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Limited protective role of V-PYRRO/NO against cholestasis produced by alpha-naphthylisothiocyanate in mice ☆

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Abstract

O^2-vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO) is a liver-selective nitric oxide donor that has been shown to protect against hepatotoxic effects of endotoxin, acetaminophen and cadmium. This study examined the effects of V-PYRRO/NO on alpha-naphthylisothiocyanate (ANIT)-induced hepatotoxicity in mice. Mice were given V-PYRRO/NO via osmotic pumps (5.4 mg/ml; 0.5 μl/h) starting 24 h before receiving a hepatotoxic dose of ANIT (150 mg/kg in olive oil, i.g.), and continuing for additional 48 h (3-day pumps). V-PYRRO/NO administration partially ameliorated ANIT-induced hepatotoxicity, as evidenced by reduced serum alanine aminotransferase and alkaline phosphatase, markers of liver cell death, and by improved liver pathology. However, V-PYRRO/NO had no effect on ANIT-induced cholestasis, as ANIT-increased serum bilirubin levels and gamma-glutamyl transpeptidase activity were not ameliorated. Microarray and real time RT-PCR analysis revealed that ANIT intoxication altered expression of various genes, including genes encoding metabolic enzymes, transporter proteins, acute phase proteins, inflammation- and, apoptosis-related genes, as well as other genes related to liver injury. V-PYRRO/NO treatment attenuated ANIT-induced elevations in certain inflammation- and apoptosis-related genes, but had no effect on ANIT-induced disturbance on the expression of genes related to metabolism, transport, and acute phase proteins. Thus, the liver-selective NO donor, V-PYRRO/NO, was partially protective against ANIT-induced liver injury, without affecting ANIT-induced cholestasis and cholestasis-related gene expression.
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Keywords: V-PYRRO/NO; ANIT hepatotoxicity; Cholestasis; Inflammation; Gene expression

1. Introduction

O^2-Vinyl 1-(pyrrolidin-1-yl) diazen-1-ium-1,2-diolate (V-PYRRO/NO), is a nitric oxide (NO) prodrug that has been shown to protect against hepatotoxicity produced by endotoxin/TNF-α [1–3], acetaminophen [4], and cadmium [5]. In addition, V-PYRRO/NO is also effective in protecting against liver damage from ischemia/reperfusion [6], in protecting against bile-duct ligation induced portal hypertension and liver fibrosis [7], and is beneficial in improving hepatic microcirculation and preservation of sinusoid endothelial cell integrity in moncrotaline-induced hepatic sinusoid obstruction syndrome [8]. Thus, the actions of this liver-selective NO donor appears to improve the hepatic microvascular blood flow and to suppress hepatocellular apoptosis [1–9,25].

Alpha-naphthylisothiocyanate (ANIT) is a toxicant that targets the bile ducts and causes an intrahepatic cholestasis that models human chronic cholangitic diseases [10]. ANIT is thought to be bioactivated in the liver by cytochrome P450s, and the bioactivated ANIT is detoxified in hepatocytes by conjugation with glutathione catalysed by glutathione S-transferases. ANIT–glutathione complexes are transported into bile, but they are unstable and rapidly dissociate. The released ANIT then damages bile-duct

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epithelial cells, causing cholangiolitis that leads to intrahepatic biliary obstruction and increased accumulation of toxic bile acids in the liver [11]. ANIT-induced cholestasis is a strong chemotactic stimulus for neutrophil adhesion, and extravasation from sinusoid into parenchyma [12]. The activated neutrophils and/or macrophages then release inflammatory cytokines and other substances that are toxic to hepatocytes [13]. Thus, ANIT-induced hepatotoxicity involves metabolism, transport, cholestasis, inflammation, and hepatocellular death [11].

During acute cholestasis, there is evidence that NO regulates the level of hepatic microvascular perfusion. In bile duct-ligated (BDL) rats, serum nitrite/nitrate levels and liver iNOS protein were decreased [14]. Treatment with L-arginine, a precursor of NO synthesis, partially prevents BDL-induced liver injury [15]. On the other hand, N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of inducible nitric oxide synthase (iNOS), reduces NO production and abolishes beneficial effects of L-arginine against BDL-induced hepatotoxicity [15]. These data suggest a role for NO to protect against intrahepatic cholestasis. The NO donor molsidomine has been shown to reduce neutrophil infiltration, to block hepatocellular death in BDL-induced liver injury [16], and to decrease BDL-induced mortality [17]. Another liver-selective NO donor NCX-1000, which will release NO within the liver, can protect against ANIT-induced liver injury with reduction of neutriphil chemoattractant-1 (CINC-1) [18]. These observations prompted us to hypothesize that V-PYRRO/NO, a liver-selective NO donor, could be beneficial in decreasing ANIT-induced liver injury. Thus, the goals of the present study were to examine the effects of V-PYRRO/NO on ANIT-induced liver injury in mice, and to examine gene expression alterations associated with ANIT hepatotoxicity. V-PYRRO/NO was used as an experimental tool to help our understanding the role of NO in ANIT-induced cholestasis and liver injury at both biochemical and molecular levels.

2. Materials and methods

2.1. Chemicals

V-PYRRO/NO was synthesized as previously described [1]. The structure of V-PYRRO/NO and the mode of NO release have been demonstrated in previous publications [1,3,9,19]. Alpha-naphthylisothiocyanate (ANIT) was purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals were commercially available and of reagent grade.

2.2. Animals

Male Crl:CD-1 mice, weighing 25–30 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 (#5002, Ralston Purina Co., St. Louis, MO) and tap water. All procedures involving the use of laboratory animals were reviewed and approved by the Institutional IACUC. Osmotic pumps were implanted sc in the dorsal thoracic region under anaesthesia and auto clips were used to close the incision.

2.3. Experimental design

Mice were anesthetized with 3% isoflurane and oxygen, and 3-day osmotic pumps containing 5.4 mg/ml of V-PYRRO/NO were implanted (Alzet, model 1007D, Cupertino, CA). V-PYRRO/NO was released at a continuous perfusion rate of 0.5 μl/h) upon pump implanation. The use of continuous osmotic pump infusion was based on data showing that V-PYRRO/NO has a short plasma half-life in mice [19], and that continuous V-PYRRO/NO infusion at the same highest allowable concentration produces protective effects against acetaminophen hepatotoxicity [4]. The dose of ANIT was selected based on our pilot dose-range finding experiments. Twenty-four hours after implanting the pumps, mice were given a single oral dose of ANIT by gavage (150 mg/kg, 10 ml/kg in olive oil). Forty-eight hours after ANIT administration (72 h after NO pump implantation), mice were anesthetized with 3% isoflurane and oxygen, blood was collected by decapitation and livers were removed and frozen at −70 °C until analysis.

2.4. Evaluation of hepatotoxicity

All serum biochemistry was analyzed using commercially available kits (Sigma Chemical Company). Serum alanine aminotransferase (ALT) activity was assayed as a marker of hepatotocellular death. Serum bilirubin levels and gamma-glutamyl transpeptidase (γ-GGT) activity were assayed as markers for cholestasis. Serum alkaline phosphatase (ALP) activity was assayed as a marker for both cell death and cholestasis. A portion of the liver was fixed in 10% neutral formalin, processed by standard histological techniques, stained with haematoxylin and eosin, and examined for morphological evidence of liver injury.

2.5. Microarray analysis

The customer-designed mouse cancer arrays (588 genes, Clontech, Palo Alto, CA) were used for cDNA microarray analysis [4]. Total RNA was isolated from liver samples with TRIzol agent (Invitrogen, Carlsbad, CA), followed by purification and DNase-I digestion with RNeasy columns (Qiagen, Valencia, CA). Approximate 5 μg of total RNA was converted to [α-^{32}P]-dATP-labelled cDNA probe

Table 1
Sequences of primers used for real-time RT-PCR analysis

Gene	GenBank#	Forward	Reverse
beta-actin	M12481	GTATGACTCCACTCACGGCAAA	GGTCTCGCTCCTGGAAGATG
BSEP	AF186585	AGTGGTGGGCAGAAGCAAAG	CCATGTCCAGAAGCAGGATCTT
c-Jun	J04115	ACTCCGAGCTGGCATCCA	CCCACTGTTAACGTGGTTCATG
c-myc	X01023	CGCCGCTGGGAAACTTT	TCCTGGCTCGCAGATTGTAA
CYP2B9	M21855	TCTCTGTGGCAAGCCCTGTT	GGTGTGCTGGAGGTATTTTTCC
CYP2E1	L11650	CACAGCCAAGAACCCATGTACA	CAGGAGCCCATATCTCAGAGTTG
CYP2F2	M77497	CCTTTGACCCCGTGTTTATCC	TCGAAGCGACTTCCGAAGAC
CYP4A14	Y11638	GGTGAGGCTGATTGAGTCTTGAG	CTCCAGATTGATCCAGGATGGA
CYP8B1	NM_010012	AGTACACATGGACCCCGACATC	GGGTGCCATCAGGGTTGAG
EGR1	M20157	AGGTTCCCATGATCCCTGACT	GGTACGGTTCTCCAGACCCTG
GST theta	X98055	CTTGTTGGGCCCCACATCT	CTGGGATGCCCTTCAAAGACT
HO-1	M33203	CCTCACTGGCAGGAAATCATC	CCTCGTGGAGACGCTTTACATA
mKC	NM_008176	TGGCTGGGATTCACCTCAAG	GTGGCTATGACTTCGGTTTGG
MDR2	U46840	TGGCCGATGTGTGTGAGTACA	TGCCTGGCACCAAAAGGT
MIP2	NM_009140	CCTCAACGGAAGAACCAAAGAG	CTCAGACAGCGAGGCACATC
TSP-1	M87276	GCCGGATGACAAGTTCCAA	GCCTCAAGGAAGCCAAGAAGA
TNF-alpha	XM_110221	GACCCTCACACTCAGATCATCTTCT	CCTCCACTTGGTGGTTTGCT

using MMLV reverse transcriptase and the Atlas customer array specific cDNA synthesis primer mix, and then purified with a NucleoSpin column (Clontech, Palo Alto, CA). The membranes were prehybridized with Expresshyb from Clontech for 2 h at 68 °C, followed by hybridization with the cDNA probe overnight at 68 °C. The membranes were then washed four times in 2× SSC/1% SDS, 30 min each, and two times in 0.1× SSC/0.5% SDS for 30 min. The membranes were then sealed with plastic wrap and exposed to a Molecular Dynamics Phosphoimage Screen. The images were analyzed densitometrically using Atlas-Image software (version 2.01). The gene expression intensities were first corrected with the external background and then globally normalized.

2.6. Real-time RT-PCR analysis

Total RNA was extracted and purified as described above and reverse transcribed with MuLV reverse transcriptase and oligo-dT primers. The forward and reverse primers for selected genes were designed using ABI Primer Express software (Applied Biosystems, Foster City, CA) and listed in Table 1. The SYBR green PCR master mix (Applied Biosystems, Foster City, CA) 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 two-fold difference in starting copy.

2.7. Statistics

Data represent means and standard errors of ($n = 6$–10). For comparisons between two groups, Students' t-test was performed. The level of significance was set at $p < 0.05$.

3. Results

3.1. Protection by V-PYRRO/NO against ANIT-induced hepatotoxicity

As expected in CD-1 mice, ANIT (150 mg/kg, i.g.) produced profound hepatotoxicity 48 h after administra-

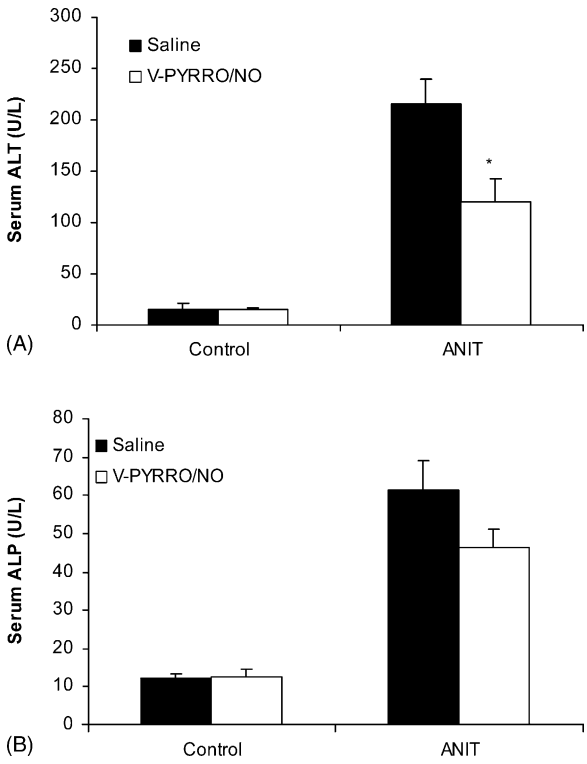

Fig. 1. Effect of V-PYRRO/NO on ANIT-induced liver injury in mice. Mice were given ANIT (150 mg/kg, i.g.) with or without the administration of V-PYRRO/NO via osmotic pumps (5.4 mg/ml, 0.5 μl/h for 72 h). Liver injury was evaluated by serum alanine aminotransferase (ALT) activity (A) and alkaline phosphotase (ALP) activity (B). Data are mean ± S.E.M. of 8–12 mice. *, significantly different from ANIT-only group, $p < 0.05$.

tion, as evidenced by increases in serum markers for hepatotoxicity: 14-fold increases in alanine aminotransferase (ALT, top) and seven-fold increases in alkaline phosphatase (ALP, bottom) (Fig. 1). V-PYRRO/NO (5.4 mg/ml) delivered via osmotic pump at a rate of 0.5 μl/h starting 24 h before a hepatotoxic dose of ANIT and continuing for a total 72 h, significantly reduced ANIT-induced elevations in serum ALT by ~50% (15 ± 6, 215 ± 24, and 120 ± 23 U/L for control, ANIT, and ANIT + NO, respectively). ANIT-induced increases in serum ALP tended to be reduced by ~26% with V-PYRRO/NO treatment but this reduction was not statistically significant (12 ± 2, 61 ± 8, and 46 ± 5 U/L for control, ANIT, and ANIT + NO, respectively).

Histologically, ANIT-induced liver injury showed sinusoid congestion, neutrophil infiltration, hepatocellular degeneration, and cell death (Fig. 2B). Consistent with serology, ANIT-induced sinusoid congestion and hepatocelluar death were partially reduced in animals also receiving

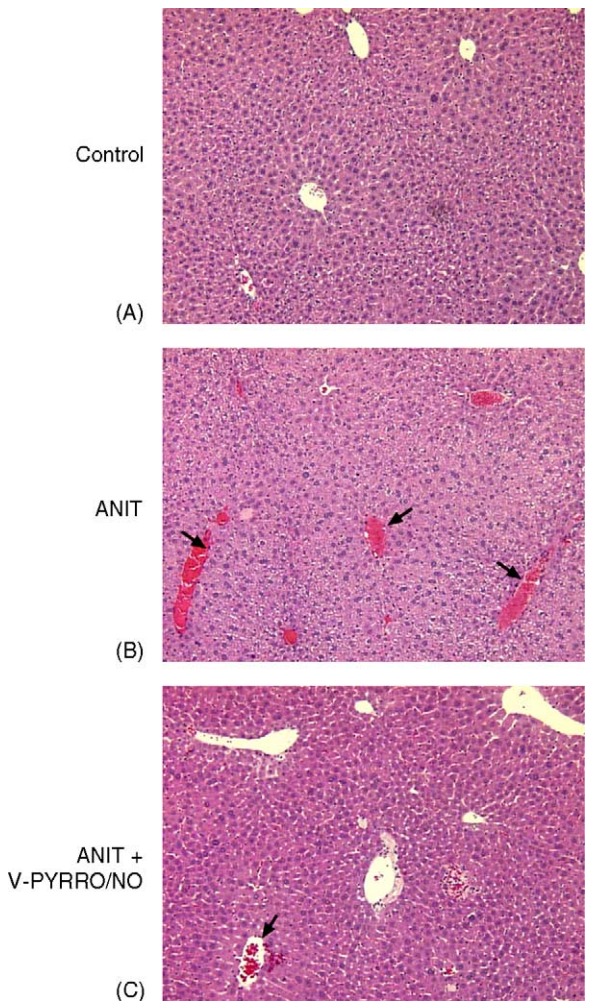

Fig. 2. Photomicrographs of the mouse liver. (A) Control; (B) ANIT alone, foci of necrotic parenchymal cells, neutrophil infiltration and sinusoid congestion are evident; and (C) ANIT plus V-PYRRO/NO, hepatic congestion and are mild, and necrotic cells are rare. Sections were stained with hematoxylin and eosin, 100× magnification.

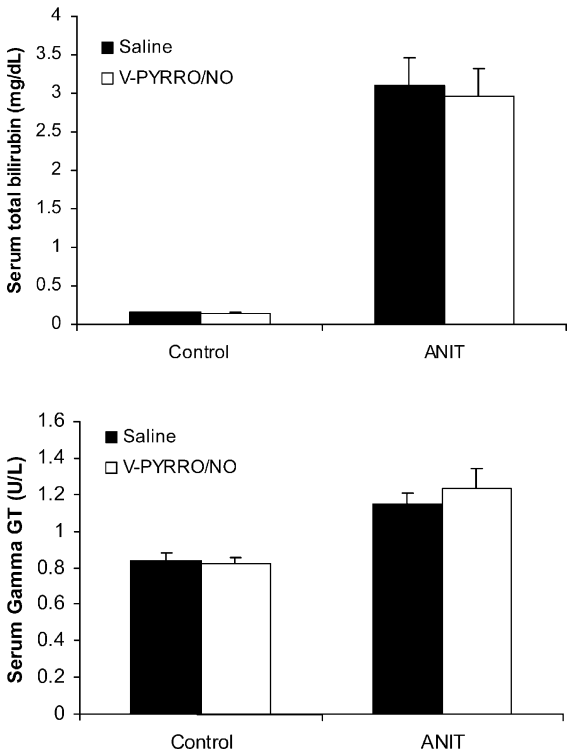

Fig. 3. Effect of V-PYRRO/NO on ANIT-induced elevation of serum bilirubin and serum gamma-glutamyl aminotransferase (γ-GGT) activity in mice. Mice were given ANIT (150 mg/kg, i.g.) with or without the administration of V-PYRRO/NO via osmotic pumps (5.4 mg/ml, 0.5 μl/h for 72 h). Data are mean ± S.E.M. of 8–12 mice.

V-PYRRO/NO infusion (Fig. 2C). Arrows indicate sinusoid congestion and necrosis in ANIT-treated animals, while in ANIT plus V-PYRRO/NO-treated animals there was minimal congestion and areas of cell loss were also reduced.

Serum bilirubin levels and γ-glutamyl transpeptidase (γ-GGT) activity are markers for cholestasis. ANIT alone significantly increased serum bilirubin levels (19-fold; top) and γ-GGT activity (1.5-fold; bottom) (Fig. 3). V-PYRRO/NO administration, however, did not alter serum levels of ANIT-induced markers of cholestasis, even with doubled doses using two pumps (data not shown).

3.2. Gene expression analysis

Mouse customized arrays relatively specific for liver genes (588 genes) were used in the present study. Means and S.E.M. of gene hybridization intensity from three hybridizations were calculated for statistical analysis as described previously [3,4]. Under the criteria of the >1.5-fold difference together with statistical significance ($p < 0.05$), ANIT-treated animals had 28 genes up-regulated and 32 genes down-regulated as compared to controls (gene alteration rate: 10.2%). To confirm the gene array results, real-time RT-PCR was performed on selected genes. In general, the RT-PCR results were in agreement with microarray results but were more sensitive. Table 2 shows the selected gene alterations associated with ANIT-

Table 2
Microarray analysis of aberrant gene expression in mouse liver 48 h after ANIT (150 mg/kg, i.g.)

Protein/gene	GenBank#	Control mean intensity	ANIT/control ratio	ANIT + NO/control ratio
Inflammation acute phase proteins				
TSP-1	M87276	317	5.68	1.38
MIP	X12531	1079	3.91	2.23
TNF-alpha	L26349	7533	1.51	1.04
IL-1R	M20658	1466	1.78	1.85
IL-6	X51975	3481	0.94	1.19
HO-1	M33203	8159	2.25	2.38
c-jun	J04115	3364	2.32	2.03
c-myc	X00195	406	5.86	4.45
c-fos	V00727	240	6.17	6.68
EGR1	M20157	2888	4.25	3.25
LPS-BP	X99347	6773	2.46	2.77
HSP90	M36830	9740	1.33	0.76
HSP110	D85904	1241	2.28	1.47
Stat3	U06922	3266	1.55	1.26
Ceruloplasmin	U49430	18430	1.78	1.68
Apoptosis-related protein genes				
Fas/APO1	U25995	2257	1.80	1.08
FAS	AF006040	420	2.19	1.38
Bax	L22472	1442	1.97	1.47
FDDA	AF006040	420	2.19	1.38
cyclin D1	M64403	6093	1.85	1.22
NMO1	U12961	2626	1.59	1.41
Catalase	L25069	13520	0.45	0.55
SOD1	M35725	45994	0.77	0.87
Metabolic enzymes and transporters				
CYP1A1	Y00071	8755	0.59	0.75
CYP2B9	M21855	38145	0.56	0.50
CYP2E1	L11650	49780	0.29	0.39
CYP2D9	M27168	34015	0.68	0.75
CYP2F2	M77497	43009	0.49	0.59
CYP2J5	U62294	28573	0.65	0.61
CYP4A14	NM_007822	10423	1.75	1.59
Odc	M10624	4726	1.66	1.47
UGT1A	S64760	27971	0.76	0.90
UGT2B5	X06358	26938	0.73	0.46
GST-pi	D30687	23293	1.56	1.64
GST-alpha	J03958	2491	2.22	1.80
GST-mu	U24428	2401	1.69	1.34
GPx1	X03920	29017	0.76	1.18
GR	X76341	6573	2.62	1.87
Lx1	U38652	12097	0.38	0.36
MDR2	J03398	1297	3.23	2.36
MRP1	AF022908	2072	1.85	1.90
PXR	AF031814	12905	1.83	2.12
Oatp2	AJ006036	1921	1.95	1.18
Others				
IGF-1	X04480	49090	0.72	0.79
IGFBP1	X81579	4812	1.93	1.50
EGFR	X78987	16520	0.58	0.60
VEGF	M95200	7621	0.51	0.64
MMP3	X66402	746	2.30	2.34
MMP14	X83536	1881	2.14	1.39
K-8	X12789	8928	2.61	1.88
K-18	M11686	2836	2.79	3.99
Dnmt3a	AF068625	1045	3.44	1.63
HSD17beta-1	D45850	15058	0.18	0.14
FMO1	D16215	15210	0.46	0.55

induced liver injury in four categories: inflammation and acute phase proteins, apoptosis-related protein genes, genes encoding metabolic enzymes and transporter, and others. V-PYRRO/NO treatment attenuated the alterations in gene expression associated with inflammation and apoptosis, but was ineffective in other categories.

Neutrophil extravasations and inflammatory mediators are known to play a role in ANIT-induced liver injury [11,20]. Thus, real-time RT-PCR was used to confirm the gene expression changes associated with inflammatory responses (Fig. 4). V-PYRRO/NO attenuated the ANIT-induced elevations in the expression of CXC chemokines (mKC), macrophage inflammatory protein-2 (MIP-2), thrombospondin-1 (TSP-1), and tumor necrosis factor alpha (TNF-α), but was ineffective in ANIT-induced increases in the expression of heme oxygenase 1 (HO-1), c-jun, c-myc, and early growth response protein 1 (EGR1) (Fig. 4, top). ANIT-induced liver injury is related to cytochrome P450 enzymes, glutathione S-transferases, and transporters [11], and real-time RT-PCR was also performed on selected genes. Consistent with the microarray analysis, V-PYRRO/NO did not affects ANIT-induced down-regulation of CYP2B9, CYP2E1, CYP2F2, CYP8B, or on ANIT-increased expression of CYP4A14, GST, BSEP, and MDR2 (Fig. 4, bottom).

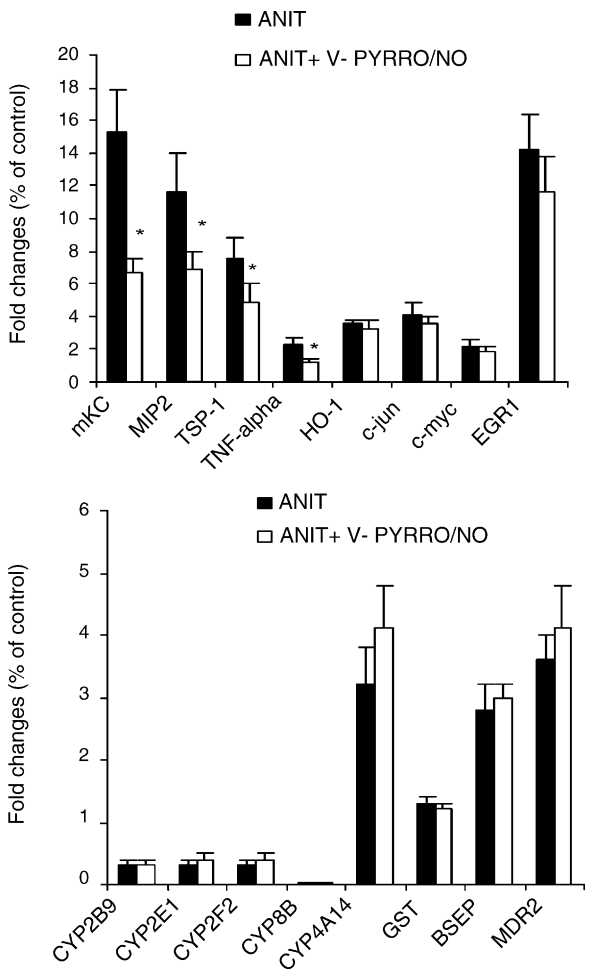

Fig. 4. Real-time RT-PCR analysis of ANIT-induced aberrant expression of genes encoding imflammation and acute phase proteins (top), and genes encoding metabolic enzymes and transport proteins (bottom). Data are mean ± S.E.M. of six mice. *, Significantly different from ANIT alone group, $p < 0.05$.

4. Discussion

V-PYRRO/NO is a stable diazeniumdiolate that can circulate freely throughout the body until it is presumably metabolized to NO by cytochromes P450 in the liver [1,19,21]. The release of NO from V-PYRRO/NO into hepatocytes has been confirmed by detection of increased nitrite/nitrate levels, and by the stimulation of hepatic cyclic guanosine 3′,5′-monophosphate (cGMP) production, a key second message of NO [1,21,25]. In the present study, it was demonstrated that V-PYRRO/NO only offered a partial protective effect against ANIT-induced hepatotoxicity in mice, as evidenced biochemically by decreased serum ALT activity, a tendancy towards reduced ALP activity, and by histopathology showing reduced hepatic lesions. However, V-PYRRO/NO is ineffective in preventing ANIT-induced cholestasis and cholestais-related gene expression. Thus, it appears that ANIT-induced toxic effects on the vascular system, other than cholestasis, could be ameliorated by V-PYRRO/NO treatment.

ANIT-intoxication is widely used as an animal model for intrahepatic cholestasis, and the bile-duct ligation (BDL) is used as a model for extrahepatic cholestasis; both can lead to hepatocellular death [10]. In the present study, V-PYRRO/NO has little impact on ANIT-induced cholestasis. This observation is consistent with the study using the NO donor molsidomine, which reduces neutrophil infiltration into the hepatic parenchyma and reduces hepatocellular death but does not impact serum bilirubin levels from BDL rats [16]. It should be noted that although cholestasis and hepatocellular death can occur sequentially, they are toxicologically different events that are not always linked. Intrahepatic cholestasis triggers cell death via a cascade of events including increased neutrophil adhesion, extravasation, activation, and subsequent release of toxic cytokines and/or other substances [11,20], and toxic bile acids can directly induce apoptotic cell death [20]. Indeed, depletion of blood neutrophils protect against ANIT hepatotoxicity without affecting ANIT-induced cholestasis [12], and the NO donor, Spermine NONOate, ameliorates hydrophobic bile acid-induced apoptosis in isolated rat hepatocyes [22]. V-PYRRO/NO has been shown to improve hepatic circulation and to reduce liver apoptosis produced by TNF-α [1,2], endotoxin [3], acetaminophen [4], and cadmium [5]. Thus, V-PYRRO/NO mediated protection could be due, at least in part, to the reduction of neutrophil infiltration and the suppression of apoptosis pathways. In addition, since V-PYRRO/NO protected against only a portion of the overall toxic lesions induced by ANIT, it appears unlikely

that this was due to alterations of hepatic ANIT deposition by enhancing hepatic blood flow.

Little is known about gene expression alterations associated with ANIT hepatotoxicity, and our second goal is to profile ANIT-induced gene expression changes and the modifying effects of V-PYRRO/NO. V-PYRRO/NO has little effect on gene expression changes associated with ANIT-induced cholestasis, such as the reduction of CYP1A1, CYP2E1, CYP8B, CYP2F2, CYP2J5, and FMO1, the increases in GST-alpha, GST-mu, GST-pi, CYP4A14 and ornithine decarboxylase (Odc), the acute phase protein HO-1, HSP90, c-jun, c-myc, and EGR1, as well as the increases in PXR, MDR2, and BSEP. In contrast, V-PYRRO/NO attenuated the expression of genes associated with neutrophil infiltration such as mKC, MIP2, and TSP-1, and the apoptotic-related genes such as TNF-α, FAS, Bax, and FDDA. ANIT-induced gene alterations in vivo are largely in agreement with ANIT-induced gene alteration profiles in cultured hepatocytes [23], and are consistent with hepatic histopathology and biochemical changes in the present study. Thus, the gene expression profiles associated with ANIT hepatotoxicity in intact animals include increases in inflammatory marker genes, acute phase protein genes, apoptosis genes, and genes encoding glutathione S-transferases and transporters; as well as the decreases in cytochrome P450 genes, certain antioxidant genes, and genes encoding drug metabolizing enzymes. To examine the effects of NO release on these gene expressions would clearly add to our understanding of the molecular mechanisms of ANIT-induced liver injury.

The constant NO release to the liver via osmotic pumps appear to be able to maintain hepatic vascular integrity, and thus to reduce chemoattractant stimuli from ANIT-induced cholestasis. Two major CXC chemokines that attract neutrophils are macrophage inflammatory protein-2 (MIP-2) and mKC [20]. Both MIP-2 and mKC interact with CXCR2 [24]. However, in the CXCR−/− mice, the CXC chemokines and neutrophil infiltration was reduced, but only had limited role in ANIT-induced liver injury [24]. This could explain the reduced chemokine expression by V-PYRRO/NO only has a limited role against ANIT hepatotoxicity.

In summary, this study demonstrates that the liver-selective NO donor, V-PYRRO/NO, partially reduced ANIT-induced hepatotoxicity. This protection by NO release does not extend to prevention of ANIT-induced cholestasis, but appears to be due to the maintenance of hepatic vasculature and subsequent reduction of inflammatory responses and apoptotic cell death.

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