Catecholamines and water retention in cirrhosis.

PATHOGENESIS OF SOLUTE-FREE WATER RETENTION IN EXPERIMENTAL

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ABSTRACT

Background. Catecholamines trigger proximal tubular fluid retention and reduce renal excretion of solute-free water. In advanced cirrhosis, non-osmotic hypersecretion of vasopressin (ADH) is considered the cause of dilutional hyponatremia, but ADH V_2 receptor antagonists are not beneficial in long-term treatment of ascites. **Aim.** To test the hypothesis that water retention in experimental ascitic cirrhosis might depend primarily on adrenergic hyperfunction. **Methods.** Hormonal status, renal function and tubular free-water reabsorption (TFWR) were assessed in six groups of rats with ascitic cirrhosis: rats with cirrhosis due to 13-week CCl_4 administration (group G1); cirrhotic rats receiving daily diuretics (0.5 mg/kg furosemide plus 2 mg/kg K^+-canrenoate) from 11^{th} to 13^{th} week of CCl_4 (G2), diuretics associated with guanfacine oral prodrug (α_{2A} adrenergic receptor agonist and sympatholytic agent) 2 (G3), 7 (G4), or 10 mg/kg (G5), or with SSP-004240F1 (V_2 receptor antagonist) 1 mg/kg (G6). **Results.** Natriuresis was lower in G1 than in G2, G4 and G6 (all $P<0.05$). Guanfacine, added to diuretics (i.e. G3 vs. G2), reduced serum norepinephrine from 423 ± 22 to 211 ± 41 ng/L ($P<0.05$), plasma renin activity from 35 ± 8 to 9 ± 2 ng/mL/h ($P<0.05$), and TFWR from 45 ± 8 to 20 ± 6 microL/min ($P<0.01$). TFWR correlated with plasma aldosterone ($r=0.51$, $P<0.01$) and urinary potassium excretion ($r=0.90$, $P<0.001$). **Conclusion.** In ascitic cirrhosis, reduced volaemia, use of diuretics (especially furosemide), and adrenergic hyperfunction cause tubular retention of water. Suitable doses of sympatholytic agents are effective aquaretics.

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SUMMARY STATEMENT

Adrenergic hyperfunction reduces renal excretion of water.
In advanced cirrhosis, hypersecretion of vasopressin is considered the cause of dilutional hyponatremia.
We show that in experimental cirrhosis sympatholytic agents (α_{2A}-adrenoceptor agonists) are as effective as V_2-antagonists to blunt water retention.

Short title. Catecholamines and water retention in cirrhosis.

Keywords. α_2-adrenoceptor agonists; experimental cirrhosis; ascites; cirrhosis complications; dilutional hyponatremia.

Abbreviations used in this paper: A, aldosterone; ADH, vasopressin; CCl_4, 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; E, epinephrine; EABV, effective arterial blood volume; FEK, fractional excretion of potassium; FENa, fractional sodium excretion; FF, filtration fraction; FlNa, filtered sodium load; GFR, glomerular filtration rate; HRS, hepatorenal syndrome; IN, inulin; MAP, mean arterial pressure; N, norepinephrine; PAH, para-aminohippurate; Posm, plasma osmolality; PRA, plasma renin activity; RPF, renal plasma flow; SD, standard deviation; TFWR, tubular free-water reabsorption; Uosm, urine osmolality.

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INTRODUCTION

Impairment in body water homeostasis is common in patients with advanced cirrhosis and ascites. A higher rate of renal retention of water in relation to sodium, due to a reduction in solute-free water clearance, leads to a positive balance between water ingestion and excretion and to dilutional hyponatremia [1]. In turn, the severity of dilutional hyponatremia affects cirrhotic patients' survival rate significantly [2].

The inability of ascitic cirrhotic patients to excrete an adequate amount of solute-free water in the urine is related to the following mechanisms: i) baroreceptor-mediated non-osmotic stimulation of vasopressin (ADH) release due to arterial splanchnic vasodilatation and reduction of effective arterial blood volume (EABV) [3]; ii) reduced production of solute-free water in the ascending limb of the loop of Henle (where solute-free water is generated through reabsorption of Na^+, K^+ and Cl^- without water) as a consequence of reduced fluid delivery due to decreased glomerular filtration rate and/or increased sodium reabsorption in the proximal tubule [4-6]. In addition to the above mechanisms of dilutional hyponatremia, true hypovolemic hyponatremia should be mentioned: it accounts for only 10% of all cases of hyponatremia in patients with cirrhosis, is due to over-diuresis, loss of fluids from the gastrointestinal tract, or decreased fluid intake, and is clinically characterized by signs of dehydration, little ascites, and prerenal azotemia [7].

Significant reduction of effective arterial blood volume (EABV) triggers both excess isosmotic proximal tubular sodium retention (with reduced delivery of fluid to the Henle's loop) and non-osmotic hyper-secretion of ADH.

These two homeostatic mechanisms, which aim at preserving the size of functional volaemia by means of tubular solute-free water retention [8], have common cause and purpose. Nonetheless, excess isosmotic reabsorption of fluid in the proximal convoluted tubule, where normally 70% of the glomerular filtrate is reabsorbed, is clearly a limiting factor for the water-retentive action that hyper-secreted vasopressin might exert in the terminal nephron (i.e. in the collecting duct, where normally only 5% of the glomerular filtrate is reabsorbed) [8].

This assumption seems even more convincing when the behavior of tubular reabsorption of sodium and water in patients with liver cirrhosis is pondered. Lithium clearance (an established index of delivery of tubular fluid to the loop of Henle) is already significantly reduced in standing pre-ascitic cirrhotic patients [9-10]. Moreover, in advanced cirrhosis, systemic arterial pressure is maintained, despite the splanchnic arterial vasodilatation, through the activation of the systemic renin-angiotensin system (i.e. systemic generation of angiotensin II), the sympathetic nervous system and, later, the non-osmotic release of ADH [11]. Increased systemic and renal levels of catecholamines augment dramatically the isosmotic reabsorption of sodium and water in the proximal renal tubule, which leads further to negligible response to diuretics, refractory ascites, tubular water retention, and hyponatremia [12]. Indeed, progressive decrease in lithium clearance and fractional excretion (i.e. progressive reduction of fluid delivery to the Henle's loop) accompanies the worsening of cirrhotic disease from clinical compensation to the ascitic stage and eventually to refractory ascites [13]. This means that patients with severe cirrhosis, ascites and hyponatremia have but a minimal amount of fluid, less that 5% of glomerular filtrate, still reaching the collecting duct, where hyper-secreted vasopressin should exert its water-retentive effects.

Despite the above considerations, in recent literature and clinical practice, the use of vasopressin V_2 receptor antagonists has become the standard of care in order to try and treat dilutional hyponatremia in ascitic cirrhosis [14-15], but there is no evidence of

beneficial effects of these drugs on patients' survival or long-term management of ascites [16].

In the present study we intend to compare, in rats with liver cirrhosis and gross ascites due to 13-week carbon tetrachloride (CCl_4) administration, the aquaretic effects of a vasopressin V_2 receptor antagonist and of a sympatholytic agent, the oral prodrug of guanfacine, a selective α_{2A}-adrenoceptor agonist. In this setting, the contribution to tubular solute-free water retention of non-osmotic hyper-secretion of vasopressin and of excess proximal tubular sodium retention due to adrenergic hyper-function is dissected and analyzed both qualitatively and quantitatively.

MATERIALS AND METHODS

Studies were performed on sixty male adult Wistar rats with ascitic liver cirrhosis. All rats were fed with standardized chow and water [17-20]. Cirrhosis was induced by CCl_4 (Riedel-de Haën, Sigma-Aldrich, Seelze, Germany) administered by gavage twice a week for 13 weeks [17]. The hepatotoxic effects of this method are quite predictable: after 9 weeks of CCl_4, micronodular cirrhosis is histologically 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 (i.e. more than 75% of rats receiving CCl_4 are ascitic) and their mean portal pressure is about 24 mmHg; after 13 weeks more than 90% of treated rats are ascitic and roughly 1 in 10 rats are lost prior to experiments or scheduled sacrifice; after 14 weeks, rats develop renal failure and eventually die [17-20]. 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 paragraph: furosemide, Henle's loop diuretic (Sanofi-Aventis, Milano, Italy); potassium canrenoate, aldosterone receptor antagonist (Teofarma, Pavia, Italy); SSP-002021R, oral prodrug of guanfacine, selective α_{2A}-adrenoceptor agonist (Shire, Basingstoke, U.K.); SSP-004240F1, selective vasopressin V_2 receptor antagonist (Shire, Basingstoke, U.K.).

Animal groups. Furosemide, canrenoate, SSP-002021R, and SSP-004240F1 were dissolved in distilled water to obtain different solutions to be administered orally to the rats in 400 µl of fluid. The animals were divided into six groups of ten rats: rats with ascitic cirrhosis due to 13-week CCl_4 administration and receiving no active drug (group G1); cirrhotic rats treated daily with oral furosemide (0.5 mg/Kg b.w.) plus oral potassium canrenoate (2 mg/Kg b.w.) between the beginning of the 11th and the end of the 13th week of CCl_4 (three-week drug intervention study) (G2); cirrhotic rats treated with oral furosemide, oral potassium canrenoate (see above dosage), associated with the oral prodrug of guanfacine 2 (in G3), 7 (in G4), or 10 mg/kg (in G5) each day between the beginning of the 11th and the end of the 13th week of CCl_4; cirrhotic rats treated with oral furosemide, oral canrenoate, and oral SSP-004240F1, vasopressin V_2 receptor antagonist (1 mg/kg each day) between the beginning of the 11th and the end of the 13th week of CCl_4 (G6). Dosage of furosemide and potassium canrenoate was patterned on respective standard daily human dosage. The doses of SSP002021R and SSP-004240F1 were established by the provider of the drug (Shire, Basingstoke, U.K.); in this study the presence of ascites was evident at laparotomy, even if its amount was not quantified, in all rats studied after 13 weeks of CCl_4 administration (groups G1-G6).

Study protocol. Rats belonging to G1-G6 were weighed, studied and finally sacrificed at the end of 13 weeks of CCl_4 administration, with or without the above active drugs, which were administered according to schedule only over weeks 11 through 13 of CCl_4. The focus of this study was not to monitor the diuretic performance of these ascitic rat groups during different pharmacologic treatments, otherwise we would have used metabolic cages and assessed with ultrasound the presence of ascites at the beginning of the administration of active drugs (i.e. after 11 weeks of CCl_4). The true aim of this study was the exhaustive evaluation of renal function at the end of different three-week treatment periods. As such, on the final day of the study, i.e. after 13 weeks of CCl_4, 8 hours after the latest administration of active drugs, 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 [20]; laparotomy was performed 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) [21, 22]. When 90 minutes of IN and PAH infusion had elapsed (i.e. once their steady-state plasma concentrations had been reached), 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), epinephrine (E), 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. All anesthetized rats in each group had their mean arterial pressure evaluated through tail sphygmomanometry, as described elsewhere [18], before performing laparotomy.

Plasma and urine analyses. Plasma and urinary concentrations of electrolytes, and plasma concentrations of IN and PAH were measured as described elsewhere [19, 23, 24]. Plasma A, ADH, N, E, and PRA were determined according to standard procedures [18, 25].

Calculations. Sodium and potassium clearances (CNa and CK) were calculated through the usual formula [25]. Inulin clearance (CIN) and para-aminohippurate clearance (CPAH) were calculated through the steady-state plasma clearance formula as:

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

where ssP-x is the steady-state plasma concentration of x. CIN and CPAH were taken as measures of GFR and RPF, respectively [21, 22]. Filtration fraction (FF) and filtered sodium load (FlNa) were calculated through the usual formulae [25].

Fractional sodium excretion (FENa) and fractional potassium excretion (FEK) were also calculated [20].

Tubular free-water reabsorption (TFWR) was calculated, following Rose and Post [26], through the formula:

$$TFWR = Cosm - V$$

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

$$Cosm = (Uosm \times V)/Posm$$

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

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

1/3 (systolic blood pressure – diastolic blood pressure) + 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. Correlation coefficients were derived using Spearman's rank correlation. Results are expressed as means ± SD. Significance is accepted at the 5 % probability level.

RESULTS

Increased natriuretic and aquaretic efficiency of guanfacine and V_2 receptor antagonist plus diuretics vs. treatment with sole diuretics. (Table 1). In these ascitic cirrhotic rats, the administration of guanfacine 7 mg/kg plus diuretics (G4) prompted the highest values of urinary sodium excretion rate and fractional sodium excretion (i.e. the strongest tubular diuretic effects) among all experimental groups. Unexpectedly, cirrhotic rats treated with diuretics and the vasopressin V_2 receptor antagonist (G6) showed a similar natriuretic performance, and, indeed, the highest values of absolute urinary flow rate. The addition of high-dose guanfacine to diuretics (G5) was ineffective in order to achieve increased natriuresis or urine volume because of considerable arterial hypotensive effects. Remarkably, guanfacine 7 mg/kg plus diuretics (G4), alongside the above natriuretic effects, caused actual improvement of the parameters reflecting renal circulation (i.e. renal plasma flow and GFR), at variance with standard diuretics (G2) or the association of high-dose guanfacine and diuretics (G5). The sizeable increase in filtration fraction found in the cirrhotic group treated with diuretics alone (G2) was related to renal autoregulation (i.e. efferent glomerular arteriolar vasoconstriction to preserve GFR) following effective arterial blood volume loss and secondary adrenergic hyper-function (read later). Tubular free-water reabsorption was reduced to a very similar extent in groups G3 (low-dose guanfacine plus diuretics) and G6 (vasopressin V_2 receptor antagonist plus diuretics) vs. G1 (untreated ascitic cirrhosis) or G2 (ascitic cirrhosis treated with sole diuretics). As a consequence, the dilutional hyponatremia found in G1-G2 was corrected in G3 and G6. No statistically significant correlation was found between tubular free-water reabsorption (TFWR) and ADH plasma levels. Conversely, in the whole group of 60 rats TFWR did correlate significantly with plasma aldosterone levels ($r=0.51$, $P<0.01$), urinary potassium excretion rate ($r=0.90$, $P<0.001$), and osmolar clearance ($r=0.93$, $P<0.001$). Liver enzymes, total bilirubin and liver histology (after 13-week CCl_4 treatment) were not significantly affected by scheduled pharmacological treatments (Table 1 and Figure 1).

Hormonal status (Table 2). Guanfacine, in combination with diuretics, blunted the adrenergic hyper-function of advanced liver cirrhosis, as shown by reduced levels of serum catecholamines in ascitic cirrhotic rats belonging to groups G3, G4, and G5 vs. untreated cirrhotic rats (G1) and, mostly, cirrhotic rats treated with sole diuretics (G2), which showed the highest adrenergic activation. Partly due to improved renal plasma flow (i.e. renal arterial perfusion) and partly dependent on the above blunting of adrenergic function, PRA and plasma aldosterone were significantly lower in G3-G4 than in ascitic cirrhotic rats, whether treated or not with diuretics (G1 and G2). The peak value of secondary aldosteronism was found in the group of cirrhotic rats treated with sole diuretics (G2) or with the highest, hypotensive dosage of guanfacine plus diuretics (G5). Plasma levels of ADH went largely unaffected by α_{2A}-adrenergic agonists or V_2 receptor antagonists, but non-osmotic secretion of ADH was further stimulated by the treatment of cirrhotic rats with sole diuretics (in G2).

Mean arterial pressure (Table 1). When compared to absolute cirrhotic controls (G1), significantly lower values of MAP ($P<0.05$) were measured in the group of cirrhotic rats receiving diuretics alone (G2) and high-dose guanfacine plus diuretics (G5).

DISCUSSION

In patients with cirrhosis and refractory ascites, the addition to diuretics of aspecific α_2-adrenoceptor agonists has been attempted to improve urinary sodium excretion, since clonidine reduces central sympathetic outflow, systemic release of catecholamines [27], and portal pressure [28]. Indeed, clonidine improves the diuretic effects of spironolactone alone or the combination of furosemide and spironolactone in patients and experimental animal models with advanced liver cirrhosis and ascites [29, 30, 31].

Guanfacine, a distinct α_2-adrenoceptor agonist, has approximately 60-fold more selectivity than clonidine for α_{2A}-receptors [32], which are located in the proximal tubular nephron in the inner stripe of the renal cortex [33, 34]. Guanfacine does not lower arterial pressure in patients with arterial hypertension [35] and, through specific stimulation of renal α_{2A}-adrenoceptors, increases osmolar clearance and sodium excretion in a peculiar naltrexone (opioid receptor antagonist)-sensitive manner [32]. Unlike clonidine, guanfacine cannot enhance vascular production of nitric oxide through stimulation of endothelial α_{2D}-receptors [36], and cannot stimulate, in the basolateral membrane of the proximal renal tubule, α_{2B}-adrenoceptors, which accelerate sodium reabsorption [37]. Therefore, among α_2-adrenoceptor agonists, which behave as sympatholytic agents, guanfacine is a more promising candidate drug than clonidine in order to improve the effects of diuretics in ascitic cirrhosis, at least on pharmacological basis.

In this study, all doses of guanfacine, associated with diuretics, attenuated systemic release and plasma levels of catecholamines. Not unexpectedly, the peak dose of 10 mg/kg of guanfacine (G5), which caused arterial hypotension, led to significant stimulation of the renin-angiotensin system (RAS) (Table 2) and therefore aggravated sodium retention (Table 1). Conversely, a lower dose of guanfacine (7 mg/kg in G4) increased sodium absolute and fractional excretions (Table 1). Interestingly, this drug, when used in small amount (2 mg/kg in G3), showed aquaretic properties to the same extent as vasopressin V_2 receptor antagonists plus diuretics (G6) (Table 1) in this model of CCl_4-dependent ascitic cirrhosis with dilutional hyponatremia. This experimental model reproduces most of the histological, hemodynamic, renal, and neurohumoral abnormalities observed in cirrhotic patients, including sodium retention, decreased systemic vascular resistance, and increased circulating levels of catecholamines, renin, aldosterone and ADH [3].

The above dose-dependency of guanfacine's pharmacodynamics (Tables 1-2), especially when the natriuretic and aquaretic properties of this adrenolytic drug are considered, is easy to comprehend. At the lowest dose of 2 mg/kg, the aquaretic effect of guanfacine is maximal and the hyponatremia found in ascitic cirrhotic rats treated or not with diuretics (G1 and G2) is corrected (in G3); the natriuretic effect of guanfacine peaked at the dose of 7 mg/kg (G4), and this, albeit favourable in the ascitic stage of disease, cancelled the capacity of the kidney to excrete solute-free water and to correct hyponatremia (Table 1). At the highest dose of 10 mg/kg (G5), guanfacine resulted in arterial hypotension and the natriuretic and aquaretic effects vanished.

These results unmask the following issue: a considerable amount of solute-free water retention occurs, in ascitic cirrhosis, not in the collecting duct through non-osmotic hyper-secretion of ADH, but as a consequence of adrenergic hypertone and ensuing isosmotic fluid retention in the proximal tubular nephron. Of course, this reduced delivery of fluid to the ascending limb of Henle's loop (where otherwise solute-free water would be generated inside the tubular lumen due to reabsorption of electrolytes without water) cannot be impeded by vasopressin V_2 receptor antagonists.

In this experimental model, V_2 receptor antagonists still exerted an aquaretic action, but this specific effect is clearly limited by the avid sodium and water retention in the tubular segments that precede the collecting duct (i.e. mostly in the proximal convoluted tubule). This is an unfortunate event since ADH and its receptor antagonists work only in the collecting duct.

The occurrence of multiple mechanisms of tubular water retention in cirrhotic patients with dilutional hyponatremia and refractory ascites or hepatorenal syndrome (HRS) is corroborated by a host of clinical observations. First, alongside the undisputed attenuation of dilutional hyponatremia caused by vasopressin V_2 receptor antagonists [14, 15], there is no evidence of beneficial effects of these drugs on patients' survival rate or long-term management of difficult-to-treat ascites [16]. Second, the administration of satavaptan (selective vasopressin V_2 receptor antagonist) is associated with reduction of ascites only in patients with moderately severe cirrhosis (mean Child-Pugh score of 8) without hyponatremia (i.e. with no evidence of fluid retention in the proximal tubular nephron) [38]. Third, dilutional hyponatremia associated with HRS ameliorates with vasoconstrictors and albumin, which restore the effective arterial blood volume: among vasoconstrictors the most effective ones are vasopressin analogues, and of course not vasopressin antagonists [39, 40]. Finally, in patients with refractory ascites, vasopressin analogues, while improving sodium and lithium clearances, do not exacerbate the already reduced solute-free water retention [13].

Accordingly, it seems unlikely that non-osmotic hyper-secretion of ADH might represent the most important mechanism of water retention in advanced cirrhosis. In fact, catecholamine- and angiotensin II-driven isosmotic sodium retention in the proximal tubule leads to minimal delivery of fluid to collecting ducts and reduced delivery even to the loop of Henle, where free-water is generated inside the tubular lumen (if furosemide is not used). Moreover, the direct correlations, found in these ascitic rats, between TFWR, on the one hand, and plasma aldosterone levels, urinary potassium excretion rate, or osmolar clearance, on the other hand, further suggest that reduced effective arterial blood volume with secondary aldosteronism, especially when exacerbated by the use of kaliuretic agents (i.e. furosemide), is the actual trigger of solute-free water retention.

The following paradox, sometimes neglected, still holds in current medical literature: the successful treatment of hyponatremia in HRS or refractory ascites is achieved with vasopressin analogues, while the treatment of simple dilutional hyponatremia, as such, should be based on vasopressin V_2 receptor antagonists. Moreover, if ADH hyper-secretion represented the key cause of water retention in advanced cirrhosis, such an ADH hyper-secretion should expand the EABV and cause paradoxical urinary sodium loss, and V_2-antagonists should exacerbate sodium retention rather than relieving it. Instead, some relief to sodium retention by V_2-antagonists was observed in this (Table 1) and other studies [14-16].

After the evaluation of the renal pharmacodynamic profile of guanfacine, further important issues remain to be addressed. First, attempts to increase the delivery of tubular fluid to the collecting duct during the administration of V_2 receptor antagonists have been made by the addition of traditional diuretics: furosemide, active in the ascending limb of Henle's loop, and anti-aldosterone drugs, active mostly in the distal convoluted tubule. Unfortunately, these diuretics do not affect isosmotic sodium and water retention in the proximal convoluted tubule, while an adrenolytic agent like guanfacine could. Moreover, furosemide itself is not a good choice in order to increase solute-free water excretion since it causes paradoxical solute free-water retention by inhibiting reabsorption of sodium, potassium and chloride in a water-impermeable segment of the nephron [41]. Second, a

promising strategy, in order to treat dilutional hyponatremia in advanced cirrhosis, could be the association of a V_2 receptor antagonist with an adrenolytic agent like guanfacine, the latter being able to reduce the adrenergic drive that leads to water retention in the proximal tubule. Third, consistent beneficial effects of V_2 receptor antagonists alone or in combination with common diuretics could be predicted only in the rare cirrhotic patients with non-osmotic hyper-secretion of ADH and little or no adrenergic hyper-function, which instead leads to proximal tubular fluid retention. This would be a rare patient since shrinking of EABV is followed first by secondary aldosteronism and adrenergic hyper-function, and later by non-osmotic hyper-secretion of vasopressin [11].

In conclusion, this paper shows the usefulness of the addition to common diuretics of α_2-adrenoceptor agonists, especially those selective for the α_{2A} adrenoceptors (guanfacine), in order to improve the management of the so called difficult-to-treat ascites. This is a clinical condition further complicated by dilutional hyponatremia, adrenergic hyperfunction, and early decrease in GFR, which may go unnoticed at least when GFR is evaluated through the measurement of creatinine plasma levels or systemic clearance [42]. We have also shown that, in advanced experimental cirrhosis, roughly 50% of solute free-water retention occurs by excess fluid reabsorption in the proximal convoluted tubule (under adrenergic drive) rather than in the collecting duct through non-osmotic hyper-secretion of ADH. This paves the way to alternative strategies of treatment of dilutional hyponatremia in ascitic cirrhosis, perhaps less “cosmetic”, so to speak, than the exclusive use of vasopressin V_2 receptor antagonists.

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Clinical perspectives. (i) Dilutional hyponatremia in ascitic cirrhosis is usually treated with vasopressin V_2 receptor antagonists, but these drugs do not improve the management of ascites or patients’ survival rate. Adrenergic hyperfunction triggers proximal tubular fluid retention and reduce renal excretion of solute-free water. (ii) we provide experimental evidence that in experimental ascitic cirrhosis sympatholytic agents (α_{2A}-adrenoceptor agonists) are at least as effective as V_2-antagonists to blunt water retention. (iii) α_{2A}-adrenoceptor agonists (e.g. guanfacine), which are effective adrenolytic agents, do not cause arterial hypotension, and do not trigger nitric oxide production (at variance with clonidine), may be a promising adjunct to diuretics in order to treat patients with advanced ascitic cirrhosis, once the most suitable dosage is established for human disease.

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Catecholamines and water retention in cirrhosis.

FIGURE LEGENDS

Figure 1. Morphological analysis with Gomori trichrome of liver cirrhosis due to 13-week CCl_4 administration: slides of rat livers from group G1 (untreated ascitic cirrhotic controls) and G4 (ascitic cirrhotic rats treated with daily diuretics plus guanfacine 7 mg/kg). No appreciable difference in liver histology between the groups.

Table 1. Body weight, liver enzymes and renal function in the rat groups.

	Group G1 (n = 10)	G2 (n = 10)	G3 (n = 10)	G4 (n = 10)	G5 (n = 10)	G6 (n = 10)
MAP (mm Hg)	88 ± 4	79 ± 2*	86 ± 5	89 ± 7	77 ± 3*$^{\Psi}$	90 ± 9
Body weight (g)	401 ± 17	392 ± 87	372 ± 10*	351 ± 18*	394 ± 47	369 ± 15*
AST (U/l)	102 ± 81	91 ± 77	112 ± 56	89 ± 81	121 ± 100	99 ± 70
ALT (U/l)	78 ± 61	80 ± 56	86 ± 61	62 ± 56	87 ± 55	72 ± 59
Bilirubin (mg/dl)	2.8 ± 0.5	2.4 ± 0.4	3.0 ± 0.9	2.7 ± 1.0	3.3 ± 1.1	3.1 ± 0.6
CPAH (ml/min)	3.4 ± 0.2	2.7 ± 0.3*	4.1 ± 0.3*$^{¥}$	5.1 ± 0.09*$^{¥}$	4.0 ± 1.2	3.8 ± 0.25$^{¥\Psi}$
CIN (ml/min)	1.5 ± 0.3	1.4 ± 1.0	1.42 ± 1.0	1.84 ± 0.2*$^{¥}$	0.8 ± 0.3*$^{¥}$	1.56 ± 0.1$^{\Psi}$
FF (%)	34 ± 10	72 ± 10*	33 ± 10$^{¥}$	37 ± 11$^{¥}$	24 ± 9*$^{¥}$	42 ± 10$^{¥}$
Urine volume (ml/h)	0.62 ± 0.15	0.83 ± 0.13*	0.82 ± 0.11*	1.38 ± 0.76*$^{¥}$	0.8 ± 1.05	1.48 ± 0.7*$^{¥}$
Natriuresis (µmol/h)	62 ± 21	92 ± 21*	73 ± 31	119 ± 15*$^{¥}$	39 ± 24*$^{¥}$	112 ± 13*$^{¥}$
FENa (%)	1.3 ± 0.3	2.1 ± 0.3*	1.9 ± 0.5*	2.6 ± 0.2*	0.7 ± 0.2*	2.1 ± 0.3*
Kaliuresis µmol/h)	37 ± 14	53 ± 12	25 ± 14	59 ± 15	44 ± 20	23 ± 10
FEK (%)	9.2 ± 2.7	8.1 ± 1.7	9.1 ± 2	8.3 ± 2	9.6 ± 2.1	8 ± 1.8
Plasma Na (mEq/l)	130 ± 4	132 ± 5	140 ± 4*$^{¥}$	133 ± 4	133 ± 7	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
TFWR (microl/min)	32 ± 9	45 ± 8*	20 ± 6*$^{¥}$	32 ± 12	28 ± 18	21 ± 7*$^{¥}$

Rat groups: G1, untreated ascitic cirrhotic controls; G2, ascitic cirrhotic rats treated with daily diuretics (0.5 mg/kg b.w. furosemide plus 2 mg/kg b.w. K^+-canrenoate); G3, ascitic cirrhotic rats treated with daily diuretics plus guanfacine 2 mg/kg; G4, ascitic cirrhotic rats treated with daily diuretics plus guanfacine 7 mg/kg; G5, ascitic cirrhotic rats treated with daily diuretics plus guanfacine 10 mg/kg; G6, ascitic cirrhotic rats treated with daily diuretics plus SSP-004240F1, vasopressin V_2 receptor antagonist, 1 mg/kg.
Data are means ± SD. *P<0.05 versus G1, cirrhotic control group; $^{\Psi}$P<0.05 versus G4; $^{¥}$P<0.05 versus G2 (One-way ANOVA followed by Tukey's LSD post-hoc comparisons). AST, aspartate aminotransferase; ALT, alanine aminotransferase; CIN: steady-state plasma clearance of inulin; 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; TFWR: tubular free-water reabsorption.

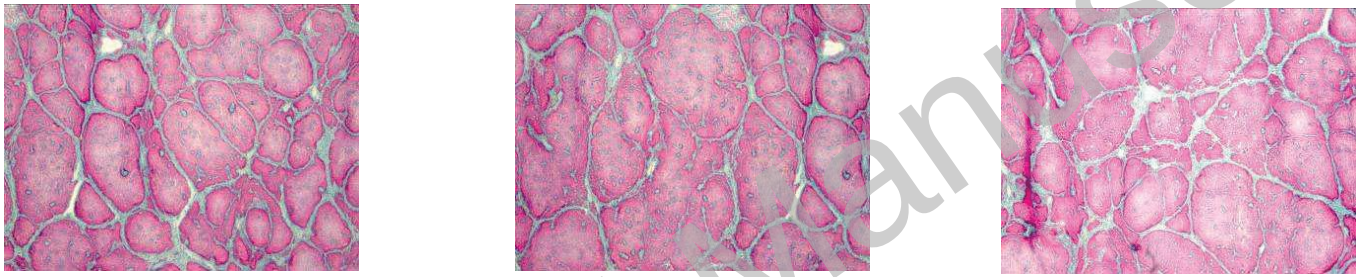
Table 2. Hormonal status in the rat groups.

	Group G1 (n = 10)	G2 (n = 10)	G3 (n = 10)	G4 (n = 10)	G5 (n = 10)	G6 (n = 10)
PRA (ng/ml/h)	24 ± 7	35 ± 8*	9 ± 2*¥	17 ± 3*¥	34 ± 7*	19 ± 5
Plasma A (pg/ml)	390 ± 101	930 ± 101*	187 ± 56*¥	263 ± 57*¥	496 ± 85*¥	322 ± 98¥
Plasma N (ng/l)	296 ± 39	423 ± 22*	211 ± 41*¥	238 ± 22*¥	242 ± 38*¥	347 ± 190
Plasma ADH (pg/ml)	69 ± 11	85 ± 11*	73 ± 55	84 ± 8*	76 ± 71	79 ± 61
Plasma E (ng/l)	37 ± 9	56 ± 12*	25 ± 7¥	21 ± 9*¥	32 ± 9¥	41 ± 21

Rat groups: G1, untreated ascitic cirrhotic controls; G2, ascitic cirrhotic rats treated with daily diuretics (0.5 mg/kg b.w. furosemide plus 2 mg/kg b.w. K^+-canrenoate); G3, ascitic cirrhotic rats treated with daily diuretics plus guanfacine 2 mg/kg; G4, ascitic cirrhotic rats treated with daily diuretics plus guanfacine 7 mg/kg; G5, ascitic cirrhotic rats treated with daily diuretics plus guanfacine 10 mg/kg; G6, ascitic cirrhotic rats treated with daily diuretics plus SSP-004240F1, vasopressin V_2 receptor antagonist, 1 mg/kg.
Data are means ± SD. *P<0.05 versus G1, cirrhotic control group; ¥P<0.05 versus G2 (one-way ANOVA followed by Tukey's LSD post-hoc comparisons). A: aldosterone; ADH: vasopressin; N: norepinephrine; PRA: plasma renin activity; E: epinephrine.

FIGURE 1

Group G1 (untreated ascitic cirrhotic controls)



Group G4 (ascitic cirrhotic rats treated with daily diuretics plus guanfacine 7 mg/kg)