ROLE OF PLASMA PROTEIN BINDING ON RENAL METABOLISM AND DYNAMICS OF FUROSEMIDE IN THE RABBIT

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ABSTRACT:

To investigate the influence of furosemide plasma protein binding on its kinetics and dynamics, the kinetics of furosemide was studied in the presence of a protein binding displacer, warfarin, and in hypoalbuminemic rabbits. Compared with controls, in anesthetized rabbits pretreated with warfarin, the unbound fraction of furosemide increased from 1.8 ± 0.4% to 7.0 ± 0.4% (p < .001), and its metabolic clearance increased by 30%, whereas furosemide urinary excretion decreased by 48% (p < .05). Experiments in nephrectomized rabbits showed that the increase in metabolic clearance was secondary to an increase in its renal metabolic clearance (p < .05). Compared with controls, in warfarin pretreated rabbits, sodium excretion and diuresis were decreased by 30% (p < .05). However, when furosemide was injected mixed with albumin, warfarin-induced kinetic and dynamic alterations of furosemide were reversed. Compared with control rabbits, in conscious hypoalbuminemic rabbits, furosemide unbound fraction was enhanced from 1.2 ± 0.1% to 5.5 ± 0.5% (p < .001), and its urinary excretion, diuresis, and sodium excretion were reduced by 22% (p < .05). The administration of warfarin to hypoalbuminemic rabbits further increased the fraction of unbound furosemide, and diminished its urinary excretion and diuretic effect. In conclusion, 1) binding of furosemide to plasma proteins, and not albumin per se, facilitates its renal secretion and pharmacological response; 2) the decrease in furosemide binding, secondary to drug displacement and/or hypoalbuminemia, can be a cause of resistance to the diuretic; and 3) when furosemide binding is decreased, the administration of furosemide mixed with albumin enhances its renal secretion and diuretic effect.

In mutant analbuminemic rats, the renal secretion of mercapturic acid, phenolsulphophthaleic acid, and furosemide is diminished, whereas their volume of distribution is increased (Okajima et al., 1985; Inoue et al., 1985, 1987), suggesting that albumin serves as a vector for the transport of drugs to the kidney (Inoue et al., 1987). On the other hand, according to results obtained with isolated perfused kidneys or proximal tubules, it has been proposed that albumin directly facilitates the cellular uptake and secretion of organic anions (Depner et al., 1984; Besseghir et al., 1989), promotion that appears unrelated to the extent of organic ion binding to albumin (Besseghir et al., 1989).

Furosemide is eliminated by renal excretion, proximal tubular secretion, and biotransformation to approximately the same extent (Hammarlund-Udenaes and Benet, 1989; Pinto and Schoenwald, 1990a,b). In the rabbit, the major site for the biotransformation of furosemide is the kidney (Pichette and du Souich, 1996). Moderate hypoalbuminemia is associated with a decrease in the renal proximal tubular secretion of furosemide and an increase in its renal metabolic clearance, suggesting that in vivo albumin or the binding to albumin facilitates the renal secretion of furosemide but limits its renal metabolism (Pichette et al., 1996).

The modifications in the pharmacokinetics of furosemide induced by hypoalbuminemia or analbuminemia are associated with significant alterations in the pharmacodynamics of furosemide, i.e., a reduction in the excretion of sodium and in the urinary volume (Inoue et al., 1987; Pichette et al., 1996). In analbuminemic rats, the decrease in the renal secretion of furosemide and in its natriuretic and diuretic effects is prevented when furosemide is administered mixed with albumin (Inoue et al., 1987), suggesting that the binding of furosemide to plasma proteins is the limiting factor and not albumin plasma levels as proposed by others (Depner et al., 1984; Besseghir et al., 1989).

To determine whether the changes in the pharmacokinetics and pharmacodynamics of furosemide induced by hypoalbuminemic conditions are associated with the binding of furosemide to albumin or to the concentration of albumin itself, the disposition and dynamics of furosemide have been studied in 1) control rabbits and rabbits pretreated with warfarin, a known displacer of furosemide from its binding sites to albumin; 2) functionally nephrectomized rabbits with and without pretreatment with warfarin; 3) rabbits pretreated with warfarin but receiving furosemide mixed with albumin; and 4) rabbits with moderate hypoalbuminemia with and without warfarin pretreatment.

Materials and Methods

Experimental Model. Male New Zealand rabbits (Ferme Cunicole, Mirebel, 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. The rabbits were segregated into eight groups. The rabbits in five groups were anesthetized, and in the remaining three groups, the rabbits were conscious. All the experiments were conducted...
according to the Canadian Council on Animal Care guidelines for care and use of laboratory animals.

**Effect of Warfarin on Furosemide Disposition and Dynamics in Anesthetized Rabbits.** Rabbits were fasted for at least 12 h before surgery. A lateral vein of an ear was cannulated with a Butterfly-25 (Venetronic; Abbot Ireland, Sligo, Ireland) for the infusion of 0.9% NaCl at the rate of 30 ml/h to compensate for the loss of water and allow for blood sampling. Urinary losses secondary to the injection of furosemide were replaced with a solution of 0.9% NaCl. Anesthesia was induced by injecting 30 mg/kg sodium pentobarbital through a cannula (Butterfly-25) inserted into the lateral vein of the opposite ear; the trachea was exposed, and an endotracheal tube (CDMV; ST-Hyacinthe, Quebec, Canada) was inserted between the fourth and fifth tracheal rings, caudally to the thyroid cartilage, for artificial ventilation (21 ml/cycle, 48 cycles/min) (Harvard Apparatus, Boston, MA). The right femoral artery was dissected, and a polyethylene tube (P-60; Intramedic, Becton Dickinson, 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 anesthetized, in sham and in rabbits with functional nephrectomy, the abdomen was opened by a midline incision to have access to the kidneys by clearing the surrounding tissues. Functional nephrectomy was produced by ligating both renal pedicles as described elsewhere (Pichette and du Souich, 1996). The surgical procedure was completed in less than 20 min. Throughout the experiment, pH, PaO₂, and PaCO₂ were measured in arterial blood samples with an automated, computerized 1312 pH and oxygen analyzer (Instrumentation Laboratory, Lexington, MA), and arterial blood pressure was monitored with an automated, computerized 1312 pH and oxygen analyzer (Instrumentation Laboratory, Lexington, MA).

Furosemide was injected through a cannula (Butterfly-25) inserted into a lateral vein of an ear and warfarin through another cannula inserted into the lateral vein of the opposite ear. A vesical catheter (Bardex Foley 8 Ch/Fr; Mississauga, Ontario, Canada) was installed to collect urine. Urinary losses induced by furosemide were replaced with 0.9% NaCl. At 3 min, 4 ml of blood was withdrawn at the rate of five exchanges daily for 2 days, a technique that does not induce changes in blood pressure or GFR (Pichette et al., 1996). Furosemide was injected through a cannula (Butterfly-25) inserted into a lateral vein of an ear and warfarin through another cannula inserted into the lateral vein of the opposite ear.

**Effect of Hypoalbuminemia and Warfarin on Furosemide Dynamics in Conscious Rabbits.** To determine whether a smaller dose of warfarin could displace furosemide from its binding sites to albumin in conscious hypoalbuminemic rabbits, the dynamics of furosemide (2.5 mg/kg) were studied in 12 conscious rabbits, 4 controls, 4 with moderate hypoalbuminemia, and 4 with severe hypoalbuminemia who were pretreated with warfarin (5 mg/kg).

Hypoalbuminemia was induced by repeated plasmapheresis of 10 ml/kg at a rate of five exchanges daily for 2 days, a technique that does not induce changes in blood pressure or GFR (Pichette et al., 1996). Furosemide was injected through a cannula (Butterfly-25) inserted into a lateral vein of an ear and warfarin through another cannula inserted into the lateral vein of the opposite ear. A vesical catheter (Bardex Foley 8 Ch/Fr; Mississauga, Ontario, Canada) was installed to collect urine. Urinary losses induced by furosemide were replaced with 0.9% NaCl. At 3 min, 4 ml of blood was withdrawn at the rate of five exchanges daily for 2 days, a technique that does not induce changes in blood pressure or GFR (Pichette et al., 1996). Furosemide was injected through a cannula (Butterfly-25) inserted into a lateral vein of an ear and warfarin through another cannula inserted into the lateral vein of the opposite ear.

**Data Analysis.** Furosemide terminal half-life (T½), area under its plasma concentration-time curve (AUC<sub>0–150</sub> or AUC<sub>0–t</sub>), systemic clearance, and predicted apparent volume of distribution at steady state were estimated according to noncompartmental analysis based on statistical moment theory (Gibaldi and Perrier, 1982) with the computer program Pharmacokinetic Data Analysis Program included in Lotus 1-2-3, Version 2.2 (Lotus Development Corporation, Cambridge, MD). The urinary clearance of furosemide (C<sub>u</sub>) was calculated with the following equation: C<sub>u</sub> = Xu<sub>u</sub> / AUC<sub>0–t</sub> where Xu<sub>u</sub> is the amount of furosemide excreted unchanged in the urine during the experiment. Furosemide metabolic clearance was estimated by subtracting C<sub>u</sub> from systemic clearance. In anesthetized rabbits, GFR was assumed to be equal to the clearance of inulin, which was calculated as follows: C<sub>IN</sub> = inulin infusion rate and steady-state plasma concentration. On the other hand, in conscious rabbits, GFR was estimated by dividing the urinary excretion rate of creatinine by creatinine plasma concentration. Fractional excretion of sodium (FeNa) was estimated by the ratio of the sodium excreted over the sodium filtered (GFR × sodium plasma concentration).

The results are expressed as mean ± S.E. Differences between groups were assessed with an unpaired Student’s t test or an analysis of variance test with Fisher’s correction for multiple comparisons. The threshold of significance was p < .05.
Anesthetized Rabbits. Hemodynamic and biochemical parameters. After anesthesia and surgical manipulations, mean arterial pressure was not affected by the administration of warfarin or albumin, i.e., 64 ± 2, 62 ± 4, and 66 ± 3 mm Hg in the control, warfarin, and albumin groups, respectively. After the injection of furosemide, there was a small drop in blood pressure (5 mm Hg) in each group, although thereafter blood pressure remained constant during the experiment. Functional nephrectomy did not modify arterial blood pressure in either control or warfarin pretreated rabbits.

Compared with baseline values of the rabbits, the coadministration of furosemide with human albumin (187.5 mg/kg) raised plasma albumin concentration from 33.7 ± 0.9 to 36.2 ± 1.0 g/liter (p < .05). However, the biochemical parameters (including albumin) and GFR did not differ between the groups (Table 1).

Furosemide kinetics in control and warfarin-pretreated rabbits with or without albumin coadministration. In rabbits pretreated with warfarin, mean plasma concentrations of furosemide administered alone or with albumin were essentially identical with those estimated in control rabbits (Fig. 1). However, peak plasma concentrations of furosemide were lower in the group treated with warfarin than in the other two groups (Table 2). In plasma of control rabbits, the unbound fraction of furosemide was 1.8%, and pretreatment with warfarin increased the unbound fraction of furosemide to 7% (p < .05). The administration of furosemide mixed with albumin restored the unbound fraction to control values (Table 2). The furosemide apparent volume of distribution tended to increase (p > .05) in rabbits being pretreated with warfarin.

In the control group, systemic clearance of furosemide was 11.2 ± 0.8 ml-min⁻¹·kg⁻¹, of which 54% corresponded to urinary and 46% to metabolic clearance (Table 2). Compared with control rabbits, pretreatment with warfarin did not modify the value of the systemic clearance of furosemide; however, furosemide metabolic clearance increased by 31% (p < .05), and furosemide urinary clearance decreased by 54% (p < .05). The changes in furosemide metabolic and urinary clearances were almost totally precluded when the rabbits received the diuretic mixed with albumin (Table 2). Compared with control rabbits, warfarin reduced the urinary recovery of furosemide by 46%. When the diuretic was injected mixed with albumin, furosemide urinary recovery increased to reach baseline control values (Table 2). The urinary excretion of furosemide was negatively correlated with its unbound fraction in all groups (r = 0.65, p < .05).

Furosemide kinetics in rabbits with functional nephrectomy. In control nephrectomized rabbits, plasma concentrations of albumin, creatinine, and urea did not differ from normal values. Functional nephrectomy reduced the slope of the decline of furosemide plasma concentrations in both control and warfarin pretreated rabbits to a similar extent (Fig. 1). Compared with control rabbits (Table 2), functional nephrectomy decreased furosemide volume of distribution by about 30% and systemic clearance of furosemide by 85% (p < .05), secondary to the abolition of its renal excretion and the reduction in its metabolic clearance (p < .05) (Table 3). In rabbits with functional nephrectomy, warfarin did not modify furosemide pharmacokinetics.
Furosemide dynamics in control and warfarin pretreated rabbits. Compared with control rabbits, in rabbits pretreated with warfarin, the urinary excretion rate of furosemide, decreased by almost 50% (from 2784 ± 214 to 1451 ± 111 mg/h; p < .05). Consequently, the rate of sodium excretion as well as the diuresis were diminished by the same proportion (Fig. 2). In control animals, fractional excretion of sodium was 15.7 ± 1.8%, and decreased to 10.9 ± 1.0% (p < .05) in rabbits pretreated with warfarin. When furosemide was injected mixed with albumin to rabbits pretreated with warfarin, furosemide excretion rate and fractional excretion of sodium increased to values similar to those observed in control rabbits, i.e., 2682 ± 283 mg/h and 15.4 ± 1.1%, respectively. Moreover, sodium excretion and diuresis attained control values (Fig. 2). Natriuresis and diuresis were positively correlated with furosemide excretion (r = 0.77, p < .001; r = 0.73, p < .001).

**Conscious Rabbits.** Furosemide dynamics in conscious rabbits with hypoalbuminemia and warfarin. Compared with control rabbits, hypoalbuminemia increased the fraction of unbound furosemide in plasma (p < .05) and decreased the urinary recovery of furosemide, but the GFR remained unchanged (Table 4). As a consequence, in hypoalbuminemic rabbits, furosemide response, as assessed by the sodium excretion rate and the urinary volume, was decreased by 21%. Pretreatment of hypoalbuminemic rabbits with 5 mg/kg warfarin further enhanced the fraction of unbound furosemide and reduced its urinary recovery (Table 4). Consequently, sodium excretion rate and urinary volume were decreased by 33% and 19%, respectively (p < .05), compared with hypoalbuminemic rabbits not pretreated with warfarin. Natriuresis and diuresis were positively correlated with furosemide excretion (r = 0.78, p < .001; r = 0.75, p < .001).

**Discussion**

This study demonstrates that warfarin increases the unbound fraction of furosemide by almost 300%, leading to an increase in its renal metabolic clearance and to a decrease in its urinary excretion. Moreover, the administration of warfarin is associated with a marked reduction in natriuretic and diuretic effects of furosemide. The effect of warfarin on the kinetics of furosemide is essentially elicited at the renal level, because in rabbits with functional nephrectomy, warfarin also enhanced furosemide unbound fraction by 300% but did not affect furosemide extrarenal clearance or its volume of distribution.

It is unlikely that the decrease in furosemide urinary clearance may be secondary to a direct interaction with warfarin at the level of tubular secretion, because this drug is neither secreted by the proximal tubule nor metabolized in the kidney (Ganeval et al., 1985; Shetty et al., 1989). Further support of such a statement is the fact that the warfarin-induced changes in furosemide kinetics and dynamics were completely reversed when the diuretic was injected mixed with albumin, despite similar plasma concentration of warfarin. Therefore, the changes in the kinetics of furosemide reported in this study appear to be secondary to the displacement of furosemide from its binding sites to albumin and to an increase in its unbound fraction.

Warfarin induced changes in the kinetics of furosemide similar to those triggered by moderate hypoalbuminemia, i.e., increase in the renal metabolic clearance and reduction in the urinary excretion of furosemide (Pichette et al., 1996). In studies with isolated proximal tubules, it has been reported that albumin promotes the proximal secretion of two organic anions, para-aminophenylphosphate acid and methotrexate, an effect that appears unrelated to the binding of the anions to albumin (Besseghir et al., 1989). On the other hand, the results of the present study indicate that in vivo, albumin per se does not influence the proximal secretion of furosemide, because the levels of albumin in control rabbits were similar to those measured in rabbits pretreated with warfarin. Further support that the binding of furosemide to albumin influences its renal metabolism and secretion rather than albumin per se is the fact that when furosemide binding to albumin is enhanced to control values by the administration of the diuretic mixed with albumin, the changes in furosemide kinetics induced by warfarin are reversed.

The present results indicate that binding of furosemide to albumin reduces its renal metabolism, whereas it enhances its secretion. To be excreted in the urine or metabolized by the kidney, furosemide must reduce its renal metabolism, whereas it enhances its secretion. To be excreted in the urine or metabolized by the kidney, furosemide must reduce its renal metabolism, whereas it enhances its secretion. To be excreted in the urine or metabolized by the kidney, furosemide must reduce its renal metabolism, whereas it enhances its secretion. It is evident that furosemide excretion rate and the urinary volume are decreased by 21% (p < .05), compared with hypoalbuminemic rabbits not pretreated with warfarin. Natriuresis and diuresis were positively correlated with furosemide excretion (r = 0.77, p < .001; r = 0.73, p < .001).

**TABLE 4**

Dynamic parameters of furosemide injected (2.5 mg/kg) to conscious rabbits, control rabbits, and rabbits with moderate hypoalbuminemia in absence or in presence of warfarin (5 mg/kg).

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 4)</th>
<th>Hypoalbuminemia (n = 4)</th>
<th>Hypoalbuminemia + Warfarin (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma albumin (g/l)</strong></td>
<td>31.5 ± 0.5</td>
<td>24.5 ± 1.3*</td>
<td>24.3 ± 1.1*</td>
</tr>
<tr>
<td><strong>GFR (ml/min * kg⁻¹)</strong></td>
<td>5.1 ± 0.6</td>
<td>4.8 ± 0.5*</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td><strong>Unbound drug (%)</strong></td>
<td>1.2 ± 0.1</td>
<td>5.5 ± 0.5*</td>
<td>9.7 ± 1.4*</td>
</tr>
<tr>
<td><strong>Urinary recovery (%)</strong></td>
<td>48.8 ± 3.0</td>
<td>40.0 ± 2.3*</td>
<td>31.4 ± 2.1*</td>
</tr>
<tr>
<td><strong>Sodium excretion (mmol/h)</strong></td>
<td>18.3 ± 1.9</td>
<td>14.5 ± 0.4*</td>
<td>9.7 ± 0.1*</td>
</tr>
<tr>
<td><strong>Urinary volume (ml/h)</strong></td>
<td>141 ± 8</td>
<td>111 ± 1*</td>
<td>90 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± S.E. *p < .05 compared with control rabbits. **p < .05 compared with control and hypoalbuminemic rabbits.
explaining an increase in its renal metabolism when its binding to albumin is decreased. Further studies are needed to confirm this hypothesis.

The results of the present study may tentatively be extrapolated to humans. Indeed, it has been reported that in patients with heart failure who are treated with furosemide and who received a vitamin K anticoagulant concomitantly, furosemide volume of distribution and metabolic clearance were greater than the values reported in patients not receiving the anticoagulant (Andreasen and Mikkelsen, 1977). Pretreatment of rabbits with warfarin produces a marked decrease in the diuretic response of furosemide, decrease that is prevented by the administration of furosemide mixed with albumin, i.e., by correcting the warfarin-induced reduction in furosemide binding to plasma proteins. These results suggest that the displacement of furosemide from its binding sites to albumin could be a cause of diuretic resistance. Moreover, it could be a frequent mechanism of diuretic resistance, because there is a long list of drugs commonly used that are potential competitive displacers of furosemide binding to albumin, i.e., phenytoin, tolbutamide, chloropromazine, nonsteroidal anti-inflammatory agents, and sultonamides (Sjöholm et al., 1979). For example, it has been shown that phenytoin reduces the efficiency of furosemide by an unknown mechanism (Tongia, 1981). In light of our results, it is tempting to speculate that phenytoin decreased furosemide binding to albumin. The results of the present study may have clinical implications, because they suggest that a condition associated with an increase in the unbound fraction of furosemide, i.e., hypoalbuminemia, drug–drug interactions, and disease states, could lead to a significant decrease in furosemide response.

The studies with anesthetized rabbits were conducted using a dose of warfarin (50 mg/kg) that generated plasma concentrations of warfarin (182 ± 21 µg/ml) much greater than those usually attained in humans (Chan et al., 1994). Because hypoalbuminemia, i.e., albumin less than 35 g/liter, is a very frequent clinical condition affecting 3.1% of subjects older than 71 years (Salive et al., 1992), it was of interest to document the effect of moderate hypoalbuminemia combined with smaller doses of warfarin on the natriuretic and diuretic response to furosemide in conscious rabbits. The results show that moderate hypoalbuminemia combined with doses of warfarin yielding plasma concentrations of 17.1 ± 1.7 µg/ml, which are close to those obtained in humans (Chan et al., 1994), increased the unbound fraction of furosemide and decreased its pharmacological response. These results also suggest that in patients with moderate hypoalbuminemia, the administration of one or several acidic drugs that may potentially displace furosemide from its binding sites could be a cause of resistance to diuretics.

The rationale of combining furosemide with albumin in patients with severe hypoalbuminemia to promote its renal secretion and natriuretic response (Inoue et al., 1987) is reinforced by the results of the present study. However, the success of this practice may depend on the cause and/or severity of the hypoalbuminemia. For instance, in nephrotic patients with plasma concentrations of albumin of 17.3 g/liter, the infusion of 0.5 g/kg of albumin did not increase the natriuretic or the diuretic effect of a high dose of furosemide (Akcicek et al., 1995). On the other hand, in nephrotic patients with plasma concentrations of albumin of 27 g/liter, the administration of 40 g of albumin increased the diuresis to furosemide (Sjostrom et al., 1989). Several factors may explain the differences between these reports, such as the mode of administration of albumin and furosemide (whether furosemide was premixed with albumin or not), the severity of hypoalbuminemia, and the importance of the proteinuria known to bind furosemide in the tubular fluid (Kirchner et al., 1991).

In conclusion, the displacement of furosemide from its binding sites to albumin by warfarin enhances its unbound fraction. Consequently, there is an increase in the renal metabolism and a decrease in the proximal tubular secretion of furosemide, with a diminution in its natriuretic and diuretic response. Albumin appears to promote the renal secretion of furosemide by its role as a ligand and not by a mechanism associated to its presence. From the results of this study we may postulate that the decrease in furosemide binding to albumin, secondary to hypoalbuminemia and/or binding displacement, may be a frequent cause of resistance to the diuretic that could be reversed by injecting the diuretic mixed with albumin.

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References


