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Adrenergic agonists and experimental ascitic cirrhosis.

**DOSE-DEPENDENCY OF CLONIDINE'S EFFECTS IN ASCITIC CIRRHOTIC RATS:  
COMPARISON WITH  $\alpha$ 1-ADRENERGIC AGONIST MIDODRINE.**

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**List of abbreviations:** A, aldosterone; ADH, vasopressin; BDL, bile duct-ligated; CCl<sub>4</sub>, carbon tetrachloride; CIN, steady-state plasma clearance of inulin; CK, potassium clearance; CNa, sodium clearance; Cosm, osmolar clearance; CPAH, steady-state plasma clearance of para-aminohippurate; EABV, effective arterial blood volume; FEK, fractional excretion of potassium; FENa, fractional excretion of sodium; FF, filtration fraction; FIna, filtered sodium load; GFR, glomerular filtration rate; IN, inulin; MAP, mean arterial pressure; N, norepinephrine; NO, nitric oxide; PAH, para-aminohippurate; PVL, portal vein-ligated; Posm, plasma osmolality; PRA, plasma renin activity; RAS, renin-angiotensin system; RPF, renal plasma flow; SD, standard deviation; SMT, standard medical therapy; SNS, sympathetic nervous system; TF-WR, tubular free-water reabsorption; Uosm, urine osmolality.

**Keywords.**  $\alpha$ -adrenoceptor agonists; experimental cirrhosis; ascites; cirrhosis complications.

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## ABSTRACT

**Background.** Sympathetic nervous system (SNS) activation decreases fluid delivery to the Henle's loop and response to diuretics; paradoxically, both  $\alpha$ 1-adrenoceptor agonists and sympatholytic  $\alpha$ 2-adrenoceptor agonists are recommended in the management of ascitic cirrhosis. **Aims & Methods.** We assessed the effects of increasing doses of clonidine ( $\alpha$ 2-agonist) vs. midodrine ( $\alpha$ 1-agonist) on renal function, mean arterial pressure (MAP), and hormonal status in rats with ascitic cirrhosis due to 13-week CCl<sub>4</sub> administration (groups G1-G5), in comparison with control rats (Gc), rats with ascitic cirrhosis untreated (G6) or treated with daily diuretics (0.5 mg/kg furosemide plus 2 mg/kg K<sup>+</sup>-canrenoate during the 11<sup>th</sup>-13<sup>th</sup> weeks of CCl<sub>4</sub>) (G7). G1-G5 cirrhotic rats received daily, during the 11<sup>th</sup>-13<sup>th</sup> CCl<sub>4</sub> weeks: clonidine 0.3 mcg only (G1), diuretics + clonidine 0.2 (G2), 0.5 (G3), or 1 mcg (G4), diuretics + midodrine 1 mg/kg b.w. (G5). **Results.** Cirrhotic rats in G1 or G2 had higher glomerular filtration rate, renal plasma flow, and natriuresis than cirrhotic rats treated with diuretics (G7) (all P<0.05). The addition of clonidine 0.2 mcg to diuretics (G2 vs. G7) reduced serum norepinephrine (169 ± 71 vs. 523 ± 88 ng/L) and plasma renin activity (12 ± 3 vs. 25 ± 5 ng/mL/h) (all P<0.05). Midodrine did not improve the renal performance in ascitic rats treated with diuretics. In comparison to absolute cirrhotic controls (G6), MAP was lower in G4 and higher in G5 (all P<0.05). **Conclusions.** Low-dose  $\alpha$ 2-agonists improve natriuresis and reduce SNS function and hyper-aldosteronism without affecting arterial pressure in experimental ascitic cirrhosis treated with diuretics.

### KEY POINTS BOX

- Current literature advances the paradoxical notion that both adrenolytic vasodilators and vasoconstricting adrenergic agonists are useful in the management of refractory cirrhotic ascites.
- This study shows that only a non-hypotensive dosage of clonidine ( $\alpha_2$ -adrenoceptor agonist and adrenolytic agent) potentiates the effects of diuretics, and reduces adrenergic hypertone and hyperreninism in experimental ascitic cirrhosis.
- Midodrine ( $\alpha_1$ -adrenoceptor agonist and vasoconstrictor agent) does not add any advantage to the diuretic treatment of ascites.
- The sole diuretics are followed by deleterious renal effects due to the adrenergic hypertone and secondary aldosteronism they invariably elicit.

## INTRODUCTION

Cirrhotic patients with advanced liver disease develop renal sodium retention, accumulation of ascites and, in turn, refractory ascites. The latter is defined as ascitic fluid that can no longer be treated with standard diuretics, either because of intolerable side effects (diuretic-intractable ascites) or because of genuine unresponsiveness to the use of natriuretic agents (diuretic-resistant ascites) [1-4].

Several factors contribute to this chain of clinical events. Peripheral arterial vasodilatation and portal hypertension reduce the effective arterial blood volume (EABV) and cause a permanent activation of endogenous anti-natriuretic and vasoconstrictor mechanisms (renin-angiotensin [RAS] and sympathetic nervous [SNS] systems, non-osmotic hypersecretion of vasopressin [ADH]) [5, 6]. Difficult-to-treat ascites occurs as a result of extreme vascular underfilling with maximal activation of these systems [1]. Among the above hormonal factors, the activated SNS stimulates kidney arterial vasoconstriction, with ensuing decrease in renal blood flow and glomerular filtration rate. Moreover, norepinephrine directly increases reabsorption of sodium in the proximal renal tubule, which leads to slight, if any, response to standard diuretics and to further secretion of renin, aldosterone and ADH [7].

In advanced ascitic cirrhosis, different vasoconstrictors have been tried out in order to counter the splanchnic arterial vasodilatation that contributes considerably to portal hypertension and renal sodium retention. This is paradoxical, because, in advanced cirrhosis, systemic vasoconstrictor systems (catecholamines, ADH and renin) are naturally overactive [5-7], and because impaired arterial reactivity to endogenous and exogenous vasoconstrictors has been repeatedly described [8]. Alongside intravenous catecholamines themselves [9] and vasopressin analogues [10, 11], generally used in association with plasma expanders or albumin, midodrine, an oral selective  $\alpha$ 1-

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adrenoceptor agonist [12], has been employed in several studies to cause arterial vasoconstriction and to improve systemic hemodynamics, effective arterial blood volume and renal function.

Midodrine plus standard medical therapy (SMT) has been reported to improve the systemic hemodynamics and to be superior to SMT alone for the control of ascites [13]. In addition, midodrine, associated with octreotide and intravenous albumin, has been suggested to reverse even type 1 hepatorenal syndrome and its severe sodium-retention [14]. However, the results of the administration of midodrine, with or without octreotide, remain still controversial [11], when not disputed openly [15, 16].

Recently, in patients with cirrhosis and gross ascites a different approach to sodium retention has been undertaken with the aim of improving the efficacy of traditional diuretics: clonidine,  $\alpha_2$ -adrenoceptor agonist, has been used on the grounds that this drug reduces central sympathetic outflow, efferent sympathetic neuronal firing, and release of norepinephrine from vascular neuroeffector junctions [17]. Indeed, inhibition of sympathetic nervous activity with clonidine decreases portal pressure in alcoholic cirrhosis [18]. Moreover, clonidine restored the effects of diuretics (spironolactone alone or the combination of furosemide and spironolactone) in patients with cirrhosis and refractory ascites [19, 20].

Nonetheless, some drawbacks might emerge with the use of clonidine in patients with advanced cirrhosis. First, clonidine, an arterial vasodilator, through stimulation of endothelial  $\alpha_{2D}$ -receptors enhances vascular production of nitric oxide (NO) [21], which may lead to worsening of the hyperdynamic circulation of cirrhotic patients. Second, the stimulation of NO synthesis may increase the expression of apical  $\text{Na}^+\text{-K}^+\text{-2Cl}^-$  cotransporters and reabsorption of sodium in the loop of Henle [22]. Finally, stimulation of  $\alpha_{2B}$ -adrenoceptors in the basolateral membrane of the proximal renal tubule, which cannot

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be avoided when clonidine is used, can accelerate sodium reabsorption in this tubular segment [23].

In the present study, performed on rats with carbon tetrachloride (CCl<sub>4</sub>)-induced advanced cirrhosis and ascites, we aimed: a) to investigate renal pharmacodynamics of different doses of clonidine associated with standard diuretics; b) to compare clonidine's effects to those obtained by a standard dosage of the vasoconstrictor midodrine and c) to try to shed light on the current paradoxical opinion that both adrenolytic vasodilators and vasoconstricting adrenergic agonists may be of some help in the management of advanced ascitic cirrhosis.

## MATERIALS AND METHODS

Studies were performed on seventy male adult Wistar rats with advanced liver cirrhosis and ten male adult Wistar control rats. All rats were fed *ad libitum* with standard chow and water. Cirrhosis was induced by CCl<sub>4</sub> (Riedel-de Haën, Sigma-Aldrich, Seelze, Germany) administered by gavage twice weekly for 13 weeks [24]. The pathophysiological progression of this model is predictable and reproducible: after 9 weeks, micronodular cirrhosis is evident, rats are devoid of ascites (as assessed by laparotomy) and portal pressure is increased to about 10 mmHg; after 11 weeks, rats are ascitic and their mean portal pressure is 24 mmHg; after 14 weeks, generally rats develop renal failure and eventually die [25, 26]. Rats were cared for in compliance with the European Council directives (No. 86/609/EEC) and with the Principles of Laboratory Animal Care (NIH No. 85–23, revised 1985). This scientific project was approved by the Ethical Committee of the University of Torino (permit number: D.M. 94/2012-B). In this study, the following active drugs were administered to the rats according to the protocol described in the next paragraphs: furosemide, Henle's loop diuretic (Sanofi-Aventis, Milan, Italy); potassium canrenoate, aldosterone receptor antagonist (Teofarma, Pavia, Italy); clonidine,  $\alpha_2$ -adrenoceptor agonist (Boehringer Ingelheim, Milan, Italy); midodrine,  $\alpha_1$ -adrenoceptor agonist (Lusofarmaco S.p.A., Milan, Italy).

**Animal groups.** Furosemide, K<sup>+</sup>-canrenoate, clonidine, and midodrine were dissolved in distilled water to obtain different solutions to be administered orally to the rats in 400  $\mu$ l of fluid. The animals were divided into eight groups of 10 rats: healthy control rats (G<sub>c</sub>), rats with ascitic cirrhosis due to 13-week CCl<sub>4</sub> administration receiving only oral clonidine (0.3 mcg daily between the beginning of the 11<sup>th</sup> and the end of the 13<sup>th</sup> week of CCl<sub>4</sub> (G1); cirrhotic rats treated daily with oral diuretics (0.5 mg/kg b.w. furosemide plus 2 mg/kg b.w. K<sup>+</sup>-canrenoate) plus clonidine 0.2 (G2), 0.5 (G3), or 1 mcg (G4) during the 11<sup>th</sup>-

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13<sup>th</sup> weeks of CCl<sub>4</sub>; cirrhotic rats treated daily with oral standard diuretics plus midodrine 1 mg/kg b.w. over the 11<sup>th</sup>-13<sup>th</sup> weeks of CCl<sub>4</sub> (G5); cirrhotic rats receiving CCl<sub>4</sub> for 13 weeks but no pharmacological intervention at all (G6); cirrhotic rats treated only with oral furosemide and potassium canrenoate (see above dosage) during the 11<sup>th</sup>-13<sup>th</sup> weeks of CCl<sub>4</sub> (G7). Dosage of diuretics was patterned on respective standard daily human dosage. Clonidine was administered in increasing doses, from sub-pressor (0.2 mcg) to arterial hypotensive dosage (1 mcg) (Table 1). The dosage of midodrine 1 mg/kg b.w. was chosen because in previous experiments in our laboratory it demonstrated to improve mean arterial pressure (MAP) by at least 10 mm Hg in rats with experimental cirrhosis, a vasopressor effect that was confirmed in this study (Table 1).

**Study protocol.** Rats belonging to G<sub>c</sub> and G1-G7 were weighed, studied and finally sacrificed at the end of week 13 of observation or CCl<sub>4</sub> administration, respectively, with or without the above respective active drug treatments and within 8 hours after the latest drug administration. Each day of study, rats were anesthetized with a mixture of Ketavet 100 (Farmaceutici Gellini, Sabaudia, Italy) and Rompum (Xilazina, Bayer A.G., Leverkusen, Germany) (4:1 v:v) by intraperitoneal injection (0.5 ml mixture/200 g b.w.), as described elsewhere [27]; laparotomy was performed, ascites was evident, even if not quantified, in all rats studied after 13 weeks of CCl<sub>4</sub> administration, and the urinary bladder was emptied before clamping the urethral orifice for further urine collection. Shortly thereafter, inulin (IN) 10% (w/v) (Laevosan-Gesellschaft, Linz/Donau, Austria) plus para-aminohippurate (PAH) 20% (w/v) (Nephrotest, BAG GmbH, Munich, Germany) were administered into the caudal vein as a priming bolus followed by a continuous infusion, in order to assess glomerular filtration rate (GFR) and renal plasma flow (RPF) by means of their respective steady-state plasma clearances (CIN and CPAH) [28, 29]. When 90 minutes of IN and PAH infusion had elapsed (i.e. once their steady-state plasma concentrations had been reached),

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cardiac blood was sampled to assess plasma osmolality and concentrations of inulin, PAH, sodium, and potassium. Blood samples withdrawn at this time were also used to measure plasma concentrations of vasopressin (ADH), plasma renin activity (PRA), aldosterone (A), and norepinephrine (N). Finally, urinary bladder was emptied to collect the urine volume produced during the 90 min of IN and PAH venous infusion. This urine was used to determine its osmolality and the excretion of sodium and potassium. Rats were then killed by exsanguination through the aorta. Anesthetized [27] rats in each group had their mean arterial pressure evaluated through tail sphygmomanometry [25], before performing laparotomy.

**Plasma and urine analyses.** Plasma and urinary concentrations of electrolytes, and IN and PAH plasma concentrations were measured as described elsewhere [26, 30, 31]. Plasma A, ADH, N, and PRA were determined according to standard procedures [25, 32].

**Calculations.** Sodium and potassium clearances (CNa and CK) were calculated through the usual formula [32]. Inulin clearance (CIN) and para-aminohippurate clearance (CPAH) were calculated through the steady-state plasma clearance formula as:

$$C_x = \text{Infusion rate (x)} / \text{ssP-x}$$

where ssP-x is the steady-state plasma concentration of x. CIN and CPAH were taken as measures of GFR and RPF, respectively [28, 29]. Filtration fraction (FF) and filtered sodium load (FINa) were calculated through the usual formulae [32].

Fractional sodium excretion (FENa) and fractional potassium excretion (FEK) were also calculated [27].

Tubular free-water reabsorption (TF-WR) was calculated, following Rose and Post [33], through the formula:

$$\text{TF-WR} = \text{Cosm} - V$$

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where V is the urinary output (ml/min) and Cosm is the osmolar clearance, which was computed via the usual formula:

$$\text{Cosm} = (\text{Uosm} \times \text{V}) / \text{Posm}$$

where Uosm and Posm are urine and plasma osmolalities, respectively.

Mean arterial pressure (MAP) was calculated from the formula:

$$1/3 (\text{systolic blood pressure} - \text{diastolic blood pressure}) + \text{diastolic blood pressure}.$$

**Statistical analysis.** Comparisons among groups of rats were made by one-way analysis of variance (ANOVA) followed by Tukey's LSD post-hoc comparisons. Results are expressed as means  $\pm$  SD. Significance is accepted at the 5 % probability level.

## RESULTS

**Increased natriuretic efficiency of low-dose  $\alpha$ 2-adrenergic agonists versus the other treatments. (Table 1).** In these cirrhotic rats, in all of which at laparotomy ascites was evident, even if its amount was not quantified, clonidine alone (group G1) or low-dose clonidine plus daily diuretics (G2) prompted significantly higher values of urinary sodium excretion rate and fractional sodium excretion than midodrine plus diuretics (G5) or high-dose clonidine plus diuretics (G3 and G4) or the sole diuretics (G7) did. In particular, clonidine 0.2 mcg (plus diuretics) and clonidine 0.3 mcg (not associated with diuretics) resulted in larger tubular natriuretic effects than in the other treatment groups. The highest values of absolute urinary flow rate were found in groups G1 (clonidine alone), G2 (clonidine 0.2 mcg plus diuretics) and G7 (diuretics alone) (all values significantly higher than among untreated cirrhotic rats in G6). The addition of a vasopressor dose of midodrine to standard diuretics (in G5) was ineffective in achieving increased natriuresis or urine volume, due to a significant derangement of GFR and despite the measured increase in sodium fractional excretion with respect to untreated ascitic rats (G6). Remarkably, compared with G6 cirrhotic control rats, clonidine alone (G1) and low-dose clonidine plus diuretics (G2) caused actual improvement of the parameters reflecting renal circulation (i.e. renal plasma flow and GFR), at variance with standard diuretics and, mostly, the association of midodrine and diuretics. The physiological increase in filtration fraction found in the cirrhotic group treated with diuretics alone (G7) versus G6, expression of effective autoregulation (i.e. efferent glomerular arteriolar vasoconstriction to preserve GFR in the face of effective arterial blood volume loss), did not occur in the rat group receiving  $\alpha$ 1-adrenoceptor agonists (this time expression of afferent glomerular arteriolar vasoconstriction and GFR loss). Tubular free-water reabsorption was reduced in group G2 vs. cirrhotic rats receiving diuretics (G7) (ancillary aquaretic effects of  $\alpha$ 2-adrenoceptor

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agonists) (Table 1). Healthy control rats (group G<sub>c</sub>) had significantly higher values of CPAH, CIN, urinary sodium excretion rate, fractional sodium excretion, and urine volume than untreated cirrhotic rats (G6).

**Hormonal status (Table 2).** Clonidine, in combination with diuretics or not, blunted the adrenergic hyperfunction of advanced liver cirrhosis, as shown by reduced levels of serum norepinephrine in ascitic cirrhotic rats belonging to groups G1, G2, G3 and G4 vs. cirrhotic rats, whether untreated (G6) or treated with diuretics (G7). PRA and plasma aldosterone (in G1, G2 and G3) were significantly lower than in ascitic cirrhotic rats, whether treated or not with diuretics (G6 and G7). Indeed, the peak value of plasma aldosterone (i.e. secondary aldosteronism) was found in the group of cirrhotic rats treated with sole diuretics (G7). Plasma levels of ADH went largely unaffected by the current dosage of diuretics and/or  $\alpha$ 2- and  $\alpha$ 1-adrenergic agonists. Healthy control rats (group G<sub>c</sub>) had significantly lower levels of PRA, plasma aldosterone, norepinephrine, and ADH than untreated cirrhotic rats (G6) and cirrhotic rats treated with standard diuretics (G7).

**Mean arterial pressure (Table 1).** When compared to absolute cirrhotic controls (G6), significantly lower values of MAP ( $P < 0.05$ ) were measured in the group of cirrhotic rats receiving high-dose clonidine plus diuretics (G4), while significantly higher MAP values ( $P < 0.05$ ) were found in the cirrhotic group receiving midodrine and diuretics (G5).

## DISCUSSION

This pathophysiological study shows that low-dose clonidine,  $\alpha_2$ -adrenoceptor agonist endowed with adrenolytic properties, used alone or in addition to standard diuretics, is effective in increasing the natriuresis produced by the use of standard diuretics in experimental ascitic cirrhosis. Conversely, the vasoconstrictor midodrine, used with diuretics in a dosage capable of increasing arterial pressure significantly, is far less effective when used to improve sodium excretion parameters, with the only exception of the beneficial effects exerted on fractional sodium excretion (tubular diuretic effect of midodrine).

The larger natriuretic effects of clonidine plus diuretics vs. diuretics alone was found only when a low, non-hypotensive dosage of clonidine was employed (0.2 mcg daily in G2). Amazingly enough, a natriuresis was elicited even when low-dose clonidine (0.3 mcg daily in G1) was used without diuretics. This suggests the obvious need to avoid any hypotensive dosage when this adrenolytic agent is used in liver cirrhosis.

The only dosage of midodrine we decided to test (1 mg/kg b.w.), still in the range of doses able to increase arterial pressure significantly (Table 1), as suggested in the papers that recommend this vasoconstrictor as a valid adjunct to standard medical therapy to treat ascites or hepatorenal syndrome [13, 14], was not able (in association with diuretics) to improve urinary sodium excretion or renal function (Table 1). This is shown by the detrimental effects of midodrine on GFR (Table 1), which was not accompanied by a parallel decrease in RPF, leading to a significant decrease in filtration fraction (FF). This further suggests that midodrine 1 mg/kg b.w. (associated with diuretics) either led to a definite squeezing of glomerular afferent arteriole or to a redistribution of RPF towards the renal medulla, according to the vasoconstrictive activity of the drug.

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The lack of relevant beneficial effects of midodrine in our model of ascitic liver cirrhosis at least echoes the results found with the use of this drug alone in patients with hepatorenal syndrome [34], or when midodrine was used with octreotide to treat the sodium-retentive complications of portal hypertension [15], or as sole treatment of post-paracentesis circulatory dysfunction [16]. Actually, midodrine proved to be of some help in relieving the circulatory dysfunction of advanced liver cirrhosis only when associated with the infusion of albumin (plasma expander) and octreotide [14]. Indeed, the mere combination of the renal vasoconstrictive effects of midodrine with the loss of extracellular fluid volume caused by diuretics led, in our study, to further loss of glomerular perfusion and effective arterial blood volume (EABV) or both, as witnessed by the midodrine-induced increase in PRA and systemic catecholamine levels (Table 2).

Recently, Coll and colleagues described the beneficial effects on mesenteric arterial circulation and renal function exerted by droxidopa, an oral synthetic precursor of catecholamines, in portal vein-ligated (PVL) and bile duct-ligated (BDL) rats [35]. Both experimental models employed by Coll are different in every way from the CCl<sub>4</sub> model used in this paper because PVL and BDL rats do not produce ascites but in rare cases.

The beneficial effects of clonidine on renal function and sodium excretion in experimental ascitic cirrhosis are dose-dependent. All doses of clonidine used in this study, even the arterial hypotensive daily dosage of 1 mcg, caused significant decrease of adrenergic function, as expected due to the pharmacological properties of this  $\alpha_2$ -adrenoceptor agonist (Table 2). However, PRA values increased in proportion with the doses of clonidine and showed actual derangement of the effective arterial perfusion of the kidney when a hypotensive dosage of this adrenolytic agent (1 mcg daily) was used (Table 2).

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The above beneficial effects of low-dose clonidine and some limitations of this experimental study deserve further discussion. First, the significant increase in FENa observed in G1 and G2 (Table 1) underlines a direct tubular diuretic effect of clonidine in these groups, when compared to the control cirrhotic group (G6). This may be the consequence of either clonidine adrenolytic effects (i.e. lower systemic catecholamine levels lead to lesser sodium retention in the proximal convoluted tubule) [7] or specific activation of renal  $\alpha_{2A}$ -adrenoceptors, which increase osmolar clearance and sodium excretion in a peculiar naltrexone (opioid receptor antagonist)-sensitive manner [36]. Indeed, a natriuretic function for the renal tubular  $\alpha_{2A}$ -adrenoceptors has been clearly established. Second, clonidine-dependent decrease in PRA (Table 2) shows a beneficial effect of this drug on the blood perfusion of the macula densa or on effective arterial blood volume (EABV), but only when used in non-hypotensive dosage. The putative increase in EABV, which followed the decreased systemic levels of catecholamines due to low-dose clonidine, might resemble the splanchnic vasoconstrictive effects of the  $\beta$  adrenergic block due to carvedilol in cirrhotic patients [37]. Anyway, these similar effects of clonidine and  $\beta$ -blockers cannot be demonstrated in this study. Third, inhibition of sympathetic nervous activity with clonidine decreases portal pressure in human liver cirrhosis [18]. This effect should have occurred also in our rats with experimental ascitic cirrhosis, but the measurement of portal pressure was not the focus of this study and was not performed. Finally, our results, which were achieved with an adrenolytic agent, suggest that the degree of spontaneous activation of the adrenergic system in ascitic cirrhosis might be too strong for the body needs and detrimental to renal function. This overwhelming activation of adrenergic function has already been demonstrated in patients with severe congestive heart failure, who are now effectively treated with  $\beta$ -blockers [38].

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In summary, this study confirms, in the experimental model, the findings of previous papers that showed the potentiation of the effects of standard diuretics by the addition of clonidine in advanced liver cirrhosis [19, 20]. What's more, our study shows that only non-hypotensive doses of clonidine may be useful. The role of midodrine,  $\alpha$ 1-adrenoceptor agonist, remains debatable in the setting of cirrhotic ascites. Potential therapeutic opportunities might arise, in future studies dealing with experimental ascitic cirrhosis, from the assessment of the renal effects of more specific  $\alpha$ <sub>2A</sub>-adrenoceptor agonists [36]. Finally, diuretics, while exerting their natriuretic effects, further reduce the effective arterial blood volume and stimulate counter-natriuretic forces (i.e. adrenergic function and renin-angiotensin system). The addition of low-dose clonidine to diuretics may avert these adverse hormonal effects (Table 2). Finally, the traditional treatment with diuretics only, which further reduce the effective arterial blood volume, may be regarded as a somewhat rudimentary approach to ascites.

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**Table 1.** Body weight and renal function in the different rat groups.

|                      | Group G <sub>c</sub> | G1                     | G2                     | G3                     | G4                     | G5           | G6         | G7           |
|----------------------|----------------------|------------------------|------------------------|------------------------|------------------------|--------------|------------|--------------|
| MAP (mm Hg)          | 93 ± 5               | 90 ± 5                 | 92 ± 7                 | 87 ± 6                 | 83 ± 3*                | 97 ± 4*      | 89 ± 4     | 87 ± 6       |
| Body weight (g)      | 401 ± 97             | 352 ± 37*              | 352 ± 40*              | 331 ± 38*              | 384 ± 47               | 405 ± 45     | 401 ± 27   | 357 ± 25*    |
| CPAH (ml/min)        | 4.4 ± 1.0**          | 5.4 ± 1.3**            | 3.2 ± 0.3**            | 2.9 ± 0.29             | 2.4 ± 0.22             | 2.4 ± 0.25   | 2.3 ± 0.21 | 2.1 ± 0.31   |
| CIN (ml/min)         | 2.2 ± 0.3**          | 1.8 ± 0.3**            | 1.62 ± 0.3**           | 1.54 ± 0.45*           | 1.17 ± 0.35            | 0.56 ± 0.25* | 1.0 ± 0.31 | 1.1 ± 0.47   |
| FF (%)               | 53 ± 10*             | 33 ± 10                | 60 ± 10*               | 54 ± 11*               | 40 ± 6                 | 23 ± 6*      | 34 ± 8     | 49 ± 6*      |
| Urine volume (ml/h)  | 1.7 ± 0.15*          | 1.83 ± 0.25*           | 1.73 ± 0.31*           | 1.31 ± 0.46            | 1.27 ± 1.05            | 1.38 ± 0.07  | 1.4 ± 0.15 | 1.87 ± 0.51* |
| Natriuresis (µmol/h) | 112 ± 21*            | 120 ± 21 <sup>ψ</sup>  | 128 ± 31 <sup>ψ</sup>  | 96 ± 15 <sup>¥</sup>   | 65 ± 14 <sup>¥</sup>   | 74 ± 13      | 62 ± 12    | 92 ± 11*     |
| FENa (%)             | 2.3 ± 0.3*           | 2.1 ± 0.2 <sup>ψ</sup> | 3.1 ± 0.5 <sup>ψ</sup> | 1.3 ± 0.2 <sup>¥</sup> | 1.4 ± 0.2 <sup>¥</sup> | 1.7 ± 0.3*   | 1.0 ± 0.22 | 1.8 ± 0.2*   |
| Kaliuresis µmol/h)   | 37 ± 14              | 33 ± 12                | 45 ± 14                | 59 ± 15                | 44 ± 20                | 33 ± 10      | 40 ± 12    | 52 ± 18      |
| FEK (%)              | 9.2 ± 2.7            | 8.1 ± 1.7              | 9.1 ± 2                | 8.3 ± 2                | 9.6 ± 2.1              | 8 ± 1.8      | 8.2 ± 1.8  | 8.5 ± 2.2    |
| Plasma Na (mEq/l)    | 139 ± 4              | 141 ± 5                | 140 ± 4                | 136 ± 4                | 133 ± 7                | 137 ± 4      | 138 ± 4    | 137 ± 4      |
| Plasma K (mEq/l)     | 3.9 ± 0.9            | 4 ± 0.7                | 4.1 ± 0.7              | 3.2 ± 0.3              | 3.3 ± 0.3              | 3.6 ± 0.3    | 3.7 ± 0.4  | 3.6 ± 0.3    |
| Cosm (ml/h)          | 1.98 ± 0.5*          | 1.91 ± 0.6*            | 1.55 ± 1.2             | 2.03 ± 0.5*            | 1.77 ± 1.13            | 1.51 ± 1.11  | 1.34 ± 0.3 | 1.91 ± 0.6*  |
| TF-WR (microl/min)   | 87 ± 20**            | 50 ± 21                | 34 ± 5***              | 35 ± 12                | 39 ± 18                | 32 ± 15      | 32 ± 11    | 45 ± 6       |

Rat groups: G<sub>c</sub>, control rats; G1, ascitic cirrhotic rats treated with daily clonidine 0.3 mcg; G2, ascitic cirrhotic rats treated with daily diuretics (0.5 mg/kg b.w. furosemide plus 2 mg/kg b.w. K<sup>+</sup>-canrenoate) plus clonidine 0.2 mcg; G3, ascitic cirrhotic rats treated with daily diuretics plus clonidine 0.5 mcg; G4, ascitic cirrhotic rats treated with daily diuretics plus clonidine 1 mcg; G5, ascitic cirrhotic rats treated with daily diuretics plus midodrine 1 mg/kg b.w.; G6, untreated rats with ascitic cirrhosis (absolute cirrhotic controls); G7, ascitic cirrhotic rats treated with daily diuretics.

Data are means ± SD. \*P<0.05 versus G6, cirrhotic control group; <sup>ψ</sup> P<0.05 versus G5, G6 and G7; <sup>¥</sup>P<0.05 versus G1 and G2; \*\*P<0.05 versus G6 and G7; \*\*\*P<0.05 versus G7 (One-Way ANOVA followed by Tukey's LSD post-hoc comparisons). CIN: steady-state plasma clearance of inulin; Cosm: osmolar clearance; CPAH: steady-state plasma clearance of para-aminohippurate; FEK: fractional excretion of potassium; FENa: fractional excretion of sodium; FF: filtration fraction; MAP: mean arterial pressure; TF-WR: tubular free-water reabsorption.

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**Table 2.** Hormonal status in the different rat groups.

|                    | Group G <sub>c</sub> | G1         | G2         | G3         | G4        | G5        | G6        | G7          |
|--------------------|----------------------|------------|------------|------------|-----------|-----------|-----------|-------------|
| PRA (ng/ml/h)      | 4.5 ± 0.7*           | 9 ± 0.9*   | 12 ± 1.6*  | 16 ± 1.4*  | 19 ± 2.7  | 27 ± 2.1  | 24 ± 2.4  | 25 ± 2.1    |
| Plasma A (pg/ml)   | 390 ± 101*           | 630 ± 201* | 687 ± 156* | 663 ± 117* | 796 ± 85  | 874 ± 298 | 940 ± 82  | 1163 ± 65** |
| Plasma N (ng/l)    | 122 ± 39*            | 182 ± 39*  | 169 ± 71*  | 304 ± 102* | 131 ± 98* | 547 ± 190 | 488 ± 102 | 523 ± 88    |
| Plasma ADH (pg/ml) | 27 ± 21***           | 97 ± 61    | 93 ± 55    | 88 ± 68    | 80 ± 71   | 85 ± 61   | 69 ± 57   | 65 ± 61     |

Rat groups: G<sub>c</sub>, control rats; G1, ascitic cirrhotic rats treated with daily clonidine 0.3 mcg; G2, ascitic cirrhotic rats treated with daily diuretics (0.5 mg/kg b.w. furosemide plus 2 mg/kg b.w. K<sup>+</sup>-canrenoate) plus clonidine 0.2 mcg; G3, ascitic cirrhotic rats treated with daily diuretics plus clonidine 0.5 mcg; G4, ascitic cirrhotic rats treated with daily diuretics plus clonidine 1 mcg; G5, ascitic cirrhotic rats treated with daily diuretics plus midodrine 1 mg/kg b.w.; G6, untreated rats with ascitic cirrhosis (absolute cirrhotic controls); G7, ascitic cirrhotic rats treated with daily diuretics.

Data are means ± SD. \*P<0.05 versus both G6, cirrhotic control group, and G7, cirrhotic group treated with diuretics; \*\*P<0.05 versus G6, cirrhotic control group; \*\*\*P<0.05 versus every other group (One-Way ANOVA followed by Tukey's LSD post-hoc comparisons). A: aldosterone; ADH: vasopressin; N: norepinephrine; PRA: plasma renin activity.