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Failure of glutathione and cysteine prodrugs to block the chlorpropamide-induced inhibition of aldehyde dehydrogenase *in vivo*

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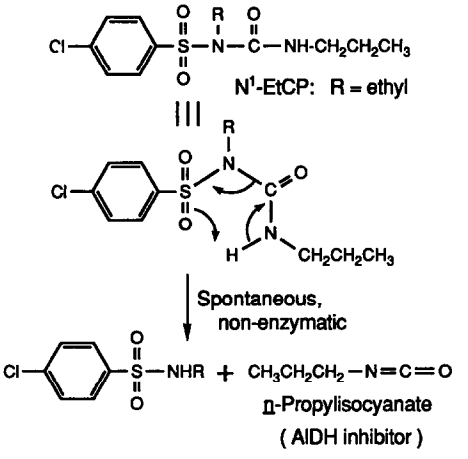
Abstract—Augmentation of cellular L-cysteine or glutathione (GSH) levels *in vivo* by the administration of prodrugs of L-cysteine or GSH, viz. 2(*R*,*S*)-methylthiazolidine-4(*R*)-carboxylic acid (MTCA), 2(*R*,*S*)-D-ribo-(1′,2′,3′,4′-tetrahydroxybutyl)thiazolidine-4(*R*)-carboxylic acid (RibCys) and GSH monoethyl ester (GSH-OEt), did not block the inhibition of aldehyde dehydrogenase (AlDH) by chlorpropamide (CP) or N^1-ethylchlorpropamide (N^1-EtCP), as shown by their inability to protect AlDH and thereby prevent the elevation of blood acetaldehyde (AcH) in ethanol-treated rats. Since the formation of an alkylcarbamoylating species by conjugation of *n*-propylisocyanate, a potential metabolite of CP or N^1-EtCP, with GSH or L-cysteine is possible, intervention by GSH or cysteine may not produce a detoxified product. Evaluation of the two products that could theoretically be produced *in vivo*, viz. *S*-(*n*-propylcarbamoyl)-L-cysteine and *S*-(*n*-propylcarbamoyl)-GSH, indicated that these compounds inhibit rather than spare AlDH in rats. Indeed, the latter were as effective as N^1-EtCP, a direct acting inhibitor of AlDH, and all three were better inhibitors of AlDH *in vivo* than CP itself. Thus, formation of *S*-conjugates of the active CP metabolite produced *in vivo* may not be a detoxication process, but may in fact represent redistribution of a transportable form of this highly reactive metabolite.

An as yet unidentified metabolite [1, 2] of the oral hypoglycemic agent chlorpropamide (CP*) appears to be responsible for the CP-induced hypersensitivity to ethanol experienced by susceptible individuals [3]. This alcohol sensitivity is a direct consequence of the inhibition of hepatic aldehyde dehydrogenase (AlDH, EC 1.2.1.3) by a metabolite of CP, and the individuals afflicted present a characteristic CP–alcohol flush and elevated blood acetaldehyde (AcH) levels [4, 5]. Formation of this inhibitor from CP does not involve the enzyme catalase (EC 1.11.1.6) [2] or the oxidative metabolism of the side chain of CP by hepatic microsomal P450 enzymes, suggesting that a unique type of metabolic functionalization reaction must be involved. Recently, we showed that substitution on the N^1-nitrogen of the CP molecule with an alkyl group, e.g. as in N^1-ethylchlorpropamide (N^1-EtCP), led to a direct acting CP derivative which inhibited yeast as well as rat liver mitochondrial AlDH *in vitro* and which was three times as active as CP in inhibiting AlDH *in vivo* [6, 7]. Since other chemical substitutions at the N^1-position of the CP molecule also produced CP derivatives that appeared to obviate the need for bioactivation, a similar metabolic substitution at this position was postulated to occur *in vivo* [7] (Scheme 1). This mechanism predicts the release of *n*-propylisocyanate, a known inhibitor of yeast AlDH [7].

The ability of isocyanates to react with endogenous sulfhydryl compounds such as glutathione (GSH) is well known. If *n*-propylisocyanate is indeed the inhibitory species formed from CP as it is from N^1-EtCP, then the presence of GSH or other sulfhydryl compounds which can serve to sequester the isocyanate should prevent the CP- and N^1-EtCP-induced inhibition of AlDH *in vivo*. The present study was designed to examine the possibility that the reactive CP-metabolite or the *n*-propylisocyanate derived from N^1-EtCP could be trapped by enhancing the levels of intracellular cysteine and GSH, thereby sparing hepatic AlDH. We therefore pretreated rats with prodrugs



Scheme 1. Proposed formation of *n*-propylisocyanate from N^1-EtCP or from a possible N^1-substituted metabolite of CP.

that are known to release L-cysteine non-enzymatically by an equilibrium-based mechanism and stimulate GSH biosynthesis or to release GSH directly by esterase action on its mono ester, in order to evaluate their effect on inhibition of AlDH by CP and N^1-EtCP.

Materials and Methods

2(*R*,*S*)-Methylthiazolidine-4(*R*)-carboxylic acid (MTCA) [8], 2(*R*,*S*)-D-ribo-(1′,2′,3′,4′-tetrahydroxybutyl)thiazolidine-4(*R*)-carboxylic acid (RibCys) [9], CP [2], N^1-EtCP [2], and GSH monoethyl ester (GSH-OEt) [10, 11] were synthesized by known procedures. *S*-(*n*-Propylcarbamoyl)-L-cysteine (PC-CYS) [12] and *S*-(*n*-propylcarbamoyl)-glutathione (PC-GSH) were prepared using a general synthetic procedure for the preparation of alkylthiocarbamates [13]. Elemental analyses for C,H,N,S agreed with calculated values within ± 0.4% for all of the above compounds synthesized.

Animal studies were performed in adherence with the guidelines established in the *Guide for the Care and Use of Laboratory Animals* published by the Department of Health and Human Resources. Animal care facilities are

* Abbreviations: CP, chlorpropamide; AlDH, aldehyde dehydrogenase; N^1-EtCP, N^1-ethylchlorpropamide; GSH, glutathione; MTCA, 2(*R*,*S*)-methylthiazolidine-4(*R*)-carboxylic acid; RibCys, 2(*R*,*S*)-D-ribo-(1′,2′,3′,4′-tetrahydroxybutyl)thiazolidine-4(*R*)-carboxylic acid; GSH-OEt, GSH monoethyl ester; PC-CYS, *S*-(*n*-propylcarbamoyl)-L-cysteine; PC-GSH, *S*-(*n*-propylcarbamoyl)-glutathione; CMC, carboxymethylcellulose; and AcH, acetaldehyde.

accredited by the American Association of the Accreditation of Laboratory Animal Care. Male rats of Sprague–Dawley descent (BioLab, Inc., St. Paul, MN), weighing 132–166 g and maintained on a pellet diet, were fasted 24 hr before drug administration. All drugs were administered as a 100 mM suspension in 2% (w/v) aqueous carboxymethylcellulose (CMC). At zero time, the sulfhydryl prodrug (1.0 mmol/kg, i.p.) or saline was administered. At 1.0 hr, the animals were given N^1-EtCP (0.5 mmol/kg, i.p.), CP (1.0 mmol/kg, i.p.) or 2% (w/v) CMC, and at 3.0 hr, ethanol (2.0 g/kg, i.p.) was administered. One hour later, duplicate samples of blood (0.2 mL) were removed from each animal for assay of acetaldehyde by headspace gas chromatography as previously described [14]. Control groups received saline followed by 2% (w/v) CMC and ethanol. In a second experiment, PC-CYS or PC-GSH (1.0 mmol/kg, i.p.) was administered at zero time. At 2.0 hr, ethanol (2.0 g/kg, i.p.) was given. One hour later, duplicate samples of blood (0.2 mL) were removed from each animal for determination of blood AcH [14]. Control groups received 2% (w/v) CMC in place of drug. Results are expressed as means ± SEM. Statistical significance was determined using a one-way analysis of variance. When significance was indicated, the Neuman–Keuls criteria was used to compare the means of multiple groups. Statistical significance was set at $P < 0.05$.

Results and Discussion

To ascertain whether enhancement of cellular cysteine or GSH levels would sequester the active inhibitor derived from CP *in vivo*, we selected two cysteine prodrugs (MTCA) [8] and RibCys [9], based on their ability to elevate hepatic GSH levels and to protect mice from the reactive metabolite generated from acetaminophen, thus preventing hepatotoxicity. GSH-OEt [10, 11, 15] was selected for its ability to penetrate cells and release GSH intracellularly by esterase action. To ensure adequate GSH levels, RibCys, MTCA, and GSH-OEt were administered 1 hr prior to CP or its N^1-ethyl analog and the effect on blood AcH was measured 1 hr later. Hepatic GSH levels are known to be maximally elevated 2 hr after administration of MTCA [16] or GSH-OEt [15] to fasted mice, even when challenged with GSH-depleting hepatotoxins [15, 17], while RibCys administration prevents the depletion of liver GSH [18].

The results (Fig. 1) indicated that, contrary to expectation, pretreatment with these sulfhydryl prodrugs did not produce a significant change in the sensitivity of animals treated with CP to ethanol, as indicated by their inability to prevent the elevation of ethanol-derived blood AcH. Similar results were obtained when animals, that were pretreated with the sulfhydryl prodrugs, were challenged with N^1-EtCP, a direct acting inhibitor of AlDH that releases *n*-propylisocyanate non-enzymatically [19]. All four treated groups had significantly higher blood AcH levels compared to control groups receiving vehicle (CMC) alone or prodrug alone. In previous studies with N^1-EtCP [7], cyanamide [14], and pargyline [20], the elevation of blood AcH following ethanol administration to rats that were treated previously with these substances was shown to directly reflect the inhibition of the hepatic low K_m mitochondrial (class II) AlDH. No significant differences were found in blood ethanol levels between treated groups and groups receiving vehicle alone.

In view of the lack of effect of these sulfhydryl prodrugs on the inactivation of AlDH by administered CP or N^1-EtCP and the known propensity of compounds such as *S*-(methylcarbamoyl)-GSH, a metabolite of methylisocyanate, to participate in transcarbamoylation reactions with other nucleophiles, we were led to conclude that the formation of *S*-conjugates of *n*-propylisocyanate from CP or N^1-EtCP *in vivo* may not provide a detoxication mechanism; indeed, these conjugates may represent depot

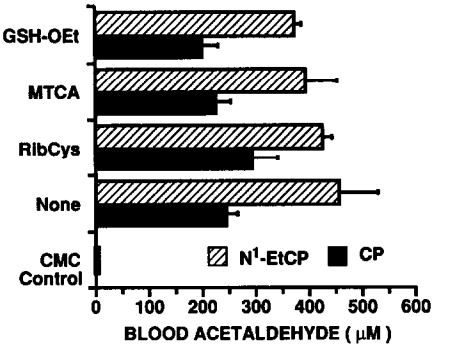


Fig. 1. Effect of a GSH prodrug (GSH-OEt) and the cysteine prodrugs (MTCA and RibCys) on the CP- or N^1-EtCP-induced elevation of ethanol-derived blood acetaldehyde in rats. Data represent the means ± SEM of three animals except for the CP and N^1-EtCP groups which received no prodrug (N = 5). No significant differences were found when prodrug-treated groups were compared with CP- or N^1-EtCP-treated groups receiving no prodrug: $P > 0.05$.

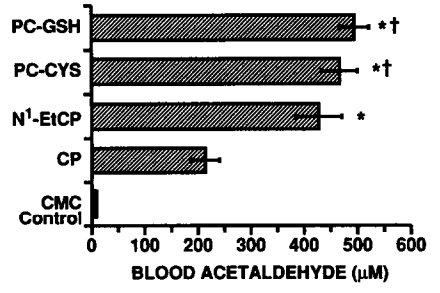


Fig. 2. Blood acetaldehyde levels after administration of the cysteine or GSH conjugates of *n*-propylisocyanate, *S*-(*n*-propylcarbamoyl)-L-cysteine (PC-CYS) and *S*-(*n*-propylcarbamoyl)-glutathione (PC-GSH), in ethanol-treated rats. Data represent the means ± SEM of five animals except for the PC-CYS and PC-GSH groups (N = 6). Key: Newman–Keuls test: (*) significantly different when compared to CP-treated group: $P < 0.05$; and (†) not significantly different when compared to N^1-EtCP-treated group: $P > 0.05$.

or transport forms of the isocyanate itself [21]. Accordingly, we synthesized and examined the effect of the postulated *S*-conjugates, viz. PC-CYS and PC-GSH, on AlDH *in vivo*. As shown in Fig. 2, blood AcH levels attained after administration of these *S*-conjugates of *n*-propylisocyanate following ethanol administration were comparable to values obtained with the direct acting CP analog N^1-EtCP. In contrast, substantially lower blood AcH values were obtained with CP itself. These results suggest that even though the reactive metabolite formed from N^1-EtCP (or possibly from CP itself), i.e. *n*-propylisocyanate, may have been sequestered by cysteine or GSH *in vivo*—although direct evidence for this is still lacking (we are presently attempting to identify the mercapturic acid conjugate of this substance in rat urine)—this reaction may not represent a detoxication process. Such *S*-conjugates may in fact act as depot or transportable forms of this highly reactive metabolite [21], carrying it to sites where it can react with other cellular nucleophiles, e.g. with the sulfhydryl group located at the active site of AlDH.

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