Effect of the nitric oxide donor V-PYRRO/NO on portal pressure and sinusoidal dynamics in normal and cirrhotic mice

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Edwards C, Feng HQ, Reynolds C, Mao L, Rockey DC. Effect of the nitric oxide donor V-PYRRO/NO on portal pressure and sinusoidal dynamics in normal and cirrhotic mice. Am J Physiol Gastrointest Liver Physiol 294: G1311–G1317, 2008. —Reduced sinusoidal endothelial nitric oxide (NO) production contributes to increased intrahepatic resistance and portal hypertension after liver injury. We hypothesized that V-PYRRO/NO, an NO donor prodrug metabolized “specifically” in the liver, would reduce portal venous pressure (PVP) without affecting the systemic vasculature. Liver injury was induced in male BALB/c mice by weekly CCl4 gavage. PVP and mean arterial pressure were recorded during intravenous administration of V-PYRRO/NO. In vivo microscopy was used to monitor sinusoidal diameter and flow during drug administration. Mean PVP was increased in CCl4-treated mice compared with sham-treated mice. In dose-response experiments, the minimum dose of PYRRO/NO required to acutely lower PVP by 20%, the amount believed to yield a clinically meaningful outcome, was 200 nmol/kg. This dose decreased portal pressure in cirrhotic (23.4 ± 2.0%, P < 0.001 vs. vehicle) and sham-treated (19.5 ± 2.3%, P < 0.001 vs. vehicle) animals by a similar magnitude. This concentration also led to dilation of hepatic sinusoids and an increase in sinusoidal venules, consistent with a reduction of intrahepatic resistance. The effect of V-PYRRO/NO on mean arterial pressure was significant at all concentrations tested, including the lowest, 30 nmol/kg (P < 0.001 vs. vehicle for all doses). We conclude that V-PYRRO/NO had widespread vascular effects and, as such, is unlikely to be suitable for treatment of portal hypertension. As the potential of this or other similar compounds for treatment of portal hypertension is evaluated, effects on the systemic vasculature will also need to be considered.

LIVER CIRRHOSIS ACCOUNTED for the deaths of over 27,000 people in the United States in 2002 (12). Much of the mortality from cirrhosis is attributable specifically to complications resulting from portal hypertension, such as bleeding esophageal varices and spontaneous bacterial peritonitis. Reduction of portal pressure, as measured by the hepatic venous pressure gradient, in patients with cirrhosis by ≥20% (and/or to <12 mmHg) has been associated with decreased incidence of variceal hemorrhage (5). Moreover, reduction of portal pressure may be beneficial with respect to other complications, such as ascites, spontaneous bacterial peritonitis, and hepatic encephalopathy and, furthermore, may improve overall survival (1). Therefore, therapies targeting portal hypertension are of particular importance in the treatment of liver disease.

From a pathophysiological standpoint, available data indicate that intrahepatic nitric oxide (NO) is reduced after liver injury and that this deficit plays a role in the increased intrahepatic resistance typical of portal hypertension (20). The mechanism underlying the reduction in NO is tied to dysfunction of the enzyme responsible for NO production by the sinusoidal endothelium, endothelial NO synthase (eNOS) (14). The activity of eNOS, in turn, is regulated by a complex array of molecular events, including extensive posttranslational modification (8). Critical regulatory interactions include activation by Akt, a serine/threonine kinase (15), and inhibition by caveolin-1 (4, 9), an integral component of membrane caveolae. After liver injury, changes in eNOS regulation, including decreased interaction with Akt (14) and increased caveolin-1 binding due to upregulated levels of the caveolar protein (23), lead to decreased production of NO in the liver vascular endothelium. Therefore, therapeutic replenishment of NO within the portal system is a particularly attractive target.

Importantly, the vasodilatory effects of NO are not unique to the portal vasculature. Nonspecific NO donors, such as sodium nitroprusside and glyceryl trinitrate, cause vasodilation throughout the body. In fact, these drugs prominently influence systemic hemodynamics, perhaps more so than portal pressure, even when given intraportally (25). Excessive systemic vasodilation is a highly undesirable effect of a therapy for portal hypertension, especially because of the typical systemic circulatory dysfunction found in cirrhosis. This “vasodilatory syndrome” is marked by decreased systemic vascular resistance and hypotension, and there is extensive evidence that these changes are mediated by increased NO production outside the portal system (16). Increased NO in the splanchic arterial bed results in increased blood flow (the “hyperdynamic circulation”), contributing further to portal hypertension (26). These findings emphasize the importance of targeting any NO-based therapy to the liver specifically.

One strategy for liver-specific NO delivery is to take advantage of the NO-donating properties of the diazeniumdiolate ion and the enzymatic properties of liver-specific enzymes. V-PYRRO/NO is a stable NO donor fashioned from a pyrrolidine diazeniumdiolate ion with attached prodrg groups predicted to be metabolized by cytochrome P-450 and hepatic epoxidase (22). The drug is protective against several types of hepatotoxicity, presumably because of the cytoprotective effects of NO (10, 13). We hypothesized that V-PYRRO/NO should have

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specific effects within the liver, in particular at the level of the hepatic sinusoid. Thus we explored the potential of V-PYRRO/NO as a selective therapy for portal hypertension by testing the effects of the drug on portal pressure, systemic pressure, and sinusoidal diameter ($D_s$) in a mouse model of cirrhosis. We specifically postulated that, in a mouse model of cirrhosis and portal hypertension, intravenous administration of V-PYRRO/NO would selectively replenish NO in the liver sinusoids, leading to sinusoidal dilation and, therefore, decreased hepatic vascular resistance. We further hypothesized that this liver-selective drug would not direct NO systemically and, therefore, would not affect systemic vascular hemodynamics.

**MATERIALS AND METHODS**

*Animal model of cirrhosis.* CCl₄ (2 ml/kg in a 1:1 corn oil mix) was administered to 4- to 6-wk-old male BALB/c mice by gavage for 8 wk (1 dose/wk). Such treatment resulted in grossly nodular livers, which, on histological examination, demonstrated substantial bridging fibrosis. Age-matched mice given corn oil alone served as controls. All animals received humane care according to National Institutes of Health guidelines; studies were performed after approval by local Institutional Animal Care and Use Committees.

*Portal and systemic pressure measurement.* Mice were anesthetized with pentobarbital sodium (Nembutal; 50 mg/kg ip), and the portal vein was catheterized with a 24-gauge intravenous catheter (Becton Dickinson). A low-pressure transducer (LPA, Micromed, Louisville, KY) was connected to the catheter via polyethylene tubing (Clay Adams). For mean arterial pressure (MAP) measurement, the axillary artery of each anesthetized mouse was cannulated with polyethylene tubing connected to a blood pressure transducer (BPA, Micromed). Pressures were recorded using Digimed System Integrator software (Micromed). V-PPYRRO/NO (Cayman Chemical) in 0.9% saline was administered as a bolus by tail vein injection at 30–400 nmol/kg.

*In vivo microscopy.* Mice were anesthetized, and the jugular vein was catheterized for administration of experimental substances. The liver was freed and placed onto a glass coverslip embedded in a petri dish in which the animal was placed. For immobilization of the liver, another glass coverslip was placed on top of the liver. The liver vasculature was visualized using an inverted microscope (Nikon) equipped with a ×20 Plan Apo objective and a charge-coupled device camera (DAGE-MTI). Time-lapse video recordings of liver sinusoids were created before and after intravenous administration of specific compounds (i.e., 0.9% saline, endothelin-1, methoxamine, and V-PYRRO/NO); $D_s$ was analyzed with image analysis software (MetaVue, Universal Imaging). Baseline images were obtained for 2 min before drug infusion, the preparation and hemodynamics were allowed to stabilize for 8 min, and images were obtained for 2 min. Random fields were chosen, and then a threshold level of color pixels corresponding to sinusoids was assigned. The thresholded area was systematically quantitated in a blinded fashion. Data were analyzed as described below.

FITC-labeled red blood cells (RBCs) were prepared as described elsewhere (2, 27). Briefly, RBCs from 1 ml of heparinized blood from a donor mouse were washed three times in Alsever’s buffer [in g/l: 20.5 glucose, 8.0 citric acid trisodium salt, 0.55 citric acid, and 3.766 NaCl (pH 6.2)] and once in bicine-saline buffer [in g/l: 3.264 bicine, 0.399 Ca(OH)₂, and 7.288 NaCl (pH 8.5)]. FITC at 4 mg/ml RBC suspension was dissolved in 0.1 ml of N,N-dimethylformamide and added to 1:1-diluted RBCs in the bicine-saline buffer. After incubation for 3 h at room temperature on a rocking plate, cells were washed five times by centrifugation in bicine-saline buffer, resuspended, and injected.

Video images were digitized and analyzed (frame by frame) with image analysis software (MetaVue, Universal Imaging). $D_s$ (in μm) and RBC velocity ($V_{RBC}$, in μm/s) were measured in the same sinusoidal segment (~200 μm long) of at least five different sinusoids within each liver. The velocity of two FITC-labeled RBCs was measured within each sinusoid, and $V_{RBC}$ values were expressed as the average of the two individual $V_{RBC}$ values per sinusoid. Volumetric flow ($Qv$, in μl/s) was calculated from $V_{RBC}$ and sinusoidal cross-sectional area ($πD_s^2$) according to the following equation: $Qv = V_{RBC} × π × D_s × D_s$ (11).

*Statistics.* Values are means ± SE. Statistical analysis was conducted using Microsoft Excel and SigmaStat (SPSS). Student’s two-tailed t-test or ANOVA was used for statistical comparisons. Statistical analysis was performed by using an independent Student’s t-test or one-way ANOVA with Tukey’s post hoc test when appropriate. $P < 0.05$ was considered statistically significant. For calculation of mean values and statistical variation, $n$ indicates the number of experiments.

**RESULTS**

*Portal and arterial pressure in a mouse model of cirrhosis.* Continued CCl₄ treatment resulted in grossly nodular livers, which, on histological examination, demonstrated bridging fibrosis. Mean portal pressure in CCl₄-treated mice was increased compared with mice treated with vehicle (corn oil) alone (4.03 ± 0.2 vs. 3.21 ± 0.2 mmHg, $P = 0.05$) and untreated mice. A small difference in baseline portal venous pressure (PVP) between oil-treated and untreated mice was also noted (3.21 ± 0.2 vs. 2.96 ± 0.2 mmHg), but this was not statistically significant. MAP was decreased in cirrhotic animals compared with controls (86.9 ± 4.7 vs. 105.8 ± 3.3 mmHg, $P = 0.019$).

*Vascular responses to V-PYRRO/NO.* Administration of a bolus of V-PYRRO/NO (200 nmol/kg iv) resulted in a marked, acute drop in portal pressure in CCl₄- and oil-treated animals, with a gradual return toward baseline (Fig. 1). The drop from baseline pressure to the minimum pressure occurred within 1 min of exposure to V-PYRRO/NO in nearly all cases. The drop in MAP after bolus V-PYRRO/NO followed a similarly acute time course (Fig. 2). The time required for pressure to return to baseline was variable in all groups but was always longer than the time course of the pressure drop.

Because of the substantial effect of 200 nmol/kg V-PYRRO/NO, we examined a series of doses in dose-response experiments (Figs. 3 and 4). Administration of vehicle had no effect; therefore, we quantified the baseline level of pressure variation as a control in “vehicle” experiments. V-PYRRO/NO at 30–400 nmol/kg resulted in a percent decrease in portal pressure that was significantly greater than baseline variation ($P < 0.001$ vs. vehicle). The minimum dose of V-PYRRO/NO required to decrease portal pressure in cirrhotic animals by 20% was 200 nmol/kg; >200 nmol/kg V-PYRRO/NO did not consistently increase the effect.

To assess the response to 200 nmol/kg V-PYRRO/NO in injured mice, normal and injured mice were exposed to 200 nmol/kg V-PYRRO/NO or vehicle (Table 1). Interestingly, 200 nmol/kg V-PYRRO/NO decreased portal pressure in cirrhotic (23.42 ± 2.0% vs. 34.72 ± 6.7% vs. vehicle) and sham-treated (19.59 ± 2.3% vs. 0.001 vs. vehicle) animals by a similar magnitude. Indeed, the difference in the magnitude of the response between groups was not significant. At 200 nmol/kg, V-PYRRO/NO also significantly decreased MAP in cirrhotic (21.21 ± 3.9% vs. 0.001 vs. vehicle) and control (34.02 ± 7.6% vs. 0.001 vs. vehicle) mice. The difference in response magnitude between groups was not significant.
As with portal pressure, reduction in MAP in response to V-PYRRO/NO was dose dependent, and V-PYRRO/NO decreased MAP at all doses, even the lowest tested, 30 nmol/kg (\(P < 0.001\) vs. vehicle for all doses; Fig. 4). At most doses, there was no statistically significant difference between the effect of the drug on portal pressure and MAP, except at 400 nmol/kg, which had a greater effect on MAP than on PVP (\(P = 0.035\)).

By comparison, administration of 200 nmol/kg sodium nitroprusside, a nonspecific NO donor, had larger effects on portal pressure and MAP in CCl\(_4\)-treated animals. Sodium nitroprusside decreased portal pressure by an average of 30.1% and decreased MAP by 55.3% (data not shown). The difference in the magnitude of the portal pressure response to V-PYRRO/NO vs. sodium nitroprusside was not statistically significant, but the arterial pressure response to 200 nmol/kg sodium nitroprusside was significantly greater than the response to 200 nmol/kg V-PYRRO/NO (\(P = 0.001\)).

In vivo microscopy. To test the hypothesis that V-PYRRO/NO decreases intrahepatic resistance by releasing NO within sinusoids, we went to great length to design a protocol for in vivo microscopy in mice. Using previous studies in rats (3) as a guide, we were able develop a protocol that allowed us to monitor acute changes in \(D_s\) in real time (Fig. 5). We captured baseline data, and, after exposing the liver to specific agents, we allowed the system to equilibrate; postdrug exposure data were obtained 8 min later (we noticed subtle changes in \(D_s\) during the “equilibration phase” and, therefore, chose to obtain data from the period after drug infusion). Importantly, infusion of endothelin-1 led to a reduction in \(D_s\) in many sinusoids and some larger vascular structures (Fig. 5, A and B). Saline had no effect on sinusoidal volume (Fig. 5C), whereas endothelin-1 led to a reduction in sinusoidal volume (Fig. 5D). Similar to changes in portal pressure, administration of V-PYRRO/NO led to a relatively rapid increase in \(D_s\) (Fig. 5E).

V-PYRRO/NO increased the volume occupied by sinusoids (Figs. 5E and 6), consistent with the postulated sinusoidal dilation. In control experiments, administration of endothelin-1 (Fig. 5D) and methoxamine (Fig. 6), both known vasoconstric-
tors, decreased the volume occupied by sinusoids, consistent with sinusoidal constriction, and saline caused little change in the sinusoidal area.

To verify that the change in $D_s$ was consistent with a change in intrahepatic resistance, we next evaluated the effect of V-PYRRO/NO (and endothelin-1) on sinusoidal flow in normal BALB/C mice. We chose to assess flow by measuring FITC-labeled $V_{RBC}$. In Fig. 7A, FITC-labeled $V_{RBC}$ was recorded over a 2-s period. Changes in $D_s$ in the same livers are shown in Fig. 7B. As previously shown, $D_s$ was significantly decreased after endothelin-1 infusion and significantly increased after V-PYRRO/NO perfusion (Fig. 7B). No significant change was noted after saline perfusion. Quantitation of $V_{RBC}$ showed a significant reduction by endothelin-1 but a modest increase by V-PYRRO/NO (Fig. 7C). Changes in $Q_s$ were readily detected after infusion of endothelin-1 and V-

Table 1. V-PYRRO/NO decreases portal and mean arterial pressure in normal mice and mice with liver injury

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<th>Baseline</th>
<th>V-PYRRO/NO-Induced Decrease, %</th>
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<tr>
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<td>Portal Pressure, mmHg</td>
<td>Arterial Pressure, mmHg</td>
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<tr>
<td>Sham</td>
<td>3.2 ± 0.2</td>
<td>105.8 ± 3.3</td>
</tr>
<tr>
<td>CCl$_4$</td>
<td>4.03 ± 0.2</td>
<td>86.9 ± 4.7</td>
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Values are means ± SE ($n = 6$). Baseline portal and mean arterial pressures were measured in sham-treated and CCl$_4$-injured mice, which were then treated with V-PYRRO/NO (200 nmol/kg iv). Portal pressure was significantly higher ($P < 0.007$) and arterial pressure was significantly lower ($P < 0.011$) in CCl$_4$-injured than in sham-treated mice. Difference between baseline pressure before V-PYRRO/NO and the lowest pressure after V-PYRRO/NO was quantified. In all groups, V-PYRRO/NO had a significant effect ($P < 0.001$ vs. vehicle). Magnitude of the effect of V-PYRRO/NO was not significantly different between sham-treated and CCl$_4$-injured mice, nor was magnitude of the effect on arterial pressure significantly different from magnitude of the effect on portal pressure.

**DISCUSSION**

Experimental strategies that increase NO in the portal vasculature have been shown to decrease PVP. An ideal safe and effective intervention for portal hypertension would be a liver-specific NO donor drug that could decrease PVP without affecting systemic hemodynamics. V-PYRRO/NO was designed to be just such a drug, with prodrug groups chosen on the basis of their selective metabolism by liver enzymes (22). The drug has generated great interest, inasmuch as it has been demonstrated to be protective against several types of hepatotoxicity, presumably because of the cytoprotective effects of NO (10). In the present study, we have demonstrated that intravenous bolus administration of V-PYRRO/NO acutely decreased PVP over a range of doses in normal mice and mice with liver injury. However, V-PYRRO/NO also reduced MAP over the same dose range, and the effect of the drug on portal and arterial pressure was nearly identical in magnitude of change compared with baseline. For these reasons, we conclude that V-PYRRO/NO is unlikely to be an ideal candidate for acute treatment of portal hypertension in patients with cirrhosis.

We were surprised by the apparent nonselectivity of V-PYRRO/NO; the reason for this, despite the careful selection of prodrug groups to be metabolized only by liver-specific enzymes, is unclear. The drug is hypothesized to go through two stages of metabolism: 1) cytochrome P-450 metabolizes the drug to an epoxide intermediate (cytochromes P450 2E1 and 2B1 have been specifically implicated (10), and 2) an epoxide hydrolase converts the intermediate to a hemiacetal, which spontaneously degenerates, producing NO (22). If these two steps could be accomplished without the use of these liver-specific enzymes, it is possible that NO could be released by the drug outside the liver. The drug might actually be metabolized by similar enzymes [e.g., there exists a soluble, as well as a liver microsomal, form of mammalian epoxide hydrolase (7)]. Alternatively, it might undergo nonenzymatic breakdown in the peripheral vasculature [the drug’s inventors have also speculated that the epoxide intermediate might be
able to undergo spontaneous hydrolysis (24)]. Evidence arguing against this idea is that when the metabolism of V-PYRRO/NO in various cell monolayers, including vascular smooth muscle and endothelial cells, was compared, V-PYRRO/NO was metabolized only in hepatocytes (22). It could be possible that the drug truly is processed only in hepatocytes but circulates to the peripheral vasculature efficiently enough in vivo to affect MAP.

It has been previously reported that 30 nmol/kg V-PYRRO/NO had a minimal effect on MAP in rats, which leads to the conclusion that the drug does not affect systemic resistance (22). In contrast, we found that 30 nmol/kg V-PYRRO/NO elicited only a small change in MAP in cirrhotic mice (Fig. 4). Although 30 nmol/kg V-PYRRO/NO induced a statistically significant decrease in MAP, perhaps more important is the observation that a much larger dose (200 nmol/kg) was required to obtain the therapeutic goal of a 20% decrease in portal pressure, and, at this dose, MAP was greatly affected (i.e., decreased by 21%) by administration of the drug (Figs. 4 and 5).

Previous studies have examined the effect of continuous infusion of V-PYRRO/NO (by pump, in contrast to bolus injection used in our study). In a study of bile duct-ligated rats, continuous infusion of V-PYRRO/NO decreased portal pressure by 27% on average but had little effect on systemic pressure (17). Similarly, continuous infusion of V-PYRRO/NO decreased portal pressure by an average of 2.8 mmHg in pigs but had no effect on systolic blood pressure (19). The acute decrease in portal pressure we observed was consistent with pharmacokinetic studies showing that V-PYRRO/NO is rapidly cleared from the plasma, with a mean residence time of only 3.4 min (24). Interestingly, the effect of V-PYRRO/NO on PVP was not permanently sustained in our study: portal pressure gradually returned to baseline. The published results of continuous infusion studies, then, suggest that this decrease in portal

![Figure 5](image_url)

Fig. 5. Quantification of sinusoidal diameter changes by in vivo microscopy. Representative images were obtained by in vivo microscopy. A: representative magnified (×20) view of a mouse liver shown in pseudocolor to emphasize branching anatomy of sinusoid. Capsule can be seen at far right (bright blue). B: representative in vivo images of hepatic sinusoids before and after administration of endothelin-1 (200 nmol/kg in a volume of 120 μl, horizontal bar), demonstrating sinusoidal constriction (bar = 50 μm). C: percentage of each image area occupied by sinusoids was quantified during intravenous infusion of saline (used as a control). Delivery of saline (also in a volume of 120 μl, horizontal bar) had minimal effect on sinusoidal area. D: endothelin-1 (ET-1) was used as an additional control. Endothelin-1 caused sinusoidal constriction, decreasing sinusoidal area. E: administration of 200 nmol/kg V-PYRRO/NO (also in a volume of 120 μl, horizontal bar) caused sinusoidal dilation, increasing sinusoidal area. Results in C–E are representative of data from 10 experiments.

![Figure 6](image_url)

Fig. 6. Change in sinusoidal diameter after exposure of the liver to vasoactive compounds. In vivo microscopy images of liver sinusoids were obtained during administration of saline, V-PYRRO/NO (200 nmol/kg), or methoxamine (2 nmol/kg), and percentage of each image area occupied by sinusoids was quantified before and after administration of drug (data for peak change during an observation period of 10 min is shown). Effects of methoxamine and V-PYRRO/NO were significantly different from effect of saline (n = 6). *P < 0.05; †P < 0.01.
pressure becomes stable with pump administration. The fact that the published results differ in the case of MAP, which is apparently not stably decreased, is intriguing. It is possible that tolerance to the hypotensive effects of the drug develops over time, so that a decrease in MAP is seen with bolus administration, but not with continuous infusion.

Liver-selective NO donor drugs other than V-PYRRO/NO have been described. The drug NCX-1000 comprises an NO-releasing moiety attached to ursodeoxycholic acid, a compound metabolized solely by hepatocytes (6). In one study, rats were pretreated with NCX-1000 or ursodeoxycholic acid given orally and then treated with CCl₄ to induce cirrhosis. Baseline PVP was lower in those pretreated with NCX-1000 than in the control group. Although no bolus or intravenous administration was attempted to rule out the possibility that the drug could cause an acute decrease in MAP, the authors speculate that the stability of the drug causes very slow release of NO into the bloodstream and, thereby, minimizes its effects on MAP. On the other hand, NCX-100 appeared to reduce fibrogenesis, and this may have contributed to reduced portal pressure (6).

V-PYRRO/NO has a slightly longer half-life when given intraperitoneally than when given intravenously, and a large proportion of the drug may be retained in the liver at first pass. This suggests that the MAP drop caused by V-PYRRO/NO might also be mitigated by oral or intraperitoneal, rather than intravenous, administration of the drug. Nevertheless, the precipitous drop in MAP that we observed after bolus V-PYRRO/NO administration is concerning. For any potentially liver-selective NO donors, the potential to cause acute hypotension if administered acutely must be addressed before it can be asserted that any such drug truly has “no effect” on systemic hemodynamics.

Liver specificity is important in NO donor therapies for portal hypertension, because eNOS is actually upregulated and NO is overproduced (the vasodilatory syndrome) in the systemic circulation in this disease (16, 21). Administration of a nonspecific NO donor to a cirrhotic individual with the vasodilatory syndrome, therefore, could dangerously exacerbate systemic hypotension (16). Nonselective NO donors also worsen renal function and sodium retention in patients with cirrhosis and ascites (6). Aside from systemic hypotension, a major component of the vasodilatory syndrome is an increased hemodynamic response to endothelium-dependent vasodilators (21). In our cirrhotic mice, baseline MAP was decreased compared with controls, but they did not demonstrate an increased MAP response to V-PYRRO/NO relative to controls. This suggests that the drug operates in an endothelium-independent manner. Nevertheless, any additional systemic NO could potentially further contribute to the abnormalities in vascular tone seen in cirrhosis.

Increased intrahepatic resistance is an important factor in portal hypertension and one that we hypothesized could be ameliorated by V-PYRRO/NO. However, data from animal models have shown that increased portal venous inflow also is
a key factor in the pathogenesis and maintenance of the portal hypertensive state (21, 26). For this reason, β-blockers, such as propranolol, have been extensively investigated and used clinically to ameliorate portal hypertension by constricting the splanchnic vasculature, thereby decreasing portal vein inflow. However, the decrease in MAP accompanying V-PYRRO/NO administration suggests that it would be more likely to cause splanchnic vasodilation and increased portal vein inflow. This indicates that decreased intrahepatic resistance (rather than effects on flow) is the mechanism whereby V-PYRRO/NO decreases PVP. It further suggests that the pairing of the drug with a vasoconstrictive agent, such as a β-blocker, might positively modulate its hemodynamic effects and should be investigated. Pairing of a β-blocker with a nonspecific NO donor has previously been shown to be effective in lowering portal pressure (i.e., the hepatic venous pressure gradient) and appeared to be more effective than an NO donor alone (18).

In the present study, we monitored the physiological response to an NO donor in real time, through continuous pressure measurement and through correlation of changes in $D_s$ and sinusoidal flow using in vivo microscopy. Furthermore, the data suggest that the mechanism for the effect of V-PYRRO/NO (and endothelin-1) was alteration of intrahepatic resistance to blood flow, presumably at the level of the sinusoid. The strategies used here could be applied to the evaluation of other NO donor and vasoactive molecules and to the study of responsiveness to such molecules in disease states. For example, animals with experimentally induced portal hypertension have enhanced systemic response to some vasodilators and impaired response to vasoconstrictors. Exploration of the responsiveness of these animals to NO donor drugs could yield insight into the factors that determine sinusoidal endothelial responsiveness to exogenous NO. This, in turn, will help clarify the potential effectiveness of NO replacement in disease states, such as portal hypertension, that involve eNOS dysfunction.

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