N-Acetyl-DL-penicillamine and Acetaminophen Toxicity in Mice

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Abstract □ *N*-Acetyl-DL-penicillamine (III*b*), a structural analog of *N*-acetyl-L-cysteine (III*a*), did not protect mice from lethal doses of acetaminophen (I), whereas III*a* offered protection. This lack of efficacy of III*b* probably is due to the decreased nucleophilicity of its sulfhydryl group compared to that of III*a*, the probable involvement of cysteine in any conjugate addition to the reactive intermediates of I, and the absence of metabolic conversion of III*b* to inorganic sulfate.

Keyphrases □ *N*-Acetyl-DL-penicillamine—evaluation for protection against acetaminophen toxicity in mice □ Acetaminophen—toxicity, evaluation of protection offered by *N*-acetyl-DL-penicillamine, mice □ Toxicity—acetaminophen, evaluation of protection offered by *N*-acetyl-DL-penicillamine, mice

Acetaminophen (I) is hepatotoxic in large doses (1, 2) and can be toxic even in therapeutic doses in patients with impaired liver function (3). Renal necrosis also has been associated with acetaminophen abuse (1, 2, 4).

THEORY

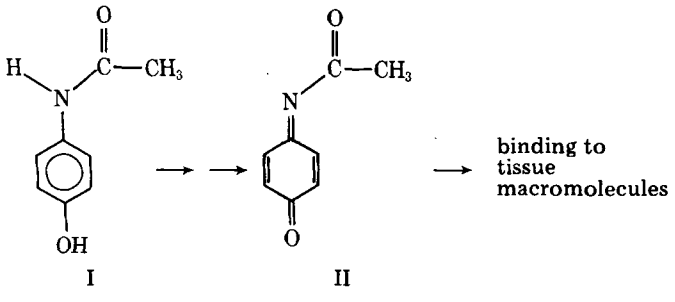
Animal studies have demonstrated that both the hepatotoxicity (5, 6) and renal toxicity (7) follow cellular glutathione depletion and subsequent covalent binding of a highly reactive metabolic product of I, presumed to be the *p*-quinoneimide (II), to tissue macromolecules (Scheme I).

Numerous sulfhydryl-containing agents, including cysteamine, L-methionine, D-penicillamine, and *N*-acetyl-L-cysteine (III*a*), have been tested and shown to ameliorate acetaminophen toxicity in varying degrees (8–15), ostensibly by covalent intervention with the reactive metabolite, II. Of these compounds, III*a* has become the currently accepted therapy (16, 17). The use of *N*-acetyl-DL-penicillamine (III*b*), a structural analog of III*a*, has not been reported. Compound III*b*, which is effective for the treatment of mercuric chloride poisoning (18, 19), is not catabolized extensively and theoretically could form a pseudomercapturic acid conjugate (IV*b*) by interaction with the quinoneimide (II) *via* its sulfhydryl group. Compound IV*b* then would be expected to be excreted as an analog of the mercapturic acid (IV*a*) (Scheme II).

Accordingly, the effectiveness of III*b* as a protective agent against the hepatotoxicity elicited by I in mice was evaluated and compared to the protection afforded by III*a*.

EXPERIMENTAL

Animals—Randomly bred male Swiss–Webster mice[1], 20–30 g, were



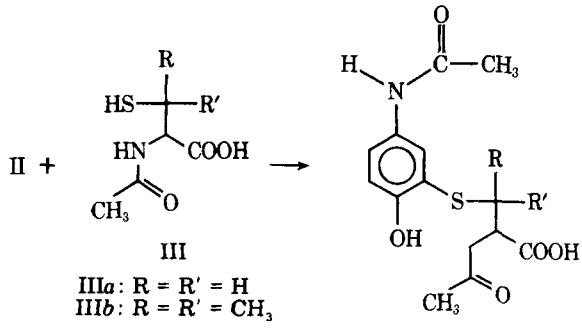
Scheme I

housed individually and fed a standard diet of rat chow and water *ad libitum*.

Chemicals—*N*-Acetyl-DL-penicillamine[2] (III*b*) was recrystallized from hot water. Injection solutions were prepared by dissolving 0.15 g in 15.0 ml of water (10 mg/ml). *N*-Acetyl-L-cysteine (III*a*) was a 20% sterile solution[3]. Solutions for injection were prepared by taking 1.0 ml of the 20% solution and diluting it to 20 ml with 0.9% sterile saline (10 mg/ml). Acetaminophen[4] (I) was recrystallized from hot water. Injection solutions were prepared by dissolving 1.25 g in 50.0 ml of sterile 0.9% saline. The solution was kept at 35–40° for injections.

Treatment Protocol and Histological Criteria—All drugs were administered intraperitoneally. Mice were treated with 750 mg of I/kg and 30 min later were given 400 mg of III*b* or III*a*/kg. Control mice received III*b*, III*a*, or 1.0 ml of sterile 0.9% saline. The mice then were observed for 48 hr with food and water allowed *ad libitum*. Mice that died during this period were examined grossly, and the liver and kidneys were excised, sliced, and fixed in 10% formalin for histological examination.

Survivors were sacrificed by cervical dislocation after the 48-hr period, and the liver and kidneys of all mice were processed as described. Paraffin sections of these tissues were stained with hematoxylin and eosin. Liver injury was graded according to the criteria described by Mitchell *et al.* (20) and quantitated as follows: 0, necrosis absent; 1+, necrosis of <6% of hepatocytes; 2+, necrosis of 6–25% of hepatocytes; 3+, necrosis of 25–50% of hepatocytes; and 4+, necrosis of >50% of hepatocytes.



Scheme II

RESULTS

The results of these experiments are summarized in Table I. Mice given 750 mg of I/kg without sulfhydryl treatment did poorly. At 48 hr, 40% had died and 40% had 3–4+ liver necrosis. Mice that were given I and treated with III*b* did not fare much better. At 48 hr, 40% of the animals had died and 50% had 3–4+ necrosis. However, mice given I and III*a* responded well to the latter treatment, as expected. No deaths were noted at 48 hr; no animal had 3–4+ necrosis, and only three of 10 animals had any evidence of liver injury characterized by mild 1+ necrosis. Although the doses of III*b* and III*a* were not exactly equimolar due to differences in their molecular weights, the results clearly indicated that III*b* was ineffective as a protective agent. Some mice in the control groups given III*a* or III*b* alone apparently sustained a low-grade liver injury, an observation noted previously with III*a* (21).

Liver necrosis was of the centrilobular hemorrhagic type in the more severe examples and of the centrilobular type in the less severe cases.

[1] Biolabs, St. Paul, Minn.

[2] Lot 2998, Nutritional Biochemical Corp., Chagrin Falls, Ohio.
[3] Mucomyst (Lot UGH38), Mead-Johnson Co., Evansville, Ind.
[4] Lot A730-2, Aldrich Chemical Co., Milwaukee, Wis.

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Table I—Effect of *N*-Acetyl-DL-penicillamine (III*b*) and *N*-Acetyl-L-cysteine (III*a*) Treatment on the Hepatotoxicity Induced by Acetaminophen (I) in Mice

Treatment	Number of Animals	Deaths (48 hr)	Percent of Animals with Necrosis				
			4+	3+	2+	1+	0
Saline	10	0	0	0	0	0	100
I	10	4	40	0	10	0	50
I + III*b*	10	4	50	0	10	10	30
III*b*	10	0	0	10	0	10	80
I + III*a*	10	0	0	0	0	30	70
III*a*	10	0	0	0	0	20	80

Centrilobular hemorrhagic necrosis was reported previously as a component of acetaminophen overdose in the mouse (21). The histological pattern of centrilobular hemorrhagic necrosis is more typically seen with rapidly progressive congestive heart failure (21). Centrilobular necrosis differs from centrilobular hemorrhagic necrosis only in the absence of extravasated red cells and also was observed after toxic exposure to carbon tetrachloride, chloroform, or naphthalene (22).

DISCUSSION

In a study of the relative nucleophilicities of the sulfhydryl groups in thiol amino acids, Friedman *et al.* (23) observed that the mercaptide ion of III*b* was approximately 50 times less reactive with α,β-unsaturated systems than was III*a*. Since the pK values of the two sulfhydryl groups are similar (9.90 and 9.52, respectively), it was concluded that this differential nucleophilicity was due to steric hindrance in III*b* by the presence of the *gem*-dimethyl groups adjacent to the sulfhydryl group. The failure of III*b* to protect mice against toxic doses of I may reflect this low relative nucleophilicity of its sterically hindered sulfhydryl group.

Chasseaud (24) showed that enzymatic deacetylation of III*a* occurs prior to reaction with electrophiles. This finding suggests that cysteine itself and not III*a* may be involved in any addition reactions with II *in vivo*, a premise supported by the observation that cysteine is approximately 2.5 times more nucleophilic than its acetylated derivative, III*a* (23). Moreover, III*a* is rapidly and nearly quantitatively deacetylated to cysteine in the rat (25). When [^{35}S]acetylcysteine was administered to rats, the major metabolites were cysteine and cystine at 2 hr. At 24 hr, inorganic sulfate was the major product in the urine, with minor amounts of mixed disulfide also present. Thus, III*a* may act as a prodrug form of cysteine and release the latter in the liver as a trapping agent or as a biosynthetic precursor of glutathione.

In contrast, other investigators (26–28) presented indirect evidence for the absence of deacetylation of orally administered III*b* in rats and humans. The present investigators also observed that rat liver homogenates did not deacetylate III*b* under conditions where III*a* was completely deacetylated. In any event, D-penicillamine is not a particularly effective protective agent for I (14). Since the administration of sodium sulfate itself significantly reduced the toxicity of I in mice (29), the efficacy of III*a* treatment in acetaminophen overdoses has been suggested to be due to increased sulfate generation. D-Penicillamine is not catabolized appreciably *in vivo*, and inorganic sulfate has not been demonstrated as a metabolite (30, 31). These observations and the present results with III*b* suggest that the direct formation of conjugates such as IV (as in Scheme II) may not be operative *in vivo*.

Based on metabolic (32) and pharmacokinetic considerations (33), a dual therapeutic regimen designed to (*a*) eliminate much of the excess acetaminophen represented by the overdose *via* conjugation mechanisms on the preformed phenolic hydroxyl group and (*b*) sequester the reactive metabolite(s) generated during the oxidative metabolism of I by administration of a suitable trapping agent appears to be superior to a single-treatment regimen. An obvious choice would be the combination therapy of III*a* with sodium sulfate.

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