Effects of Varying Doses of Spironolactone Without and With Nitrates on Portal Vein Pressure and Kidney Function in Partial Portal Vein Ligated Rats

MARC VAN DE CASTEELE, GUY VAN ROEY, FREDERIK NEVENS, AND JOHAN FEVERY

The optimal dose of spironolactone to reduce portal vein pressure, alone or in combination with isosorbide-5-mononitrate (IsMn), has not been identified. We studied the effects of 8 days oral treatment with spironolactone, IsMn or both on portal pressure, plasma volume and renal sodium handling in rats with partial portal vein ligation. At daily doses of 0.33; 0.50; 1, and 1.50 mg/kg, spironolactone reduced portal pressure (all $P < .05$) as compared with placebo. Only the highest dose significantly lowered plasma volume (10.1 ± 0.7 vs. 13.0 ± 0.3 mL; $P < .03$) and enhanced urinary fractional sodium excretion (0.73 ± 0.04 vs. 0.58 ± 0.03%; $P < .03$) and the $(Na^+)/(K^+)$ ratio in urine (0.43 ± 0.04 vs. 0.30 ± 0.03; $P < .02$). IsMn at doses of 0.25; 0.50, and 1 mg/kg decreased portal pressure (all $P < .05$) without a change in plasma volume but with a tendency (not significant) to lower fractional sodium excretion. IsMn impaired free water clearance at doses of 0.5 and 1 mg/kg ($P < .05$). Combinations of spironolactone 1 mg/kg with IsMn 0.5 or 1 mg/kg have no additive effect on portal pressure compared with spironolactone or IsMn alone. The higher the dose of IsMn in the combination, the more the natriuretic effect of spironolactone is opposed. Low doses of spironolactone are as effective as a higher dose to reduce portal pressure. This reduced portal pressure was independent of changes in plasma volume and diuretic effect, which suggests that spironolactone might have a direct vasoactive effect on the splanchnic circulation. To counteract sodium retention of nitrovasodilators, combination with high doses of spironolactone seems advantageous. (HEPATOLOGY 1996;24:1492-1496.)

Pharmacological therapy in the primary and secondary prevention of variceal bleeding aims to lower the portal vein pressure.[1] Noncardioselective β-blockers lower portal pressure by a reduction in cardiac output and by direct splanchnic vasoconstriction.[2] These β-blocking agents, however, fail to significantly lower the hepatic venous pressure gradient (HVPG)[3] or the variceal pressure[4] in up to 40% of cirrhotic patients. This has stimulated the search for alternative drugs in the treatment of portal hypertension. Recent data have shown that spironolactone lowers portal pressure even in patients without ascites.[5-7] Spironolactone is assumed to lower portal pressure by reducing the enhanced circulating plasma volume in portal hypertension and hence by reducing the splanchnic flow.[5,6] However, a correlation between the decline in plasma volume and portal pressure could not be shown.[5-7] Acute and chronic administration of nitrovasodilators lowers HVPG[8,9] but a concomitant reduction of the glomerular filtration rate and activation of the renine-aldosterone system with reduced water and sodium excretion have been observed.[10,11] This might explain the worsening or development of ascites observed in some patients.[12] The combination of nitrates with spironolactone might offer the advantage that spironolactone could counteract the activation of the renine-aldosterone system and the development of ascites. Furthermore, if spironolactone lowers portal pressure by a reduction in plasma volume, it might enhance the portal pressure lowering effect of nitrates. Therefore, the aim of the present study, using a portal hypertensive rat model, was to investigate the effects of different doses of spironolactone, isosorbide-5-mononitrate (IsMn), and their combination, on portal pressure, plasma volume, water, and sodium household.

MATERIALS AND METHODS

Treatment Schedules. Male Wistar rats, with a body weight between 209 and 240 g (mean 226 g) underwent partial portal vein ligation, as described previously.[13] Three weeks postoperatively, animals were randomly assigned to one of the following treatments given by daily gavage: placebo, i.e., 2 mL saline (n = 8); spironolactone, 0.33 mg/kg body weight (n = 6), 0.50 mg/kg (n = 6), 1 mg/kg (n = 6), and 1.50 mg/kg (n = 6); IsMn, 0.25 mg/kg (n = 6), 0.50 mg/kg (n = 8), and 1 mg/kg (n = 11); spironolactone, 1 mg/kg in combination with IsMn, 0.5 mg/kg (n = 8) and 1 mg/kg (n = 6). All medication was dissolved in 2 mL saline and given by gavage for 8 days. Rats were housed under standard conditions in metabolic cages (Tecniplast, Varese, Italy) to allow separate measurements of food and fluid intake and of urinary and faecal output; they were housed 3 days for adaptation before treatment and during the 8 days of treatment. They had free access to food and drinking water. The mixed rodents' food (Trouw, Gent, Belgium) contained 0.18 g Na^+ or 8.2 meq/g of food.

All animals received humane care and this study protocol complied with the Guidelines for Laboratory Animals of the Catholic University of Leuven, Leuven, Belgium.

Measurements. On day 0, the day before the first gavage, and on day 7 of treatment, blood and 24-hour urine sampling were performed to determine (Na^+), (K^+), (creatinine), and osmolality. Hence creatinine clearance, free water clearance, fractional Na^+ excretion (FE), and urinary $(Na^+)/(K^+)$ ratio were calculated. Creatinine clearance was calculated as: (creatinine) urine × diuresis/(creatinine) serum and expressed as mL/min. Free water clearance was calculated as diuresis minus osmolar clearance and the FE of sodium as urinary Na^+ clearance divided by creatinine clearance or (Na^+) serum × (creatinine) urine/(Na^+) urine × (creatinine) serum and expressed as percentage. FE of Na^+ was used as a parameter of natriuresis in addition to 24-hour urinary Na^+ output. Although all rats were housed under stable conditions, 24-hour urinary Na^+ output is prone to biological variation, eg, because of dietary sodium intake. This intraindividual variation in sodium intake was limited, however, because statistically significant differences in sodium intake by food were not present within a group nor between different groups. Fur-

Abbreviations: HVPG, hepatic venous pressure gradient; IsMn, isosorbide-5-mononitrate; FE, fractional Na^+ excretion.

From the Laboratory of Hepatology, University Hospital Gasthuisberg, Catholic University of Leuven, B-3000 Leuven, Belgium.

Received May 13, 1996; accepted August 5, 1996.

Address reprint requests to: Johan Fevery, M.D., Department of Liver and Pancreas Diseases, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium.

0270-9139/96/2406-0032$3.00/0

TABLE 1. Hemodynamic and Renal Effects of Increasing Doses of Spironolactone and Placebo

			Spironolactone (mg/kg/d)			
		Placebo (n = 8)	0.33 (n = 6)	0.50 (n = 6)	1 (n = 6)	1.50 (n = 6)
PP (mm Hg)	day 8	10.4 ± 1.2	5.6 ± 0.9*	6.3 ± 0.5*	5.7 ± 0.7*	5.1 ± 0.4*
AP (mm Hg)	day 8	76 ± 10	62 ± 8	72 ± 6	67 ± 5	66 ± 7
Na^+ in (meq/24 h)	day 0	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.2 ± 0.2
	day 7	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.3	1.1 ± 0.3	1.5 ± 0.2
Ur Na^+ (meq/24 h)	day 0	1.1 ± 0.1	1.1 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1
	day 7	1.2 ± 0.1	1.4 ± 0.2	1.0 ± 0.2	1.5 ± 0.1	1.3 ± 0.1
Ur K^+ (meq/24 h)	day 0	4.2 ± 0.2	3.5 ± 0.3	3.9 ± 0.2	3.5 ± 0.5	3.5 ± 0.4
	day 7	4.1 ± 0.3	3.9 ± 0.5	3.9 ± 0.4	4.0 ± 0.2	3.5 ± 0.5
CreaCl (mL/min)	day 0	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
	day 7	0.9 ± 0.1	1.3 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.1

NOTE. Data are expressed as means ± SEM.

Abbreviations: PP, portal venous pressure; AP, mean arterial pressure; Na^+ in, 24-hour sodium intake by food; Ur Na^+, 24-hour urinary sodium excretion; Ur K^+, 24-hour urinary potassium excretion; CreaCl, creatinine clearance; Cl_{H_2O}, free water clearance.

* $P < .05$ as compared with placebo treatment. Collections on day 0 represent the pretreatment 24-hour and on day 7 the 24-hour period during the 7th day of treatment.

thermore, by relating Na^+ clearance to creatinine clearance, the FE of Na^+ is considered as rather stable in intact kidneys (intact glomerulotubular balance).[14]

The urinary $(Na^+)/(K^+)$ ratio was used to assess aldosterone antagonism by spironolactone, because the higher the $(Na^+)/(K^+)$ ratio under spironolactone treatment, the more pronounced aldosterone receptor blocking is present in the distal tubule of the kidney.

On day 8, day of the last gavage, portal and arterial pressure and plasma volume were measured under mild general anesthesia with sodium pentobarbital 30 mg/kg intraperitoneally. Portal vein pressure was measured by cannulation via an ileocolic vein with an Insyte-W catheter (Becton Dickinson Benelux, Erembodegem, Belgium). Plasma volume was assessed by dilution of intravenously administered Evans blue.[15] Briefly, a weighed volume of 300 μL Evans blue (1.67 mg/mL) was injected via a PE 50 catheter (Becton Dickinson Benelux) in the jugular vein and flushed with 50 μL saline. After 10 minutes, arterial pressure was measured and blood was sampled through an Insyte-W catheter (Becton Dickinson Benelux) at the aortic bifurcation. The concentration of Evans blue in plasma was measured as follows: 300 μL of plasma was diluted 11-fold with a 10% sodium dodecyl sulfate solution to precipitate plasma proteins. Following centrifugation at 3,000 rpm for 10 minutes, photometric absorption of the supernatant was measured at 610 nm in comparison with a blank sodium dodecyl sulfate solution. The concentration of Evans blue was calculated from a standard dilution curve, made the same day. Portal and arterial pressure were recorded on a 2-channel pressure transducer (Servocorder SR 6255, Watanabe, Tokyo, Japan). After the measurements, the animal was killed with an overdose of sodium pentobarbital.

Statistics. To compare effects between groups, ANOVA and multiple comparison test (Scheffé) were performed. When appropriate ($P < .05$), unpaired Student's t test was performed to compare between groups on the same day. To compare effects within a group on day 0 and day 7, paired Student's t test was used. Two-tailed significance in the t tests was established at $P < .05$. All values are expressed as means ± SEM.

RESULTS

Effects of Increasing Doses of Spironolactone. In PVL rats spironolactone given at doses of 0.33 to 1.5 mg/kg/d for 8 days, induced a marked decrease in mean portal pressure compared with placebo: minus 44 ± 20%, 39 ± 12%, 45 ± 16%, 51 ± 10%, respectively (all $P < .05$; Table 1 and Fig. 1). The decreases in portal pressure induced by doses of 0.33 to 1 mg/kg/d were independent of any significant change in plasma volume when compared with placebo (13.0 ± 0.7 mL): minus 3 ± 10%, 8 ± 9%, 10 ± 6% (all NS; Fig. 2). A significant decrease in plasma volume was only observed with the 1.50 mg/kg/d dose (10.2 ± 1.9 vs. 13 ± 0.7 mL; $P < .03$; Fig. 2).

Similarly, an increase in the urinary FE of Na^+ (0.76 ± .04% vs. .58 ± .03%; $P < .03$; Fig. 3), as well as an enhanced urinary $(Na^+)/(K^+)$ ratio (.43 ± .04 vs. .30 ± .03; $P < .02$; Fig.

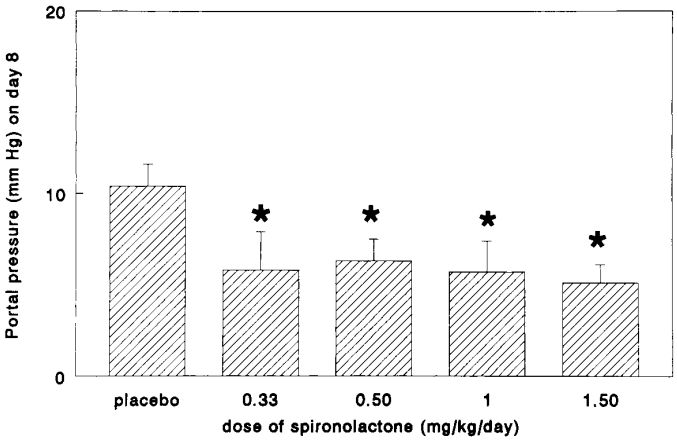

FIG. 1. Effect of placebo and varying doses of spironolactone on portal pressure (mm Hg) on day 8 of treatment. The portal pressures under spironolactone were significantly below that of placebo-treated animals (*$P < .05$) but did not differ significantly between each other. Values are expressed as means ± SEM.

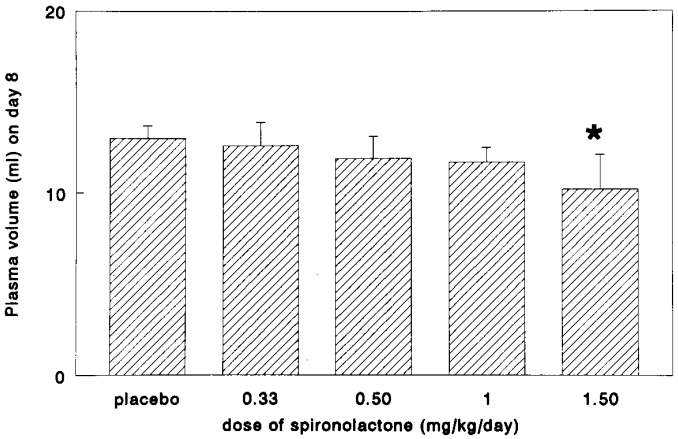

FIG. 2. Effect of placebo or spironolactone on plasma volume (mL) on day 8 of treatment. Only the highest dose of spironolactone resulted in a significant reduction of circulating plasma volume; *$P < .03$ compared with placebo. Values are expressed as means ± SEM.

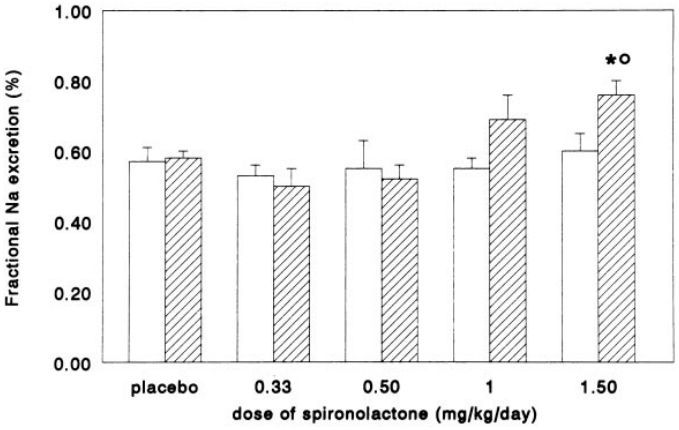

FIG. 3. Fractional excretion rate of sodium (%) before i.e., day 0 (□) and on day 7 (▨) of treatment with placebo and spironolactone. Only the highest dose of spironolactone enhanced natriuresis on day 7, as compared with placebo (*$P < .03$) and to day 0 within the same group (°$P < .05$). Values are expressed as means ± SEM.

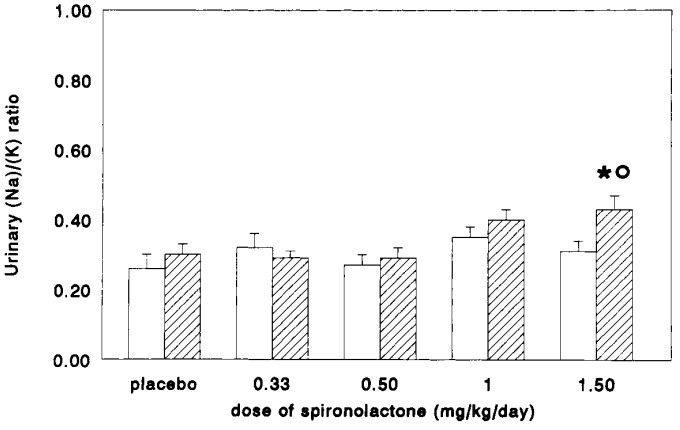

FIG. 4. Urinary $(Na^+)/(K^+)$ ratio before i.e., on day 0 (□) and on day 7 (▨) of treatment with placebo and spironolactone. The highest dose of spironolactone increased the urinary $(Na^+)/(K^+)$ ratio on day 7, as compared with placebo (*$P < .02$) and to day 0 within the group (°$P < .03$). Values are expressed as means ± SEM.

4) were only observed when the highest dose of spironolactone was given. Furthermore, plasma volumes and portal pressures in the individual animals did not correlate with each other. Daily Na^+ intake, 24-hour urinary Na^+ and K^+ excretion and creatinine clearance were unaltered by the different doses of spironolactone (Table 1).

Effects of Increasing Doses of IsMn. These results are given in Table 2. IsMn, 0.25; 0.50 and 1 mg/kg/d given during 8 days significantly decreased mean portal pressure: 7.1 ± 1.4, 7.8 ± 0.6, and 6.2 ± 0.5 mm Hg, respectively, compared with placebo 10.4 ± 1.2 mm Hg (all $P < .05$). The mean arterial pressure was decreased only with the highest dose of IsMn namely 1 mg/kg (49 ± 6 vs. 76 ± 12 mm Hg; $P < .05$). Significant changes in circulating plasma volume were not observed with any of these doses used after 1 week. The FE of Na^+ on day 7 tended to decrease as compared with placebo (0.49 ± 0.05, 0.50 ± 0.01, 0.51 ± 0.02 vs. placebo 0.58 ± 0.03; ANOVA $P < .09$) suggesting a tendency toward Na^+ retention. Free water clearance became significantly impaired with the higher doses of 0.5 and 1 mg/kg ($P < .001$ and $P < .05$). Creatinine clearance did not change under any of these nitrovasodilator treatments after 1 week.

Combinations of Spironolactone With IsMn. The combination of spironolactone 1 mg/kg with IsMn .5 and 1 mg/kg lowered the mean portal pressure when compared with placebo (6.9 ± 1.0 and 7.0 ± 0.5 vs. 10.4 ± 1.2; $P < .05$) but resulted in similar portal pressures when compared with spironolactone alone or nitrates alone (Table 3). The mean plasma volumes with these two combinations were reduced when compared with placebo (minus 35 ± 2% and 25 ± 3%; both $P < .001$) and to nitrates alone (both $P < .001$). Sodium retention clearly developed when spironolactone was combined with the higher dose of IsMn 1 mg/kg: both FE of Na^+ (0.38 ± 0.07) and urinary $(Na^+)/(K^+)$ ratio (0.22 ± 0.01) were reduced significantly in comparison to placebo ($P < .03$ and $P < .001$, respectively). This indicates that the natriuretic effect of spironolactone is opposed by combining with a high dose of nitrates. Free water clearance under these combination therapies was higher than with the corresponding monotherapies of IsMn alone (both $P < .05$).

DISCUSSION

The beneficial effect of spironolactone on portal hypertension was at first believed to result from its ability to lower the circulating plasma volume because of its diuretic and natriuretic effect.[5,6] Reduction of an enhanced circulating plasma volume in the case of portal hypertension might attenuate the splanchnic hyperdynamic circulation.[6] However,

TABLE 2. Hemodynamic and Renal Effects of a Treatment With IsMn 0.25, 0.50, and 1 mg/kg/d

			IsMn (mg/kg/d)		
		Placebo (n = 8)	0.25 (n = 6)	0.50 (n = 8)	1 (n = 11)
PP (mm Hg)	day 8	10.4 ± 1.2	7.2 ± 1.4*	7.8 ± 0.6*	6.2 ± 0.5*
PV (mL)	day 8	13.0 ± 0.3	11.1 ± 1.0	13.1 ± 0.4	10.9 ± 0.9
AP (mm Hg)	day 8	76 ± 12	71 ± 7	68 ± 8	49 ± 6*
FE Na^+ (%)	day 0	0.54 ± 0.06	0.62 ± 0.08	0.56 ± 0.04	0.58 ± 0.05
	day 7	0.58 ± 0.03	0.49 ± 0.05	0.50 ± 0.01	0.51 ± 0.02
Ur $(Na^+)/(K^+)$	day 0	0.26 ± 0.04	0.24 ± 0.02	0.27 ± 0.02	0.27 ± 0.01
	day 7	0.30 ± 0.03	0.38 ± 0.05	0.27 ± 0.02	0.33 ± 0.02
Cl_{H_2O} (mL/min)	day 0	−62 ± 14	−73 ± 4	−69 ± 5	−68 ± 5
	day 7	−52 ± 5	−64 ± 4	−81 ± 4†	−76 ± 3*
CreaCl (mL/min)	day 0	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
	day 7	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1

NOTE. Data are expressed as means ± SEM.

Abbreviations: PP, portal pressure; PV, plasma volume; AP, mean arterial pressure; FE Na^+, urinary fractional sodium excretion; Cl_{H_2O}, free water clearance; CreaCl, creatinine clearance.

* $P < .05$ compared with placebo.

† $P < .001$ compared with placebo.

TABLE 3. Hemodynamic and Renal Effects of a Treatment With SP (1 mg/kg/d) Alone or in Combination With IsMn 0.5 and 1 mg/kg/d IsMn 1

		Placebo (n = 8)	SP (n = 6)	SP + IsMn 0.5 (n = 8)	SP + IsMn 1 (n = 6)
PP (mm Hg)	day 8	10.4 ± 1.2	5.7 ± 0.7†	6.9 ± 1.0*	7.0 ± 0.5*
PV (mL)	day 8	13.1 ± 0.3	11.6 ± 0.3	8.5 ± 0.4‡	9.8 ± 0.4‡
AP (mm Hg)	day 8	76 ± 12	68 ± 5	69 ± 9	65 ± 10
FE Na^+ (%)	day 0	0.54 ± 0.06	0.55 ± 0.03	0.63 ± 0.03	0.54 ± 0.04
	day 7	0.58 ± 0.03	0.69 ± 0.07*	0.64 ± 0.05	0.38 ± 0.07*§
Ur $(Na^+)/(K^+)$	day 0	0.26 ± 0.04	0.35 ± 0.03	0.31 ± 0.02	0.27 ± 0.02
	day 7	0.30 ± 0.03	0.40 ± 0.03*	0.30 ± 0.02	0.22 ± 0.01‡‖
Cl_{H_2O} (mL/min)	day 0	−62 ± 14	−70 ± 5	−72 ± 3	−66 ± 4
	day 7	−52 ± 5	−69 ± 7	−71 ± 5*	−52 ± 4
CreaCl (mL/min)	day 0	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.0 ± 0.1
	day 7	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.2

NOTE. Data are expressed as means ± SEM.

Abbreviations: PP, portal pressure; PV, plasma volume; AP, mean arterial pressure; FE Na^+, urinary fractional sodium excretion; Cl_{H_2O}, free water clearance; CreaCl, creatinine clearance; SP, spironolactone.

* $P < .05$.

† $P < .01$.

‡ $P < .001$ compared with placebo treatment.

§ $P < .03$.

‖ $P < .001$ compared with spironolactone 1 mg/kg alone.

a correlation between the decrease in HVPG or in variceal pressure and a diminution of plasma volume has not been shown.[5,7] Furthermore, only spironolactone and not furosemide appeared to reduce HVPG in chronic conditions in man.[5] In the present study with portal hypertensive rats, low doses of the spironolactone decreased portal pressure as effectively as a higher dose and this occurred without any effect on systemic hemodynamics, circulating plasma volume or on renal Na^+ handling. Our data suggest that spironolactone or its metabolites may have a direct vasoactive effect on splanchnic and/or portal circulation. Such a direct effect of spironolactone on the splanchnic vascular system, not mediated by aldosterone antagonism, has in fact previously been suggested by studies *in vitro.*[16,17] Dacquet et al.[17] showed the calcium antagonistic properties of spironolactone on smooth muscle cells in the portal vein in rats with vasodilatation as a result. Spironolactone seemed also to exert a direct and dose-dependent vasodilatory effect on the mesenteric vascular bed in rats.[16] The drug counteracted vasopressor effects of noradrenaline and acetylcholine, and this action was not mediated by aldosterone antagonism. A calcium channel blocking effect of spironolactone *in vitro* was later confirmed.[18,19] The question arises, however, whether the effect of spironolactone observed *in vitro,* can be extrapolated to the *in vivo* situation, taking into account the short plasma half-life of spironolactone.[20,21] Metabolites of spironolactone with a longer plasma half-life might also cause vasodilatation. The vasodilatory effect *in vivo* has only been shown till now in patients with arterial hypertension.[22] Further studies will have to clarify whether this calcium channel blocking activity of spironolactone can explain the portal pressure reducing effect observed, especially because calcium antagonists such as nifedipine failed to reduce portal pressure in man.[23,24]

It will be of particular interest to investigate whether low doses of spironolactone will also reduce portal hypertension in man. Indeed, with somewhat higher doses of spironolactone (1.50 mg/kg), a high drop-out rate was observed in our male patients under chronic spironolactone therapy because of painful gynecomastia.[7] The latter side-effect of spironolactone is known to be dose-dependent.[25]

Nitrates have been proposed in the treatment of portal hypertension in patients[8,9,12] and different dose-dependent mechanisms were proposed to explain the reduction in portal pressure.[26] Since it was suggested that nitrates can lead to plasma volume expansion,[27] spironolactone could provoke an additional effect on portal venous pressure. However, in the present study, an additional decrease in portal pressure was not observed. A disadvantage of treatment with nitrates is represented by the known deleterious effect on ascites formation.[12] Indeed, also in the present study in rats, a reduced free water excretion and a tendency to retain Na^+ were observed. These effects of nitrates have been ascribed to activation of the renin-angiotensin-aldosterone system.[11,27] To counteract this water and Na^+ retention, nitrates might have to be combined with a dose of spironolactone (1-1.5 mg/kg) higher than the one needed to lower portal pressure. These combination treatments did not impair glomerular filtration rate, as was reasonably but not fully accurately measured by the creatinine clearance.

In conclusion, low doses of spironolactone reduce portal pressure in portal hypertensive rats as efficiently as higher doses, but without significant changes in plasma volume. This suggests a direct effect of the drug or of its metabolites on splanchnic and/or portal venous circulation. Low doses of spironolactone, which have less side-effects than the usual doses should therefore be explored in the prevention of variceal bleeding in man. Spironolactone counteracts the water and salt retention induced by IsMn, without deleterious effects on renal function. Spironolactone could therefore be advocated to be used with nitrates when the latter are given to patients with portal hypertension.

Acknowledgment: We are grateful to Mr. R. Michiels for technical assistance and Mrs. R. Santy for secretarial assistance.

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