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BASIC RESEARCH

Hemodynamic and antifibrotic effects of a selective liver nitric oxide donor V-PYRRO/NO in bile duct ligated rats

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Abstract

AIM: To assess whether a liver specific nitric oxide (NO) donor (V-PYRRO/NO) would prevent the development of portal hypertension and liver fibrosis in rats with bile duct ligation (BDL).

METHODS: Treatment (placebo or V-PYRRO/NO 0.53 μmol/kg per hour) was administered i.v. to rats 2 d before BDL (D-2) and maintained until the day of hemodynamic measurement (D26). Intra-hepatic NO level was estimated by measuring liver cGMP level. Effects of V-PYRRO/NO on liver fibrosis and lipid peroxidation were also assessed.

RESULTS: Compared to placebo treatment, V-PYRRO/NO improved splanchnic hemodynamics in BDL rats: portal pressure was significantly reduced by 27% ($P < 0.0001$) and collateral circulation development was almost completely blocked (splenorenal shunt blood flow by 74%, $P = 0.007$). Moreover, V-PYRRO/NO significantly prevented liver fibrosis development in BDL rats (by 30% in hepatic hydroxyproline content and 31% in the area of fibrosis, $P < 0.0001$ respectively), this effect being probably due to a decrease in lipid peroxidation by 44% in the hepatic malondialdehyde level ($P = 0.007$). Interestingly, we observed a significant and expected increase in liver cGMP, without any systemic hemodynamic effects (mean arterial pressure, vascular systemic resistance and cardiac output) in both sham-operated and BDL rats treated with V-PYRRO/NO. This result is in accordance with studies on V-PYRRO/NO metabolism showing a specific release of NO in the liver.

CONCLUSION: Continuous administrations of V-PYRRO/NO in BDL rats improved liver fibrosis and splanchnic hemodynamics without any noxious systemic hemodynamic effects.

Key words: Nitric oxide; Portal hypertension; Liver fibrosis; Hemodynamics; Rats

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INTRODUCTION

Chronic liver diseases generally progress slowly from inflammation to fibrosis and in many cases to portal hypertension (PHT) and cirrhosis. PHT is responsible for the development of collateral venous circulation and esophageal varices which may bleed and are life-threatening[1,2]. PHT results from increased intrahepatic vascular resistance due to not only hepatic architectural changes inherent to fibrosis but also contraction of liver vascular smooth muscle cells, myofibroblasts and hepatic stellate cells (HSCs). Indeed, not only are HSCs involved in collagen deposition and development of liver fibrosis, but they also regulate intrahepatic blood flow by sinusoid contraction/constriction[3-5]. Therefore, liver fibrosis and PHT are usually two major therapeutic targets in chronic liver diseases that might be prevented by a single agent[6].

Nitric oxide (NO), a potent vasodilator was recently shown to modulate the intrahepatic vascular tone in normal rats[7,8]. Interestingly, NO has anti-fibrotic potentials which are related to its reactive oxygen species (ROS) scavenging capabilities. Cumulative evidences suggest that PHT in cirrhosis is partly due to a decreased liver NO production from the liver sinusoidal endothelial cells[9-11]. The deficiency of NO in the cirrhotic liver may also be involved in liver fibrosis and, inversely, intrahepatic NO supplementation may prevent liver fibrosis. Several NO donors (nitroglycerin, S-nitroso-N-acetyl penicillamine and sodium nitroprusside) were shown to exert a direct anti-

fibrogenic effect by inhibiting proliferation, motility, and contractility of HSCs induced by several ROS generating systems or by platelet-derived growth factor[12-14].

Contrasting with liver NO level, systemic NO level is increased in chronic liver diseases causing deleterious effects on systemic hemodynamics[11]. Thus, NO-based drug therapy in PHT should target the liver without systemic delivery of NO. Recently, it has been shown that transduction of liver with recombinant adenovirus carrying the neuronal NOS gene significantly reduced intrahepatic resistance and portal pressure in bile duct ligation and CCl_4 models of cirrhosis[15]. A promising pharmacological approach has also been reported with NCX-1000, a selective liver NO-releasing derivative of ursodeoxycholic acid (UDCA)[16]. NCX-1000, but not UDCA, was shown to reduce intrahepatic resistance in the CCl_4 model and to inhibit HSC contraction *in vitro*, suggesting that NCX 1000-derived NO was responsible for these effects. In contrast, both NCX 1000 and UDCA reduced liver collagen deposition in that study and it was unclear whether NCX 1000-derived-NO could have antifibrotic effects by itself. Beneficial effects of selective liver NO release need to be further assessed since UDCA derivative NCX 1000 yielded encouraging results for PHT and liver fibrosis treatment[16].

V-PYRRO/NO (O^2-vinyl 1-(pyrrolidin-1-yl) diazen-1-ium-1, 2-diolate) was reported to specifically deliver NO in the liver[17-19]. These (presumably cytochrome P450 and epoxide hydrolase) convert V-PYRRO/NO into PYRRO/NO by removal of the O-substituted vinyl ether group[17]. In contrast to V-PYRRO/NO, PYRRO/NO is an unstable anionic diazeniumdiolate with a very short half-life (3 s at pH 7.4, 37℃) and spontaneously decomposes into NO[17]. In other words, intra-hepatic production of NO from V-PYRRO/NO is due to the hepatic enzyme-dependent metabolism of V-PYRRO/NO. Using Alzet minipumps to deliver V-PYRRO/NO into rat systemic circulation for up to 24 h, Saavedra *et al* found no significant change in the mean arterial pressure, while liver protective effects were observed in TNFalpha / galactosamine hepatotoxic model[17]. This study suggested that NO derived from V-PYRRO/NO was present mainly in the liver rather than in the systemic circulation[17]. *In vitro* studies showed consistently that V-PYRRO/NO was specifically metabolized by hepatocytes and not by other cell types examined (endothelial cells plus Kupffer cells, pulmonary artery smooth muscle cells, pulmonary artery endothelial cells and the macrophage cell line Raw 264.7)[17].

The aim of our study was to determine the effects of an early and continuous administration of V-PYRRO/NO on liver fibrogenesis and on systemic/splanchnic hemodynamics in an appropriate model of liver injury. The bile duct ligation (BDL) model was chosen for several reasons. Like the CCL_4 model, BDL is one of the most common models of liver fibrosis and PHT. However, using BDL rather than CCl_4 to induce liver fibrosis and PHT should be preferable in our study because V-PYRRO/NO and CCl_4 both require cytochrome P450 to be metabolized. CCL_4 would therefore introduce a bias in our study. The main aim of the present study was to assess the effects of V-PYRRO/NO when administered continuously two days before BDL operation, for a total of four weeks. We found that V-PYRRO/NO was efficient in decreasing both hemodynamic disorders and liver fibrosis associated with BDL and that V-PYRRO/NO anti-fibrotic effect may be due to a decrease in lipid peroxidation.

MATERIALS AND METHODS

Animal model of cirrhosis

Male Sprague-Dawley rats (Faculty of Medicine, Angers, France) with an initial body weight of 210 to 350 g underwent BDL under ether anesthesia. The surgical procedure was performed on day 0, as previously described[20]. Under the same conditions, sham-operated rats with an initial body weight of 220 to 320 g had a laparotomy without ligation of bile duct and served as controls. According to published recommendations[21], all rats received weekly subcutaneous injections of vitamin K1 (50 μg) to decrease mortality from hemorrhagic diathesis. Protocols performed in this laboratory were approved by the French Agriculture Office in conformity with the European legislation for research involving animals.

Therapeutic regimen

Our study aimed to assess the effects of early and continuous administration of V-PYRRO/NO in rats with BDL. For that purpose, treatment (V-PYRRO/NO or placebo) was administered two days before BDL or sham-surgical operation (D2) and maintained until the day of hemodynamic measurement four weeks later (D26). Continuous treatment was made using Alzet osmotic minipumps (model 2ML2, Alzet®, USA). Because of their two-week half-life characteristic, these pumps were replaced on D12 with new pumps filled with freshly diluted solutions of V-PYRRO/NO, prepared from stock solutions of V-PYRRO/NO (100 mg in 2 mL ethanol) diluted 1:10 in NaCl 0.9%. Final concentration of V-PYRRO/NO at 5 mg/mL was chosen in order to obtain a delivery rate of 0.53 μmol/kg per hour into the rat circulation using Alzet minipumps with a 2 mL volume capacity and a 5 μL/h delivery rate (giving a two-week half-life to these minipumps). The rat average weight was around 300 g. The minipumps were inserted subcutaneously to the back of the rats under anesthesia (ether) and connected to the left femoral vein of the animals with a polyethylene catheter (PE-60, Clay Adams, NJ, USA). The study included 4 groups of rats treated either with V-PYRRO/NO (0.53 $\mu mol.kg^{-1}.h^{-1}$) or with placebo (ethanol diluted 1:10 in NaCl 0.9 %) from D2 to D26. The groups were as follows: sham with V-PYRRO/NO (n = 13), sham with placebo (n = 9), BDL with V-PYRRO/NO (n = 21), and BDL with placebo (n = 22).

Rat conditioning

Hemodynamic measurement was performed on rats anesthetized with an intraperitoneal injection of 1mL/100 kg pentobarbital (Nesdonal® 0.5 g, Rhône-Poulenc, Paris, France). All rats were given free access to food and water until 14 to 16 h before the study. Food was withdrawn to avoid digestive influences on splanchnic hemodynamics[22].

During hemodynamic measurement, body temperature was maintained at 37℃ with a homeothermic blanket system (Homeothermic Blanket Control Unit, Harvard Apparatus Inc, Natick, USA). Hemodynamic measurement was performed 30 min after manipulation, i.e. when values had stabilized, which included: mean arterial pressure (MAP), heart rate (HR), portal pressure (PP), cardiac output (CO) or index (CI), systemic vascular resistance (SVR), and splenorenal shunt (SRS) blood flow. After hemodynamic measurement, the animals were killed by exsanguination under anesthesia. Body weight and liver mass were recorded at the time of death. Gains in body mass in each group were calculated as: (final body mass - initial body mass)/initial body mass.

Hemodynamic and ascites measurement

MAP, HR, PP, SRS blood flow and CO were measured in anesthetized rats as described previously[23-25]. CO and SRS blood flow, an accurate index of collateral circulation blood flow, (as shown before[24]) were measured using the transit time ultrasound (TTU) devices. Amounts of ascites were estimated roughly and indicated with the following grades: 0: no ascites, 1: ascites absorbing less than half a compress, 2: ascites absorbing a compress, 3: ascites absorbing more than one compress.

Liver fibrosis evaluation

Area of liver fibrosis. The area of liver fibrosis was measured by image analysis as described in our laboratory[26]. Briefly, three liver sections (> 1 cm^2 each) were randomly taken from the right, median and left liver lobes of each rat. Liver sections were stained in 0.1% picrosirius red solution and histomorphometric analysis was performed on a Leica Quantimet Q570 image processor. Total liver area of fibrosis was expressed as the mean fibrosis percentage in the 3 liver sections. In each of them, 30 fields were evaluated.

Hepatic hydroxyproline content. As a liver fibrosis marker[27], hepatic hydroxyproline-content was measured using a modified version of the method described by Jamall *et al*[28] and Seifert *et al*[29]. Briefly, 3 liver fragments (250 mg) from each rat were homogenized in 6N HCl and then hydrolyzed at 110℃ for 18 h. After cooling, the hydrolysate was filtered through a 0.45-μm Millipore filter and aliquots of the hydrolysate were neutralized with NaOH 6N. Chloramine T was added to a final concentration of 2.5 mmol/L. After 5 min, 410 mmol/L paradimethyl-aminobenzaldehyde was added and the mixture was incubated for 30 min at 60℃. After cooling to room temperature, the samples were read at 560 nm with a control reagent which contained the complete system without added tissues.

The concentration of hydroxyproline in each sample was determined from a standard curve generated from known quantities of hydroxyproline. Mean hepatic hydroxyproline for each rat corresponds to three liver samples analyzed. The final result was expressed as μg of hydroxyproline/g liver protein. Liver protein content was measured in the fourth fragment of liver with a BCA Protein Kit (Pierce, Rockford, USA).

Other biochemical measurements

Liver and kidney function tests. At the end of the hemodynamic measurement, blood samples (from the femoral artery) were immediately centrifuged at 4℃, and sera were kept at -80℃ until the biochemical assays were performed. Each rat underwent blood liver function tests including total bilirubin, alkaline phosphatases (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities and blood renal function tests including urea and creatinine.

Liver cGMP assay. Hepatic cGMP levels, reflecting hepatic NO level, were measured by enzyme immunoassay using a commercially available kit (Amersham-Pharmacia Biotech, Uppsala, Sweden).

Total liver NOS activity. NOS activity was measured by converting L[^{14}C] arginine into L[^{14}C] citrulline based on the modified method from Cahill *et al*[30]. Tissues were minced and homogenized by sonication (Vibracell, Bioblock, Illkirch, France) at 4℃ in a buffer containing 320 mmol/L sucrose, 50 mmol/L Tris-HCl, 1 mmol/L ethylendiaminetetra-acetic acid, 1 mmol/L DTT, 100 mg/L PMSE 10 mg/L leupeptin, 10 μg/ml trypsin-inhibitor and 2 mg/L aprotinin. After centrifugation (1000 × *g* for 5 min at 4℃), 50 μL of the supernatant (50-100 μg protein) was incubated in a total volume of 160 μL Tris-HCI (pH 7.4) containing 0.1 mmol/L ethylendiaminetetra-acetic acid, 3 μmol/L tetrahydrobiopterin, 1 mmol/L NADPH, 5 mmol/L valine, 5 mmol/L of ethylene glyco-bi-aminoethylether-N-tetra-acetic acid (EGTA), and 1 37 KBq/Ml [^{14}C] arginine. L[^{14}C] citrulline was separated by applying the samples to columns containing pre-equilibrated DOWEX AGW-X8 and eluting them with 1 mL of 1 mmol/L citrulline. The amount of radioactivity was measured by scintillation counting (Beckmann, LS 3801, Irvine, CA, USA). Enzyme activity was expressed as pmol of citrulline formed per mg of proteins per hour. Protein concentration was measured in the supernatant using a commercially available protein kit (BCA Protein Kit, Pierce, Rockford, USA).

Serum nitrate/nitrite levels. The systemic NO production was evaluated through the measurement of serum nitrate/nitrite levels using a commercially available colorimetric assay (Nitrate/Nitrite endpoint determination colorimetric assay kit, Cayman Chemical, Ann Arbor, USA).

Liver malonedialdehyde assay (MDA). Hepatic MDA level, a marker of lipid peroxidation, was determined by the thiobarbituric acid reaction and quantified by fluorometry (excitation wavelength: 515 nm, emission wavelength: 548 nm), using 1, 1, 3, 3-tetraethoxypropane as standard[31]. Hemodynamic, biochemical assays and morphometric studies of the liver were performed by two observers unaware of the treatment given.

Statistical analysis

Quantitative variables were expressed as mean ± SD. Multiple quantitative variables were compared using the analysis of variance or by the Kruskall and Wallis test when variances were heterogeneous. Post hoc comparisons were performed using parametric tests for homogeneous (Tukey) or heterogeneous (Tamhame) variances or non-parametric test (Mann-Whitney). Box plots indicate median, interquartile range and extremes. An α risk < 5% was considered to be statistically significant. The qualitative variable 'mortality' has been studied using the χ^2 test. The statistical software

Table 1 General characteristics of rats

Characteristics	Sham		BDL		*P*
	Placebo *n* = 9	V-PYRRO/NO *n* = 12	Placebo *n* = 12	V-PYRRO/NO *n* = 10	
Initial body weight (g)	299 ± 34	274 ± 29	288 ± 56	293 ± 67	NS
Body weight gain (%)	33 ± 21	22 ± 9	14 ± 12[a]	21 ± 17	0.04
Liver / body weight (%)	2.3 ± 0.4	2.8 ± 0.3	5.9 ± 1.5[a]	5.6 ± 2.0[c]	< 0.0001
Ascites score	0	0	0.3 ± 0.9	0	NS

[a]*P* < 0.05 *vs* sham, [c]*P* < 0.05 *vs* V-PYRRO/NO sham; NS: Not significant.

Table 2 Liver and kidney serum function tests of rats

Parameter	Sham		BDL		*P*
	Placebo *n* = 9	V-PYRRO/NO *n* = 12	Placebo *n* = 12	V-PYRRO/NO *n* = 10	
Urea (mmol/L)	10.2 ± 2.1	7.9 ± 1.7	9.2 ± 1.6	9.5 ± 2.7	NS
Creatinine (μmol/L)	77 ± 25	63 ± 24	66 ± 11	73 ± 18	NS
AST (UI/L)	172 ± 52	123 ± 60	1150 ± 1480[a]	1164 ± 1349[c]	< 0.0001
ALT (UI/L)	48 ± 18	55 ± 36	88 ± 47	82 ± 20	NS
Alkaline phosphatases (UI/L)	160 ± 103	148 ± 80	380 ± 197[a]	367 ± 141[c]	< 0.001
Bilirubin (μmol/L)	1.0 ± 0.0	1.3 ± 0.5	117.0 ± 22.0[a]	101.0 ± 30.0[c]	< 0.0001

[a]*P* < 0.05 *vs* sham placebo, [c]*P* < 0.05 *vs* sham V-PYRRO/NO; NS: Not significant.

Table 3 Hemodynamic data of rats

	Sham		BDL		*P*
	Placebo *n* = 9	V-PYRRO/NO *n* = 12	Placebo *n* = 12	V-PYRRO/NO *n* = 10	
MAP (mm Hg)	119 ± 7	113 ± 11	104 ± 8[a]	98 ± 13[b]	< 0.001
Heart rate (beat/min)	441 ± 29	428 ± 44	411 ± 29	422 ± 26	NS
Cardiac index (mL/min·100 g)	14 ± 5	15 ± 4	39 ± 11[a]	33 ± 6[a,c]	< 0.0001
SVR (dyn.s.cm^{5}·100 g^{1}·10^{3})	782 ± 355	636 ± 184	236 ± 89[a]	248 ± 78[c]	< 0.0001
Portal pressure (mmHg)	9.2 ± 0.8	8.0 ± 1.2	16.3 ± 2.2[a]	11.9 ± 2.7[a,c,e]	< 0.0001
SRS BF (mL/min)	0.22 ± 0.08	0.31 ± 0.12	1.33 ± 1.34[a]	0.42 ± 0.22[e]	0.007

[a]*P* < 0.05 *vs* Sham, [c]*P* < 0.05 *vs* sham V-PYRRO/NO, [e]*P* < 0.05 *vs* BDL placebo. MAP: Mean arterial pressure, SRS BF: Spleno-renal shunt blood flow; SVR: Systemic vascular resistance; NS: Not significant.

used was SPSS version 11.5.1 (SPSS Inc., Chicago, IL, USA).

RESULTS

General characteristics of rats

Initial body weight was not significantly different among the groups. As expected in placebo-treated rats, body mass gain was significantly lower in BDL rats than in sham rats. Moreover, liver mass/body weight ratio was significantly higher in BDL rats than in sham rats. V-PYRRO/NO treatment did not influence liver mass in any group but seemed to reduce the presence of ascites in BDL rats (no ascites was observed in the V-PYRRO/NO BDL rats, in contrast with placebo BDL rats) (Table 1).

Liver and kidney serum function tests

BDL rats had a significant increase in AST and ALP activities and bilirubin level compared to their respective control groups. V-PYRRO/NO had no effect on liver or renal tests in sham or BDL rats (Table 2).

Hemodynamics

As expected, BDL rats had a significant reduction in MAP and SVR, and a marked increase in CI and portal pressure as compared to their respective control groups. In the placebo groups, SRS blood flow was significantly increased in BDL rats *vs* sham rats. V-PYRRO/NO did not significantly change the systemic hemodynamics (MAP, HR, CI and SVR) in both BDL and sham rats. In contrast, V-PYRRO/NO significantly affected portal hemodynamics in BDL rats: a 27% decrease in PP and a 74% decrease in SRS blood flow (Table 3).

Liver fibrosis and lipid peroxidation

Liver fibrosis was quantified in each group of rats based

Table 4 Liver fibrosis of rats

	Sham		BDL		*P*
	Placebo *n* = 9	V-PYRRO /NO *n* = 12	Placebo *n* = 12	V-PYRRO /NO *n* = 10	
Area (%)	2.5 ± 0.5	3.1 ± 0.8	12.1 ± 4.3[a]	8.4 ± 3.6[c,e]	< 0.0001
Liver hydroxyproline (μg/g protein)	164 ± 26	171 ± 49	381 ± 88[a]	268 ± 83[c,e]	< 0.0001

[a]$P < 0.05$ *vs* Sham, [c]$P < 0.05$ *vs* sham V-PYRRO/NO, [e]$P < 0.05$ *vs* BDL placebo.

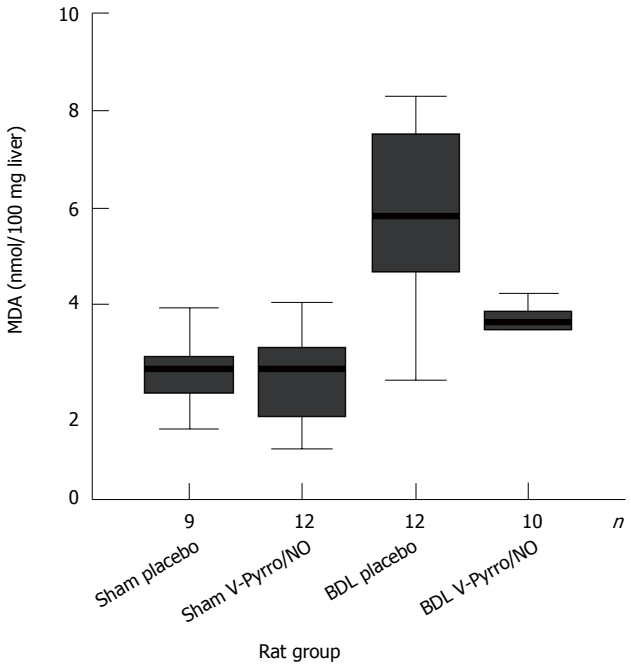

Figure 1 Liver malonedialdehyde content (Kruskal-Wallis test: $P = 0.002$).

on the area of liver fibrosis and hepatic hydroxyproline content (Table 4). As expected, BDL operation induced a dramatic increase in both markers of liver fibrosis. V-PYRRO/NO significantly reduced the area of liver fibrosis and hepatic hydroxyproline content in BDL rats (by 31% and 30%, respectively). Since NO has ROS scavenging capabilities and lipid peroxidation products are known to stimulate fibrogenesis, we measured liver MDA levels in each group of rats to determine whether the anti-fibrotic effect of V-PYRRO/NO was related to the inhibition of lipid peroxidation. V-PYRRO/NO significantly decreased liver MDA level in BDL rats by 44%, $P = 0.007$ (Figure 1).

Measurements reflecting NO level

V-PYRRO/NO significantly increased liver cGMP levels in BDL rats ($P = 0.04$) (Figure 2) but decreased liver total NOS activity ($P = 0.04$) (Figure 3). V-PYRRO/NO did not significantly change the serum nitrate/nitrite levels in BDL rats (82 ± 19 *vs* 72 ± 16 μmol/L, NS) (data not shown).

DISCUSSION

V-PYRRO/NO had no noxious effects on body and liver

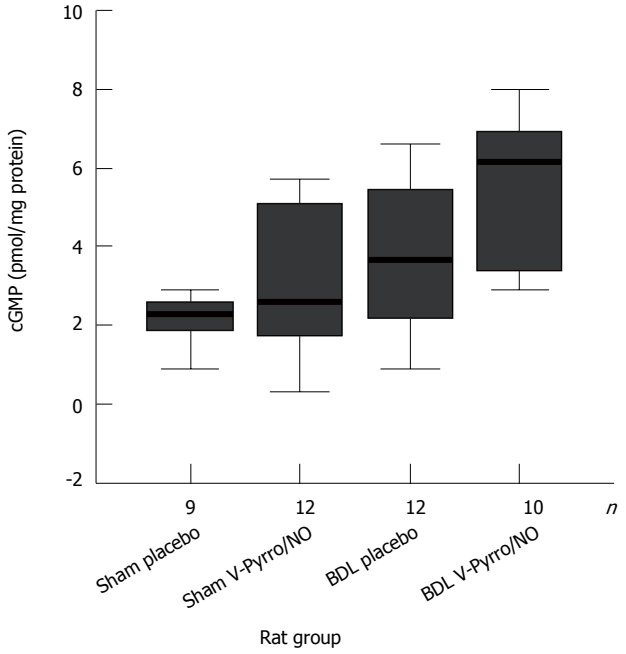

Figure 2 Liver cGMP level (Kruskal-Wallis test: $P = 0.003$).

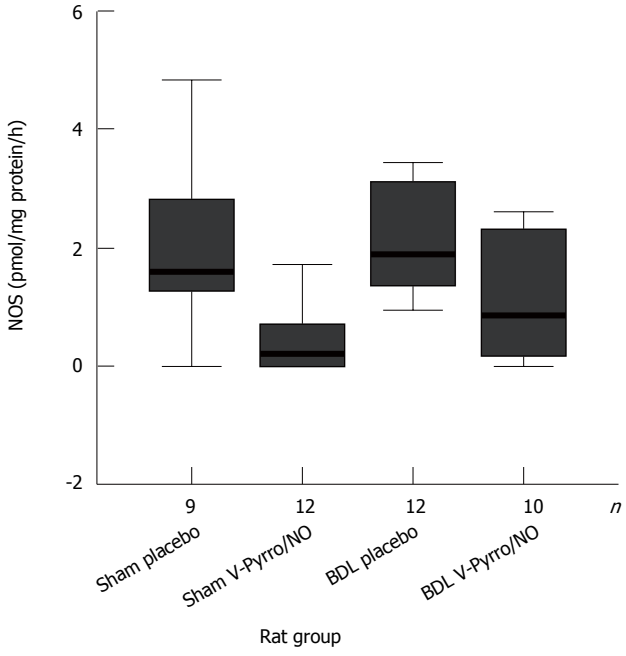

Figure 3 Liver total NOS activity (Kruskal-Wallis test: $P = 0.001$).

weight gains, hepatic and renal functions, and did not significantly alter systemic hemodynamics (MAP, CI, or SVR) in both sham and BDL rats. Our data on V-PYRRO/NO systemic effects after continuous administration complete those of Saavedra *et al* showing that acute systemic administration of V-PYRRO/NO had no effect on MAP in the normal rats[17,19]. On the other hand, V-PYRRO/NO significantly decreased PP and SRS blood flow (an index of collateral circulation) in BDL rats. The decrease in PP can be attributed to reduced hepatic vascular resistance and/or diminished fibrogenesis. The decrease in SRS blood flow might be partly due to the reduction in PP or to other

mechanisms. Concerning the effect of liver-specific NO donor on PP, Fiorucci *et al*[32] reported a decrease in PP upon NCX-1000 administration for 5 d in four-week BDL rats whereas Loureiro-Silva *et al*[33] did not document such a decrease in their 10-12 week CCl_4 rats treated for 14 d with the same dosage of NCX-1000. The reason for NCX-1000 effect on PP is not obvious but may be model-related[34]. Our findings support this concept in the BDL model where V-PYRRO/NO did decrease PP.

Our study showed that V-PYRRO/NO limited the development of liver fibrosis in BDL rats (there was a significant decrease in the area of liver fibrosis and liver hydroxyproline content). Products of lipid peroxidation are known to stimulate fibrogenesis. To determine whether the anti-fibrotic effect of V-PYRRO/NO was related to the inhibition of lipid peroxidation, we measured MDA level (a marker of lipid peroxidation) in the livers of each group. Consistently with a study in the acetaminophen-induced hepatotoxicity murine model[35], we found that V-PYRRO/NO decreased hepatic MDA level in BDL rats. The decrease in liver lipid peroxidation in our V-PYRRO/NO rats may result from a reduction in hepatocyte death as suggested by several *in vitro* studies[17-19,35]. Thus, the mechanism underlying the anti-fibrotic effect of V-PYRRO/NO may be a NO-mediated protection against lipid peroxidation.

In the present study, V-PYRRO/NO treatment increased hepatic cGMP levels (reflecting hepatic NO generation) but decreased hepatic total NOS activity. This may be due to NO feedback inhibition on NOS activity, as suggested in a recent study[36]. As discussed in the introduction section, V-PYRRO/NO was designed to be a stable molecule until metabolized into PYRRO/NO by enzymes predominant in the liver. Hepatocytes contain high levels of enzymes capable of metabolizing V-PYRRO/NO and incubation with V-PYRRO/NO (24 h, 1 mmol/L) resulted in increased nitrite/nitrate levels in the supernatant of these cells in contrast to four other cell types examined[17]. Confirming other studies, we observed no systemic hemodynamic change and unaltered nitrate/nitrite levels in the serum of our BDL and sham rats after V-PYRRO/NO treatment (0.53 μmol/L per hour for 24 d), supporting the absence of systemic diffusion of NO from V-PYRRO/NO[17-19]. Moreover, in agreement with a study using acute administration of V-PYRRO/NO[17], we found that cGMP increased in the liver but not in a non-liver tissue (such as lungs, data not shown) of the rats chronically treated with V-PYRRO/NO. Our study also suggests that exogenous NO production in the liver is beneficial to the treatment of liver fibrosis and PHT. Additional experiments are however needed to rule out an effect of the bio-converted drug (i.e. the carrier compound without NO group).

In conclusion, the present *in vivo* study showed that early and continuous administration of V-PYRRO/NO via osmotic minipumps resulted in a decrease in liver lipid peroxidation and fibrosis, as well as splanchnic hemodynamic improvement (decrease in portal pressure and in collateral circulation development), without systemic hemodynamic effects. This result supports other studies showing that NO derived from V-PYRRO/NO was delivered preferentially to the liver rather than in the systemic circulation. Taken together, our study suggests that targeting NO delivery to the liver might be an interesting option for the treatment of liver fibrosis or early cirrhosis.

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