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Effect of 2-Thiotriazone (TTZ) on Hepatic and Pulmonary Glutathione (GSH) Concentrations in Rats

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2-thiotriazone (TTZ) is a thiourea derivative that produces marked pulmonary toxicity in rats. Previous studies have shown TTZ toxicity to be both age and sex dependent (Tate et al., 1991). Immature rats (30-40 days of age) are innately resistant to the effects of TTZ. Female rats are also resistant with only 40% mortality being produced at dosages up to 100 mg TTZ/Kg bw. Resistance or protection against TTZ can also be produced by prior exposure to a single sublethal dose of TTZ (1 mg/kg), prior pretreatment with N-acetyl-L-cysteine and induction with beta-napthoflavone (BNF) (Tate et al., 1991).

The effects of TTZ, like other thiourea compounds is potentiated by diethylmaleate (glutathione depletor) pretreatments. Diethylmaleate (DEM) pretreatments also abolished resistance in immature rats, female rats, TTZ induced and BNF induced resistant male rats. Based on previous results, interest in the glutathione concentration in rats dosed with TTZ developed because, glutathione seemed to play a major role in the toxicity of TTZ.

Previous investigations with thiourea indicated that sulfhydryl compounds, including cysteine, and 2-aminoethylisothiouronium bromide protected rats against the lethal effects of thiourea derivatives (Van Den Brenk et al., 1976). Giri and Combs (1970), found that cysteine and reduced glutathione inhibited both pulmonary endematogenic effects and hyperglycemic effect of pheylthiourea in rats. Several experiments suggested that sulfhydryl compounds could modulate susceptibility to the toxicity of thiourea. Because of this, the effect of diethylmaleate (DEM) pretreatment on the toxicity and covalent binding of ^{35}S ANTU was studied by Boyd and Neal (1976). DEM depleted liver and lung GSH levels by 80%. Both pulmonary toxicity and *in vivo* covalent binding was strikingly increased after DEM pretreatment. These findings showed that GSH could possibly detoxify reactive species involved in the toxicities of thiourea by forming non-toxic conjugates (Hollinger et al., 1976).

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This study was developed to determine if the glutathione concentration was affected in TTZ exposed rats and to determine if glutathione plays a role in the metabolism and mechanism of action of TTZ.

MATERIALS AND METHODS

Glutathione content was analyzed in adult male and female rats (3 rats per group). All animals were dosed intraperitoneally and sacrificed by decapitation at 1 hr post-dosing. Control animals were administered distilled water (10 ml/kg) and treated animals were administered TTZ at doses of 1.25, 2.5, 5, and 10 mg/kg. Diethylmaleate (DEM) control animals were administered DEM (0.5 ml/kg), and animals pretreated with DEM and subsequently dosed with TTZ were administered DEM (0.5 ml/kg) 30 minutes prior to dosing with TTZ (10 mg/kg). N-Acetyl-L-cysteine (NAC) control animals were administered NAC (150 mg/kg) alone and animals pretreated with NAC (150 mg/kg) and subsequently dosed with TTZ (10 mg/kg) were administered NAC 30 minutes prior to the administration of TTZ.

Livers and lungs were removed from each of the animals and quick frozen in liquid nitrogen. Duplicate sample pieces weighing approximately 0.1 g were broken off from frozen liver and lung tissues, placed on ice in 2 ml of 2% sulfosalicylic acid and homogenized. The homogenate was centrifuged at 10,000 g for 15 minutes. Fifty microliter aliquots of the supernatant were added to cuvettes containing 1 ml of 0.1M phosphate buffer (pH 7.4; 10 ml 0.2M monobasic sodium phosphate, 81 ml 0.2M dibasic sodium phosphate, diluted to 200 ml with distilled water). A 1000 uM stock standard solution of glutathione was prepared with distilled water. Other standards were prepared by diluting the stock glutathione (100 uM and 500 uM). A 20 ul aliquot of 5,5'-dithiobis-(2-nitobenzoic acid) (DTNB) (0.4M in phosphate buffer pH 7.4), was added to each tube, mixed and incubated for 15 minutes at room temperature (22-25^{0}C). Glutathione concentrations were determined by measuring absorbances of samples and standards at a wavelength of 400 nm (according to the method of Mitchell et al. 1973 as modified by Droy 1987). All results are presented as mean ± SD. Statistical comparison between groups are by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

2-Thiotriazone produced a dose-dependent decrease in glutathione (GSH) concentration in the lung of adult male rats. The decrease in GSH (non-protein sulfhydryl) was observed at 1 hr following exposure of male rats to TTZ (Table 1). All treatment values were depressed from control values. TTZ also decreased non-protein sulhydryl (NPSH) concentration in the lungs of adult female rats but, in a dose-

Table 1. Effect of 2-thiotriazone (TTZ) on rat liver and lung glutathione (GSH) concentrations[a].

TTZ (mg/kg)	Sex	LIVER GSH(μm/g)	% Control	Lung GSH(μm/g)	% Control
Control	Male	5.81 ± 0.68	100	2.19 ± 0.36	100
1.25	"	5.17 ± 0.71*	88.9	2.04 ± 0.29	93.5
2.5	"	6.39 ± 0.81	117.7	1.75 ± 0.29*	80.1
5.0	"	6.91 ± 0.36*	118.9	1.37 ± 0.43*	62.7
10.0	"	5.83 ± 0.00	103.3	0.49 ± 0.14*	22.5
Control	Female	5.53 ± 0.34	100	1.79 ± 0.33	100
1.25	"	5.13 ± 1.51	92.7	0.44 ± 0.64*	24.2
2.5	"	6.39 ± 0.81	117.7	0.18 ± 0.28*	9.83
5.0	"	5.29 ± 0.41	95.7	0.55 ± 0.33*	30.7
10.0	"	3.55 ± 0.18*	64.2	0.96 ± 0.15*	53.8

[a] Data represents Mean ± SD for n=3 rats.
* Level of significance (ANOVA) $p < 0.05$.

independent manner. TTZ did not affect the glutathione concentrations in the liver of neither adult male or female rats, except that, 1.25 mg TTZ/kg caused a significant decrease in male liver GSH concentration when compared to controls and 10 mg TTZ/kg caused a significant decrease in female liver GSH concentration. These results suggest that glutathione probably plays a protective role in the metabolism of TTZ in both male and female rats and that glutathione concentration in the lungs is very important in the detoxification of TTZ. The exact reasoning for the abrupt decrease in glutathione concentration at 1.25 mg TTZ/kg in male liver and at 10 mg/kg in female liver may be due to increased absorption or availability of TTZ to exert an effect on the glutathione concentration in the liver.

The importance of GSH in TTZ induced pulmonary toxicity was tested by pretreatment of adult male and female rats with diethylmaleate (DEM). Both liver and lung GSH levels were depleted by DEM (Table 2). DEM depleted liver GSH in male rats to 10.5% of control and 25.9% of control in female rats. Lung GSH levels were depleted to 7.1% of control in males and 22.1% of control in females. DEM-TTZ (combined treatment) depleted liver GSH in male rats to 1.09% of control and 18.3% of control in females; lung GSH concentration in male rats was depleted to 27% of control and 43.7% of control in female rats. These results show that DEM depleted GSH stores in in both the liver and lung of both male and female rats. However, GSH was depleted more in the liver than in the lungs. The increase in GSH in the lungs of both male and female rats may be due to subsiding effects of DEM, as resynthesis of GSH in the lung is faster than resynthesis in the liver. Glutathione concentrations in the lung of female rats were much higher than those of males administered DEM-TTZ and DEM alone. The fact that higher concentrations of GSH were present in the lungs of female rats after depletion with DEM suggest that more GSH was available, which could deactivate reactive metabolites formed by metabolism of TTZ. Previous studies by Tate et al. 1991 showed that DEM pretreatment potentiates the toxicity of TTZ in adult and immature rats producing 90-100% mortality at sub-lethal doses.

The effect of N-acetyl-L-cysteine (NAC), on GSH levels in liver and lungs of rats treated with TTZ is shown in Table 3. Administration of NAC maintained liver GSH concentrations significantly above control values even in the presence of a toxic dose of TTZ (10 mg/kg) in male rats (152.2% of control for NAC treated rats and 149.5% of control for NAC-TTZ treated rats). Also, liver GSH concentrations were maintained at 89.8% of control values for NAC treated female rats (Table 3). Lung GSH concentrations were maintained in male rats at 96.9% of controls in rats treated with NAC alone and 87% of control for NAC-TTZ treated animals. However, female lung GSH concentrations were significantly decreased to 33-43% of control.

Table 2. Effect of diethylmaleate (DEM) on rat liver and lung glutathione (GSH) concentrations[a].

Treatment	Sex	Liver GSH (μm/g)	% Control	Lung GSH (μm/g)	% Control
Control[b]	Male	5.81 ± 0.68	100	2.19 ± 0.26	100
DEM-Control[c]	"	0.61 ± 0.72*	10.5	0.16 ± 0.03*	7.1
DEM-TTZ[d]	"	0.06 ± 0.11*	1.09	0.59 ± 0.91*	27.1
Control[b]	Female	5.53 ± 0.38	100	1.79 ± 0.33	100
DEM-Control[c]	"	1.44 ± 0.27*	25.9	0.39 ± 0.04*	22.1
DEM-TTZ[d]	"	1.02 ± 0.29*	18.3	0.78 ± 0.12*	43.7

[a] Data represent Mean ± SD for n=3 rats.
* Level of significance (ANOVA), $P < 0.05$.
[b] Control - animals were dosed with distilled water (10 ml/kg ip).
[c] Diethylmaleate control - rats were dosed with DEM (0.5 ml/kg ip).
[d] Diethylmaleate + 2-thiotriazone - rats were dosed with DEM (0.5 ml/kg ip) 30 min prior to being dosed with TTZ(10 mg/kg ip).

Table 3. Effect of N-acetyl-L-cysteine (NAC) on rat liver and lung glutathione (GSH) concentrations[a].

Treatment	Sex	Liver GSH (μm/g)	% Control	Lung GSH (μm/g)	% Control
Control[b]	Male	5.81 ± 0.68	100	2.19 ± 0.26	100
NAC-Control[c]	"	9.79 ± 0.40*	158.2	2.12 ± 0.42	96.9
NAC-TTZ[d]	"	8.68 ± 0.22*	149.5	1.91 ± 0.19	87.2
Control[b]	Female	5.53 ± 0.38	100	1.79 ± 0.33	100
NAC-Control[c]	"	4.96 ± 1.45	89.8	0.79 ± 0.34*	43.3
NAC-TTZ[d]	"	5.04 ± 0.48	91.2	0.59 ± 0.58*	33.1

[a] Data represents Mean ± SD for n=3 rats.
* Level of significance (ANOVA), $p < 0.05$.
[b] Control - rats were dosed with distilled water (10 ml/kg ip).
[c] N-acetyl-L-cysteine control - rats were dosed with NAC (150 mg/kg ip).
[d] N-acetyl-L-cysteine + 2-thiotriazone - rats were dosed with NAC (150 mg/kg ip) 30 min prior to being dosed with TTZ(10 mg/kg ip).

These results show that NAC maintains glutathione concentration to much higher levels when animals are exposed to TTZ, which would in turn protects the rats from TTZ toxicity. These results also suggest that GSH synthesis in females may be high enough to keep up with the depletion caused by TTZ (Previous studies by Tate et al. 1991 showed that female rats were more resistant to TTZ toxicity than male rats). Also instead of increasing the concentration of glutathione in the lungs of female rats NAC had a negative effect, possibly because of the overabundance of glutathione.

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REFERENCES

Boyd MR, Neal RA (1976) Studies on the mechanism of toxicity and development of tolerance to the pulmonary toxin, alpha-napthylthiourea (ANTU). Drug metab Dispos 4: 314-322.

Droy BF (1988) Mechanism of allyl formate hepatotoxicity in rainbow trout. Dissertation Research.

Giri SN, Combs AB (1970) Sulfur containing compounds and tolerance in the prevention of certain metabolic effects of phenylthiourea. Toxicol Appl Pharmacol 16: 709.

Hollinger MA, Giri SN, Budd E (1976) A pharmacodynamic study of (^{14}C) thiourea toxicity in mature, immature, tolerant and non-tolerant rats. Toxicol Appl Pharmacol 37:545-556.

Mitchell JR, Jollow DJ, Potter WZ, Gillete JR, Brodie BB, (1973) Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther 187: 211-217.

Tate T, Henk WG, Flory W (1991) Acute toxicity of 2-thiotriazone (TTZ) in rats. Arch Environ Contam Toxico 20: 380-384.

Van Den Brenk, HAS, Stone MG (1976) Innate and drug induced resistance to acute lung damage caused in rats by alphanapthylthiourea (ANTU) and related compounds. Br J Exp Pathol 57:621-636.

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