Journal of Pharmacokinetics and Biopharmaceutics, Vol. 10, No. 6, 1982

Plasma Protein Binding of Furosemide in Kidney Transplant Patients

David E. Smith[1,2] and Leslie Z. Benet[1,3]

Received June 3, 1982—Final October 11, 1982

The present investigation was undertaken in order to determine the in vivo plasma protein binding of furosemide in kidney transplant patients and its possible consequence on furosemide effect. Using an equilibrium dialysis technique, serial plasma samples of furosemide taken after intravenous administration were dialyzed against an equal volume of isotonic Krebs Ringer bicarbonate buffer (pH 7.4). Dialysis was performed at 37°C for 5 hr, and furosemide concentrations (total as well as free) were analyzed by HPLC using fluorescence detection. It was observed that kidney transplant patients on concomitant sulfisoxazole treatment (KT+) had a significantly greater value for percent free of furosemide as compared to transplant patients not on sulfisoxazole (KT−) (4.4 ± 0.8 for KT+ vs. 1.7 ± 0.3% for KT−; $p < 0.01$) as well as to healthy volunteers (4.4 ± 0.8 for KT+ vs. 1.2 ± 0.2% for controls; $p < 0.01$). In addition, kidney transplant patients not on concomitant sulfisoxazole treatment had a significantly higher value for percent free of furosemide with respect to healthy volunteers ($p < 0.05$). Nonlinear plasma protein binding was also observed for one patient, who had values for percent free of furosemide ranging from 1.3 to 12.9%. However, no significant correlation was found between the fraction of the dose excreted unchanged in the urine and percent free of furosemide.

KEY WORDS: furosemide; protein binding; kidney transplant; renal transplant.

INTRODUCTION

It is generally recognized that the binding of a drug to plasma proteins can affect its distribution, elimination, and ultimately its therapeutic or toxic response since only the unbound drug is pharmacologically active. It has also been established that renal impairment may alter drug binding to plasma proteins (1–5), particularly with respect to acidic drugs (3). Possible explanations for reduced drug binding in patients with renal dysfunction

This work was supported in part by NIH Grant AM 20884 and by the Earl C. Anthony Fund.
[1]Department of Pharmacy, School of Pharmacy, 926–S, University of California, San Francisco, California 94143.
[2]Present Address: College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109.
[3]Address correspondence to L.Z.B.

0090-466X/82/1200-0663$03.00/0

include hypoalbuminemia (2), the presence of irreversible and competitive inhibitors in the plasma (3,5), and altered albumin composition (1).

Furosemide is a valuable diuretic in kidney transplant patients for the treatment of volume overload. It is highly bound to plasma proteins (6–12) and gains access to its site of action in the kidney lumen primarily through active secretion via the nonspecific organic acid secretory pathway (13–15). Previous studies have shown that renal disease can effect dramatic changes in the pharmacokinetics of furosemide (6,11,16–20), including impaired plasma protein binding in uremics (7,11), nephrotics (10,11), and anephric patients (6). The degree of binding of furosemide to plasma proteins in kidney transplant patients has not been reported. Since only the free drug is presumed to be transported by the kidney into the tubular fluid, it may be important to understand the role of plasma protein binding with respect to the natriuretic and diuretic response to furosemide.

METHODS

Patient Studies

The characteristics of nine kidney transplant patients were previously described and the pharmacokinetics and pharmacodynamics of furosemide in this patient population were evaluated (19). Patients were titrated to, and studied at, a dose capable of inducing an adequate pharmacodynamic response. In addition, transplant patients were subdivided into responder (R; 40–80 mg/day) and nonresponder (NR; $\geq$120 mg/day) populations as previously described (19).

After fasting overnight, the intravenous dose of furosemide was infused over a 10 min period at approximately 8 a.m. Blood samples (3 ml) were obtained from an indwelling heparinized scalp vein needle at 0, 10, 15, 20, 30, 45, 60, 80, 100; 120, 180, 240, 360, 480, and 1440 min; the end of the infusion period being 10 min. All patients signed the consent form approved by the Human Research Committee of the University of California, San Francisco.

Measurement of Furosemide

Plasma samples were assayed for total furosemide (bound and free) by a high performance liquid chromatographic method as described by Smith *et al.* (19). The analytical procedure for determination of free furosemide concentrations in dialyzed buffer has also been previously reported (12). However, chlorpromazine hydrochloride (0.02%) was substituted as the internal standard for the analysis of furosemide in those

patients concomitantly taking sulfisoxazole (19). This was necessary since sodium phenobarbital, our usual internal standard for furosemide, and sulfisoxazole have similar retention times and will interfere with each other.

Protein Binding

The *in vivo* binding of furosemide to plasma proteins was determined using an equilibrium dialysis method (12). One-half ml of plasma was dialyzed against an equal volume of isotonic Krebs Ringer bicarbonate buffer (*p*H 7.4). Dialysis was performed at 37°C for 5 hr using Spectrapor 2 membrane tubing (Spectrum Medical Industries, Inc., Los Angeles, Calif.).

Calculations

The percent free or percent of furosemide unbound to plasma proteins (fu) was calculated as

$$fu = 100 \times Cf'/(Cp - Cf') \tag{1}$$

where Cp represents the measured total plasma concentration of furosemide prior to dialysis and Cf' represents the measured unbound or free concentration of furosemide in buffer after dialysis. Equation (1) assumes that the initial plasma and buffer volumes are equal prior to dialysis, that there is negligible binding of drug to the dialysis membrane, and that protein binding is linear. In cases of nonlinear plasma protein binding, equation (1) is inappropriate and will underestimate the true value for the percent free of drug in the original plasma sample. Patient EH displayed nonlinear binding of furosemide to plasma proteins and values for percent free were determined in this patient accordingly (21,22).

The appropriate bound (Cb'') and free (Cf') equilibrium concentrations of furosemide were best fitted to a conventional protein binding model for a single Langmuir term plus a linear term:

$$Cb'' = Pl \cdot Cf'/(P2 + Cf') + P3 \cdot Cf' \tag{2}$$

where Cb'' represents the concentration of bound drug in the plasma compartment at dialysis equilibrium assuming no volume change (a hypothetical concentration which cannot be measured). This representation is necessary in order to correct for the osmotic water shift that occurs (10–15% for furosemide) during equilibrium dialysis, resulting in lower protein and drug concentrations in the postdialysis plasma compartment (22). Other protein binding models were tested (single Langmuir and double Langmuir), but the data did not fit them as well, as determined by the values for the coefficient of determination and the residual sum of squares.

Since the initial volumes of the plasma and buffer compartments were equal prior to dialysis, Cb'' was calculated (22) and is given by Eq. (3):

$$Cb'' = Cp - 2 \cdot Cf' \tag{3}$$

where Cp and Cf' were experimentally determined. The above Langmuir-type protein binding model (Eq. 2) can be modified (21) to give the quadratic equation given by Eq. (4):

$$(1+P3) \cdot Cf^2 + (P1+P2+P2 \cdot P3-Cp) \cdot Cf - P2 \cdot Cp = 0 \tag{4}$$

where the binding parameters $P1$, $P2$, and $P3$ were obtained from a computer fit to the Langmuir-type model (Eq. 2). Values for the free plasma concentrations of furosemide prior to dialysis or in the original plasma sample (Cf) were obtained by finding the positive root of the quadratic equation (4) for a given value of Cp.

The percent free of drug in the original plasma sample (fu) can now be calculated using Eq. (5):

$$fu = 100 \times Cf/Cp \tag{5}$$

The above equations were used for patient EH, who demonstrated nonlinear binding of furosemide to plasma proteins. Values for percent free of furosemide in plasma for all other kidney transplant patients were determined using Eq. (1).

RESULTS

The plasma protein binding of furosemide in kidney transplant patients is presented in Table I. Serial plasma samples taken after intravenous administration ranged from 0.32 to 124 μg/ml total furosemide. The variability between patients in percent free of furosemide was substantial as evidenced by an approximate 50% coefficient of variation.

Patient EH demonstrated nonlinear protein binding as displayed in Fig. 1. The appropriate bound and free equilibrium plasma concentrations were fitted to Eq. (2) by nonlinear least squares using the "Multi-fun" procedure of Prophet (a specialized computer resource developed by the Chemical/Biological Information Handling Program of the National Institutes of Health). The parameters obtained by computer fitting were $P1 = 4.02$, $P2 = 0.0525$, and $P3 = 4.54$ ($r^2 = 0.980$). Using the above parameters, and the original total plasma concentrations, the corresponding free concentrations of furosemide were estimated and appropriate values for percent free were obtained. Patient EH had an approximate 10-fold range in percent free over the total furosemide plasma concentrations studied (Fig. 2).

Table I. Plasma Protein Binding of i.v. Furosemide in Kidney Transplant Patients

Patient	Status	Treatment (mg i.v.)	Serum albumin conc. (gm %)	Percent[a] free	SD[b]	*CV* (%)
CT	NR	160	4.4	1.6	0.3	18.8
EH	NR	120	3.4	1.3–12.9[c]	—[c]	
DH	NR	120	4.2	1.6	0.2	12.5
LT	NR	120	3.9	2.2	0.1	4.6
VW	R	80	4.4	—[d]	—[d]	
SJ	R	120	4.1	5.2	2.2	42.3
PD	R	40	3.7	3.7	1.2	32.4
WJ	R	80	4.5	1.5	0.1	6.7
FR	R	80	3.5	4.2	1.4	33.3

Intersubject variability: 2.9 ± 1.5
(Total, $n = 7$)

[a]The percent free for each patient represents the mean value for at least eight serial plasma samples taken after i.v. administration.
[b]Intrasubject variability.
[c]The range of values for percent free in patient EH are reported due to nonlinear plasma protein binding. These values were excluded from the intersubject variability results.
[d]Insufficient plasma sample.

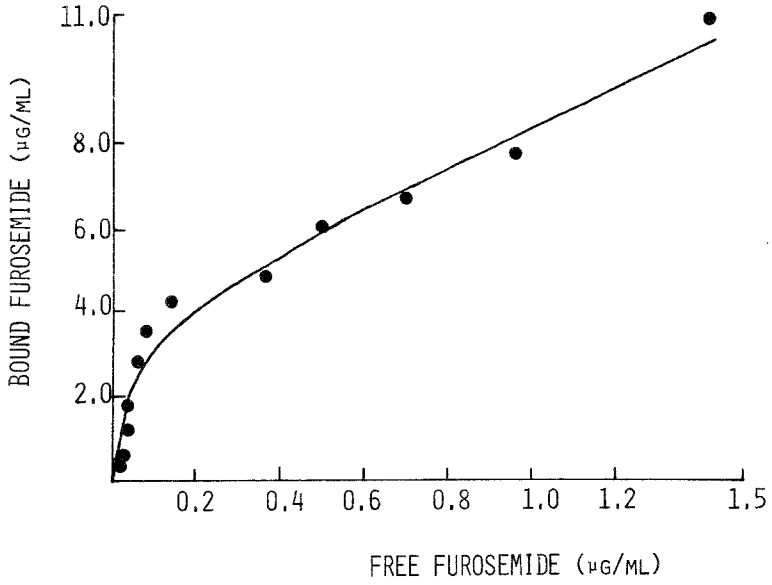

Fig. 1. Relationship between bound and free equilibrium plasma concentrations of furosemide in kidney transplant patient EH.

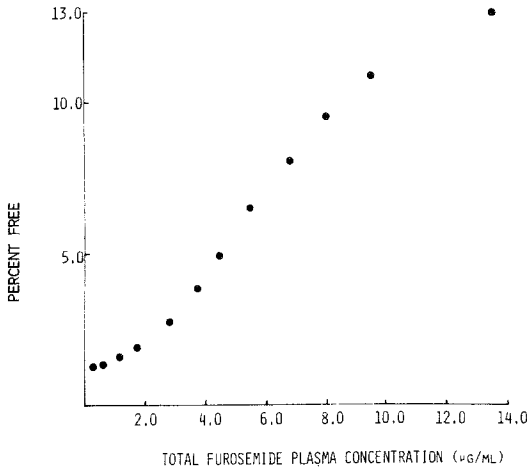

Fig. 2. Relationship between percent free and total furosemide plasma concentrations in kidney transplant patient EH.

Table II compares the values for percent free of furosemide between healthy volunteers (controls), kidney transplant patients not taking sulfisoxazole concomitantly (KT−) and transplant patients taking sulfisoxazole concomitantly (KT+) with furosemide. The results demonstrate that those kidney transplant patients who are on concomitant sulfisoxazole treatment have a significantly greater percent free of furosemide as compared to transplant patients not on sulfisoxazole (4.4 ± 0.8 for KT+ vs. 1.7 ± 0.3% for KT−; $p < 0.01$) as well as to healthy

Table II. Comparison of Plasma Protein Binding of i.v. Furosemide in Healthy Volunteers and Kidney Transplant Patients

	Controls[a] ($n = 9$)	KT−[b] ($n = 4$)	KT+[c] ($n = 3$)
Percent free	1.2	1.7	4.4
SD	0.2	0.3	0.8
Level of significance[d]		($p < 0.01$)	

Comparison	*Inference*[e]
KT+ vs. controls	$p < 0.01$
KT+ vs. KT−	$p < 0.01$
KT− vs. controls	$p < 0.05$

[a] Values were previously reported (12).
[b] Kidney transplant patients not concomitantly taking sulfisoxazole.
[c] Kidney transplant patients concomitantly taking sulfisoxazole.
[d] Determined by single factor analysis of variance.
[e] Level of significance determined by Newman–Keuls multiple range test.

volunteers (4.4 ± 0.8 for KT+ vs. $1.2 \pm 0.2\%$ for controls; $p < 0.01$). In addition, kidney transplant patients not on concomitant sulfisoxazole treatment had a significantly higher value for percent free of furosemide with respect to healthy volunteers (1.7 ± 0.3 for KT− vs. $1.2 \pm 0.2\%$ for controls; $p < 0.05$).

DISCUSSION

Impaired binding of furosemide to plasma proteins has been reported in uremics (7,11), nephrotics (10–11), and anephric patients (6). In the present study, the percent free of furosemide in plasma was significantly greater in kidney transplant patients (KT− and KT+) than in healthy volunteers. This was probably due to the presence of endogenous and exogenous (drugs) substances which compete with furosemide for binding sites on the plasma proteins. Since furosemide is exclusively bound to albumin (7,10), the presence of hypoalbuminemia has been postulated (11) as a possible cause for reduced drug binding of furosemide in patients with renal dysfunction. However, the kidney transplant patients in this study were normal with respect to serum albumin levels, and no significant correlation was observed between percent free of furosemide and albumin concentration (Fig. 3). This lack of correlation was in agreement with results from a previous study in anephric patients (6). Nevertheless, it should be noted that the lack of correlation between percent free of furosemide and albumin concentration may have been obscured by the inhomogenous patient population studied (3 out of the 7 patients were receiving concomitant sulfisoxazole therapy).

Although the mean values for percent free were two-fold greater in responder than in nonresponder kidney transplant patients, the difference was not statistically significant (3.6 ± 1.6 for R vs. $1.8 \pm 0.3\%$ for NR; $p > 0.10$). This was due to the large variability in this parameter between responders ($CV = 44.4\%$) and probably reflects the effect of concomitant sulfisoxazole administration in three out of the four patients studied in this population. The effect of sulfisoxazole on furosemide protein binding was assessed by comparing the percent free of those patients concomitantly taking sulfisoxazole with those patients on furosemide without sulfisoxazole (Table II). Not only were these two groups (KT− and KT+) different from one another with respect to percent free, but their within group variability ($CV = 17.6\%$ for KT−; $CV = 18.2\%$ for KT+) was substantially reduced, compared to the total transplant population, and in good agreement with the variability observed for percent free in healthy volunteers ($CV = 16.7\%$). This displacing effect by sulfisoxazole has previously been demonstrated *in vitro* and results in significantly reduced binding of furosemide to human albumin (10).

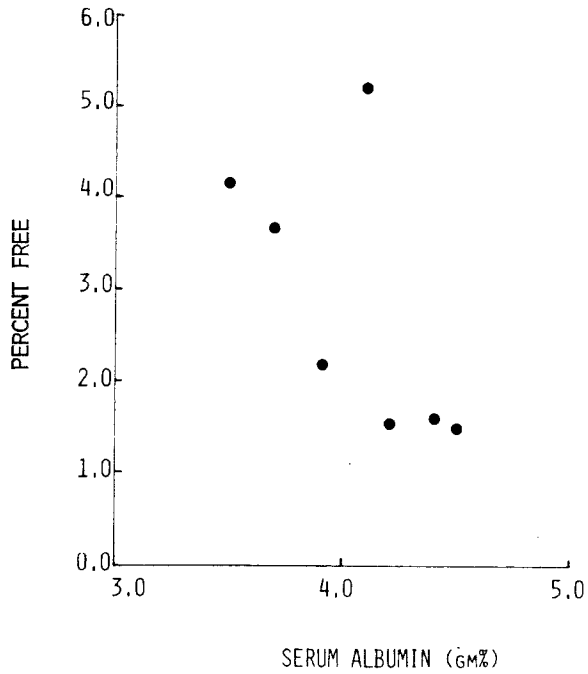

Fig. 3. Correlation between percent free of furosemide in plasma and albumin concentration in kidney transplant patients ($r = -0.629$, $p > 0.10$).

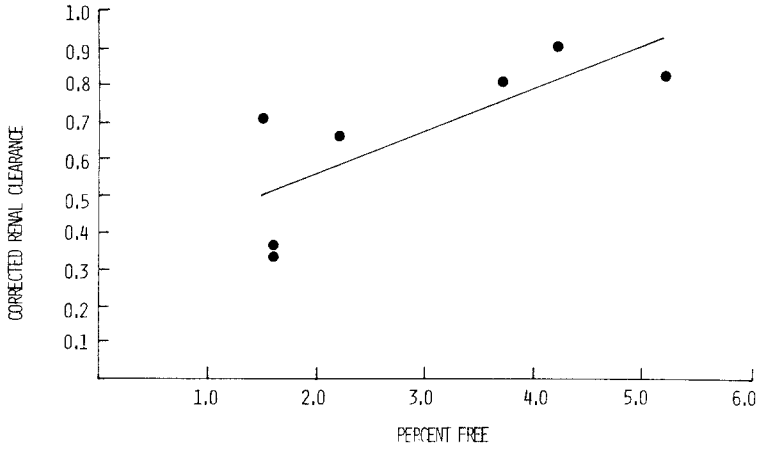

Fig. 4. Correlation between the corrected renal clearance of furosemide (CL_r/CL_{cr}) and percent free in plasma in kidney transplant patients ($r = 0.762$, $p < 0.05$).

Table III. Incremental Renal Clearances for Patient EH[a]

Time (min)	ΔCp (μg/ml)	Percent free	CL_r (ml/min)
0–40	7.5–13.6	9.1–12.9	18.5
40–94	3.8–7.5	3.9–9.1	33.9
94–130	2.6–3.8	2.5–3.9	29.6
130–208	1.6–2.6	1.8–2.5	18.9
208–328	0.8–1.6	1.5–1.8	14.5
328–480	0.3–0.8	1.3–1.5	16.9

[a]Determined following 120 mg intravenous dose of furosemide. ΔCp represents the range of total plasma concentrations of furosemide during the specified time interval. CL_r represents the incremental renal clearance.

A positive correlation was observed in kidney transplant patients between the corrected renal clearance of furosemide (CL_r/CL_{cr}) and the percent free in plasma (Fig. 4; $r = 0.762$, $p < 0.05$). This finding is consistent with a study by Yacobi and Levy (23) who found the renal clearance of sulfisoxazole in rats to be positively correlated with the serum free fraction of the drug. In addition, Table III reveals that, except for the initial collection interval (0–40 min), a significant positive correlation exists between the median percent free and the incremental renal clearance of furosemide in patient EH ($r = 0.906$, $p < 0.05$). Considering the short urinary collection interval (0–40 min) and the usual time lag in drug elimination from the bladder, it is not surprising that the initial incremental renal clearance is somewhat low. This observation for patient EH and the relationship between corrected renal clearance of furosemide and the percent free in plasma for all of the kidney transplant patients (Fig. 4) suggest that plasma protein binding plays a significant role in the renal clearance of furosemide. A positive correlation was also observed between the nonrenal clearance of furosemide and the percent free in plasma ($r = 0.841$, $p < 0.02$). However, since the changes in both the renal and nonrenal clearances of furosemide were proportional to percent free of drug, no significant correlation was observed between the fraction of the dose excreted in the urine unchanged and percent free of furosemide in the kidney transplant patients (Fig. 5; $r = -0.106$, $p > 0.50$).

It is interesting to note that three out of the five kidney transplant patients designated as responders were concomitantly taking sulfisoxazole with furosemide. However, it is doubtful that this drug interaction was a factor in these patients being more responsive to furosemide treatment. Although responder patients on concomitant sulfisoxazole had reduced binding of furosemide to plasma proteins, this effect was not translated

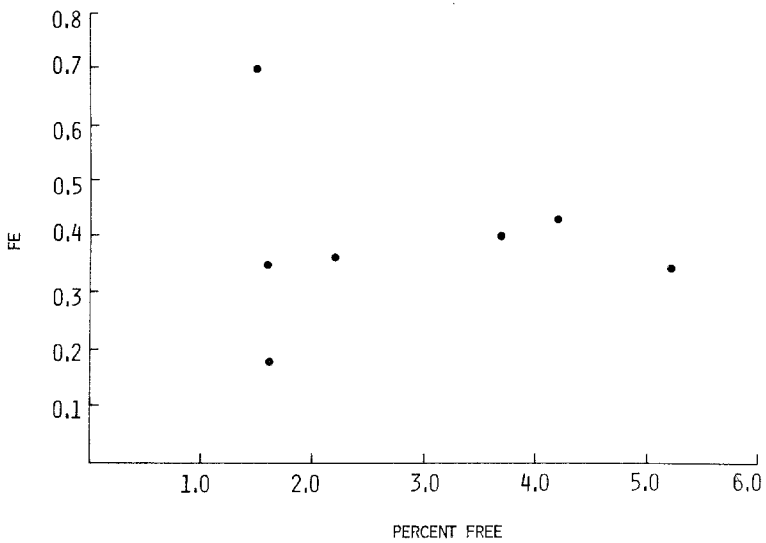

Fig. 5. Correlation between the fraction of the dose excreted in the urine unchanged (FE) and percent free of furosemide in plasma in kidney transplant patients ($r = -0.106$, $p > 0.50$).

into greater values for fraction of the dose excreted unchanged in the urine (compare patients SF, PD, and FR to patients VW and WJ; Table II of ref. 19). In fact, responder patient WJ (KT−) excreted over 70% of the unchanged drug in the urine with a percent free for furosemide of only 1.5%.

The large number and wide variety of concomitant drugs received by the kidney transplant patients (19) could potentially complicate the interpretation of the protein binding and renal clearance data. However, when one analyzes the percent free data (Tables I and II), the variability ($CV\%$) about the mean in each subject is relatively small except for those patients receiving concomitant sulfisoxazole therapy (compare CT = 19%, DH = 13%, LT = 5%, and WT = 7% vs. SF = 42%, PD = 32%, and FR = 33%). In addition, sulfisoxazole is the only drug that is consistent in the three patients showing large intrasubject variability in percent free of furosemide. Since plasma samples in each subject were obtained over time when concentrations of concomitant drugs would be changing, we believe that the potential effects of these other drugs on the parameters reported here would be minimal. The substantial variability in percent free exhibited by patients SJ, PD, and FR is most probably due to changing concentrations of both sulfisoxazole and furosemide as a function of time. However, no consistent trend in the percent free values was observed. Therefore, the data were treated as following linear protein binding.

In conclusion, the present study has shown that the binding of furosemide to plasma proteins is significantly reduced in kidney transplant patients as compared to healthy volunteers. This binding is further reduced in those patients concomitantly on sulfisoxazole. In addition, furosemide may exhibit nonlinear protein binding as evidenced by patient EH. Although differences may exist in the percent free of furosemide in kidney transplant patients, the fraction of the dose excreted in the urine unchanged does not appear to be influenced by its protein binding.

ACKNOWLEDGMENTS

We are indebted to Dr. John G. Gambertoglio and Dr. Flavio Vincenti for their initiative and provision of the patient population for these studies. During the course of this work, D.E. S. was supported as an NIH Predoctoral Scholar on NIH Training Grant GM07175.

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