Cytotoxicity of $S$-(1,2-Dichlorovinyl)glutathione and $S$-(1,2-Dichlorovinyl)-L-cysteine in Isolated Rat Kidney Cells*

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The cytotoxicity of $S$-(1,2-Dichlorovinyl)glutathione (DCVG) and $S$-(1,2-dichlorovinyl)-L-cysteine (DCVC) produced time- and concentration-dependent cell death in isolated rat kidney proximal tubular cells. AT-125 blocked and glycylglycine potentiated the ionic toxicity, indicating that metabolism by $\gamma$-glutamyltransferase is required. $S$-(1,2-Dichlorovinyl)-L-cysteine (DCVG), a putative metabolite of DCVG, also produced cell death, which was prevented by 1,10-phenanthroline, phenylalanine, glycine, and aminoxyacetic acid, inhibitors of aminopeptidase M, cysteine conjugate $\beta$-lyase, and cysteine conjugate $\beta$-lyase, respectively. Aminoxyacetic acid and probenecid protected against DCVG toxicity, indicating a role for metabolism by cysteine conjugate $\beta$-lyase and organic anion transport, respectively. DCVG produced a small decrease in cellular glutathione concentrations and did not change cellular glutathione disulfide concentrations or initiate lipid peroxidation. DCVG caused a large decrease in cellular glutamate and ATP concentrations with a parallel decrease in the total adenine nucleotide pool; these changes were partially prevented by aminoxyacetic acid. Both DCVG and DCVC inhibited succinate-dependent oxygen consumption, but DCVC had no effect when glutamate + malate or ascorbate + N,N,N,N'-tetramethyl-p-phenylenediamine were the electron donors. DCVC inhibited mitochondrial, but not microsomal, Ca** sequestration. These alterations in mitochondrial function were partially prevented by inhibition of DCVG and DCVC metabolism and were strongly correlated with decreases in cell viability, indicating that mitochondria may be the primary targets of nephrotoxic cysteine $S$-conjugates.

The glutathione and cysteine $S$-conjugates of trichloroethylene, $S$-(1,2-dichlorovinyl)glutathione (DCVG) and $S$-(1,2-dichlorovinyl)-L-cysteine (DCVC), respectively, have been employed as model compounds to study halogenated hydrocarbon-induced nephrotoxicity (1). Both conjugates are potent and specific nephrotoxins and produce proximal tubular necrosis and increases in blood urea nitrogen and urine glucose concentrations (1-4).

The hypothesis that the nephrotoxicity of glutathione $S$-conjugates is dependent on their metabolism to the corresponding cysteine $S$-conjugates, which are metabolized to the ultimate toxic species by cysteine conjugate $\beta$-lyase (EC 4.4.1.13), has been validated by the elucidation of the metabolic pathway of DCVG and DCVC bioactivation and by use of specific enzyme inhibitors and nonmetabolizable analogues (1, 4, 5). DCVG, like other glutathione $S$-conjugates, is converted to the corresponding cysteine $S$-conjugate, DCVC, by $\gamma$-glutamyltransferase (EC 2.3.2.2) and cysteinylglycine dipeptidase (EC 3.4.13.6) or aminopeptidase M (EC 3.4.11.2), which are also responsible for degradation of GSH to cysteine (1, 6). Subsequent metabolism of DCVG, however, may follow two routes: N-acetylation, catalyzed by N-acetyltransferase, to form the mercapturic acid or a $\beta$-lyase-catalyzed $\alpha$,$\beta$-elimination to produce pyruvate, ammonia, and a reactive thiol (1, 7). A role for the $\beta$-lyase reaction in DCVG- and DCVC-induced nephrotoxicity has been established (1, 4).

Little is known about the mechanism by which DCVG and DCVC produce nephrotoxicity. Both compounds inactivate the organic ion transport system in rabbit renal proximal tubules (5, 8). DCVC causes a 50% decrease in nonprotein sulfhydryl groups in rabbit renal proximal tubules, and DCVC toxicity is potentiated by GSH depletion and is diminished by exogenous GSH (9). In rat kidney slices and in rat kidney and liver mitochondria, DCVC is a potent inhibitor of respiration (10, 11), indicating that mitochondria are targets within the cell. Specific interactions with individual enzymes also occur (12, 13) and may partially explain DCVC toxicity. What is lacking, however, is an analysis of the biochemical processes in the cells affected by DCVG and DCVC and the relationship of these effects to cytotoxicity.

To study the mechanism of $S$-conjugate nephrotoxicity in more detail, we have employed isolated rat kidney proximal tubular cells as an in vitro system. We report herein that DCVG and DCVC produced both time- and concentration-dependent cell death and that inhibition of mitochondrial function correlated with the observed cytotoxicity, indicating that mitochondria are primary targets. A preliminary report of some of this work has been presented (1).

EXPERIMENTAL PROCEDURES

Materials—Collagenase (type IV), aminoxyacetic acid (AOAA), 1,10-phenanthroline, phenylalanine, glycine, probenecid, digitoxin (recrystallized twice from hot ethanol), bovine serum albumin (fraction V), Percol, 1-fluoro-2,4-dinitrobenzene, A23187, and arsenazo III (purified as described by Kendrick (15)), TMPD, lactate
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dehydrogenase (EC 1.1.1.27), pyruvate kinase (EC 2.7.1.40), hexoki-

nase (EC 2.7.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and adenylate kinase (EC 2.7.4.3) were purchased from Sigma. AT-

25 (Aevicin, lot number 10985-DGM-153-15) was kindly provided by Dr. Ruth D. Devis, National Cancer Institute (Bethesda, MD). FCCP, a mem-

brane-permeant derivative of carbonyl cyanide 4-(trifluoromethoxy)phenol (FCCP), was obtained from Chemical Dynamics Corp. (South Plainfield, N.J.). DCVG and DCVC were synthesized by the method of McKinney et al. (16); S-[1,2-dichlorovinyl]-L-

cysteinylglycine (DCVCG) was synthesized by an analogous proce-

dure with 1-cystinyl-bis-glycine as a starting material. A more complete description of the synthesis and a chemical characterization of DCVCG will be reported elsewhere. BTC was synthesized by the method of Dohn and Anders (17).

Cell Isolation—Isolated rat kidney proximal tubular cells were prepared by the collagenase perfusion method of Jones et al. (18) from male Fischer 344 rats (200-300 g; Charles River Laboratories, Wilmington, MA). Cell concentration was determined in the presence of 0.2% (w/v) trypsin blue in a hemacytometer, and cell viability was measured by trypsin blue exclusion or by lactate dehydrogenase leakage into the medium. Typically, 85-95% of the cells excluded trypsin blue, and 10-18% of the total lactate dehydrogenase activity leaked from the cells. Cell yield was approximately 20 x 106 cells/kidney. Cells were incubated at 37°C in Krebs-Henseleit buffer, pH 7.4, supplemented with 25 mM Heps, 2.5 mM CaCl2, 25 mM NaHCO3, and 2% (w/v) bovine serum albumin. All buffers were equilibrated with 95% O2, 5% CO2.

Assays—β-Lyase activity was measured by the formation of 2-

maleonitrilotetrazolone from BTC, as previously described (17) except that substrate was dissolved in supplemented Krebs-Henseleit buffer, pH 7.4. Cells (1 x 106/ml) were incubated at 37°C with 0.1-2 mM BTC. After 2.5 min, 0.3 ml of 30% (w/v) trichloroacetic acid was added to 1.5 ml of cell suspension, and the absorbance of the acid extract was measured at 291 nm.

Cellular GSH, GSSG, and glutamate concentrations were measured by ion-exchange high-pressure liquid chromatography on a 10-μm Ul-

trasil-NH2 column (4.6 x 250 mm; Beckman Instruments) with a methanol-acetate solvent system according to the method of Reed et al. (19). After 30-35 min (at 37°C in supplemented Krebs-Henseleit buffer, pH 7.4, with the indicated additions. At various times, 1-ml samples of the cell suspension were centrifuged in an Eppendorf microcentrifuge for 1 min. The cells were resuspended in 0.5 ml of 0.9% (w/v) NaCl, and 0.1 ml of 30% (v/v) trichloroacetic acid was added. A sample (0.5 ml of the acid extract was neutralized with approximately 0.1 ml of 1 M K2CO3, and 0.1 ml of 40 mM iodoacetic acid was added. After 1 h of incubation at room temperature, 0.5 ml of 1.5% (v/v) 1-fluoro-2,4-dinitrobenzene in absolute ethanol was added, and the samples were incubated for 4 h in the dark. Derivatives were separated by gradient elution, were detected at 365 nm, and were quantified by comparison to standards. GSH (10 nmol/106 cells) was added during acid extraction to assess recovery; 9.4 nmol of GSH (69.0%) was recovered, and 8.6 nmol (91.5%) was in the form of GSSG (in GSH equivalents), indicating little autooxidation of GSH during the derivatization procedure.

For measurement of total cellular adenine nucleotide concentra-

tions, 0.2 ml of 30% (w/v) trichloroacetic acid was added at various times to 1-ml samples of cell suspensions (4-5 x 106 cells/ml) incubated at 37°C with supplemented Krebs-Henseleit buffer, pH 7.4, with the indicated additions. Acid extracts, neutralized with 5 M K2CO3 were analyzed for ATP (20) and ADP and AMP (21) by pyridine nucleotide-linked enzyme assays.

Cellular oxygen consumption was measured polarographically with a Clark-type electrode at 37°C. The electrode was calibrated with air-saturated buffer at 37°C, and zero oxygen concentration was obtained by addition of sodium sulfite. Measurements were begun by addition of 3.3 mM succinate, 4 mM glutamate + 2 mM malate, or 1 mM ascorbate + 0.2 mM TMPD as respiratory substrates to 1 x 106 cells. The endogenous rate of cellular oxygen consumption (i.e., in the absence of added respiratory substrates) in control cells was 15 nmol of O2/min/106 cells. At all time points examined (up to 3 h), endogenous rates of cellular oxygen consumption in the absence and presence of S-conjugates were 30-40% of succinate-stimulated rates.

For the measurement of cellular Ca2+ sequestration, isolated kidney cells (1 x 106/ml) were separated from the incubation medium by rapid centrifugation through a solution of Ca2+- and Mg2+-free Hanks’ solution (22) and Percoll (20%, v/v). The cells were then resuspended in the modified Hanks’ solution. Intracellular Ca2+ compart-

ementation was determined by dual-wavelength spectroscopy (654-685 nm) with an Amino DW-2C spectrophotometer and the metallochromic indicator arszena III (30 μM final concentration), as described by Di Monte et al. (23). FCCP (10 μM) and A23187 (15 μM) were then added sequentially, and the absorbance changes were recorded. The FCCP-releasable Ca2+ pool is equivalent to Ca2+ se-

questrated within mitochondria, and Ca2+ releases from mitochondria by addition of A23187, after FCCP-induced Ca2+ release, represents mainly the pool sequestered by the endoplasmatic reticulum (22, 24-26). In some experi-

ments, digitonin (12.5 μg/ml) was added together with FCCP to examine the function of plasma membrane Ca2+ transport (23). This low concentration of digitonin permeabilizes the plasma membrane without affecting intracellular membranes.

RESULTS

Isolated kidney cells were incubated with DCVG and cell viability, determined by trypan blue exclusion and lactate dehydrogenase leakage, was assessed at various times and at various DCVG concentrations (Fig. 1). DCVG (1 mM) reduced cell viability from approximately 90% at the start of the incubation to less than 40% at 4 h. Addition of 0.25 mM AT-

15, a potent inhibitor of γ-glutamyltransferase (27), protected the cells from DCVG toxicity. In contrast, addition of 1 mM glyoxyllic acid, a γ-glutamyl acceptor, caused a slight, but statistically significant, increase in DCVG cytotoxicity at 0.5 and 1 h. These results show that metabolism of DCVG by γ-glutamyltransferase is required for the expression of toxicity and indicate that the cysteinylglycine analogue DCVCG is the product of the above reaction. To test this point, the effects of 1,10-phenanthroline, an inhibitor of aminopeptidase M (28), and phenylalanine, an inhibitor of cysteinylglycine dipeptidase (6), on DCVG cytotoxicity were examined (data not shown). Both inhibitors protected the cells from DCVG toxicity, indicating that the ultimate toxin is generated by DCVCG metabolism. This conclusion was supported by studies with DCVCG itself; DCVCG produced cell death comparable to that produced by DCVG, and 1,10-phenanthroline and phenylalanine protected against DCVCG-induced cell death (data not shown).

After metabolism of DCVG to DCVCG, the next reaction in the bioactivation pathway is removal of the glycol residue to form the cysteine conjugate DCVC (1). Isolated kidney cells were incubated with DCVC, and cell viability was ass-

essed at various times and at various DCVC concentrations (Fig. 2). DCVC, at a concentration of 1 mM, reduced cell viability from approximately 90% to 35% during a 4-h incubation. Addition of the β-lyase inhibitor AOAA (1, 4) or the renal anion transport inhibitor probenecid protected the cells from DCVC toxicity, indicating that probenecid-sensitive

![Figure 1. Effect of DCVG on viability of isolated kidney cells.](image-url)

**A**, 100% viability; **B**, effects of various concentrations of DCVG on cell viability measured after 3 h.
was measured with BTC as the substrate. The apparent bation of isolated kidney cells with 1 mM DCVC for 30 min tubules to DCVC produced a 50% decrease in the content of DCVG and DCVCG toxicity, but had no effect on y-glutathionylation. Results are the mean ± S.E. of 4 cell preparations.

**TABLE I**

Effect of DCVC on cellular GSH, GSSG, and glutamate concentrations

| Addition          | GSH  | GSSG  | Glutamate |
|-------------------|------|-------|-----------|
|                   | nmol/10^6 cells |
| None              | 19.7 ± 2.4 | 1.7 ± 0.8 | 19.4 ± 3.4 |
| 0 min             | 15.8 ± 1.1 | 2.4 ± 0.3 | 26.2 ± 3.2 |
| 30 min            | 15.8 ± 1.1 | 2.4 ± 0.3 | 26.2 ± 3.2 |
| 1 mM DCVC, 30 min | 10.5 ± 1.6 | 1.9 ± 0.3 | 13.7 ± 2.6 |
| 1 mM DCVC + 0.1 mM AOAA, 30 min | 12.8 ± 2.8 | 18 ± 0.4 | 21.0 ± 2.4 |

transport and y-lyase-catalyzed metabolism are important in DCVC cytotoxicity. Furthermore, AOAA protected against DCVG and DCVC toxicity, but had no effect on y-glutamyltransferase activity (data not shown). AOAA (0.1 mM) or AT-125 (0.25 mM) alone produced less than a 10% loss in cell viability relative to control cells over the 4-h incubation. These results supported the hypothesis that DCVC bioactivation occurs by this sequence of reactions:

\[
\text{Glu} \xrightarrow{\text{DCVG}} \text{Gly} \xrightarrow{\text{DCVCG}} \text{DCVC} \xrightarrow{\text{pyruvate}} \text{NH}_2 + \text{reactive thiol}
\]

Because the y-lyase plays a central role in DCVG and DCVC nephrotoxicity, y-lyase activity in isolated kidney cells was measured with BTC as the substrate. The apparent \(K_m\) and \(V_{max}\) values were 0.7 mM BTC and 9.7 nmol of 2-mercaptoethanol formed/min/10^6 cells, respectively. \(K_m\) for AOAA inhibition of the y-lyase was 2 \(\mu M\).

Hassall et al. (9) reported that exposure of rabbit renal tubules to DCVC produced a 50% decrease in the content of nonprotein sulfhydryl groups. Hence the effect of DCVC on total cellular GSH, GSSG, and glutamate concentrations in rat kidney cells was determined (Table I). Glutamate concentrations were measured, because glutamate is normally present at relatively high concentrations in the kidney and serves as a good substrate for cellular energy production (29). Incubation of isolated kidney cells with 1 mM DCVC for 30 min caused a 34% reduction in the GSH concentration, a 48% reduction in the glutamate concentration, and no significant change in the GSSG concentration. Incubations up to 2 h produced no further decreases in GSH concentrations, but glutamate concentrations declined further to 35% of control (data not shown). The addition of 0.1 mM AOAA prevented most of the loss in glutamate and nearly half of the loss in GSH concentration. The modest decline in GSH concentration and the lack of a change in GSSG concentration due to exposure to DCVC indicate that production of an oxidative stress is probably not a primary mechanism of cysteine S-conjugate-induced cytotoxicity. In agreement with this conclusion, lipid peroxidation, as assessed by formation of thiobarbituric acid-reactive material, was not detected (data not shown).

The decrease in the glutamate concentration indicates that DCVC affects cellular energy metabolism. Therefore, the effect of DCVC on three parameters of mitochondrial function in isolated cells was examined: adenine nucleotide status, oxygen consumption, and \(Ca^{2+}\) compartmentation. Several nephrotoxic agents, such as aminoglycosides and heavy metals, and pathological conditions, such as renal ischemia, cause mitochondrial dysfunction (30). A prominent and early effect of these diverse agents is a dramatic fall in ATP concentrations with a parallel decline in total adenine nucleotide content.

Similarly, isolated kidney cells incubated with 1 mM DCVC for 30 min exhibited a 67% decline in both ATP and total adenine nucleotide concentrations (Table II). The large decrease in ATP concentration, combined with the moderate (30–40%) increase in ADP and AMP, caused the cellular ATP/ADP ratio and energy charge to fall, indicating that the ability of the cells to maintain ATP-dependent functions will be severely impaired. Addition of AOAA to inhibit DCVC bioactivation provided substantial protection against DCVC-induced alterations in adenine nucleotide status.

DCVG caused a time-dependent inhibition of succinate-dependent oxygen consumption, and addition of AT-125 prevented this effect (Fig. 3). Similarly, DCVC inhibited succinate-dependent oxygen consumption, and inhibition of DCVC metabolism by inclusion of AOAA partially prevented this effect (Fig. 4A). The concentration-response curve for DCVC inhibition of oxygen consumption was relatively steep; DCVC concentrations as low as 0.1 mM caused substantial inhibition (Fig. 4B).

The effect of DCVC on cellular oxygen consumption with respiratory substrates other than succinate was also studied (Table III). Incubation times of 2 h were chosen to compare the substrate specificity of DCVC-induced inhibition of cellular respiration because earlier incubation times, while giving qualitatively similar results, produced only modest inhibition of oxygen consumption. In contrast to metabolite levels (cf. Tables I and II), changes involving integrated cellular function, such as maintenance of cell viability and cellular respiration, require longer to occur. In contrast to the inhibitory effect of DCVC with succinate as respiratory substrate, little effect was observed when glutamate + malate or ascorbate + TMPD were the electron donors. This shows that DCVC specifically inhibits succinate oxidation.

Mitochondria and the endoplasmic reticulum play major roles in regulation of hepatic (22, 31) and renal (25, 32) intracellular \(Ca^{2+}\) homeostasis by sequestering \(Ca^{2+}\), thus buffering changes in cytosolic \(Ca^{2+}\) concentrations. Therefore, the effect of DCVC on cellular \(Ca^{2+}\) compartmentation was investigated (Fig. 5). Incubation of isolated kidney cells with 1 mM DCVC reduced mitochondrial \(Ca^{2+}\) sequestration by 62% in 2 h. In contrast, microsomal \(Ca^{2+}\) sequestration was not impaired by DCVC. The plasma membrane is also involved in intracellular \(Ca^{2+}\) homeostasis. Addition of a low
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TABLE II

| Addition             | ATP (nmol/10^6 cells) | ADP (nmol/10^6 cells) | AMP (nmol/10^6 cells) | ATP + ADP + AMP (nmol/10^6 cells) | ATP/ADP | Energy charge |
|----------------------|------------------------|------------------------|------------------------|----------------------------------|---------|--------------|
| None                 | 3.54 ± 0.60            | 1.20 ± 0.16            | 0.21 ± 0.02            | 4.95 ± 0.64                      | 2.95    | 0.84         |
| 0 min                | 2.83 ± 0.53            | 1.15 ± 0.06            | 0.31 ± 0.04            | 4.28 ± 0.58                      | 2.66    | 0.80         |
| 1 mM DCVC, 30 min    | 0.93 ± 0.18            | 1.53 ± 0.13            | 0.43 ± 0.06            | 2.88 ± 0.29                      | 0.61    | 0.59         |
| 1 mM DCVC + 0.1 mM AOAA, 30 min | 2.01 ± 0.59          | 1.31 ± 0.10            | 0.31 ± 0.03            | 3.63 ± 0.62                      | 1.53    | 0.73         |

FIG. 3. Inhibition of cellular oxygen consumption by DCVC. Cellular oxygen consumption was measured polarographically with a Clark-type electrode at 37 °C. Isolated cells (1 × 10^6/μl) were incubated in the absence or presence of 1 mM DCVC for 2 h. Measurements were begun by addition of respiratory substrates to the cell suspension. Results are the mean ± S.E. of 3 cell preparations. Standard errors were less than 10% of the means.

FIG. 4. Inhibition of cellular oxygen consumption by DCVC. Cellular oxygen consumption was measured polarographically with a Clark-type electrode at 37 °C. Measurements were begun by addition of 3.3 mM succinate to 1 × 10^6 cells. A, time course: control cells (○), 1 mM DCVC (●), 1 mM DCVC + 0.1 mM AOAA (▲). B, effects of various concentrations of DCVC on cellular oxygen consumption. Results are the mean of 4-5 cell preparations. Standard errors were less than 10% of the means.

concentration of digitonin, which permeabilizes the plasma membrane without affecting the mitochondrial and endoplasmic reticular membranes, caused only a slight increase (<10%) in FCCP-releasable Ca^2+ (data not shown). Thus, as was found in isolated hepatocytes (23), plasma membrane Ca^2+ transport mechanisms efficiently extruded Ca^2+ mobilized from mitochondria by FCCP. In cells treated with 1 mM DCVC, the amount of Ca^2+ released by FCCP, but not released from the cells in the absence of digitonin, was unchanged at

FIG. 5. Effects of DCVC on mitochondrial (A), microsomal (B), and total cellular (C) Ca^2+ sequestration. Cells (4-5 × 10^6/ml) were incubated in the absence (●) or presence (○) of 1 mM DCVC and were analyzed as described under "Experimental Procedures" for spectrophotometric measurement of the intracellular Ca^2+ pools released by the sequential addition of 10 μM FCCP and 15 μM A23187. Total cellular Ca^2+ is that released by FCCP and A23187. Results are the mean of 4 cell preparations. Standard errors were less than 10% of the means.
and aminopeptidase M, and the lyase verifies the suggested particularly relevant model.

Under certain conditions, DCVC produces an oxidative stress.

...concentrations without concomitant oxidation of GSH to... particularly active transport... an acute decline in ATP supply is not, however, associated with irreversible cell damage (39), indicating that perturbations in adenine nucleotide metabolism are not key events in DCVC-induced nephrotoxicity. Of greater significance than the decrease in ATP concentration produced by DCVC is the decline in the ATP/ADP ratio and the cellular energy charge (Table II). Concentration ratios, rather than concentrations of the individual components of the adenylate system, are important in regulation of energy metabolism (40). The energy charge is maintained within a narrow range in normal cells due to the kinetics of adenine nucleotide metabolism (30, 39, 40). As both ATP concentrations and the energy charge decrease, AMP deaminase and 5'-nucleotidase are activated, which leads to increases in IMP and adenosine concentrations and to an irreversible loss of AMP; the activation of these enzymes supports maintenance of the energy charge and thus normal cell function. The decrease in the size of the total adenine nucleotide pool observed in the present study indicates that similar processes occur in response to DCVC exposure.

The selective inhibitory effect of DCVC on oxygen consumption with different electron donors indicates that succinate oxidation is specifically inhibited in these cells. In agreement with this conclusion, DCVC inhibits succinate-cytochrome c oxidoreductase activity in rat kidney mitochondria. DCVC also inhibited the ability of mitochondria to regulate intracellular Ca**+** distribution, providing further support for the conclusion that DCVC-induced toxicity is primarily associated with mitochondrial dysfunction. Comparison of the rapid changes in the adenine nucleotide pool (Table II) with the slower time course of inhibition of mitochondrial Ca**+** sequestration (Fig. 5) indicates the importance of maintenance of Ca**+** homeostasis in the renal proximal tubule cell. Moreover, Ca**+** uptake takes priority over oxidative phosphorylation in the hierarchy of mitochondrial function, thereby effectively uncoupling oxidative phosphorylation (41).

Much recent work has focused on the role of Ca**+** in cellular injury and cell death. Maintenance of a low cytosolic Ca**+** concentration is essential for normal cell function (25, 31, 32). Farber and colleagues (42, 43) proposed that influx of extracellular Ca**+** leading to Ca**+** overload is the final common mediator of irreversible cell injury, although more recent studies support a critical role for redistribution of endogenous Ca**+** among the several pools within the cell as an important

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**FIG. 6. Relationship between effects of DCVC on renal cell viability and mitochondrial function.** Data from Figs. 2, 3, and 5 are replotted and analyzed by linear regression to examine the relationship between DCVC cytotoxicity and effects of DCVC on mitochondrial function. A, relationship between effects of 1 mM DCVG, DCVCG, and DCVC on cell viability and oxygen consumption; r = 0.989, slope = 1.45, y intercept = 34.5. B, relationship between effects of 1 mM DCVC on cell viability and mitochondrial Ca**+** sequestration; r = 0.999, slope = 21.90, y intercept = 30.4.
event in chemical-induced cytotoxicity (44, 45). Therefore, inhibition of mitochondrial Ca\(^{2+}\) sequestration may be a key component of the mechanism of DCVC nephrotoxicity.

Correlation analysis revealed a firm relationship between cell viability and cellular oxygen consumption or mitochondrial Ca\(^{2+}\) sequestration; hence DCVC-induced alterations in intracellular Ca\(^{2+}\) distribution can be attributed to a mitochondrial effect. Moreover, mitochondria account for most of the cellular oxygen consumption. These observations are consistent with the finding that DCVC lowered the cellular energy charge, which is highly dependent on mitochondrial respiration, and did not appear to produce an oxidative stress in renal proximal tubular cells.

In conclusion, this study demonstrates the usefulness of isolated rat kidney proximal tubular cells in the investigation of the mechanism of glutathione and cysteine S-conjugate-induced nephrotoxicity. More importantly, the studies reported herein validate and extend the hypothesis asserted above that the cytotoxicity of glutathione S-conjugates is dependent on metabolism to the corresponding cysteine S-conjugates, which undergo bioactivation by cysteine conjugate \(\beta\)-lyase to produce a reactive thiol, the presumed ultimate reactive species. Finally, the observation that the cytotoxicity of cysteine S-conjugates is associated with mitochondrial dysfunction, rather than with the production of oxidative stress, warrants detailed investigation.

Acknowledgment—We thank Lori J. Mittelstaedt for typing the manuscript.

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