Biochemical Pharmacology, Vol. 59, pp. 531–543, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(99)00374-3

Role of Cytochrome P450 and Glutathione *S*-Transferase α in the Metabolism and Cytotoxicity of Trichloroethylene in Rat Kidney

Brian S. Cummings, Jean C. Parker† and Lawrence H. Lash*‡*

*Department of Pharmacology, Wayne State University School of Medicine, Detroit, MI 48201; and †National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC 20460, U.S.A.

ABSTRACT. The toxicity and metabolism of trichloroethylene (TRI) were studied in renal proximal tubular (PT) and distal tubular (DT) cells from male Fischer 344 rats. TRI was slightly toxic to both PT and DT cells, and inhibition of cytochrome P450 (P450; substrate, reduced-flavoprotein:oxygen oxidoreductase [RH-hydroxylating or -epoxidizing]; EC 1.14.14.1) increased TRI toxicity only in DT cells. In untreated cells, glutathione (GSH) conjugation of TRI to form *S*-(1,2-dichlorovinyl)glutathione (DCVG) was detected only in PT cells. Inhibition of P450 transiently increased DCVG formation in PT cells and resulted in detection of DCVG formation in DT cells. Formation of DCVG in PT cells was described by a two-component model (apparent V_{max} values of 0.65 and 0.47 nmol/min per mg protein and K_m values of 2.91 and 0.46 mM). Cytosol isolated from rat renal cortical, PT, and DT cells expressed high levels of GSH S-transferase (GST; RX:glutathione *R*-transferase; EC 2.5.1.18) α (GSTα) but not GSTπ. Low levels of GSTμ were detected in cortical and DT cells. Purified rat GSTα2–2 exhibited markedly higher affinity for TRI than did GSTα1–1 or GSTα1–2, but each isoform exhibited similar V_{max} values. Triethyltinbromide (TETB) (9 μM) inhibited DCVG formation by purified GSTα1–1 and GSTα2–2, but not GSTα1–2. Bromosulfophthalein (BSP) (4 μM) only inhibited DCVG formation by GSTα2–2. TETB and BSP inhibited approximately 90% of DCVG formation in PT cytosol but had no effect in DT cytosol. This suggests that GSTα1–1 is the primary isoform in rat renal PT cells responsible for GSH conjugation of TRI. These data, for the first time, describe the metabolism of TRI by individual GST isoforms and suggest that DCVG feedback inhibits TRI metabolism by GSTs. BIOCHEM PHARMACOL **59**;5:531–543, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. trichloroethylene; kidney; GSH conjugation; nephron heterogeneity; cytochrome P450; proximal tubular; distal tubular

TRI§ is a major environmental contaminant and is an occupational concern because of its widespread industrial use [1, 2]. It produces toxicity and tumors in several tissues, with target organ specificity varying significantly among species, among different strains of the same species, and between males and females of the same strain. Most TRI toxicity is dependent on its metabolism, which occurs by two pathways: P450 (substrate, reduced-flavoprotein:oxygen oxidoreductase [RH-hydroxylating or -epoxidizing]; EC 1.14.14.1)-dependent oxidation and GSH conjugation. The nephrotoxicity and nephrocarcinogenicity of TRI have been attributed to formation of reactive, sulfur-containing metabolites generated by GSH conjugation and subsequent metabolism by GGT ([5-L-glutamyl]-peptide:amino-acid 5-glutamyltransferase; EC 2.3.2.2), dipeptidase (aminopeptidase M; EC 3.4.11.2), and β-lyase (L-cysteine-*S*-conjugate thiol-lyase [deaminating]; EC 4.4.1.13) [3, 4]. The initial step of this pathway is catalyzed by GST (RX:glutathione *R*-transferase; EC 2.5.1.18) isoforms, which catalyze the formation of DCVG (Fig. 1). This is followed by hydrolysis reactions catalyzed by GGT and dipeptidase, which cleave the glutamyl and glycyl residues to form DCVC. Then DCVC can undergo either *N*-acetylation to form the mercapturate NAcDCVC or a β-elimination reaction catalyzed by the β-lyase to form a reactive thiol. This thiol, in turn, can rearrange to form potent acylating species. Subsequent acylation of proteins and DNA may lead to cytotoxicity and mutagenesis [3, 4]. Although NAcDCVC is a detoxification product, it can be deacetylated to regenerate DCVC. Whereas marked sex- and species-dependent differences also exist in P450-dependent metab-

* ‡Corresponding author: Lawrence H. Lash, Ph.D., Department of Pharmacology, Wayne State University School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201. Tel. (313) 577-0475; FAX (313) 577-6739; E-mail: l.h.lash@wayne.edu

§ *Abbreviations*: β-lyase, cysteine conjugate β-lyase; BSP, bromosulfophthalein; CDNB, 1-chloro-2,4-dinitrobenzene; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DT, distal tubular; F344, Fischer 344; GGT, γ-glutamyltransferase; GST, GSH *S*-transferase; LDH, lactate dehydrogenase; NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine; P450, cytochrome P450; PT, proximal tubular; TETB, triethyltinbromide; and TRI, trichloroethylene (also known as trichloroethene).

Received 4 March 1999; accepted 12 August 1999.

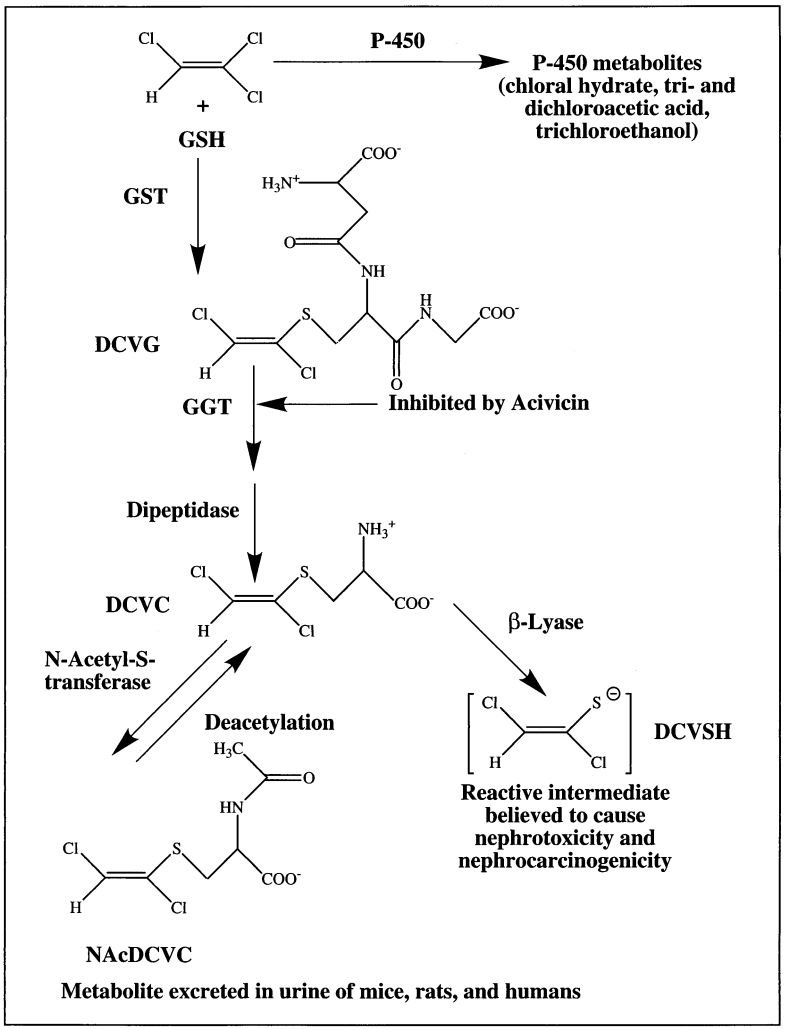

FIG. 1. Scheme of TRI metabolism by the GSH-conjugation pathway. *Abbreviations*: P450, cytochrome P450; GST, GSH *S*-transferase; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DCVSH, dichlorovinylthiol; GGT, γ-glutamyltransferase; and NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine.

olism of TRI, there is no evidence that metabolism by P450 plays a role in the renal effects of TRI.

GSH conjugation of TRI occurs predominantly in the liver. DCVG formed in the liver is secreted rapidly into the bile and/or plasma and eventually is delivered to the kidneys (in the form of DCVG, DCVC, or NAcDCVC) through enterohepatic and renal–hepatic translocation pathways [5]. Because of the tissue distribution of membrane transport systems and GGT, subsequent reactions of the pathway occur within the kidneys, thereby generating the reactive and toxic species. However, the entire pathway also can occur within the kidney, removing the need for translocation of DCVG and its derivatives through the circulation [5–7]. Differences in TRI-induced nephrotoxicity correlate positively with differences in rates of DCVG formation and GST activity [5–7].

The human health hazard of TRI toxicity and the relevance of the kidney as a target organ in humans have been subject to controversy, mostly because the flux of TRI through the GSH conjugation pathway is thought to represent only a small fraction of TRI metabolism [1]. However, humans form DCVG at detectable levels after exposure to small doses of TRI [8]. Three studies of workers exposed to TRI demonstrated some degree of kidney dysfunction [9–11]. However, in one of these studies [11], workers were exposed to multiple solvents, and only a relatively small fraction of the workers were known to be exposed solely to TRI. In another study of metal degreasers in Sweden [12], no indication of nephrotoxicity was observed. In contrast to these somewhat equivocal studies, a recent report [13] of a case involving acute TRI poisoning demonstrated that the products of the GSH-conjugation pathway can be formed in humans and that a single, large dose of TRI can produce renal injury in humans.

The controversy over the human health hazard of TRI recently came to the forefront when the International Agency for Research on Cancer (IARC) issued a report in 1995 evaluating the epidemiologic evidence on kidney cancer incidence and mortality due to TRI exposure [14]. The IARC committee reclassified TRI as a Group 2A

carcinogen ("Probably Carcinogenic to Humans"). Most of the studies on which the evaluation was based were occupational exposures or case-control studies of kidney cancer. The IARC review placed greater weight on observations from three cohort studies [15–17] where no elevations in kidney cancer risks were noted and on two case-control studies that reported divergent results. Of special note was a study of German cardboard manufacturing workers [18], which reported five cases of renal cancer compared with none in the comparison population. This study prompted much debate in the literature [19–21], and the IARC considered these findings a cluster; however, it was concluded that the incidence of kidney cancer among these workers warranted further study [14]. A subsequent review [22] of the epidemiology of TRI concluded that there is neither consistent nor convincing evidence to support a causal relationship between TRI exposure and renal-cell cancer. On the other hand, three more recent studies of occupationally exposed workers [23–25] concluded that an association between renal-cell cancer and TRI exposure does exist.

The rat has been used as an animal model for TRI exposure, and these studies have provided significant mechanistic information. Rat renal cortical cells are composed primarily of PT and DT cells [26]. Both *in vitro* and *in vivo* studies have shown that DCVC is toxic to these cells. However, PT and DT cells differ significantly in terms of their susceptibility to many nephrotoxicants [27]. Differences in the nephrotoxicity of several chemicals have been attributed to differences in key drug-metabolizing enzymes, including P450 and GSH-dependent enzymes. We have shown recently that freshly isolated PT and DT cells differ significantly in the expression of P450 isoforms [28]. Both rat and human PT and DT cells express different GST isoforms [29, 30]. Given the differences in both P450 and GST expression between rat renal PT and DT cells, these cells would appear to be good models in which to study mechanisms of TRI toxicity and metabolism.

TRI oxidation by P450 has been studied extensively [31, 32], and previous studies showed that CYP2E1, CYP1A1/2, CYP2B1/2, and CYP2C isoforms can metabolize TRI. However, the GST isoforms responsible for the metabolism of TRI have never been determined. This would greatly aid in the analysis of the susceptibility of humans to TRI toxicity. We reported previously on the conjugation of TRI with GSH in rat renal microsomes, cytosol, and cortical cells [5, 6]. These reports demonstrated that TRI can be conjugated with GSH solely within the kidney and that the level of DCVG formation correlates positively with TRI cytotoxicity. Furthermore, differences in TRI metabolism correlate positively with differences in GST and GGT activity. We also reported that DCVC is toxic both *in vitro* and *in vivo* in rat PT and DT cells, with DCVC being slightly more toxic in PT cells [33]. Hence, there are nephron cell-type differences in the handling of and response to metabolites of the GSH conjugation pathway.

The objective of the present study was to determine the differences in TRI toxicity and metabolism between rat renal PT and DT cells so that factors that determine susceptibility to TRI can be identified. These differences were correlated with differences in P450 and GST expression in these cells. In doing so, we report herein, for the first time, data on the ability of individual rat GST isoforms to conjugate TRI with GSH. The kinetics of TRI conjugation with GSH by rat PT and DT cells and by individual GST isoforms was also determined. The results indicated that differences in TRI toxicity and metabolism in PT and DT cells are a consequence of differences in expression of both P450 and GST isoforms. Because many similar isoforms are also present in human kidney, this information can be extrapolated to humans to help make predictions for human health risk assessment of TRI.

MATERIALS AND METHODS

Chemicals

TRI (reported to be 99.9% pure, as judged by electron ionization mass spectrometry), collagenase type IV, bovine serum albumin (fraction V), acivicin [L-(αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid], BSP, L-γ-glutamyl-L-glutamate, and CDNB were purchased from the Sigma Chemical Co. DCVG was synthesized as previously described [34]. Purity (>95%) was determined by HPLC analysis, and identity was confirmed by proton NMR spectroscopy. TETB was purchased from the Aldrich Chemical Co. Individual isoforms of rat GSTα, GSTπ, and GSTμ and polyclonal antibodies to human GSTA1 and rat GSTπ and GSTμ were purchased from Oxford Biomedical and Detroit R & D. Polyclonal antibody to rat GSTα2 was purchased from Biotrin (Newton, MA).

Animals

Male F344 rats (150–250 g; Charles River Laboratories) were used in these studies. They were housed in a controlled room on a 12-hr light/dark cycle and were given commercial rat chow and water *ad lib*.

Preparation of Isolated Rat Renal Cortical, PT, and DT Cells

Isolated renal cortical cells were obtained by collagenase perfusion [35] from male F344 rats. To obtain enriched populations of renal PT and DT cells, cortical cells were subjected to density-gradient centrifugation in Percoll as described previously [26]. Marker enzyme activities and functional assays were used to confirm the identity and purity of the two cell populations [26]. Cell concentrations were determined in the presence of 0.2% (w/v) trypan blue in a hemacytometer, and cell viability was estimated by measuring the fraction of cells that excluded trypan blue.

Isolation of Rat Renal Cortical, PT, and DT Cytosol

Cytosol was prepared from freshly isolated rat renal cortical, PT, and DT cells by homogenization of the cells in a Polytron ultrasonic homogenizer (Brinkmann Instruments), followed by centrifugation at 11,000 *g* for 20 min to spin down nuclei, mitochondria, and cellular debris. Supernatant from this step was centrifuged in a tabletop ultracentrifuge at 105,000 *g* for 90 min at 4°. The resulting supernatant was the cytosolic fraction; the pellet was the microsomal fraction.

Toxicity of TRI in Isolated Rat Renal PT and DT cells

The toxicity of TRI in isolated rat renal PT and DT cells was determined by release of LDH [(*S*)-lactate:NAD^+ oxidoreductase; EC 1.1.1.27] in the medium, and total cellular LDH activity was determined spectrophotometrically by measuring the oxidation of NADH at 340 nm. For P450 inhibition studies, cells were preincubated with 0.25 mM SKF-525A or 0.25 mM metyrapone.

Assay of TRI Metabolism by the GSH Conjugation Pathway in PT and DT Cells

All incubations were performed in 25-mL polypropylene Erlenmeyer flasks on a Dubnoff metabolic shaking incubator (60 cycles/min) at 37°. Isolated renal PT and DT cells were preincubated for 15 min with 0.25 mM acivicin to inhibit GGT activity before performing incubations to measure DCVG formation. Previous studies [36] showed that this concentration of acivicin inhibits GGT activity by $> 95\%$. Then cells were incubated with 0–20 mM TRI by the addition of TRI (dissolved in 1%, v/v, acetone) to the liquid phase. This concentration of acetone, which was present in all incubations, does not affect GST activity (with CDNB as substrate) in renal cells (data not shown) and has only a modest effect on P450 activity [28]. Triton X-100 (0.1%, v/v, final concentration) was added to solubilize the plasma membrane. After incubations for the indicated times, reactions were terminated by the addition of perchloric acid (10%, w/v, final concentration), and samples were processed for analysis of DCVG as described previously [5], using the HPLC method described by Fariss and Reed [37]. For P450 inhibition studies, cells were preincubated in the presence or absence of the general P450 inhibitor SKF-525A (0.25 mM) or the CYP2E1 competitive substrate chlorzoxazone (1 mM) for 15 min. Reactions were allowed to proceed as described above.

Assay of TRI Metabolism by the GSH Conjugation Pathway with Purified Rat GST Isoforms and PT and DT Cytosol

Assays were performed as described above except that incubations were done in 1.5-mL polypropylene microcentrifuge tubes at 30° instead of 37°. The lower temperature was chosen for assays with subcellular fractions and purified enzyme because more consistent results were obtained than at the higher temperature used with intact cells. Neither purified enzyme (2–9 μg protein per incubation) nor PT or DT cytosol (5–10 mg protein per incubation) was preincubated with acivicin because these biological samples do not contain significant GGT activity [6]. Reactions were performed in Krebs–Henseleit buffer (pH 7.4) minus bovine serum albumin, as this was the buffer used for the cellular reactions. In some experiments, purified enzymes or cytosol from PT or DT cells were preincubated for 15 min with one of two GSTα-selective inhibitors before the addition of TRI. Reactions were allowed to proceed for the indicated times, stopped, and processed for HPLC analysis as described above.

Western Blot Analysis of Individual GST Isoforms in Rat Renal PT, DT, and Liver Cytosol

The expression of individual forms of GST in rat renal cortical, PT, DT, and liver cytosol was demonstrated by SDS–PAGE on a 10% gel followed by transfer of the gel to nitrocellulose. Then nitrocellulose membranes were exposed to polyclonal antibodies to goat anti-rat GSTα and GSTμ and rabbit anti-human GSTπ. Alkaline phosphatase-conjugated secondary antibodies and substrate were used to detect protein bands.

Protein Determination and Data Analysis

Protein concentrations were measured with the bicinchoninic acid protein determination kit from the Sigma Chemical Co. All values are means ± SEM of measurements made on the indicated number of separate preparations. Significant differences between means for data were assessed first by a one-way ANOVA. When significant "F values" were obtained, Fisher's protected least significance *t*-test was performed to determine which means were significantly different from one another, with two-tail probabilities < 0.05 considered significant.

RESULTS

Cytotoxicity of TRI in Freshly Isolated Rat Renal PT and DT Cells

Freshly isolated rat renal PT and DT cells were incubated in the presence of various concentrations of TRI, ranging from 0.1 to 10 mM (data not shown). TRI was modestly cytotoxic to PT and DT cells only at the highest concentration tested (i.e. 10 mM), causing approximately a 20% increase in LDH release, whereas concentrations of TRI of 5 mM or below had no effect on LDH release.

Preincubation of PT and DT cells with metyrapone (0.25 mM), a general P450 inhibitor, increased TRI cytotoxicity in DT cells only (Fig. 2). The same results were seen when 0.25 mM SKF-525A, another general P450 inhibitor, was used instead of metyrapone (data not shown). These data

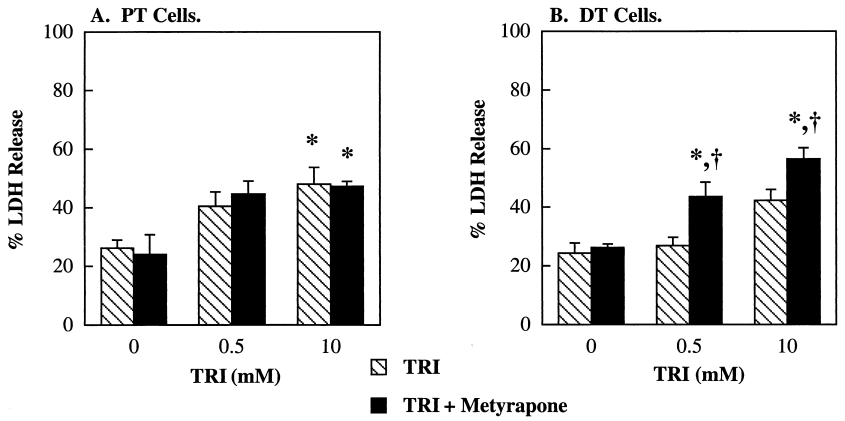

FIG. 2. Effect of P450 inhibition on the cytotoxicity of TRI in freshly isolated rat renal PT and DT cells. Freshly isolated rat renal PT (A) and DT (B) cells (0.5–1.0 × 10^6 cells/mL) were preincubated in the presence of either solvent control (i.e. 1.0%, v/v, acetone) or metyrapone (0.25 mM) for 15 min prior to the addition of TRI. At the indicated time points, the percent of LDH release was determined. Results are the means ± SEM of at least 3 experiments. Key: (*) significant difference ($P < 0.05$) from control; and (†) significant difference ($P < 0.05$) from the same concentration without inhibitor.

suggested that the cytotoxicity of TRI in rat renal PT and DT cells was influenced by the expression of P450 isoforms within these cells.

Metabolism of TRI to DCVG in Freshly Isolated Rat Renal PT and DT Cells

TRI (10 mM) conjugation with GSH was assessed in freshly isolated rat renal PT and DT cells by measuring the formation of DCVG by HPLC analysis. In the absence of P450 inhibition, DCVG formation was detected only in PT cells, and occurred at a rate of approximately 1–2 nmol/min per mg protein (Fig. 3). DCVG formation in PT cells was approximately linear throughout 60 min of incubation. Preincubation of cells with SKF-525A resulted in significant increases in DCVG formation in both PT and DT cells. Inhibition of P450 in DT cells resulted in DCVG formation at levels comparable to those of PT cells. DCVG formation decreased significantly after 60 min of incubation in the presence of P450 inhibitors. Data from our laboratory have shown that DT cells express higher levels of CYP2E1 than PT cells [28]. Preincubation of both cell types with chlorzoxazone, a competitive substrate for CYP2E1, significantly increased DCVG formation in both PT and DT cells. These increases were measured only after 30 min and were larger in DT cells than in PT cells, and DCVG content decreased to control levels by 60 min. The larger increase in DCVG formation in DT cells as compared with that in PT cells correlates positively with the levels of expression of CYP2E1 in these cells [28]. Furthermore, the increase in DCVG formation in DT cells after P450 inhibition with SKF-525A correlates positively with the increases in TRI cytotoxicity in DT cells after P450 inhibition.

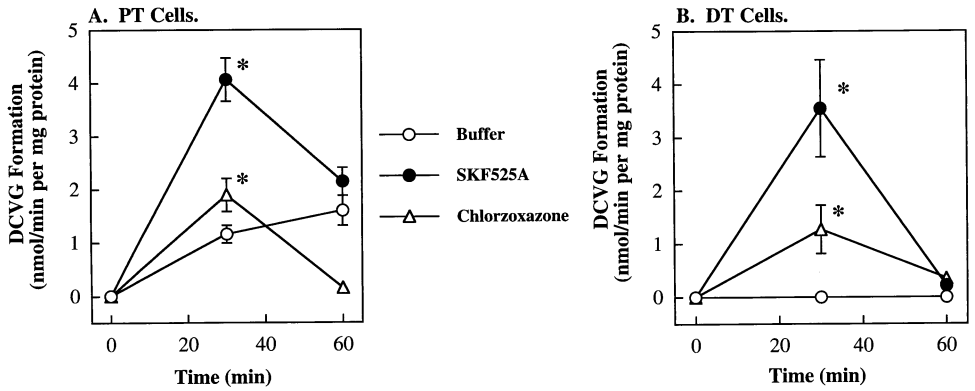

FIG. 3. Metabolism of TRI to DCVG in freshly isolated rat renal PT and DT cells. Isolated renal PT (A) and DT (B) cells (0.5 to 2 mg protein/mL) were lysed and preincubated with either solvent control (i.e. 1.0%, v/v, acetone), SKF-525A (0.25 mM), or chlorzoxazone (1 mM) for 15 min prior to the addition of 10 mM TRI in the presence of 5 mM GSH at 37° for either 30 or 60 min. Metabolism was measured by quantitation of DCVG formation by HPLC after derivatization. Controls lacked TRI. Results are the means ± SEM of at least 3 experiments. Key: (*) significant difference ($P < 0.05$) from control.

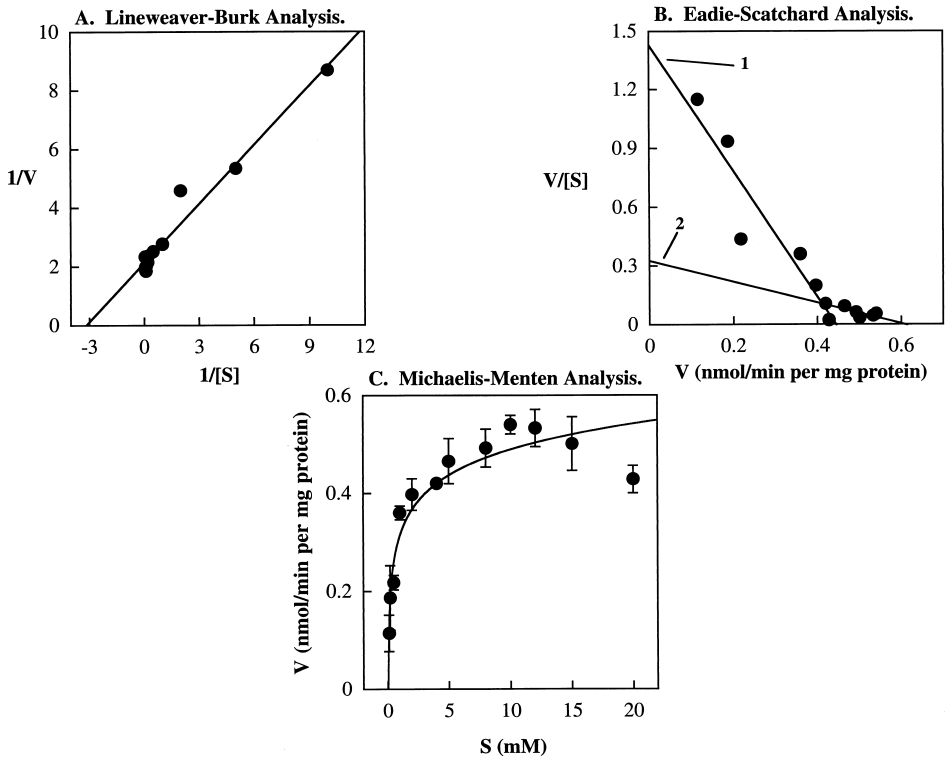

FIG. 4. Kinetic analysis of TRI conjugation with GSH in freshly isolated rat renal PT cells. Isolated renal PT cells (0.5 to 2 mg protein/mL) were lysed and incubated in the presence of 0–20 mM TRI and 5 mM GSH at 37° for 30 min. Metabolism was measured by quantitation of DCVG formation by HPLC after derivatization. Controls included the absence of TRI. Results were subjected to Lineweaver–Burk (A), Eadie–Scatchard (B), and Michaelis–Menten (C) analysis, and are the means (A and B) or means ± SEM (C) of at least 3 experiments. K_m and V_{max} values are given in Table 1. The line in panel A was obtained by linear regression (y = 0.671x + 2.127, r^2 = 0.965). The two lines in panel B were obtained by linear regression (line 1: y = −3.204x + 1.427, r^2 = 0.878; line 2: y = −0.530x + 0.326, r^2 = 0.713). The curve in panel C was obtained by a logarithmic regression (y = 0.175 log(x) + 0.314).

Kinetics of DCVG Formation in Freshly Isolated Rat PT Cells

The kinetics of TRI conjugation with GSH to form DCVG was determined in freshly isolated rat renal PT cells (Fig. 4). DCVG formation was detected with all concentrations of TRI used in PT cells, but was detected in DT cells only at the highest concentrations used (i.e. 15 and 20 mM). Hence, kinetic analysis could be performed only on data obtained in PT cells. Lineweaver–Burk analysis of DCVG formation in PT cells resulted in a single line (Fig. 4A). In contrast, Eadie–Scatchard (or Eadie–Hofstee) analysis of DCVG formation in PT cells resulted in a two-component model that yielded a high-affinity, low-capacity and a low-affinity, high-capacity process (Fig. 4B). Kinetic parameters derived from these two analyses are summarized in Table 1. Cells incubated with higher concentrations of TRI (>15 mM) exhibited significantly decreased DCVG levels (Fig. 4C), suggesting end-product inhibition of GSH conjugation.

Expression of Individual GST Isoforms in Freshly Isolated Rat Renal PT and DT Cells

Western blot analysis of cytosol isolated from rat renal cortical, PT, and DT cells using polyclonal antibodies to rat GSTα and GSTμ, and human GSTπ, revealed that GSTα was expressed in all cell types at very high levels (Fig. 5, panel labeled α, lanes 2–4). A small amount of GSTμ was detected in cortical and DT cells, whereas no GSTπ was detected in any cell type (Fig. 5, panels labeled μ and π, respectively). All of these antibodies detected bands in rat liver cytosol (lane 1). The polyclonal GSTμ and GSTπ antibodies did not cross-react with purified rat GSTα2–2. However, a strong band was detected when GSTα2–2 was

TABLE 1. Kinetics of TRI metabolism to DCVG in freshly isolated PT cells

Method	K_m-1 (mM)	K_m-2 (mM)	V_{max}-1 (nmol/min per mg protein)	V_{max}-2 (nmol/min per mg protein)
Lineweaver-Burk	0.31		0.47	
Eadie-Scatchard	0.46	2.91	0.47	0.65

Freshly isolated PT cells were incubated in the presence of acivicin (0.25 mM) for 15 min to inhibit GGT. Then TRI (0.1 to 20 mM) and GSH (5 mM) were added, and reactions were allowed to proceed for 30 min. The reactions were stopped by the addition of 100 μL of 70% (v/v) perchloric acid followed by 50 μL of bathophenanthroline disulfonate. Samples were derivatized with 1-fluoro-2,4-dinitrobenzene and injected onto a Waters 10 μm μBondapak C_{18} amine column (8 × 100 mm) and were separated by a methanol/acetate mobile phase and gradient elution at 365 nm. Kinetic parameters were derived from linear transformations shown in Fig. 4 and are from measurements of at least 3 separate experiments.

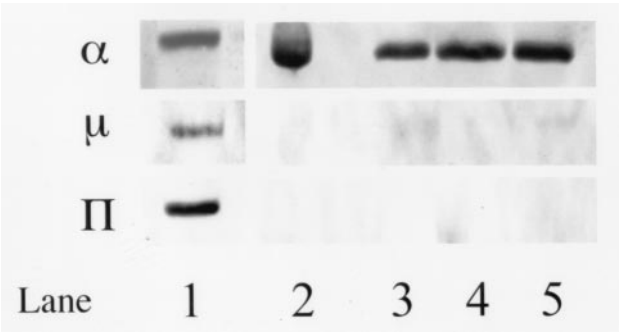

FIG. 5. Expression of GST isoforms in cytosol from freshly isolated rat renal PT and DT cells. Cytosol was isolated as described in Materials and Methods, and approximately 50 μg of protein was subjected to SDS–PAGE, transferred to a nitrocellulose membrane, and exposed to polyclonal antibodies to rat GSTα, rat GSTμ, or human GSTπ. Lane 1: cytosol from liver homogenates used as a positive control; lane 2: purified rat GSTα2–2 used as a positive and negative control; lane 3: cytosol from freshly isolated rat renal cortical cells; lane 4: cytosol from freshly isolated rat renal PT cells; and lane 5: cytosol from freshly isolated rat renal DT cells.

cross-reacted with the polyclonal rat GSTα antibody (Fig. 5, panels α, μ, and π; lane 2).

The class of rat GSTα isoforms is made up of multiple members; GSTα1 and GSTα2 were chosen for study, as they are two of the best characterized isoforms and are readily available commercially. These members can form homo- and heterodimers with each other, resulting in three specific isoforms: GSTα1–1, GSTα1–2, and GSTα2–2; the ability of these isoforms to conjugate TRI with GSH *in vitro* was determined. Kinetic analysis of TRI conjugation with GSH by purified GSTα isoforms revealed that all of these isoforms were capable of conjugating TRI with GSH *in vitro*, although no DCVG formation was detected at 0.1 and 0.2 mM TRI with GSTα1–1. Lineweaver–Burk analysis (Fig. 6) of these data showed that GSTα1–1 and GSTα1–2 exhibited similar, high K_m values for TRI, whereas the K_m value for GSTα2–2 was 50- and 100-fold lower than that for GSTα1–1 and GSTα1–2, respectively (Table 2). In contrast, all three isoforms exhibited similar V_{max} values.

Data on the time-dependence of DCVG formation catalyzed by these three isoforms revealed that TRI formation was linear up to 20 min with 10 mM TRI. After this time, DCVG formation decreased significantly (Fig. 7). Thus, whereas all GSTα isoforms were capable of conjugating TRI with GSH, GSTα2–2 had the highest affinity for TRI.

Inhibition of TRI Conjugation with GSH by GSTα Inhibitors

The ability of the GSTα inhibitors TETB and BSP to inhibit TRI conjugation with GSH by both purified rat

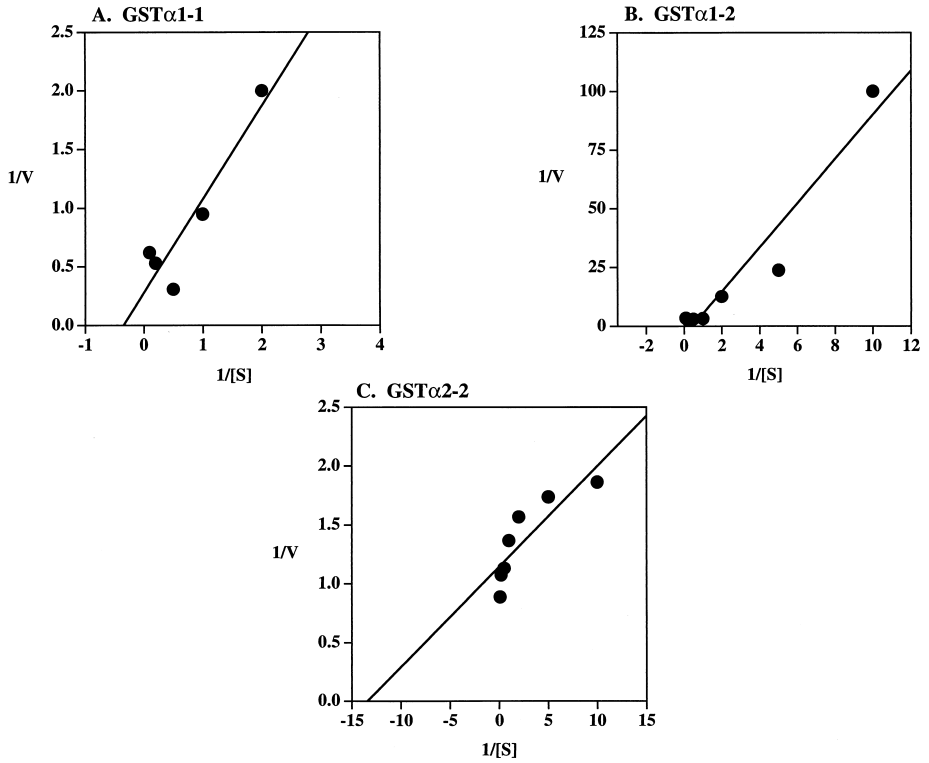

FIG. 6. Kinetic analysis of TRI conjugation with GSH to form DCVG by purified rat GSTα isoforms. Purified rat GSTα1–1 (A), GSTα1–2 (B), or GSTα2–2 (C) (2–9 μg per preparation) were incubated with 0–10 mM TRI in the presence of 5 mM GSH for 10 min at 30°. Metabolism was measured by quantitation of DCVG formation by HPLC after derivatization. Controls included the absence of enzyme, TRI, or GSH. Results were subjected to Lineweaver–Burk analysis. Results are the means of at least 3 experiments. The K_m and V_{max} values for each GST isoform are given in Table 2. Lines in panels A–C were obtained by linear regression (panel A: y = 0.796x + 0.276, r^2 = 0.861; panel B: y = 9.403x −4.006, r^2 = 0.928; panel C: y = 0.085x + 1.146, r^2 = 0.737).

TABLE 2. Kinetics of TRI metabolism to DCVG by purified rat GSTα isoforms

Isoform	K_m (mM)	V_{max} (μol/per mg protein)
GSTα1-1	3.99	0.75
GSTα1-2	7.51	1.50
GSTα2-2	0.074	0.87

Purified enzyme plus TRI and GSH (5 mM) were mixed, and reactions were allowed to proceed for 30 min. The reactions were stopped by the addition of 100 μL of 70% (v/v) perchloric acid followed by 50 μL of bathophenanthroline disulfonate. Samples were derivatized with 1-fluoro-2,4-dinitrobenzene and injected onto a Waters 10 μm μBondapak C_{18} amine column (8 × 100 mm) and were separated by a methanol-acetate mobile phase and gradient elution at 365 nm. Kinetic parameters were derived form linear transformations shown in Fig. 6 and are from measurements of at least 3 separate experiments.

GSTα isoforms and in cytosol from rat renal PT and DT cells was tested. TETB and BSP are noncompetitive inhibitors of CDNB metabolism by GSTα2–2 and GSTα1–1, respectively, with EC_{50} values of 3 and 2 μM, respectively [38]. No studies could be found discussing the ability of these compounds to inhibit TRI conjugation with GSH by any enzyme or in any tissue. TRI (1 mM) conjugation with GSH by rat GSTα1–1 was inhibited significantly by TETB (9 μM) but not by BSP (4 μM) (Fig. 8). DCVG formation catalyzed by rat GSTα2–2 was inhibited significantly by both TETB (9 μM) and BSP (4 μM), with BSP causing more inhibition. Neither TETB nor BSP was able to inhibit the ability of GSTα1–2 to conjugate TRI with GSH. It should be noted that both TETB and BSP can inhibit CDNB metabolism by GSTα1–2 if the concentration of each is raised (EC_{50} values of 100 and 10 μM, respectively [38]). The concentrations of both TETB and BSP were kept below those needed to inhibit rat GSTα1–2 to better elucidate the effects of these inhibitors on the individual rat GSTα isoforms. Thus, as expected based on its ability to inhibit CDNB metabolism, TETB inhibited TRI conjugation with GSH that was catalyzed by rat GSTα1–1. Rather surprisingly, both TETB and BSP inhibited TRI conjugation with GSH that was catalyzed by rat GSTα2–2.

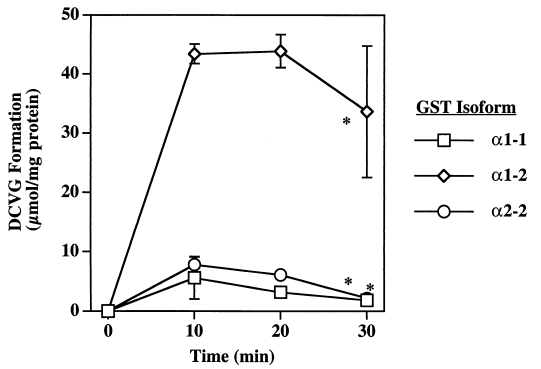

FIG. 7. Time-dependence of TRI conjugation with GSH by purified rat GSTα isoforms. Purified rat GSTα1–1, GSTα1–2, or GSTα2–2 (2–9 μg per preparation) was incubated with 10 mM TRI in the presence of 5 mM GSH for the indicated times at 30°. Metabolism was measured by quantitation of DCVG formation by HPLC after derivatization. Controls included the absence of enzyme, TRI, or GSH. Results are the means ± SEM of at least 3 experiments. Key: (*) significant difference ($P < 0.05$) from DCVG levels at 10 min.

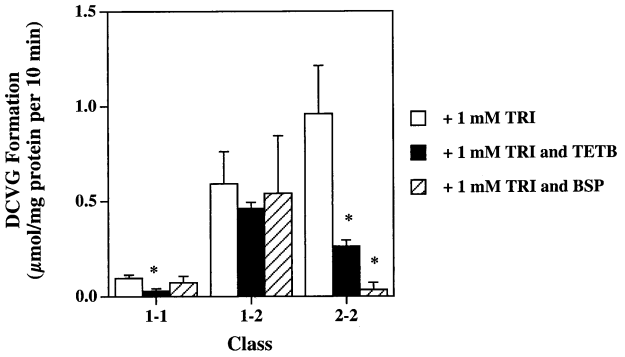

FIG. 8. Effect of GSTα inhibitors on GSH conjugation with TRI to form DCVG by rat GSTα isoforms. Purified rat GSTα1–1, GSTα1–2, or GSTα2–2 (2–9 μg per preparation) was preincubated for 15 min with solvent control, 9 μM TETB, or 4 μM BSP. Then TRI (1 mM) and 5 mM GSH were added, and the reactions were allowed to proceed for 15 min at 30°. Metabolism was measured by quantitation of DCVG formation by HPLC after derivatization. Controls included the absence of enzyme, TRI, or GSH. Results are the means ± SEM of at least 3 experiments. Key: (*) significant difference ($P < 0.05$) from enzyme with TRI alone.

Inhibition of TRI Conjugation with GSH in Rat Renal PT and DT Cytosol

Data from the above study were used to test the ability of TETB and BSP to inhibit TRI conjugation with GSH in cytosol isolated from rat renal PT and DT cells. Cytosol was used instead of intact cells to enable study of TRI metabolism in the absence of P450. Both TETB and BSP inhibited DCVG formation in rat renal PT cytosol at high levels of TRI (10 mM) (Fig. 9A) but were unable to inhibit DCVG formation in cytosol isolated from rat renal DT cells at any concentration of TRI tested (Fig. 9B).

Expression of GSTα1 and GSTα2 in Rat Renal PT and DT Cytosol

Data from Fig. 9 suggested that the inability of TETB and BSP to inhibit DCVG formation in DT cells could be caused by a lack of GSTα2 expression. To test this hypothesis, the expression of GSTα1 and GSTα2 was determined in rat renal PT and DT cells by western blot analysis (Fig. 10). The migration of GSTα1–1 and GSTα1–2 was determined by subjecting the proteins to 10% SDS–PAGE and allowing the gel to run until the 20-kDa band was approximately 5 mm from the bottom of the gel. Then the gel was stained with Coomassie Blue G dye for 1 hr. Analysis of these proteins revealed that GSTα1–1 contained a single band and GSTα1–2 contained two bands: one that corresponded to the band for GSTα1–1 and one higher band that did not migrate as far

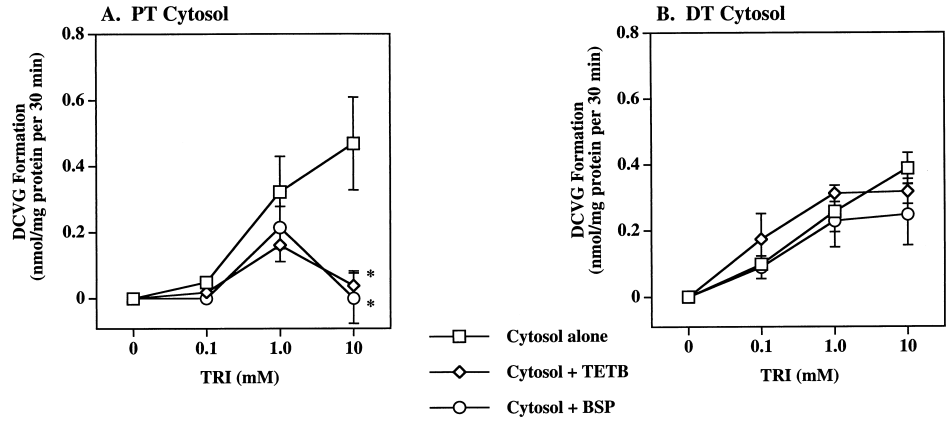

FIG. 9. Effect of GSTα inhibitors on GSH conjugation with TRI by cytosol isolated from rat renal PT (A) and DT (B) cells. Cytosol (5–10 mg/mL) from freshly isolated rat renal PT and DT cells was preincubated for 15 min with solvent control, 9 μM TETB, or 4 μM BSP. TRI (0, 0.1, 1.0, or 10 mM) and 5 mM GSH were then added, and reactions were allowed to proceed for 30 min at 30°. Metabolism was measured by quantitation of DCVG formation by HPLC after derivatization. Results are the means ± SEM of at least 3 experiments. Key: (*) significant difference ($P < 0.05$) from cytosol with TRI alone.

(Fig. 10A). Thus, the lower band was identified as GSTα1 and the higher band was identified as GSTα2. Western blot analysis of rat renal PT and DT cytosol using a polyclonal rabbit anti-rat GSTα1 antibody, followed by overexposure of the blots, revealed the presence of two bands (Fig. 10B). A dark, lower band whose migration corresponded with that seen with purified GSTα1 was detected along with a lighter band whose migration was equal to that seen with GSTα2. Western blot analysis of rat renal PT and DT cytosol using a polyclonal rabbit anti-rat GSTα2 antibody revealed the presence of only one band whose migration corresponded with that of GSTα2 (Fig. 10C). The expression of GSTα2 in rat renal DT cells appeared to be slightly less than that seen in rat renal PT cells.

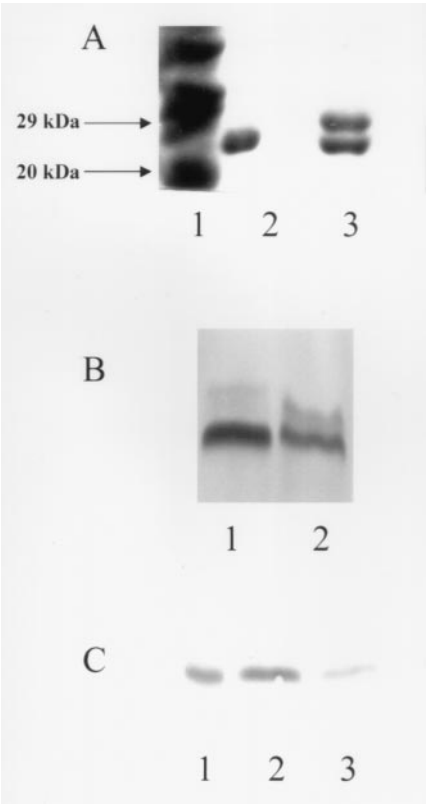

FIG. 10. Expression of GSTα1 and GSTα2 isoforms in rat renal PT and DT cells. (A) Purified GST isoforms were subjected to 10% SDS–PAGE on a gel that was allowed to run until the 20-kDa band was approximately 5 mm from the bottom of the gel. Lane 1 = molecular weight standards; lane 2 = purified rat GSTα1–1; lane 3 = purified rat GSTα1–2. (B) Cytosol from PT cells (lane 1) and DT cells (lane 2) was probed with an antibody to rat GSTα1. (C) Cytosol from renal cortical cells (lane 1), PT cells (lane 2), and DT cells (lane 3) was probed with antibody to rat GSTα2. Blots exposed to polyclonal rabbit anti-rat GSTα1 antibody were overexposed to visualize the upper band, which represents a relatively weak reactivity towards GSTα2.

DISCUSSION

The objective of the present study was to determine differences in TRI cytotoxicity and metabolism between rat renal PT and DT cells and to use these differences to assess factors that are determinants of susceptibility to TRI. Freshly isolated cells were used so that susceptibility and enzyme expression and activity would be similar to that found in the kidneys *in vivo*. With knowledge of how renal metabolism and handling of TRI and its GSH-derived metabolites differ in rats and humans, these data can be used to improve the assessment of the human health hazard of TRI. As TRI-induced nephrocarcinogenicity is believed to be a result of long-term exposure, it was not surprising that TRI was not very toxic to freshly isolated rat renal PT and DT cells after acute exposures. TRI undergoes oxidative metabolism by P450 primarily in the liver. When metabolism in all tissues is taken into account, the P450 pathway predominates over the GSH-conjugation pathway in overall TRI metabolism [1, 2, 4]. An important point, however, is that the GSH-conjugation pathway generates highly reactive metabolites, whereas the P450 pathway

generates chemically stable metabolites [3]. Hence, a simple comparison of total flux through each pathway does not provide a useful indication of the importance of each pathway in TRI-induced toxicity.

The recovery and identification of NAcDCVC as a urinary metabolite of TRI in rats, mice, and humans [39–42] demonstrate the function of the GSH-conjugation pathway and indicate that the kidneys are a primary site for the accumulation of DCVG and formation of its subsequent metabolites. The amount of P450-derived, urinary metabolites of TRI recovered exceeds that of NAcDCVC by a factor of between 10 and 3000. This has often been interpreted to indicate that flux through the GSH-conjugation pathway is insignificant in overall metabolism of TRI [4, 43]. It is critical to understand that NAcDCVC is a stable detoxification product of DCVC metabolism and does not necessarily reflect flux through the β-lyase pathway, which is primarily responsible for generation of the reactive, sulfur-containing metabolite [3]. However, urinary NAcDCVC can be used validly as a marker or indicator of TRI exposure.

Kinetics of GSH Conjugation of TRI in Rat Renal PT and DT Cells

As indicated in the scheme of TRI metabolism in Fig. 1, the GST-catalyzed formation of DCVG competes directly with P450-dependent metabolism of TRI. Determination of the kinetic characteristics of this pathway and the effects of modulation of P450 activity can provide information on the role of GSH conjugation in the toxic effects induced by TRI.

The rates of DCVG formation in PT cells compare favorably with those that we reported previously for rat renal cortical cells [5, 6]. Furthermore, the proposal of a two-component model for metabolizing TRI, as supported by the Eadie–Scatchard plot in Fig. 4, also is supported by data on DCVG formation generated from human liver and kidney cytosol [7]. The two apparent K_m values reported in this study for DCVG formation in rat renal PT cells (0.46 and 2.91 mM) are about an order of magnitude higher than those reported for DCVG formation in human kidney cytosol and microsomes (0.026 and 0.16 mM, respectively). The V_{max} values reported here are within the same range reported in human kidney cytosol (0.65 and 0.47 vs 0.81 nmol/min per mg protein, respectively).

Two possible explanations for the existence of two separate K_m values are that more than one enzyme metabolizes TRI (i.e. multiple GST isoforms) or that DCVG may inhibit TRI metabolism by GST at high concentrations of TRI. The latter would cause feedback inhibition, resulting in a biphasic line in the kinetic analysis. The decrease in DCVG levels after 60 min in PT and DT cells after P450 inhibition (cf. Fig. 3), in PT cells after exposure to high concentrations of TRI (cf. Fig. 4C), and in preparations containing purified rat GSTα after exposure to 10 mM TRI for 30 min is somewhat surprising. GSH *S*-conjugates can bind to GST *in vitro* with higher affinity than the second substrate or GSH has for GST *in vivo* [44, 45]. It has been hypothesized that the binding of GSH *S*-conjugates to GST may prevent toxicity or stabilize the conjugates [44]. Thus, the decreases in DCVG levels measured in Figs. 3, 4, and 7 may be a result of DCVG binding to GST. This would result in lower levels of DCVG being available for derivatization. It is unlikely that DCVG is being processed further in PT and DT cells, as the concentration of acivicin used almost completely inhibits GGT [36]. Furthermore, no further processing could occur in the experiments shown in Fig. 7, where only TRI, GSH, and purified GST were present. The possibility that DCVG is metabolized further by GST is also doubtful; the size of DCVG would hinder its access to GST, and GST metabolism would result in a species that would be detected by HPLC analysis. No unidentified peaks were detected in any samples. The fact that decreases in DCVG are seen only at high levels of TRI after long periods of incubation may be explained by the requirement for high levels of DCVG to affect TRI conjugation with GSH. The levels of DCVG formed in Fig. 7 after 20 min are in the micromolar range. The possibilities that DCVG is exhibiting feedback inhibition of TRI metabolism or is binding to GST, and the physiological relevance of either possibility, need to be explored further. If the above hypothesis is correct, then it would mean that the amount of TRI being metabolized by the GSH-conjugation pathway may be underestimated, as a significant portion of the GSH *S*-conjugate may be binding to GST and thus is not available for detection.

Expression of GST Isoforms and Their Role in Renal Metabolism of TRI

Western blot analysis of cytosol isolated from rat renal cortical, PT, and DT cells revealed the presence of high levels of GSTα, very low levels of GSTμ, and no detectable GSTπ (cf. Fig. 5). Previous studies with Sprague–Dawley rats showed that PT cells only express GSTα, whereas DT cells express GSTμ and GSTπ but not GSTα [30]. Our results differ significantly from these, as GSTα was detected in all tissues tested, whereas GSTπ was not. Our results agree with the above studies in that GSTμ was detected only in DT cells. One possible reason for these differences is the different strain of rat used in this study. Therefore, care should be taken when choosing rat strains for study of toxicants metabolized by GST isoforms.

Individual members of the GSTα family differed significantly in their ability to conjugate TRI with GSH (cf. Fig. 6 and Table 2). GSTα2–2 had a significantly higher affinity for TRI as compared with GSTα1–1 and GSTα1–2. The V_{max} values for these enzymes were all comparable, varying only 2-fold from highest to lowest value. The K_m values for both GSTα1–1 and GSTα1–2 were within the same order of magnitude determined in kinetic studies using freshly isolated rat renal PT cells and Eadie–Scatchard analysis for Line 1 (3.99 and 7.51 vs 2.91 mM, respectively). The K_m

value of 0.074 mM for GSTα2–2 is very close to that reported in kinetic studies with human kidney cytosol (K_m = 0.026 mM) [7]. Although only a single line is shown in the kinetic analysis of GSTα2–2 (Fig. 6C), the linear fit was not as good for this isoform as it was for the other two. In fact, the data points could readily be fit to two slopes. Such a biphasic, linear transformation typically is interpreted as indicating the function of multiple enzymes. Because this is a purified protein, however, another explanation is likely, namely that relatively high amounts of DCVG inhibit the enzyme. Data from kinetic studies in cytosol from PT cells are also consistent with end-product inhibition of GST activity.

Based on the K_m values reported for purified rat GSTs, we hypothesize that GSTα1–2 should be the major isoform to metabolize TRI at a concentration of 10 mM. This was demonstrated subsequently in the studies shown in Fig. 7. However, use of relatively selective GST inhibitors in cytosol from PT and DT cells (Fig. 9) and the determination of GSTα1 and GSTα2 expression (Fig. 10) indicate that in the intact renal cells, GSTα1–1 is likely the primary isoform responsible for GSH conjugation of TRI. The relatively high activity of purified GSTα1–2 suggests that its role in TRI metabolism in the intact renal cells needs further study. Data reported in this study are the first to describe the conjugation of TRI with GSH by individual GST isoforms. It should be noted that only three of the five known isoforms of rat GSTα were studied, so that we cannot exclude a role for the other two isoforms in TRI metabolism.

Role of P450 in Renal Metabolism and Cytotoxicity of TRI

Although P450-dependent metabolism of TRI in the kidneys has not been reported in the literature, we have found that several of the P450 isoforms that are capable of metabolizing TRI are expressed in rat renal PT and/or DT cells [28]. Hence, it was not surprising that inhibition of P450 altered TRI cytotoxicity in PT and DT cells. What was surprising was that inhibition of P450 significantly increased TRI cytotoxicity only in DT cells. In the absence of P450 inhibition, DCVG formation was detected only in PT cells and occurred at rates comparable to those measured in rat renal cortical cells (cf. Fig. 3) [5, 6]. Inhibition of P450 resulted in increases in DCVG formation in PT cells and detection of DCVG formation in DT cells at levels comparable to those in PT cells. The formation of DCVG in DT cells after inhibition of P450 is consistent with the increases in cytotoxicity after P450 inhibition. Inhibition of P450 in PT cells did not increase TRI cytotoxicity at any concentration despite increased DCVG formation. Reasons for this remain to be investigated, but other factors controlling DCVG metabolism were not studied.

While both PT and DT cells express CYP2E1 and CYP2B, CYP2C11 is detected only in PT cells, and expression of CYP2E1 is higher in DT cells [28]. Inhibition of CYP2E1 in PT and DT cells using the CYP2E1 competitive substrate chlorzoxazone resulted in a slight increase in DCVG formation in PT cells and a larger increase in DT cells. This increase was transient, being measured at 30 min and decreasing back to control values, or lower, after 60 min. These data are the first to show the involvement of P450 in TRI metabolism in individual cells of the rat kidney. The fact that CYP2E1 is involved in TRI metabolism in these cells is not surprising, as several studies have shown that CYP2E1 has a high affinity for TRI in the liver [31]. The role of other P450 isoforms, such as CYP2C11 and CYP2B, in TRI metabolism and toxicity needs to be explored further. Indeed, the metabolism data from the chlorzoxazone-pretreated cells provide support for a role for other P450 isoforms besides CYP2E1 in TRI metabolism. Inhibition of P450 with chlorzoxazone slightly increased GSH conjugation of TRI in PT cells but markedly increased GSH conjugation of TRI in DT cells. Thus, because CYP2E1 expression is higher in DT cells [28] and the effect of chlorzoxazone was greater in DT cells, P450 isoforms other than CYP2E1 must play significant roles in TRI metabolism, particularly in PT cells.

Extrapolation of Results from Rat Renal Cells to Human Kidney

Human kidneys under normal physiological conditions express GSTα and GSTπ (called GSTA and GSTP in humans [46]), and similar to the situation in rat kidney, GSTA is the predominant form [47]. Hence, the present findings on the role of rat GSTα in TRI metabolism and cytotoxicity should be applicable to humans. In contrast to this similarity, humans do not express CYP2E1 in the kidney [48]. It is unknown whether or not this would cause increased relative flux of TRI metabolism by the GSH-conjugation pathway in human kidney cells or whether other P450 isoforms are present in human kidney that could metabolize TRI. However, we have recently measured very low rates of P450-dependent metabolism of TRI in freshly isolated human PT cells [49], suggesting that renal P450 has little involvement in the metabolism and disposition of TRI in humans. Hence, prior or simultaneous exposure to P450 substrates, inducers, or inhibitors may have little direct effect on the potential nephrotoxicity of TRI in humans.

Based on the greater potential for competition between P450 and GST in rat kidney as compared with human kidney, one would expect a greater flux of TRI through the GSH-conjugation pathway and, hence, a greater potential for nephrotoxicity. As summarized in Table 3, although approximate rates of GST activity with TRI as substrate are actually modestly higher in human kidney than in rat kidney, rates of GGT and β-lyase activity in human kidney are only about 30 and 10%, respectively, of those in rat kidney. This suggests that the risk for humans from TRI-induced nephrotoxicity or nephrocarcinogenicity is significantly less than would be indicated from the rat data.

TABLE 3. Relative rates of TRI metabolism by key enzymes of the GSH-conjugation pathway

Enzyme	Rat	Human
GST	20	30
GGT	200	60
β-Lyase	10	1

Approximate, relative rates of TRI metabolism by key steps of the GSH-conjugation pathway are based on comparison of data in the present study and in Refs. 5–7 for rat and human GST activity, in Ref. 36 for rat GGT activity, unpublished data for human GGT activity, in Refs. 33 and 50 for rat β-lyase, and in Ref. 51 for human β-lyase.

Note, however, that it would be incorrect to conclude that there is little or no risk because ample evidence from clinical and epidemiological studies confirms the occurrence of renal injury in humans from TRI exposure.

In summary, the toxicity and metabolism of TRI in renal PT and DT cells and cytosol from male F344 rats were determined to gain insight into the mechanism of TRI toxicity and metabolism. TRI was acutely cytotoxic to both PT and DT cells only at high concentrations, but, under conditions in which P450 was not inhibited, DCVG formation was detected only in PT cells. Inhibition of P450 resulted in increases in both cytotoxicity and metabolism of TRI. Inhibition of CYP2E1 resulted in a small, transient increase in DCVG formation in PT cells and a larger, transient increase in DCVG formation in DT cells, indicating that P450, and possibly CYP2E1 in particular, plays a role in TRI toxicity and metabolism in individual cells of the rat kidney. The kinetics of TRI conjugation with GSH were measured in freshly isolated rat renal PT cells, and the apparent K_m and V_{max} values were within the range reported in other studies for rats and humans. This suggests that these cells are useful as models to study the toxicity and metabolism of TRI. These results report on the expression of GST isoforms in the rat kidney, and for the first time, the metabolism of TRI by individual members of the GST superfamily was studied. Members of the rat GSTα class conjugated TRI with GSH with different affinities.

Furthermore, for the first time, the effect of various inhibitors of rat GSTα members on DCVG formation by both purified rat GSTα class members and in cytosol isolated from rat renal PT and DT cells was studied. These data suggest a possible explanation for differences in TRI conjugation with GSH between rat renal PT and DT cells. Comparison of these data with those on other key enzymes involved in GSH-dependent bioactivation of TRI and similar data in human kidney tissue suggests that use of information obtained with rat kidney tissue overestimates the risk for humans.

This work was supported by National Institutes of Diabetes and Digestive and Kidney Diseases Grant R01-DK40725 and by a Cooperative Agreement with the U.S. Environmental Protection Agency (CR-824183). The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

References

1. Davidson IWF and Beliles RP, Consideration of the target organ toxicity of trichloroethylene in terms of metabolite toxicity and pharmacokinetics. *Drug Metab Rev* **23:** 493–599, 1991.
2. Maull EA and Lash LH, Trichloroethylene: A current review of metabolism, mode of action, and regulatory considerations. *Toxic Substance Mech* **17:** 153–169, 1998.
3. Anders MW, Lash LH, Dekant W, Elfarra AA and Dohn DR, Biosynthesis and metabolism of glutathione conjugates to toxic forms. *Crit Rev Toxicol* **18:** 311–341, 1988.
4. Goeptar AR, Commandeur JNM, Van Ommen B, van Bladeren PJ and Vermeulen NPE, Metabolism and kinetics of trichloroethylene in relation to toxicity and carcinogenicity. Relevance of the mercapturic acid pathway. *Chem Res Toxicol* **8:** 3–21, 1995.
5. Lash LH, Xu Y, Elfarra AA, Duescher RJ and Parker JC, Glutathione-dependent metabolism of trichloroethylene in isolated liver and kidney cells of rats and its role in mitochondrial and cellular toxicity. *Drug Metab Dispos* **23:** 846–853, 1995.
6. Lash LH, Qian W, Putt DA, Jacobs K, Elfarra AA, Krause RJ and Parker JC, Glutathione conjugation of trichloroethylene in rats and mice: Sex-, species-, and tissue-dependent differences. *Drug Metab Dispos* **26:** 12–19, 1998.
7. Lash LH, Lipscomb JC, Putt DA and Parker JC, Glutathione conjugation of trichloroethylene in human liver and kidney: Kinetics and individual variation. *Drug Metab Dispos* **27:** 351–359, 1999.
8. Lash LH, Putt DA, Brashear WT, Abbas R, Parker JC and Fisher JW, Identification of *S*-(1,2-dichlorovinyl)glutathione in the blood of human volunteers exposed to trichloroethylene. *J Toxicol Environ Health* **56:** 1–21, 1999.
9. Davis NJ, Wolman R, Milne FJ and van Niekerk I, Acute renal failure due to trichloroethylene poisoning. *Br J Ind Med* **46:** 347–349, 1989.
10. Nagaya T, Ishikawa N and Hata H, Urinary total protein and β-2-microglobulin in workers exposed to trichloroethylene. *Environ Res* **50:** 86–92, 1989.
11. Rasmussen K, Brogen CH and Sabroe S, Subclinical effects on liver and kidney function and solvent exposure. *Int Arch Occup Environ Health* **64:** 445–448, 1993.
12. Selden A, Hultberg B, Ulander A and Ahlborg G Jr, Trichloroethylene exposure in vapor degreasing and the urinary excretion of *N*-acetyl-beta-D-glucosaminidase. *Arch Toxicol* **67:** 224–226, 1993.
13. Brüning T, Vamvakas S, Makropoulos V and Birner G, Acute intoxication with trichloroethylene: Clinical symptoms, toxicokinetics, metabolism, and development of biochemical parameters for renal damage. *Toxicol Sci* **41:** 157–165, 1998.
14. IARC, Dry cleaning, some chlorinated solvents and other industrial chemicals. *IARC Monog Eval Carcinog Risks Hum* **63:** 75–158, 1995.
15. Anttila A, Pukkala E, Sallmen M, Hernberg S and Hemminki K, Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. *J Occup Med* **37:** 797–806, 1995.
16. Axelson O, Selden A, Andersson K and Hogstedt C, Updated and expanded Swedish cohort study of trichloroethylene and cancer risk. *J Occup Med* **36:** 556–562, 1994.
17. Spirtas R, Stewart P and Lee S, Retrospective cohort mortality study of workers at an aircraft maintenance facility. II. Exposures and the assessment. *Br J Ind Med* **48:** 531–537, 1991.
18. Henschler D, Vamvakas S, Lammert M, Dekant W, Kraus B, Thomas B and Ulm K, Increased incidence of renal cell tumors in a cohort of cardboard workers exposed to trichloroethene. *Arch Toxicol* **69:** 291–299, 1995.

19. Bloemen LJ and Tomenson J, Increased incidence of renal cell tumours in a cohort of cardboard workers exposed to trichloroethylene (Letter to the Editor). *Arch Toxicol* **70:** 129–130, 1995.
20. Henschler D, Vamvakas S, Lammert M, Dekant W, Kraus B, Thomas B and Ulm K, Increased incidence of renal cell tumours in a cohort of cardboard workers exposed to trichloroethene (Reply). *Arch Toxicol* **70:** 131–133, 1995.
21. Swaen GMH, Increased incidence of renal cell tumours in a cohort of cardboard workers exposed to trichloroethylene (Letter to the Editor). *Arch Toxicol* **70:** 127–128, 1995.
22. McLaughlin JK and Blot WJ, A critical review of epidemiology studies of trichloroethylene and perchloroethylene and risk of renal-cell cancer. *Int Arch Occup Environ Health* **70:** 222–231, 1997.
23. Blair A, Hartge P, Stewart PA, McAdams M and Lubin J, Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: Extended follow up. *Occup Environ Med* **55:** 161–171, 1998.
24. Morgan RW, Kelsh MA, Zhao K and Heringer S, Mortality of aerospace workers exposed to trichloroethylene. *Epidemiology* **9:** 424–431, 1998.
25. Vamvakas S, Brüning T, Thomasson B, Lammert M, Baumüller A, Bolt HM, Dekant W, Birner G, Henschler D and Ulm K, Renal cell cancer correlated with occupational exposure to trichloroethene. *J Cancer Res Clin Oncol* **124:** 374–382, 1998.
26. Lash LH and Tokarz JJ, Isolation of two distinct populations of cells from rat kidney cortex and their use in the study of chemical-induced toxicity. *Anal Biochem* **182:** 271–279, 1989.
27. Lash LH and Tokarz JJ, Oxidative stress and cytotoxicity of 4-(2-thienyl)butyric acid in isolated rat renal proximal tubular and distal tubular cells. *Toxicology* **103:** 167–175, 1995.
28. Cummings BS, Zangar RC, Novak RF and Lash LH, Cellular distribution of cytochromes P-450 in the rat kidney. *Drug Metab Dispos* **27:** 542–548, 1999.
29. Otieno MA, Baggs RB, Hayes JD and Anders MW, Immunolocalization of microsomal glutathione *S*-transferases in rat tissues. *Drug Metab Dispos* **25:** 12–20, 1997.
30. Rozell B, Hansson H-A, Guthenberg C, Tahir MK and Mannervik B, Glutathione transferases of classes α, μ and π show selective expression in different regions of rat kidney. *Xenobiotica* **23:** 835–849, 1993.
31. Miller RE and Guengerich FP, Metabolism of trichloroethylene in isolated hepatocytes, microsomes, and reconstituted enzyme systems containing cytochrome P-450. *Cancer Res* **43:** 1145–1152, 1983.
32. Nakajima T, Wang R-S, Elovaara E, Park SS, Gelboin HV and Vainio H, Cytochrome P450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethylene in liver microsomes. *Biochem Pharmacol* **45:** 1079–1085, 1993.
33. Lash LH, Sausen PJ, Duescher RJ, Cooley AJ and Elfarra AA, Roles of cysteine conjugate β-lyase and *S*-oxidase in nephrotoxicity: Studies with *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide. *J Pharmacol Exp Ther* **269:** 374–383, 1994.
34. Elfarra AA, Jakobson I and Anders MW, Mechanism of *S*-(1,2-dichlorovinyl)glutathione-induced nephrotoxicity, *Biochem Pharmacol* **35:** 283–288, 1986.
35. Jones DP, Sundby G-B, Ormstad K and Orrenius S, Use of isolated kidney cells for study of drug metabolism. *Biochem Pharmacol* **28:** 929–935, 1979.
36. Visarius TM, Putt DA, Schare JM, Pegouske DM and Lash LH, Pathways of glutathione metabolism and transport in isolated proximal tubular cells from rat kidney. *Biochem Pharmacol* **52:** 259–272, 1996.
37. Fariss MW and Reed DJ, High-performance liquid chromatography of thiols and disulfides: Dinitrophenol derivatives. *Methods Enzymol* **143:** 101–109, 1987.
38. Mannervik B, The isoenzymes of glutathione transferase. *Adv Enzymol Relat Areas Mol Biol* **57:** 357–417, 1985.
39. Birner G, Vamvakas S, Dekant W and Henschler D, Nephrotoxic and genotoxic *N*-acetyl-*S*-dichlorovinyl-*l*-cysteine is a urinary metabolite after occupational 1,1,2-trichloroethene exposure in humans: Implications for the risk of trichloroethene exposure. *Environ Health Perspect* **99:** 281–284, 1993.
40. Commandeur JNM and Vermeulen NPE, Identification of *N*-acetyl-(2,2-dichlorovinyl)- and *N*-acetyl-(1,2-dichlorovinyl)-L-cysteine as two regioisomeric mercapturic acids of trichloroethylene in the rat. *Chem Res Toxicol* **3:** 212–218, 1990.
41. Dekant W, Metzler M and Henschler D, Identification of S-(1,2-dichlorovinyl)-N-acetyl-cysteine as a urinary metabolite of trichloroethylene: A possible explanation for its nephrocarcinogenicity in male rats. *Biochem Pharmacol* **35:** 2455–2458, 1986.
42. Dekant W, Koob M and Henschler D, Metabolism of trichloroethylene—*In vivo* and *in vitro* evidence for activation by glutathione conjugation. *Chem Biol Interact* **73:** 89–101, 1990.
43. Green T, Dow J, Ellis MK, Foster JR and Odum J, The role of glutathione conjugation in the development of kidney tumours in rats exposed to trichloroethylene. *Chem Biol Interact* **105:** 99–117, 1997.
44. Hayes JD and Pulford DJ, The glutathione *S*-transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *CRC Crit Rev Biochem Mol Biol* **30:** 445–600, 1995.
45. Meyer DJ, Significance of an unusually low K_m for glutathione in glutathione transferases of the α, μ and π classes. *Xenobiotica* **23:** 823–834, 1993.
46. Armstrong RN, Structure, catalytic mechanism and evolution of the glutathione transferases. *Chem Res Toxicol* **10:** 2–18, 1997.
47. Rodilla V, Benzie AA, Veitch JM, Murray GI, Rowe JD and Hawksworth GM, Glutathione *S*-transferases in human renal cortex and neoplastic tissue: Enzymatic activity, isoenzyme profile and immunohistochemical localization. *Xenobiotica* **28:** 443–456, 1998.
48. Amet Y, Berthou F, Fournier G, Dreano Y, Bardou L, Cledes J and Menez J-F, Cytochrome P450 4A and 2E1 expression in human kidney microsomes. *Biochem Pharmacol* **53:** 765–771, 1997.
49. Cummings BS and Lash LH, Metabolism and toxicity of trichloroethylene and *S*-(1,2-dichlorovinyl)-L-cysteine in freshly isolated human proximal tubular cells. *Toxicol Sci*, in press.
50. Lash LH, Elfarra AA and Anders MW, Renal cysteine conjugate β-lyase: Bioactivation of nephrotoxic cysteine *S*-conjugates in mitochondrial outer membrane. *J Biol Chem* **261:** 5930–5935, 1986.
51. Lash LH, Nelson RM, Van Dyke RA and Anders MW, Purification and characterization of human kidney cytosolic cysteine conjugate β-lyase activity. *Drug Metab Dispos* **18:** 50–54, 1990.