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# The influence of moderate hypoalbuminaemia on the renal metabolism and dynamics of furosemide in the rabbit

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**1** The present study aimed to investigate the influence of hypoalbuminaemia on the pharmacokinetics and pharmacodynamics of furosemide. Hypoalbuminaemia was produced by repeated plasmapheresis, to attain plasma albumin concentrations of $21.6 \pm 0.9$ g $l^{-1}$, compared with $33.0 \pm 0.6$ g $l^{-1}$ in controls ($P<0.001$). The per cent of bound furosemide in hypoalbuminaemic rabbits ($90.8 \pm 0.7\%$) was lower than that in control rabbits ($97.4 \pm 0.5\%$, $P<0.001$). The kinetics of intravenous furosemide (2.5 mg $kg^{-1}$) were studied in control ($n=6$) and hypoalbuminaemic rabbits ($n=6$).

**2** To assess the effect of hypoalbuminaemia on extrarenal clearance of furosemide, functional anephria was induced by ligating the renal pedicles of 12 rabbits, which were segregated in two groups, with and without hypoalbuminaemia.

**3** In the control group, total, urinary and metabolic clearances of furosemide were $11.8 \pm 1.0$, $5.0 \pm 0.4$ and $6.8 \pm 0.6$ ml $min^{-1}$ $kg^{-1}$, respectively. Compared with control rabbits, in hypoalbuminaemic rabbits, total clearance of furosemide increased by 40% ($P<0.001$), result of the enhancement of furosemide metabolic clearance ($Cl_m$) from 5 to 10 ml $min^{-1}$ $kg^{-1}$ ($P<0.01$). In hypoalbuminaemic rabbits, urinary excretion of furosemide was reduced by 26% ($2451 \pm 115$ vs $1818 \pm 134$ $\mu g$ $h^{-1}$, $P<0.01$). In anephric rabbits, furosemide total clearance was $1.77 \pm 0.12$ ml $min^{-1}$ $kg^{-1}$, value not affected by hypoalbuminaemia, confirming that the increase in $Cl_m$ induced by hypoalbuminaemia occurs in the kidneys.

**4** Compared with controls, in hypoalbuminaemic rabbits, the rate of urinary excretion ($142 \pm 9$ vs $74 \pm 8$ ml $h^{-1}$, $P<0.001$) and the rate of excretion of sodium ($18.6 \pm 0.9$ vs $9.9 \pm 0.9$ mmol $h^{-1}$, $P<0.001$) were very much reduced. However, the dose-response curves were not different.

**5** In conclusion, hypoalbuminaemia is associated with an increase in renal metabolic clearance of furosemide, possibly because of the increase in furosemide unbound concentration, and a decrease in the diuretic/natriuretic effect of furosemide, secondary to a reduction in furosemide tubular secretion. Thus, albumin facilitates the renal secretion of organic anions but not their metabolism.

**Keywords:** Albumin; furosemide; metabolism; nephrectomy; hypoalbuminaemia

## Introduction

In the systemic circulation, most organic anions are bound to plasma albumin, and although it is generally admitted that only the unbound drug will be able to penetrate into the cells, the role of albumin as a modulator of the access of drug into the cell remains controversial. Similarly, the repercussions of hypoalbuminaemia on the pharmacokinetics and pharmacodynamics of drugs are not fully characterized.

Compared to normal rats, in mutant analbuminaemic rats, bile excretion of sulphobromophthalein (BSP) is reduced by 30% (Inoue *et al.*, 1985b). On the other hand, in analbuminaemic rats, the renal secretion of furosemide, phenolsulphophthalein and mercapturic acid is diminished, while their volume of distribution is increased (Inoue *et al.*, 1985a; 1987; Okajima *et al.*, 1985). These studies suggest that albumin serves as a vector for the transport of drugs to the organs and also, albumin prevents their random distribution (Inoue *et al.*, 1987). In analbuminaemic rats, the systemic clearance of the different organic anions studied is markedly increased, even though their renal or biliary excretion is reduced. Similar changes in the pharmacokinetics of furosemide were described in patients with severe hypoalbuminaemia associated with nephrotic syndrome (Keller *et al.*, 1982; Smith *et al.*, 1985).

In *in vitro* studies, with isolated perfused kidneys or proximal tubules, it has been shown that albumin favours the secretion of organic anions such as paraaminohyppuric acid and methotrexate but not cations (Depner *et al.*, 1984; Besseghir *et al.*, 1989). Thus, beside a carrier role, it has been proposed that albumin could also facilitate the cellular uptake and renal secretion of organic anions (Besseghir *et al.*, 1989).

Furosemide is rapidly eliminated by renal extraction, via the tubular organic anion system transport, and by biotransformation to approximately the same extent (Hammarlund-Udenaes & Benet, 1989; Boles Ponto & Schoenwald, 1990a,b). In the rabbit, the kidneys are the major site of furosemide metabolism, being responsible for at least 75% of its metabolism (Pichette & du Souich, 1996). However, the organ(s) where albuminaemia increases furosemide metabolism (Inoue *et al.*, 1987) has not been defined.

It has been suggested that resistance to loop diuretics, especially to furosemide, occurs more frequently in patients with severe hypoalbuminaemia (Inoue *et al.*, 1987). The mechanism leading to a decrease in response might be related to the reduction in urinary excretion of the diuretic (Inoue *et al.*, 1987), or to pharmacodynamic alterations. This question has not been addressed and, moreover, the effect of moderate hypoalbuminaemia on the response to a diuretic has not been documented. Since moderate hypoalbuminaemia is of frequent presentation among patients, it was of interest to determine *in vivo*, in the rabbit, the effects of moderate hypoalbuminaemia on the pharmacokinetics, including renal and extrarenal metabolism, and on the pharmacodynamics of furosemide.

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## Methods

### *Experimental model*

Male New Zealand rabbits (Ferme Cunicole, Les Lapins Léonard, Mirabel, Canada) weighing 2.2 to 2.8 kg were individually housed in ventilated metabolic cages and maintained on Purina Pellets and water *ad libitum*. An acclimatization of at least 7 days was allowed for the animals before any experimental work was undertaken. All the experiments were conducted in accord with the Canadian Council on Animal Care guidelines for care and use of laboratory animals.

Hypoalbuminaemia was produced by plasmapheresis. Blood (10 ml $kg^{-1}$) was withdrawn from a central ear artery and centrifuged at 2500 r.p.m. Plasma was discarded and was replaced volume per volume by sterile Lactate Ringer (Abbot Laboratories, Montréal, Québec, Canada) and both the red cells and Lactate Ringer were reinfused. Five exchanges daily for two days were done. Pharmacokinetic studies were performed on the third day.

Rabbits were fasted for at least 12 h before surgery. A lateral vein of an ear was cannulated with a Butterfly-25 (Venisystem, Abbot Ireland, Sligo, Ireland) for the infusion of 0.9% NaCl, at a rate of 30 ml $h^{-1}$ to compensate for losses of water and blood sampling. Urinary losses following the administration of furosemide were also replaced with a solution of 0.9% NaCl. The lateral vein of the opposite ear was also cannulated with a Butterfly-25 for furosemide and pentobarbitone administration. Anaesthesia was induced by injecting 30 mg $kg^{-1}$ sodium pentobarbitone, the trachea was exposed, and an endotracheal tube (CDMV, ST-Hyacinthe, Québec, Canada) was inserted between the fourth and fifth tracheal rings, caudally to the thyroid cartilage, for artificial ventilation (21 ml per cycle, 48 cycles $min^{-1}$) (Harvard Apparatus, Boston, MA). The right femoral artery was dissected, and a polyethylene tube (P-60, Intramedic, Becton, Dickinson and Co., Parsippany, NJ) was inserted into the abdominal aorta above the renal arteries, for blood sampling and arterial blood pressure measurement. Finally, a vesical catheter (Bardex Foley 8 Ch/Fr, Mississauga, Ontario, Canada) was installed to collect urine.

Once the rabbits were anaesthetized, the abdomen of all rabbits was opened by a midline incision to have access to the kidneys by clearing the surrounding tissues. Functional anephria was produced by ligating both renal pedicles. The surgical procedure was completed in less than 20 min.

Throughout the experiment, pH, $PaO_2$, and $PaCO_2$ were measured in arterial blood samples with an automated and computerized 1312 pH/oxygen analyzer (Instrumentation Laboratory, Lexington, MA), and arterial blood pressure was monitored via a three-way stopcock (Seamless, Division of Professional Medical Products, Inc., Ocala, Florida) connected to a pressure transducer (E and M Instruments, Houston, TX) and a physiograph (E and M Instruments).

### *Experimental protocol*

Four groups of 6 anaesthetized rabbits each were used. Rabbits of the first group were control, those of the second group were functionally anephric, and the rabbits of the third and fourth groups presented a hypoalbuminaemia without and with a functional nephrectomy, respectively. All the rabbits received furosemide 2.5 mg $kg^{-1}$, i.v. This dose was based upon the fact that the kinetics of furosemide injected intravenously are first order up to doses of 10 mg $kg^{-1}$ (Homsy *et al.*, 1995).

Glomerular filtration rate was assessed in groups 1 and 3 by measuring the clearance of inulin. Rabbits received an intravenous dose of 20 mg $kg^{-1}$ inulin, followed by an infusion at the rate of 1 mg $min^{-1}$. After an equilibration period of at least 90 min, blood (0.75 ml) was withdrawn at 0, 20, 30, 40 and 50 min and inulin was assayed in plasma by spectrophotometry (Schreiner, 1950).

In all rabbits, immediately after the sham laparotomy or the functional nephrectomy, furosemide was injected in 1 min. In groups 1 and 3, blood samples (1.0 ml) were withdrawn at 0, 6, 9, 12, 15, 20, 25, 30, 35, 40, 50 and 60 min. In groups 2 and 4 (anephric rabbits), blood samples were withdrawn at 0, 6, 10, 15, 20, 25, 30, 45, 60, 90, 120 and 150 min. In addition, from each rabbit at 3 min, 3 ml of blood were withdrawn to assess furosemide protein binding. Urine was collected from control and hypoalbuminaemic rabbits for 60 min. Plasma and urine was stored at −20°C in tubes protected from light until furosemide was assayed. Furosemide in plasma and urine was assayed by high performance liquid chromatography as described previously (Lambert *et al.*, 1982).

In the samples of blood withdrawn at 3 min, plasma protein binding of furosemide was assessed by use of the ultrafiltration method. The 3 min blood sample was selected since furosemide plasma concentrations were at their highest value, and because the experimental procedure (anaesthesia and surgery) does not modify furosemide plasma protein binding (Pichette & du Souich, 1996). Plasma (1.0 ml) was centrifuged at 3500 r.p.m. in Centrifree System devices (Amicon, W.R. Grace & Co., Beverly, MA) for 30 min at −25°C. The concentration of unbound furosemide was assayed in 250 μl of the resulting ultrafiltrate.

Plasma and urinary sodium, urea, creatinine and albumin were determined with a Hitachi 717 analyzer (Boehringer Mannheim Canada, Laval, Québec, Canada). Urinary sodium was assayed with an automatic flame photometer (Model 11943, Instrumentation Laboratory Inc., Lexington, MA, U.S.A.). Urinary pH was measured in each urine collection with an Acumet model 230 pH/ion meter (Fisher Scientific Ltd, Fairlawn, NJ).

To assess the effect of hypoalbuminaemia on the dynamics of furosemide, 12 anaesthetized rabbits, 6 controls and 6 with hypoalbuminaemia, received 20 mg $kg^{-1}$ i.v. of inulin, followed by an infusion at the rate of 1 mg $min^{-1}$. After an equilibration period of at least 90 min, furosemide (5 mg $kg^{-1}$) was injected i.v. in 1 min, and blood (1 ml) was withdrawn at 0, 9, 19, 29, 39, and 49 min to assay inulin. At 3 min, 3 ml of blood were withdrawn to assess furosemide binding to plasma proteins. Urine was collected from control and hypoalbuminaemic rabbits at the following intervals: 0–10, 10–20, 20–30, 30–40, 40–50 and 50–60 min. Furosemide, inulin and biochemical parameters were determined as above. The 5 mg $kg^{-1}$ dose of furosemide was selected because the response elicited was close to the predicted maximal effect.

### *Drugs used*

Furosemide was purchased from Sabex (Montréal, Québec, Canada). Methyl esther of furosemide was donated by Hoechst-Roussel Canada. Inulin was obtained from Sigma Chemical Company (St. Louis, MO).

### *Data analysis*

Furosemide kinetic parameters were estimated assuming non-compartmental kinetics. The area under the curve of furosemide plasma concentrations as a function of time ($AUC_{0-60}$ or $AUC_{0-150}$) was estimated by means of the trapezoidal method. Total clearance ($Cl_t$), terminal half-life ($t_{1/2}$), and predicted volume of distribution at steady state ($Vd_{ss}$) of furosemide were calculated as described by Gibaldi & Perrier (1982). The urinary clearance of furosemide ($Cl_u$) was calculated from the following equation: $Cl_u = Xu_{0-t}/AUC_{0-t}$. Where $Xu_{0-t}$ is the amount of furosemide excreted unchanged in the urine during the experiment. Furosemide metabolic clearance ($Cl_m$) was estimated by subtracting $Cl_u$ from $Cl_t$. The metabolic clearance of furosemide is the sum of the metabolism of furosemide in the kidneys, i.e. renal metabolic clearance ($Cl_{rm}$), and the metabolism of furosemide in organs other than the kidneys, i.e. extrarenal metabolic clearance ($Cl_{erm}$). The $Cl_{erm}$

corresponds to furosemide total clearance in rabbits with a functional nephrectomy. Average $Cl_{rm}$ was calculated by subtracting the mean $Cl_{erm}$ of rabbits with a functional nephrectomy from mean $Cl_m$ of control rabbits. The rate of glomerular filtration was assumed to be equal to the clearance of inulin, which was calculated as follows: Cl = insulin infusion rate/steady state plasma concentration. Fractional excretion of sodium (FeNa) was estimated by the ratio of the sodium excreted over the sodium filtered. Pharmacodynamic parameters ($E_{max}$ and $EC_{50}$) were calculated according to the $E_{max}$ model by use of a PCNONLIN least squares nonlinear regression analysis programme (Holford & Sheiner, 1981).

### *Stastistical tests*

The results are expressed as mean ± s.e.mean. Differences between groups were assessed by using an unpaired Student's *t* test or an ANOVA test. The threshold of significance was $P<0.05$.

## Results

### *Haemodynamic and biochemical parameters*

In the groups of rabbits used to assess the effect of hypoalbuminaemia on furosemide kinetics and dynamics, plasmapheresis resulted in the reduction in plasma albumin of the order of 35% (Tables 1 and 3) with no modification in other biochemical parameters. Compared with control rabbits, hypoalbuminaemia did not affect the glomerular filtration rate (Table 1) nor the mean arterial pressure.

Following anaesthesia and surgical manipulations, mean arterial pressure in rabbits with hypoalbuminaemia was similar to that measured in control rabbits, i.e. 66 ± 4 vs 68 ± 3 mmHg, respectively. After the injection of furosemide there was a small drop in blood pressure (≈5 mmHg), which remained constant during the experiment. At the end of the experiments, mean blood pressure was 61 ± 2 mmHg in control, and 59 ± 3 mmHg in hypoalbuminaemic rabbits. Functional nephrectomy did not modify arterial blood pressure in either control or hypoalbuminaemic rabbits.

### *Furosemide pharmacokinetics in control and hypoalbuminaemic rabbits*

In hypoalbuminaemic rabbits, mean plasma concentrations of furosemide were consistently lower than those measured in control rabbits (Figure 1). As a consequence, the $AUC_{0-60}$ of furosemide in hypoalbuminaemic rabbits was 33% smaller ($P<0.05$) than in control animals (Table 2).

In the control group, total clearance of furosemide was 11.8 ± 0.9 ml $min^{-1}$ $kg^{-1}$, of which 42% corresponded to urinary and 58% to metabolic clearance (Table 2, Figure 2). In hypoalbuminaemic rabbits, total clearance of furosemide was 35% greater than in control animals ($P<0.05$), secondary to an increase in furosemide metabolic clearance ($P<0.01$) (Table 2). On the other hand, compared with control rabbits, hypoalbuminaemia reduced the urinary recovery at furosemide (Table 2). In hypoalbuminaemic rabbits, furosemide volume of distribution was greater than in control animals ($P<0.05$).

In plasma of control rabbits, unbound furosemide per cent was 2.6%. Hypoalbuminaemia increased the unbound per cent of furosemide to 9.2% (Table 2). The urinary excretion of furosemide and the metabolic clearance were positively ($r=0.78$, $P<0.005$) and negatively ($r=-0.79$, $P<0.005$) correlated, respectively, with plasma albumin levels. Urinary excretion and metabolic clearance of furosemide were also correlated with the unbound per cent of furosemide.

### *Furosemide pharmacokinetics after functional nephrectomy in control and hypoalbuminaemic rabbits*

By comparison with control rabbits, functional nephrectomy decreased the total clearance of furosemide by 85% ($P<0.001$), secondary to the abolition of furosemide renal excretion and to the reduction in its metabolic clearance ($P<0.001$) (Tables 2 and 3). In addition, compared with rabbits with normal renal function (Table 2), functional nephrectomy (Table 3) reduced furosemide volume of

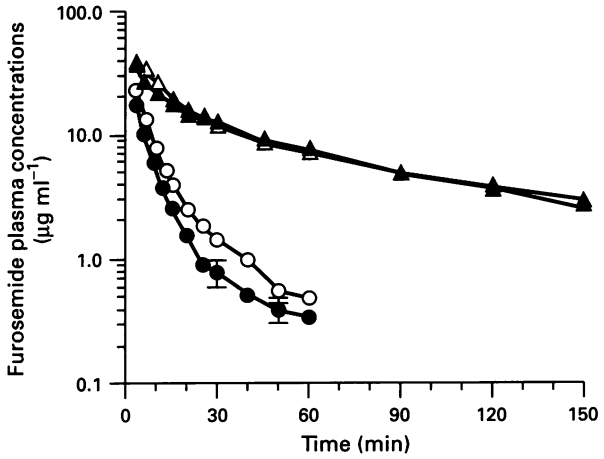


**Figure 1** Mean plasma concentrations of furosemide as a function of time following the i.v. administration of 2.5 mg $kg^{-1}$ of the diuretic to control rabbits without (○) and with (●) hypoalbuminaemia, and functionally anephric rabbits without (△) and with (▲) hypoalbuminaemia. Vertical lines show s.e.mean.

**Table 1** Biochemical renal function parameters in control and hypoalbuminaemic rabbits

| | *Control* (n=6) | *Hypo-albuminaemia* (n=6) |
|---|---|---|
| Plasma albumin (g $l^{-1}$) | 33.0 ± 0.6 | 21.6 ± 0.9[a] |
| Creatinine (μmol $l^{-1}$) | 92.7 ± 4.6 | 86.2 ± 4.8 |
| Urea (mmol $l^{-1}$) | 5.8 ± 0.6 | 5.0 ± 0.6 |
| Plasma sodium (mmol $l^{-1}$) | 142 ± 0.9 | 145 ± 1.8 |
| GFR (ml $min^{-1}$ $kg^{-1}$) | 5.4 ± 0.5 | 4.7 ± 0.5 |
| Urinary pH | 8.1 ± 0.1 | 7.8 ± 0.4 |

Values are means ± s.e. [a]$P<0.001$ compared with control rabbits.

**Table 2** Pharmacokinetic parameters of furosemide (2.5 mg $kg^{-1}$, i.v.) in control and hypoalbuminaemia rabbits

| | *Control* (n=6) | *Hypo-albuminemia* (n=6) |
|---|---|---|
| Bound drug (%) | 97.4 ± 0.5 | 90.8 ± 0.7[b] |
| $t_{½}$ (min) | 14.2 ± 1.2 | 11.7 ± 1.1 |
| $C_{max}$ (μg $ml^{-1}$) | 22.7 ± 1.3 | 17.1 ± 1.6[a] |
| $AUC_{0-60}$ (μg min $ml^{-1}$) | 210 ± 17 | 141 ± 15[a] |
| $Vd_{ss}$ (ml $kg^{-1}$) | 170 ± 7 | 235 ± 33[a] |
| $Cl_t$ (ml $min^{-1}$ $kg^{-1}$) | 11.8 ± 0.9 | 18.0 ± 1.9[a] |
| $Cl_u$ (ml $min^{-1}$ $kg^{-1}$) | 5.0 ± 0.4 | 6.0 ± 0.7 |
| $Cl_m$ (ml $min^{-1}$ $kg^{-1}$) | 6.8 ± 0.6 | 11.9 ± 1.3[b] |
| % urinary recovery | 39.6 ± 2.3 | 31.9 ± 2.3[a] |

Values are means ± s.e. [a]$P<0.05$; [b]$P<0.01$ compared with control rabbits. $t_{½}$ is furosemide terminal half-life, $C_{max}$ is maximal furosemide plasma concentration; $AUC_{0-60}$ is the area under furosemide plasma concentrations curve-time from time 0 to 60 min; $Vd_{ss}$ is furosemide apparent volume of distribution; $Cl_t$, $Cl_u$ and $Cl_m$ are furosemide total, urinary and metabolic clearances.

distribution by 23% and 43% in rabbits without or with hypoalbuminaemia, respectively, on the other hand, the increase in furosemide volume of distribution induced by hypoalbuminaemia was precluded by the nephrectomy.

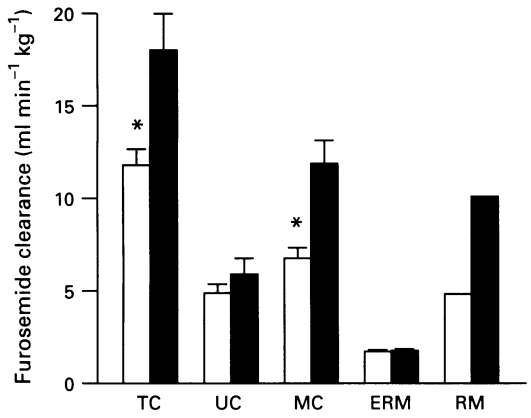


**Figure 2** Average values of total clearance (TC), urinary clearance (UC) and metabolic clearance (MC) of furosemide calculated in control rabbits with normal renal function, without (open columns), and with hypoalbuminaemia (solid columns). Mean extrarenal metabolic clearance (ERM) estimated in functionally anephric rabbits without (open column), and with hypoalbuminaemia (solid column); and renal metabolic clearance (RM) in rabbits without (open column), and with hypoalbuminaemia (solid column) was calculated by subtracting mean values of ERM from mean values of MC. Vertical lines show s.e.mean. *$P<0.05$ compared with the hypoalbuminaemic rabbits.

Functional nephrectomy reduced the slope of the decline of furosemide plasma concentrations in both control and hypoalbuminaemic rabbits to a similar extent (Figure 1). In rabbits with functional nephrectomy, hypoalbuminaemia did not modify furosemide pharmacokinetic parameters, i.e. mean values of total clearance and volume distribution of furosemide were identical to those estimated in anephric rabbits (Table 3).

### *Furosemide pharmacodynamics in control and hypoalbuminaemic rabbits*

In hypoalbuminaemic rabbits receiving 5 mg kg$^{-1}$ furosemide, plasma albumin was lower than in control rabbits, i.e. $23.6\pm0.5$ vs $32.4\pm0.4$ g l$^{-1}$ ($P<0.05$), as was furosemide plasma protein binding, i.e. $93.8\pm1.5$ vs $98.6\pm0.1\%$ ($P<0.05$).

Compared with control rabbits, after the administration of furosemide 2.5 mg kg$^{-1}$, i.v. to rabbits with hypoalbuminaemia, the rate of sodium excretion in urine was reduced by almost 50% (Table 4). As a consequence, the volume of urine excreted diminished in the same proportion. The fractional excretion of sodium was also decreased by 34% ($P<0.05$). Doubling the dose of furosemide (5 mg kg$^{-1}$) did not increase the natriuretic and diuretic effect of furosemide in control rabbits, although in hypoalbuminaemic rabbits, the 5 mg kg$^{-1}$ dose of furosemide succeeded to increase both its natriuretic and diuretic effect (Table 4). However, in hypoalbuminaemic rabbits, sodium excretion was still 25% lower than in control animals.

The graphical representation of the rate of urinary excretion of sodium, as a function of the rate of urinary excretion of furosemide of control rabbits (Figure 3) showed that the

**Table 3** Pharmacokinetic parameters of furosemide (2.5 mg kg$^{-1}$, i.v.) after functional nephrectomy in control and hypoalbuminaemic rabbits

| | *Anephric* (n=6) | *Anephric + hypoalbuminaemia* (n=6) |
|---|---|---|
| Plasma albumin (g l$^{-1}$) | 32.2±0.5 | 22.8±1.0[a] |
| Bound drug (%) | 97.7±0.6 | 89±1.5[a] |
| $t_{½}$ (min) | 56.6±2.2 | 56.9±2.8 |
| $C_{max}$ (μg ml$^{-1}$) | 36.0±2.5 | 35.9±2.8 |
| $AUC_{0-150}$ (μg min ml$^{-1}$) | 1223±62 | 1291±113 |
| $Vd_{ss}$ (ml kg$^{-1}$) | 130±8 | 134±13 |
| $Cl_t$ (ml min$^{-1}$ kg$^{-1}$) | 1.77±0.1 | 1.82±0.1 |

Values are means±s.e. [a]$P<0.001$ compared with control rabbits. $t_{½}$ is furosemide terminal half-life; $C_{max}$ is maximal furosemide plasma concentration; $AUC_{0-150}$ is the area under the furosemide plasma concentrations curve-time 0 to 150 min; $Vd_{ss}$ is furosemide apparent volume of distribution; $Cl_t$ is furosemide total clearance.

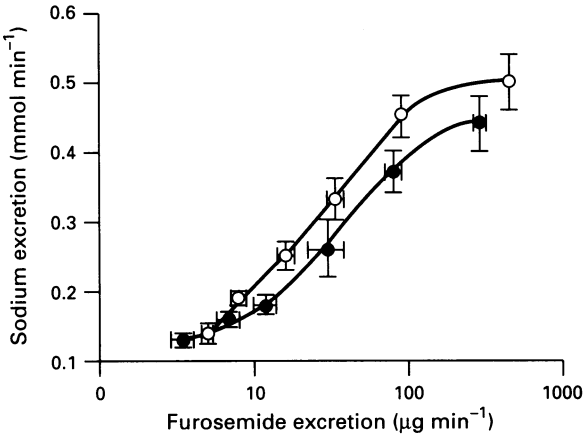


**Figure 3** Rate of urinary sodium excretion as a function of the rate of urinary furosemide excretion rate in control (○) and hypoalbuminaemic (●) rabbits following the i.v. administration of furosemide 5 mg kg$^{-1}$. Vertical and horizontal lines show s.e.mean.

**Table 4** Pharmacodynamic parameters of furosemide (2.5 and 5 mg kg$^{-1}$, i.v.) in control and hypoalbuminaemic rabbits

| | *Furosemide* (2.5 mg kg$^{-1}$) | | *Furosemide* (5.0 mg kg$^{-1}$) | |
|---|---|---|---|---|
| | *Control* (n=6) | *Hypoalbuminaemic* (n=6) | *Control* (n=6) | *Hypoalbuminaemic* (n=6) |
| $X_u$ (μg h$^{-1}$) | 2451±115 | 1818±134* | 5965±277 | 4288±450**¶ |
| Sodium excretion (mmol h$^{-1}$) | 18.6±1.0 | 9.9±1.0* | 18.7±1.0 | 14.5±0.8**¶ |
| Urinary volume (ml h$^{-1}$) | 142±9 | 74±8* | 137±9 | 105±8**¶ |
| FeNa (%) | 17.3±2.0 | 11.4±2.0* | 21.3±2.0 | 15.6±1.5**¶ |

Values are means±s.e. *$P<0.05$ compared with control (2.5 mg kg$^{-1}$); **$P<0.05$ compared with control rabbits (5 mg kg$^{-1}$); ¶$P<0.05$ compared with hypoalbuminaemic rabbits (2.5 mg kg$^{-1}$). $X_u$ is the amount of furosemide excreted in urine. FeNa is the fractional excretion of sodium.

maximal effect was practically attained at the dose of 5 mg $kg^{-1}$ of furosemide. The predicted maximal rate of urinary excretion of sodium induced by furosemide ($E_{max}$) was $0.52 \pm 0.04$ mmol $min^{-1}$, and the rate of urinary excretion of furosemide eliciting a natriuresis half the $E_{max}$ ($ED_{50}$) was $15.3 \pm 2.6$ $\mu g$ $min^{-1}$. Hypoalbuminaemia induced a slight rightwards and downwards shift displacement of the dose-response curve (Figure 3). However, the estimated $E_{max}$ ($0.49 \pm 0.04$ mmol $min^{-1}$) and $ED_{50}$ ($22.1 \pm 8.3$ $\mu g$ $min^{-1}$) did not differ ($P > 0.05$) from the values estimated in control rabbits.

## Discussion

The volume of distribution and total clearance of furosemide are greater in analbuminaemic than in control rats, and on the other hand, the renal excretion of furosemide is slower in analbuminaemic rats (Inoue *et al.*, 1987). In the present study, moderate hypoalbuminaemia, i.e. a decrease in serum albumin by 35%, induced very similar changes in the kinetics of furosemide. The volume of distribution of furosemide was increased by 28%, although this increase was prevented in rabbits with functional nephrectomy. These observations suggest that the hypoalbuminaemia-induced expansion of furosemide volume of distribution does not result from a random distribution in extra-renal organs (Inoue *et al.*, 1987), but rather from the accumulation of the diuretic in the kidneys.

The results of the present study confirm that the kidneys account for nearly 85% of the total clearance of furosemide, either via excretion or via biotransformation (Pichette & du Souich, 1996). Moderate hypoalbuminaemia induced an increase in total clearance of furosemide secondary to a rise in its metabolic clearance. Functional nephrectomy reduced furosemide total clearance to the same extent in both control and hypoalbuminaemic rabbits, indicating that hypoalbuminaemia does not elicit any effect on the extrarenal metabolism of furosemide. Therefore, the enhancement of furosemide metabolic clearance in hypoalbuminaemia occurs in the kidneys.

In hypoalbuminaemic rabbits there was a 21% reduction in the renal excretion of furosemide. Theoretically, the rate of glomerular filtration of furosemide is modulated primarily by furosemide binding to plasma proteins; since, in hypoalbuminaemic rabbits the rate of glomerular filtration was not affected, and furosemide unbound fraction was increased, we can exclude that a decrease in furosemide glomerular filtration is at the origin of the reduction in renal excretion of furosemide. An increase in furosemide tubular reabsorption may also decrease its renal excretion. However, furosemide tubular reabsorption is pH dependent (Green & Mirkin, 1981), and hypoalbuminaemia did not affect urinary pH (Table 1). Therefore, hypoalbuminaemia decreased the renal excretion of furosemide by reducing the proximal tubular secretion of furosemide (Odlind, 1979; van Ginneken & Russel, 1989).

Cellular entry of unbound furosemide into the epithelial cells of the proximal tubule by passive diffusion should be very limited, since with a $pK_a$ of 3.8, most of the furosemide is ionized at pH 7.4 in the blood. We have shown that high doses of probenecid, an inhibitor of the proximal anion transport system, reduce both the renal secretion and metabolism of furosemide to the same extent as does the functional nephrectomy (Homeida *et al.*, 1977; Sommers De *et al.*, 1991; Pichette & du Souich, 1996), indicating that passive diffusion into the tubular cell is negligible. Therefore, to be excreted in the urine or metabolized by the kidneys, furosemide must enter the proximal tubular cell via an anion carrier. In the present study, despite the fact that furosemide urinary excretion was reduced in hypoalbuminaemia, it is evident that furosemide could penetrate into the proximal tubular epithelial cell since furosemide renal metabolism, as well as its volume of distribution, were enhanced. These results indicate that binding of furosemide to albumin, or albumin *per se*, reduced furosemide renal metabolism, whereas it enhances its secretion.

The heterogeneity of proximal tubular cells concerning their morphology and functions is well documented (Grantham & Chonko, 1991). For instance, the secretion of *p*-aminohippurate (an organic anion) occurs predominantly in the S2 segment of the proximal tubule (Woodhall *et al.*, 1978). On the other hand, the S1 segment has a greater ability to conjugate drugs, i.e. to conjugate morphine (Schali & Roch-Ramel, 1982). Thus, the results of the present study could be explained on the basis that distinct organic anion carriers are present along the different segments of the proximal tubule. Since the cells of these segments differ in their function to handle organic substances (either metabolism or secretion), the basolateral carriers could be modulated differently by albumin. If this hypothesis holds true, organic anion carriers located in the S2 segment, where the ability to secrete drugs appears higher, will be facilitated by albumin, whereas, those located in the S1 segment, where the ability to conjugate drugs appears higher, will not. These functional differences can be explained assuming that the organic anion transporters have distinct affinity constants for furosemide. In such a way, that the efficiency of carriers with low affinity (primarily located in S1 segment and associated to metabolism) will increase whenever furosemide binding to plasma proteins decreases. Conversely, the efficiency of carriers with high affinity (primarily located in S2 segment and coupled to secretion) will increase when drug binding increases. Further studies are needed to understand fully the role of albumin in the renal excretion and metabolism of organic anions.

Besides pharmacokinetic modifications, hypoalbuminaemia also altered significantly the pharmacodynamics of furosemide. Urinary volume, sodium excretion rate, as well as fractional sodium excretion were all reduced in rabbits with hypoalbuminaemia, confirming previous results in analbuminaemic rats (Inoue *et al.*, 1987). Under the present experimental conditions, compared with control rabbits, hypoalbuminaemia did not modify significantly the dose-response curve of furosemide, suggesting that the tubule responds normally to furosemide reaching the active site. Therefore, we postulate that the mechanism underlying the hypoalbuminaemia-induced decrease in furosemide natriuresis and diuresis is primarily due to modification in the kinetics of the diuretic.

In conclusion, moderate hypoalbuminaemia modifies the kinetics of furosemide by producing an increase in its volume of distribution and renal metabolic clearance, and a decrease in its renal secretion. These results indicate that binding of furosemide to albumin, or albumin *per se*, promotes its urinary excretion although it is a limiting factor to its renal metabolism. In addition, moderate hypoalbuminaemia reduces the natriuresis and diuresis of furosemide, essentially due to the reduction in urinary furosemide excretion. The decrease in response may be partially overcome by increasing the dose of furosemide. Since the dual role of albumin appears to be a common phenomenon for several anions (Keller *et al.*, 1982; Inoue *et al.*, 1985a,b; Okajima *et al.*, 1985; Smith *et al.*, 1985), the results of the present study may be inferred to other xenobiotics. Furthermore, we are tempted to extrapolate the actual results to man, since the present changes of 35% in plasma albumin reflects a decrease from 45 g $l^{-1}$ to 30 g $l^{-1}$ in man, suggesting that moderate hypoalbuminaemia may be a cause of decreased response to diuretics, and may also alter significantly the kinetics of other organic anions.

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