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ScienceDirect
Toxicology and Applied Pharmacology 221 (2007) 349–362
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Interactive toxicity of inorganic mercury and trichloroethylene in rat and human proximal tubules: Effects on apoptosis, necrosis, and glutathione status

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Received 7 January 2007; revised 19 March 2007; accepted 22 March 2007
Available online 30 March 2007

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. 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

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doi:10.1016/j.taap.2007.03.023

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,b). 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, 2001a,b). 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,