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Does Albumin Preinfusion Potentiate Diuretic Action of Furosemide in Patients with Nephrotic Syndrome?

The aim of this cross-over study was to investigate whether albumin infusion before furosemide administration could potentiate the diuretic action of furosemide. Seven patients with nephrotic syndrome were given the following infusions in random order on two separate days: 1) a sham solution followed by 160 mg of furosemide, 2) 100 mL of 20% human albumin followed by 160 mg of furosemide. Urine and serum furosemide concentrations were measured by high-performance liquid chromatography. The increment of urine volume was greater in albumin preinfusion than in furosemide alone. However, the increments of sodium and chloride excretions between furosemide alone and albumin preinfusion were not different. No significant differences in the pharmacokinetic parameters between the two treatments were observed: area under the concentration-time curve (AUC: 12.7±2.2 vs 15.1±4.4 μg/mL·hr), total plasma clearance (253±41 vs 256±54 mL/min), volume of distribution (341±34 vs 494±153 mL/kg), elimination half life (4.0±1.1 vs 4.6±0.8 hr), and urine furosemide excretion of the administered amount (16.5±7.3 vs 7.5±1.6%). In conclusion, these data show that albumin preinfusion potentiated diuresis, but not natriuresis, of furosemide without any change in the pharmacokinetics of the agent in patients with nephrotic syndrome.

Key Words : *Albumin Preinfusion; Furosemide; Diuresis; Natriuresis; Nephrotic Syndrome*

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INTRODUCTION

Resistance to diuretics, including furosemide, is frequently encountered in patients with nephrotic syndrome. The overall response to a diuretic is determined by delivery of drug to its site of action, delivery of solute to the site of action, the dynamics of the drug with its receptor and whether or not solute is reclaimed distal to the site of action (1). Both pharmacokinetic and pharmacodynamic mechanisms have been proposed as explanations for the resistance to loop diuretics (2-4).

In plasma, furosemide is extensively bound to proteins, mainly to albumin. The albumin-bound fraction of furosemide reaches the proximal tubule cells and is secreted into the tubular lumen. Hypoalbuminemia diminishes the amount of albumin-bound furosemide and diminishes the furosemide delivery to the ascending limb of the loop of Henle (5, 6). A reduction in the amount of pharmacologically active drug and an impairment of tubular reabsorption due to urinary albumin-furosemide binding could ultimately diminish the diuretic effect (4, 7). Absolute or relative hypovolemia in nephrotic syndrome may enhance NaCl reabsorption and contribute to diuretic resistance (8).

Intravenous infusion of albumin in combination with furosemide has been advocated as an effective method of treating edema due to the nephrotic syndrome, but its clinical efficacy is controversial (2, 3, 9, 10). Coadministration of albumin with furosemide may improve furosemide delivery and hence diuretic effect by changing the pharmacokinetics of furosemide. Parallel infusion method of albumin and furosemide was refuted by other opinion that optimal efficacy of the combination could be achieved when furosemide was given after infusion of albumin, as the peak furosemide concentrations would then coincide with maximal expansion of plasma volume (3, 11). However, we demonstrated previously that a mixed infusion of albumin and furosemide, compared with furosemide alone, did not enhance the diuretic action of furosemide in six nephrotic patients, either pharmacodynamically or pharmacokinetically (12). Therefore, we tried to demonstrate some potentiation by sequential infusion.

The present study was therefore undertaken to investigate

whether albumin preinfusion to expand plasma volume could improve the diuretic action of furosemide in patients with nephrotic syndrome.

MATERIALS AND METHODS

Patients

This study was approved by the institutional review board of Seoul National University Hospital. Seven patients were enrolled after having given informed consent. They exhibited generalized edema and the excretion of urinary protein exceeded 3.5 g/1.73 m^2/24 hr. Patients who were on steroid treatments or who had taken diuretics within 48 hr before the start of this study were excluded. Edematous patients, with causes other than nephrotic syndrome (liver cirrhosis, congestive heart failure, malnutrition, constrictive pericarditis) were also excluded. Characteristics of the patients are given in Table 1.

Study design

The study was performed as two way cross-over with a washout period of two days. On each occasion, patients were given no treatments as baseline. On the next day, they were randomly given one of the two following infusions: 1) 100 mL of 5% dextrose water as a sham solution for albumin for 1 hr followed by 160 mg of furosemide (Lasix®; Handok Inc., Seoul, Korea) dissolved in 50 mL of dextrose water for 30 min, 2) 100 mL of 20% human albumin (Green Cross Co., Yong-In, Korea) for 1 hr followed by 160 mg of furosemide dissolved in 50 mL of dextrose water solution for 30 min. All these infusions were given at 8 a.m. over a period of 90 min.

All the patients were hospitalized and were placed on a low-salt diet (salt < 5 g/day) through the study period. For the infusion and blood sampling, an indwelling cannula was inserted into a vein in the left arm of each patient.

Urine was collected sequentially over 24 hr, from 9 a.m. to 10 a.m. (0-1 hr), from 10 a.m. to noon (1-3 hr), from noon to 3 p.m. (3-6 hr), from 3 p.m. to 9 p.m. (6-12 hr), and from 9 p.m. to 9 a.m. of the next day (12-24 hr). An effort was made to prevent undue exposure of urine samples to light to prevent degradation of furosemide. Urine volume, osmolality, sodium and chloride excretions, and furosemide excretion were measured. Blood samples were taken for the determination of furosemide concentration at 10 a.m. (1 hr), noon (3 hr), 3 p.m. (6 hr), 9 p.m. (12 hr), and 9 a.m. of the next day (24 hr). Serum and urine samples for the measurement of furosemide concentration were stored at -70℃ immediately after sampling.

Measurements and Calculations

Urine osmolality was measured with a cryoscopic osmometer (Osmomat 030-D-M; Gonotec, Berlin, Germany). Urine sodium and chloride concentrations were measured using an ion-selective method (System E4A; Beckman Coulter Inc., Fullerton, CA, U.S.A.). The concentrations of furosemide in urine and serum were measured with high-performance liquid chromatography (HPLC) according to the method described by Farthing et al. (13). The HPLC system consisted of a pump (model 305; Gilson Inc., Villiers Le Bel, France), a fluorescence detector (model S-3350; Soma optics, Ltd., Japan), an integrator (Shimadzu Co., Kyoto, Japan), and an injector (model 7725i; Rheodyne, Cotati, CA, U.S.A.). The analytic column was a 4-μm-particle-sized Nova-Pak® C_{18} column (300 × 3.9 mm, i.d.) from Waters (Milford, MA, U.S.A.). The mobile phase of 10 mM monobasic potassium phosphate buffer:acetonitrile (70:30, v/v) was delivered at a flow rate of 1 mL/min. Acetonitrile (HPLC grade) was used as received (Burdick and Jackson, Muskegon, MI, U.S.A.). Monobasic potassium phosphate (HPLC grade) was obtained from Merck KgaA (Darmstadt, Germany). To measure the concentration of free furosemide in urine, urine samples were centrifuged at 1,000 *g* for 10 min (GS-6 R; Beckman Coulter Inc., Fullerton, CA, U.S.A.), after filtration through 14-mm diameter ultrafiltration membranes (Diaflo® 40420; Amicon Division, W.R. Grace & Co., Conn. Berverly, MA, U.S.A.). The lower limit of quantification (LLOQ) of furosemide concentration in serum and urine was 0.025 μg/mL. The day-to-day coefficient of variations of serum and urine furosemide concentrations were 13% and 20%, respectively.

Osmolal clearance was calculated using the following for-

Table 1. Patients characteristics

Patient	Age (yr)	Sex	Diagnosis	Cr (mg/dL)	Albumin (g/dL)	24-hr protein excretion (mg)
1	53	M	FSGS	2.7	1.8	13475
2	23	M	MC	1.2	1.6	17869
3	17	M	FSGS	2.7	1.7	14965
4	74	M	MPGN	1.6	2.1	7474
5	65	M	MC	1.1	1.7	6972
6	20	M	MC	1.2	1.4	10874
7	36	F	FSGS	0.6	1.6	12060

FSGS: focal segmental glomerulosclerosis; MC: minimal change; MPGN: membranoproliferative glomerulonephritis

Table 2. Pharmacodynamic data in furosemide alone and furosemide after albumin infusions

	Furosemide alone		Furosemide after albumin infusions	
	Basal	Post-treatment	Basal	Post-treatment
Urine volume (mL/day)	996±230	1730±199[a]	646±91	2051±199[b]
C_{osm}* (mL/day)	ND	1304±140	ND	1488±210
C_{H2O}† (mL/day)	ND	403±136	ND	506±167
Urine Na (mEq/day)	19.0±7.2	129.5±29.6[c]	19.8±7.9	121.4±38.2[d]
Urine Cl (mEq/day)	39.1±12.4	166.4±29.2[e]	30.7±8.9	148.9±37.0[f]

Data are expressed as mean±SEM (standard error of the mean). [a-f]$p<0.05$ by Wilcoxon signed rank test. *Osmolal clearance: †Free water clearance. ND: not done

mula.

Osmolal clearance=urine osmolality×urine volume/plasma osmolality.

Free water clearance was calculated from urine volume minus osmolal clearance. Noncompartmental pharmacokinetic analysis was performed using the WinNonlin standard version 2.1 program (Pharsight Co., Mountain View, CA, U.S.A.). The area under the concentration-time curve (AUC), total plasma clearance, elimination half life, and volume of distribution were all calculated from the sequential change in the concentration of furosemide. The urinary excretion rate of furosemide was calculated from the urine volume and the concentration in urine, and was expressed as a ratio (%) of the total administered dose.

Statistics

Differences between the effects of different treatments were analyzed with Statview software (Abacus Concepts Inc., Berkeley, CA, U.S.A.) using Wilcoxon signed rank test. Values were presented as mean±SEM. Values of $p<0.05$ were considered as indicative of statistical significance.

RESULTS

Pharmacodynamic data

Both furosemide alone and furosemide after albumin infusions increased urine volume significantly as compared with each basal state. However, the volumes of urine between furosemide alone and furosemide after albumin infusions were not different. The osmolal clearance and the free water clearance between the two groups were not significantly different, either. Sodium and chloride excretions were significantly increased in both groups, compared with each basal state. However, the amounts of sodium and chloride excretions between the two groups were not different (Table 2).

The increment of urine volume from basal state to post-treatment state was greater in furosemide after albumin infusions (1406±190 mL) than in furosemide alone (734±224 mL). However, there was no difference in the increments of sodium excretions (110.5±23.6 mEq vs. 101.6±33.3 mEq) and chloride excretions (127.4±26.1 mEq vs. 132.3±36.8 mEq) between the two groups (Fig. 1).

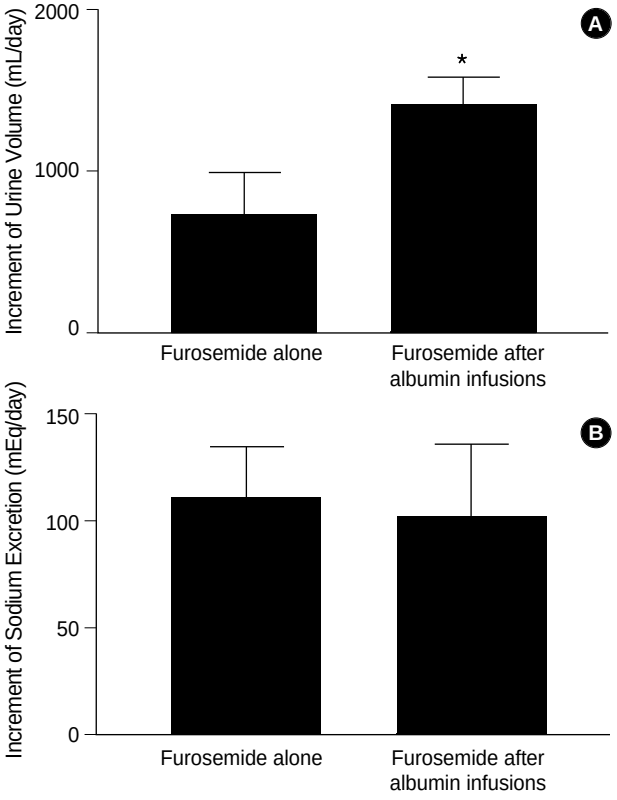

Fig. 1. Changes in 24-hr urine volume and sodium excretion in the patients. The values depicted represent the differences between basal and post-treatment states (mean±SEM). A: Increment of urine volume. The increment of urine volume is greater in furosemide after albumin infusions than in furosemide alone ($p<0.05$). B: Increment of urinary sodium excretion. No difference is observed between the two treatments.

Fig. 2 depicts the time course of sodium excretion. Albumin preinfusion had no effect on sodium excretion rate. The urinary excretion rate of furosemide, which reflects the amount reaching the site of action, is related to sodium

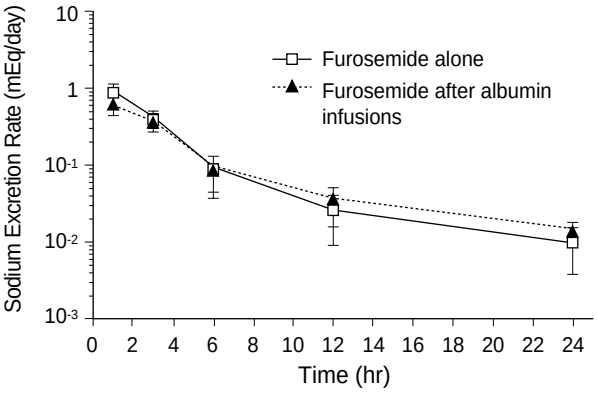

Fig. 2. Urinary sodium excretion rates in furosemide alone and furosemide after albumin infusions.

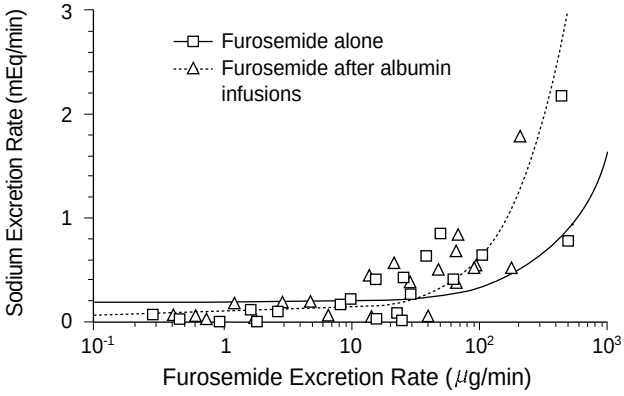

Fig. 3. Relationship between furosemide excretion rate and sodium excretion rate in furosemide alone and furosemide after albumin infusions.

Table 3. Pharmacokinetic data in furosemide alone and furosemide after albumin infusions

	Furosemide alone	Furosemide after albumin infusions
AUC (μg/mL-hr)	12.7±2.2	15.1±4.4
Total plasma clearance (mL/min)	253±41	256±54
Volume of distribution (mL/kg)	341±34	494±153
Elimination half life (hr)	4.0±1.1	4.6±0.8
Urine furosemide excretion (%)	16.5±7.3	7.5±1.6

AUC, area under the concentration-time curve. Data are expressed as mean±SEM (standard error of the mean)

excretion rate (Fig. 3). Fig. 3 shows that this relationship was not changed by albumin preinfusion.

Pharmacokinetic data

Table 3 shows the pharmacokinetic parameters from furosemide alone versus furosemide after albumin infusions. There was no significant differences of AUC, total plasma clearance, volume of distribution, and elimination half life between the two treatments. The amount of furosemide delivered into the urine is more directly related to response than is serum concentration of furosemide (14). Urine furosemide excretions between the two treatments were not different. Fig. 4 shows that albumin preinfusion had no effect on the excretion rate of furosemide.

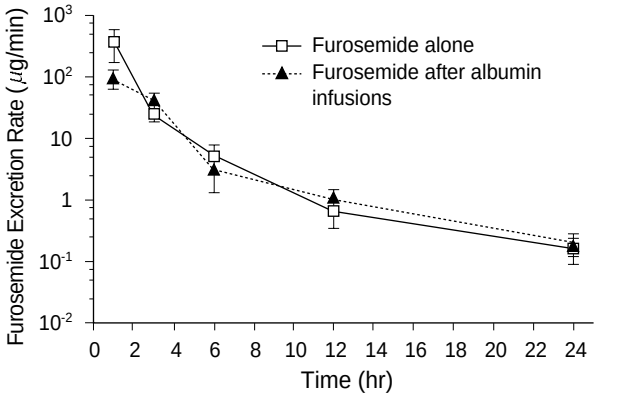

Fig. 4. Furosemide excretion rates in furosemide alone and furosemide after albumin infusions.

DISCUSSION

In this study, a significant increase in the volume of urine was observed in patients with nephrotic syndrome when albumin was infused before furosemide administration as compared with administration of furosemide alone. The potentiation of diuretic effect by albumin preinfusion was not accompanied by any increase in sodium and chloride excretion. This potentiation was not related to a change in pharmacokinetics of furosemide, either. We speculate that albumin preinfusion might expand plasma volume, which might therefore have improved the diuretic action of furosemide.

In an attempt to overcome diuretic resistance in patients with nephrotic syndrome, many trials of albumin coadministration have been conducted. Many of these studies revealed that albumin had an additive diuretic effect. Davison et al. and Eadington et al. reported positive results, but they did not perform a cross-over study or show any pharmacokinetic data (15, 11). Inoue et al. reported that effective diuresis was achieved by infusion of the albumin-furosemide complex in analbuminemic rats and hypoalbuminemic patients (2). All the patients in their experiment were hypoalbuminemic due to causes other than nephrotic syndrome. It is not certain therefore, whether their data could apply to the study of nephrotic syndrome. Recently, it was reported that coadministration of albumin moderately potentiated the action of furosemide in patients with nephrotic syndrome (10). In this study, the mean serum albumin concentration

of patients was 2.9 g/dL and the mean 24-hr urine volume, without administration of furosemide, was approximately 2 liters. The results of this experiment cannot be extrapolated to morbid diuretic-resistant patients with more extreme degrees of hypoalbuminemia. In our study, the mean serum albumin concentration of the patients was 1.7 g/dL. In an attempt to overcome the effect of diuretic-resistance, a much higher dose of furosemide, 160 mg, was administered to the patients. Furthermore, we measured pharmacokinetic parameters of furosemide and found that potentiation of diuretic effect was mediated by the changes of pharmacodynamics, but not pharmacokinetics.

The cross-over study performed by Akcicek et al. demonstrated that potentiation of the furosemide effect by infusion of albumin did not occur (9). They simultaneously infused furosemide 60 mg bolus followed by 40 mg/hr for 4 hr, and 20% albumin solution 0.5 mg/kg for 4 hr, to their patients. At maximal doses of furosemide, albumin did not facilitate any further increments in diuresis or natriuresis. These results concur with the findings of our previous study (11). We used maximal dose of furosemide, 160 mg, to overcome diuretic-resistance. To improve delivery of the drug, we mixed 160 mg of furosemide and 100 mL of 20% albumin solution at room temperature before administration. This method of administration was previously used by other investigators (2, 16). The timing of administration of albumin and furosemide varied according to the investigators.

This study showed that albumin preinfusion increased diuresis but not natriuresis that was provoked by furosemide. There have been some reports that plasma volume expansion, induced by albumin or dextran, caused an increase in urine flow rate but not in sodium excretion in healthy volunteers and in nephrotic patients (3, 17-19). This is important given that an acute rise in blood volume suppresses vasopressin secretion (20). The kidney shows of considerable variations in urine flow in response to changing levels of vasopressin. The large changes in urine flow are achieved without substantial changes in osmolal clearance (21). We calculated free water clearance and osmolal clearance to see whether the increase of urine volume was due to water diuresis or not. We found that the free water clearance as well as the osmolal clearance in furosemide after albumin infusions was slightly greater than in furosemide alone, albeit without any statistical significance. Measurement of vasopressin levels in patients could have given the clear information about suppression of vasopressin secretion.

The diuretic effect of furosemide is directly related to the amount and rate of the drug excreted in urine. The presence of massive proteinuria and hypoalbuminemia in patients with nephrotic syndrome alters the pharmacokinetics of furosemide. The normal values of pharmacokinetic parameters of furosemide are as follows: total plasma clearance, 200 mL/min; volume of distribution, 170-270 mL/kg; and elimination half life, 45-60 min (22). In patients with nephrotic syndrome, the binding of furosemide to plasma proteins decreases in proportion with the reduction of plasma albumin levels. At the same time, the volume of distribution is apparently increased (23). In our analysis of pharmacokinetic parameters from patients with nephrotic syndrome, total plasma clearance slightly increased (253 mL/min), volume of distribution increased (341 mL/kg), and elimination half life was prolonged (4 hr). However, there was no significant difference in clearance, volume of distribution, and urinary furosemide excretion between furosemide alone and furosemide after albumin infusions. These results indicate that the potentiation of diuresis by albumin preinfusion is not relevant to the pharmacokinetics of furosemide.

In animal studies, albumin in renal tubule fluid attenuated the effect of furosemide because the amount of pharmacologically active drug was reduced due to albumin-furosemide binding (4, 24). This was suggested as one of the possible mechanisms to explain diuretic-resistance in patients with nephrotic syndrome. However, this suggestion was argued against by the observation that unbound furosemide in urine corresponded to the active furosemide at its site of action. Furthermore, Agarwal et al. demonstrated that displacement of urinary protein binding with sulfisoxazole in nephrotic patients did not enhance response to furosemide (25). Urinary protein binding of furosemide is not a major mechanism of diuretic-resistance in nephrotic syndrome. To measure the exact concentration of unbound furosemide in urine, we filtered urine samples through ultrafiltration membranes, which removed the substances larger than 30 kDa.

The resistance of the nephron to loop diuretics is proportional to the degree of hypoalbuminemia. This is due to an overreabsorption of sodium by a stimulated and anatomically expanded basal labyrinth and an increased number of sodium pumps in distal convoluted tubule, connecting tubule, and cortical collecting duct (26), despite the deficient proximal reabsorption of the molecule. Therefore, it does not count whether sodium reabsorption in the proximal tubule or is blocked in the thick ascending limb by furosemide, because the distal tubules adapt to this overload. Since thiazides inhibit the furosemide-induced overreabsorption of sodium in distal tubule, coadministration of furosemide and thiazide is commonly practiced to overcome diuretic resistance (8).

There are several limitations in our study. Firstly, we did not evaluate the exact plasma volume status by using radioisotopes (e.g. ^{125}I albumin) in the patients. It was therefore not possible to assess any sequential changes in plasma volume after albumin infusions. Secondly, as measurements of glomerular filtration rate and effective renal plasma flow using inulin and paraaminohippurate were not performed, the renal hemodynamic changes in our patients

remained unknown. Thirdly, had the findings of the renal pathology been more uniform, the experiment would have produced more consistent data. However, it was not practically easy to recruit patients with nephrotic syndrome and with homogeneous pathology. Fourthly, three out of the seven patients had reduced renal function. Complication of azotemia is not uncommon in nephrotic patients. Because abnormal renal function itself can alter the diuretic action of furosemide, it is therefore possible that our data was confounded by the presence of azotemia. Lastly, when the distribution of furosemide is plotted, a two-compartmental open model is usually used. In this study, however, we plotted the pharmacokinetics using a noncompartmental model because not all the individual data could be plotted adequately using a two-compartment model. Noncompartmental analysis requires more frequent sampling intervals than the intervals in our study. We are of an opinion, however, that these limitations had little adverse effects on the reliability of our data.

As to the clinical management of patients with nephrotic syndrome, it is more reasonable to increase the dose of furosemide than to administer albumin (3, 9, 10). From these results, taken together with our previous data (12), we claim that albumin infusion before furosemide administration can be used to overcome diuretic-resistance patients with nephrotic syndrome unresponsive to maximal doses of furosemide.

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