The Nitric Oxide Donor, O\textsuperscript{2}-Vinyl 1-(Pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO), Protects against Cadmium-Induced Hepatotoxicity in Mice

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Received December 29, 2003; accepted March 9, 2004

ABSTRACT

The nitric oxide (NO) donor, O\textsuperscript{2}-vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO), is metabolized by P450 enzymes to release NO within the liver and is effective in protecting against hepatotoxicity of endotoxin and acetonaminophen. This study examined the effects of V-PYRRO/NO on cadmium (Cd) hepatotoxicity in mice. Mice were given multiple injections of V-PYRRO/NO (10 mg/kg, s.c. at 2-h intervals) before and after a hepatotoxic dose of Cd (3.7 mg/kg Cd as CdCl\textsubscript{2}, i.p.). V-PYRRO/NO administration reduced Cd-induced hepatotoxicity as evidenced by reduced serum alanine aminotransferase activity, improved pathology, and reduced hepatic lipid peroxidation. The protection by V-PYRRO/NO was not mediated by altered Cd distribution to the liver or within hepatic subcellular fractions. Similar inductions of metallothionein, a metal-binding protein, were observed in mice receiving Cd alone or Cd plus V-PYRRO/NO. Real-time reverse transcription-polymerase chain reaction analysis revealed that V-PYRRO/NO administration suppressed the expression of inflammation-related genes such as macrophage inflammatory protein-2, CXC chemokine, thrombospondin-1, intracellular adhesion molecular-1, and interleukin-6. V-PYRRO/NO also suppressed the expression of acute phase protein genes and genes related to cell-death pathways, such as c-jun/AP-1, nuclear factor-κB, early response growth factor-1, heme oxygenase-1, caspase-3, growth arrest, and DNA-damaging protein-153. In summary, the liver-selective NO donor, V-PYRRO/NO, protects against Cd hepatotoxicity in mice. This protection is not mediated through altered distribution of Cd but may be related to reduced hepatic inflammation, reduced acute phase responses, and the suppression of cell-death-related components.

O\textsuperscript{2}-Vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO), a nitric oxide (NO) prodrg that targets the liver, is created by adding a vinyl functional group to the terminal oxygen of pyrrolidine diazeniumdiolate (Saavedra et al., 1997). V-PYRRO/NO is a stable diazeniumdiolate that can circulate freely throughout the body until it is metabolized to release NO by enzymes, presumably cytochromes P450, in the liver (Saavedra et al., 1997). V-PYRRO/NO administration reduced Cd-induced hepatotoxicity as evidenced by reduced serum alanine aminotransferase activity, improved pathology, and reduced hepatic lipid peroxidation. The protection by V-PYRRO/NO was not mediated by altered Cd distribution to the liver or within hepatic subcellular fractions. Similar inductions of metallothionein, a metal-binding protein, were observed in mice receiving Cd alone or Cd plus V-PYRRO/NO. Real-time reverse transcription-polymerase chain reaction analysis revealed that V-PYRRO/NO administration suppressed the expression of inflammation-related genes such as macrophage inflammatory protein-2, CXC chemokine, thrombospondin-1, intracellular adhesion molecular-1, and interleukin-6. V-PYRRO/NO also suppressed the expression of acute phase protein genes and genes related to cell-death pathways, such as c-jun/AP-1, nuclear factor-κB, early response growth factor-1, heme oxygenase-1, caspase-3, growth arrest, and DNA-damaging protein-153. In summary, the liver-selective NO donor, V-PYRRO/NO, protects against Cd hepatotoxicity in mice. This protection is not mediated through altered distribution of Cd but may be related to reduced hepatic inflammation, reduced acute phase responses, and the suppression of cell-death-related components.
Protection against Cd Hepatotoxicity by V-PYRRO/NO

Klaassen, 1984). Unlike most hepatotoxins, Cd produces liver damage without biotransformation as it does not undergo enzymatic conjugation, and there is no possibility of degradation. Endothelial cells are thought to be the initial target of Cd in the liver (Nolan and Shaikh, 1986; Liu et al., 1992). Following acute Cd administration, hepatic congestion, ischemia, and hypoxia occur very rapidly (Dudley et al., 1982; Dudley and Klaassen, 1984; Habeebu et al., 1998). The resultant ischemic hypoxia leads to neutrophil infiltration, Kupffer cell activation, and inflammation, which could potentially contribute to the widespread hepatocellular apoptosis and necrosis observed with Cd (Dudley and Klaassen, 1984; Habeebu et al., 1998; Rikans and Yamano, 2000).

Inflammatory mediators, such as TNF-α, IL-1, IL-6, IL-8, and NO, are thought to play diverse roles in acute Cd-induced hepatotoxicity (Kayama et al., 1995; Horiguchi et al., 2000). The same work indicates these inflammatory mediators may be the actual basis of Cd hepatotoxicity (Yamano et al., 2000), whereas other data point toward production of these mediators as an adaptive mechanism for Cd tolerance (Min et al., 2002). In this regard, knockout mice, TNF-α−/− or iNOS−/−, are not resistant to Cd toxicity (Harstad and Klaassen, 2002a,b). Thus, NO, like other mediators of inflammation, could have dual effects on progression of chemically induced hepatic pathology depending on experimental conditions and type of toxicants used (Kim and Billiar, 2001; Liu et al., 2003).

This study examined the effect of V-PYRRO/NO on acute Cd-induced hepatotoxicity in mice using pharmacological doses of the NO donor produg. The results demonstrate that V-PYRRO/NO protected against Cd-induced hepatotoxicity including congestion, apoptosis, and necrosis. This protection is not due to altered distribution of Cd but potentially may be related to the vasodilatory and antiapoptotic properties of V-PYRRO/NO.

Materials and Methods

Chemicals. V-PYRRO/NO was synthesized as previously described (Saavedra et al., 1997). The structure of V-PYRRO/NO and the mode of NO release have been demonstrated in previous publications (Saavedra et al., 1997; Liu et al., 2002b; Stinson et al., 2002). Cadmium chloride (CdCl₂) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were commercially available and of reagent grade.

Animals. Male CD-1 mice, weighing 25 to 30 g, were obtained from Charles River Laboratories (Wilmington, MA). Animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care at the National Institute of Environmental Health Sciences at 20–22°C with a 12-h light/dark cycle for at least 1 week before treatment. Animals were allowed free access to Rodent Laboratory Chow (#9502;Ralston Purina Co., St. Louis, MO) and tap water. All procedures involving the use of laboratory animals were reviewed and approved by the Institutional Animal Care and Use Committee.

Experimental Design. Mice were given repeated s.c. injections with 10 mg/kg b.wt. V-PYRRO/NO in saline at 1 h before and again at 1, 3, and 5 h after Cd (3.7 mg/kg Cd as CdCl₂ i.p., in saline). Controls received vehicle injections at the same time points. Hepatotoxicity was evaluated at 1, 3, and 5 h after cadmium injection. The use of multiple s.c. injections of V-PYRRO/NO was based on data showing that this compound has a short plasma half-life in mice (Stinson et al., 2002) and our previous work using the same multiple injection protocol to protect against d-galactosamine/endotoxin-induced hepatotoxicity in mice without producing toxicity (Liu et al., 2002b). Thus, these doses are considered "pharmacologically relevant".

Evaluation of Hepatotoxicity. Serum alanine aminotransferase (ALT) activity was assayed as a marker of hepatocellular death using a commercially available kit (Infinite ALT, Sigma-Aldrich). A portion of the liver was fixed in 10% neutral formalin, processed by standard histological techniques, stained with hematoxylin and eosin, and examined for morphological evidence of liver injury.

Lipid Peroxidation Assay. The concentrations of 4-hydroxynoneals, 4-hydroxy-2(E)-nonenal (4-HNE), and malondialdehyde (MDA) were measured in liver homogenates using a lipid peroxidation assay kit (#43674) from EMD Biosciences (San Diego, CA). Approximately 200 mg of liver tissue was homogenized in 9 vol of ice-cold 20 mM Tris-HCl buffer (pH 7.4) and then centrifuged at 3000g for 10 min at 4°C. Supernatants (200 µl) were mixed with 650 µl of methanol/acetone (1:3, v/v) and 150 µl of the chromogenic agent, N-methyl-2-phenylindole, to yield a purple chromophore, which is measured spectrophotometrically at the absorbance of 586 nm using 4-hydroxynonenal as standard. The level of lipid peroxidation was expressed as the amount of 4-HNE + MDA (nanomoles) per gram of tissue.

Subcellular Distribution of Cadmium. Four hours after Cd (3.7 mg/kg, i.p.) treatment, liver was removed and homogenized in 0.25 M sucrose and 10 mM Tris-HCl (pH 7.4) with a Teflon pestle glass homogenizer. Various fractions were prepared by differential centrifugation at 4°C. The resultant pellets were defined as nuclei (600g, 10 min), mitochondria (10,000g, 10 min), microsome (22,000g, 30 min), and cytosol (final supernatant). The Cd content in the various fractions was analyzed by atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 100; PerkinElmer Life and Analytical Sciences, Boston, MA) after digestion with nitric acid.

Hepatic Metallothionein Determination. A portion of liver was homogenized in 10 mM Tris-HCl buffer (1.5, w/v%) followed by centrifugation at 20,000g for 10 min. Metallothionein (MT) concentrations in the hepatic cytosol were determined by the Cd/hemoglobin assay (Eaton and Teal, 1982).

Real-Time RT-PCR Analysis. Expression of the selected genes was quantified using real-time RT-PCR analysis as described by Walker (2001). Briefly, total RNA was reverse transcribed with murine leukemia virus reverse transcriptase and oligo-dT primers. The forward and reverse primers for selected genes were designed using Primer Express software (Applied Biosystems, Foster City, CA) and are listed in Table 1. The SYBR green DNA PCR kit (Applied Biosystems) was used for real-time PCR analysis. The relative differences in expression between groups were expressed using cycle time (Ct) values, and the relative differences between groups were expressed as relative increases setting control as 100%. Assuming that the Ct value is reflective of the initial starting copy and that there is 100% efficacy, a difference of one cycle is equivalent to a 2-fold difference in starting copy.

Statistics. Data represent means and standard errors of n = 5 to 20. For comparisons between two groups, a Student’s t test was performed. For multiple comparisons, data were analyzed using a one-way analysis of variance followed by Duncan’s multiple range test. The level of significance was set at p < 0.05.

Results

Protection by V-PYRRO/NO against Cd-Induced Hepatotoxicity. In CD-1 mice, Cd (3.7 mg/kg, i.p.) treatment produced serological evidence of liver injury starting 4 h after administration (Fig. 1), as evidenced by increased serum activity of ALT. Serum ALT increased ~25-fold over control values by 8 h after Cd exposure. V-PYRRO/NO administration alone did not alter ALT activity (Liu et al., 2002b) but significantly decreased Cd-induced release of hepatic ALT, as evidenced by a 58% decrease in serum ALT activity at 8 h after...
Oxidative stress induced by hepatotoxic doses of Cd. This is clear evidence that V-PYRRO/NO has decreased the induced lipid peroxidation at 8 h after Cd treatment (Fig. 3). Treatments significantly reduced these increases in Cd-induced lipid peroxidation by 80% over control. The V-PYRRO/NO treatments (data not shown) did not alter the distribution of Cd to various subcellular fractions (nuclear, mitochondrial, and cytosolic). Similar patterns for the subcellular distribution of Cd were also observed at 1 and 8 h of Cd exposure and these were similarly not altered by the V-PYRRO/NO treatments (data not shown).

Altered subcellular distribution of Cd has been observed as an important mechanism for reduction of toxicity. The amount of Cd reaching critical organelles is thought to be a key factor in Cd-induced hepatotoxicity. Thus, subcellular distribution of Cd was determined at 4 h after Cd administration. As shown in Fig. 5, V-PYRRO/NO treatment did not alter the distribution of Cd to various subcellular fractions (nuclear, mitochondrial, and cytosolic). Similar patterns for the subcellular distribution of Cd were also observed at 1 and 8 h of Cd exposure and these were similarly not altered by the V-PYRRO/NO treatments (data not shown).

Real-Time RT-PCR Analysis of Expression of Genes of Interest. To examine the potential mechanism by which V-PYRRO/NO protects the liver from Cd-induced injury, pertinent gene expression changes were examined with real-time RT-PCR (Table 2). Cd toxicity is initially associated with endothelial cell damage resulting in congestion, hypoxia, ischemia, and inflammation (Dudley and Klaassen, 1984; Nolan and Shaikh, 1986; Habeebu et al., 1998). Thus, the expression of genes associated with hypoxia, ischemia, and inflammation was assessed. As shown in Table 2, 8 h after a hepatotoxic dose of Cd, there were dramatic increases in the expression of mouse macrophage inflammatory protein (MIP-2, 45-fold), thrombospordin-1 (TSP1, 55-fold), mouse chemokine (mKC, 11-fold), intracellular cell adhesion molecule-1 (ICAM-1, 2-fold), IL-6 (58-fold), and TNF-α (16-fold) genes. V-PYRRO/NO significantly diminished the Cd-induced enhanced expression for the MIP-2, TSP1, and ICAM-1 genes. However, V-PYRRO/NO had no significant effects on Cd-increased expression of mKC, IL-6, TNF-α, and iNOS.

Cd is thought to produce toxicity by the activation of transcription factors, such as the AP-1 complex (Liu et al., 2002b), NF-κB (Hart et al., 1999), and activation of caspase-3 (Habeebu et al., 1998). As a result of Cd toxicity, the DNA damage responsible proteins, such as GADD45 and...
GADD153, are also increased (Liu et al., 2002b). Consistent with previous observations, acute Cd hepatotoxicity greatly enhanced the expression of c-jun/AP-1 (187-fold) and NF-κB (36-fold), whereas the expression of both genes was diminished to 17- and 13-fold, respectively, with cotreatment with V-PYRRO/NO. There was also a significant suppression of Cd-induced acute phase protein genes by V-PYRRO/NO including heme oxygenase-1 (HO-1, 19-fold versus 82-fold) and early response growth factor-1 (ERG1, 60-fold versus 106-fold). V-PYRRO/NO administration also suppressed the expression of Cd-induced caspase-3 (1.5-fold versus 2.5-fold), growth arrest, and DNA damage responsible protein-153 (GADD153, 3-fold versus 6-fold). However, V-PYRRO/NO had no effect on the induction of MT-1 mRNA (45-fold versus 50-fold) consistent with the MT protein assay (see Fig. 4).

**Discussion**

This study demonstrates that V-PYRRO/NO is effective in protecting against the hepatotoxicity of Cd in mice as evidenced biochemically by decreased serum ALT activity and histologically by diminished liver pathology. In addition, Cd-induced lipid peroxidation in the liver was reduced by the NO-releasing prodrug. Furthermore, Cd-induced aberrant expression of genes related to inflammation and cell death was greatly ameliorated by V-PYRRO/NO. Thus, this liver-selective NO donor clearly blocks the hepatotoxicity induced by inorganic Cd. This is consistent with V-PYRRO/NO-induced reductions in hepatotoxicity by a variety of organic hepatotoxic chemicals (Liu et al., 2002b, 2003). This observation with Cd is of particular importance since the metallic element, unlike many organic hepatotoxicants, is not metabolized in the liver and, there is currently no effective therapy for Cd intoxication.
Oxidative stress has been implicated as a key event in Cd toxicity (Liu et al., 2002b). Indeed, acute Cd hepatotoxicity is associated with the production of free radicals in the liver as evidenced by production of free radicals and increased lipid peroxidation (Liu et al., 2002b). In the present study, Cd-induced lipid peroxidation was suppressed by V-PYRRO/NO suggesting that the reduced oxidative stress and/or lipid peroxidation could be an important aspect of the hepatoprotective effects of V-PYRRO/NO. Various other NONOates can act as antioxidants against chemically induced oxidative stress (Fitzhugh and Keefer, 2000). Thus, antioxidant capacity of V-PYRRO/NO likely contributes to reduction of Cd-induced hepatotoxicity.

One mechanism by which Cd induces radical production is mediated through inflammatory cells (Rikans and Yamano, 2000). In this regard, the Kupffer cell inhibitor gadolinium chloride blocks Cd-induced hepatotoxicity (Sauer et al., 1998; Harstad and Klaassen, 2002c) and diminishes Cd-induced hepatic radical formation (J. Liu, Q. Guo, M. Waalkes, C. Klaassen, S. Qian, R. Mason, and M. Kadiiska, unpublished data). Cd-induced activation of Kupffer cells could result from endothelial cell damage leading to subsequent ischemia/congestion, hypoxia, and inflammation (Nolan and Shaikh, 1986; Liu et al., 1992). In the present study, Cd-induced hepatic congestion and inflammation were greatly attenuated by V-PYRRO/NO. Accordingly, Cd-induced over-expression of thrombospondin-1, ICAM-1, mKC, MIP-2, IL-6, and TNF-α, all mediators of inflammatory response, are greatly suppressed by V-PYRRO/NO. This effect may be related to vasodilatory effects of V-PYRRO/NO (Ricciardi et al., 2001; Moal et al., 2002), which potentially reduce hepatic congestion/ischemia. In addition, exogenous NO has also been shown to suppress the expression of thrombospondin-1 (Wang et al., 2002), ICAM-1 (Liu et al., 1998), and proinflammatory cytokines such as IL-6 and TNF-α (Menger et al., 1999; Liu et al., 2002b). Thus, V-PYRRO/NO treatment appeared to maintain vascular integrity in the face of Cd insult by delivering NO to the liver (Ricciardi et al., 2001). Thus, initial protection against Cd-induced hepatic vascular damage by the NO-releasing prodrug appears to sharply limit the subsequent inflammatory response and ensuing cascade of ischemic hypoxia and hepatocellular death.

Cd-induced endothelial cell damage, hypoxia, and inflammation can activate transcription factors such as AP-1, NF-kB, and hypoxia-inducible transcription factor-1 (HIF-1) (Faller, 1999; Hart et al., 1999; Liu et al., 2002b). These, in turn, increase the expression of acute phase proteins such as HO-1 and EGR1 (Liu et al., 2002b). Cd has been proposed to suppress pro-oxidant transcription factor activation (Buzard and Kaspzak, 2000). In the present study, Cd-induced expression of c-Jun/AP-1, NF-kB, and hypoxia-inducible transcription factor-1 (HIF-1) was significantly suppressed by V-PYRRO/NO suggesting that NO may also exert its protective effects through the inhibition of the signal transduction pathways leading to cell death.

Cd produces both apoptosis and necrosis in the mouse liver (Habeebu et al., 1998). In the present study, Cd-induced hepatocellular death, regardless of specific type, was clearly ameliorated by V-PYRRO/NO. Similarly, Cd-increased expressions of caspase-3 (a key enzyme in the execution of apoptosis) and GADD153 (an indicator of DNA damage) were also suppressed by V-PYRRO/NO. NO is thought to play a role...
TABLE 2
Real-time RT-PCR analysis of liver tissue in control, Cd-, and Cd + V-PYRRO/NO-treated mice
Mice were given CdCl₂ (3.7 mg/kg Cd, i.p.) or Cd + V-PYRRO/NO (10 mg/kg, s.c., ×4). Liver samples were taken at 8 h after Cd administration, and total RNA was isolated for real-time RT-PCR analysis. In each individual sample, the expression level of each gene was first normalized with that of G3PDH, and then the relative differences between groups were expressed as relative increases setting controls as 1.0. Data represent means ± S.E. of n = four to six animals per group.

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th>Control</th>
<th>Cd Alone</th>
<th>Cd + NO</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1.0 ± 0.3</td>
<td>44.6 ± 12.0*</td>
<td>9.3 ± 0.9‡</td>
</tr>
<tr>
<td>MIP-2</td>
<td>1.0 ± 0.2</td>
<td>55.4 ± 7.2*</td>
<td>17.6 ± 8.5‡</td>
</tr>
<tr>
<td>Thrombospondin-1</td>
<td>1.0 ± 0.2</td>
<td>10.8 ± 2.2*</td>
<td>6.8 ± 4.4*</td>
</tr>
<tr>
<td>MRC</td>
<td>1.0 ± 0.1</td>
<td>2.1 ± 0.3*</td>
<td>1.0 ± 0.1*</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>1.0 ± 0.1</td>
<td>65.4 ± 17.0*</td>
<td>38.1 ± 7.0*</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.0 ± 0.4</td>
<td>5.4 ± 4.5*</td>
<td>11.7 ± 5.5*</td>
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<tr>
<td>TNF-α</td>
<td>1.0 ± 0.2</td>
<td>121.0 ± 0.7*</td>
<td>11.8 ± 1.7*</td>
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<tr>
<td>Acute phase protein genes and cell death markers</td>
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<tr>
<td>e-Jun/AP-1</td>
<td>1.0 ± 0.3</td>
<td>187 ± 14.2*</td>
<td>17.7 ± 5.4**</td>
</tr>
<tr>
<td>NF-κB</td>
<td>1.0 ± 0.2</td>
<td>36.0 ± 9.7*</td>
<td>13.3 ± 3.1**</td>
</tr>
<tr>
<td>Heme oxygenase-1</td>
<td>1.0 ± 0.3</td>
<td>82.0 ± 9.9*</td>
<td>18.8 ± 6.8**</td>
</tr>
<tr>
<td>EGR1</td>
<td>1.0 ± 0.3</td>
<td>106 ± 14.7*</td>
<td>60.6 ± 16.8**</td>
</tr>
<tr>
<td>GADD153</td>
<td>1.0 ± 0.4</td>
<td>6.4 ± 1.8*</td>
<td>2.7 ± 0.9*</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>1.0 ± 0.4</td>
<td>2.4 ± 0.3*</td>
<td>1.5 ± 0.3*</td>
</tr>
<tr>
<td>Metallothionein-1</td>
<td>1.0 ± 0.2</td>
<td>50.2 ± 14*</td>
<td>44.6 ± 13*</td>
</tr>
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* P < 0.05 (compared with control); ‡ P < 0.05 (compared with Cd alone).

in directly reducing liver apoptosis (Kim and Billiar, 2001). Indeed, V-PYRRO/NO protection against the hepatotoxicity of β-galactosamine/endotoxin and acetaminophen appears to be related to suppression of apoptotic pathways (Liu et al., 2002, 2003). Thus, the protective effect of V-PYRRO/NO against Cd hepatotoxicity could also be due, at least in part, to direct suppression of cell death pathways leading to apoptosis and necrosis. Again this would block progression of liver pathology despite the occurrence of initial Cd lesions. Indeed, V-PYRRO/NO was effective in decreasing Cd-induced apoptosis and suppressed Cd-induced e-Jun NH₂-terminal kinase activation in cultured rat liver cells (Qu et al., 2002).

Tolerance to acute Cd hepatotoxicity has been attributed to the induction of MT (Goering and Klaassen, 1984). MT is a low-molecular weight, cysteine-rich, metal-binding protein that can bind Cd in the cytosol and renders it inert and thereby reduces the amount of Cd at critical cellular targets (Klaassen et al., 1999). We examined the effect of V-PYRRO/NO on hepatic MT induction and subcellular distribution of Cd. V-PYRRO/NO had no effect on Cd induction of hepatic MT both at the transcript and the protein levels. Thus, MT is not likely a major factor in V-PYRRO/NO-induced resistance to Cd. NO released from V-PYRRO/NO likely blocked Cd-induced hepatic endothelial damage and preserved microvascular circulation. The presumed accumulation improvement by V-PYRRO/NO (Riccieri et al., 2001; Moal et al., 2002, De leve et al., 2003) may have reduced Cd distribution to the liver; however, V-PYRRO/NO administration had no effects on distribution of Cd to the liver, nor the altered subcellular distribution of Cd within the liver. Thus, induction of MT and altered toxicokinetics of Cd are not the primary mechanisms of V-PYRRO/NO-mediated protection. However, this does not exclude the potential role of presynthesized MT in Cd tolerance following V-PYRRO/NO pretreatment in cultured rat liver cells (Qu et al., 2002).

In summary, this study demonstrates that the liver-selective NO donor, V-PYRRO/NO, is effective in protecting against Cd-induced liver injury in mice. This protective effect does not appear to be due to altered subcellular Cd distribution or induction of MT but does involve the inhibition of oxidative stress and resulting apoptosis probably through the maintenance of hepatic vasculature to prevent ischemia and hypoxia.

Acknowledgments
We thank Drs. Larry K. Keefer, Lamia Benbratim-Tallalaa, and Jingbo Pi for critical review during preparation of this manuscript.

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