Renal and Hepatic Toxicity of Trichloroethylene and Its Glutathione-Derived Metabolites in Rats and Mice: Sex-, Species-, and Tissue-Dependent Differences

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ABSTRACT

Acute cytotoxicity (lactate dehydrogenase release) of trichloroethylene (TRI), S-(1,2-dichlorovinyl)-L-cysteine (DCVC), and S-(1,2-dichlorovinyl)glutathione (DCVG) in freshly isolated renal cortical cells and hepatocytes from male and female rats was evaluated to test the hypothesis that the assay provides a valid indicator of sex- and tissue-dependent differences in sensitivity to TRI and its metabolites. We then determined mitochondrial toxicity (inhibition of state-3 and/or stimulation of state-4 respiration) in renal cortical and hepatic mitochondria from male and female rats and mice to assess sex-, tissue-, and species-dependent susceptibility. TRI was moderately cytotoxic in renal and female rats and mice to assess sex-, tissue-, and species-dependent differences. Two prominent target organs were the kidneys and liver, but also in the kidneys (Lash et al., 2000b). DCVG is subsequently processed by glutathione (GSH) conjugation (Lash et al., 2000b). Metabolites of TRI that are derived from the P450 pathway are associated with the liver as a target organ, whereas those derived from the GSH-conjugation pathway are associated with the kidneys and the hematopoietic system (Lock et al., 1996) as a target organ.

The first step in the GSH-conjugation pathway is catalyzed by GSH S-transferases (EC 2.5.1.18) and forms S-(1,2-dichlorovinyl)glutathione (DCVG) (Fig. 1). This reaction occurs predominantly in the liver, but also in the kidneys (Lash et al., 1995, 1998, 2000b). DCVG is subsequently processed by renal γ-glutamyltransferase (EC 2.3.2.2; GGT) and dipeptidase (EC 3.4.13.19) to form S-(1,2-dichlorovinyl)-L-cysteine (DCVC). DCVC may undergo bioactivation by the cysteine conjugate β-lyase (EC 4.4.1.13; β-lyase) to form a reactive intermediate, or detoxification by the cysteine conjugate N-acetyltransferase (EC 2.3.1.80) to form the mercapturate

Trichloroethylene (TRI; also known as trichloroethene) is a major environmental contaminant that is both an occupational concern and a potential concern for the general population because of its widespread use and designation as a "probable human carcinogen" (International Agency for Research on Cancer, 1995; Maull and Lash, 1998). TRI produces acute toxicity or tumors in several tissues, with the target organ specificity and sensitivity exhibiting species-, strain-, and sex-dependent differences. Two prominent target organs in humans and other animal species are the kidneys and liver (Bull, 2000; Lash et al., 2000a). Most TRI toxicity is dependent on bioactivation, which occurs by two pathways, cytochrome P450 (P450)-dependent oxidation and glutathione (GSH) conjugation (Lash et al., 2000b). Metabolites of TRI that are derived from the P450 pathway are associated with the liver as a target organ, whereas those derived from the GSH-conjugation pathway are associated with the kidneys and the hematopoietic system (Lock et al., 1996) as a target organ.

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ABBREVIATIONS: TRI, trichloroethylene; P450, cytochrome P450; GSH, glutathione; DCVG, S-(1,2-dichlorovinyl)glutathione; GGT, γ-glutamyltransferase; DCVC, S-(1,2-dichlorovinyl)-L-cysteine; β-lyase, cysteine conjugate β-lyase; NAcDCVC, N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine; FMO, flavin-containing monooxygenase; P344, Fischer 344; LDH, lactate dehydrogenase; RCR, respiratory control ratio.
N-acetyl-L-cysteine (NAcDCVC). DCVC may be regenerated from NAcDCVC by deacetylation, which is catalyzed by aminoacylase (EC 3.5.1.14) (Uttamsingh et al., 1998; Uttamsingh and Anders, 1999).

Besides bioactivation by the β-lyase, DCVC may also undergo sulfoxidation to form a reactive intermediate (RSS) that is nephrotoxic, or be N-acetylated by cysteine conjugate N-acetyltransferase (NAT) to the mercapturate NAcDCVC. NAcDCVC may also be deacetylated by a deacetylase (DA-case) activity to regenerate DCVC.

**Experimental Procedures**

**Materials.** TRI (reported to be 99.9% pure, as judged by electron ionization mass spectrometry), collagenase (EC 3.4.24.3) type I and type IV, and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO). DCVG and DCVC were synthesized from TRI and GSH or L-cysteine, respectively, using sodium thiosulfate and liquid ammonia as described previously (Elfarra et al., 1986). Purity of DCVG and DCVC was >95% and was determined by high performance liquid chromatography analysis with identity confirmed by proton NMR spectroscopy. All other chemicals were of the highest purity available and were obtained from commercial sources.

**Animals.** Male and female F344 rats (150–300 g) and male and female B6C3F1 mice (18–27 g) were purchased from Charles River Laboratories (Wilmington, MA). Animals were housed in a temperature- and humidity-controlled room in the Wayne State University vivarium on a 12-h light/dark cycle and were given commercial food and water ad libitum.

**Preparation of Isolated Renal Cortical Cells and Isolated Hepatocytes from Rats.** Suspensions of isolated renal cortical cells from rats were prepared by collagenase perfusion, as described previously (Jones et al., 1979; Lash, 1989; Lash et al., 1998). Cells were suspended in Krebs-Henseleit buffer supplemented with 25 mM HEPES, pH 7.4, 0.2% (w/v) bovine serum albumin, 2.5 mM CaCl₂, 5 mM glucose, and 5 mM glutamine. Suspensions of isolated hepatocytes from rats were prepared by collagenase perfusion, as described previously (Lash et al., 1998; Malddeus et al., 1978). Cells were suspended in Krebs-Henseleit buffer supplemented with 25 mM HEPES, pH 7.4, 0.2% (w/v) bovine serum albumin, and 2.5 mM CaCl₂. Cell suspensions were maintained in 25-mL polypropylene Erlenmeyer flasks in an atmosphere of 95% O₂, 5% CO₂. Cell concentrations were estimated by counting on a hemacytometer, and cell viabilities were estimated by determining the fraction of cells that excluded trypan blue (0.2%, w/v) on a hemacytometer or by determining the percentage of LDH release. By either method, initial cell viability was at least 85 and 90% for renal cortical cells and hepatocytes, respectively.

**Isolation of Mitochondria from Hepatic and Renal Cortical Homogenates from Rats and Mice.** Mitochondria were isolated essentially as described by Johnson and Lardy (1967), except that the buffer used (referred to as “mitochondrial buffer”) was 20 mM triethanolamine/HC1 (pH 7.4) containing 225 mM sucrose, 10 mM potassium phosphate (pH 7.4), 5 mM MgCl₂, 20 mM KC1, and 0.1 mM phenylmethylsulfonyl fluoride to inhibit proteolysis (kidney only). EGTA (2 mM) was included in all preparatory stages, except the final resuspension, to remove calcium ions.
Assay of Cytotoxicity in Isolated Hepatocytes and Kidney Cells. Isolated renal cortical or hepatocytes (2 x 10^6 cells/ml) were incubated in 25-ml polypropylene Erlenmeyer flasks with various concentrations of TRI, DCVG, or DCVC at 37°C in a Dubnoff metabolic shaking water bath (60 cycles/min) for up to 3 h (renal cells) or 4 h (hepatocytes). Flasks were sealed with rubber serum bottle stoppers, and the atmosphere in each flask was equilibrated with 95% O2, 5% CO2. Cell viability was determined by measurement of LDH release (Lash, 1989). Solvent vehicle for TRI (acetone, 1%, v/v) was added to cells as a control, and these cells exhibited no differences in LDH release or trypan blue exclusion from cells incubated only with buffer.

Assay for Hepatic and Renal Mitochondrial Toxicities of TRI and Its Metabolites. Isolated renal cortical mitochondria (1.5–5 mg of protein/ml for rats; 1–2 mg of protein/ml for mice) and hepatic mitochondria (8–10 mg of protein/ml for rats; 3–5 mg of protein/ml for mice) were incubated at 25°C for 30 min with various concentrations of TRI, DCVG, or DCVC. Incubations were performed in 25-ml polypropylene Erlenmeyer flasks sealed with rubber serum bottle stoppers. Mitochondrial oxygen consumption was measured using a Gilson 5/6H oxygraph. Aliquots (0.5 ml) of incubation mixtures were added to a thermostated, airtight, 1.6-ml chamber containing 1 ml of mitochondrial buffer. State-3 rates were measured by addition of 3.3 mM succinate and 0.3 mM ADP in the presence of 5 μM rotenone in ethanol (final ethanol concentration = 0.3%, v/v). State-4 rates were then measured as the rate of oxygen consumption after exhaustion of the ADP supply. Respiratory control ratio (RCR) values (state-3 rate/state-4 rate) were calculated as well.

Experimental Design. Isolated renal and hepatic cells from rats or isolated renal and hepatic mitochondria from rats and mice were exposed to a broad spectrum of concentrations of TRI, DCVG, or DCVC, ranging from 0.2 mM up to 10 mM. Comments and qualifications are needed concerning two issues, the use of isolated cells from rats only and the physiological relevance of the concentrations of toxicants that were used. First, isolated renal and hepatic cells were only used from the rat because it was impractical to use cells from mice, particularly for renal cells, because of inadequate yield of material. The low amount of material obtainable from mouse kidney or liver would make it difficult to perform a reasonable number of incubations with toxicant paired with controls. Second, although concentrations of TRI or its principal GSH-derived metabolites up to 10 mM were used in these investigations, only data from incubations with ≤1 mM toxicant are shown. This is because the attainable concentrations of TRI or its metabolites in the kidneys in vivo are likely to be in the micromolar range, although concentrations as high as 200 to 500 μM are reasonably attainable under conditions of accidental poisonings or industrial accidents. Some of the results from incubations with toxicant concentrations >1 mM will be discussed, although limitations on the interpretation of such results will be clearly stated.

Data Analysis. All values are means ± S.E. of measurements made on the indicated number of separate cell or mitochondrial isolations. Significant differences between means for data were first assessed by a one-way or two-way analysis of variance. When significant F values were obtained with the analysis of variance, the 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

Acute Renal Cytotoxicity in Rats

Suspensions of isolated renal cortical cells from male and female F344 rats were incubated for up to 3 h with a series of concentrations of TRI, ranging from 0.2 to 10 mM, to assess the acute cytotoxicity of the parent chemical; data for incubations with up to 1 mM TRI are shown (Fig. 2). Kidney cells from male rats exhibited moderate cytotoxicity from exposure to TRI, with the first significant increase in LDH release occurring with 0.5 mM TRI at the 2-h time point (33.4% for TRI-treated versus 22.1% for control). In contrast to these results, renal cells from female rats were much less susceptible than cells from male rats to TRI-induced cytotoxicity. No significant increases in LDH were observed over the 3-h incubations with up to 1 mM TRI; significant increases in LDH release were observed only at the 3-h time point with concentrations of TRI of ≥1 mM (data not shown).

Previous studies of ours (Lash et al., 1995) showed that both DCVG and DCVC produced significant increases in LDH release when these conjugates were incubated with suspensions of isolated renal cortical cells from male F344 rats. Male rat kidney cells exhibited maximal LDH release of
55.1% in 3-h incubations with 1 mM DCVG, compared with 29.1% for the respective control cells (Fig. 3A). Female rat kidney cells exhibited modestly lower sensitivity toward DCVG, with maximal LDH release of 50.4% in 3-h incubations with 1 mM DCVG, compared with 32.0% for the respective control cells (Fig. 3B).

Acute cytotoxicity of DCVC was markedly higher than that due to DCVG, as would be expected inasmuch as DCVC is a more proximate toxic metabolite (Lash and Anders, 1986). Male and female rat kidney cells exhibited maximal LDH release of 86.8 and 73.2% in 3-h incubations with 1 mM DCVC using cells from male and female rats, respectively, compared with 29.9% for the respective control cells from both male and female rats (Fig. 4). Besides the extent of cytotoxicity being greater for incubations with DCVC compared with those with DCVG, significant cytotoxicity was also observable for shorter incubation times (2 h) at lower concentrations of toxicant (0.2 mM). Incubations of rat kidney cells with 10 mM DCVC produced nearly complete cell death in 2 h in cells from male rats (data not shown).

**Acute Liver Cytotoxicity in Rats**

Although acute hepatotoxicity is not commonly observed with TRI, we examined the effect of incubations of up to 4 h with up to 10 mM TRI, DCVG, or DCVC on LDH release from male and female rat hepatocytes. This was done to provide a direct comparison with data from isolated kidney cells, which is the target cell for TRI metabolites derived from the GSH conjugation pathway. Furthermore, the liver is the primary site of formation of DCVG, although this first step in the pathway that leads to nephrotoxic metabolites can also occur in the kidneys (Lash et al., 1995, 1998). In contrast to results in isolated kidney cells, no significant increases in LDH release were observed in hepatocytes from either male or female rats incubated for up to 4 h with up to 10 mM TRI (data not shown).

Previous studies of ours (Lash et al., 1995) showed that DCVG produced a very modest increase in LDH release in male rat hepatocytes, whereas DCVC produced nearly 100% cell death in 4-h incubations with as low a concentration as 0.2 mM. In the present study, neither 0.2 nor 1 mM DCVG produced any significant increases in LDH release in incubations with male rat hepatocytes (Fig. 5A). However, incubations of female rat hepatocytes with 0.2 mM DCVG produced a modest, but statistically significant increase in LDH release after 3 and 4 h of incubation (36.1 and 43.0% for DCVG-treated cells at 3 and 4 h, respectively, versus 24.6 and 27.1% for control cells at 3 and 4 h, respectively) (Fig. 5B). In contrast to this apparent higher susceptibility of female rat hepatocytes to DCVG-induced cytotoxicity, incubations with 0.2 or 1 mM DCVC produced markedly greater increases in LDH release in male rat hepatocytes (Fig. 5, A and B).

**Mitochondrial Toxicity in Rats and Mice**

Inasmuch as previous work showed that the mitochondria are early and sensitive targets of nephrotoxic cysteine S-conjugates such as DCVC (Lash and Anders, 1986, 1987; Lash et al., 1986, 1995), effects of TRI, DCVG, and DCVC on respiratory function were compared in kidney and liver mitochondria from male and female rats and mice. These measurements were performed to determine whether tissue-, sex-, and species-dependent differences in susceptibility are reflected in acute effects on mitochondrial respiration. Mitochondrial toxicity was determined by measurement of effects on rates of state-3 and state-4 respiration and on the calculated RCR values.

**Rat Kidney and Liver Mitochondria.** Control respiratory rates were modestly, but significantly lower in isolated renal mitochondria from female rats compared with those in isolated mitochondria from male rats, although RCR values did not differ between the sexes (Fig. 6). Incubation of renal mitochondria of either sex for 30 min with 1 mM TRI had no effect on state-3 respiration. In contrast, 1 mM TRI significantly increased rates of state-4 respiration in renal mitochondria from both male and female rats (53.9 and 48.0% increase, respectively), leading to a 39.2 and 34.6% decrease in RCR in renal mitochondria from male and female rats, respectively. Both DCVG and DCVC produced significant decreases in rates of state-4 respiration, but no effects on rates of state-3 respiration, in renal mitochondria from both sexes of rats. Renal mitochondria from male rats exhibited...
larger decreases in rates of state-3 respiration with both conjugates (26.7 and 14.6% decrease in renal mitochondria from males and females, respectively, with 1 mM DCVG; 45.6 and 24.4% decrease in renal mitochondria from males and females, respectively, with 1 mM DCVC). RCR values showed similar decreases, with DCVG producing a 20.1 and 10.4% decrease in renal mitochondria from male and female rats, respectively, and DCVC producing a 44.3 and 25.7% decrease in renal mitochondria from male and female rats, respectively. As expected, DCVC was a significantly more potent inhibitor of state-3 respiration than either DCVG or TRI, consistent with DCVC being the more proximate toxic metabolite.

Incubation of liver mitochondria from male and female rats with 1 mM TRI caused a modest, 23.6% decrease in rates of state-3 respiration with both conjugates (26.7 and 14.6% decrease in renal mitochondria from males and females, respectively, with 1 mM DCVG; 45.6 and 24.4% decrease in renal mitochondria from males and females, respectively, with 1 mM DCVC). RCR values showed similar decreases, with DCVG producing a 20.1 and 10.4% decrease in renal mitochondria from male and female rats, respectively, and DCVC producing a 44.3 and 25.7% decrease in renal mitochondria from male and female rats, respectively. As expected, DCVC was a significantly more potent inhibitor of state-3 respiration than either DCVG or TRI, consistent with DCVC being the more proximate toxic metabolite.

Incubation of liver mitochondria from male and female rats with 1 mM TRI caused a modest, 23.6% decrease in rates of state-3 respiration in males but no significant change in females (Fig. 7). No significant effects on rates of state-4 respiration in liver mitochondria of either male or female rats were observed in incubations with 1 mM TRI. RCR values in liver mitochondria from male and female rats incubated with 1 mM TRI declined by 37.2 and 15.3%, respectively. Incubation with 1 mM DCVG produced no significant effects on rates of either state-3 or state-4 respiration or in the calculated RCR values in liver mitochondria from rats of either sex. This is consistent with the absence of significant DCVG metabolism in rat liver (Hinchman and Ballatori, 1990), which is required for DCVG to elicit toxicity. In contrast, 1 mM DCVC produced marked inhibition of state-3 respiration and decreases in RCR values in both male and female rat liver mitochondria that were comparable to those observed in renal mitochondria. Liver mitochondria from male rats were more sensitive to DCVC, as judged by the 61.6% decrease in RCR value in mitochondria form males and the 34.6% decrease in RCR value in mitochondria from females.

**Mouse Kidney and Liver Mitochondria.** To assess the sensitivity of mice to TRI, DCVG, and DCVC, respiratory function in isolated kidney (Fig. 8) and liver (Fig. 9) mitochondria from male and female mice were measured. Renal mitochondria from male mice were much less sensitive than those from male rats to DCVG- and DCVC-induced inhibition of state-3 respiration (Fig. 8A). Neither 1 mM TRI nor 1 mM
DCVG produced any significant effect on state-3 respiration in renal mitochondria from male mice, and 1 mM DCVC inhibited state-3 respiration by only 37.6%. Unlike results in renal mitochondria from male rats, 1 mM TRI had no effect and 1 mM DCVG and 1 mM DCVC each modestly (22.6 and 23.3%, respectively) increased rates of state-4 respiration in renal mitochondria from male mice (Fig. 8B). Consequently, although TRI had no effect on RCR values in renal mitochondria from male mice, DCVG and DCVC both significantly decreased RCR values (27.7 and 49.5% decrease, respectively) (Fig. 8C). In contrast to the pattern in renal mitochondria from rats, rates of state-3 respiration in renal mitochondria from female mice were significantly more sensitive than those from male mice and markedly more sensitive than those from female rats to DCVG and DCVC. Because neither DCVG nor DCVC affected rates of state-4 respiration, the decrease in RCR values induced by both conjugates was similar in renal mitochondria from mice of both males and females.

Liver mitochondria from both male and female mice appear to be much less sensitive than the sex-matched liver mitochondria from rats to inhibition of respiratory function by TRI and DCVG (Fig. 9). Rates of state-3 and state-4 respiration in control liver mitochondria from female mice were significantly lower (30.3 and 23.6% lower for state-3 and state-4, respectively) than those in control liver mitochondria from male mice. In the case of DCVC treatment, rates of state-3 and state-4 respiration and RCR values in liver mitochondria from male or female mice were decreased by similar fractions compared with the response in liver mitochondria from rats of the same sex. With the exception of a small (15.1%), but significant decrease in RCR value in liver mitochondria from female mice incubated with 1 mM DCVG, neither 1 mM TRI nor 1 mM DCVG had any effects on respiratory function in liver mitochondria from either male or female mice.

Discussion

Toxicity of TRI exhibits significant sex-, tissue-, and species-dependent differences (Davidson and Beliles, 1991). With the kidney as target organ, male rats are the most sensitive sex and species, and toxicity is associated with GSH-derived metabolites (Lash et al., 2000b). For the liver as target organ, male mice are the most sensitive sex and species, and toxicity is associated with P450-derived metabolites (Bull, 2000). To assess the sex, species, and tissue dependence of acute toxicity of TRI and its GSH-derived metabolites, two types of in vitro model systems for kidney and liver were used in this study, freshly isolated cells and isolated mitochondria. In the acute cytotoxicity experiments, toxicant concentrations of 0.2 to 1 mM were used, whereas a toxicant concentration of 1 mM was used in the mitochondrial respiration experiments. Although 1 mM is certainly at the upper
limit of concentrations that are attainable in environmental or occupational exposures, concentrations in the range of 0.2 to 0.5 mM are reasonably attained with various types of exposures.

The cytotoxic potencies of TRI, DCVG, and DCVC are summarized in Table 1. TRI itself is not a particularly potent, acute cytotoxicant. However, kidney cells from male rats are clearly more sensitive to TRI than are kidney cells from female rats or hepatocytes from rats of either sex. Because acute renal cellular injury from TRI is believed to be associated solely with metabolites derived from the GSH conjugation pathway and because the majority of flux of TRI metabolism is through the P450 pathway, particularly in the liver, the modest degree of cytotoxicity of TRI is expected. Although it is possible that some toxicity from TRI is due to reactive metabolites generated by P450 or FMO, this is unlikely to play a major role because TRI was not very toxic, even at high doses. This is particularly true in the kidneys, where P450 activity is very low. The requirement for DCVG to undergo additional metabolism to DCVC is consistent with DCVC being more potent and/or having a more rapid onset of toxicity in renal cortical cells than DCVG. Although kidney cells from male rats are only modestly more sensitive than kidney cells from female rats to DCVG, the difference in sensitivity between renal cells from male and female rats toward DCVC is much greater. The greater cytotoxic potency of DCVC compared with DCVG and the greater sensitivity of male compared with female rat kidney cells to DCVC may be partially accounted for by the pattern of sensitivity of isolated renal mitochondria to the S-conjugates, whereby more inhibition of state-3 respiration in renal mitochondria isolated from male rats than from either female rats or male or female mice was observed. Additional factors, such as sex- and species-dependent differences in rates of metabolism also likely play a role in determining sensitivity.

Although TRI exhibited no cytotoxicity in isolated hepatocytes from either male or female rats, DCVG exhibited a small degree of cytotoxicity in hepatocytes from female rats and DCVC was a potent cytotoxicant in hepatocytes from rats of both sexes (Table 1). In fact, DCVC produced a similar amount of LDH release from hepatocytes and kidney cells. Hepatotoxicity from DCVG or DCVC is normally not observed when either is administered in vivo (Elfarra et al., 1986). This is likely due to low GGT activity in the liver of most mammals (Hinchman and Ballatori, 1990) and the tissue distribution of transporters that effectively deliver GSH and cysteine conjugates to the kidneys (Lash et al., 1988). The modest degree of cytotoxicity of DCVG in isolated hepatocytes from female rats is likely due to the higher activity of GGT in the liver of female rats compared with that in male rats (Lash et al., 1998).

Because it was not practical to assess susceptibility of isolated cells from mice, suspensions of isolated mitochondria from renal cortical and hepatic homogenates were used to

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Fig. 7. Effects of TRI and its GSH-derived metabolites on respiratory function in isolated hepatic mitochondria from male and female F344 rats. Hepatic mitochondria (8–10 mg of protein/ml) from male or female F344 rats were incubated at 25°C for 30 min with either buffer or 1% (v/v) acetone as controls or 1 mM of either TRI, DCVG, or DCVC. Mitochondrial respiration was measured as state-3 (A) and state-4 (B) rates of oxygen consumption. RCR values (C; state-3/state-4) were then calculated. Results are means ± S.E. of measurements from three separate experiments. *Significantly different (P < 0.05) from the corresponding control value. †Significantly different (P < 0.05) from the correspondingly treated sample from male rats.
assess toxicity and compare sensitivity of rats and mice. We showed previously that TRI and DCVC produced potent, concentration-dependent inhibition of state-3 respiration in isolated mitochondria from both liver and kidney of male rats (Lash et al., 1995). Additionally, Stonard and Parker (1971a,b) used isolated mitochondria from the livers of male rats to study the metabolism and mechanism of toxicity of DCVC. Because the liver is not a normal, in vivo target organ for nephrotoxic cysteine S-conjugates, the hepatic mitochondrial toxicity of DCVC does not directly reflect on events that occur in the intact animal. However, these effects can provide mechanistic insight concerning toxicity.

The order of potency of inhibition of state-3 respiration in mitochondria from male rats was the same as that of acute cytotoxicity in isolated kidney cells from male rats, namely, that DCVC was the most inhibitory, followed by DCVG, and then TRI. Renal mitochondria from male rats were also more sensitive to DCVC-induced inhibition of state-3 respiration than renal mitochondria from female rats. This enhanced sensitivity may be a determinant of the sex dependence of DCVC-induced cytotoxicity in intact renal cells. The parent chemical TRI produced only a small decrease in state-3 respiration. However, unlike its S-conjugates, TRI significantly increased rates of state-4 respiration in renal mitochondria of both male and female rats, indicating uncoupling of oxidative phosphorylation. The absence of NADPH in incubations with mitochondria suggest that the modest inhibition of state-3 respiration and marked stimulation of state-4 respiration by TRI are metabolism-independent. In contrast, the presence of β-lyase activity in renal (Dohn and Anders, 1982; Lash et al., 1986; Stevens et al., 1988) and hepatic (Dohn and Anders, 1982; Stevens, 1985) mitochondria and the ability of aminooxyacetic acid, an inhibitor of pyridoxal phosphate-dependent enzymes, including the β-lyase, to prevent DCVC from inhibiting mitochondrial respiration (Lash et al., 1986), indicate that the mitochondrial toxicity of DCVC is dependent on its metabolism. The mitochondrial toxicity of DCVG is due to the presence of some contaminating GGT activity in the mitochondrial fraction (Lash et al., 1995, 1998).

Sensitivity of state-3 respiration to inhibition by DCVC in isolated mitochondria from male and female B6C3F1 mice was similar. In contrast, state-3 respiration in isolated mitochondria from female mouse kidneys was more sensitive than state-3 respiration in isolated mitochondria from both male mouse kidneys and female rat kidneys. In liver, in contrast, state-3 respiration in isolated mitochondria from male or female mice was much less sensitive to TRI and DCVG but was similarly sensitive to DCVC than that in isolated mitochondria from male or female rats. Thus, although mitochondrial dysfunction in rats correlates with acute cytotoxicity and the known, in vivo susceptibility, the same is not true for the mouse, although the relative insensitivity of mitochondria from male mouse kidney does agree

**Fig. 8.** Effects of TRI and its GSH-derived metabolites on respiratory function in isolated renal cortical mitochondria from male and female B6C3F1 mice. Renal cortical mitochondria (1–2 mg of protein/ml) from male or female B6C3F1 mice were incubated at 25°C for 30 min with either buffer or 1% (v/v) acetone as controls or 1 mM of either TRI, DCVG, or DCVC. Mitochondrial respiration was measured as state-3 (A) and state-4 (B) rates of oxygen consumption. RCR values (C; state-3/state-4) were then calculated. Results are means ± S.E. of measurements from three separate experiments. *Significantly different (P < 0.05) from the corresponding control value. †Significantly different (P < 0.05) from the correspondingly treated sample from male rats.
with the sex and species patterns of sensitivity observed in vivo.

Both TRI and DCVC are also cytotoxic to freshly isolated proximal tubular cells from human kidney (Cummings and Lash, 2000), suggesting that similar processes are occurring in human and rodent kidneys. Although human kidney cytosol contains β-lyase activity, albeit at much lower levels than rat kidney (Lash et al., 1990), our recent study (Cummings and Lash, 2000) suggested that a significant portion of DCVC-induced cytotoxicity is independent of β-lyase-dependent bioactivation. FMO-dependent bioactivation of DCVC may be another determinant of sex and species dependence of toxicity. FMOs, and in particular, the FMO3 isoenzyme, exhibit significant sex and species differences (Ripp et al., 1999a,b). Human FMO3 also exhibits population-specific polymorphisms (Cashman et al., 2000), suggesting that genetic polymorphisms may play a role in determining susceptibility to TRI- and DCVC-induced nephrotoxicity in humans.

**Fig. 9.** Effects of TRI and its GSH-derived metabolites on respiratory function in isolated hepatic mitochondria from male and female B6C3F1 mice. Hepatic mitochondria (3–5 mg of protein/ml) from male or female B6C3F1 mice were incubated at 25°C for 30 min with either buffer or 1% (v/v) acetone as controls or 1 mM of either TRI, DCVG, or DCVC. Mitochondrial respiration was measured as state-3 (A) and state-4 (B) rates of oxygen consumption. RCR values (C; state-3/state-4) were then calculated. Results are means ± S.E. of measurements from three separate experiments. *Significantly different (P < 0.05) from the corresponding control value. †Significantly different (P < 0.05) from the correspondingly treated sample from male rats.

**TABLE 1**
Summary of selected data on acute cytotoxicity of TRI and its GSH-derived metabolites in isolated kidney cells and hepatocytes from male and female F344 rats

Acute cytotoxicity of TRI, DCVG, and DCVC in freshly isolated kidney cells and hepatocytes from male and female F344 rats was determined by measurement of LDH release. Selected data are summarized in tabular form to fully illustrate sex- and tissue-dependent differences in sensitivity, and are taken from Figs. 2–5. Results are expressed as the difference of values from control incubations with buffer, and are means ± S.E. of measurements from three separate cell preparations. Net LDH release values are for 3-h incubations for kidney cells and 4-h incubations for hepatocytes.

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<tr>
<td>0.2 mM</td>
<td>24.2 ± 1.8a</td>
<td>12.4 ± 0.8a</td>
</tr>
<tr>
<td>1 mM</td>
<td>61.4 ± 3.6a</td>
<td>43.3 ± 3.1a</td>
</tr>
</tbody>
</table>

*a* Significantly different (P < 0.05) from 0-mM control.

*b* Significantly different (P < 0.05) from the corresponding sample from male rats.
Although neither the cellular nor mitochondrial toxicity of, nor the rates of formation of, DCVC sulfoxide were determined in the present study, it can be assumed that any sex-, tissue-, or species-dependent differences in these processes would be reflected in differences observed with DCVC. This is because DCVC serves as the penultimate, toxic metabolite that undergoes either $\beta$-lyase- or S-oxidase-dependent bioactivation, the former being predominant.

In conclusion, we have validated the hypothesis that the LDH release assay can be used to assess the relative sensitivity of isolated kidney and liver cells from male and female rats to TRI and its GSH-derived metabolites. Male rat kidney cells were significantly more sensitive than female rat kidney cells to acute cytotoxicity induced by both the parent chemical TRI and its GSH-derived metabolites. Somewhat surprisingly, the sensitivity to acute cytotoxicity induced by DCVC of isolated hepatocytes from either male or female rats was similar to that of the respective isolated kidney cells. This provides further evidence of the importance of the distribution of metabolites to target tissues and that the tissue-specific distribution of transporters is critical in determining target organ specificity in vivo. Studies in isolated mitochondria showed a similar pattern of sensitivity of state-3 respiration to inhibition by TRI, DCVG, and DCVC but also showed that TRI can produce significant uncoupling of respiration by a mechanism-independent mechanism. Thus, mitochondrial toxicity can occur by multiple mechanisms, both direct, physicochemical effects of TRI or by the specific interaction of reactive species with selected target macromolecules.

References

Stonard MD and Parker VH (1971a) 2-Oxocarbox dehydrogenases of rat liver mitochondria as the site of action of S-(1,2-dichlorovinyl)-L-cysteine and S-(1,2-dichlorovinyl)-3-mercaptopropionic acid. Biochem Pharmacol 202417–2427.

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