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Effect of chronic administration of sildenafil on sodium retention and on the hemodynamic complications associated with liver cirrhosis in the rat

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Abstract

Previous studies demonstrated increased phosphodiesterase-5 (PDE5) activity and expression in the kidneys of rats with liver cirrhosis. Acute intravenous administration of PDE5 inhibitors enhanced sodium excretion in these rats. The aim of the present study was to examine the effects of chronic administration of sildenafil on renal sodium handling and hemodynamics in rats with liver cirrhosis. Male Sprague–Dawley rats underwent bile-duct ligation and excision or sham operation and were housed in metabolic cages throughout the study. Body weight, food intake, water intake and urine volume were measured daily, and plasma samples were obtained twice weekly. Fourteen days following surgery sildenafil or its vehicle (dimethylsulfoxide) were administered (20 mg/kg subcutaneously 3 times/day). Two weeks later, systemic hemodynamics were measured under general anesthesia. Sildenafil enhanced the systemic vasodilatation associated with liver cirrhosis and reduced the arterial pressure. There was no reduction in the glomerular filtration rate, however. Despite these hemodynamic changes, sildenafil prevented the decrease in sodium excretion observed in the bile-duct-ligated group receiving vehicle and markedly increased fractional sodium excretion relative to the other groups. These results suggest that chronic sildenafil administration may help prevent or ameliorate sodium retention in cirrhosis, but that hemodynamic adverse effects may ensue.

Keywords: Cirrhosis; Vasodilation; Phosphodiesterase 5; cGMP; Kidney; Sildenafil

1. Introduction

The major cardiovascular complication of liver cirrhosis is a hyperdynamic circulation with peripheral and splanchnic vasodilatation, increased cardiac output and varying degrees of hypotension. The peripheral arterial vasodilation hypothesis suggests that primary peripheral (mainly splanchnic) vasodilation induces activation of compensatory mechanisms such as the rennin–angiotensin–aldosterone system, antidiuretic hormone and the sympathetic nervous system to result in sodium and water retention in order to restore effective arterial volume and maintain arterial pressure (Schrier et al., 1988). The primary mesenteric vasodilatation leading to the hyperdynamic circulation has been attributed to increased production of various vasodilator substances such as nitric oxide (NO) (Vallance and Moncada, 1991; Wiest and Groszman, 1999), prostacyclin (Oberti et al., 1993), endothelium derived hyperpolarizing factor (Barriere et al., 2000), glucagon (Lin et al., 1996), substance P and calcitonin-gene-related peptide (Gines et al., 1997). Among all, nitric oxide has gained the most attention as the major cause of the decreased vascular resistance in cirrhosis (Martin et al., 1998). Nitric oxide, formed by endothelial cells from its precursor L-arginine raises intracellular cGMP levels, its second messenger, leading to vasodilatation (Wiest and Groszman, 2002).

Another abnormality associated with cirrhosis is a decreased responsiveness of the kidney to the natriuretic effect of the atrial natriuretic factor, a key component of blood pressure regulation and homeostasis. Atrial natriuretic factor, like nitric oxide, acts by increasing intracellular levels of cGMP, which is then hydrolyzed by numerous phosphodiesterase isoforms (Beavo, 1995). Among those isoforms which are specific to cGMP hydrolysis is phosphodiesterase 5 (PDE5), located in the smooth muscle cells of blood vessels. PDE5 was found to be

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present in the mesenteric artery (Sampson et al., 2001; Wallis et al., 1999) in pulmonary arteries (Ahn et al., 1991) and in other vascular beds (Medina et al., 2000). It has been shown that the blunted renal response to atrial natriuretic factor in cirrhosis is due, at least partially, to upregulation of PDE5, and that inhibition of PDE5 in the kidney can enhance the responsiveness to atrial natriuretic factor by restoring intracellular levels of cGMP producing an increase in renal plasma flow, in the glomerular filtration rate and in sodium excretion (Shah et al., 1999; Angeli et al., 2000; Ni et al., 2001). These results suggest that inhibition of PDE-5 may be a therapeutic strategy in patients with liver cirrhosis and ascites. However, it should be noted that inhibition of PDE5 in the splanchnic area may increase the vasodilator effect associated with nitric oxide by raising intracellular levels of cGMP. Inhibition of PDE5, therefore, may have a beneficial effect in the kidney and on the response to atrial natriuretic factor, but may worsen the hemodynamic picture in cirrhosis. In preliminary studies we demonstrated that acute administration of a PDE5 selective inhibitor, DMPPO (1,3 dimethyl-6-(2-propoxy-5-methane sulphonylamidophenyl)-pyrazolo[3,4-*d*]pyrimidin-4-(5H)-one), led to worsening in the vasodilation and the decrease in arterial pressure; despite this, however, it induced an increase in sodium excretion by the kidney (Tahseldar-Roumieh et al., 2006). Others showed that the acute administration of sildenafil in the splanchnic bed increased mesenteric blood flow and portal venous pressure and decreased mean arterial pressure (Colle et al., 2004). While results of acute drug administration may be interesting, they do not reflect the possible clinical relevance of such an intervention. The vasodilation induced by sildenafil in cirrhosis may in the long term enhance sodium retention by the kidney as a result of more severe activation of the compensatory mechanisms mentioned above. Therefore, this study was designed to explore the effect of chronic administration of a PDE5 inhibitor, sildenafil, on the hemodynamic and renal functional consequences of liver cirrhosis in rats; specifically, the aim was to examine whether the beneficial effect on sodium excretion, observed in acute studies, will also be observed after chronic administration.

2. Materials and methods

2.1. Animal preparation and treatment

The animals used in the present study were reared, treated and euthanized in accordance with the provisions for animal welfare of the Institutional Animal Care and Use Committee of the American University of Beirut. All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals, National Academy of Sciences. Adult male Sprague–Dawley rats weighing between 230 and 290 g were randomized to undergo either sham operation (Sham) or bile-duct ligation and excision (BDL). Two days prior to surgery, rats were housed in metabolic cages for adaptation and allowed access to normal rat chow and water. For surgery, rats were anesthetized with pentobarbital (40 mg/kg body weight intraperitoneally) and their abdomen was opened through a midline incision. The common hepatic bile duct was isolated and transected between two ligatures in bile-duct-ligated animals. Sham rats underwent the same procedure but without ligation and excision of the bile duct. Following surgery, rats were housed individually in metabolic cages. Two weeks after surgery, rats were started on either sildenafil (Pfizer Ltd, Sandwich, Kent, UK) (30 mg/ml in dimethylsulfoxide) or its vehicle (dimethylsulfoxide) at a dose of 20 mg/kg subcutaneously 3 times daily. This dose was chosen based on the following: according to the manufacturer, a dose of 20 mg/kg/day in the rat is approximately equivalent to 1.2 times the maximal recommended human dose on a mg/m^2 basis (Viagra®, package insert). However, since the half-life of sildenafil in rats is at least 3 times shorter than in humans (0.4–1.3 h in rats v 4 h in humans) (Walker, 1999), repeated administration of that dose is recommended at a rate not less than three times per day in order to maintain plasma concentrations. Thus, 4 groups resulted: sham-operated with vehicle (Sham-V) or sildenafil (Sham-S) treatment, or bile-duct-ligated with similar treatment (BDL-V and BDL-S, respectively).

2.2. Metabolic studies

Several parameters were measured on a 24-h basis including: body weight, food intake, water intake and urine volume. Sodium excretion was calculated after measurement of sodium concentrations in the urine over 24 h. During the first 2 weeks, 0.3–0.4 ml of blood was withdrawn from the rats' tail veins, under light ether anesthesia, once weekly then twice weekly for the remaining 2 weeks. Plasma and urine were used to measure sodium and creatinine levels from which sodium excretion rate, creatinine clearance, and fractional sodium excretion were calculated. On day 26, urine samples were used to measure cGMP concentration using a commercially available immunoassay kit (Sigma-Aldrich, Inc. USA); daily cGMP excretion rates were then calculated for each rat.

2.3. Hemodynamic studies

Four weeks following surgery, animals were anesthetized with pentobarbital (25 mg/kg for bile-duct-ligated and 50 mg/kg for Sham, intraperitoneally) and their tracheas were cannulated with PE-205 tubing. A PE-50 catheter was introduced into the right carotid artery and connected to a blood pressure transducer (TDX-310) for measurement of arterial pressure using a BPA-100 blood pressure analyzer (Micro-Med Louisville, KY, USA), while another PE50 catheter was inserted in the right jugular vein and advanced to the right atrium. A thermistor probe was advanced to the aortic arch through a left carotid approach to monitor the cardiac output by the thermodilution technique using a cardiac output computer (Cardiotherm® Columbus Instruments, Columbus, OH, U.S.A.). Three consecutive measurements of cardiac output were obtained at 5-min intervals and the peripheral vascular resistance was calculated as the ratio of mean arterial pressure to cardiac index, which is the cardiac output expressed per 100 g body weight. The bladder was then exposed through a supra-pubic incision and

the urethra catheterized using PE 190 tubing for urine collection. ^{3}H-Inulin was then administered (0.75 μCi loading dose followed by a continuous infusion of 0.05 μCi/min at 50 μl/min). After 30 min of stabilization, a urine sample was collected over a 30-min period and a blood sample withdrawn at the midpoint of the collection. The urine and plasma samples were used to measure ^{3}H counts and the glomerular filtration rate was estimated from the clearance of inulin.

2.4. Statistical analysis

Data are presented as mean ± standard error of the mean. Comparison of values between two groups was conducted using the Students' unpaired *t*-test; comparisons among more than two groups was performed by one-way or two-way analysis of variance (ANOVA) followed by the Newman–Keul test or the Bonferroni post-test for multiple comparisons. The responses of variables over time in one group were compared to other groups by 2-way ANOVA with repeated measures and followed by Bonferroni post-test for multiple comparisons. Analyses were performed using the SigmaStat statistical Software (Jandel Corporation, San Rafael, California, U.S.A.), or the GraphPad Prism software (GraphPad Software, Inc., San Diego, California, U.S.A.). Differences in values were considered statistically significant at a P level of <0.05.

3. Results

Survival following bile-duct-ligation surgery was determined over the 4 weeks period. Of those randomized to the sildenafil group, 5 of 17 rats died during the first 2 weeks of the study, before sildenafil or its vehicle were administered. However, none of the rats in those randomized to the vehicle group ($n=9$) died during the first 14 days. Analysis of mortality in bile-duct-ligated rats during the last 14 days of the study (after the start of sildenafil or vehicle treatment) revealed that none of the remaining 12 rats which received sildenafil died, compared with 3 of 9 (33%) in those receiving its vehicle. This difference was not statistically significant. Since 2 rats died on day 27 and one died on day 22 of the study, this meant that data was available from 8 rats for the metabolic studies during the last week and from only 6 rats for the hemodynamic studies. None of the rats in the two sham-operated groups ($n=11$ for vehicle and $n=10$ for sildenafil treatment) died during the study period.

In the following description of results, the detailed statistical analysis for each figure is provided in Table 1.

3.1. Body weight

All rats sustained a reduction in body weight on the first few days after surgery. After that, rats started to regain weight; however, the rate of weight gain was slower in the bile-duct-ligated groups compared with the sham-operated groups, irrespective of the treatment received, such that it remained lower in the bile-duct-ligated relative to the sham-operated group of rats especially during the last 2 weeks (Fig. 1A and B).

Table 1
Results of two-way analysis of variance for Figs. 1–8, showing only $P<0.1$ values

Parameter	Comparisons	Time factor	Group factor	Interaction of time and group
Body weight	Sham-V v BDL-V	<0.0001	0.076	<0.0001
	Sham-S v BDL-S	<0.0001	0.028	<0.0001
	Sham-V v Sham-S	<0.0001	–	–
	BDL-V v BDL-S	<0.0001	–	–
Urine volume	Sham-V v BDL-V	<0.0001	0.0001	0.019
	Sham-S v BDL-S	<0.0001	<0.0001	<0.0001
	Sham-V v Sham-S	<0.0001	–	<0.0001
	BDL-V v BDL-S	<0.0001	0.004	<0.0001
Water intake	Sham-V v BDL-V	<0.0001	0.099	0.026
	Sham-S v BDL-S	<0.0001	0.002	<0.0001
	Sham-V v Sham-S	<0.0001	0.012	–
	BDL-V v BDL-S	<0.0001	0.005	<0.0001
Na excretion	Sham-V v BDL-V	–	0.007	–
	Sham-S v BDL-S	0.027	–	–
	Sham-V v Sham-S	0.014	–	–
	BDL-V v BDL-S	–	0.041	–
Food intake	Sham-V v BDL-V	–	0.065	0.033
	Sham-S v BDL-S	–	0.002	0.083
	Sham-V v Sham-S	–	–	-
	BDL-V v BDL-S	0.002	–	-
Na balance	Sham-V v BDL-V	–	–	0.065
	Sham-S v BDL-S	0.054	0.004	0.094
	Sham-V v Sham-S	–	–	–
	BDL-V v BDL-S	0.004	0.054	–
Creatinine CL	Sham-V v BDL-V	–	–	–
	Sham-S v BDL-S	0.010	0.074	0.010
	Sham-V v Sham-S	0.083	–	–
	BDL-V v BDL-S	–	–	–
FE Na	Sham-V v BDL-V	–	–	0.031
	Sham-S v BDL-S	0.085	0.053	–
	Sham-V v Sham-S	–	–	0.066
	BDL-V v BDL-S	–	0.015	–

There was no difference, however, between the two bile-duct-ligated groups or the two sham-operated groups (Table 1).

3.2. Urine volume, water intake and sodium excretion

Urine volume increased in all groups over time (Fig. 2). Both bile-duct-ligated groups had significantly higher urine volumes than their sham-operated counterparts ($P<0.0001$). Furthermore, the bile-duct-ligated group treated with sildenafil had significantly higher values than the vehicle-treated bile-duct-ligated group especially during the last 2 weeks of the study ($P=0.0039$). Water intake was also different among the groups, with the sildenafil-treated bile-duct-ligated group having higher values than the others (Fig. 3).

Urinary sodium excretion during the last week of the study is presented in Fig. 4. Previous studies have shown that at this time sodium retention is manifested in rats with bile-duct ligation (Wensing and Branch 1990; Jonassen et al., 2003). Statistical analysis showed that the vehicle-treated bile-duct-ligated group had lower values than the vehicle-treated sham-operated group ($P=0.0066$) and the sildenafil-treated bile-duct-ligated group ($P=0.04$) during this period. These findings were particularly significant considering the fact that food intake and, consequently, sodium intake, were not significantly different between the bile-

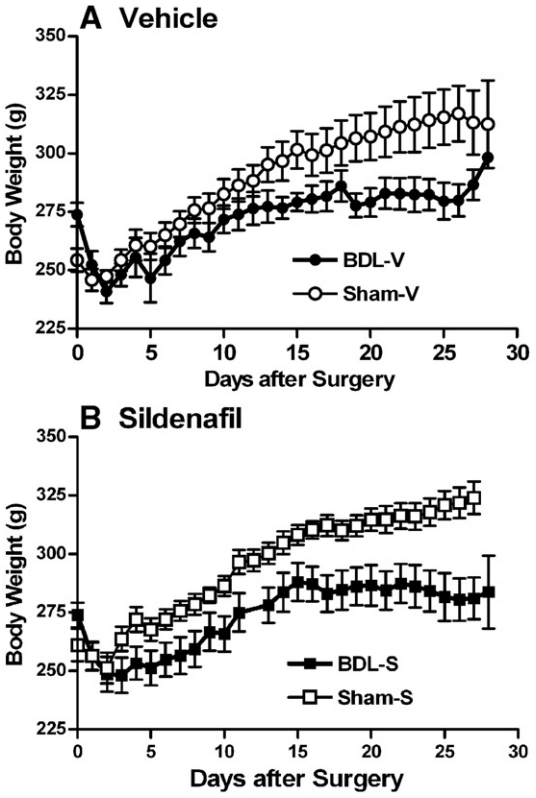

Fig. 1. Body weight over 28 days in bile-duct-ligated (BDL-V, $n=8$) and sham-operated ($n=11$) rats treated with vehicle (Sham-V) (A) and in sham-operated (Sham-S, $n=10$) and bile-duct-ligated (BDL-S, $n=12$) rats treated with sildenafil (B). S or V was started on day 14 after surgery. Values are means±SE. There was a significant difference between Sham-V and Sham-S and between BDL-V and BDL-S over time. Refer to Table 2 for detailed statistical analysis.

duct-ligated group treated with sildenafil and the bile-duct-ligated group treated with vehicle, during the same periods of time (Fig. 5), both of which were lower than the values in the corresponding sham-operated groups. Neither sodium excretion nor food intake was different between the 2 sham-operated groups.

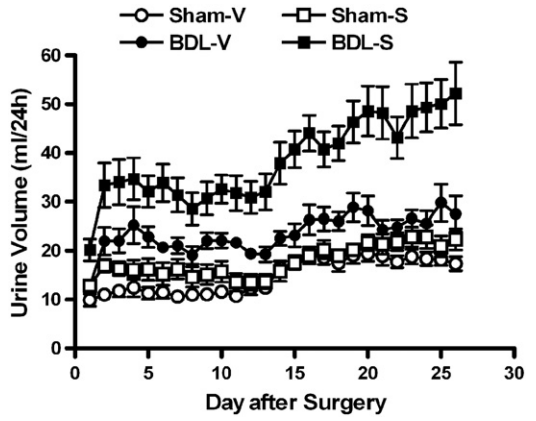

Fig. 2. Urine volume over 28 days of the study in the BDL-V ($n=8$) Sham-V ($n=11$), BDL-S ($n=12$) Sham-S ($n=10$) groups. The values represent the mean±SE. The results of 2-way ANOVA with repeated measures are shown in Table 2 and revealed significant differences between the BDL-S group and both the Sham-S and BDL-V.

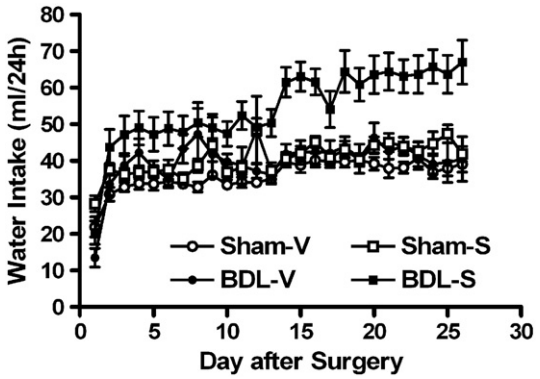

Fig. 3. Water intake over 28 days of the study in the BDL-V ($n=8$) Sham-V ($n=11$), BDL-S ($n=12$) Sham-S ($n=10$) groups. The values represent the mean±SE. The results of 2-way ANOVA with repeated measures are shown in Table 2 and revealed significant differences between the BDL-S group and both the Sham-S and BDL-V.

Consistent with these findings, calculation of sodium balance showed that the sildenafil-treated bile-duct-ligated group had a significantly lower sodium balance than both the sildenafil-treated sham-operated group and the vehicle-treated bile-duct-ligated group (Fig. 6).

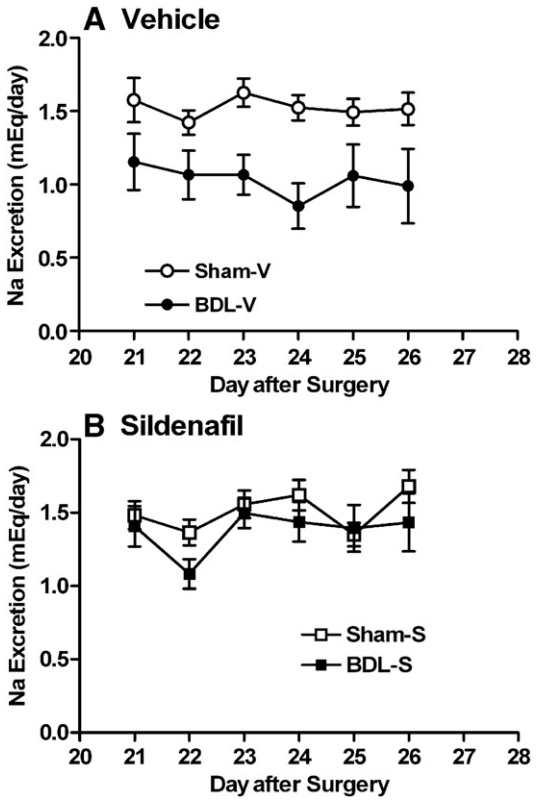

Fig. 4. Sodium excretion during the last week of the study in the BDL-V ($n=8$) Sham-V ($n=11$), BDL-S ($n=12$) and Sham-S ($n=10$) groups. The values represent the mean±SE. The results of 2-way ANOVA with repeated measures revealed that BDL-V had a significantly lower value than Sham-V and than BDL-S, the latter being similar to its control Sham-S. Details of statistics are shown in Table 2.

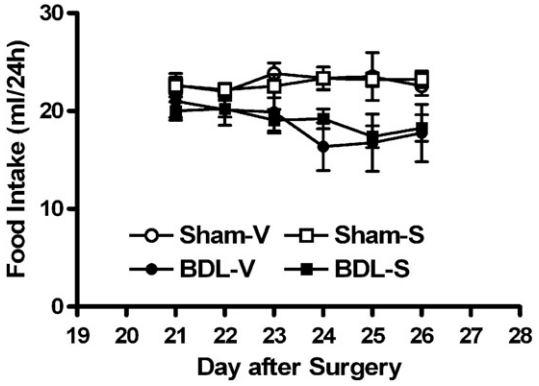

Fig. 5. Food intake during the last week of the study in the BDL-V ($n=8$) Sham-V ($n=11$), BDL-S ($n=12$) Sham-S ($n=10$). The values represent the mean±SE. The results of 2-way ANOVA with repeated measures revealed that both BDL groups ate less than their controls over time, especially the last 2 weeks of the study. Details are shown in Table 2.

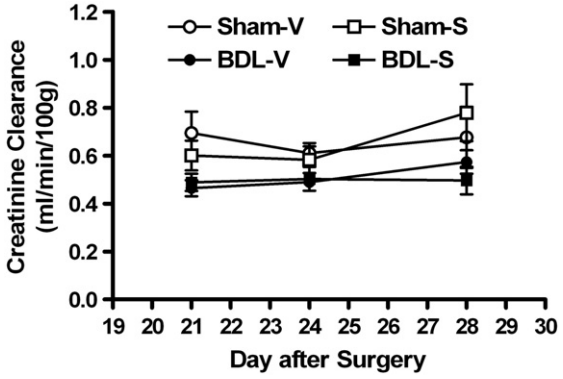

Fig. 7. Creatinine clearance on three days during the last week of the study in the BDL-V ($n=8$) Sham-V ($n=11$), BDL-S ($n=12$) Sham-S ($n=10$). The values represent the mean±SE. The results of 2-way ANOVA with repeated measures did not show major differences among groups or treatments except for the fact that over time the Sham-S group had a higher creatinine clearance than the BDL-S group. Details are shown in Table 2.

3.3. Creatinine clearance

Fig. 7 represents the creatinine clearance during the last week of the study. The values were not different among the 4 groups of rats. The mean creatinine clearance during the last week (average of the 3 values in each rat) was slightly but not significantly lower in both bile-duct-ligated groups relative to their sham-operated controls (Sham-V:0.73±0.09; BDL-V: 0.52±0.02; Sham-S: 0.65±0.09; BDL-S: 0.43±0.02 ml/min/100 g). Furthermore, there was no significant difference in creatinine clearance between the 2 bile-duct-ligated groups, or between the 2 sham-operated groups.

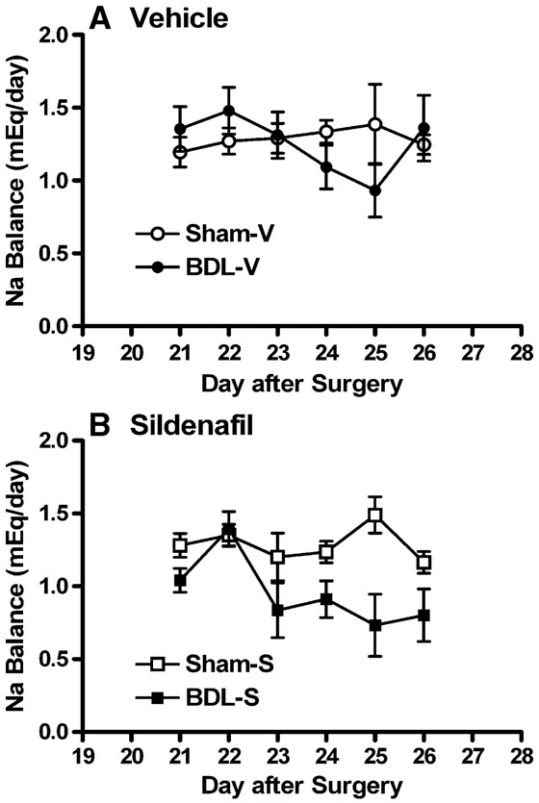

Fig. 6. Sodium balance during the last week of the study in the BDL-V ($n=8$) Sham-V ($n=11$), BDL-S ($n=12$) Sham-S ($n=10$). The values represent the mean±SE. The results of 2-way ANOVA with repeated measures revealed the BDL-S group to have a significantly lower sodium balance compared with both its control (Sham-S) and the BDL-V group. Details of statistics are shown in Table 2.

3.4. Fractional sodium excretion (FE_{Na})

In the bile-duct-ligated group treated with vehicle, FE_{Na} was similar to the one obtained in the corresponding sham-operated group. Treatment of sham-operated rats with sildenafil did not alter this parameter. In the bile-duct-ligated group treated with sildenafil, however, there was a significantly higher FE_{Na} compared with all the other groups (Fig. 8). The average FE_{Na} during the last week of the study provided similar results with an overall higher value in the sildenafil-treated bile-duct-ligated group that all the other groups (Sham-V: 0.37±0.03%; BDL-V: 0.30±0.05%; Sham-S: 0.42±0.03%; BDL-S: 0.53±0.04%,

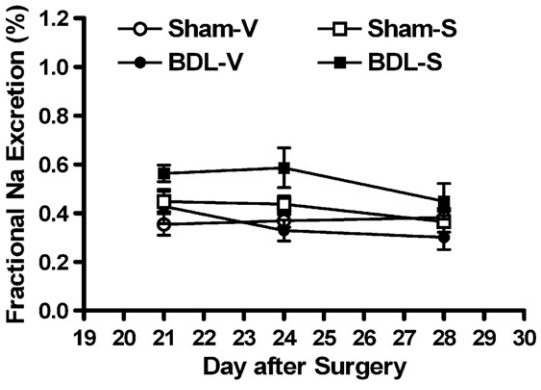

Fig. 8. Fractional excretion of sodium on three days during the last week of the study in the BDL-V ($n=8$) Sham-V ($n=11$), BDL-S ($n=12$) Sham-S ($n=10$) groups. The values represent the mean±SE. The results of 2-way ANOVA with repeated measures revealed that the BDL-V group had a different response than its control Sham-V with a decline in FENa over time. The BDL-S group had a significantly higher FE_{Na} than both the BDL-V and Sham-S groups. Details are shown in Table 2.

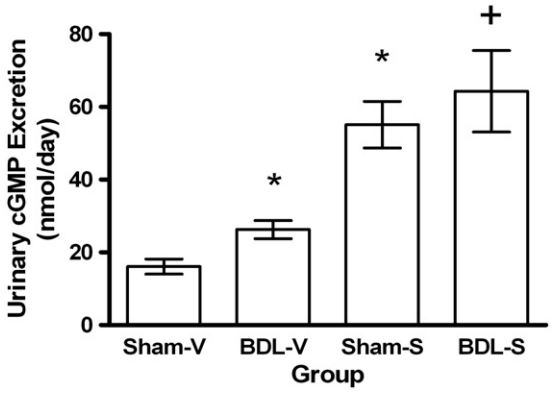

Fig. 9. cGMP excretion on the last day of the experiment, just before the hemodynamic studies were conducted in BDL-V ($n=8$) Sham-V ($n=10$) BDL-S ($n=10$) and Sham-S ($n=10$) groups. The values represent the mean±SE. The results of 2-way ANOVA revealed an influence of Treatment ($P<0.0001$) on cGMP but no influence of Group. Individual comparisons as shown in the figure: *$P<0.01$ compared with Sham-V; $^{+}P=0.009$ compared with BDL-V.

$P=0.002$ by ANOVA, and $P<0.05$ comparing BDL-S with each of the other groups).

3.5. cGMP excretion rate

The rates of cGMP excretion at week 4 after surgery are shown in Fig. 9. Vehicle-treated rats with cirrhosis had a significantly higher cGMP excretion rate than sham-operated controls. Treatment with sildenafil raised cGMP excretion in both sham- and bile-duct-ligated operated groups, and the difference between the bile-duct-ligated and sham-operated groups was eliminated. The two-way analysis of variance indicated a significant effect of treatment with sildenafil per se on the cGMP urinary excretion rate, but no group effect as such.

3.6. Systemic hemodynamics and glomerular filtration rate

At the end of the study, bile-duct-ligated rats treated with vehicle had a lower peripheral vascular resistance than their sham-operated controls. Treatment with sildenafil per se reduced the vascular resistance significantly in the sham-operated group (Table 2). Bile-duct-ligated rats receiving sildenafil sustained further vasodilation relative to their sham-operated sildenafil-treated controls, and relative to the bile-duct-ligated group treated with vehicle. Cardiac index was elevated in the bile-duct-ligated rats receiving vehicle such that mean arterial pressure was unchanged relative to their sham-operated control. In contrast, despite the enhancement of the vasodilation in the bile-duct-ligated group receiving sildenafil, there was no further increase in cardiac index relative to the bile-duct-ligated group receiving vehicle; as a result, the mean arterial pressure tended to decrease further relative to that in both the sham-operated group receiving sildenafil, and the bile-duct-ligated group receiving vehicle. The two-way analysis of variance clearly showed an effect of treatment with sildenafil per se on the arterial pressure.

The measurement of the glomerular filtration rate using inulin clearance revealed the same trends as those that were observed using creatinine clearance, with a decrease in both bile-duct-ligated groups relative to their controls. However, the differences did not reach significant levels. It should be pointed out that the power of the analysis was not sufficient for the glomerular filtration rate results due to the small number of animals reaching that stage and the wide variability in the figures; hence these results cannot be considered reliable (Table 2).

4. Discussion

The present study is the first to investigate the chronic effects of sildenafil on the hemodynamic and renal functional alterations associated with liver cirrhosis. Administration of sildenafil to rats with liver cirrhosis caused a reduction in arterial pressure by enhancing the peripheral vasodilation associated with this condition. Despite these unfavorable hemodynamic effects, sildenafil increased total and fractional sodium excretion and did not worsen the glomerular filtration rate.

In the present study, the increase in cGMP excretion in both sildenafil-treated groups demonstrates the efficacy of the dose of sildenafil used in this study in inhibiting PDE5 activity. It is interesting to note that cGMP excretion was higher in vehicle-treated rats with cirrhosis compared with the sham-operated group. If PDE5 is indeed increased in these rats, as others have suggested, one would expect a lower level of cGMP. However, this increase in cGMP may reflect the concomitant increase in systemic NO production in these rats, especially in the splanchnic vasculature.

Table 2
Hemodynamic parameters 4 weeks after bile-duct ligation and excision (BDL) or sham operation (SHAM) in vehicle- or sildenafil-treated rats

	Vehicle		Sildenafil		2-Way ANOVA		
	SHAM ($n=9$)	BDL ($n=6$)	SHAM ($n=10$)	BDL ($n=10$)	G	T	I
MAP (mm Hg)	142±11	141±7	126±8	111±11	–	0.038	–
Cardiac index (ml/min/100 g)	27.6±1.4	41.1±3.1a	35.2±2.5	46.5±3.9a	–	0.022	<0.0001
PVR (mm Hg min 100 g/ml)	5.2±0.5	3.5±0.2a	3.7±0.2b	2.2±0.3ac	–	0.0001	<0.0001
GFR (ml/min/100 g)	1.13±0.11	1.01±0.22	0.95±0.11	0.82±0.19	–	–	–

Sildenafil or its vehicle was started on day 14 after surgery. Comparison of values among the groups was made by two-way analysis of variance (ANOVA), where one factor was Group (G) and the other Treatment (T) and where (I) represents the effect of the interaction between the two factors on the parameter.
Values represent the mean±SE.
a: $P<0.05$ compared with the sham-operated group receiving the same treatment.
b: $P<0.05$ compared with the Sham-Vehicle group.
c: $P<0.05$ compared with the BDL-Vehicle group.

The results clearly show that at the time the bile-duct-ligated group treated with vehicle was showing a reduction in sodium excretion, the bile-duct-ligated group treated with sildenafil had a sodium excretion similar to the sham-operated groups, and hence, did not exhibit sodium retention. This occurred despite the fact that food intake (and consequently sodium intake) and the glomerular filtration rate in this group were reduced to levels similar to that in the bile-duct-ligated group treated with vehicle. Consequently, the sildenafil-treated bile-duct-ligated group had a significantly and markedly higher fractional sodium excretion relative to all the other groups. It is noteworthy that sildenafil, per se, did not alter renal handling of sodium (compare Sham-V with Sham-S); it was only in the context of liver cirrhosis that its effect on sodium excretion was evident. This is likely due to the fact that in liver cirrhosis, PDE5 activity is stimulated, as previously shown (Angeli et al., 2000; Ni et al., 2001), thus the effect of PDE5 inhibition becomes more evident.

It is important to note that this salutary effect on sodium handling occurred despite worsening of the hemodynamic picture. It was clear that sildenafil treatment per se induced hemodynamic changes including a decrease in peripheral vascular resistance and an increase in cardiac output, with no change in arterial pressure, as evident by comparing the two sham-operated groups receiving vehicle and sildenafil. In the bile-duct-ligated group treated with sildenafil, there was a further vascular dilation (reflecting the effect of bile-duct ligation per se). However, this was not met by a further increase in cardiac output over and above that observed in the bile-duct-ligated vehicle-treated group; this is likely due to the lack of significant sodium and water retention, thus resulting in a reduction in mean arterial pressure.

The results of this study are consistent with those from previous studies that showed an increase in sodium excretion following administration of two different PDE5 inhibitors, zaprinast and DMPPO (Angeli et al., 2000; Ni et al., 1996, 2001). A more recent study by Colle et al. showed that sildenafil administration intravenously or into the mesenteric artery of rats with bile-duct ligation increased mesenteric blood flow and portal venous pressure, but decreased arterial pressure (Colle et al., 2004). The authors suggested that the rise in portal pressure may pose a risk for hemorrhagic complications and cautioned against prescription of sildenafil to patients with cirrhosis before further studies were conducted. Administration of a single oral dose of sildenafil to patients with liver cirrhosis caused an elevation in plasma renin activity, angiotensin II and aldosterone concentrations in the plasma at 60 min, and a fall in sodium excretion at 120 and 180 min after the dose (Thiesson et al., 2005). Thus, in contrast to animal studies, single administration of sildenafil in humans did not induce natriuresis but sodium retention. This appeared to be due to activation of the renin–angiotensin aldosterone system as a result of the hypotension. In that study, however, the authors failed to detect any change in urinary cGMP levels, which suggests that renal PDE5 activity was not effectively inhibited by that single dose of sildenafil.

The present study suggests that under conditions of chronic administration of sildenafil to rats with liver cirrhosis, the drug's direct effect in the kidney to increase sodium and water excretion overcomes its indirect effect to activate sodium retaining mechanisms, which can be triggered by sildenafil's enhancement of NO-mediated vasodilation and hypotension. The clinical significance of these findings cannot be ascertained from this study, which used a single dose regimen of the drug. Future studies should attempt to selectively inhibit cGMP in the kidney, in order to minimize hypotension. Preliminary results from our laboratory suggest that PDE1 is the predominant cGMP hydrolyzing isoform in the kidney, whereas PDE5 is predominant in the vasculature (unpublished observations). Hence, better elucidating the role of PDE5 and PDE1 in the kidney and the vasculature in liver cirrhosis may provide a better means to differentially target cGMP hydrolysis in different tissues. In conclusion, the present study offers a possibility for a novel therapeutic strategy in liver cirrhosis using sildenafil specifically, and PDE-5 inhibitors in general, that should be further explored in animal and human studies.

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