide and response. Symbols are as in previous figures.

ecid indicates that the mechanism of renal handling of furosemide appears to be uniform among the species studied.

Two other important aspects of renal pharmacology were demonstrated in this study. First, the effects of probenecid on organic acid transport were long-lasting. A constant degree of inhibition was observed for up to 6 hours. The relationship between the time course of this effect and the kinetics of probenecid and/or its metabolites cannot be determined from this study but are consistent with prior studies of the time course of the inhibitory effect of probenecid on transport of other organic acids [33, 34].

Second, our data clearly demonstrate that the amount of furosemide delivered into the urine is more directly correlated to response than is serum concentration of furosemide. This fact is dramatically illustrated in Fig. 7. Probenecid caused a significant shift to the right of the relationship between serum concentration and response but caused no change in the relationship between concentration of furosemide in urine and response. This analysis confirms in vitro and in vivo animal studies showing that probenecid interferes with renal tubular secretion of furosemide but not with furosemide action at its active site. A similar phenomenon appears to occur in moderate renal insufficiency in which an accumulation of endogenous organic acids decreased furosemide secretion and, consequently, decreased response to furosemide [35, 36]. In such conditions, urinary furosemide excretion predictably would better correlate with diuretic response than would serum concentration of furosemide.

It is clear from Fig. 7 that the relationship between furosemide concentration and response is not linear but follows the "typical" sigmoid shape of a dose-response curve. Other investigators have graphically demonstrated a similar nonlinear relationship between concentrations of furosemide [6, 7] and piretanide [37] in serum and response. The investigators concluded, however, that the response was linear [6, 7] or did not define the upper plateau of the relationship [37]. Additionally, the importance of furosemide in the urine compared to serum has not been demonstrated. An implication of this analysis is that studies of the relationship between the pharmacokinetics and the pharmacodynamics of furosemide (and presumably other diuretics) cannot a priori assume a linear relationship and must include data assessing the spectrum of the dose-response curve. For example, the attainment of an upper plateau in response most likely accounts for reports that a number of normal subjects failed to show an increase in overall response with higher doses of furosemide [38].

In addition, in this study probenecid increased response without affecting the total amount of furosemide reaching the urine (19.67 ± 2.21 and 16.03 ± 1.45 mg/8 hr) before and after probenecid pretreatment, respectively ($P \approx 0.252$). Therefore, probenecid increased the overall response to furosemide by affecting the time course of delivery of furosemide to its site of action such that concentrations of furosemide in urine after probenecid pretreatment were more often at the "steep" portion of the dose-response curve than after furosemide alone. Attaining this steep portion of the dose-response curve in patients treated with diuretics would be an important determinant of the most efficacious dosing regimen in an individual patient. Application of this approach to treating patients resistant to diuretics warrants further study.

Summary. Probenecid alters the pharmacokinet-

ics of furosemide in man and causes an increase in natriuresis in contrast to the decreased response observed in animal studies. Our data are consistent with the tenet that furosemide acts at the luminal side of the nephron, and we have shown that urinary furosemide rather than serum furosemide concentrations should be used in assessing the diuretic response. It is also clear that the relationship between furosemide concentration and natriuresis is not linear through the range of concentrations achieved in man. This relationship probably also holds for other diuretics, and the full range of the dose-response curve must be considered in assessing the determinants of the response to a diuretic. This relationship, now well characterized in normal man, should provide a valuable tool in assessing response to diuretics in various disease states in which resistance to diuretics occurs.

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