Mechanism of furosemide resistance in analbuminemic rats and hypoalbuminemic patients

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Mechanism of furosemide resistance in analbuminemic rats and hypoalbuminemic patients. To elucidate the mechanism of resistance of hypoalbuminemic patients to furosemide, the effect of this diuretic on urine volume of normal and analbuminemic rats (NAR) and of hypoalbuminemic patients was studied. Intravenous administration of furosemide rapidly enhanced sodium diuresis in normal rats but not in NAR. Total plasma clearance and distribution volume of furosemide were much larger in NAR than in normal rats, while no significant difference in these pharmacokinetic parameters was observed for the unbound fraction of the diuretic between the two animal groups. In contrast, urinary secretion of furosemide was significantly lower in NAR than in normal rats. Injected furosemide bound to albumin markedly promoted diuresis in NAR, while the same dose of albumin alone had no effect, indicating that binding to albumin is essential for the delivery of furosemide to the kidney, the site for its action. Injection of the complex rapidly increased the urine volume of hypoalbuminemic patients who showed a marked resistance to this diuretic. Thus, the resistance to furosemide in both NAR and hypoalbuminemic patients may be explained on the same basis.

Edema is frequently associated with hypoalbuminemia caused by various diseases, such as nephrotic syndrome, cirrhosis and neoplasm. Furosemide is commonly used to treat patients with severe fluid retention, and induces diuresis predominantly by blocking the luminal reabsorption of sodium and chloride in renal tubules [1]. Resistance to diuretics, including furosemide, is frequently encountered in patients with severe hypoalbuminemia [2, 3] but the mechanism of resistance is not known.

Many hydrophobic organic anions, such as bilirubin, bile acids, fatty acids, sulfobromophthalein (BSP), and phenolsulfophthalein (PSP), circulate by being bound to plasma albumin. Previous studies using mutant analbuminemic rats (NAR) revealed that binding of these amphipathic organic anions with circulating albumin is important for vectorial transport of these ligands to liver and kidney [4–7]. Furosemide is an organic anion that binds strongly to albumin [8, 9]. This drug is known to undergo transtubular secretion at proximal tubules via the transport system for amphipathic organic anions [10]. Thus, the

resistance of hypoalbuminemic patients to furosemide may possibly result from an impaired delivery of this diuretic to the renal peritubular space because of a deficiency in albumin. In support of this view, a preliminary experiment showed that NAR exhibited a marked resistance to furosemide.

The present work reveals that intravenous injection of furosemide bound to albumin caused a remarkable diuresis in NAR otherwise resistant to this drug. Based on this finding, effective diuresis was achieved by infusion of the albumin–furosemide complex in hypoalbuminemic human subjects who were resistant to the diuretic.

Methods

Furosemide and rat albumin (fraction V) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Human albumin (25% solution) and 1% solution of furosemide (Lasix) were obtained from Chemo-Sero Research Institute (Kumamoto, Japan) and Hoechst–Japan (Tokyo, Japan), respectively. Other reagents used were of analytical grade.

Animals were fed laboratory chow and water *ad libitum* and were used for experiments without fasting. Unless otherwise stated, experiments with animals in vivo were performed between 9:00 a.m. and 12:00 noon under pentobarbital anesthesia (50 mg/kg). During experiments, the body temperature of pentobarbital treated rats was kept constant at 37°C by using tungsten lamp. Urine samples were collected from unanesthetized rats in a metabolism cage over a period of one hour. Urine samples were also obtained from anesthetized animals for the consecutive 10 minute collection periods by urinary bladder cannulation before and after administration of drugs. Intravenous administration of 0.5 ml saline solution containing furosemide and other reagents was performed over a period of 10 seconds.

Urine samples were also obtained from hypoalbuminemic patients for the consecutive one–hour collection periods by urinary bladder cannulation before and after the administration of drugs. In general, patients were given furosemide (20 to 60 mg) with 25 ml of saline solution intravenously between 9:00 a.m. and 10:00 a.m., over a period of 10 minutes. Patients who were resistant to furosemide were again given the diuretic, albumin or albumin furosemide complex between 4:00 p.m. and 6:00 p.m. of the day. Furosemide was mixed with albumin solution to make an equimolar mixture 10 minutes before the
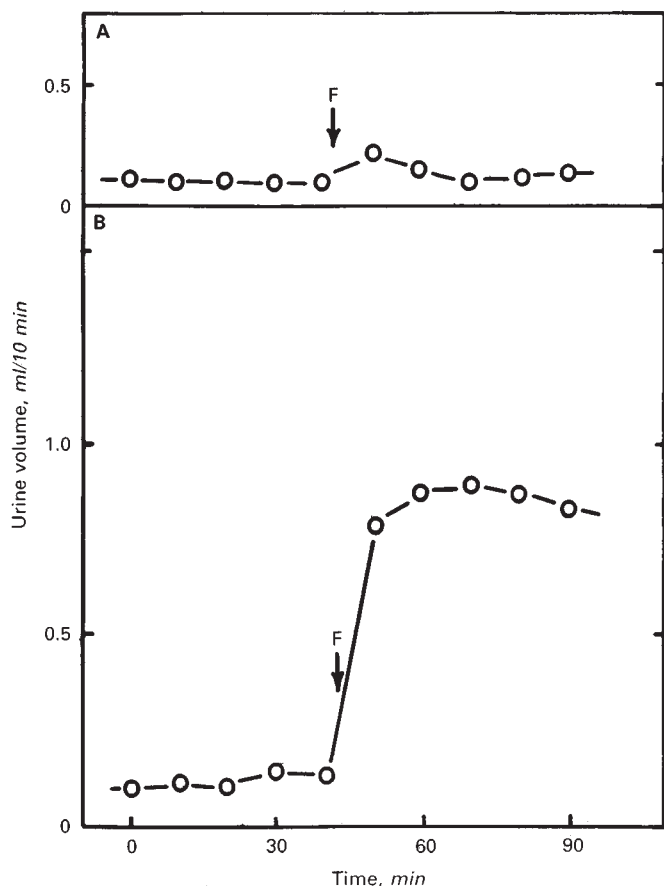

Fig. 1. *Effect of furosemide on urine volume of NAR and normal rats.* Under pentbarbital anesthesia, animals were administered furosemide intravenously (0.5 mg/kg body wt) over a period of 10 seconds. Urine samples were collected by urinary bladder cannulation for the consecutive 10 minute collection period. Arrow (F) indicates the time of furosemide administration. Other conditions were as described under the text. A typical result of six experiments. **A**, NAR; **B**, normal rats.

Fig. 2. *Dose dependent response of natriuresis in NAR and normal rats.* After intramuscular injection of furosemide, urine samples were collected in a metabolism cage for one hour. Each point represents mean ± SD derived from six animals. Symbols are (O) normal rats; (●) NAR.

Fig. 3. *Sensitivity of the kidney to furosemide in NAR and normal rats.* After intramuscular administration of various dose of furosemide, urine samples were collected in a metabolism cage. Urine volume and sodium excretion over a period of one hour after administration of furosemide were plotted against the amount of furosemide found in the urine. Symbols are: (O) normal rats; (●) NAR.

administration. Clinical diagnosis and other conditions of patients with marked hypoalbuminemia are described in the **Results**.

Furosemide concentrations in urine and plasma were determined by using high–performance liquid chromatography according to Hammarlund and Paalzow [11] in a Hitachi 655 Liquid Chromatograph equipped with a column of ODS-Hypersil C_{18}, 5 μm (Shandon). The mobile phase consisted of 40% acetonitrile in 20 mM phosphate buffer, pH 2.5. Urinary concentrations of Na^+ were determined by an ion–selective electrode (Technicon-SMAC). Binding of furosemide to plasma proteins was determined by an ultrafiltration method as described previously [12].

Results

Resistance of NAR to furosemide

Figure 1 shows the effect of furosemide on urine volume in normal and analbuminemic rats. The rate of urine formation was markedly increased by a single dose of furosemide in normal rats and continued for more than 50 minutes. The same
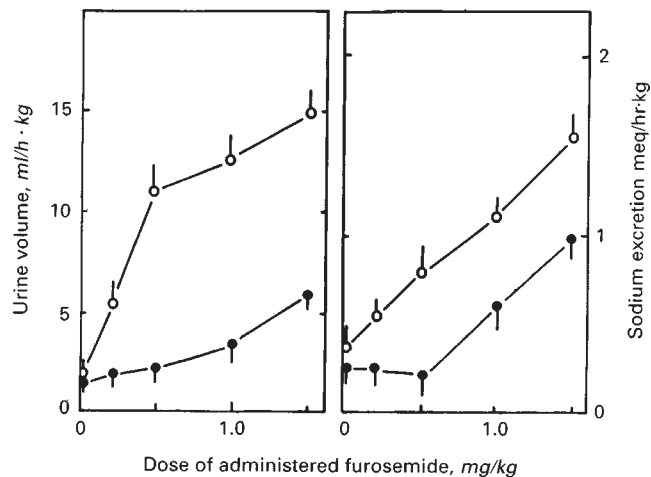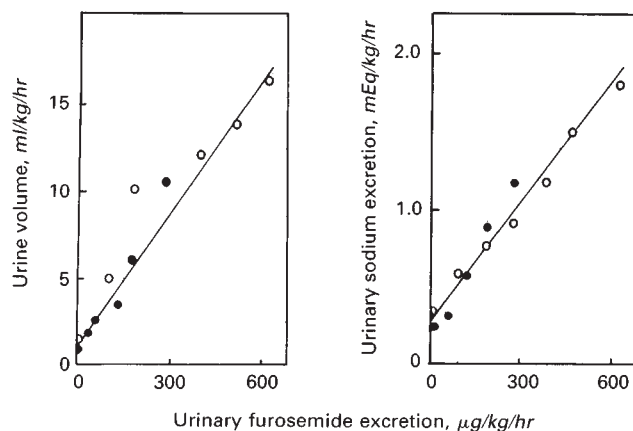
dose of furosemide failed to enhance diuresis in NAR. To elucidate the mechanism for furosemide resistance in NAR, the natriuretic response was compared between the two animal groups at different furosemide concentrations. Both urine volume and sodium excretion increased in normal rats with the increase in the dose of furosemide (Fig. 2). Furosemide induced increase in urine volume and sodium excretion was much less pronounced in NAR than in normal rats. When urine volume and sodium excretion were plotted as a function of the urinary level of furosemide after administration of various doses of this diuretic, no significant difference was observed between NAR and normal rats (Fig. 3), indicating that the renal sensitivity to furosemide is identical and the same mechanism of natriuresis is operating in the two animal groups. However, a noticeable finding elicited from this series of experiments was that the
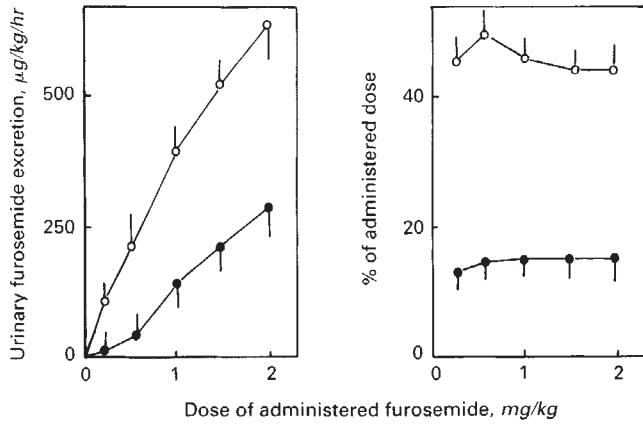
Fig. 4. *Urinary recovery of furosemide in NAR and normal rats.* After intramuscular administration of various dose of furosemide, the amount of the diuretic excreted in urine within one hour was determined. Each point represents mean ± SD derived from six animals. Symbols are (○) normal rats; (●) NAR.
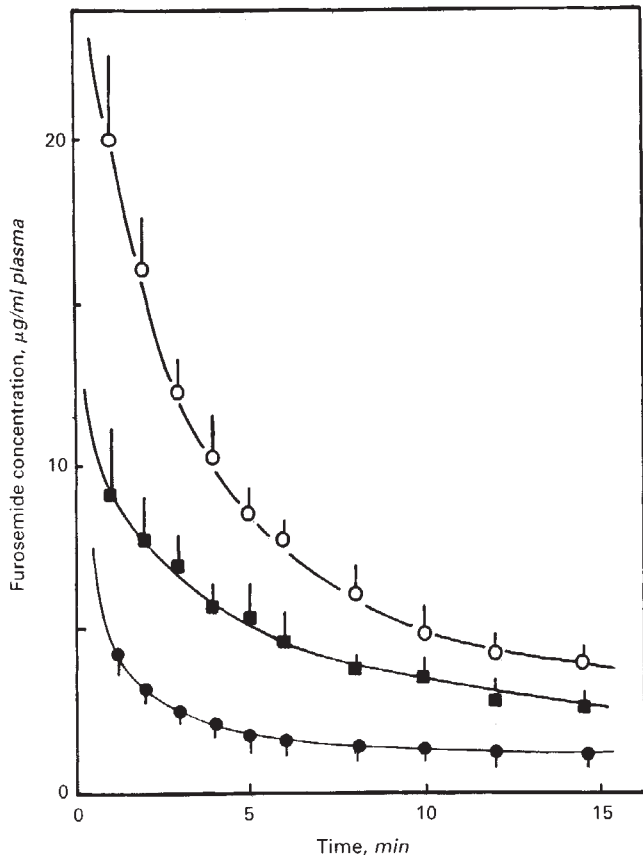
Table 1. Pharmacokinetic parameters of furosemide in normal and analbuminemic rats

Parameters	Normal rats	NAR	NAR + albumin
In vitro protein binding	98.9%	12.0%	–
20 μg furosemide/ml plasma			
Plasma clearance			
total furosemide ml/min × kg	9.4	24.4	13.3
unbound fraction liter/min × kg	0.65	0.67	0.64
Distribution volume			
total furosemide ml/kg	59.6	545.5	121.2
unbound fraction l/kg	4.7	5.1	5.3
Urinary recovery % dose	26	7	18

Details of experimental procedures were as described in Figure 5. Binding of furosemide to plasma proteins was determined as described previously [13]. Plasma clearance and distribution volume were calculated for the total (free form + protein–bound) and the unbound fraction of furosemide as described previously [6]. Urinary secretion of the injected furosemide was determined for the urine samples collected for 30 minutes after the administration.

urinary recovery of furosemide was about three times lower in NAR than in normal rats irrespective of the dose of the diuretic (Fig. 4). Thus, the resistance of NAR to furosemide appeared to result from an impaired accumulation of this diuretic in the kidney.

Pharmacokinetic analysis of furosemide in NAR and normal rats

Plasma clearance of intravenously administered furosemide was compared between NR and normal rats. The intravenously administered furosemide disappeared from the circulation much faster in NAR than in normal rats (Fig. 5). Both total plasma clearance and distribution volume of furosemide (free + protein–bound) were significantly larger in NAR than in normal rats (Table 1). However, urinary recovery of furosemide was markedly lower in NAR than in normal rats. Thus, a significant amount of furosemide injected to NAR underwent random distribution to extrarenal tissues presumably due to insufficient binding of the ligand to plasma protein(s). Although total plasma clearance and distribution volume of furosemide (free + protein–bound) was larger in NAR than in normal rats, no significant difference in the two parameters for the unbound furosemide was observed between the two animal groups. Administration of furosemide with an equimolar amount of albumin markedly decreased the rate of disappearance of the diuretic from the circulation. Thus, when furosemide was injected bound to albumin, total plasma clearance and distribution volume of furosemide were markedly decreased in NAR while those for the unbound ligand remained unchanged. When furosemide was intravenously injected bound to albumin, the urine volume of NAR increased rapidly to reach a maximum at 10 min after administration (Fig. 6). Urinary excretion of furosemide was also greater in NAR when the diuretic was injected bound to albumin than when the ligand alone was administered. A single dosage of furosemide (Fig. 1) or albumin alone (Fig. 6) did not promote diuresis in NAR. These results indicate that binding of furosemide to circulating albumin may be important for an efficient delivery of this drug to the kidney, the site for its diuretic action.

Fig. 5. *Plasma clearance of furosemide in NAR and normal rats.* Under pentobarbital anesthesia, animals were intravenously injected with furosemide (2 mg/kg) with (closed squares) or without equimolar albumin (circles) in the right femoral vein. At indicated times, blood samples (0.1 ml) were withdrawn from the left femoral vein and centrifuged at 13,000 × g for one minute in a Kubota microcentrifuge. Plasma samples thus obtained were determined for amounts of furosemide as described in the text. Symbols are: (○) normal rats; (●, ■) NAR.
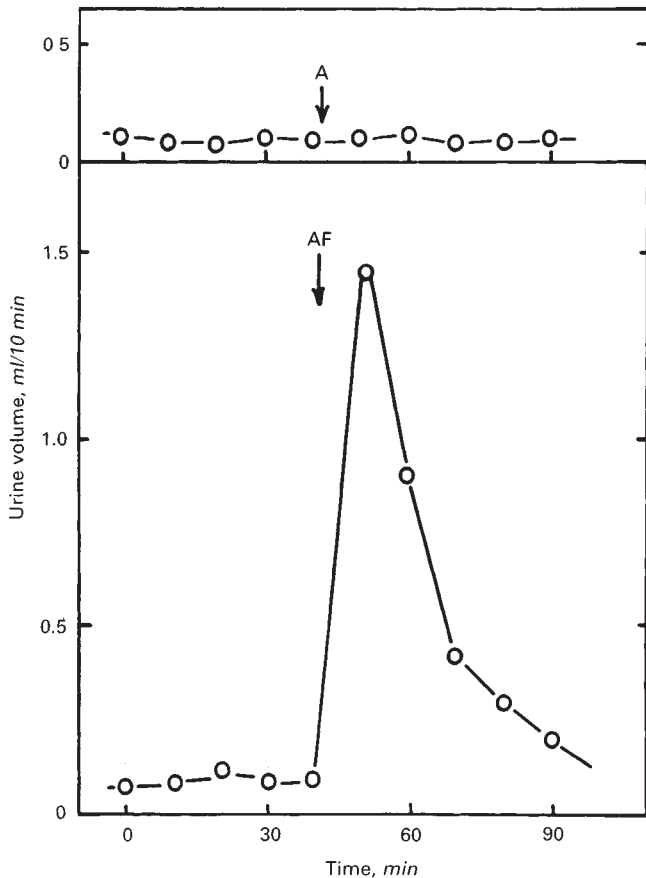
Fig. 6. *Effect of furosemide–albumin complex on urine volume of NAR.* Under pentobarbital anesthesia, NAR were intravenously injected with furosemide (0.5 mg/kg) mixed with equimolar albumin. Urine samples were collected as described in Figure 1. A, albumin (0.1 g/kg); AF, albumin–furosemide complex. Data show a typical result of five experiments.

Fig. 7. *Effect of furosemide–albumin complex on urine volume of hypoalbuminemic human subjects.* Hypoalbuminemic and furosemide resistant patients were intravenously administered 30 mg of furosemide alone or the diuretic bound to albumin (6 g) over a period of 10 minutes. Before and after administration of furosemide alone (F) or the furosemide–albumin complex (AF), urine samples were collected by uretheral catheterization for the consecutive one hour collection periods. Plasma albumin levels were 1.0 g/dl (O, cholangiocarcinoma), 2.2 g/dl (X, chronic renal failure), 2.2 g/dl (△, pulmonary edema), and 1.9 g/dl (□, brain tumor).

Diuresis by furosemide–albumin complex in furosemide–resistant hypoalbuminemic patients

The finding with NAR suggested that the furosemide–albumin complex might also promote diuresis in furosemide–resistant hypoalbuminemic patients. Thus, we tested this on 20 patients who showed a marked resistance to this diuretic. Figure 7 shows the diuretic response of several hypoalbuminemic patients to a single dose of furosemide or the furosemide–albumin complex. Administration of a therapeutic dose of furosemide as an admixture with equimolar albumin increased the rate of urine formation in these patients. The same dose of either furosemide (Fig. 7) or albumin (data not shown) alone failed to promote diuresis in these patients. The diuretic response was maximal one hour after administration and decreased thereafter. HPLC analysis revealed that about 7 to 12% of the injected dose was excreted in the patients urine within three hours after the administration of furosemide. Administration of furosemide with equimolar albumin increased the urinary recovery of the diuretic to about 24 to 30%. Thus, administration of furosemide–albumin complex efficiently promoted diuresis by increasing the urinary recovery of the diuretic in both NAR and
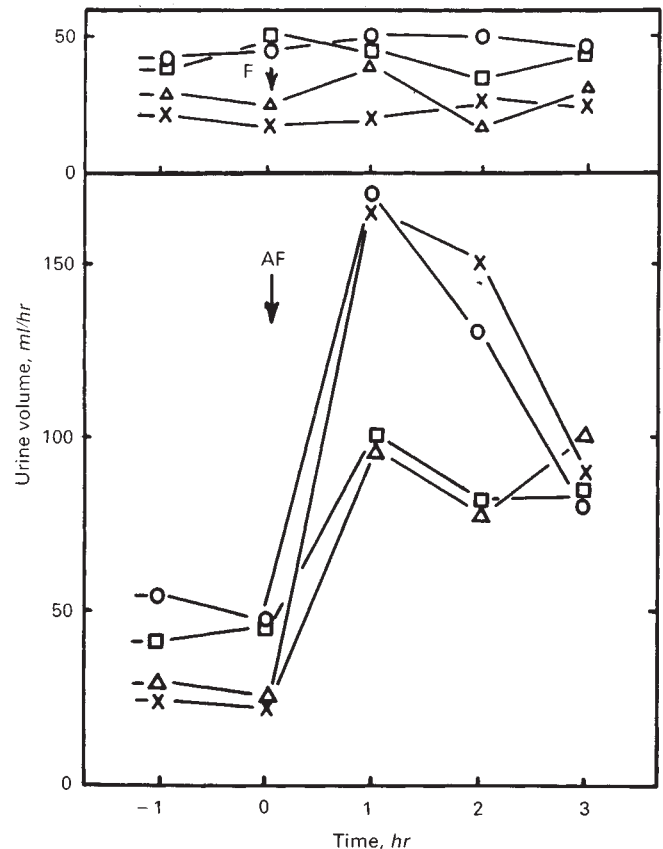
hypoalbuminemic patients. Table 2 summarizes the diuretic effect of furosemide and the furosemide–albumin complex on additional hypoalbuminemic patients who showed a marked resistance to furosemide. Most of these patients showed a marked increase in the rate of urine formation. The diuretic response of hypoalbuminemic patients to furosemide and its albumin complex thus mimiced that of analbuminemic rats.

Discussion

Analbuminemic mutant rats showed a marked "resistance" to furosemide. Intravenous administration of the furosemide–albumin complex, but not furosemide alone, markedly increased the rate of urine formation in NAR. These findings motivated us to test the effect of furosemide–albumin complex in hypoalbuminemic patients who showed a marked "resistance" to the diuretic. The present work reveals that the furosemide–albumin complex is also effective in promoting diuresis in the hypoalbuminemic patients who showed a marked "resistance" to the diuretic. Previous studies [6] revealed that the renal transtubular secretion of PSP, an amphipathic organic

Table 2. Effect of furosemide and furosemide–albumin complex on urine volume of hypoalbuminemic patients

Patient/age/sex/ diagnosis	Plasma protein Albumin/total g/dl plasma	Urine volume[a] ml/hr Furosemide alone	Furosemide + albumin
S.F. /74/f/chronic renal failure	1.6/5.3	24[b]	55[b]
F.N.[1]/52/m/liver cirrhosis	3.2/5.4	267[b]	397[b]
Y.Y. /74/m/brain hemorrage	2.2/5.2	110[b]	222[b]
T.T. /88/f/lung cancer	1.7/5.2	58[b]	178[b]
S.H. /37/f/amyloidosis	2.1/5.0	120[b]	260[b]
F.N.[1]/52/m/liver cirrhosis	3.2/5.4	330[c]	467[b]
I.K.[2]/70/m/chronic renal failure	2.3/5.0	60[c]	305[c]
I.K.[2]/70/m/chronic renal failure	2.2/4.8	25[c]	135[c]
K.S.[3]/47/f/cholangio-carcinoma	1.0/4.7	50[c]	130[c]
K.S.[3]/47/f/cholangio-carcinoma	2.0/4.9	50[c]	128[c]
G.M. /81/m/brain tumor	1.9/4.4	50[d]	100[c]
S.K. /78/m/pulmonary edema	2.2/4.6	25[e]	100[c]
F.N. /65/m/liver cirrhosis	1.5/4.3	183[d]	291[d]
O.T. /48/f/ovalian cancer	2.7/7.0	61[d]	150[d]
S.Y. /73/m/Japanese encepharitis	3.5/6.5	0[e]	0[b]
K.K. /77/m/malignant lymphoma	2.4/5.4	0[d]	5[b]

One to three experiments were performed with the same patients at different days. A different dose of furosemide (20[b], 30[c], 40[d], and 60[e] mg/patient) was intravenously administered with or without equimolar albumin.

anion that binds to albumin with high affinity, is lower in NAR than in normal rats due to random distribution of the ligand to extrarenal tissues in NAR. Administration of PSP with equimolar albumin resulted in marked increase in the urinary secretion of the dye, suggesting that binding to circulating albumin may enhance the vectorial transport of ligand from plasma into urine by preventing random distribution of PSP among extrarenal tissues. This argument would hold true for the present finding that the administration of furosemide with equimolar albumin increased the urinary excretion of this diuretic in NAR (Table 1). Since NAR shows no remarkable pathology and retains normal function of liver and kidney [13], the resistance to furosemide could be primarily explained by the decreased accumulation of the drug in the kidney, the site for its diuretic action. Pretreatment of NAR with the same dose of albumin as that used in the experiments in Figure 6 did not increase the rate of urine formation. Assuming 36 ml/kg of body weight for the plasma volume of NAR, an initial concentration of the circulating albumin would be about 0.04 mM immediately after intravenous administration of 0.1 g of albumin per kg of body weight. Since a large fraction of furosemide remains unbound at this concentration of plasma albumin [8], it is not surprising that pretreatment of NAR with such a small dose of albumin did not elicit the diuretic action of furosemide. Similarly, treatment of the furosemide–resistant patients with a small dose of albumin had no effect on the diuretic response to furosemide. The unbound fraction of furosemide markedly increases when plasma albumin level is lower than 2 g/dl [9]. Assuming the

plasma volume of 3 1/60 kg of body weight, administration of 4 to 6 g albumin increased the plasma concentration only by 0.13 to 0.2 g/dl. Such a small increase in plasma albumin level did not significantly contribute to the protein binding of the injected furosemide. High concentration of both a ligand and its binding protein favor the complex formation. Thus, when injected bound to albumin, a large fraction of furosemide circulates bound to albumin for a short period after the administration in NAR and hypoalbuminemic human subjects. During the initial passage through the kidney, a significant fraction of albumin–associated furosemide would be taken up by renal tubular cells and secreted into the luminal compartment to exhibit its diuretic action.

Although administration of furosemide–albumin complex significantly decreased the total plasma clearance and distribution volume of furosemide in NAR (Table 1 and Fig. 5), they did not return to the values in normal rats. Albumin samples used in the present experiments were the fraction V of rat albumin, which are known to retain a significant amount of free fatty acids [15]. It has been known that free fatty acids and other amphipathic anions, such as furosemide, compete the binding site on albumin and the binding capacity of the fraction V albumin for these anionic ligands is lower than that of the defatted albumin [12] and albumin in non-heparinized plasma [11]. Preliminary experiments in NAR revealed that the rate of furosemide disappearance was slower when the diuretic was injected with defatted albumin than with fraction V albumin. Furthermore, the furosemide–albumin complex was diluted about 20-fold immediately after its administration, which might affect he equilibrium binding of the ligand and increase the unbound fraction of the diuretic. These factors might explain, at least in part, why the pharmacokinetic parameters in NAR did not return to those in normal rats even if furosemide was injected bound to albumin. That the diuretic action of the complex in NAR was transient and its duration was shorter than that of furosemide in normal animals is consistent with this notion.

The furosemide–albumin complex effectively increased the urine volume in 14 of 16 hypoalbuminemic patients but did not promote diuresis in two hypoalbuminemic subjects (S.Y. and K.K. in Table 2). Renal function and general health of both patients were very poor; blood pressure was 40 to 60 mm Hg and anuria continued for two to three days before treatment with the complex. Thus, the furosemide–albumin complex appears to exhibit a desired therapeutic effect in hypoalbuminemic patients whose general conditions and renal transport functions still bring the diuretic in sufficient amounts to the site of its action and to respond to its diuretic action.

Although the efficient diuresis induced by furosemide–albumin complex in NAR and hypoalbuminemic patients could be explained on the similar basis as described above, the present experiments in hypoalbuminemic patients are rather preliminary. More detailed studies in human subjects should be performed under well controlled experimental conditions to understand the full scope of the mechanism for furosemide resistance in hypoalbuminemic patients.

Huge amounts of human albumin have frequently been used for therapeutic purposes to complement the decreased serum albumin in many hypoalbuminemic patients. Since purified human albumin preparations are very expensive and their sources are highly limited, the minimal use of albumin as a

biovehicle for a diuretic as designed in the present study should be strongly recommended for correcting the retention of extracellular fluids and improving the relevant complications in these patients. The present study may also open the possibility that many other drugs that bind strongly to albumin could excert their pharmacological actions in a combined use of albumin as a biovehicle protein in hypoalbuminemic human subjects.

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