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PROTECTION AGAINST α-NAPHTHYLISOTHIOCYANATE-INDUCED LIVER INJURY BY DECREASED HEPATIC NON-PROTEIN SULFHYDRYL CONTENT

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Abstract—α-Naphthylisothiocyanate (ANIT) injures bile duct epithelium and hepatic parenchymal cells in rats. It is commonly believed that ANIT must undergo bioactivation by hepatic, cytochrome P450-dependent mixed-function oxidases (MFO), since agents which are inducers or inhibitors of hepatic MFO activity enhance or attenuate, respectively, the liver injury associated with ANIT. Several of these agents also affect hepatic glutathione (GSH) content and/or GSH *S*-transferase activity in a manner to suggest a causal role for GSH in ANIT-induced hepatotoxicity. To determine whether GSH might be involved in the mechanism of injury, buthionine sulfoximine (BSO), diethyl maleate (DEM), or phorone was used to reduce hepatic non-protein sulfhydryl (NPSH) content, an indicator of GSH content. Twenty-four hours after ANIT treatment, rats exhibited cholestasis and elevations in serum of total bilirubin concentration, total bile acid concentration, aspartate aminotransferase (AST) activity, and γ-glutamyltransferase activity. Cotreatment of rats with BSO decreased NPSH content by 70% at 24 hr and prevented the cholestasis and elevations in serum markers of liver injury caused by ANIT. Likewise, cotreatment of rats with DEM afforded protection against markers of liver injury. Phorone treatment attenuated ANIT-induced elevations in serum total bilirubin concentration and AST activity. Although BSO treatment afforded protection against ANIT-induced liver injury at 24 hr, the injury was evident at 48 hr, and it appeared to coincide with a return of hepatic NPSH content. These results suggest that GSH plays a causal or permissive role in the liver injury caused by ANIT.

α-Naphthylisothiocyanate (ANIT)‡ is a model cholestatic agent. Sixteen to twenty-four hours after a single administration to rats, ANIT causes an intrahepatic cholestasis [1]. This is associated with necrosis of bile duct epithelium and focal injury to hepatocytes, primarily in periportal regions of the liver [2–4]. The mechanism of toxicity of ANIT is unknown, although much attention has focused on the role of hepatic, cytochrome P450-dependent, mixed-function oxidases (MFO). ANIT may require bioactivation, since induction of hepatic MFO activity with phenobarbital treatment in rats or mice enhances ANIT-induced liver injury whereas acute pretreatment with the MFO inhibitor diethyl-aminoethyl-2,2-diphenylvalerate (SKF 525-A) affords protection [5, 6]. Certain other agents which are inducers or inhibitors of hepatic MFO also increase or decrease, respectively, the liver injury associated with ANIT [6, 7]. Presumably, ANIT is bioactivated by hepatic MFO to one or more species toxic to bile duct epithelium and hepatic parenchymal cells [5, 8].

The site of the ANIT lesion in the hepatic lobule does not fit easily into the bioactivation hypothesis; ANIT does not cause centrilobular necrosis, as do other agents for which bioactivation by MFO is required. Instead, ANIT causes focal necrosis of periportal hepatocytes [2–4]. One possible explanation is that the injury to hepatocytes is a result of toxic biliary products, such as bile salts, released from damaged bile ducts [8]. In this scenario, ANIT is bioactivated to a metabolite which is toxic specifically to bile duct epithelium [8]. This mechanism might explain why periportal hepatocytes are primarily affected. Another possibility is that cycling of a metabolite(s) in the enterohepatic circulation may be required for toxicity [9]. After absorption from the intestinal tract into mesenteric and portal venous blood, the metabolite(s) may cause toxicity to hepatocytes first exposed, i.e. periportal hepatocytes. Either of the two proposed mechanisms is consistent with the bioactivation hypothesis of ANIT hepatotoxicity and might explain the zonal toxicity.

An alternative explanation is that ANIT may not require bioactivation by hepatic MFO to cause hepatocellular injury. ANIT is toxic to ARL 3 cells derived from adult rat liver *in vitro*, and the toxicity is not reduced by pretreatment with SKF 525-A [10]. Furthermore, addition to the culture system of a 10,000 *g* supernatant from liver to provide a source of MFO does not enhance the cytotoxicity of ANIT, although it does enhance the toxicity of agents (i.e.

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‡ Abbreviations: ANIT, α-naphthylisothiocyanate; CO, corn oil; GSH, glutathione; NPSH, non-protein sulfhydryl; SAL, saline; BSO, buthionine sulfoximine; DEM, diethyl maleate; MFO, cytochrome P450-dependent, mixed-function oxidase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; TSBA, total serum bile acids; and DTNB, 5,5′-dithio-bis-(2-nitrobenzoic acid).

Table 1. Effects of selected agents on ANIT-induced liver injury and hepatic GSH status

Compound	ANIT injury	Hepatic GSH content	Hepatic GSH *S*-transferase activity*
Phenobarbital	Increase [6]**	Increase [11]	Increase†‡§‖ [12]
3-Methylcholanthrene	Increase [6]	Increase [11]	Increase†‡§‖ [12]
SKF 525-A	Decrease [6]	Decrease [13]	Decrease†‡¶ [14]
Piperonyl butoxide	Decrease [6]	Decrease [15]	
Diethyldithiocarbamate	Decrease [7]	Decrease [16]	Decrease§ [17]
Cyanidanol	Decrease [18]		Decrease§‖ [17]
Ethionine	Decrease [19]	Decrease [20]	

* Assayed with the following substrates: † 3,4-dichloronitrobenzene, ‡ *p*-nitrobenzyl chloride, § 1,2-epoxy-(*p*-nitrophenoxy)propane, ‖ methyl iodide, or ¶ 1-chloro-2,4-dinitrobenzene.

** Number in bracket refers to reference.

CCl_4 and dimethylnitrosamine) which require bioactivation [10]. In accord with the hypothesis of Williams [10], it is possible that certain agents used as MFO inhibitors or inducers may alter ANIT-induced liver injury by a mechanism other than interfering with hepatic MFO. For example, certain agents which alter the liver injury caused by ANIT *in vivo* also affect hepatic glutathione (GSH) status (Table 1). Included in Table 1 are agents used as MFO inhibitors, MFO inducers, and compounds which affect ANIT hepatotoxicity by unknown mechanisms. Agents that afford protection against ANIT-induced liver injury decrease hepatic GSH content and/or at least one form of GSH *S*-transferase. Conversely, agents that enhance the liver injury increase hepatic GSH content and/or at least one form of GSH *S*-transferase. Thus, these various agents appear to affect hepatic GSH metabolism in a manner to suggest a causal role for GSH in ANIT-induced liver injury.

The hypothesis tested in this study was that GSH plays a causal role in the liver injury produced by ANIT. Accordingly, we administered agents to rats that decreased hepatic NPSH content, an indicator of GSH content, and determined whether they afforded protection against ANIT hepatotoxicity.

MATERIALS AND METHODS

Materials. ANIT, GSH, 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), L-buthionine-*S*,*R*-sulfoximine (BSO), Kit 605-D for bilirubin determination, and Kit 505 for aspartate aminotransferase (AST) activity were purchased from the Sigma Chemical Co. (St. Louis, MO). Diethyl maleate (DEM, 98%) and phorone (93%) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). All other reagents were of the highest grade commercially available. Polyethylene (PE) 10 tubing was purchased from Clay Adams (Parsippany, NJ).

Animals. Male, Sprague–Dawley rats (CF:CD(SD)BR) (Charles River, Portage, MI) weighing 210–290 g were housed in plastic cages on aspen chip bedding under conditions of controlled temperature (18–21°) and humidity (55 ± 5%). A 12-hr light/12 hr-dark cycle was maintained. Rats were allowed tap water and rat chow (Wayne Lab Blox, Allied Mills, Chicago, IL) *ad lib.* prior to experimentation.

Treatment protocol for decreasing hepatic nonprotein sulfhydryl (NPSH) content. Rats were fasted for 24 hr prior to experimentation and for the remainder of the study. They were treated intraperitoneally (i.p.) with BSO (890 mg/kg) or an equivalent volume of saline (SAL) vehicle 2.5 hr prior to treatment with either ANIT (100 mg/kg, *per os* [p.o.]) or an equivalent volume of corn oil (CO) vehicle (time zero). At 9.5 hr, they received a second treatment of either BSO or SAL. Hepatic NPSH content was measured 24 hr after ANIT treatment as an indicator of GSH content. In a separate experiment, DEM (642 mg/kg, 1:1 in CO, i.p.) or an equivalent amount of CO vehicle was given to rats 30 min prior to either ANIT (100 mg/kg, p.o.) or an equivalent volume of CO vehicle. In another experiment, rats were treated with phorone (125 mg/kg, 1:3 in CO, i.p.) or an equivalent amount of CO vehicle 30 min prior to ANIT (50 mg/kg, p.o.). In these studies with DEM and phorone, rats were killed 24 hr after ANIT treatment. In either naive or CO-treated rats, decreases in hepatic NPSH content were confirmed 2.5 and 2 hr after treatment with DEM or phorone, respectively.

Twenty-four hours after ANIT treatment, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed on a heating pad to maintain body temperature. A midline incision was made in the rat, and the common bile duct was isolated and cannulated with PE 10 tubing. Bile was collected for a 30-min period. Bile samples were weighed, and bile flow was calculated assuming a density of 1 g/mL.

A blood sample was taken from the descending aorta for measurements of serum markers of liver injury. Total bilirubin concentration (Sigma Kit 605-D) was measured spectrophotometrically by coupling bilirubin to *p*-diazobenzenesulfonic acid to form azobilirubin. Total serum 3α-hydroxy bile acid (TSBA) concentration was determined by measuring the fluorescence of resorfin [21]. γ-Glutamyltransferase (GGT) activity was measured spectrophotometrically by monitoring GGT-catalyzed formation of *p*-nitroanilide from L-γ-glutamyl-*p*-nitroanilide [22]. Serum AST activity (Sigma Kit

505) was measured spectrophotometrically by monitoring formation of the phenylhydrazone of oxalacetate.

NPSH content in liver was measured by a modification of the method of Ellman [23] as described by Costa and Murphy [24]. Briefly, sulfhydryl groups were reacted with DTNB at pH 8.0 to produce the *p*-nitrothiophenol anion, which was detected spectrophotometrically at 412 nm. NPSH content was calculated from a standard curve of reduced GSH.

Treatment protocol to determine whether decreased hepatic NPSH content delayed the onset of injury. Rats were fasted for 24 hr prior to experimentation and for the remainder of the study. All received ANIT and either BSO or SAL vehicle as described above. Half of the rats were killed 24 hr after ANIT treatment, similar to the BSO study described above. The other half received no additional BSO after the second treatment and were killed 48 hr after ANIT treatment. Markers of liver injury and hepatic NPSH content were measured 24 and 48 hr after ANIT treatment as described above.

A separate study was performed with DEM to determine whether DEM afforded complete protection or delayed the onset of injury. Rats were fasted 24 hr prior to experimentation and for the remainder of the study. They were treated with either DEM or CO vehicle 30 min prior to ANIT as described above, and several markers of liver injury were measured 48 hr later.

Statistical analysis. Results are expressed as means ± SEM. Homogeneity of variance was tested using the F-max test. Log transformations were performed on nonhomogeneous data. If the variances were homogeneous, data were analyzed using Student's *t*-test or a completely randomized factorial analysis of variance, as appropriate. Individual comparisons between treatment means were made with Tukey's ω test [25]. When the variances were nonhomogeneous after log transformation of data, the data were analyzed with the nonparametric, distribution-free, multiple comparison test [26]. The criterion for significance was $P < 0.05$ for all comparisons.

RESULTS

Effects of decreased NPSH content on markers of liver injury 24 hr after ANIT treatment. Administration of ANIT to rats caused cholestasis and elevations in serum of total bilirubin concen-

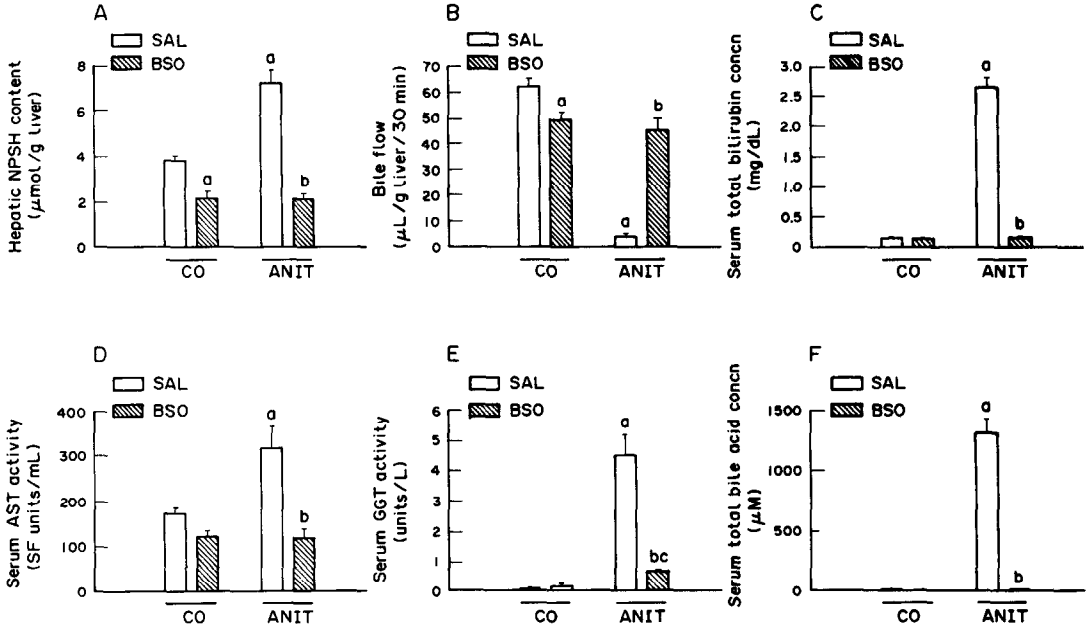

Fig. 1. Effects of BSO on ANIT-induced changes in hepatic NPSH content (A), bile flow (B), serum total bilirubin concentration (C), serum AST activity (D), serum GGT activity (E), and serum total bile acid concentration (F). Rats were fasted prior to treatments. They received either BSO (890 mg/mg, i.p.) or SAL vehicle 2.5 hr before treatment with either ANIT (100 mg/kg, p.o.) or CO vehicle (time zero). They received a second treatment of either BSO or SAL at 9.5 hr. Hepatic NPSH content and other markers of liver injury were measured 24 hr after ANIT or CO treatment. Values are means ± SEM, N = 4–7. Total serum bile acid concentrations for CO/SAL, CO/BSO, and ANIT/BSO groups were 9 ± 1, 8 ± 1, and 12 ± 2 μM, respectively. Abbreviations: SF, Sigma Frankel; and concn., concentration. Key: (a) significantly different from group treated with CO and SAL, $P < 0.05$; (b) significantly different from group treated with ANIT and SAL, $P < 0.05$; and (c) significantly different from group treated with CO and BSO, $P < 0.05$.

Table 2. Effects of DEM on liver injury 24 hr after ANIT treatment

Treatment*	Bile flow (μL/g liver/30 min)	Serum total bilirubin concentration (mg/dL)	Serum AST activity (SF units/mL)	Serum GGT activity (U/L)	Serum total bile acid concentration (μM)
CO/CO	54 ± 2	0.2 ± 0	138 ± 9	0 ± 0.1	4 ± 0
CO/DEM	55 ± 5	0.2 ± 0	156 ± 15	0.2 ± 0.1	4 ± 0
ANIT/CO	0 ± 0†	2.7 ± 0.2†	498 ± 63†	5.0 ± 0.4†	1388 ± 91†
ANIT/DEM	54 ± 2‡	0.4 ± 0.1‡	201 ± 26‡	0.6 ± 0.2‡	54 ± 22‡

* Rats were fasted for 24 hr and were treated with either DEM (642 mg/kg, i.p.) or CO vehicle 30 min prior to either ANIT (100 mg/kg, p.o.) or CO vehicle. Markers of liver injury were measured 24 hr after ANIT treatment. Values are means ± SEM, N = 4-7.
† Significantly different from CO/CO group, $P < 0.05$.
‡ Significantly different from ANIT/CO group, $P < 0.05$.

tration, total bile acid concentration, AST activity, and GGT activity (Fig. 1). Treatment of rats with ANIT increased hepatic NPSH content nearly 2-fold when compared to vehicle controls. Cotreatment of rats with BSO decreased hepatic NPSH content by 70% at 24 hr after ANIT treatment and prevented the cholestasis and elevations in markers of liver injury caused by ANIT (Fig. 1). BSO treatment had minimal effects on markers of liver injury in rats treated with CO, although it caused a slight reduction in bile flow (Fig. 1).

As with BSO, cotreatment of rats with DEM (642 mg/kg) prevented the cholestasis and elevations in markers of liver injury 24 hr after ANIT treatment (Table 2). DEM treatment had minimal effects on markers of liver injury to rats treated with CO. In a separate experiment in CO-treated rats, this dose of DEM decreased hepatic NPSH content by 56% 2.5 hr later, but NPSH content returned to control levels by 6 hr (data not shown).

Phorone treatment (125 mg/kg) afforded partial protection against ANIT-induced elevations in serum total bilirubin concentration and AST activity, although it did not affect the cholestasis and elevation in serum GGT activity (Table 3). In a separate experiment in naive rats, phorone decreased hepatic NPSH content by 88% at 2 hr after treatment. As in the case with DEM, hepatic NPSH content returned to control by 6 hr (data not shown). A larger dose of phorone (250 mg/kg, i.p.) reduced hepatic NPSH content by >80% for at least 7 hr in naive rats, and it afforded near-complete protection against ANIT-induced elevations in serum total bilirubin concentration, total bile acid concentration, and GGT activity (data not shown). However, this dose of phorone was hepatotoxic, as indicated by a reduction in bile flow and elevation in serum AST activity (data not shown).

Effects of BSO administration on liver injury 48 hr after ANIT treatment. Rats treated with ANIT had reduced bile flow and elevations in serum total bilirubin concentration and GGT activity at 24 and 48 hr (Fig. 2). Cotreatment of rats with BSO decreased hepatic NPSH content at 24 hr, and it prevented the cholestasis and elevations in serum total bilirubin concentration and GGT activity (Fig. 2). These results confirm those described above and shown in Fig. 1.

Although BSO administration prevented the cholestasis and elevations in serum total bilirubin concentration and GGT activity 24 hr after ANIT treatment, rats exhibited cholestasis and elevations in serum markers of liver injury by 48 hr. Rats treated with ANIT and BSO had values for bile flow, serum total bilirubin concentration, and serum GGT activity similar to those of rats treated with ANIT and the SAL vehicle (Fig. 2). The onset of liver injury by 48 hr coincided with a return of hepatic NPSH content (Fig. 2).

Effects of DEM administration on liver injury 48 hr after ANIT treatment. A reduction in bile flow and elevations in serum total bilirubin concentration and AST activity occurred in rats 48 hr after ANIT treatment (Table 4). The protection by DEM seen 24 hr after ANIT treatment (Table 2) was not apparent at 48 hr (Table 4).

Table 3. Effects of phorone on markers of liver injury 24 hr after ANIT treatment

Treatment*	Bile flow (μL/g liver/30 min)	Serum total bilirubin concentration (mg/dL)	Serum AST activity (SF units/mL)	Serum GGT activity (U/L)
CO	3 ± 2	3.3 ± 0.2	705 ± 59	5.1 ± 0.4
Phorone	7 ± 2	2.1 ± 0.2†	354 ± 38†	5.0 ± 0.2

* Rats were fasted for 24 hr and were treated with either phorone (125 mg/kg, i.p.) or CO vehicle 30 min prior to ANIT (50 mg/kg, p.o.). Markers of liver injury were measured 24 hr after ANIT treatment. Values are means ± SEM, N = 5–6.

† Significantly different from respective CO group, $P < 0.05$.

DISCUSSION

Results from this study indicate that NPSHs may play a causal or permissive role in ANIT-induced liver injury, since agents which decreased hepatic NPSH content (BSO, DEM, and phorone) afforded protection. It seems likely that GSH is involved in the pathogenesis, since it comprises >95% of the NPSH pool in rat liver [27]. Furthermore, using specific assays for GSH, others have shown that BSO, DEM, and phorone reduce hepatic GSH content to a similar extent as NPSH content in this study [28, 29]. Therefore, measurement of hepatic NPSH content is a valid indicator of GSH content, at least under the conditions described above. Although all three agents reduced hepatic NPSH content in the present study, one cannot rule out the possibility that these agents provided protection by an NPSH-independent mechanism and that changes in NPSH content were coincidental.

The strongest evidence for NPSH involvement is that provided by BSO. BSO blocks GSH synthesis by inhibiting γ-glutamylcysteine synthetase [30]. Administration of BSO to animals causes a decrease

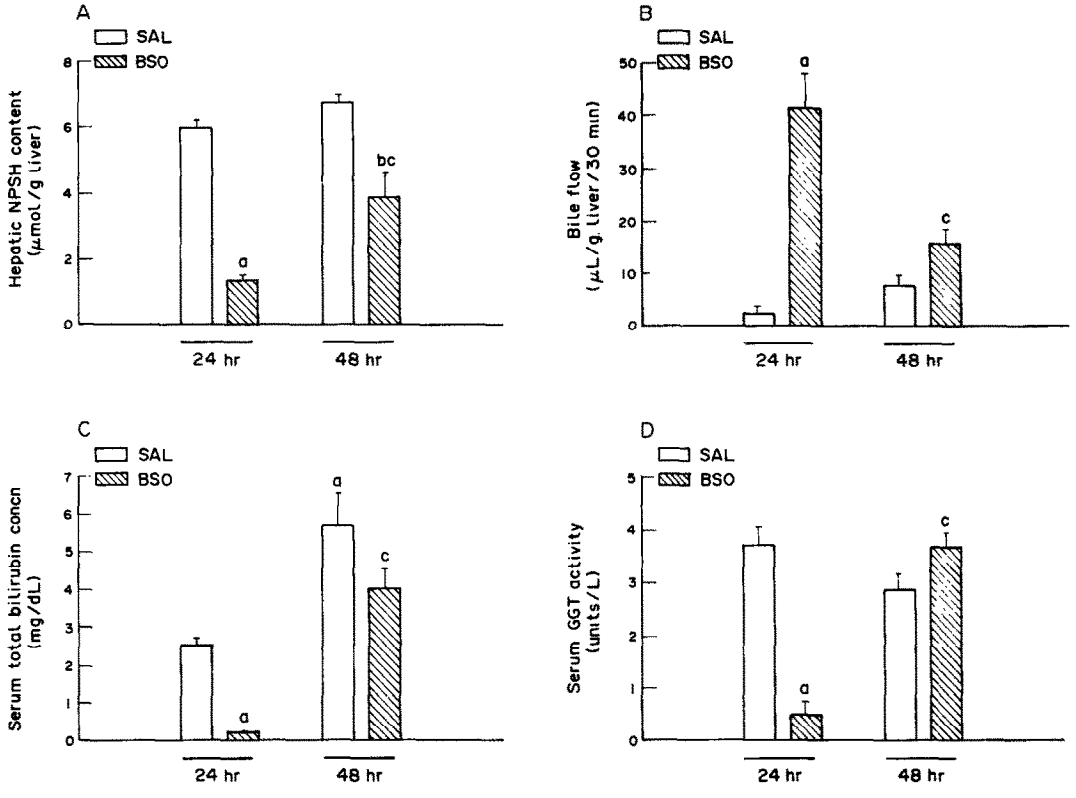

Fig. 2. Effects of BSO on hepatic NPSH content (A), bile flow (B), serum total bilirubin concentration (C), and serum GGT activity (D) 24 and 48 hr after ANIT treatment. Rats were fasted prior to treatments. All received ANIT (100 mg/kg, p.o.) and either BSO or SAL as described in the legend to Fig. 1. No additional BSO or SAL was given after the treatment at 9.5 hr. Values are means ± SEM, N = 5–8. Key: (a) significantly different from 24-hr SAL group, $P < 0.05$; (b) significantly different from 48-hr SAL group, $P < 0.05$; and (c) significantly different from 24-hr BSO group, $P < 0.05$.

Table 4. Effects of DEM on markers of liver injury 48 hr after ANIT treatment

Treatment*	Bile flow (μL/g liver/30 min)	Serum total bilirubin concentration (mg/dL)	Serum AST activity (SF units/mL)
CO	2 ± 1	5.4 ± 0.7	2053 ± 274
DEM	3 ± 1	7.1 ± 0.4	1396 ± 165

* Rats were fasted for 24 hr and were treated with either DEM (642 mg/kg, i.p.) or CO vehicle 30 min prior to ANIT (100 mg/kg, p.o.). Markers of liver injury were measured 48 hr after ANIT treatment. Values are means ± SEM, N = 5–6.

in GSH content in liver and other tissues with a high GSH turnover, i.e. kidney. The advantage of BSO as a means to reduce hepatic GSH content is that it is rather specific and apparently does not affect hepatic MFO activity or enzymes involved in phase II metabolism [31–33]. Therefore, the protection afforded by BSO against ANIT-induced liver injury is likely related to its effects on GSH. BSO decreased hepatic NPSH content and afforded protection when markers of liver injury were measured at 24 hr after ANIT treatment, although injury was evident by 48 hr. Since the onset of injury appeared to coincide with a return of hepatic NPSH content, these data provide additional support for the role of NPSHs in the pathogenesis. Presumably, the liver injury occurs as hepatic NPSH content increases to a critical level and triggers an unidentified, NPSH-dependent process leading to toxicity.

Protection against ANIT-induced liver injury with DEM or phorone at 24 hr after treatment provides further support for NPSH involvement in ANIT's mechanism of toxicity. DEM and phorone are α,β-unsaturated carbonyl compounds which decrease hepatic GSH content by conjugating to GSH [34]. In naive rats or those treated with CO, these agents decrease hepatic NPSH content for only 2–6 hr after treatment, suggesting that hepatic NPSHs, participate in the hepatotoxic response during the first few hours after ANIT administration.

DEM and phorone are effective in decreasing hepatic NPSH content, but their use as experimental tools is complicated by their other biological effects. For example, DEM alters hepatic MFO activity *in vitro* [35], and it decreases body temperature [24], reduces cytochrome P450 content [36], and causes a transient choleresis [37] when administered *in vivo*. Phorone itself is hepatotoxic to rats at doses close to those needed to deplete hepatic NPSH content. Thus, interpretation of results of experiments employing these agents is complicated by their other effects. However, taken together, the observations that BSO, DEM, and phorone afforded protection against ANIT-induced liver injury support a causal or permissive role for NPSHs, i.e. GSH, in the pathogenesis.

Certain agents which are MFO inducers or inhibitors alter hepatic GSH content and/or GSH *S*-transferase activity (Table 1), suggesting that their effects on ANIT-induced liver injury might result, at least in part, from changes in GSH status. It is tempting to speculate that other manipulations that alter ANIT injury act via an effect on GSH. For example, Indacochea-Redmond *et al.* [19] suggested that unimpaired protein or ribonucleic acid synthesis may be required in the mechanism of injury, because ethionine, actinomycin D, and cycloheximide afford protection. Since ethionine decreases hepatic GSH content [18], it, and perhaps other inhibitors of protein and ribonucleic acid synthesis, may afford protection against ANIT hepatotoxicity by a mechanism involving a decrease in hepatic GSH content.

Participation of NPSHs in ANIT-induced liver injury likely involves GSH, although the mechanism is presently unknown. One possible explanation is that GSH may form hepatotoxic thiol ether leukotrienes, which have been implicated in cholestasis [38], liver injury caused by frog virus 3 [39], and fulminant hepatitis caused by a combination of galactosamine and endotoxin [40].

A second possibility is that ANIT forms a GSH *S*-conjugate which is involved somehow in the pathogenesis. Knowledge on ANIT metabolism is limited, and evidence against GSH *S*-conjugation *in vivo* has been presented. For example, ANIT, unlike allyl isothiocyanate and benzyl isothiocyanate, does not form a mercapturic acid detectable in urine or bile [41–43]. However, recent evidence *in vitro* indicates that ANIT forms a reversible GSH *S*-conjugate in hepatocytes [44]. Because isothiocyanates form reversible GSH *S*-conjugates [44–46], conjugation of ANIT with GSH may serve as a transport mechanism to target ANIT to bile duct epithelium after biliary secretion. Alternatively, like certain nephrotoxic GSH *S*-conjugates, a GSH *S*-conjugate of ANIT may require further metabolism to cause toxicity [reviewed in Ref. 47]. That ANIT injures bile duct epithelium is also consistent with this hypothesis, since this tissue has high GGT activity [48] necessary in processing GSH *S*-conjugates to toxic species. The issue of whether or not GSH *S*-conjugation plays a role in the metabolism of ANIT *in vivo* and how this contributes to the mechanism of toxicity remain unclear at present.

In summary, we have shown that ANIT hepatotoxicity was prevented by agents that decreased hepatic NPSH content, an indicator of GSH content. Agents which are inducers or inhibitors of hepatic MFO also affect hepatic GSH content and/or GSH *S*-transferase activity in a manner to suggest a causal or permissive role for GSH in ANIT-induced liver injury. Taken together, these observations call into question the need for MFO bioactivation in ANIT hepatotoxicity. However, they do not rule out, necessarily, a role for hepatic MFO in the pathogenesis of injury, since both GSH

and metabolism by MFO may be required. Additional studies are needed to determine how GSH may be involved in the liver injury caused by ANIT.

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