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# COMMENTARY

# RENAL PROCESSING OF GLUTATHIONE CONJUGATES

## ROLE IN NEPHROTOXICITY

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The toxicity of many chemicals is associated with biotransformation to highly reactive metabolites. The reactive metabolites may become covalently bound to cellular macromolecules or may stimulate cellular lipid peroxidation, which may lead to cell injury or death or to neoplastic transformation [1, 2].

Because of its extensive xenobiotic metabolic capacity, the liver is frequently the target organ for many toxic chemicals that require bioactivation, such as carbon tetrachloride, bromobenzene, and acetaminophen [3]. Extrahepatic tissues are, however, selectively injured by some toxic chemicals, such as benzene, which causes bone marrow toxicity [4], and 4-ipomeanol, which causes pulmonary injury [5]. Studies with enzyme preparations from many extrahepatic tissues have identified drug-metabolizing systems that are capable of activating toxic chemicals to reactive intermediates [6, 7]. Such activation may also occur *in vivo* and may result in extrahepatic toxicity. Conclusive evidence for toxicity associated with metabolism in extrahepatic tissues is often difficult to obtain; this may be due to the cellular heterogeneity of extrahepatic tissues and to the major role the liver plays in the overall *in vivo* metabolism of most chemicals.

The kidneys are susceptible to the toxicity of a large number of chemicals, but the mechanisms of renal damage are poorly understood [8]. Because of their structure and physiological functions, the kidney may be exposed to higher concentrations of chemicals than other organs. Nephrotoxic chemicals, such as chloroform, 2-furanamide, and cephaloridine, are believed to be activated within the kidneys by cytochrome P-450-dependent monooxygenases (Cyt. P-450) [9–11]. These studies are complicated, because inducers and inhibitors of renal Cyt. P-450 also modify renal transport systems [12, 13] as well as affect enzyme concentrations in other organs [14]. On the other hand, hexachloro-1,3-butadiene (HCBD) is a potent nephrotoxin that is not activated by Cyt. P-450 [15]. Female rats are more susceptible to the nephrotoxic effects of HCBD than are male rats [16, 17]. Treatment of female rats with HCBD lowers hepatic and renal glutathione concentrations [16]; in contrast, treatment of male rats with HCBD depletes only hepatic glutathione concentrations [18]. These findings suggest that activation mechanisms that do not depend on Cyt. P-450 may play a role in producing renal damage.

*Glutathione conjugation reactions and toxicity.* A variety of chemicals, including many halogenated hydrocarbons, form glutathione conjugates [19]. Glutathione conjugates are frequently less toxic than their parent compounds and are eliminated in the bile or, after biotransformation to mercapturic acids, in the urine. Glutathione conjugation has been implicated in the activation of 1,2-dihaloethanes to potentially carcinogenic electrophiles [20–22]. Glutathione *S*-transferase-catalyzed displacement ($S_N2$) reactions between 1,2-dihaloethanes and glutathione may yield sulfur half-mustards, which may form electrophilic episulfonium ions by the internal displacement of the second halogen atom by the sulfur atom. The episulfonium ion can then react with nucleophilic groups of biological macromolecules to produce toxicity. *In vitro* incubation of the carcinogen 1,2-dibromoethane with calf thymus DNA in the presence of glutathione and glutathione *S*-transferases results in the formation of the adduct *S*-[2-($N^7$-guanyl)ethyl]glutathione [23]. Thus, glutathione-dependent conjugation reactions are associated with toxicity.

Gluthathione conjugate formation is catalyzed by cytosolic, microsomal, and mitochondrial glutathione *S*-transferases present in several tissues [24–26]. Of particular interest are the reports that microsomal proteins are more effective than cytosolic proteins in catalyzing the addition reaction of glutathione and chlorotrifluoroethylene (CTFE) [27] as well as the substitution reaction of glutathione and HCBD [15]. Glutathione *S*-transferase activity in the kidney is much lower than in the liver [26], which suggests that conjugates formed in the liver may be responsible for extrahepatic effects. In contrast, the activities of the enzymes that catalyze mercapturic acid formation [γ-glutamyl transpeptidase (γ-GT), cysteinylglycine dipeptidase, and cysteine conjugate *N*-acetyl transferase] are higher in the kidney than in the liver [28, 29].

*Glutathione conjugation and nephrotoxicity.* Recently, evidence has been presented showing that

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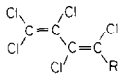
Table 1. Glutathione and cysteine *S*-conjugates of halogenated hydrocarbons that are nephrotoxic

| | COMPOUND | A R=Glutathione | B R=Cysteine | Ref. |
|---|---|---|---|---|
| I. |  | + | + | [33, 34] |
| II. |  | + | + | [35, 36] |
| III. |  | N.D. | + | [37] |
| IV. |  | + | + | [*] |
| V. |  | + | + | [38] |
| VI. |  | + | N.D. | [†] |

N.D. = Not Determined.

* D. R. Dohn and M. W. Anders; unpublished observations.

† T. Monks, S. Lau, R. Highet and J. R. Gillette; personal communications.

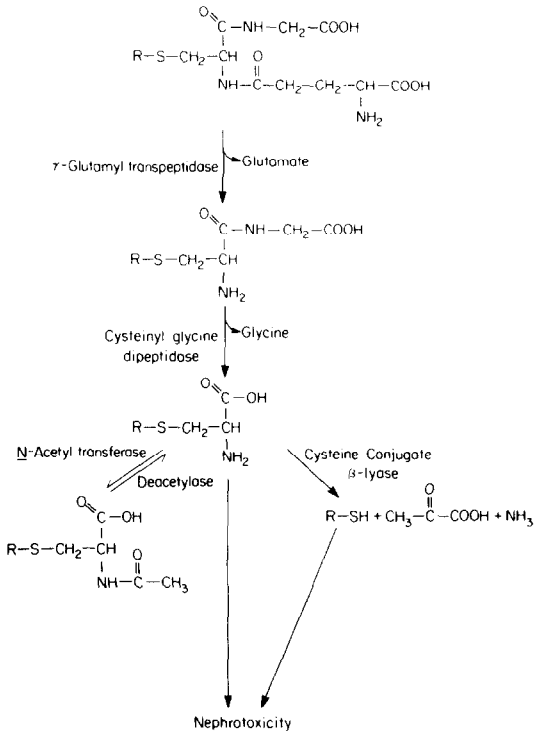



Fig. 1. Metabolism of *S*-substituted glutathione conjugates.

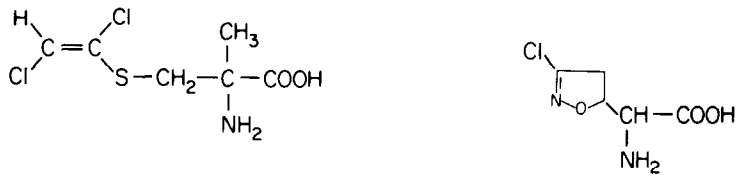
glutathione conjugates, as well as the corresponding cysteine conjugates, of several halogenated hydrocarbons are nephrotoxic (Table 1). The glutathione conjugates IIA, IVA, VA, and VIA (Table 1) are known metabolites of the nephrotoxins HCBD [15], CTFE [27], tetrafluoroethylene [38] and 2-bromohydroquinone* respectively. In addition, the cysteine conjugate *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) (IIB, Table 1) was identified as the toxic factor causing aplastic anemia in cattle fed soya bean meal that had been extracted with trichloroethylene [30, 31]. In the rat, as well as in several other species, only renal damage was found after giving DCVC, but, in the calf, recovery from the renal insult is followed by the onset of aplastic anemia [31, 32, 34]. Finally, sulfur-containing metabolites, which could arise from *S*-(2-chloroethyl)glutathione (IIIA, Table 1) or the nephrotoxic *S*-(2-chloroethyl)-DL-cysteine (CEC) (IIIB, Table 1), have been isolated from urine of rats treated with the nephrotoxin 1,2-dichloroethane [39, 40]. Thus, the nephrotoxicity of glutathione conjugates has been established, and metabolites arising from glutathione conjugates have been identified.

*Renal processing of glutathione conjugates and its role in nephrotoxicity.* Results of studies conducted in this laboratory, as well as those obtained by others [33–38], support the concept that renal processing of glutathione conjugates to produce the corresponding *S*-substituted cysteine conjugates (Fig. 1) plays a key role in glutathione conjugate-induced nephrotoxicity. The *S*-substituted cysteine conjugates may directly form reactive episulfonium ions that cause renal damage. Alternatively, the *S*-substituted cysteine conjugates may be metabolized by renal cysteine conjugate $\beta$-lyase ($\beta$-lyase) to yield reactive sulfur-containing moieties that cause renal toxicity. The renal selectivity of the toxic glutathione conjugates is attributed to processing of glutathione conjugates by $\gamma$-GT and cysteinylglycine dipeptidase, which convert glutathione conjugates to cysteine conjugates. These peptidases are brush-border enzymes and are present in high concentrations in the renal tubule [7, 28, 29].

*Nephrotoxicity of glutathione conjugates and the role of $\gamma$-GT.* To study to the role of $\gamma$-GT in the nephrotoxicity of glutathione conjugates, the $\gamma$-GT inactivator AT-125 [L-($\alpha S$,5*S*)$\alpha$-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid] (Fig. 2) [41] was used. Treatment of rats with AT-125 inhibits renal $\gamma$-GT and is accompanied by glutathionuria [33, 41]. Rats given AT-125 1 hr before being given *S*-(1,2-dichlorovinyl)glutathione (DCVG) (IA, Table 1) show reductions in DCVG-induced nephrotoxicity, as assessed by blood urea nitrogen concentrations (BUN) and by urinary glucose excretion rates [33]. In addition, AT-125 treatment protects against 2-bromohydroquinone-induced increases in BUN concentrations†. AT-125 treatment did not block completely the nephrotoxicity of DCVG or 2-bromohydroquinone. These results and the report that AT-

* T. J. Monks, S. S. Lau and J. R. Gillette, *Abstracts of the First International Symposium on Foreign Compound Metabolism*, p. 48 (1983).

† T. J. Monks, S. S. Lau and J. R. Gillette, *Proceedings of the Sixth International Symposium on Microsomes and Drug Oxidations*, Brighton, Sussex, U.K., 5–10 August 1984.



DCVMC AT-125

Fig. 2. Structures of *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine (DCVMC) and AT-125.

125 does not protect against HCBD-induced nephrotoxicity [42] suggest that some glutathione conjugates may undergo nonenzymatic hydrolysis or that the γ-GT activity remaining after AT-125 treatment allows sufficient processing of glutathione conjugates to produce cysteine derivatives in nephrotoxic amounts. The enterohepatic circulation of glutathione and cysteine conjugates [35, 43] suggests that metabolism by intestinal microflora may also contribute to the renal toxicity of glutathione conjugates.

*Nephrotoxic cysteine conjugates that are directly acting.* The *in vivo* covalent binding to renal cortical macromolecules and the nephrotoxicities of the β-lactam antibiotics cephaloglycin, cephalothin and benzylpenicillin correlate with the instability of these molecules [44]. Thus, the ability of a glutathione or cysteine conjugate to form a reactive episulfonium ion may be an important determinant of its nephrotoxicity. Treatment of rats with CEC (IIIB, Table 1) produces elevations in BUN and urinary glucose concentrations, which are accompanied by acute proximal tubular and punctate glomerular necrosis [37]. CEC is not a substrate for β-lyase [45], but is capable of reacting with chemical nucleophiles by a mechanism consistent with the formation of an episulfonium ion [46]. Equimolar doses of analogues of CEC, in which the chlorine atom is replaced by a hydrogen atom [*S*-ethyl-L-cysteine] or a hydroxyl group [*S*-(2-hydroxyethyl)-DL-cysteine], fail to produce nephrotoxicity [37]. These results suggest that the reactive episulfonium ion formed from CEC may be responsible for the renal damage.

*Nephrotoxicity of cysteine conjugates and the role of β-lyase.* Several cysteine conjugates are metabolized by β-lyase to produce thiols, ammonia, and pyruvate [47, 48]. The thiols can be methylated by thiol *S*-methyltransferase [49], which accounts for the *in vivo* formation of methylthio derivatives of several xenobiotics [50].

β-Lyase has been isolated and purified from bovine kidney, from bovine and rat liver, and from intestinal microflora [45, 47, 51–53]. The potent nephrotoxins *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (VB, Table 1) and DCVC are good substrates for β-lyase [38, 45]. The products identified from the cleavage of DCVC by β-lyase were pyruvic acid, ammonia, and an unidentified sulfur-containing reactive metabolite [51]. The reactive metabolite generated *in vitro* from the cleavage of DCVC combines with proteins, glutathione, and nucleic acids [54, 55].

To ascertain the role of β-lyase in the nephrotoxicity of DCVC, *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine (DCVMC, Fig. 2), which cannot be cleaved by the pyridoxal phosphate-dependent β-lyase, was synthesized. Treatment of rats with DCVC at doses of 25 or 50 mg/kg, i.p., produced elevations in BUN and urinary glucose concentrations, but equimolar doses of DCVMC failed to produce any change in these renal function parameters [33]. Additional evidence for the role of β-lyase in DCVC-induced nephrotoxicity has been obtained. Aminooxyacetic acid, an inhibitor of pyridoxal phosphate-dependent enzymes, inhibits renal β-lyase, both *in vivo* and *in vitro*, and protects against DCVC-induced nephrotoxicity (A. A. Elfarra and M. W. Anders, unpublished observations). These results clearly implicate β-lyase in DCVC-induced nephrotoxicity.

Cysteine conjugates are *N*-acetylated to form mercapturic acids by cysteine conjugate *N*-acetyl transferase [56]. *N*-Acetylated cysteine conjugates are not substrates for β-lyase [47, 53], but deacetylase activity may lead to recycling of mercapturic acids back to the non-acetylated form [56]. This probably explains the nephrotoxicity of *N*-acetyl *S*-(1,1,2,3,4-pentachloro-1,3-butadienyl)-L-cysteine [35].

*Concluding remarks.* The data summarized above clearly show that both glutathione and cysteine conjugates are nephrotoxic. The target organ selectivity of glutathione conjugates is probably due to their renal processing to yield the corresponding cysteine conjugates (Fig. 1), which may be direct-acting nephrotoxins (CEC) or which may require bioactivation by β-lyase (DCVC) to produce toxicity.

DCVMC was designed to test directly the role of β-lyase in the nephrotoxicity of DCVC. The finding that DCVMC is not nephrotoxic strongly suggests a role for renal β-lyase in the nephrotoxicity of DCVC and, presumably, other cysteine conjugates (IIB, IVB, VB, Table 1). β-Lyase is a pyridoxal phosphate-dependent enzyme [45, 47, 53], which shares a common reaction mechanism with several related enzymes [50]. Liver cytosolic β-lyase can utilize either kyurenine or 3-hydroxykyurenine as substrates, which suggests that β-lyase and kyureninase are similar, if not identical, catalytic proteins [50]. The nature of the renal enzyme has not been investigated in detail. It is possible that renal β-lyase activity is not attributable to a single enzyme but is a composite of activities of several enzymes. Future investigations should clarify the identity and nature of renal β-lyase activity.

β-Lyase catalyzes the conversion of cysteine conjugates to thiols, pyruvate, and ammonia. The thiol formed is probably responsible for the toxicity. With

DCVC, the thiol would be expected to be 1,2-dichlorovinyl mercaptan, which may rearrange to yield a thioketene or a thiono acyl halide. Elucidation of the structures of the thiols formed and their interactions with renal macromolecules should clarify the cellular and biochemical effects of nephrotoxic cysteine conjugates.

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