Interaction of Metals during Their Uptake and Accumulation in Rabbit Renal Cortical Slices

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The uptake and accumulation of metals occurs in the kidney, which is a key site for interaction between metal nephrotoxins. The uptake/accumulation and interaction of CdCl2, HgCl2, K2Cr2O7, and NaAsO2 was examined in precision-cut rabbit renal cortical slices. Slices were incubated with 10−8 to 10−3 M of a single metal toxicant or combinations of metal toxicants for 12 hr in DME-F12 media. Slices were blotted and sandwiched between two mylar films stretched across XRF sample cups. Quantitation of the metal in the slices was performed by proton-induced X-ray emission analysis (PIXE). The uptake of the metals was rapid, often reaching a maximum between 3 to 6 hr; the accumulation of Hg was highest, followed in order by Cd, Cr, and As. When two metals were present together, substantial alterations were observed in the uptake of the metals in the slices. HgCl2 hindered the uptake of K2Cr2O7, NaAsO2, CdCl2 (in this order), whereas these metals facilitated the uptake of HgCl2. However, a decreased uptake of both metals was often noted after exposure to other combinations of metals. PIXE analysis of metal content in slices is attractive since all elements (atomic number >20) can be determined simultaneously. This information will be particularly useful in studying potential toxic interactions. — Environ Health Perspect 103(Suppl 1):77–80 (1995)

Key words: HgCl2, CdCl2, K2Cr2O7, NaAsO2, kidney toxicants, interaction, uptake, accumulation, analysis PIXE (proton-induced X-ray emission)

Introduction

Many of the metals associated with hazardous waste sites can leach into the surrounding environment, raising the potential for human exposure. Following exposure, many of these metals tend to accumulate in the kidney. The uptake and accumulation of metals in the kidney make it a key site for metal–metal interactions (1). Previous studies have shown that coexposures of more than one metal can result in potentiation of their renal toxicity (2,3). Coexposure of metal compounds has been shown to shift the usual renal uptake of the individual metal compound (4–7). Studying the interaction of metals in the kidney presents a problem due to the number of animals required for the permutations and combinations of metal compounds and the difficulty in measuring multiple metals in the same tissue. By using a representative valid in vitro system, such as precision-cut renal cortical slices (8), various combinations of exposures can be more readily performed. In addition, proton-induced X-ray emission (PIXE) has proven to be a technique that allows for quantitation of multiple elements in these renal slices (9).

This study examines the effects of four metal compounds (HgCl2, CdCl2, NaAsO2, K2Cr2O7) in each others’ uptake in precision-cut rabbit renal cortical slices using PIXE analysis to measure accumulation of the metals.

Materials and Methods

Renal Positional Slices

After male New Zealand white rabbits (1.5–2.5 kg) were killed, their kidneys were removed and placed in chilled Krebs-HEPES buffer. Renal slices were prepared using a Krumdieck tissue slicer (10). Cylindrical cores (diameter = 0.6 cm) were taken through each kidney along the cortico-papillary axis. The cortical areas were trimmed from the medullary regions and subsequently cut with the slicer. The slices obtained were approximately 275 μm thick and 10 mg in weight.

Incubation System

Renal slices were incubated at room temperature in an incubation vessel consisting of 20 wells through which 95% O2/5% CO2 was bubbled (10). Each well was filled with 20 ml of DME-F12 media containing 2 mM valeric acid. Four slices were placed on a screen located at the base of each well. Metals (HgCl2, NaAsO2, CdCl2, K2Cr2O7) were added at the desired concentrations for 12-hr time periods. While one metal compound was held at a single concentration, the other was varied over a predetermined range. All buffers and media used in the studies were aerated and pH adjusted to 7.4.

Emission Spectroscopy Analysis

Renal slices were blotted dry and placed on 3.5 μm Mylar film stretched across XRF sample cups (diameter = 3.2 cm, open ended). Another piece of film was placed on top to create a sandwich. Two holes were punched in the sandwich to allow air to escape and the sample to dry. Samples were placed in the freezer immediately after preparation until the day before the analysis, at which point they were placed in a desiccator and allowed to thaw and dry. The dried samples were then analyzed by PIXE, as described by Lowe et al. (9). Briefly, the samples were placed in an evacuated chamber where they were subjected to a proton beam produced by a Van de Graaff generator. The resulting X-rays released by the individual elements in the sample were then counted by a detector, and this raw data processed by a spectrum modeling program to clean up peak overlap and background noise. Quantities of individual elements detected were given in units of ng/cm².

From past studies the standard deviations for metals of interest averaged 13% (9). In this investigation the standard deviations were 13 to 15%.

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Results
A concentration-dependent uptake occurred with the four metal compounds (Figure 1). Accumulation already was plateauing by 4 hr of incubation, which is the first time point measured. At a fixed concentration of $10^{-4}$ M, the rank order of accumulation was: HgCl$_2$ $>$ K$_2$Cr$_2$O$_7$ $>$ CdCl$_2$ $>$ NaAsO$_2$.

HgCl$_2$ inhibited the uptake of K$_2$Cr$_2$O$_7$, NaAsO$_2$, and CdCl$_2$ in this order (Figures 2, 3). In fact, at the highest concentration, HgCl$_2$ almost completely inhibited the uptake of K$_2$Cr$_2$O$_7$ (Figure 2). Conversely, K$_2$Cr$_2$O$_7$, NaAsO$_2$, and CdCl$_2$ actually stimulated the uptake of HgCl$_2$.

While K$_2$Cr$_2$O$_7$ stimulated the uptake of HgCl$_2$ (Figure 1), it inhibited the uptake of CdCl$_2$ (Figure 3). CdCl$_2$ was only able to inhibit K$_2$Cr$_2$O$_7$ uptake when the CdCl$_2$ was present at its highest concentration. CdCl$_2$ did not inhibit the uptake of NaAsO$_2$ (Figure 4). Likewise, NaAsO$_2$ had little effect on CdCl$_2$ accumulation.

Both of the metal anions (K$_2$Cr$_2$O$_7$ and NaAsO$_2$) were able to inhibit each other’s uptake slightly (Figure 4). NaAsO$_2$ was more effective in inhibiting K$_2$Cr$_2$O$_7$ uptake, providing almost a concentration–response effect.

Discussion
Previous in vivo (1) and in vitro (11) studies have demonstrated a differential uptake of metallic compounds into renal tissue and cells. Our renal slice studies also demonstrated a preferential uptake, with HgCl$_2$, accumulating the most and NaAsO$_2$ the least (Figure 1). The reason for the plateauing and uptake at 4 hr is unclear, since the accumulated quantity represents only a small portion of the metal compound placed in the media. The plateau in uptake, despite the high concentrations in the media, could be related to the free metal concentrations inside the cell compared to outside the cell. Free unbound metal concentrations are in equilibrium and not total metal concentrations. In addition, the “intracellular” concentrations may be overly influenced by membrane-bound metal. Alternatively, the chemical species of the metal ion may be important in interpreting these data and the interaction data. Only at the highest concentrations of the metal compounds would there be concern for the viability of the tissue slices, which would indirectly affect transport processes.

![Figure 1](image1.png)  
**Figure 1.** Uptake and accumulation of metal compounds in rabbit renal cortical slices. Metal accumulation was monitored by PIXE analysis. Reprinted with permission from Lowe et al. (9).

![Figure 2](image2.png)  
**Figure 2.** Interaction of K$_2$Cr$_2$O$_7$–HgCl$_2$ and CdCl$_2$–HgCl$_2$ on each other’s uptake in rabbit renal cortical slices. See “Materials and Methods” for experimental design. Accumulation of metal in the slice was measured using PIXE analysis. Values are the mean of four determinations.
INTERACTION OF METALS DURING UPTAKE IN RENAL SLICES

Figure 3. Interaction of CdCl₂ and NaAsO₂, and NaAsO₂-HgCl₂ on each other's uptake in rabbit renal cortical slices. See "Materials and Methods" for experimental design. Accumulation of metal in the slice was measured using PIXE analysis. Values are the mean of four determinations.

Figure 4. Interaction of CdCl₂-NaAsO₂ and K₂Cr₂O₇-NaAsO₂ on each other's uptake in rabbit renal cortical slices. See "Materials and Methods" for experimental design. Accumulation of metal in the slice was measured using PIXE analysis. Values are the mean of four determinations.

Of most interest was the ability of the PIXE technique (9) to quantify the uptake of two metals simultaneously, thus being able to examine their interactive effects on accumulation processes. Most previous studies monitored only the accumulation of a single metal compound without considering the other interacting metal compound (4,5). However, a number of recent studies have demonstrated the importance of examining the effect of the metal compounds on each other's uptake (6,7,11). Even essential metals (Zn, Cu, Fe) have been shown to affect the uptake (but not efflux) of metallic nephrotoxicans such as Cd and As (7,12). These are important studies due to the acknowledged interactive nephrotoxicity that exists when more than one metal compound is present (2,3). The interactive effects on uptake we observed between the various metal compounds ranged from substantial decreases in the uptake of K₂Cr₂O₇ in the presence of HgCl₂ (Figure 2) to relatively minor effects on uptake seen with CdCl₂ and NaAsO₂ (Figure 3). Interestingly, the trend (and magnitude) of interactions between Hg and Cd seen with the renal slices are comparable to those reported with primary cultures of renal cortical epithelial cells (7).

Information on the uptake and accumulation of the metal compounds is needed to interpret interactive toxicity studies. It is well established that the level of injury in the kidney via a metal compound is related to the amount present in the renal tissue (1). Both potentiation and inhibition of toxicity are possible when studying mixtures of metal compounds. By understanding how one metal compound affects the accumulation of another metal compound, a prediction might be possible relative to the potential toxicity that would be exhibited. In future studies our in vitro system will also allow for an examination of the form of the metal compound (oxidation state, ligand, complex) responsible for the toxicity to the target tissue and what effect other metal compounds or conditions have on the expression of this toxicity.

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