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Dose and Time Relations in Hg\textsuperscript{++}– induced Tubular Necrosis and Regeneration

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Mercuric chloride is a well-known human and animal nephrotoxicant. Previous studies have demonstrated an inverse relationship between dose size and relative whole-body retention of mercury after oral administration of mercuric chloride to mice. The present study indicates that this inverse relationship is caused by a dose-related induction of kidney damage leading to increasing leakage of mercury through the kidneys. Histopathologic investigation revealed extensive necrosis of the proximal tubules in kidneys from mice exposed to 100 \( \mu \text{g}\) HgCl\textsubscript{2}/kg or higher doses. Moreover, maximum renal damage occurred between days 2 and 3 after administration. The renal damage was followed by regeneration, which was observed between days 3 and 7 at increasing dose levels up to 100 \( \mu \text{g}\) HgCl\textsubscript{2}/kg. The amount of glutathione and the glutathione peroxidase activity in kidney decreased with increasing doses of mercuric chloride. The reduced glutathione peroxidase activity was due to a reduction in selenium-dependent glutathione peroxidase activity. The level of lipid peroxidation was not changed by increasing doses of mercuric chloride, and hence was not a primary toxic mechanism in acute nephrotoxicity induced by mercuric chloride. — Environ Health Perspect 102(Suppl 3):317–320 (1994).

Key words: mercuric chloride, kidney damage, toxicokinetics, lipid peroxidation, glutathione peroxidation, mice

Introduction

Recent studies on the toxicokinetics of HgCl\textsubscript{2} in two mice strains demonstrated an inverse relationship between dose size and whole-body retention at 2 weeks after oral or intraperitoneal administration. (1,2). This inverse relationship is unlikely to be explained by a dose-relationship involving the intestinal uptake of mercury, but is more likely a dose related induction of kidney damage leading to increasing leakage of mercury through the kidneys. However, such leakage should be transient as groups of mice given up to 100 \( \mu \text{g}\)/kg (orally) or 5 \( \mu \text{g}\)/kg (intraperitoneally) of HgCl\textsubscript{2} had almost identical excretion rates at 7 days after dosage (1,2).

Zalups et al. (3) suggested that during acute HgCl\textsubscript{2} induced renal damage, the rate of urinary mercury excretion is positively related to the degree of cellular damage. The mechanism for the increased rate of mercury excretion is unknown, but might, in part, be caused by changes in concentrations of binding ligands for mercury (e.g., glutathione [GSH]) within the cell (4). Thus, previous reports demonstrated that the renal GSH content is an important factor in the progression of HgCl\textsubscript{2} nephrotoxicity since depletion of renal GSH markedly enhanced renal damage induced by HgCl\textsubscript{2} in spite of a reduced uptake of HgCl\textsubscript{2} (5,6). Further, as inorganic mercury is able to change between different oxidation steps, HgCl\textsubscript{2} is a potential stimulator of the peroxidative chain reaction, and has been shown to enhance renal lipid peroxidation in rats (7,8).

The present study aimed at studying time and dose-response relationships for the various functional, morphological, and biochemical indicators of HgCl\textsubscript{2}-induced acute renal damage in mice.

Materials and Methods

A total of 126 8-week-old female Bom:NMRI mice (Bom-mice, Ry, Denmark) were kept on beechwood bedding in a well controlled environment (50 + 5\% relative humidity, 20 air changes/hr, temperature 21 ± 1\(^\circ\)C, light/dark periods 12/12 hr with 0.5 hr twilight) with free access to standard mouse pellets (Altromin, Chr. Pedersen, Ringsted, Denmark) and water. After random assignment to experimental groups, animals were marked and weighed and given one oral dose (10 ml/kg) by stomach tube of HgCl\textsubscript{2} (Merck, Darmstadt FRG) in water (1, 25, 50, 100, or 200 \( \mu \text{g}/\text{kg} \) body weight as stated in Figures 1–4). The HgCl\textsubscript{2} solutions were labeled with \( \text{^{208}Hg} \) (Amersham, Buckinghamshire UK) to enable quantification of mercury in whole-body, kidney, liver, and blood, as well as in urinary excretion.

Experimental Design

Experiment 1. Six groups of four mice were killed on days 0, 1, 2, 3, 4, and 7, respectively, after a single oral dose of 100 \( \mu \text{g}\)/kg. The right kidney was removed for histologic investigation.

Experiment 2. Four experimental groups of 20 mice each were all killed at day 2 after a single oral dose of HgCl\textsubscript{2} (25, 50, 100, or 200 \( \mu \text{g}/\text{kg} \) body weight). An untreated group of 10 mice served as controls. The right kidney was removed for histologic investigation and the left kidney was weighed and counted in a Searle 1195R gamma counter (10 animals per experimental group). Livers as well as blood from the thoracic cavity (six animals per experimental group) were counted in the Searle 1195R gamma counter. The kidneys from the remaining ten animals per experimental group and the controls were used for measuring lipid peroxidation, concentration of GSH, total activity (T-GSH-Px) and activity of GSH-Px. All mice

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were killed between 8 and 10 A.M. to avoid influence from circadian rhythms. For each animal in the experimental groups, the amount of mercury in whole-body, kidneys, liver, and blood was calculated and expressed as nmol/kg.

**Experiment 3.** Two groups of six mice were killed at day 14 after a single oral dose of HgCl₂ (1 or 100 μmole/kg body weight). The animals were kept in metabolism cages during the 2-week experimental period, enabling calculation of the daily urinary excretion during the study period and total urinary excretion at day 14.

**Biochemical Assays**

**Lipid peroxidation.** The level of lipid peroxidation was measured by quantitation of thiobarbituric acid (TBA)-reactive substances in fresh tissue homogenates at pH 2 in accordance with the method described by Uchiyama and Miura (9) and modified by Sunderman et al. (10). The results were expressed as μmole malondialdehyde (MDA)/g tissue (dry weight). Analyses were performed in duplicate.

**Glutathione.** The concentration of total glutathione (nonprotein thiols) was determined by the method of Griffith (11). The results were expressed as μmole GSH/g tissue (dry weight).

**Glutathione Peroxidases.** The activity of the enzymes was determined by using the method of Lawrence and Burk (12) using either 12 mM β-butyryl hydroperoxide (T-GSH-Px assay) or 1.5 mM H₂O₂ (Se-GSH-Px assay) as substrate. When H₂O₂ was used as substrate, the activity of catalase was blocked by addition of sodium azide (1 mM in the assay). The results were expressed as units/mg protein.

**Histology**

The right kidney was cut into two parts through the sagittal plane and immersed into phosphate (0.075M)-buffered 4.5% formaldehyde, pH 7. Both halves of the kidneys were embedded in paraplast. Sections in the frontal plane were examined after staining with hematoxylin and eosin. Necrosis was diagnosed when the tubular epithelium was desquamated or when the nuclei were pyknotic or had disappeared. Regeneration was diagnosed when the cytoplasm of the tubular epithelium exhibited basophilia and the nuclei had increased in size. Mitoses were observed with varying frequency. The differential diagnosis between collapsed empty tubules and regenerating ones was based on the presence of mitoses. The grading of the alterations was based on the following criteria: 0— the alteration concerned was not found; 1—a few scattered tubules showed the alteration; 2—small but confluent areas of tubules showed the alteration; 3—nearly all proximal tubules were affected. In cases with simultaneous necrosis and regeneration, a grading of 3 was given to each, if about half of the tubules were necrotic and the other half regenerating.

The affected tubules were found in the cortex cortices and in the middle part of the cortex corresponding to the convoluted parts of at least the proximal tubules. In some cases altered pars rectae could be identified. The brush borders were absent in regenerating tubules. No interstitial inflammatory reaction was seen. The morphology was evaluated by one of the authors (HS) without knowledge of dose and time since exposure.

**Statistical Evaluation**

Differences in Hg deposition and biochemical parameters between experimental groups were analyzed by the nonparametric Mann-Whitney U-test. The level of significance was chosen at 0.05.

**Results**

**Experiment 1.** The necrosis in proximal tubuli was most extensive and severe in kidneys from mice killed 2 to 4 days after dosing. Necrosis of the proximal tubuli was not observed in mice killed 7 days after mercuric chloride administration. The kidneys of mice killed at 2, 3, 4, and 7 days after dosage showed extensive regeneration in the proximal tubular region, whereas regeneration in this region was absent in mice killed at an earlier time after dosing (Figure 1).

**Experiment 2.** At day 2 after dosing, whole-body retention (WBR) was increased almost 8-fold when the dose was increased from 100 to 200 μmole/kg (Figure 2). Thus, the relative mercury retention in the group given 200 μmole/kg was 4 times higher than in the group given 100 μmole/kg. At the higher doses, the kidney deposition expressed as nmole/g tissue (wet weight) was not increased proportionally with the dose administered (Figure 3).
2). The amounts of mercury in blood and liver increased proportionally with the dose, except at 200 μmole/kg. At this dose the amount of mercury in the blood was eight times higher than after a dose of 100 μmole HgCl₂/kg, whereas liver deposition was increased four times. Histologic investigation revealed necrosis of the proximal tubules in all kidneys from mice given 100 μmole/kg. In the kidneys from mice given 200 μmole/kg, more extensive necrosis and minimal regeneration were observed. Regeneration of proximal tubuli was, however, observed in all animals from the groups given 50 and 100 μmole/kg (Figure 3). No stimulation of renal lipid peroxidation, measured as TBA reactive substances, was observed 2 days after a single oral dose of HgCl₂, although the two highest doses induced extensive tubular necrosis (Figure 2). After a dose of 25 μmole HgCl₂/kg the renal concentration of GSH was enhanced compared to untreated controls. At higher doses of HgCl₂, the GSH concentration declined; and at 200 μmole HgCl₂/kg the GSH concentration was significantly lower than in untreated controls. A dose-dependent reduction in activity of both T-GSH-Px and Se-GSH-Px was observed. The activities were significantly reduced in groups receiving 50 μmole HgCl₂/kg and higher doses.

Experiment 3. Increasing the dose of HgCl₂ from the nontoxic level of 1 μmole/kg to the nephrotoxic level of 100 μmole/kg identified in experiments 1 and 2 clearly increased the relative urinary excretion (percent of administered dose) during the first 2 days (Figure 4). From day 3 the difference in percent of renal excretion between the groups diminished and eventually disappeared. The higher total relative urinary excretion during the 2-week study in the group given 100 μmole/kg compared with the group given 1 μmole/kg correlated with the differences existing already during the first 2 days after dosing. Thus, the period of enhanced Hg excretion coincided with the period of histologically visible tubular damage prior to extensive regeneration (Figure 1).

Discussion

As a significant change in the toxicokinetic behavior of mercuric chloride has been demonstrated in mice given 100 μmole/kg or higher oral doses compared with lower doses (1,2), a dose level of 100 μmole/kg was used for evaluating the time course relationship for induction and regeneration of renal tubular damage. The change in toxicokinetics was confirmed in the present study as the kidney deposition of mercury did not increase proportional to the whole-body retention of mercury (Figure 2). At day 2 after dosing, whole-body retention correlated well with the concentration of mercury in blood (Figure 2).

The dose-dependent reduction in Se-GSH-Px activity (Figure 5) is in accordance with earlier investigations. Thus, Wada et al. (13) observed an inhibition of renal Se-GSH-Px in mice exposed to HgCl₂. Simultaneous administration of an equimolar dose of sodium selenite completely prevented the inhibition of Se-GSH-Px, indicating that at normal selenium levels, interactions between selenium and mercury might reduce the amount of selenium available for Se-GSH-Px, and this could be responsible for the enzyme inhibition. The decrease in amount of renal GSH observed at higher dose levels could be due to saturation of gamma-glutamyltranspeptidase, inhibition of enzymes involved in GSH synthesis (as demonstrated by Chung et al. (14) or leakage from damaged renal tissue. As TBA-reactive substances were not increased at a time period in which renal damage was maximum as estimated from urinary Hg excretion and morphologic damage, the present results indicate that lipid peroxidation is unlikely to be the primary toxic mechanism in acute nephrotoxicity induced by HgCl₂ in this experimental setting.

In accordance with earlier published results (15), necrosis in the proximal tubules developed within 1 to 3 days after dosage. The regeneration began at day 3 and was also demonstrated at day 4 and 7. Depending on the dose, regeneration is probably completed within 4 to 7 days after exposure. This may explain the absence of necrosis in proximal tubules in mice killed at day 7.

The increased relative urinary excretion during the first 48 hr in animals given 100 μmole HgCl₂/kg compared with mice given a nontoxic dose of HgCl₂ (Figure 4) correlates with the time course of kidney damage demonstrated in experiment 1 (Figure 1). This momentary increase in relative urinary excretion at the high dose level could be caused by mercury leakage through damaged kidney tissue prior to regeneration. The lack of a proportional

![Figure 3](image-url) Effect of HgCl₂ dose size on histopathologic kidney damage at day 2 after administration of a single oral dose of HgCl₂ (group size = 10).

![Figure 4](image-url) Effect of HgCl₂ dose size on cumulative urinary mercury excretion (percent of administered dose) after a single oral dose of HgCl₂ to mice (medians, group size = 6).
increase in kidney deposition at increasing doses also supports the conclusion of mercury leakage through damaged kidneys at the higher dose levels. Mercury leakage could be due to reduced tubular resorption of Hg–GSH and other low-molecular weight Hg complexes in proximal tubuli, to cell shedding from the tubuli, or to glomerular damage. The present study demonstrates necrosis in the tubuli, but early stages of glomerular dysfunction can not be excluded, although the rapid reestablishment of normal excretion rates (2) suggests tubular damage to be the primary cause for the change in excretion.

The histologic data from experiment 2 indicate that already a dose level of 50 pmole/kg induces minor kidney damage that is rapidly regenerated. Increasing the dose will lead to aggravation of the kidney damage and regeneration may take longer or even be absent (Figure 3). The inverse relationship between administered dose and percent WBR previously reported (1,2) may thus be explained by temporary leakage in the proximal tubules leading to increased mercury excretion with the urine.

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