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Communications to the Editor

Prodrugs of L-Cysteine as Liver-Protective Agents. 2(*RS*)-Methylthiazolidine-4(*R*)-carboxylic Acid, a Latent Cysteine

Sir:

Compounds containing free sulfhydryl groups have been shown to be protective in varying degrees against experimental drug-induced liver injury. For example, L-cysteine (**1**),[1] cysteamine,[2,1b] D-penicillamine,[3,1b] and *N*-acetyl-L-cysteine (**2**)[4] have been reported to offer protection against acetaminophen (**3**) toxicity in mice. In contrast, *N*-acetyl-DL-penicillamine, a β,β-dimethyl analogue of **2**, was completely ineffective in this regard.[5] Since **2** is rapidly deacetylated by the liver—at least in rodents[6]—whereas acetylpenicillamine is not,[5] we hypothesized[5] that **2** might be acting as a prodrug of **1** and not as a *direct* scavenger of the reactive intermediate generated in the hepatic cytochrome P-450 mediated metabolism of **3**. The latter course can theoretically lead to a preformed mercapturic acid, thereby short-circuiting the conjugation/detoxication pathway initiated by glutathione *S*-transferase.

L-Thiazolidine-4-carboxylic acid (**4**), a cyclic cysteine derivative with a masked sulfhydryl group, has been reported to protect against tetracycline[7] and allyl alcohol[8] toxicity in rats and against acetaminophen-induced mortality[9,1b] and bromobenzene hepatoxicity[3,1b] in mice. Compound **4**, as the free amino acid or as its arginine salt, has also been used for the treatment of alcoholic hepathopathies in humans.[10] However, toxicity manifested by seizures on accidental ingestion by children[11] and in overdose situations[12] has been reported for **4**. Compound **4** is metabolized by liver mitochondrial proline oxidase[13] to *N*-formyl-L-cysteine.[14] The latter is then presumably hydrolyzed to **1**, since the urinary metabolites of **4** are similar to those derived from **1**;[15] moreover, **4** can substitute nutritionally for **1** in rats.[16] Thus, **4** also requires metabolism before **1** can be liberated in vivo.

We postulate that 2-substituted thiazolidine-4-carboxylic acids—a class of compounds which, unlike the parent **4**, are chemically unstable and known to dissociate[17] or mutarotate[18] in protic solvents—should be capable of liver protection without prior metabolic activation (however, vide infra). We herewith present evidence that 2(*RS*)-methylthiazolidine-4(*R*)-carboxylic acid (MTCA, **5**), the simplest member of this series, (a) can liberate **1** nonenzymatically at physiological pH and temperature, (b) is much less toxic than **4** (or **1** itself), and (c) is more effective than **4** in protecting mice against acetaminophen-induced liver necrosis.

Reaction of equimolar quantities of **1** with acetaldehyde in aqueous solution at room temperature for 1.5 h, followed by solvent evaporation in vacuo, gave crude **5** in essentially quantitative yield. Recrystallization from methanol afforded MTCA (**5**): mp 161–162 °C dec (lit.[19] mp 161–163 °C dec); $[\alpha]^{26}_{D}$ −147° (*c* 1.00, H_2O). In D_2O or in deuterated potassium phosphate buffer, pD 7.41, temperature 34 °C, the hydrogens on the methyl group at the 2 position of MTCA were found to undergo deuterium exchange as measured by the gradual diminution in intensities over time of the three-proton methyl doublet centered at δ 1.67 in the NMR spectra (sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal standard), compared to the H-4 multiplet at δ 3.34 which integrated as a constant. A similar exchange in alkaline solution has been reported

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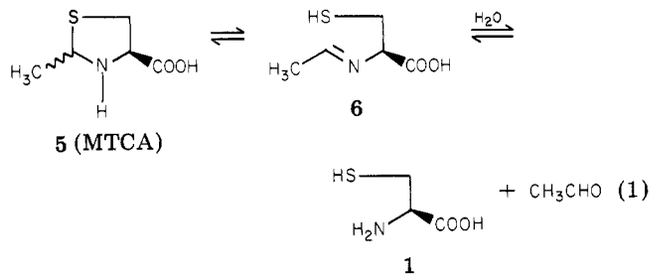
Table I. Protection from Acetaminophen-Induced Liver Necrosis in Mice by MTCA

compd(s) administered [dose, mg (mmol)/kg, ip]	no. of animals [a]	deaths (48 h)	no. of animals with liver necrosis [b]				
			4+	3+	2+	1+	0
acetaminophen (3) [750 (4.97)]	17	11	8	7	1		1
MTCA (5) [360 (2.45)]	17	0					17
3 + 5	18	1 [c]	1				17
L-thiazolidine-4-carboxylic acid (4) [100 (0.75)] [d]	10	3		1 [e]			9
3 + 4	10 [f]	4	2	4	1		1
N-acetyl-L-cysteine (2) [400 (2.45)]	17 [g]	0				2	15
3 + 2	18 [g]	1 [c]	1	1		3	13
saline	17 [g]	0					17

[a] Represents the cumulative results of two experiments conducted independently at different times, with an N of 10 and 7 (or 8), respectively. [b] Criteria of Mitchell et al.[1a] [c] Death due to hemorrhagic necrosis of the liver. It could not be ascertained whether this was due to faulty administration of the protective drug. [d] This dose produced convulsions in some mice; for this reason, the experiment was not repeated. Reported LD_{50} for mice: 164 mg/kg ip.[26] [e] Autolysis had set in and the results may be due to artifacts. [f] Histological data reported for eight mice only; liver from one mouse that died was completely autolyzed, and the liver sections from one mouse were lost. [g] Taken in part from ref 5.

previously for **5**.[20] The pseudo-first-order rate constant for this exchange at physiological pH was calculated to be $1.9 \pm 0.2 \times 10^{-5}$ s^{-1}. This deuterium exchange is only possible via a ring *seco* intermediate, such as the imine **6** (eq 1).



5 (MTCA) **6**

1

Although **1** was not detected by NMR under these conditions (added **1** was readily detectable), a dilute aqueous stock solution of MTCA was found to undergo a time-dependent dissociation at room temperature to **1** as determined by high-performance liquid chromatography. MTCA was not a substrate for a solubilized preparation of rat liver mitochondrial proline oxidase; indeed, it gave rise to artifactual color development in the assay due to its dissociation to **1** during the incubation.[13] The single-dose, 24-h LD_{50} of MTCA administered intraperitoneally to Swiss-Webster mice greatly exceeded 2.0 g/kg; solubility limitations prevented evaluation at higher doses.

2-[^{14}C]Methyl[2-^{14}C]thiazolidine-4-carboxylic acid (specific activity 0.67 mCi/mmol) was prepared from [1,2-^{14}C]acetaldehyde and **1**. A carrier-diluted sample (specific activity 15 μCi/mmol) was administered intraperitoneally to a male Swiss-Webster mouse (wt 27 g; dose 2.57 mmol/kg; 2.31×10^6 dpm) housed in an all-glass metabolism cage, and the expired CO_2 was quantitatively collected in ethanolamine/ethylene glycol monomethyl ether.[21] A total of 52.8% of the administered radioactive dose was expired as $^{14}CO_2$ within the first 4 h, as determined by liquid scintillation counting. $^{14}CO_2$ expiration continual for at least 23 h, albeit at diminished rates; for example, between 15 and 23 h only 5.5% of the radioactive dose was recovered in this form. These results indicate that [1,2-^{14}C]acetaldehyde was released from [^{14}C]MTCA in vivo and was rapidly metabolized to $^{14}CO_2$. By deduction, an equimolar quantity of **1** must have been liberated per mole of acetaldehyde released (eq 1).

The efficacy of MTCA in protecting mice against hepatic necrosis and death after LD_{90} doses of acetaminophen (**3**) was compared against the protection afforded by **4** in an experimental mouse model. A separate group given *N*-acetyl-L-cysteine (**2**) served as positive control. Male, Swiss-Webster mice weighing 18–33 g obtained from Biolab Corp. (St. Paul, MN) were randomized and administered **1**; 30 min later the mice were treated with the compounds listed in Table I. The animals were observed for 48 h, during which time food and water were available ad libitum, and the surviving animals were killed by cervical dislocation. The livers from all animals were excised, examined grossly, fixed in bufered 10% formalin, and sectioned. The sections were stained with hematoxylin and eosin, and contiguous sections were processed using Gomori's method for reticulum staining. It can be seen (Table I) that MTCA was comparable to **2** in its general lack of toxicity and in its protective action against histologically verifiable liver injury. By contrast, the parent unsubstituted thiazolidine-4-carboxylic acid (**4**) was toxic at one-third the molar dose of **5** or **2**, and this dose level offered only slight protection against **3**.

These data suggest that MTCA, a 2-alkylthiazolidine-4-carboxylic acid, is acting as a prodrug of **1**. The rapid catabolism of **1**[22] and its known toxicity [LD_{50} (mice) = 660 mg/kg ip][1b] are attenuated in this prodrug form. Since **1** stimulates hepatic glutathione biosynthesis,[23,1b] the protective effect of MTCA may be due to the released **1**, its precursor **6**, or to glutathione, which, in conjunction with glutathione *S*-transferase, can sequester the proximate electrophilic species generated in the metabolism of **3**.[2b,24] In addition, **1** can be catabolized to inorganic sulfate,[22] which in turn can be activated to 3′-phosphoadenosine 5′-phosphosulfate, the cofactor required for sulfate conjugation[25] of the phenolic **3**.

It is suggested that other *prodrug forms of* L-*cysteine* might also be therapeutically beneficial as protective agents

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against xenobiotic substances that elicit tissue injury by metabolism to highly reactive electrophilic or free-radical species. Studies on the protection by MTCA against other toxic xenobiotics and the evaluation in this regard of various other 2-substituted thiazolidine-4-carboxylic acids are in progress.

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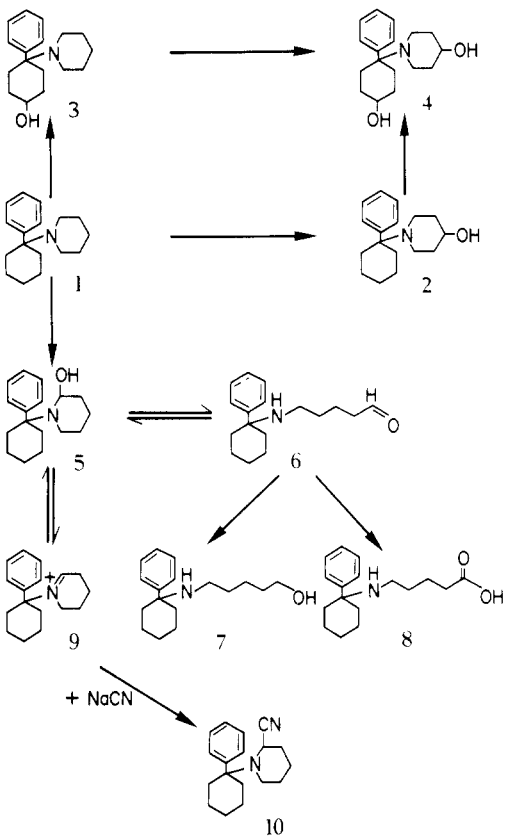
Metabolic Formation of Iminium Species: Metabolism of Phencyclidine

Sir:

Phencyclidine, 1-(1-phenylcyclohexyl)piperidine (1; Scheme I), is a commonly abused drug which displays both acute and long-term neurotoxic effects. Interest in the possibility that metabolites may contribute to the pharmacological and toxicological effects of phencyclidine has led to the identification of the ring-hydroxylated metabolites **2**–**4** in several species.[1] More recently, an amino alcohol (**7**)[2] and amino acid (**8**)[3] have been characterized as metabolites of phencyclidine. The formation of such ring-opened metabolites presumably proceeds via initial α-C-hydroxylation to yield the carbinolamine **5**, which ring opens to the corresponding amino aldehyde **6**, the intermediate leading to **7** and **8**. An unidentified metabolite of **1** has been shown to bind irreversibly to rat and rabbit liver and lung microsomal protein.[4]

Results from previous studies have established that metabolically generated carbinolamines, such as **5**, ionize to yield the corresponding iminium ions.[5,6] These electrophilic species are trapped with nucleophilic cyanide ion as the corresponding α-aminonitriles. With phencyclidine, this sequence would lead to iminium ion **9** and α-aminonitrile **10**. Given the potential reactivity of such an iminium species, it could contribute to the reported metabolically dependent covalent binding to proteins observed with radioactive phencyclidine.[4] Therefore, we were prompted to examine the metabolism of phencyclidine in an attempt to trap **9** as the corresponding α-aminonitrile **10**.

Scheme I



Rabbit liver microsomes were prepared as described previously.[7] Incubation mixtures consisted of 5–10 mg of microsomal protein in 5 mL of 0.1 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes) buffer, pH 7.6, which contained phencyclidine (1.0 mM), $Na^{14}CN$ (1.0 mM, specific activity 0.5 mCi/mmol), and an NADPH-generating system containing 0.5 mM $NADP^+$, 8 mM glucose 6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase, and 4 mM $MgCl_2$. Incubations were carried out at 37 °C for 30 min, and cold 0.1 M $NaHCO_3$, pH 8.2, was added to quench the metabolic activity of the system. This mixture was passed through a Sep-Pak C-18 reverse-phase cartridge (Waters Associates, Inc.). The Sep-Pak was washed extensively with water, and the organic material was subsequently eluted with methanol. Acid–base partitioning led to the isolation of the organic base fraction, which was subjected to thin-layer chromatography on silica gel 60 (EtOAc–CH_3CN–concentrated NH_4OH, 175:20:1). The major radioactive zone detected was extracted and analyzed by chemical-ionization mass spectrometry (130 °C, isobutane/1.0 torr). A prominent ion of mass 242 was observed, consistent with the pseudomolecular ion (MH^+) of aminonitrile **10** minus 1 mol of HCN. Further analysis of the organic base fraction by gas chromatography–electron-impact mass spectrometry (temperature programmed 130–250 °C at 6 °C min^{-1} on a 21 m, 0.2% OV-1, 0.3 mm i.d., fused silica column, 70

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