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Extrahepatic metabolism of frusemide in anaesthetized rabbits

José Vergés, Lucie Héroux, Hélène Maurice & [1]Patrick du Souich

Département de Pharmacologie, Faculté de Médecine, Université de Montréal, Québec, Canada

1 Frusemide is removed from the body by biotransformation and renal secretion, but since frusemide metabolism is not altered in patients with hepatic cirrhosis, the role of the liver may be questioned. The aim of the study was to investigate which organs contribute to the first-pass metabolism and systemic clearance of frusemide.

2 Groups of anaesthetized New Zealand rabbits were administered frusemide proximally (prox) and distally (dist) to different organs, and blood was sampled from the abdominal aorta. The area under frusemide plasma concentrations-time curve ($AUC_{0-\infty}$) was calculated and frusemide extraction by an organ was estimated from the ratio $(AUC_{dist}-AUC_{prox})/AUC_{dist}$. The small intestine extracted 83% of the absorbed dose of frusemide but the first-pass uptake by the liver and lungs was negligible.

3 To assess the contribution of the intestine and the kidneys to the systemic clearance of frusemide, it was injected into the jugular vein and blood was sampled proximal and distal to each organ. The kidneys extracted 24% of frusemide circulating in the renal arteries; on the other hand, the ability of the intestine to extract frusemide from the systemic circulation could not be detected.

4 The lungs did not metabolize frusemide *in vitro*; the rate of metabolism of frusemide *in vitro* by kidneys was similar to that estimated in the intestine, and both rates were faster ($P<0.05$) than that observed in the liver.

5 It is concluded that in rabbits, presystemic metabolism of frusemide is carried out by the intestine, and that systemic clearance of frusemide is mainly performed by the kidneys, although other organs, such as the intestine and the liver, must contribute to it.

Keywords: Frusemide; presystemic metabolism; systemic clearance; intestinal metabolism; renal metabolism; anaesthetized rabbits

Introduction

It has been assumed that most drugs undergo biotransformation in the liver by virtue of the presence of many isoforms of the cytochrome P-450 (Gonzalez, 1992; Guengerich, 1992), despite the fact that several organs, such as the gastrointestinal tract, kidneys, lungs, brain and skin, contain microsomal and cytosolic phase I and phase II isoenzymes (Krishna & Klotz, 1994). Tissue homogenate or isolated perfused organ studies have shown that these organs can mediate the phase I metabolism of a variety of xenobiotics (Krishna & Klotz, 1994). Additionally, the gastrointestinal tract and the kidneys are able to conjugate drugs with glucuronic acid and with glutathione (Pacifici *et al.*, 1988; De Waziers *et al.*, 1990).

Frusemide is a potent loop diuretic widely used in disease states where there is a retention of sodium and water. Frusemide is rapidly eliminated by renal and metabolic routes, the latter accounting for approximately 40% of the systemic clearance (Cutler *et al.*, 1974; Andreasen & Mikkelsen, 1977). About 30% of a dose of frusemide is recovered in urine conjugated with glucuronic acid (Boles Ponto & Schoenwald, 1990). Because the oral bioavailability of frusemide is around 60% (Hammarlund-Udenaes & Benet, 1989) it has been suggested that frusemide might be subjected to a first-pass metabolism. Supporting this suggestion, Zhu & Koizumi (1987) reported that the oral clearance was higher than the systemic clearance, and concluded that frusemide was extracted by the liver during its first-pass.

The role of the liver in the first-pass metabolism of frusemide is questionable, since it has been shown in the rat that between 20 and 30% of the absorbed oral dose was biotransformed in the intestine (Lee & Chiou, 1983). Furthermore, it has been consistently found that in patients with hepatic cirrhosis, the non-renal clearance of frusemide is only slightly modified (Fuller *et al.*, 1981; Sawhney *et al.*, 1981), suggesting that even in the presence of liver insufficiency, frusemide is metabolized adequately. Confirming this conclusion, Verbeeck *et al.* (1981) showed that the non-renal clearance of frusemide was not affected in functionally hepatectomized dogs; furthermore, the proportion of conjugated frusemide was not modified by the experimental condition, indicating that other organs must contribute to the biotransformation of frusemide. Based on indirect evidence, it has been suggested that the kidneys may play an important role in the metabolism of frusemide (Homeida *et al.*, 1977; Smith & Benet, 1983; Rakhit *et al.*, 1987).

The aim of the present study was to determine which organs are involved in the biotransformation of frusemide. To this purpose, the contribution of the intestinal tract, the liver and the lungs in the first-pass metabolism of frusemide, and that of the gastrointestinal tract and the kidneys in the systemic clearance of frusemide was assessed *in vivo* in anaesthetized rabbits. The ability of the intestinal tract, the liver, the lungs and the kidneys to metabolize frusemide were also assessed in *in vitro* studies.

Methods

In vivo *studies*

Experimental model Male New Zealand rabbits (2.5 ± 0.3 kg) purchased from Ferme Cunicole (Mirabel, Québec, Canada) were used throughout the study. They were maintained on Purina pellets and water *ad libitum* in individual well-ventilated cages. Animals were acclimatized in their cages for at least 8 days before any experimental work was undertaken and then fasted for 24 h before surgery. Anaesthesia was induced with 30 mg kg^{-1} of pentobarbitone intravenously and maintained with 5 mg kg^{-1} intravenous doses of pentobarbi-

[1] Author for correspondence.

tone as needed. The trachea was exposed, and an endotracheal tube (No. 3.5, CDMV, St. Hyacinthe, Québec, Canada) was inserted between the fourth and fifth tracheal rings caudally to the thyroid cartilage, for artificial ventilation (21 ml/cycle, 48 cycles min^{-1}) (Harvard Apparatus, Boston, MA, U.S.A.). A catheter Butterfly-25 (Venisystems Abbott Ireland, Sligo, Ireland) was installed into a lateral vein of the ear for the infusion of the hydrating solution. Another catheter (P-60, Intramedic, Becton, Dickinson and Co., Parsippany, NJ, U.S.A.) was inserted into the abdominal aorta, above the renal arteries, via the right femoral artery for blood sampling and to monitor arterial blood pressure via a three-way stopcock (Seamless, Division of Professional Medical Products Inc., Ocala, Florida, U.S.A.) connected to a pressure transducer (E and M Instruments, Houston, Texas, U.S.A.) and to a physiograph (E and M Instruments). Finally, a vesical catheter (Bardex Foley 8 Ch/Fr, Mississauga, Ontario, Canada) was installed to collect urine.

A laparotomy was performed on all animals, from the xiphoid appendix until 3 cm above the pubic symphysis, the duodenum was exposed, and the portal vein and a mesenteric vein were cleared of surrounding tissues for frusemide administration and/or blood sampling.

Experimental protocol In preliminary studies, we confirmed that the doses of frusemide used in the experiments (2.5 and 5 mg kg^{-1}) generate first order kinetics. To study the involvement of an organ in the first-pass metabolism of frusemide, the diuretic was injected proximally and distally to the organ under investigation, and blood was sampled from the abdominal aorta. The contribution of the kidneys and the intestine to the systemic clearance of frusemide was assessed by injecting the diuretic systemically and blood was sampled before and after the organ (Mistry & Houston, 1985). Seven groups of six rabbits each were studied. The first group was used as control to determine the effect of anaesthesia and the surgical procedure: conscious rabbits received frusemide (2.5 mg kg^{-1}) in the lateral vein of the ear, and blood was sampled from the central artery of the ear. To assess the extraction of frusemide by the lungs, groups of rabbits received frusemide (2.5 mg kg^{-1}) into the thoracic aorta via the carotid artery or into the jugular vein, respectively, and blood was sampled from the abdominal aorta.

To determine the extraction of frusemide by the liver, rabbits received frusemide (2.5 mg kg^{-1}) into the portal vein via a Jelco cannula (Critikon, Toronto, Canada), and blood was drawn from the abdominal aorta. To assess the uptake of frusemide by the intestine, a group of rabbits received frusemide (5 mg kg^{-1}) directly into the duodenal lumen (5 cm beyond the pylorus) injected with a Butterfly-25 (Venisystems Abbott Ireland, Sligo, Ireland), and blood was drawn from the abdominal aorta. Once the experiment was finished, the rabbits were killed and the intestine was clamped and removed (in sections of 30 cm) from the pylorus to the anus; each section was carefully washed with 20 ml of methanol (Fisher Scientific, Montréal, Canada) to recover frusemide not absorbed. In preliminary *in vitro* studies we observed that the recovery of frusemide previously introduced into the intestinal lumen approached 100% if a large volume of methanol (20 ml) was used to wash the mucosa.

To investigate the contribution of the intestine to the systemic clearance of frusemide, a group of rabbits received the diuretic (2.5 mg kg^{-1}) into the jugular vein, and blood was sampled simultaneously from the abdominal aorta and portal vein. Finally, to estimate the contribution of the kidneys to the systemic clearance of frusemide, a group of rabbits received the diuretic (2.5 mg kg^{-1}) into the jugular vein, and blood was sampled simultaneously from the abdominal aorta and the inferior cava vein, with the tip situated 2–3 mm above the renal veins. The position of the catheters was always confirmed at the end of the experiment.

In all cases, frusemide was injected over a period of 30 s. The same sampling protocol was used for all routes of administration, i.e. blood samples (2 ml) were drawn at 0, 3, 6, 10, 15, 20, 25, 30, 45, and 60 min after frusemide administration. Heparinized saline (100 units ml^{-1}) was kept in the arterial cannula to maintain patency. Urine was collected over the first hour after frusemide administration. Plasma and urine was stored at −20°C until frusemide was assayed.

All rabbits (with the exception of the group receiving frusemide into the intestine) received a solution of 0.9% NaCl with bicarbonate (4.2 g l^{-1}) and 5% glucose at a rate of 60 ml h^{-1} to compensate for the losses of water and blood during the experimental procedure. The rabbits receiving frusemide into the intestinal lumen were infused with a solution of 0.9% NaCl with bicarbonate (4.2 g l^{-1}) at a rate of 200 ml h^{-1}. The rate of hydration was increased because preliminary studies showed that the absorption of frusemide was slow and erratic when the rabbits received the hydration solution at the rate of 60 ml h^{-1}, due to the transient decrease in blood pressure secondary to the water and salt depletion caused by frusemide. A faster rate of water and salt delivery preserved the absorption process and the intestinal extraction of frusemide.

Frusemide in plasma and urine was assayed by high-performance liquid chromatography as described elsewhere (Lambert *et al.*, 1982).

In vitro *studies*

Rabbits ($n=6$) were killed by cerebral dislocation, and the small intestine, the right lobule of the liver, the lungs and the kidneys were removed and carefully washed with 1.15% KCl, 0.05 M phosphate buffer pH 7.4. Tissues were prepared as follows: epithelial cells of the intestinal mucosa were removed by gently scraping the surface, the liver was minced, trachea and large bronchi were removed from the lungs, and the renal cortex was obtained by manual dissection. Tissues were suspended in 1.15% KCl, 0.05 M phosphate buffer pH 7.4, to obtain a 25% dilution, and homogenized with a polytron (Brinkman, Rexdalle, Sweden) and/or a Potter-Elvehjem (Tri-R Instruments, Rockville, NY, U.S.A.). The homogenate was centrifuged at 10,000 *g*, and the supernatant was used to assess the ability of the various tissues to metabolize frusemide. The rate of disappearance of frusemide was assessed by incubating 302 μM (100 μg ml^{-1}) frusemide in the 10,000 *g* supernatant for 180 min, as previously described (Lambert *et al.*, 1982). In the controls, frusemide was incubated in the tissue homogenates but in the absence of the generating system.

Data analysis

Plasma concentration-time curves for frusemide were fitted to a biexponential equation, assuming first-order distribution and elimination. The $AUC_{0-\infty}$ of frusemide was estimated after the administration of the drug via the various routes. Plasma clearance, terminal half-life and apparent volume of distribution of frusemide following the intra-arterial administration were calculated as described by Gibaldi & Perrier (1982).

When frusemide was administered proximal to and distal to an organ, the extraction ratio of frusemide (E) by the organ was calculated according to the ratio: $(AUC_{dist}-AUC_{prox})/AUC_{dist}$. However, when frusemide was administered before the organ under investigation and blood samples were drawn before and after the organ, the extraction ratio was calculated according to the ratio: $(AUC_{prox}-AUC_{dist})/AUC_{prox}$ (Cassidy & Houston, 1980). The systemic availability of frusemide (*F*) by any route of administration was calculated by the ratio of AUC_{prox} from injection before the organ over that after intra-arterial administration. To estimate the systemic availability of frusemide after its administration into the duodenum, the dose administered was corrected for the fraction of frusemide not absorbed from the gut lumen. In *in vitro* studies, the decay of frusemide concentrations in tissue homogenates was expressed

as the rate constant of elimination, calculated by multiplying the slope of the decay of frusemide concentrations (estimated by linear regression analysis) by 2.303.

The results are expressed as mean ± s.d. The values of $AUC_{0-\infty}$ estimated after the various routes of frusemide administration were compared to the $AUC_{0-\infty}$ estimated after intra-arterial administration using a one-way analysis of variance for parallel groups. Statistical differences were evaluated using Dunnett's distribution table (Winer, 1971) and a P value <0.05 was taken as significant.

Drugs

Pentobarbitone was purchased from Abbott Laboratories (Nembutal, Montréal, Canada) as a solution containing 50 mg ml^{-1}. Frusemide in a solution of 10 mg ml^{-1} was obtained from Sabex (Lasix, Montréal, Canada).

Results

The mean arterial pressure measured in the central artery of an ear was 70 ± 5 mmHg in conscious rabbits. In anaesthetized rabbits, the mean arterial pressure measured in the abdominal aorta was 72 ± 5 mmHg before the administration of frusemide, and 59 ± 5 mmHg 60 min after frusemide administration. This decrease in arterial pressure was observed in all rabbits being hydrated at the rate of 60 ml min^{-1}, independently of the route of frusemide administration.

Compared with conscious rabbits, anaesthesia and the surgical procedure did not alter the systemic clearance or the apparent volume of distribution, but decreased the renal clearance of frusemide (Table 1). The decay of frusemide plasma concentrations as a function of time, following the administration of frusemide through various routes to anaesthetized rabbits is shown in Figure 1. The $AUC_{0-\infty}$ of frusemide injected into the thoracic aorta, the jugular vein and the portal vein were almost identical (Table 2). However, the $AUC_{0-\infty}$ of frusemide administered into the duodenal lumen was smaller than those estimated when frusemide was administered through the other routes. The amount of diuretic recovered in the intestine 1 h after the intraduodenal administration of frusemide averaged 2.2 mg, i.e. 17% of the dose.

When frusemide was injected into the jugular vein and blood was drawn simultaneously from the abdominal aorta and the vena cava, the $AUC_{0-\infty}$ of frusemide in the vena cava was significantly lower than that estimated in the abdominal aorta. Finally, when frusemide was injected into the jugular vein and blood samples were drawn simultaneously from the

Table 1 Kinetic parameters of frusemide (2.5 mg kg^{-1}) injected in a lateral vein of the ear of conscious rabbits ($n=6$), or in the jugular vein of rabbits ($n=6$) under pentobarbitone anaesthesia (30 mg kg^{-1}), artificial ventilation, and sham surgical procedures

	Conscious	*Anaesthetized*
$AUC_{0\rightarrow\infty}$ (μg min ml^{-1})	186 ± 54	205 ± 71
Vd_β (ml kg^{-1})	254 ± 83	356 ± 179
Cl_T (ml min^{-1} kg^{-1})	15 ± 5	14 ± 5
Cl_{NR} (ml min^{-1} kg^{-1})	8 ± 3	10 ± 5
Cl_R (ml min^{-1} kg^{-1})	7 ± 3	4 ± 3[a]
$t_{1/2}$ (min)	11.5 ± 4.2	17.6 ± 7.4

Values are mean ± s.d. [a]$P<0.05$ compared to conscious rabbits. Vd_β: volume of distribution; Cl_T,Cl_{NR}, Cl_R: total, non renal and renal clearance; $t_{1/2}$: half life.

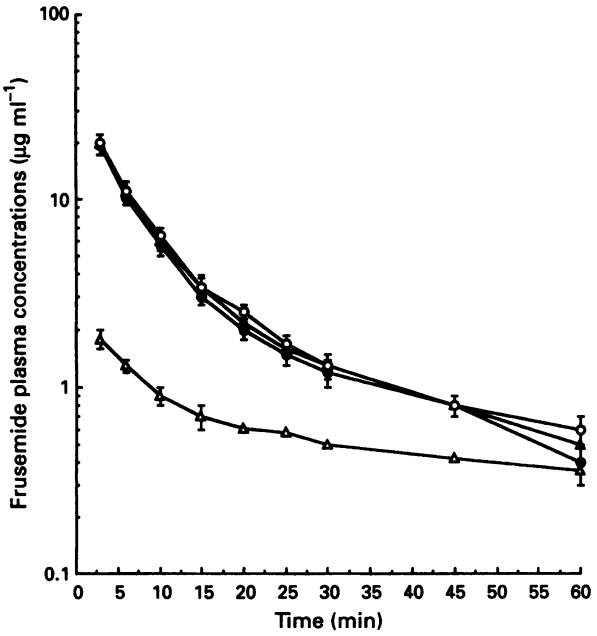

Figure 1 Frusemide plasma concentrations as a function of time in anaesthetized rabbits receiving 2.5 mg kg^{-1} of frusemide into the thoracic aorta (●) ($n=6$), the jugular vein (▲) ($n=6$), the portal vein (○) ($n=6$), and 5 mg kg^{-1} into the duodenal lumen (△) ($n=6$). Values are mean ± s.e.mean.

Table 2 Area under frusemide plasma concentrations-time curve ($AUC_{0-\infty}$), dose and dose-normalized $AUC_{0-\infty}$ following the administration of frusemide into the thoracic aorta, jugular vein, portal vein, and duodenal lumen, and blood drawn from the abdominal aorta, or after frusemide administration into the jugular vein and sampling simultaneously from the abdominal aorta and the vena cava or the portal vein of anaesthetized rabbits

Route of injection	*Site of sampling*	$AUC_{0-\infty}$ (μg min ml^{-1})	*Dose* (mg)	$AUC_{0-\infty}/Dose$ (min ml^{-1})
First-pass metabolism				
Thoracic aorta	Abdominal aorta	205 ± 71	6.2 ± 0.6	0.033 ± 0.012
Jugular vein	Abdominal aorta	190 ± 34	6.1 ± 0.7	0.031 ± 0.007
Portal vein	Abdominal aorta	206 ± 44	5.8 ± 0.3	0.036 ± 0.007
Duodenal lumen	Abdominal aorta	66 ± 10[a]	10.7 ± 0.7	0.005 ± 0.001[a,c]
Systemic clearance				
Juglar vein	Abdominal aorta	154 ± 25	6.3 ± 0.3	0.023 ± 0.004
	Vena cava	117 ± 22[b]		0.018 ± 0.003[b]
Jugular vein	Abdominal aorta	217 ± 115	6.1 ± 0.9	0.034 ± 0.006
	Portal vein	242 ± 140		0.039 ± 0.007

Values are mean ± s.d. for $n=6$ in each group.
[a]$P<0.05$ compared with values for thoracic aorta, jugular vein and portal vein routes of injection. [b]$P<0.05$ compared with abdominal aorta sampling values. [c]Oral dose has been corrected by the amount of frusemide absorbed.

abdominal aorta and the portal vein, the $AUC_{0-\infty}$ of frusemide in these sampling sites was comparable (Table 2). In other studies, observing a similar experimental protocol, it was shown that a faster rate (200 ml h^{-1}) of hydration or the infusion of frusemide (16 $\mu g\ min^{-1}\ kg^{-1}$ for 120 min) to steady state plasma concentrations did not improve the efficiency of the intestine in extracting systemic frusemide.

After intraduodenal administration of frusemide to anaesthetized rabbits, the systemic bioavailability was 0.16. Systemic availability was very low because frusemide was extracted by the intestinal wall; the first-pass metabolism of frusemide by the liver and the lungs was not quantifiable (Figure 2). When frusemide was administered through the jugular vein, the contribution of the kidneys to the systemic clearance was marked, since 24% of frusemide arriving at the kidneys was extracted by these organs. In contrast, under the experimental conditions described, the extraction of systemic frusemide by the intestine was not measurable (Figure 2).

In *in vitro* studies, in the absence of the generating system, the concentrations of frusemide did not change when incubated for 180 min with tissue homogenates. Frusemide concentrations decreased by 16, 51, 60 and 0% following 180 min of incubation with 10,000 *g* supernatants from hepatic, intestinal, renal and lung tissues, respectively (Figure 3). Consequently, the rate constant of elimination of frusemide by the liver ($0.0016 \pm 0.0012\ min^{-1}\ mg^{-1}$ protein) was slower ($P < 0.05$) than that in intestinal and renal tissue homogenates,

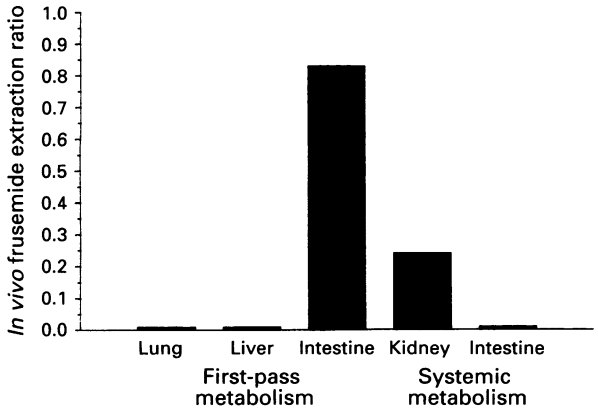

Figure 2 First-pass uptake of frusemide administered prior to the lungs ($2.5\ mg\ kg^{-1}$) ($n=6$), the liver ($2.5\ mg\ kg^{-1}$) ($n=6$) or the intestine ($5\ mg\ kg^{-1}$) ($n=6$) and blood samples drawn from the abdominal aorta, and systemic uptake by the kidneys ($n=6$) and the intestine ($n=6$) of frusemide injected into the jugular vein ($2.5\ mg\ kg^{-1}$) and blood sampled simultaneously from the abdominal aorta and the vena cava or the portal vein of anaesthetized rabbits.

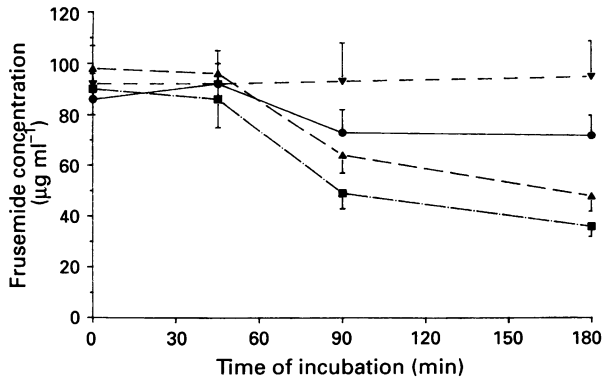

Figure 3 *In vitro* biotransformation of frusemide in 10,000*g* supernatant of the liver (●), epithelial cells of the intestinal mucosa (▲), renal cortex (■) and pulmonary parenchyma (▼) of male New Zealand rabbits ($n=6$).

i.e. 0.0052 ± 0.0010 and $0.0059 \pm 0.0017\ min^{-1}\ mg^{-1}$ protein, respectively. Frusemide concentrations did not change following incubation for 180 min in lung tissue preparations.

Discussion

The present results show that the absolute bioavailability of frusemide is 14%, taking into account the fact that 83% of the intraduodenal dose is absorbed, of which only 17% is available to the systemic circulation. The first-pass metabolism of oral frusemide is almost exclusively carried out by the intestine, since the contribution of the liver and the lungs is negligible. Assuming no extraction of frusemide by pelvic organs and hind legs, the kidneys extract 24% of frusemide entering the renal arteries. Extraction of systemic frusemide by the intestine is not quantifiable. *In vitro* studies indicate that the renal cortex and small intestine metabolize frusemide at similar rates, which is higher than the rate of frusemide metabolism in the liver.

In human subjects, most frusemide metabolism yields frusemide glucuronide (Hammarlund-Udenaes & Benet, 1989). Glucuronidation is an effective route of biotransformation in herbivores, such as rabbits (Dutton, 1980a), and liver, kidneys (Dalton, 1980a), intestinal mucosa and lungs of rabbits conjugate substrates with glucuronic acid (De Bernardi *et al.*, 1972; Gram *et al.*, 1974; Dutton, 1980b; Perreault *et al.*, 1993).

The present results show that the liver does not contribute importantly to the first-pass metabolism of oral frusemide, confirming the observations reported by Verbeeck *et al.* (1981). Frusemide is highly bound to plasma proteins, and only 1–2% of total blood concentration circulates unbound (unpublished results; Prandota & Pruitt, 1991). Therefore, only binding sites in tissues with high affinity for frusemide will overcome the rate limiting step of frusemide plasma protein binding. Compared with other tissues, the affinity of the liver for frusemide is low, since in the rat, the tissue to plasma concentration-ratio of frusemide was 9.8 for the adrenal glands, 4.3 for the lungs, 4.2 for the kidneys, 3.0 for the spleen, 2.0 for the pancreas, and only 1.9 for the liver (Prandota & Pruitt, 1991). It is possible that *in vivo*, the first-pass metabolism of frusemide in the liver is limited by frusemide high plasma protein binding.

The lungs have the ability to extract certain xenobiotics, essentially basic amines with a pK_a greater than 8, of moderate to high lipophilic nature, and with a hydrophobic moiety and a charged cationic group at physiological pH (Roth, 1985), such as chlorpromazine, imipramine, propranolol, verapamil, etc. (Tam, 1993; Krishna & Klotz, 1994). When metabolized in the lungs, these amines undergo phase I reactions. The lungs of rabbits also have the ability to extract basic amines (Wilson *et al.*, 1979), and in addition, are able to conjugate basic substances, e.g. oxazepam (de Bernardi *et al.*, 1972) and 2-aminophenol (Gram *et al.*, 1974). Frusemide was not extracted and/or metabolized by the lung *in vivo* or *in vitro*, possibly because frusemide is an acidic compound with a pK_a of 3.9, and modestly hydrophilic.

When frusemide was injected into the duodenum, the intestine absorbed 83% of the dose. The small intestine contains important amounts of uridine diphosphate-glucuronosyltransferases (Krishna & Klotz, 1994), hence absorption of frusemide from the intestine was probably followed by conjugation with glucuronic acid. When frusemide was injected into the jugular vein, systemic extraction of frusemide by the gut was not quantifiable. The mechanisms underlying such apparent discrepancies remain unknown. Frusemide may have diminished mesenteric blood perfusion (Schmitt *et al.*, 1981) and so the access of frusemide to epithelial cells of the villous tips, where most metabolic acitivity takes place (Traber *et al.*, 1988). In addition, plasma protein binding of frusemide may also be a limiting factor for intestinal extraction of systemic frusemide.

Since the muscle and testis to plasma concentration ratio for frusemide is 0.3 (Prandota & Pruitt, 1991), we may assume that the contribution of hind legs muscle, bladder and genital or-

gans to frusemide metabolism is negligible. As a consequence, the 24% difference in $AUC_{0-\infty}$ between the abdominal aorta and the vena cava probably relates to the extraction of frusemide by the kidneys. Since *in vitro* the kidneys metabolize frusemide very actively, it is likely that some of the frusemide extracted will be metabolized in the kidneys, and the remaining will be secreted into the tubule. The ability of the kidneys to metabolize frusemide can be assessed by calculating the total renal clearance (Cl_{TR}) of frusemide by multiplying plasma renal flow (Q_R) and the extraction ratio of the kidneys for frusemide (E). Cl_{TR} represents frusemide excreted plus frusemide metabolized by the kidney. Average Q_R in conscious New Zealand rabbits receiving an intravenous dose of 5 mg kg^{-1} of frusemide is 35 ml min^{-1} kg^{-1} (Babini & du Souich, 1986), and E = 0.24; therefore assuming that anaethesia and surgical procedures did not affect Q_R, Cl_{TR} = 8.4 ml min^{-1} kg^{-1}. In anaesthetized rabbits receiving frusemide through the jugular vein (Table 1), the excretion of frusemide by the kidneys, expressed as renal clearance (Cl_R), is 4 ml min^{-1} kg^{-1}. Therefore, the difference $Cl_{TR} - Cl_R$ (4.4 ml min^{-1} kg^{-1}) represents the metabolic contribution of the kidney, i.e. around 50% of the estimated non-renal clearance of frusemide (Table 1).

There is evidence suggesting that the kidneys contribute to the overall metabolism of frusemide to a similar extent in man as in the rabbit. For instance, probenecid blocks the antiluminal transport of frusemide and by impeding the entrance into tubular epithelial cells, it reduces the non-renal clearance of frusemide by 56% (Homeida *et al.*, 1977). Pentopril diminishes the renal clearance of frusemide and increases its non-renal clearance by blocking the luminal secretion of frusemide into the renal tubule and prolonging frusemide residence in the epithelial cell, and as a consequence, promoting its conjugation (Rakhit *et al.*, 1987). Based on the fact that the percentage of the dose extracted as frusemide glucuronide was positively correlated with the renal clearance of frusemide, Smith & Benet (1983) proposed that the biotransformation of frusemide occurred in the kidney. Further supporting the importance of the kidney in the biotransformation of frusemide is the fact that under conditions where renal function is decreased, i.e. in the elderly and during renal failure, frusemide non-renal clearance is reduced (Huang *et al.*, 1974; Kerremans *et al.*, 1983).

In conclusion, the present study demonstrates that in anaesthetized rabbits, oral frusemide is subjected to an important first-pass metabolism, secondary to an extensive metabolism by the intestine. On the other hand, the systemic clearance of frusemide is largely carried out by the kidneys, although other organs must also contribute to it. *In vitro* studies confirm that the kidneys metabolize frusemide at a slightly higher rate than the intestine and much faster than the liver.

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