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Interactive Toxicity of Inorganic Mercury and Trichloroethylene in Rat and Human Proximal Tubules:

Effects on Apoptosis, Necrosis, and Glutathione Status

Lawrence H. Lash, **David A. Putt**, **Sarah E. Hueni**, **Scott G. Payton**, and **Joshua Zwickl**
Department of Pharmacology, Wayne State University School of Medicine, Detroit, Michigan 48201

Abstract

Simultaneous or prior exposure to one chemical may alter the concurrent or subsequent response to another chemical, often in unexpected ways. This is particularly true when the two chemicals share common mechanisms of action. The present study uses the paradigm of prior exposure to study the interactive toxicity between inorganic mercury (Hg^{2+}) and trichloroethylene (TRI) or its metabolite *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) in rat and human proximal tubule. Pretreatment of rats with a subtoxic dose of Hg^{2+} increased expression of glutathione *S*-transferase-α1 (GSTα1) but decreased expression of GSTα2, increased activities of several GSH-dependent enzymes, and increased GSH conjugation of TRI. Primary cultures of rat proximal tubular (rPT) cells exhibited both necrosis and apoptosis after incubation with Hg^{2+}. Pretreatment of human proximal tubular (hPT) cells with Hg^{2+} caused little or no changes in GST expression or activities of GSH-dependent enzymes, decreased apoptosis induced by TRI or DCVC, but increased necrosis induced by DCVC. In contrast, pretreatment of hPT cells with TRI or DCVC protected from Hg^{2+} by decreasing necrosis and increasing apoptosis. Thus, whereas pretreatment of hPT cells with Hg^{2+} exacerbated cellular injury due to TRI or DCVC by shifting the response from apoptosis to necrosis, pretreatment of hPT cells with either TRI or DCVC protected from Hg^{2+}-induced cytotoxicity by shifting the response from necrosis to apoptosis. These results demonstrate that by altering processes related to GSH status, susceptibilities of rPT and hPT cells to acute injury from Hg^{2+}, TRI, or DCVC are markedly altered by prior exposures.

Keywords

inorganic mercury; trichloroethylene; interactive toxicity; apoptosis; necrosis; human proximal tubular cells; rat proximal tubular cells

Introduction

The study of chemical mixtures is an important, evolving subdiscipline of toxicology (Carpenter et al., 1998). Although mechanisms of toxicity are typically studied by exposure of a test system to a single chemical, the reality is that environmental contaminants rarely occur in isolation but generally exist as complex mixtures containing two or more components.

Address Correspondence To: 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

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Mechanisms of interactions between the various components of a mixture are often difficult to discern. As knowledge about specific mechanisms of action of individual chemicals increases, however, it becomes possible to test plausible hypotheses about how the presence of multiple chemicals alters cellular responses. For example, induction or inhibition of an enzyme responsible for the metabolism of one chemical by another chemical provides a mechanistic basis for understanding the cellular response to the combined or sequential presence of the two chemicals. Further, if two chemicals share a common mechanism of action (e.g., production of an oxidative stress), then prior or concurrent exposure to one chemical may be expected to alter the susceptibility of the target organ to the other chemical.

In the present studies, we assessed the interaction of the environmental contaminant trichloroethylene (TRI) and its penultimate, nephrotoxic metabolite *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) with inorganic mercury (Hg^{2+}) in rat and human proximal tubules. We hypothesize that prior exposure of rat or human proximal tubular (rPT or hPT, respectively) cells to one of the toxicants will influence the handling and acute cytotoxicity response from exposure to one of the other toxicants. The following discussion summarizes key mechanistic findings relating to Hg^{2+}-, TRI-, and DCVC-induced nephrotoxicity that provide a basis for our hypothesis.

The kidneys are the primary organs that accumulate Hg^{2+} and exhibit toxic effects after *in vivo* exposures to Hg^{2+} (Zalups, 1993). Within the kidneys, Hg^{2+} accumulates selectively along the three segments of the proximal tubule (Zalups, 1991a,1991b). Our recent studies in suspensions of freshly isolated rPT cells confirm that these cells accumulate Hg^{2+} following exposure to $HgCl_2$ or to various mercuric conjugates of thiols (Lash et al., 1998a) and these cells undergo necrotic cell death after exposure to Hg^{2+} with a very steep dose-response curve (Lash and Zalups, 1992). Both protein and non-protein sulfhydryl-containing compounds bind Hg^{2+} with high affinity and are the major ligands for Hg^{2+} in both the extracellular and intracellular space (Zalups and Lash, 1994). The cellular content of thiols, particularly that of glutathione (GSH), can modulate the intracellular uptake, cellular accumulation, and toxicity of Hg^{2+} in the renal proximal tubule (Baggett and Berndt, 1986;Berndt et al., 1985;Burton et al., 1995;de Ceaurriz et al., 1994;Girardi and Elias, 1993;Lash et al., 1998a,1999a;Zalups and Lash, 1997). Conversely, prior exposure of rats to Hg^{2+} alters cellular GSH status, with subtoxic concentrations increasing and toxic concentrations depleting GSH concentrations in the cortex and outer stripe of the outer medulla (Lash and Zalups, 1996;Zalups and Lash, 1990). Exposure of rat kidney to subtoxic doses of methyl mercury up-regulates GSH synthesis (Woods and Ellis, 1995;Woods et al., 1992). Although prior exposure of rats to Hg^{2+} also increases renal activities of several GSH-dependent enzymes and intrarenal concentrations of GSH (Lash and Zalups, 1996;Zalups and Lash, 1990), the same type of regulatory responses as shown by Woods and colleagues for methyl mercury have not been examined for Hg^{2+}. Hg^{2+} also produces oxidative stress in renal cortical mitochondria (Lund et al., 1993).

Humans may be exposed to mercury in its various forms, including Hg^{2+}, from breathing contaminated air, ingesting contaminated water and food, and by having dental and medical treatments. Hg^{2+} has been identified in at least 714 of the 1,467 SuperFund National Priorities List sites identified by the U.S. Environmental Protection Agency (ATSDR, 1999). Thus, besides being an occupational concern, due the widespread use of mercurials in various industrial processes, Hg^{2+} is a public health concern as well.

TRI is an environmental and industrial pollutant whose toxicity and carcinogenicity have been demonstrated in several animal species, including humans (Davidson and Beliles, 1991). The kidneys are one target organ for TRI, although much controversy exists about its importance for humans, as significant species differences exist in susceptibility (Lash et al., 2000a). Renal effects of TRI are generally attributed to its conjugation with GSH and subsequent metabolism

within the proximal tubules to generate DCVC, which is further metabolized to a reactive intermediate (Lash et al., 2000b). Thus, the renal disposition and toxicity of TRI are dependent on GSH status (Lash et al., 1995a,1998b). DCVC-induced cytotoxicity in rPT and hPT cells occurs by both necrosis and apoptosis, with the former being a relatively high-dose (≥ 100 μM) response and the latter being a relatively low-dose (≤ 100 μM) and early (≤ 8 h) response (Cummings and Lash, 2000;Cummings et al., 2000a;Lash et al., 1995a;Lash et al., 2001a, 2001b). Mitochondria are a prominent and early target for DCVC-induced cytotoxicity in renal proximal tubules (Lash et al., 1995a,2001a).

Hence, mechanistic studies of Hg^{2+}- and TRI- or DCVC-induced toxicity in the kidneys demonstrate several common pathways and responses, including the kidneys as a primary target organ, a role for GSH in metabolism, transport, and cellular response, production of an oxidative stress, and the potent and early targeting of mitochondria. Accordingly, we hypothesize that these common, mechanistic features provide one level of rationale that prior exposure of rat or human kidneys to either Hg^{2+}, TRI, or DCVC will influence the cellular response to a subsequent exposure to one of the chemicals. Additional rationale for studying interactions among Hg^{2+} and TRI or its metabolite DCVC, is that both Hg^{2+} and TRI are likely to be found together in many of the designated SuperFund waste sites. Thus, joint exposure is likely.

Initial studies were conducted in rat kidney and rPT cells to further establish the rationale to conduct studies in hPT cells, using the paradigm of prior exposure to one chemical followed by subsequent exposure to another chemical. All of our previous data on the metabolic and toxic effects of Hg^{2+} have been obtained with tissue or cells from rats. Although much of our previous studies with TRI and its metabolite DCVC have been conducted in rat proximal tubules, the more recent work has been done in human proximal tubules. This is important because rats are not a good surrogate, test species for humans in terms of their response to chemicals such as TRI. After demonstrating that exposure of rat kidney, both *in vivo* and *in vitro*, to Hg^{2+} produces changes in expression of enzymes or other processes that are likely to affect the metabolism and/or toxicity of TRI or DCVC, studies were then conducted in hPT cells. hPT cells were pretreated with either Hg^{2+} or TRI (DCVC) and then incubated with TRI (DCVC) or Hg^{2+}, respectively, to determine whether the order of exposure influences the cellular response. The results show that prior exposures to one of the three chemicals markedly alters susceptibility to subsequent exposures and that results vary significantly with the order by which cells are exposed to the chemicals.

Methods

Chemicals and reagents

TRI was purchased from Sigma Chemical Co. (Cat. No. T4928, ACS reagent, > 99.5% purity). DCVG, DCVC, and NAcDCVC were synthesized from TRI and GSH, L-cysteine, or *N*-acetyl-L-cysteine, respectively, in sodium metal and liquid ammonia as described previously (Elfarra et al., 1986). Purity (> 95%) was assessed by HPLC and thin layer chromatography and confirmed by ^{1}H-NMR. Antibodies for GSTα(A) and GSTP were purchased from Oxford Biomedical (Oxford, MI). Antibody for GSTT was purchased from Biotrin International (Newton, MA). Note that the convention used for naming GST isoforms is that Greek letters are used for rat enzymes whereas Arabic letters are used for the analogous human enzymes. The GSTα(A) antibody was a polyclonal goat anti-rat GST Ya antibody, prepared against affinity-purified rat liver GST Ya, is specific for rat and human GSTα(A), and cross-reacts with neither GSTμ(M) nor GSTπ(P) class isoforms; the GSTP antibody was a polyclonal rabbit anti-human GST P1-1 antibody, prepared against recombinant human GST P1-1 that was expressed in *E. coli*, is specific for rat and human GSTπ(P), and cross-reacts with neither GSTμ(M) nor GSTα(A) class isoforms; the GSTT antibody was a polyclonal rabbit anti-human

GSTT antibody, prepared against purified human liver GSTT2, is specific for rat and human GSTθ(T), and cross-reacts with neither GSTα(A), GSTπ(P), nor GSTμ(M) class isoforms. Expression of GSTM in hPT cells was not determined because previous work showed that these cells express GSTA, GSTP, and GSTT, but not GSTM (Cummings et al., 2000b). All other chemicals for cell isolation and culture and for various assays were purchased from commercial vendors and were of the highest purity available.

Studies in rats

Male Fischer 344 rats (200-250 g; Charles River Laboratories, Wilmington, MA) were used for both *in vivo* studies examining the effect of pretreatment with a subtoxic dose of Hg^{2+} on GST expression and activities of GSH-dependent activities and for isolation of rPT cells. Rats were allowed to acclimate for at least three days prior to conducting any studies, fed commercial rat chow and water *ad libitum*, and housed in a temperature-, humidity-, and light-controlled (12-hr light/dark cycle) environment in the Wayne State University vivarium. All studies were approved by the institutional Animal Investigation Committee and were conducted according to NIH guidelines. For the *in vivo* Hg^{2+}-pretreatment studies, rats were given either an ip injection of 0.5 μmol $HgCl_2$/kg body weight in normal saline, which produces no signs of nephrotoxicity (Zalups and Lash, 1990), or normal saline as a control. After 24 or 48 h, rats were anesthetized with sodium pentobarbital (50 mg/kg body weight), kidneys were removed, and cortex and outer stripe of the outer medulla were homogenized in 3 ml of buffer (250 mM sucrose, 10 mM triethanolamine/HCl, pH 7.6, 1 mM EDTA Na_2, 0.1 mM phenylmethylsulfonyl fluoride) per g tissue using a Teflon-glass device. Homogenates were initially centrifuged at 9,000 × *g* for 20 min. Supernatants were then centrifuged for 60 min at 105,000 × *g*. The resulting supernatants (cytosolic fractions) were used for enzyme assays or Western blot analyses and stored at -80°C until use.

Isolation and primary culture of rPT cells

rPT cells were isolated from male Fischer 344 rats by collagenase perfusion and Percoll density-gradient centrifugation, as described previously (Lash and Tokarz, 1989;Lash et al., 1995b). Typical yield of rPT cells from two rat kidneys is 40-50 × 10^6 cells. Only cells that exclude > 90% trypan blue or exhibit < 10% leakage of lactate dehydrogenase (LDH) were used for subsequent studies either as freshly isolated cells in suspension or for primary culture. For studies with suspensions of freshly isolated cells, cells were suspended in Krebs-Henseleit buffer containing 25 mM $NaHCO_3$, 25 mM HEPES, and 2% (w/v) bovine serum albumin. Incubations with cell suspensions were conducted in polypropylene Erlenmeyer flasks on a Dubnoff metabolic shaking incubator (60 cycles/min) under an atmosphere of 95% O_2/5% CO_2 at 37°C.

Isolation and primary culture of hPT cells

hPT cells were isolated from fresh human kidneys by a modification of the procedure described by Todd et al. (1995) as described previously (Cummings and Lash, 2000;Cummings et al., 2000b). Typical yield of hPT cells from one human kidney is 500-800 × 10^6 cells. Only cells that exclude > 90% trypan blue or exhibit < 10% leakage of LDH were used for subsequent primary culture. Human kidneys were purchased from Life Legacy Foundation (Tucson, AZ; formerly International Bioresearch Solutions). Kidney tissue was determined to be normal by a pathologist (i.e., derived from non-cancerous, non-diseased tissue) and was screened for HIV, hepatitis A, B, and C, and CMV. Kidneys were perfused with Wisconsin medium and kept on wet ice until they arrived at the laboratory, which was within 24 h of removal from the donor. Age, gender, and race information, but no other identifying information, was obtained. Because tissue was generally obtained from accident victims or those who have died from cardiovascular incidents and the tissue was rejected for use in transplants (generally because

of excessive glomerulosclerosis or arterial plaque), we restricted our donors to adults (age ≥ 21 years).

Cell culture medium and general cell culture procedures

Basal medium and supplements for rPT and hPT primary culture were as described previously (Cummings et al., 2000b;Lash et al., 1995b). Basal medium was a 1:1 mixture of Dulbecco's modified Eagle's medium : Ham's F-12 (DMEM : F-12) without serum. Medium was supplemented with 15 mM HEPES, pH 7.4, 20 mM $NaHCO_3$, antibiotics for Day-0 through Day-3 only (192 IU penicillin G/ml + 200 μg streptomycin sulfate/ml) to inhibit bacterial growth, 2.5 μg amphotericin B/ml to inhibit fungal growth, 5 μg bovine insulin/ml (= 0.87 μM), 5 μg human transferrin/ml (= 66 nM), 30 nM sodium selenite, 100 ng hydrocortisone/ml (= 0.28 μM), 100 ng epidermal growth factor/ml (= 17 nM), and 7.5 pg 3,3′,5-triiodo-DL-thyronine/ml (= 111 nM). Medium was prepared from powder using doubly-distilled, deionized water. Before addition of supplements, medium was filtered through 0.22-μm porosity cellular acetate membrane filters for sterilization.

rPT and hPT cells were seeded at densities of 1×10^6 cells/ml on Vitrogen-coated, 35-mm dishes or polystyrene culture flasks (T-25 or T-75), depending on the needs of the experiment (= Day 0). Volumes were 1.5 ml, 3 ml, and 11 ml for 35-mm dishes, T-25 flasks, and T-75 flasks, respectively. Cultures were grown at 37°C in a humidified incubator under an atmosphere of 95% air/5% CO_2 at pH 7.4. After 24 h (= Day-1), dishes or flasks were washed with PBS to remove unattached cells (generally 50 to 60%, based on protein content in dishes and media; Lash et al., 1995b) and fresh medium was added. Medium was changed every two days thereafter until confluence (generally by Day 4-6). After Day-3, antibiotics were omitted from medium as they inhibit growth. When necessary, cells were harvested from dishes by either scraping the plates with a Teflon scraper or by brief treatment with trypsin-EDTA. All experiments were performed with confluent cells.

Metabolism and enzyme assays

All incubations for metabolism and enzyme assays were performed at 37°C. GSH-dependent enzyme activities [γ-glutamylcysteine synthetase (GCS), GSH peroxidase (GPX) with 0.25 mM H_2O_2 as substrate, GSH *S*-transferase (GST) with 1 mM 1-chloro-2,4-dinitrobenzene as substrate, and glutathione disulfide reductase (GRD)] were measured by spectrophotometric assays as described previously (Cummings et al., 2000b). GSH conjugation of TRI to form DCVG was measured by HPLC (Lash et al., 1999b) after derivatization to form the *N*-dinitrophenyl derivative. Derivatives were detected by absorbance at 365 nm and were compared to authentic standards (limit of detection = 50 pmol).

Western blot analyses of GSTα expression in rat kidney cytoplasm and GSTA, GSTP, and GSTT expression in hPT cells

GST isozyme expression levels were detected by subjecting samples derived from rat kidney cytoplasm or total cell extracts of hPT cells to SDS-PAGE on a 10% slab gel followed by electrophoretic transfer of material separated on the gel to nitrocellulose membranes (Cummings et al., 2000c). The membranes were then immunostained with the appropriate antibody. Alkaline phosphatase staining intensity was determined by densitometry using Image J software.

Assay of necrosis by LDH release and activity

Cell death by necrosis was determined by measurement of either the release of LDH from cells or by decreases in total LDH activity (Lash and Zalups, 1992;Lash et al., 1995b). To measure LDH release in cell cultures, LDH activity was determined in media and, after removal of

media, washing of cells with PBS, and solubilization of cells with 0.1% (v/v) Triton X-100, in total cells, by addition of pyruvate and NADH and following the decrease in A_{340}. The fraction of LDH release was calculated by the formula: % LDH release = [LDH activity in media/(LDH activity in media + LDH activity in total cells)] × 100%. For most experiments involving pretreatment or treatment with Hg^{2+}, cellular necrosis was assessed by decreases in total LDH activity instead of LDH release, because Hg^{2+} directly inhibits LDH (Lash and Zalups, 1992). We showed in our previous study that Hg^{2+}-induced decreases in total LDH activity correspond to other indices of cell death by necrosis and could thus be used as a marker. Total LDH activity was calculated as the sum of activity in the media and that in total solubilized cells, using the extinction coefficient for NADH of 6,220 mM^{-1} cm^{-1}.

Assay of apoptosis by propidium iodide staining and flow cytometry

Cell cultures were washed twice with sample buffer (PBS plus glucose (1 g/l) filtered through a 0.22-μm filter), dislodged by trypsin/EDTA (0.1% w/v) incubation, centrifuged at 400 × *g* for 10 min, and resuspended in sample buffer. Cell concentrations were adjusted to 1 to 3 × 10^6 cells/ml with sample buffer and 1 ml of cell suspension was centrifuged at 400 × *g* for 10 min. All of the supernatant except 0.1 ml/10^6 cells was removed and the remaining cells were mixed on a vortex mixer in the remaining fluid for 10 sec. Next, 1 ml of ice-cold ethanol (70%, v/v) was added to the sample drop by drop, with samples being mixed for 10 sec between drops. The tubes were capped and fixed in ethanol at 4°C. After fixation, cells were stained in propidium iodide (50 μg/ml) containing RNase A (100 U/ml). Samples were then mixed, centrifuged at 1,000 × *g* for 5 min and all the ethanol except 0.1 ml was removed. Cells were mixed in the residual ethanol and 1 ml of the propidium iodide staining solution was added to each tube. After mixing again, cells were incubated at room temperature for at least 30 min. Samples were analyzed within 24 hr by flow cytometry using a Becton Dickinson FACSC*alibur* Flow Cytometer, which is a core facility of the NIEHS Center for Molecular Toxicology with Human Application at Wayne State University. Analysis was performed with 20,000 events per sample using the ModFit LT v. 2 for Macintosh data acquisition software package (Verity Software House, Inc., Topsham, ME; distributed by Becton Dickinson Immunocytometry System BDIS, San Jose, CA). Propidium iodide was detected by the FL-2 photomultiplier tube. Fractions of apoptotic cells were quantified by analysis of the sub-G_1 (sub-diploid) peak with ModFit cell cycle analysis. The percent distribution of cells in the various stages of the cell cycle (G_0/G_1, S, G_2/M) were also calculated. Cell aggregates were discarded in the flow cytometry analysis by post-fixation aggregate discrimination.

Protein determination and data analysis

Protein concentrations were measured with the bicinchoninic acid protein determination kit from Sigma. All values are means ± SE of measurements made on the indicated number of separate preparations. Significant differences between means for data were first assessed by a one-way analysis of variance. When significant “F values” were obtained, 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

Studies in rat kidney and primary cultures of rPT cells

Experiments were first conducted in the rat to provide further evidence for a basis for studying interactions between Hg^{2+} and TRI and its nephrotoxic metabolite DCVC. Limited studies were conducted in rats and primary cultures of rPT cells to examine the influence of pretreatments with Hg^{2+} on GSH-dependent metabolism and to establish the acute cytotoxicity of Hg^{2+} in primary cultures of rPT cells.

Effects of pretreatment of rats with a subtoxic concentration of $HgCl_2$ on renal GSH-dependent enzymes and GSH conjugation of TRI—Male F344 rats were given an ip injection of either saline (= Control) or a subtoxic dose of $HgCl_2$ (0.5 μmol/kg body weight). After 24 or 48 h, cytoplasm was isolated from kidney cortical homogenates by differential centrifugation and levels of expression of GSTα protein were determined by Western blot analysis (Figure 1). No effects were observed in GSTα1 or GSTα2 protein levels after 24 h of exposure to Hg^{2+}. In contrast, a 48-h exposure to Hg^{2+} increased GSTα1 protein levels by approximately 50% but decreased GSTα2 protein levels by approximately 50%.

To further assess effects of Hg^{2+} pretreatment on renal GSH status, rats were pretreated for 24 or 48 h with 0.5 μmol $HgCl_2$/kg body weight, renal cortical cytoplasm was isolated, and activities of four GSH-dependent enzymes were measured (Table 1). Specific activities of each of the four enzymes, GST, GRD, GPX, and GCS, were significantly increased at both time points by pretreatment with Hg^{2+}. The extent of the increases varied somewhat among the enzymes, ranging from 25% to 75%. The increase in GST activity is consistent with the increased expression of GSTα1 protein expression.

These results showing increased GSH-dependent metabolism after exposure to Hg^{2+} support the hypothesis that low or subtoxic doses of Hg^{2+} alter cellular GSH and antioxidant defense status in rat kidney. These findings then led us to the *hypothesis* that prior exposure to low or subtoxic doses of Hg^{2+} will influence the GSH-dependent metabolism of or cellular response to other chemicals whose metabolism involves GSH or whose mechanism of toxicity involves alterations of GSH status, such as TRI. To test the hypothesis that prior exposure to a sub-toxic dose of Hg^{2+} alters GSH-dependent metabolism of TRI, cytoplasm from rat kidney cortex of control and Hg^{2+}-pretreated rats were incubated with TRI for up to 60 min and formation of DCVG was measured (Figure 2). Preincubation of rats with Hg^{2+} produced 25% to 60% increases in rates of DCVG formation, depending on TRI concentration and preincubation time. Analysis of the kinetics of DCVG formation by Eadie-Hofstee plots showed that preincubation with Hg^{2+} produced little change in K_m for TRI but increased V_{max} by 55% and 34% after 24 h and 48 h of Hg^{2+} preincubation, respectively (Table 2).

Hg^{2+} increases activities of GSH-dependent enzymes in primary cultures of rPT cells—To assess whether similar responses to Hg^{2+} preincubation can be achieved in primary cell cultures, confluent, rPT cell primary cultures were preincubated for 24 h with various concentrations of $HgCl_2$ and activities of four key GSH-dependent enzymes were measured (Figure 3). At the lowest concentration of Hg^{2+} used (0.5 μM), GST specific activity was increased by 81% and GRD activity was increased by 248% as compared to control cells. Although GCS activity was not significantly increased by preincubation of rPT cells with 0.5 μM Hg^{2+}, it was significantly increased at all other concentrations of Hg^{2+}, with a maximal increase of 115% at 5 μM Hg^{2+}. GST activity exhibited a maximal increase of 165% at 1 μM Hg^{2+}. In contrast, GPX activity was not significantly changed by any concentration of Hg^{2+}.

Hg^{2+} is a potent inducer of necrosis and apoptosis in primary cultures of rPT cells—To assess the potential cytotoxicity of Hg^{2+} in primary cultures of rPT cells, cells were incubated for 24 h with 0.5 to 10 μM $HgCl_2$ and cell death was assessed by either necrosis or apoptosis. Incubation of rPT cells with $HgCl_2$ caused a concentration-dependent decrease in total LDH activity, indicating cellular necrosis (Figure 4). The lowest concentration of $HgCl_2$ reduced LDH activity by 27%. Even a Hg^{2+} concentration as low as 1 μM reduced LDH activity by > 50%, indicating a marked degree of necrosis. Consistent with these results, 0.5 μM Hg^{2+} caused a very modest increase in the proportion of apoptotic cells (Figure 5), indicating that under appropriate conditions, Hg^{2+} can also cause apoptosis in rPT cells. Moreover, 1 μM Hg^{2+} not only produced a marked increase in the proportion of subdiploid

cells but also resulted in detachment of >50% of the cells from the culture surface. Thus, primary cultures of rPT cells are very sensitive to Hg^{2+}-induced cytotoxicity.

Studies in primary cultures of hPT cells

Having established that pretreatment of both rats *in vivo* and rPT cells *in vitro* with Hg^{2+} increases activities of several GSH-dependent enzymes and GSH conjugation of TRI, and that primary cultures of rPT cells are highly sensitive to Hg^{2+}-induced necrosis and apoptosis at concentrations in the range of 0.5 to 1 μM, we extended our studies on the interactions between Hg^{2+} and TRI and its nephrotoxic metabolite DCVC to hPT cells. Although there is a large database of work on both Hg^{2+} and TRI with rat tissues, the poor ability to use data from rodents for human health risk assessment argues for the need to conduct studies with human tissue.

Effect of Hg^{2+} pretreatment on GST activity and expression in hPT cells—Unlike rPT cells, which only express GSTα (Cummings et al., 2000c), hPT cells express three isoforms of GST, GSTA, GSTP, and GSTT (Cummings et al., 2000b). In contrast to the induction of GSTα1 in rat kidney cytoplasm by pretreatment with Hg^{2+}, expression of none of the three isoforms of GST in hPT cells was altered by a 24-h pretreatment with 0.5 μM $HgCl_2$ (Figure 6).

Assessment of effects of a 24- and 48-h pretreatment of hPT cells with Hg^{2+} on activities of GSH-dependent enzymes also showed that hPT cells are less responsive than rPT cells (Table 3). Activity of none of the four GSH-dependent enzymes was altered by a 24-h pretreatment with Hg^{2+}. After a 48-h exposure to 0.5 μM $HgCl_2$, the only enzyme activity that exhibited a significant increase was GRD, which was increased by 31% as compared to that in untreated hPT cells.

Influence of Hg^{2+} pretreatment on TRI- and DCVC-induced necrosis and apoptosis in hPT cells—Although pretreatment of hPT cells with Hg^{2+} had minimal effects on GSH-dependent metabolism, effects on cytotoxicity of TRI and DCVC were examined. Confluent, primary cultures of hPT cells were pretreated with 0, 0.25, 0.5, or 1 μM $HgCl_2$ for 24 h and then cells were incubated with either media (= Control), 1 mM TRI, or 50 μM or 200 μM DCVC. Cellular necrosis was assessed by measuring either LDH release from cells (Figure 7A) or total LDH activity in cells and extracellular medium (Figure 7B).

hPT cells preincubated with media exhibited small increases in LDH release, from < 10% in media-incubated cells to 15-20% in cells incubated with either 1 mM TRI or 50 μM DCVC; incubation of media-preincubated hPT cells with 200 μM DCVC increased LDH release to nearly 30%. No effects were observed on total LDH activity in media-preincubated cells. Using LDH release as a metric of necrosis, the only significant observed effects of preincubation with Hg^{2+} was to slightly decrease LDH release from 1 mM TRI in cells preincubated with 0.25 μM $HgCl_2$ and to moderately increase LDH release from 50 μM DCVC in cells preincubated with 1 μM $HgCl_2$. Because of the ability of Hg^{2+} to directly inhibit LDH activity (Lash and Zalups, 1992), LDH inactivation is likely to be a more sensitive metric for necrosis than LDH release. Accordingly, more pronounced effects of preincubation of hPT cells with Hg^{2+} were observed when LDH inactivation was used as a metric of necrosis. Preincubation with both 0.25 μM and 1 μM $HgCl_2$ significantly increased LDH inactivation due to incubation with either 50 μM or 200 μM DCVC. Thus, preincubation of hPT cells with Hg^{2+} enhanced acute cellular necrosis due to DCVC but had no effect on that due to TRI.

Preincubation of hPT cells with Hg^{2+} also altered the induction of apoptosis induced by TRI and DCVC. As a dramatic illustration of this modulation of cytotoxicity response, sample flow cytometry histograms of cell cycle analyses of hPT cells preincubated for 24 h with either media or 0.25 μM Hg^{2+} and incubated for 1 h with either media, 1 mM TRI, or 50 μM DCVC

are shown (Figure 8). Preincubation with 0.25 μM Hg^{2+} alone resulted in a small increase in the fraction of apoptotic cells. In contrast, preincubation with Hg^{2+} markedly reduced apoptosis in cells subsequently incubated with either TRI or DCVC.

Effects of preincubation with Hg^{2+} on TRI- and DCVC-induced apoptosis were investigated in more detail by assessing both Hg^{2+}-concentration and time dependence (Figure 9). After 1-h incubations, hPT cells preincubated with media exhibited large increases in apoptosis due to either TRI or DCVC. Preincubation of cells with 0.25 μM $HgCl_2$, however, markedly reduced apoptosis due to 1-h incubation with TRI or DCVC. Preincubation of cells with 0.5 μM or 1 μM $HgCl_2$ for 1 h also decreased apoptosis due to TRI or DCVC, but the effects were less than those at the lowest concentration of Hg^{2+}. Similarly, as incubation time with TRI or DCVC was increased to 2 or 4 h, there were still decreases for most samples in the extent of apoptosis, but the amounts of decreases were less as incubation time or pretreatment concentration of Hg^{2+} increased.

To summarize the results from Hg^{2+}-pretreatment in hPT cells, those cells that were pretreated with Hg^{2+} exhibited increased LDH release or LDH inactivation due to DCVC and decreased apoptosis due to either TRI or DCVC. Thus, the cytotoxic response to TRI or DCVC is shifted from apoptosis to necrosis by prior exposure to Hg^{2+}.

Influence of TRI and DCVC pretreatments on Hg^{2+}-induced necrosis and apoptosis in hPT cells—Using the same paradigm of prior exposure, we next examined the influence of preincubations with either TRI or DCVC on the cytotoxic responses of hPT cells to Hg^{2+}. Primary cultures of hPT cells were preincubated for 24 h with either media, 1 mM TRI, or 50 μM DCVC and were subsequently incubated for 1, 2, or 4 h with either media or one of three concentrations of Hg^{2+} (0.25, 1, 5 μM). Using LDH inactivation as a metric for acute cellular necrosis, showed that hPT cells pretreated with Hg^{2+} generally exhibited less cytotoxicity from either TRI or DCVC than cells pretreated with only media (Figure 10). The response varied somewhat according to incubation time and concentration of Hg^{2+}. The only samples that showed little decrease in cytotoxicity as compared with the corresponding media-preincubated samples were those preincubated with 5 μM Hg^{2+}; this is presumably due to a significant decrease in LDH activity by the pretreatment alone.

The influence of pretreatment with TRI or DCVC on apoptosis induced by Hg^{2+} exhibited a markedly different pattern than on necrosis (Figure 11): In contrast with the results above, preincubation with 1 mM TRI had either no effect or decreased the fraction of cells undergoing apoptosis after 1-h incubations with Hg^{2+} but generally increased the fraction of cells undergoing apoptosis after 2- or 4-h incubations with Hg^{2+}. Preincubation of hPT cells with 50 μM DCVC slightly increased apoptosis due to either a 1- or 2-h incubation with 0.25 μM Hg^{2+}, had no effect on apoptosis due to either a 1- or 2-h incubation with either 1 μM or 5 μM Hg^{2+}, but markedly decreased apoptosis due to 4-h incubations with any of the three concentrations of Hg^{2+} tested.

Discussion

Based on the existence of several common pathways and mechanisms of action for renal injury induced by TRI (or its metabolite DCVC) and Hg^{2+}, we hypothesized that prior exposure to one chemical (or its metabolite) would alter the cytotoxic response of kidney cells to a subsequent exposure to the other chemical (or its metabolite). Some of the common pathways include those for metabolism and transport, and the interaction may include processes such as direct competition or induction of an enzyme involved in metabolism. The concept of prior exposure of one chemical producing either an adaptive change in cells or otherwise altering the response of the cell to subsequent exposures to potentially toxic chemicals, is not new.

Novel aspects of the present study, however, include our use of primary cultures of hPT cells as a cellular model and comparisons between rodent and human PT cells.

Calabrese and Mehendale (1996) reviewed the role of altered tissue repair as an adaptive strategy to chemical exposures. Of more direct pertinence to the present study, Mehendale and colleagues (Korrapati et al., 2005,2006;Vaidya et al., 2003a,2003b,2003c) demonstrated in a series of studies in mice that enhanced renal cell division is associated with protection of mice from DCVC-induced acute renal failure and death. Their studies have described what they have termed "auto-protection." In those studies, mice were protected from acute renal injury due to DCVC as a consequence of prior exposures to DCVC. In our study, we have examined the influence of a prior exposure to one chemical on the subsequent toxicity from exposure to another chemical. Any protection that occurs would be described as "hetero-protection." Besides protection, cells may also experience the opposite response, namely that prior exposure to one chemical may enhance sensitivity to a subsequent exposure to another chemical.

Our initial studies were conducted in rats and rPT cells to further establish effects of Hg^{2+} on cellular GSH status. *In vivo* treatment of rats with a subtoxic dose of Hg^{2+} (Zalups and Lash, 1990) produced a number of significant changes in GSH-dependent metabolism in the renal cortex. These changes included increased protein expression of GSTα1 but decreased GSTα2 increased activities of GSH synthesis and GSH-dependent enzymes, and, most significantly for the current study, a significant increase in rates of GSH conjugation of TRI. Analysis of the kinetics of DCVG formation after Hg^{2+} pretreatment showed that V_{max} was increased but the K_m for TRI was unchanged, suggesting that Hg^{2+} pretreatment led to more GST being produced. When primary cultures of rPT cells were treated with Hg^{2+}, a significant increase was observed in GST activity as well.

Assessment of acute cytotoxicity of Hg^{2+} in rPT cells showed that the dose-response relationship for Hg^{2+}-induced cell death was very steep, consistent with earlier studies (Lash and Zalups, 1992;Lash et al., 1999a). Using LDH inactivation as a measure of acute cellular necrosis, the IC_{50} was approximately 0.8 μM Hg^{2+} (cf. Figure 4). Analysis of cell cycle distribution and apoptosis by propidium iodide staining and flow cytometry showed that whereas 0.5 μM Hg^{2+} produced no effect on cell cycle distribution and no significant increase in the fraction of apoptotic cells (cf. Figure 5B) as compared with control rPT cells, 1 μM Hg^{2+} caused both a large increase in apoptosis and extensive detachment of cells from the growth surface (cf. Figures 5C and 5D).

Primary cultures of hPT cells, grown under the same conditions and with the same medium as primary cultures of rPT cells, were markedly less responsive to exposures to low concentrations of Hg^{2+} with respect to cellular GSH status. None of the three isoforms of GST in hPT cells exhibited altered expression after 24-h incubations with Hg^{2+} (cf. Figure 6). Of the GSH-dependent enzyme activities measured, only GRD exhibited a modest increase after a 48-h incubation with Hg^{2+} (cf. Table 3). In spite of these minimal to modest changes, prior exposure of hPT cells to either Hg^{2+} or TRI/DCVC had profound effects on necrosis or apoptosis due to subsequent exposures to one of the other chemicals, suggesting that factors in addition to GSH status are important in the response of hPT cells to prior exposures to cytotoxic chemicals.

Pretreatment of hPT cells with between 0.25 μM and 1 μM Hg^{2+} for 24 h caused a significant increase in necrosis (as determined by LDH inactivation) due to 50 or 200 μM DCVC, but had little effect on that due to TRI (cf. Figure 7). In contrast to this response, pretreatment of hPT cells with the same concentrations of Hg^{2+} markedly decreased apoptosis due to either DCVC or TRI (cf. Figures 8 and 9). If one views these two forms of cell death (necrosis or oncosis and apoptosis) as a continuum in the cytotoxic response of cells to toxic chemicals or pathological conditions, then we interpret the results with Hg^{2+} pretreatment as indicating that

sensitivity of hPT cells to TRI or DCVC is enhanced as the mechanism of cell death is shifted from apoptosis to necrosis. In this scenario, cells undergoing apoptosis are viewed as being less seriously compromised than cells undergoing necrosis. The energy dependence and more highly regulated nature of apoptosis would be consistent with this view. Thus, rather than protecting hPT cells from a subsequent exposure to another chemical, prior exposure to low concentrations of Hg^{2+} enhances the severity of the cellular response to TRI or DCVC.

Unlike the findings with prior exposure to Hg^{2+}, incubation first with either TRI or DCVC generally protected hPT cells from cell death due to Hg^{2+} (cf. Figures 10 and 11). Prior exposure to either TRI or its nephrotoxic metabolite DCVC markedly decreased Hg^{2+}-induced LDH inactivation. Effects on Hg^{2+}-induced apoptosis were, however, somewhat more complex. Whereas prior exposure to TRI clearly increased apoptosis due to Hg^{2+} at later incubation times, prior exposure to DCVC initially increased the occurrence of Hg^{2+}-induced apoptosis, but then led to a marked decrease at later incubation times. Again, using the view described above of apoptosis and necrosis/oncosis representing a continuum in the cytotoxic response of cells, these results are consistent with prior exposures of hPT primary cell cultures to TRI or DCVC leading to protection from subsequent exposures to Hg^{2+}.

The increased toxicity of TRI in hPT cells after pretreatment with a subtoxic concentration of Hg^{2+} can readily be rationalized by the ability of Hg^{2+} to increase bioactivation of TRI by GST and to increase renal PT cell GSH content. Understanding of the mechanism for the increased toxicity of DCVC in hPT cells due to Hg^{2+} pretreatment requires further study. One possibility is that prior exposure to Hg^{2+} may induce one or more forms of renal cysteine conjugate β-lyase, as has been shown for the mercapturate of hexachlorobutadiene (Cooper and Pinto, 2006;MacFarlane et al., 1993). Similarly, understanding of the mechanism by which pretreatment of hPT cells with TRI or DCVC decreases toxicity of Hg^{2+} also requires further study, but may involve activation of certain transcription factors and/or some of the protective mechanisms, such as activation of cellular repair and proliferation, as described by Mehendale and colleagues (Korrapati et al., 2005,2006;Vaidya et al., 2003a,2003b,2003c). Some of our earlier results in primary cultures of hPT cells, studying the effects of DCVC on expression of regulatory proteins such as heat shock protein 27 (Hsp27), p53, and nuclear factor κB (NFκB) (Lash et al., 2005), are consistent with such a potential mechanism. Expression of each of these proteins was induced severalfold by exposure to low concentrations of DCVC. Increased expression of these three proteins is associated with alterations in cellular responses to toxicants, including activation of repair mechanisms and changes in cell cycle status.

In summary, the present study has demonstrated that exposures of hPT cells to one chemical can significantly modulate the sensitivity of the cells to subsequent chemical exposures. The modulation or adaptation of the cells to the initial chemical exposure will depend on the precise mechanisms by which the subsequent chemical produces toxicity. Although changes in cellular GSH status are important, other factors may be more important to the underlying mechanisms by which pretreatment with Hg^{2+} influences TRI- or DCVC-induced toxicity in the human kidney.

Acknowledgments

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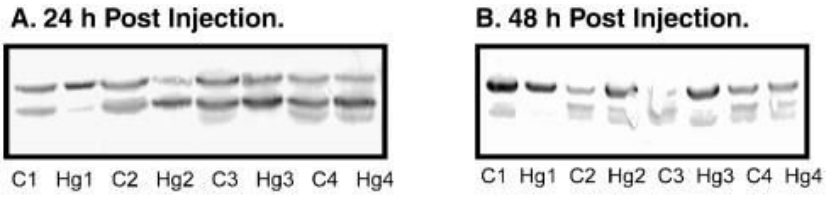


	C-24 h	Hg-24 h	C-48 h	Hg-48 h
GSTα1	42.8 ± 2.5	39.8 ± 5.7	36.3 ± 15.9	48.3 ± 6.7
GSTα2	54.7 ± 8.9	59.1 ± 16.9	30.5 ± 4.4	14.6 ± 3.6

Figure 1. Effects of pretreatment with Hg^{2+} on GSTα expression in rat kidney cytoplasm

Rats were pretreated ip with a non-toxic dose of $HgCl_2$ (0.5 μmol/kg) for 24 or 48 h. Expression of GSTα isoforms were determined by immunoblot analysis, using a polyclonal antibody to rat GSTα, which recognizes both GSTα1 (top band) and GSTα2 (lower band). Densities of bands were determined with Image J software (v. 1.34s). In some samples, a third band was visible below the GSTα2 band. This band was not identified, but was attributed to minor cross-reactivity. Band density results are means ± SE of measurements from 4 separate control rats and 4 separate Hg^{2+}-treated rats at each time point.

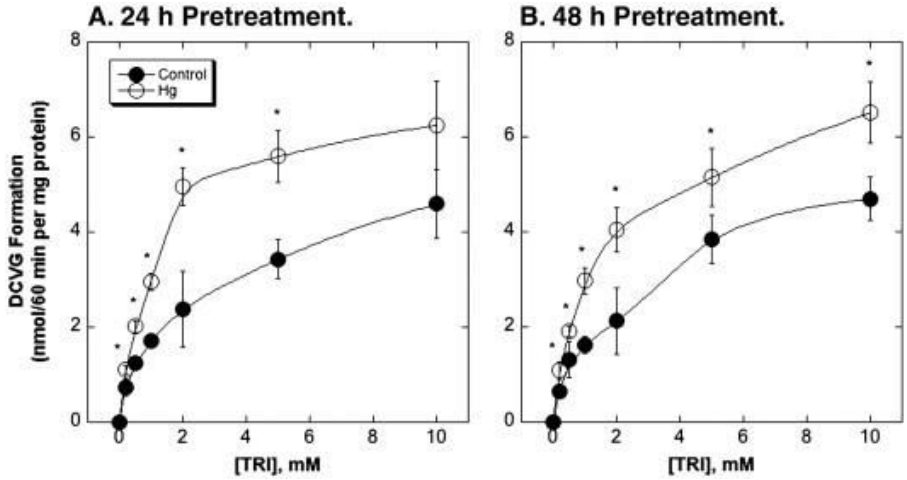


Figure 2. Effects of pretreatment with Hg^{2+} on GSH conjugation of TRI in rat kidney cytoplasm

Rats were pretreated ip with a non-toxic dose of $HgCl_2$ (0.5 μmol/kg) for 24 or 48 h. Cytoplasm was isolated from rat kidney cortex of either control or Hg^{2+}-pretreated rats by differential centrifugation. GSH conjugation of TRI was measured by incubating cytoplasm (1 mg protein/ml) with 5 mM GSH and the indicated concentrations of TRI at 37°C for up to 60 min. Reactions were stopped by addition of 10% (w/v) perchloric acid and acid extracts were analyzed for formation of DCVG by ion-exchange HPLC. Results, given for 60-min incubations, are the means ± SE of measurements from separate cytoplasm preparations from 4 control and 4 Hg^{2+}-pretreated rats. *Significantly different ($P < 0.05$) from corresponding incubations from control rat kidneys.

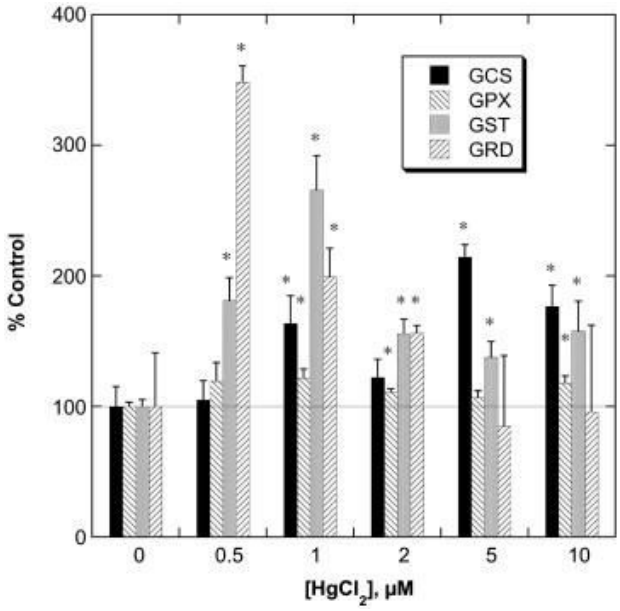


Figure 3. Effects of pretreatment of primary cultures of rPT cells with Hg^{2+} on activities of GSH-dependent enzymes

Confluent, primary cultures of rPT cells were incubated with the indicated concentrations of $HgCl_2$ for 24 h. Activities of γ-glutamylcysteine synthetase (GCS), glutathione peroxidase (GPX), glutathione *S*-transferase (GST), and glutathione disulfide reductase (GRD) were measured in cellular homogenates by spectrophotometric assays. Control activities (= 100%; in mU/mg protein) were 611 ± 93, 237 ± 8, 64.0 ± 3.4, and 2.40 ± 0.99 for GCS, GPX, GST, and GRD, respectively. Results are expressed as the percent of control ± SE for measurements from 3 separate cultures for each concentration of Hg^{2+}. *Significantly different ($P < 0.05$) from the corresponding control sample.

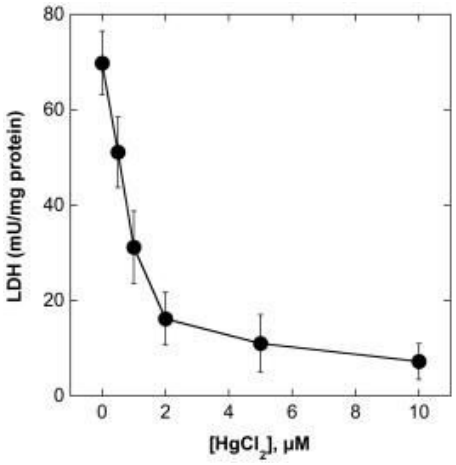
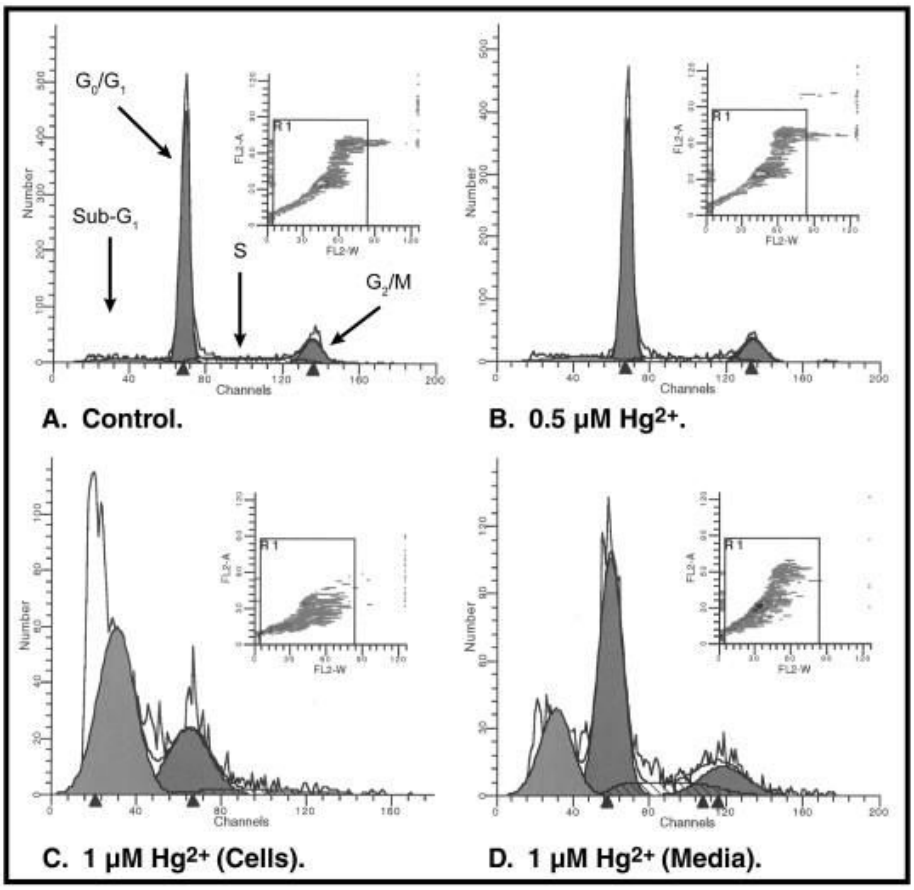


Figure 4. Hg^{2+}-induced inactivation of LDH activity in primary cultures of rPT cells

Confluent, primary cultures of rPT cells were grown on 35-mm culture dishes and were incubated with the indicated concentrations of $HgCl_2$ for 24 h. Cellular necrosis was determined by measurement of total LDH activity. Results are means ± SE of measurements from 4 to 6 separate cell cultures.



	Sub-G_1	G_0/G_1	G_2/M	S
Control	7.57	71.8	13.2	15.0
0.5 μM Hg^{2+}	9.40	74.4	14.1	11.5
1 μM Hg^{2+} (Cells)	64.4	81.1	0.5	18.4
1 μM Hg^{2+} (Media)	25.3	68.4	16.5	15.1

Figure 5. Hg^{2+}-induced apoptosis in primary cultures of rPT cells

Confluent, primary cultures of rPT cells were grown on 35-mm culture dishes and were incubated with either media (= Control) or 0.5 μM or 1 μM $HgCl_2$ for 24 h. At 1 μM $HgCl_2$, a significant portion of cells became detached from the growth surface. Accordingly, cells remaining on the culture dish and those that were detached after treatment with 1 μM $HgCl_2$ were separately analyzed. Apoptosis and cell cycle distribution were assessed by staining with propidium iodide and flow cytometry with a Becton Dickinson *FACSCalibur* flow cytometer. Peaks from left to right represent apoptotic (sub-G_1) cells and cells in G_0/G_1, G_2/M, and S phases. *Insets:* Distribution of cells according to fluorescence intensity. Cells outside the box were those excluded from analysis due to aggregation. Values in the table are the percentage of cells undergoing apoptosis and the fraction of cells in each phase of the cell cycle.

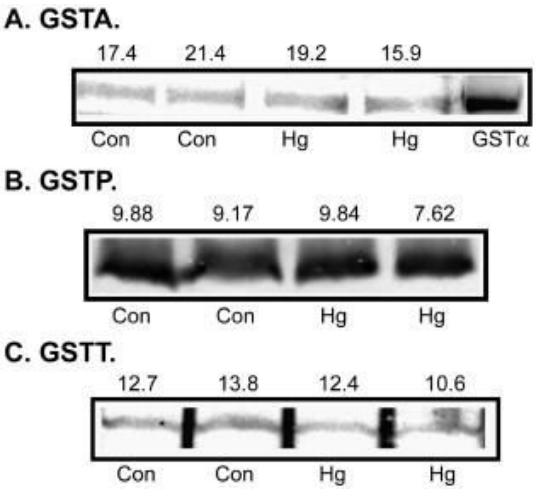


Figure 6. Effects of pretreatment of primary cultures of hPT cells with Hg^{2+} on GST isoenzyme expression

Confluent, primary cultures of hPT cells were grown on collagen-coated T-25 flasks and were incubated with 0.5 μM $HgCl_2$ for 24 h. Expression of GSTA (A), GSTP (B), and GSTT (C) were determined in total cell extracts by immunoblot analysis, using polyclonal antibodies specific for each GST isoenzyme. For GSTA, an additional sample of purified rat GSTα was run. Numbers above each panel represent total pixel densities of each band as determined with Image J software.

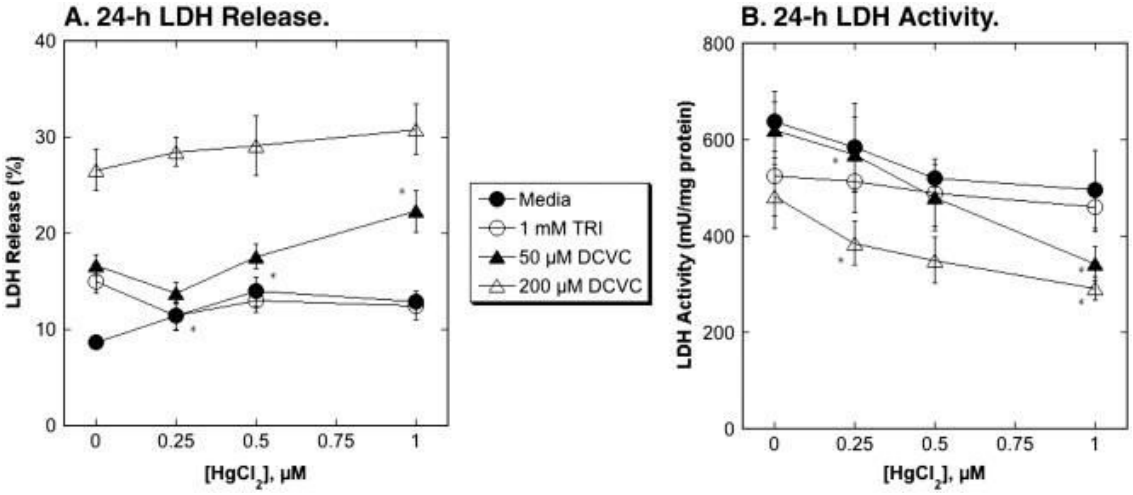


Figure 7. Effects of pretreatment of primary cultures of hPT cells with Hg^{2+} on TRI- and DCVC-induced necrosis

Confluent, hPT cell cultures were pretreated for 24 h with either media (= Control) or 0.25, 0.5, or 1 μM $HgCl_2$. Cells from the four pretreatment groups were then incubated for 24 h with either media, 1 mM TRI, or 50 or 200 μM DCVC. LDH activity was measured in media and in cells solubilized with 0.1% (v/v) Triton X-100. Results are expressed as either percent LDH release (A) or total LDH activity (B) and are means ± SE of measurements from 3 separate cell cultures. *Significantly different ($P < 0.05$) from corresponding samples preincubated with media.

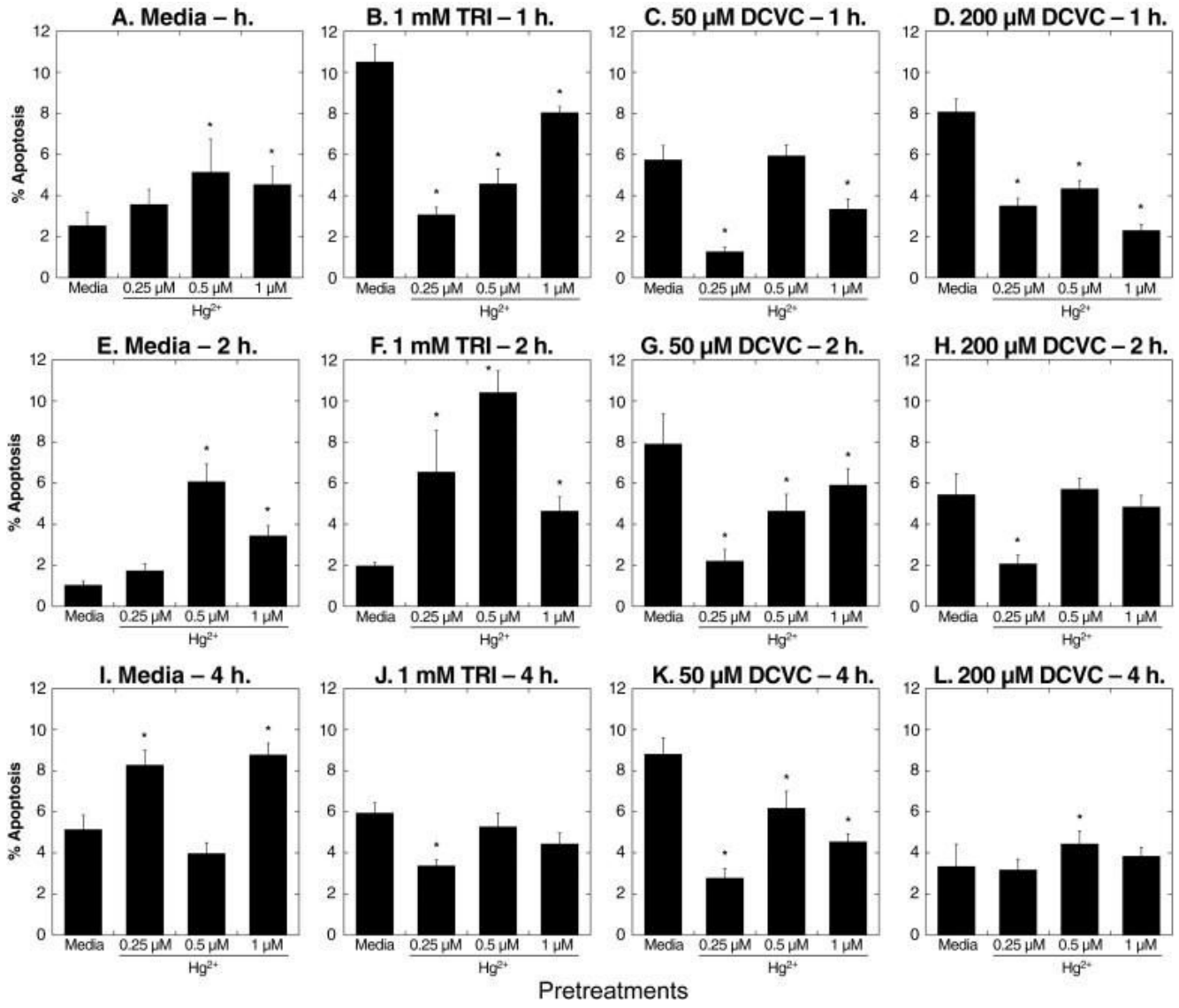


Figure 8. Effects of pretreatment of primary cultures of hPT cells with 0.25 µM Hg^{2+} on TRI- and DCVC-induced apoptosis

Confluent, hPT cell cultures were pretreated for 24 h with either media (= Control) or 0.25 µM $HgCl_2$. Cells from the two pretreatment groups were then incubated for 1 h with either media, 1 mM TRI, or 50 µM DCVC. Apoptosis was assessed by staining with propidium iodide and flow cytometry using a Becton Dickinson *FACSCalibur* flow cytometer. Peaks from left to right represent apoptotic (sub-G_1) cells and cells in G_0/G_1, G_2/M, and S phases. *Insets:* Distribution of cells according to fluorescence intensity. Cells outside the box were those excluded from analysis due to aggregation. Values above the arrows indicate the percentage of apoptotic cells in each sample.

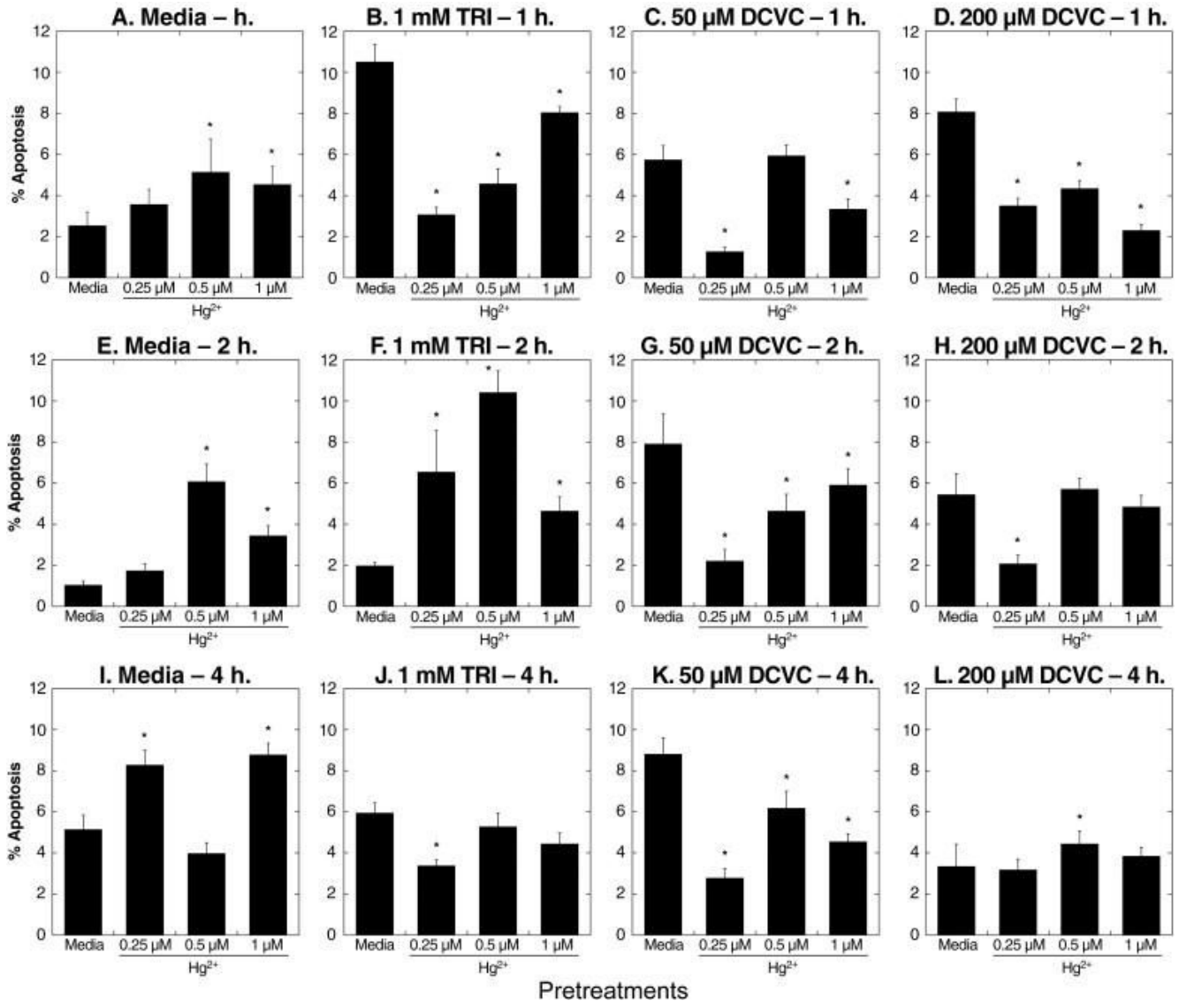


Figure 9. Time and dose dependence of effects of pretreatment of primary cultures of hPT cells with Hg^{2+} on TRI- and DCVC-induced apoptosis

Confluent, hPT cell cultures were pretreated for 24 h with 0 (= Media/Control), 0.25, 0.5, or 1 µM $HgCl_2$. Cells from the four pretreatment groups were then incubated for 1, 2, or 4 h with either media, 1 mM TRI, or 50 or 200 µM DCVC. Cells were analyzed for apoptosis by flow cytometry as described in the legend to Fig. 8. Results are means ± SE of measurements from 3 separate cell cultures. *Significantly different ($P < 0.05$) from corresponding samples preincubated with media.

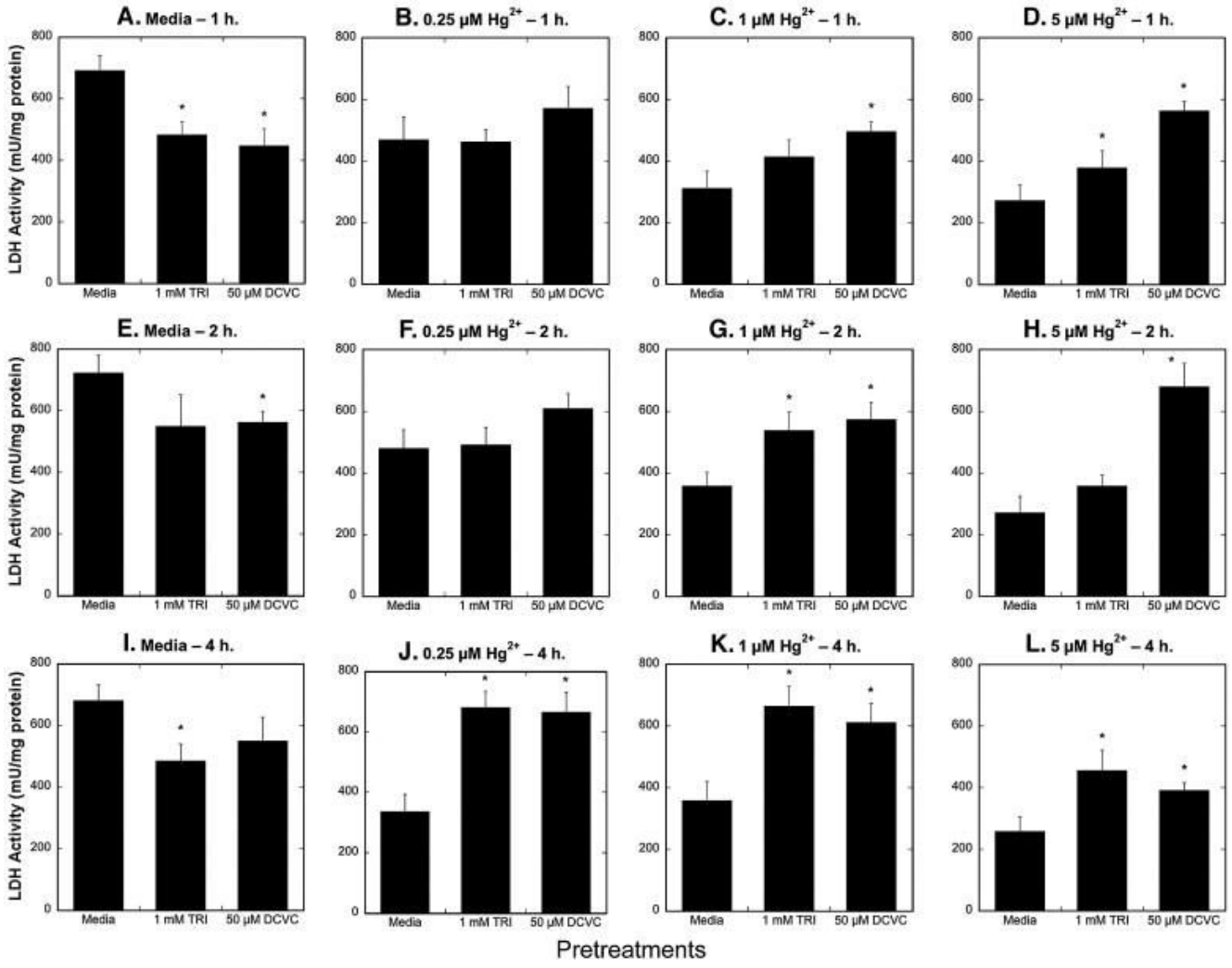


Figure 10. Effects of pretreatment of primary cultures of hPT cells with TRI or DCVC on Hg^{2+}-induced necrosis

Confluent, hPT cell cultures were pretreated for 24 h with either media (= Control), 1 mM TRI, or 50 μM DCVC. Cells from the three pretreatment groups were then incubated for 1, 2, or 4 h with either 0, 0.25, 1, or 5 μM $HgCl_2$. LDH activity was measured in media and in cells solubilized with 0.1% (v/v) Triton X-100. Results are means ± SE of measurements from 3 separate cell cultures. *Significantly different ($P < 0.05$) from corresponding samples preincubated with media.

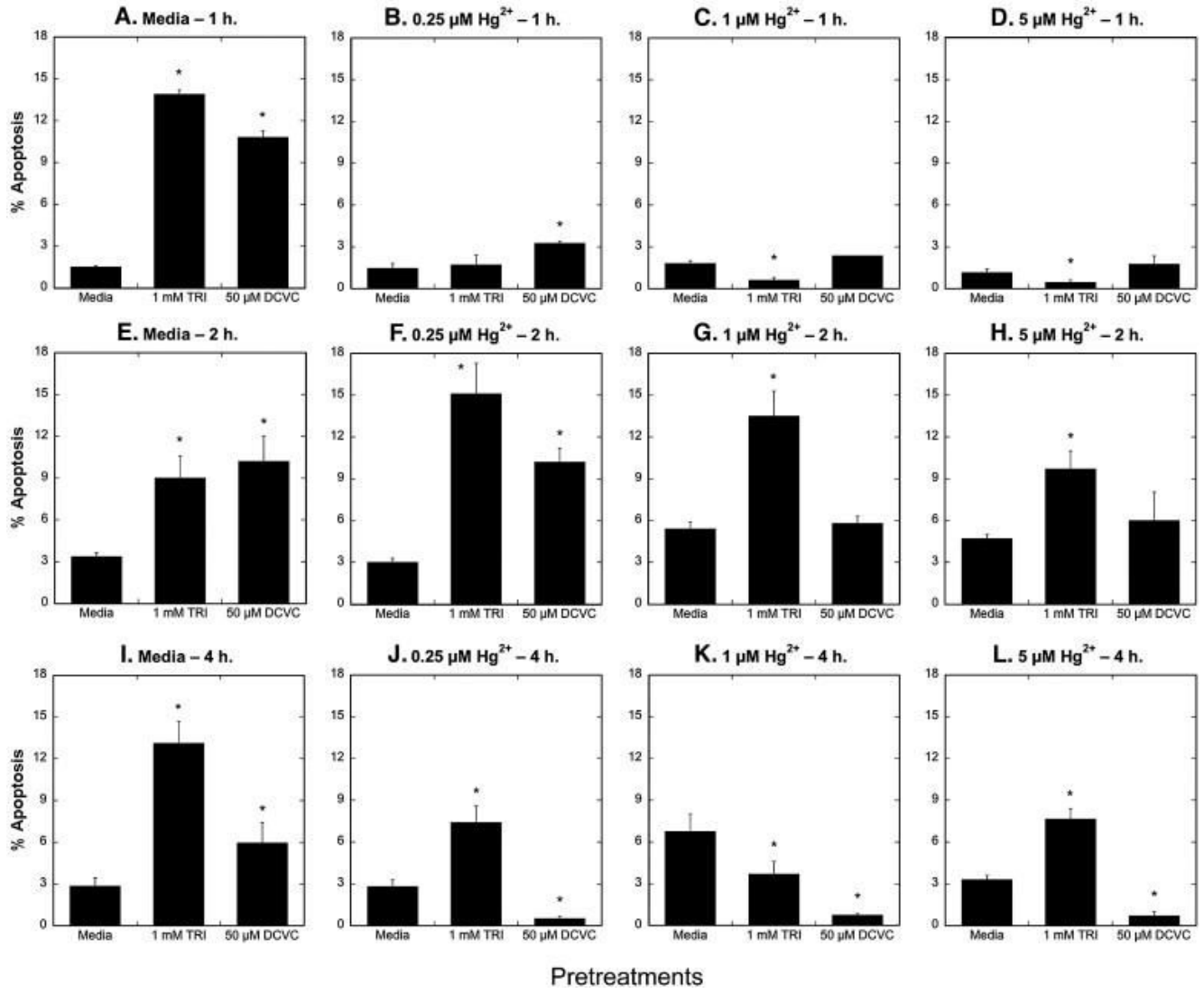


Figure 11. Effects of pretreatment of primary cultures of hPT cells with TRI or DCVC on Hg^{2+}-induced apoptosis

Confluent, hPT cell cultures were pretreated for 24 h with either media (= Control), 1 mM TRI, or 50 μM DCVC. Cells from the three pretreatment groups were then incubated for 1, 2, or 4 h with either 0, 0.25, 1, or 5 μM $HgCl_2$. Cells were analyzed for apoptosis by flow cytometry as described in the legend to Fig. 8. Results are means ± SE of measurements from 3 separate cell cultures. *Significantly different ($P < 0.05$) from corresponding samples preincubated with media.

Table 1

Effect of pretreatment with Hg^{2+} on activities of GSH-dependent enzymes in rat kidney cytoplasm.

Male Fischer 344 rats were given an ip injection of either saline (Con, control) or 0.5 μmol $HgCl_2$/kg (Hg^{2+}). After 24 or 48 h, enzyme activities (mU/mg protein) were determined in kidney cytoplasm by spectrophotometric assays. Results are means ± SE of measurements from 4 separate control and Hg^{2+}-treated rats at each time point.

	Con-O h	Con-24 h	Hg^{2+}-24 h
GST	48.0±1.7	44.9±4.0	65.5±6.3*
GRD	50.3±4.0	50.8±7.3	69.5±2.1*
GPX	40.3±3.3	38.5±3.2	57.1±2.7*
GCS	51.2±3.8	51.0±4.0	73.0±4.7*
		C-48 h	**Hg^{2+}-48 h**
GST		44.2±2.3	54.9±2.0*
GRD		50.5±3.8	69.0±7.0*
GPX		42.9±5.0	63.7±4.0*
GCS		47.5±2.1	89.1±4.2*

*Significantly different ($P < 0.05$) from the corresponding control sample.

Table 2

Kinetics of GSH conjugation of TRI in rat kidney cytoplasm from control and Hg^{2+}-pretreated rats

Male Fischer 344 rats were given an ip injection of either saline (Con, control) or 0.5 μmol $HgCl_2$/kg (Hg^{2+}). After 24 or 48 h, cytoplasm was isolated from kidney cortex of each pretreatment group and were incubated with 5 mM GSH and 0.2 to 10 mM TRI for up to 60 min. Kinetic parameters are derived from Eadie-Hofstee (E-H) plots of the V vs. S data shown in Fig. 2.

	E-H regression equation; R^2	V_{max} (nmol DCVG/60 min per mg)	K_m (mM TRI)
Control-24 h	Y=3.490-0.762X; R^2=0.831	4.58	1.31
Hg^{2+}-24 h	Y=6.045-0.852X; R^2=0.936	7.10	1.17
Control-48 h	Y=3.086-0.616X; R^2=0.799	5.01	1.62
Hg^{2+}-48 h	Y=5.744-0.857X; R^2=0.946	6.70	1.17

Table 3

Effect of pretreatment of primary cultures of hPT cells with Hg^{2+} on activities of GSH-dependent enzymes

hPT cells at ~75% confluence were incubated with either media (Con, control) or 0.25 μM $HgCl_2$ (Hg^{2+}). After 24 or 48 h, enzyme activities (mU/mg protein) were determined in total cell extracts by spectrophotometric assays. Results are means ± SE of measurements from 12-14 separate control and 8-10 separate Hg^{2+}-treated cultures at each time point.

	Con-24 h	Con-48 h	Hg^{2+}-24 h	Hg^{2+}-48 h
GST	72.9 ± 11.0	76.2 ± 18.6	64.0 ± 9.3	70.9 ± 11.5
GRD	53.9 ± 11.5	40.8 ± 3.6	44.7 ± 6.1	58.7 ± 8.6*
GPX	33.6 ± 5.6	45.9 ± 11.7	22.4 ± 5.9	31.1 ± 8.0
GCS	22.2 ± 3.0	33.4 ± 4.4	20.6 ± 4.2	28.6 ± 3.6

*Significantly different ($P < 0.05$) from the corresponding control sample.