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Effects of Taurine and Guanidinoethane Sulfonate on Toxicity of the Pyrrolizidine Alkaloid Monocrotaline

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ABSTRACT. Monocrotaline (MONO), a pyrrolizidine alkaloid, causes pulmonary arterial hypertension and right ventricular hypertrophy due to hepatic metabolism to the alkylating pyrrole dehydromonocrotaline. Taurine, a sulfonic amino acid, is hepato- and cardioprotective in a variety of conditions. We have examined the effects of taurine and its amidino analog, guanidinoethane sulfonate (GES), in rats injected i.p. with MONO (65 mg/kg). Taurine and GES were given as 1% solutions in drinking water beginning 14 days before administration of MONO and continuing for 14 days thereafter, when the rats were killed. The MONO group had right ventricular hypertrophy and pulmonary hyperplasia. Compared with control, no significant changes in the right ventricle/left ventricle weight ratio, or the right ventricle/body weight ratio occurred in rats also given taurine or GES. Lung weights in these two groups were higher than in the control group, but below that of the MONO-alone group. The lethality of MONO over 14 days was decreased by taurine (LD_{50} for MONO alone 80 mg/kg; for MONO + taurine 121 mg/kg). Rats given only MONO had lower hepatic concentrations of GSH and cysteine (Cys), and higher activities of microsomal GSH transferase and γ-glutamyl transpeptidase. In rats also receiving taurine, hepatic GSH levels and GSH transferase activity were no different from control. γ-Glutamylcysteine (Glu-Cys) synthetase and γ-glutamyl transpeptidase activities were elevated. In MONO-injected rats given GES, hepatic GSH levels were higher and Cys levels were lower than in either the MONO alone or MONO + taurine groups. γ-Glu-Cys synthetase activity was depressed. Microsomal GSH transferase, GSH peroxidase and γ-glutamyl transpeptidase activities were elevated. Livers of MONO-injected animals showed higher levels of serine (reversed by both taurine and GES) and glycine (Gly; reversed by GES) and lower levels of glutamine. Compared with control rats, the following changes occurred in serum amino acids: MONO alone: increased aspartate, taurine and lysine; taurine-supplemented: increased taurine, methionine (Met) and lysine, and decreased Gly; GES-supplemented: decreased asparagine, serine, Gly, arginine, taurine, and valine. Compared with the MONO-alone group, the taurine-supplemented group had higher glutamate (Glu), Met and alanine, and the GES-supplemented group higher alanine and lower serine, Gly, arginine and valine. We conclude that taurine protects against MONO-induced lethality and right ventricular hypertrophy. GES also protects against right ventricular hypertrophy. However, these agents act by different mechanisms, taurine preventing many of the biochemical changes induced by MONO, with GES inducing additional changes. BIOCHEM PHARMACOL 51;3:321–329, 1996.

KEY WORDS. liver; glutathione; monocrotaline; γ-glutamylcysteine synthetase; γ-glutamyl transpeptidase; GSH *S*-transferases

MONO† (Fig. 1) is a pyrrolizidine alkaloid present in various species of leguminous *Crotalaria*. It has caused numerous outbreaks of poisoning worldwide [1]. Typically, exposed people develop hepatomegaly and veno-occlusive disease of the liver. In nonhuman primates and a variety of other species, MONO also causes pulmonary arterial hypertension and right ventricular hypertrophy [2].

MONO undergoes hepatic bioactivation to the reactive pyrrole dehydromonocrotaline. It is thought that the release of dehydromonocrotaline from the liver is responsible for toxicity to extrahepatic organs, such as heart and lungs. Dehydromonocrotaline is detoxified by conjugation with GSH [3]. Thus, the toxicity of MONO is affected by the GSH status of the liver. MONO, in turn, influences the metabolism of GSH and related sulfur-containing compounds [4]. Within 24 hr of exposing rats to MONO or related pyrrolizidine alkaloids, there is a change in sulfur amino acid metabolism from the Cys → taurine axis to the Cys → GSH axis [4, 5].

Taurine (2-aminoethane sulfonic acid) is produced in rat liver via the oxidation and decarboxylation of Cys [6]. Because of its ability to maintain function in tissues with disturbed homeostasis, taurine has been named an enantiostatic agent [6]. Enantiostasis differs from homeostasis in that the consequences of a change in the chemical and physical properties of the internal milieu of a cell are ameliorated by a further, com-

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† *Abbreviations:* CDNB, 1-chloro-2,4-dinitrobenzene; Cys, cysteine; Cys-Gly, cysteinylglycine; DTT, DL-dithiothreitol; GES, guanidinoethane sulfonate; Glu, glutamate; γ-Glu-Cys, γ-glutamylcysteine; Gly, glycine; GSH, glutathione; Met, methionine; MB, monobromobimane; MONO, monocrotaline; OPA, *o*-phthaldehyde; and SSA, sulfosalicyclic acid.

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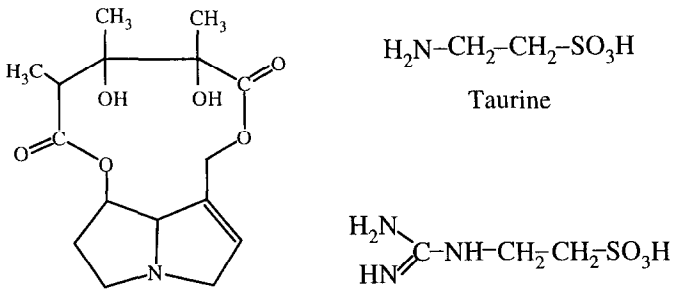

FIG. 1. Structures of MONO, taurine and GES.

pensating, change [7]. Taurine protects against insults to all three of the major organ systems affected by MONO: the liver [8, 9], heart [6, 10–14] and lungs [15–19]. We have examined, therefore, the ability of taurine to modify the toxicity of MONO and the biochemical changes it induces. In addition, we have examined the effects of a related compound, GES. GES, the amidino analog of taurine, serves as a taurine-depleting agent as it competitively antagonizes taurine transport in the rat [20] and depresses its biosynthesis in the liver [21].

MATERIALS AND METHODS

Chemicals

Taurine, GSH, L-Cys, Gly, L-glutamic acid (Glu), DTT, γ-Glu-Cys, Cys-Gly, glycylglycine, *N*-ethylmorpholine, NADPH, GSH reductase, oxidized GSH, CDNB, H_2O_2, and the kit for protein determination were purchased from the Sigma Chemical Co. (St. Louis, MO). MB (Thiolyte Reagent) was from Calbiochem (San Diego, CA). MONO was isolated from seeds of *Crotalaria spectabilis* [22] and was purified by counter-current distribution and identified by mass spectrometry. GES was synthesized according to the procedure of Huxtable *et al.* [20].

Treatment Protocol

Twenty Male Sprague-Dawley rats (body weight 53–70 g) from the University of Arizona Division of Animal Resources were housed 2–3 to a cage under 12-hr cycles of light and dark. After 3 days for adaptation, the animals were divided randomly into 4 groups of 5 animals. Rats in the control and MONO groups were maintained on fresh tap water throughout the study. Rats in the MONO plus taurine group were maintained on a solution of 1% taurine, and rats in the MONO plus GES group were maintained on a solution of 1% GES. On day 14, rats in the control group received an injection of 0.85% NaCl (i.p.). Rats in the other groups each received an injection of MONO (65 mg/kg body weight, i.p.) in the same volume as in the control group. All animals were allowed to eat and drink *ad lib*. Fourteen days after being injected, all rats were decapitated. To avoid differences due to diurnal variation in enzyme activities related to GSH metabolism, rats were killed between 9:00 and 10:00 a.m. Sera were collected for amino acid assay. Liver, heart, lung, kidney, brain and spleen were removed for determination of tissue:body weight ratios. Wet weights were recorded immediately after washing (three times) in 0.85% NaCl. Dry weights were obtained after drying the organs at 50° for 3 days. On day 11 after MONO injection, 2 rats in the MONO-alone group died.

Determination of LD_{50}

Male rats (initial body weight 60–85 g) were maintained on either tap water (30 animals) or 1% taurine solution (30 animals). After 2 weeks, animals in both groups were injected i.p. with MONO at 55, 82, 110, 165, or 220 mg/kg. Each dose was administered to a total of 5 animals in each group. Deaths of animals were recorded daily for the subsequent 2 weeks. The LD_{50} values were calculated using the Litchfield and Wilcoxon II Pharmacologic Calculation System, version 4.2, computer program.

Collection of Serum

Whole blood was collected in untreated polypropylene tubes and stood at room temperature for 3 hr. It was then centrifuged in a clinical centrifuge for 5 min, and the clear supernatant was siphoned off as serum and used for amino acid determination.

Tissue Cytosol and Microsome Preparation

Liver was removed and washed twice in saline at 4°. Then the livers were minced and homogenized in 4 vol. of 0.25 M su-

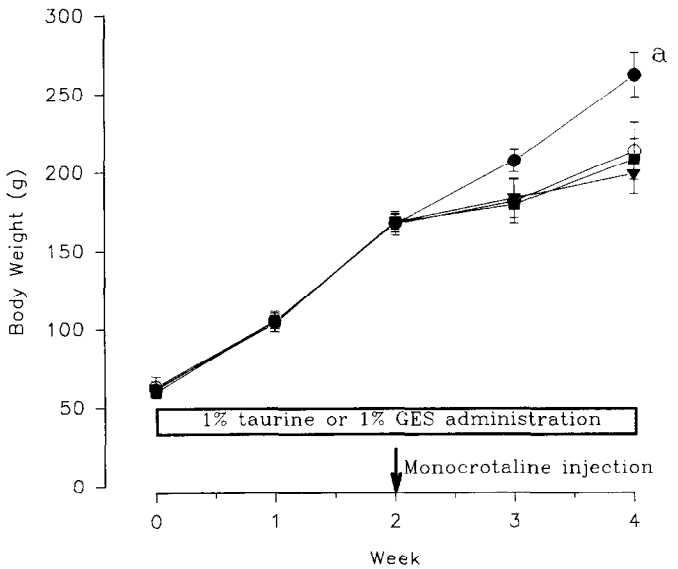

FIG. 2. Body weight gains of rats maintained on: tap water and given a saline injection (●); tap water and injected with MONO (■); 1% taurine solution and injected with MONO (▼); or 1% GES solution and injected with MONO (○). The arrow indicates the day of MONO injection (65 mg/kg, i.p.). Data are means ± SD for 5 animals (3 for the 4-week measurement on MONO-alone group). Key: $^aP < 0.05$ compared with all other groups.

TABLE 1. Effects of taurine and guanidinoethane sulfonate on dry weight of organs in rats treated with the pyrrolizidine alkaloid monocrotaline

Tissue	Control	MONO	MONO + taurine	MONO + guanidinoethane sulfonate
Right ventricular	44.1 ± 4.6	50.6 ± 2.0*	38.8 ± 5.3†	42.7 ± 2.0†
Left ventricular	154 ± 12	132 ± 13	118 ± 12*	142 ± 6‡
Lung	282 ± 22	500 ± 58*	414 ± 21*†	392 ± 30*†
Spleen	162 ± 20	163 ± 18	163 ± 21	188 ± 10
Liver	3320 ± 437	2140 ± 100*	2170 ± 466*	2700 ± 220
Brain	356 ± 27	337 ± 14	332 ± 22	346 ± 27
Kidney	517 ± 35	424 ± 36*	410 ± 59*	464 ± 37

Data are means ± SD for 5 animals per group (MONO group, 3 animals). Values are expressed in mg.
* $P < 0.05$ vs the control group.
† $P < 0.05$ vs the MONO group.
‡ $P < 0.05$ vs the MONO plus taurine group.

crose and 20 mM Tris-HCl containing 1 mM EDTA (pH 7.4). The homogenate was centrifuged at 3000 *g* for 10 min at 4°, and the resulting supernatant was centrifuged at 10,000 *g* for 20 min at 4°. Finally, the supernatant was centrifuged at 105,000 *g* for 60 min at 4°. The supernatant was used to determine GSH and amino acid concentrations and the activities of γ-Glu-Cys synthetase, GSH synthetase, cytosolic GSH S-transferase, GSH reductase, and GSH peroxidase. The microsomal pellet was used to determine the activities of dipeptidase, γ-glutamyl transpeptidase, and microsomal GSH S-transferase.

Determination of GSH Concentration

Hepatic cytosolic fraction (100 μL) was mixed with 5% SSA and centrifuged at 10,000 *g* for 5 min to precipitate protein. The supernatant was derivatized with MB and analyzed by HPLC (see below).

Enzyme Assays

Incubations were performed at 37° and terminated by the addition of 100 μL incubate to 50 μL of 5% SSA. The mixture was centrifuged for 3 min to precipitate protein. The supernatant was derivatized with MB (see below) and injected into the HPLC. Peaks were identified by comparison with the retention time of authentic peaks and quantified according to standard curves. Separation was achieved using a Beckman Ultrasphere™ ODS (250 × 4.6 mm i.d., 5 μm particle size) reversed-phase stainless-steel column (Beckman, San Ramon, CA, U.S.A.) maintained at room temperature. The analytical column was protected by a supelguard (5 × 4.6 mm, i.d.), pellicular reversed-phase cartridge precolumn (Supelco, Bellefonte, PA, U.S.A.). Elution was carried out using a gradient program with 0.25% acetic acid as solvent A (pH 3.9) and methanol: 0.25% acetic acid (30:70, v/v) as solvent B (pH 3.9). The flow rate was 1.5 mL/min, and the starting condition was 35% B for 1 min followed by a linear gradient to 62% B within 6 min. This was maintained for another 2 min. The column was then washed with 100% B for 2 min and maintained under starting conditions for 5 min. With this program, the retention times for Cys, Cys-Gly, γ-Glu-Cys, and GSH were 7.09, 8.34, 9.93, and 11.40 min, respectively.

γ-Glu-Cys Synthetase

Cytosolic γ-Glu-Cys synthetase activity was determined by following the formation of γ-Glu-Cys. Incubations were per-

TABLE 2. Effects of taurine and guanidinoethane sulfonate on the ratio of wet weight to dry weight of organs in rats treated with the pyrrolizidine alkaloid monocrotaline

Tissue	Control	MONO	MONO + taurine	MONO + guanidinoethane sulfonate
Right ventricular	4.85 ± 0.17	4.09 ± 0.03	5.01 ± 0.20	4.90 ± 0.10
Left ventricular	4.61 ± 0.07	4.55 ± 0.03	4.56 ± 0.15	4.55 ± 0.05
Lung	5.47 ± 0.71	5.35 ± 0.12	5.53 ± 0.19	5.38 ± 0.83
Spleen	4.44 ± 0.07	4.48 ± 0.04	4.47 ± 0.09	4.40 ± 0.08
Liver	3.63 ± 0.29	4.56 ± 0.45*	4.57 ± 0.28*	4.10 ± 0.32
Brain	5.07 ± 0.27	5.07 ± 0.08	5.12 ± 0.06	5.29 ± 0.34
Kidney	4.86 ± 0.46	4.71 ± 0.19	5.24 ± 0.73	4.98 ± 0.33

Data are means ± SD for 5 animals per group (MONO group, 3 animals).
* $P < 0.05$ vs the control group.

TABLE 3. Effects of taurine and guanidinoethane sulfonate on the ratio of dry organ weight to body weight in rats treated with the pyrrolizidine alkaloid monocrotaline

Tissue	Control	MONO	MONO + taurine	MONO + guanidinoethane sulfonate
Right ventricular	168 ± 26	243 ± 9*	196 ± 25	192 ± 7
Left ventricular	588 ± 63	632 ± 27	609 ± 50	670 ± 15
Lung	1076 ± 99	2412 ± 360*	1986 ± 273*	1768 ± 189*
Spleen	617 ± 88	780 ± 62	833 ± 100*	848 ± 51*
Liver	13421 ± 1645	10342 ± 1029	10423 ± 986*	11680 ± 1044
Brain	1363 ± 169	1625 ± 167	1694 ± 128*	1559 ± 142
Kidney	1979 ± 202	2031 ± 98	2074 ± 244	2082 ± 99
RV/LV‡	0.287 ± 0.037	0.385 ± 0.025*	0.327 ± 0.018†	0.312 ± 0.017†

Data are means ± SD for 5 animals per group (MONO group, 3 animals).
* $P < 0.05$ vs the control group.
† $P < 0.05$ vs the MONO group.
‡ Ratio of dry right ventricular to dry left ventricular.

formed in 1.5-mL microfuge tubes containing 10 mM Glu, 5 mM Cys, 10 mM ATP, 0.1 M Tris-HCl (pH 8.2), 0.15 M KCl, 2 mM EDTA, and 20 mM $MgCl_2$. After preincubation for 3 min at 37°, cytosolic protein (about 1 mg) was added to initiate the reaction. The total volume was 1.0 mL, and the incubation time was 20 min.

γ-Glutamyl Transpeptidase and Dipeptidase

The assay for microsomal γ-glutamyl transpeptidase was based on the release of Cys-Gly from GSH. The standard assay solution (1.0 mL) contained 0.1 M Tris-HCl buffer (pH 8.0), 75 mM NaCl, 20 mM Gly-Gly, 5 mM GSH, 50 μM DTT, and microsomal enzyme. The solution was incubated at 37° for 20 min. To correct for hydrolytic release of Cys-Gly from GSH by γ-glutamyl transpeptidase, a blank tube lacking Gly-Gly was prepared at the same time. After equilibration at 37°, the reaction was initiated by adding the enzyme.

The assay for microsomal dipeptidase was based on the release of Cys from Cys-Gly. The incubation system contained 2 mM Cys-Gly, 75 mM NaCl, 0.1 M Tris-HCl (pH 8.0), and water (total volume 1 mL). The solution was brought to 37° and incubated for 20 min. To correct for the nonenzymatic hydrolytic release of Cys from Cys-gly, a blank tube lacking microsomal protein was run.

GSH Reductase and GSH Peroxidase

Cytosolic GSH reductase activity was assayed by the method of Massey and Williams [23]. The disappearance of NADPH absorption in the incubation was monitored at 340 nm. The activity is expressed as nanomoles per milligram protein per minute, based on an extinction coefficient for NADPH of 6.22×10^6 mol/cm^{-2} [24].

Cytosolic GSH peroxidase activity was measured spectrophotometrically by a modification of the procedure of Paglia and Valentine [25], as described by Lawrence *et al.* [26].

TABLE 4. Effect of taurine on the subacute LD_{50} of monocrotaline

	LD_{50} (mg/kg)	95% Confidence limits (mg/kg)
MONO	80	62–104
MONO + taurine	121*	93–156

Deaths were recorded daily for 14 days in rats given a single i.p. injection of monocrotaline in the range of 55–220 mg/kg.
* Significantly different from MONO alone group ($P < 0.05$).

GSH S-Transferase

Cytosolic and microsomal GSH S-transferase activities were assayed with CDNB according to Habig *et al.* [27]. Briefly, the incubation system contained 1 mM GSH, 0.13 M phosphate buffer (pH 7.0), and 50 μg of cytosolic or microsomal protein. Total incubation medium was 1 mL. The mixture was preincubated at 37° for 3 min. The reaction was started by adding 1 mM CDNB (final concentration), and incubation was continued for another 5 min. Under these conditions, the product formed was linear with respect to time and protein content. The reaction was stopped by adding 5% SSA, followed by centrifugation to precipitate protein. Appropriate controls were run for determination of spontaneous (nonenzymic) conjugation.

Amino Acid Analysis

Aliquots of hepatic cytosolic fraction were removed and deproteinized with 5% SSA. Deproteinized samples were diluted and mixed with OPA solution and analyzed by HPLC as previously described [28].

Protein Assay

Protein assay was performed according to the method of Lowry (Sigma kit for protein determination) using bovine serum albumin as calibration standard.

Statistics

Statistical comparisons were obtained by MANOVA followed by a Student's *t*-test (Bonferroni comparison) (Graphpad Software, version 1.14).

TABLE 5. Effects of taurine and guanidinoethane sulfonate administration on serum amino acid concentration in rats treated with the pyrrolizidine alkaloid monocrotaline

Amino acid	Control	MONO	MONO + taurine	MONO + guanidinoethane sulfonate
Asp	64.8 ± 8.1	88.4 ± 4.3*	79.6 ± 6.7	84.9 ± 14.7
Glu	220.8 ± 17.3	185.5 ± 14.0	250.7 ± 32.4†	201.3 ± 15.4
Asn	73.6 ± 2.3	67.1 ± 1.7	72.3 ± 5.6	59.5 ± 5.1*‡
Ser	303.6 ± 9.8	298.1 ± 6.3	293.2 ± 29.0	230.5 ± 16.8*†‡
Gln	494.9 ± 35.6	429.1 ± 12.8	440.6 ± 46.5	410.8 ± 54.2
Gly	646.6 ± 36.0	598.7 ± 28.6	568.6 ± 27.5*	524.5 ± 32.3*†
Arg	305.3 ± 31.9	306.6 ± 25.8	268.6 ± 37.6	228.8 ± 25.1*†
Tau	188.5 ± 27.0	241.4 ± 12.1*	859.6 ± 41.4*†	80.9 ± 11.6*†‡
Ala	439.8 ± 32.1	380.3 ± 13.8	444.3 ± 23.4†	476.1 ± 39.4†
Met	56.4 ± 5.3	58.5 ± 2.4	70.4 ± 4.0*†	52.5 ± 5.9‡
Val	176.1 ± 14.1	153.9 ± 10.4	161.0 ± 18.7	136.9 ± 22.0*
Phe	71.6 ± 3.6	71.7 ± 3.5	79.6 ± 8.4	72.2 ± 10.1
Ile	119.8 ± 8.4	116.7 ± 6.4	125.8 ± 15.1	124.7 ± 10.5
Leu	122.0 ± 7.6	111.5 ± 6.4	115.0 ± 13.9	107.7 ± 13.7
Lys	118.1 ± 6.9	146.8 ± 8.3*	149.2 ± 15.9*	128.6 ± 12.6

Data are means ± SD for 5 animals per group (MONO group, 3 animals). Values are expressed in μmol/g protein.

* $P < 0.05$ vs control.

† $P < 0.05$ vs MONO.

‡ $P < 0.05$ vs MONO plus taurine.

RESULTS

Rats maintained on taurine or GES as a 1% solution in drinking water for 14 days before injection with MONO showed no differences in body weight gain from untreated animals. All groups injected with MONO, however, showed significant depression in body weight gain thereafter (Fig. 2). Neither taurine nor GES modified the reduced weight gain in MONO-treated animals. On day 11 following injection, 2 animals in the MONO-alone group (Group 2) died. No rats died in any of the other groups.

Despite lower body weight in the MONO group compared

TABLE 6. Effects of taurine and guanidinoethane sulfonate administration on hepatic amino acid concentration in rats treated with the pyrrolizidine alkaloid monocrotaline

Amino acid	Control	MONO	MONO + taurine	MONO + guanidinoethane sulfonate
Asp	118.0 ± 8.7	133.6 ± 17.0	120.5 ± 9.8	137.5 ± 12.5
Glu	108.6 ± 10.7	104.1 ± 12.1	114.2 ± 6.3	111.9 ± 14.3
Asn	55.0 ± 3.1	54.2 ± 4.1	57.6 ± 5.3	57.8 ± 4.7
Ser	30.7 ± 2.4	36.1 ± 0.4*	31.2 ± 1.6†	31.0 ± 4.1†
Gln	167.1 ± 11.4	148.2 ± 1.7*	180.8 ± 13.1†	166.5 ± 14.5
Gly	107.6 ± 8.9	152.0 ± 1.7*	143.4 ± 4.8*	135.6 ± 17.5
Arg	24.0 ± 2.8	26.6 ± 4.8	28.2 ± 5.9	29.5 ± 6.8
Tau	26.7 ± 3.5	28.7 ± 1.1	230.7 ± 31.6*†	16.3 ± 1.5*†‡
Ala	123.6 ± 6.1	120.7 ± 14.3	123.9 ± 5.3	120.6 ± 1.7
Met	11.8 ± 0.5	12.7 ± 1.0	12.0 ± 1.3	11.4 ± 0.5
Val	16.0 ± 0.5	17.0 ± 1.1	17.0 ± 1.9	15.2 ± 1.5
Phe	14.7 ± 0.8	15.3 ± 0.3	15.0 ± 1.1	14.1 ± 2.4
Ile	20.5 ± 1.4	21.2 ± 1.7	19.7 ± 1.6	19.9 ± 1.3
Leu	14.7 ± 2.3	14.3 ± 1.8	12.1 ± 1.4	11.9 ± 2.2
Lys	19.4 ± 1.3	21.0 ± 1.1	21.6 ± 3.0	21.5 ± 1.1

Data are means ± SD for 5 animals per group (MONO group, 3 animals). Values are expressed in μmol/g protein.

* $P < 0.05$ vs control.

† $P < 0.05$ vs MONO.

‡ $P < 0.05$ vs MONO plus taurine.

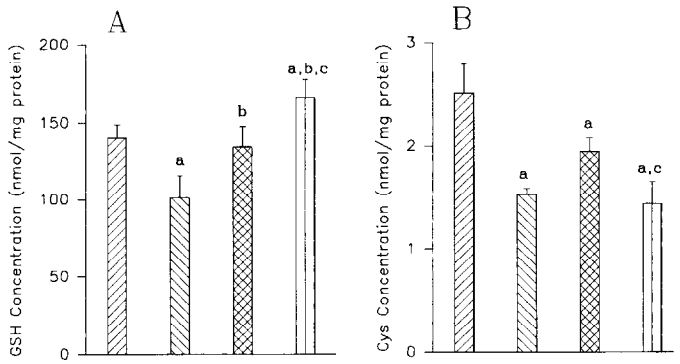

FIG. 3. (A) Hepatic GSH concentration, and (B) Cys concentration in control rats (▨) and rats treated with MONO alone (▩), MONO + taurine (▧), and MONO + GES (▥). Key: (a) $P < 0.05$ vs control; (b) $P < 0.05$ vs the MONO-alone group; and (c) $P < 0.05$ vs the MONO + taurine group.

with control, right ventricular and lung weights were increased (Table 1). Despite the similarity in final body weights of all MONO-exposed groups, rats also given either taurine or GES had significantly lower right ventricular and lung weights than the MONO-alone group (Table 1). The lung weights, however, were significantly higher than those of the control group. Wet to dry organ weight ratios, an index of edema, were increased only for the liver and only in the MONO and MONO plus taurine groups (Table 2). Two weeks after MONO injection, the MONO-alone animals showed a significant elevation in dry right ventricular to body weight ratio (indicative of right ventricular hypertrophy) and in lung weight to body weight ratio (indicative of pulmonary hyperplasia) (Table 3). MONO groups also given taurine or GES showed no right ventricular hypertrophy, but did show pulmonary hyperplasia (Table 3). Spleen to body weight ratios were increased in these two groups compared with the control group.

The right ventricular to left ventricular (including septum) weight ratio was elevated significantly in the MONO-alone group compared with the control group (Table 3). Animals given MONO together with either taurine or GES showed no elevation at the time they were killed in the right ventricular to left ventricular weight ratio.

In a separate study, the lethality of MONO was determined, based on cumulative deaths within 14 days of i.p. administration. The L_{50} was found to be 80 mg/kg. Taurine treatment significantly decreased the lethality of MONO, dropping the L_{50} to 121 mg/kg (Table 4).

As expected, the treatment with taurine or GES, respectively, increased or decreased taurine concentration in both serum and liver (Tables 5 and 6). Compared with the control group, the MONO group had significantly higher concentrations of Lys and Asp in serum. In the group also given taurine, Lys remained significantly elevated, but Asp was no different from the control. In addition, Met was elevated significantly, and Gly depressed. Compared with the MONO-alone group, serum levels of Glu, Ala, and Met were elevated.

In the MONO plus GES group, Asp and Lys were unchanged from the control. However, Asn, Ser, Gly, Arg, and Val all were depressed significantly in the serum relative to the control group. Ser, Gly, and Arg were also depressed relative to the MONO-alone group, while Ala levels were elevated. Compared with the group receiving taurine, this group showed depressed serum levels of Asn, Ser, and Met (Table 5).

In the liver, Ser and Gly were elevated in the MONO-alone group compared with the control group, while Gln levels were depressed. Gly remained elevated in the group also receiving taurine, but Ser and Gln levels were no different from control (but significantly different from the MONO-alone group). In the group receiving both MONO and GES, liver Ser levels were lower than in the MONO-alone group. No other levels in the GES group differed significantly from the other groups (Table 6).

Administration of MONO significantly decreased hepatic GSH and Cys concentration 2 weeks later (Fig. 3). Supplementation with taurine reversed the effect of MONO on hepatic GSH concentration. Supplementation with GES reversed the depletion of hepatic GSH but had no effect on the depletion of Cys concentration induced by MONO (Fig. 3).

Administration of MONO increased microsomal GSH

TABLE 7. Effects of taurine and guanidinoethane sulfonate on enzyme activities related to GSH metabolism in the liver of rats treated with the pyrrolizidine alkaloid monocrotaline

Enzyme	Control	MONO	MONO + taurine	MONO + guanidinoethane sulfonate
γ-Glutamylcysteinyl synthetase	8.55 ± 1.16	8.00 ± 0.66	10.54 ± 1.14*†	4.33 ± 0.38*†‡
Cytosolic GSH S-transferase	1050 ± 80	1070 ± 100	1100 ± 30	1120 ± 80
Microsomal GSH S-transferase	497 ± 49	842 ± 153*	597 ± 95	906 ± 98*‡
GSH reductase	6.12 ± 0.79	7.62 ± 0.38	5.67 ± 0.47†	6.51 ± 0.42
GSH peroxidase	9.57 ± 0.98	8.88 ± 0.78	10.06 ± 0.95	12.46 ± 0.85*†
γ-Glutamyl transpeptidase	2.59 ± 0.24	5.02 ± 1.28*	4.83 ± 1.02*	5.27 ± 1.11*
Dipeptidase	3.46 ± 0.35	3.86 ± 0.17	3.82 ± 0.46	4.08 ± 0.58

Data are means ± SD for 5 animals per group (MONO group, 3 animals). Values are expressed in nmol/min/mg protein.

* $P < 0.05$ vs control.

† $P < 0.05$ vs MONO.

‡ $P < 0.05$ vs MONO plus taurine.

S-transferase activity and γ-glutamyl transpeptidase activity (Table 7). Exposure of MONO-injected rats to taurine antagonized the change in the first enzyme, but not the second. In addition, taurine treatment led to an increase in γ-Glu-Cys synthetase activity compared with both the control and MONO-alone groups, and a decrease in GSH reductase activity, compared with the MONO-alone group.

In MONO-injected rats also given GES, microsomal GSH transferase and γ-glutamyl transpeptidase activities remained elevated. In addition, compared with both the control and MONO-alone groups, γ-Glu-Cys synthetase activity was depressed significantly and GSH peroxidase activity elevated significantly.

DISCUSSION

MONO is a hepatotoxic pyrrolizidine alkaloid producing, in humans and animals, hepatomegalocytosis and veno-occlusive disease of the liver. In rat, dog, mouse, nonhuman primates, and other species, MONO also produces pulmonary arterial hypertension and right ventricular hypertrophy (reviewed in Ref. 2). Following initiation of subacute administration (20 mg/L in drinking water), pulmonary hyperplasia occurs within 9 days, increased right ventricular to left ventricular weight is seen by 14 days, and pulmonary arterial blood pressure and right ventricular end diastolic pressure are elevated significantly by 22 days [29]. All these changes are presumed to be the consequence of hepatic conversion of MONO to the reactive alkylating pyrrole dehydromonocrotaline. This metabolic process was first suggested in 1968 by Mattocks [30]. Hepatic production of dehydromonocrotaline from MONO was demonstrated unequivocally by Glowaz and Huxtable [31].

The biochemical concomitants of MONO toxicity have not been explored as extensively as the pathophysiological effects. However, we have shown recently that MONO and related pyrrolizidine alkaloids produce marked alterations in sulfur amino acid metabolism in both liver and extrahepatic organs [4, 5, 32]. Hepatic GSH levels are increased within 24 hr of i.p. injection of 60% of an LD_{50} dose of MONO [4], trichodesmine [4], or retrorsine [5]. Accompanying this are acute increases in the activities of enzymes that synthesize and remove GSH and increases in Glu concentrations in the liver (a substrate for GSH synthesis). There is increased excretion of GSH, Cys, and γ-Glu-Cys into bile, and marked decreases in hepatic taurine concentrations (an alternative product of Cys metabolism) [5]. The longer term effects by pyrrolizidine alkaloids on sulfur metabolism have not been examined previously.

Taurine has been shown in other circumstances to be hepato-, pneumo- and cardio-protective. In the liver, it protects against radiation damage [33], it lowers acetaldehyde levels in ethanol-loaded rats [8], and it protects against CCl_4-induced damage [9]. A reduction in hepatic taurine levels increases CCl_4-induced damage. In the heart, taurine deficiency produces dilated cardiomyopathy [10, 11], electrophysiological abnormalities and loss of myofibrils [12]. Taurine antagonizes the inotropic actions of both high and low calcium concentrations on the heart, protects against the calcium paradox [34], and has cardiotonic actions in both experimental [14] and clinical congestive heart failure [35]. In the lungs, taurine protects against nitric oxide-induced damage [15], and the toxic effects of ozone [36], bleomycin [16], amiodarone [17], nitrogen dioxide [37], cyclophosphamide [18], and paraquat [19].

In view of these earlier findings, we examined whether taurine affords protection against the subacute changes produced by MONO in heart and liver. We found that 14 days after exposure to a dose of MONO known to cause right ventricular hypertrophy, rats maintained on taurine showed no such hypertrophy (Table 3). Although taurine-exposed rats have pulmonary hyperplasia, taurine may afford some protection, as dry lung weights were significantly below those of the MONO-alone group (Table 1). In the MONO-alone group, 2 of 5 animals died. None died in the other groups. The effects of taurine, therefore, are being compared with those of a group of survivors, in which the worst affected animals have been removed. Taurine also significantly raised the 14-day LD_{50} of MONO (Table 4). The LD_{50} value for the MONO-alone group at 14 days (80 mg/kg) compares with a value of 95 mg/kg reported for deaths within 7 days in male rats having an initial body weight of 90–200 g [38].

The effects of GES, the amidino analog of taurine, were also examined. An unanticipated finding was that GES exhibited protective properties similar to those of taurine. However, examination of the biochemical changes associated with taurine or GES administration indicates that these agents act by different mechanisms. Taurine, given to MONO-injected animals, normalizes many of the biochemical changes caused by the alkaloid (Tables 5–7). It antagonizes the changes in hepatic Ser and Gln, and the changes in serum Glu. It shows a sparing effect on serum Met, and corrects the depression in hepatic GSH by stimulating γ-Glu-Cys synthetase and antagonizing the MONO-induced changes in GSH transferase. GES, on the other hand, produces additional changes. Relative to the changes induced by taurine, it significantly increases hepatic GSH levels and depresses hepatic Cys levels. GES depresses γ-Glu-Cys synthetase activity and elevates GSH transferase and GSH peroxidase activities. Although GES has been used as a taurine-depleting agent [20, 39, 40], our findings indicate that this compound cannot be viewed simply as a taurine transport antagonist. It has a marked pharmacology of its own [41–44]. Studies with related guanidino compounds suggest that it may be a substrate for creatine phosphokinase [45, 46]. We have reported that the taurine-lowering action of GES cannot be accounted for solely by transport antagonism [47]. Recently, Ide and Murata [21] reported that GES increases hepatic GSH levels. Associated with this was a marked decrease in the activity of enzymes involved in the metabolism of Cys to taurine. This suggests, therefore, that one effect of GES is to divert Cys metabolism from taurine to GSH due to inhibition of the taurine pathway [21].

Neither taurine nor GES affected the decreased body weight gain induced by MONO. Decreased body weight gain is due to decreased eating [48]. Pair-fed animals gain the same weight, regardless of being given MONO (Yan CC and Huxtable RJ,

unpublished observations). Taurine itself, however, is known to depress eating behavior [49].

The "normalizing" effects of taurine are an example of the enantiostatic action that has been proposed as one of its major biological functions [6]. Our findings suggest that the marked decrease in hepatic taurine concentrations immediately following retrorsine administration may be one of the mechanisms of toxicity of this intensely hepatotoxic pyrrolizidine alkaloids.

The subacute actions of MONO on sulfur metabolism reported herein differ somewhat from the acute effects, observed within 24 hr of administration. Acutely, GSH concentrations are increased in liver [4], while subacutely they are decreased. Acutely, γ-Glu-Cys synthetase, dipeptidase, and cytosolic GSH *S*-transferase activities are all elevated [4], while subacutely there are no significant changes. γ-Glutamyl transpeptidase and microsomal GSH *S*-transferase activities, however, are elevated both acutely and subacutely.

Humans have a limited capacity for synthesizing taurine, deriving it instead from meat and, to a lesser extent, dairy products. Our findings that taurine protects against both the morbidity and mortality of MONO raise the possibility, therefore, that persons on a low dietary intake of taurine may be more susceptible to pyrrolizidine alkaloid toxicity. Such a possibility has obvious relevance to the outbreaks of pyrrolizidine alkaloid poisoning that occur in underdeveloped countries as a result of food contamination [1, 50, 51]. Populations in these countries typically have a low dietary intake of taurine-containing substances. Nutritional status may also be a factor in pyrrolizidine poisonings that occur in western nations as a result of herbal use [52–57].

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