Increased Oxidative DNA Damage, as Assessed by Urinary 8-Hydroxy-2’-Deoxyguanosine Concentrations, and Serum Redox Status in Persons Exposed to Mercury

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Background: Mercury is a ubiquitous and highly toxic environmental pollutant. In this study, we evaluated the relationship between mercury exposure and oxidative stress, serum and urinary mercury concentrations, oxidative DNA damage, and serum redox status in chronically mercury-exposed persons compared with healthy controls.

Methods: We measured urinary 8-hydroxy-2’-deoxyguanosine (8-OHdG), which we used as a biomarker of oxidative DNA damage in the mercury-exposed persons, by HPLC with electrochemical detection (ECD). We evaluated antioxidant status by measuring the activities of superoxide dismutase and glutathione peroxidase and the concentrations of total reduced glutathione and protein-bound thiols in serum.

Results: The significant increase in 8-OHdG concentrations in urine indicated that mercury-induced oxidative damage to DNA occurred in vivo. Differences in body mercury burden and antioxidant enzyme activities were statistically significant between the mercury-exposed persons and controls. Serum and urinary mercury concentrations in the mercury-exposed persons were more than 40-fold higher than in controls.

Conclusions: Mercury exposure can induce oxidative DNA damage, whereas the antioxidative repair systems can be expected to minimize DNA lesions caused by mercury. Measurement of urinary 8-OHdG could be useful for evaluating in vivo oxidative DNA damage in mercury-exposed populations.

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Mercury is a highly toxic environmental pollutant. The harmful impacts of mercury include neurotoxicity, neurologic problems, immunosuppression, risk of myocardial infarction, autism, Alzheimer disease, and other related problems (1–3). Even at extremely low exposures, mercury can cause permanent damage to the human central nervous system (4).

Toxicity from mercury is associated with oxidative stress in vivo, in which mercury induces the generation of reactive oxygen species (ROS)5 and alters the antioxidant defense system of cells. The detoxification process for heavy metals involves reduced glutathione (GSH), metallothioneins, and interactions with other elements such as selenium and its antioxidative selenoenzymes, e.g., glutathione peroxidase (GSH-Px) (5, 6).

ROS are considered important factors in cancer, arthritis, asthma, and heavy metal intoxication. Attacks on DNA by ROS, including the hydroxyl radical (OH), superoxide anion (O2•−), singlet oxygen, the ferryl or per-
ferroxyl ion, hydrogen peroxide, and peroxynitrate (OONO\(^-\)), frequently cause oxidative DNA damage. Recent studies have shown that DNA damage and altered intracellular redox status, such as imbalance of sulphydryl and redox enzymes, occur after exposure to mercury, iron, chromium, and cadmium (7). Occupational and environmental exposure to chemicals may increase the risk of unrepaired DNA lesions, which could become permanent mutations (7). Evaluation of the genotoxic activity of mercury in lymphocytes by single-cell gel electrophoresis assays (the comet assay), chromosome aberration analysis, and micronucleus techniques has been reported (8, 9). DNA repair products are transported through the blood and excreted into the urine without further metabolism. Although >20 different oxidative adducts of DNA bases have been identified, most attention has focused on 8-hydroxyguanine and its deoxyribonucleoside derivative, 8-hydroxy-2^{-}deoxyguanosine (8-OH\(\text{dG}\)). Because it is much easier to collect and handle a urine sample, 8-OH\(\text{dG}\) in urine has been used as a reliable biomarker of oxidative DNA damage in vivo. HPLC with electrochemical detection (ECD) is currently the most widely used method for the measurement of 8-OH\(\text{dG}\) (10–13).

The presence of mercury in the environment has increased in the past century because of fossil fuel combustion, gold and mercury mining, and industrial chemical emissions. As a pilot study, we selected Wanshan, a town located in the southeast part of Guizhou Province of China, as a typical mercury-contaminated area. The town of Wanshan was once one of the major mercury-mining areas in China. The main source of environmental mercury was the emission of elemental mercury vapor (Hg\(^0\)) from the mercury-mining plants, where large-scale production of mercury lasted for more than 50 years before ending in 2001. The mercury concentration in air is now still much higher than the US Environmental Protection Agency reference concentration for chronic mercury exposure (400 ng/m\(^3\)). Exposure of local people and animals to mercury occurs not only through inhalation of mercury vapor, but also through consumption of mercury-contaminated foodstuffs via biological accumulation and bio-transformation. In our previous study, the mean mercury concentrations in different fish species were 25- to 50-fold higher than those in non-mercury-contaminated areas (14). The local soil, water, rice, and porcine meat are also heavily contaminated (15, 16).

The relationship between oxidative DNA lesions and long-term exposure to mercury, however, has not been demonstrated. Information about ongoing or recent occupational exposure to high concentrations of mercury is scarce. Importantly, there are no published data concerning the use of 8-OH\(\text{dG}\) for evaluating DNA lesions in a mercury-exposed population. In the present study, we used urinary 8-OH\(\text{dG}\) (a metabolite of oxidized DNA) to evaluate whether mercury exposure led to oxidative damage to DNA. We also investigated the activities of serum GSH-Px and superoxide dismutase (SOD) and the concentrations of GSH and total protein-bound thiol in mercury-exposed individuals to clarify the relationship between body mercury status and oxidative stress.

**Materials and Methods**

**PARTICIPANTS AND SPECIMEN COLLECTION**

We collected serum samples from 13 residents who lived in the Wanshan area in November 2000 and from 35 workers exposed to mercury on the job in November 2003 (total age range, 32–66 year). Some of the exposed individuals showed typical symptoms of mercury toxicity, such as digestive dysfunction, hypomnesia, sleeping problems, and tremors. An additional 35 healthy residents (age range, 28–64 years) living in an area of Beijing not contaminated with mercury served as the non-contaminated group. Gender and age matching were part of the experimental design, and six women 32–45 years of age were included in each group. The mean (SD) ages of the mercury-exposed group and the controls were 45.5 (16.5) and 44.0 (14.5) years, respectively. All participants gave consent, and the study was approved by the authorized medical office and the local Ethics Committee.

First-morning void urine samples of at least 30 mL were collected from each participant. Blood samples of at least 10 mL were collected in the morning after overnight fasting, and the sera were obtained by centrifugation (10 min at 1500g) 2–4 h after blood collection. To avoid effects related to storage temperature (17), the urine and serum samples were stored at \(-70\) °C until analysis. Unfortunately, the volumes of some samples were insufficient for all biological analyses. Therefore, during the experiment, we compared the biological data with the corresponding mercury concentrations.

**REAGENTS AND INSTRUMENTATION**

Reagents and solvents were of at least analytical chromatographic grade. 8-OH\(\text{dG}\) \((C_{16}H_{13}N_2O_5\); \(M_r\) = 283.2) was obtained from Sigma. A MilliQ system (Millipore) was used to prepare ultrapure water. Hydride generation-atomic fluorescence spectrometry was performed in Beijing, China, with a Model AFS-820 dual-channel analyzer with hydride generator and quartz furnace atomizer. Bond Elut Certify solid-phase extraction (SPE) columns (10-mL capacity) were obtained from Varian. The HPLC system (ESA Inc.) included a Model 582 pump, Model 542 autosampler, Model 5600A Coularray electrochemical detector, and Coularray data station.

**MERCURY ANALYSIS**

The total mercury concentration in each sample was determined by hydride generation-atomic fluorescence spectrometry as described previously (16). The detection limit was 0.05 \(\mu\)g/L. We simultaneously evaluated the accuracy by use of several certified reference materials (NIST-bovine liver1577a, IAEA horse kidney H-8, and
Chinese bovine liver GBW (080193), and the analytical error was less than ±10%.

**MEASUREMENT OF URINARY 8-OHDG BY HPLC-ECD**

Urinary 8-OHdG was measured by SPE and HPLC-ECD as described previously (11, 12). Briefly, 1 mL of 0.1 mol/L KH_2PO_4 (pH 6.0) was added to 2 mL of urine, and the mixture was then centrifuged at 1500 g for 10 min. A 2.8-mL volume of the supernatant was added to a Bond Elut Certify SPE column that had been pretreated with 10 mL of methanol, 5 mL of deionized water, and 10 mL of 0.1 mol/L KH_2PO_4 under slightly reduced pressure. After being washed with 3 mL of deionized water, the SPE column was dried for 10 min under strongly reduced pressure. The 8-OHdG was eluted with 1 mL of 0.1 mol/L KH_2PO_4 under slightly reduced pressure and stored in a refrigerator until chromatographic analysis. Separation of 8-OHdG was carried out on a Waters Bondapak C_18 (150 × 3.9 mm; 10-µm bead size) analytical column. The mobile phase consisted of 12.5 mmol/L citric acid, 25 mmol/L sodium acetate, 10 mmol/L acetic acid, 30 mmol/L sodium hydroxide, 20 mg/L EDTA, and 30 mL/L methanol. This solution and urine samples were filtered through a Millipore 0.22 µm cellulose acetate filter. The flow rate was 1.0 mL/min, and 25 µL was injected into the HPLC system.

**CREATININE AND PROTEIN MEASUREMENTS**

Because of the variability of urinary volume among individuals, the 8-OHdG concentrations were normalized in relation to the amount of creatinine. In this experiment, the creatinine concentrations in the urine samples were measured on a Bayer Clinitek 200+ urine chemistry analyzer. Serum total protein was quantified by the Bradford method with bovine serum albumin as the protein calibrator (18).

**GSH-PX AND SOD MEASUREMENTS**

GSH-Px activity was measured according to the method of Hafeman et al. (19). One unit was defined as a decrease in the log concentration of GSH of 1 µmol/L per minute at 37 °C. Samples heated to inactivate the enzyme were used as the nonenzymatic control to eliminate interference from endogenous GSH. Total SOD activity was assayed as the inhibition of the autooxidation of pyrogallol in alkaline solution monitored at 420 nm (20), with 1 U of activity giving 50% inhibition at 10 min. The enzyme activity in the sample was expressed as U/mg of serum protein.

**MEASUREMENT OF GSH AND TOTAL PROTEIN-BOUND THIOLS**

The concentrations of GSH and the total thiol (–SH) groups in human serum proteins were measured by the Ellman method (21), with commercial GSH as the calibrator. The thiol concentration was measured at 412 nm based on the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) to form 2-nitro-5-thiobenzoate anion.

**GENERAL URINALYSIS**

The examination of urine included color, appearance, pH, specific gravity, urea nitrogen, urobilinogen, bilirubin, ketone bodies, nitrites, qualitative protein and glucose measurements, leukocyte esterase, and occult blood. These were performed on an automated biochemical analyzer (Clinitek 200+ Urine Chemistry Analyzer; Bayer Corporation).

**STATISTICAL ANALYSIS**

Statistical analyses were performed with the Student t-test. Multigroup comparisons of variables were carried out by ANOVA with the Statistical Package of Social Science (SPSS) 9.05 for Windows. All reported P values

**Table 1. Comparison of mercury concentrations in persons from the mercury-contaminated and noncontaminated areas.**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Population</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>P, t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Mercury-exposed group</td>
<td>38.5 (61.5)</td>
<td>1.85–210.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total (n = 37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local residents (n = 13)</td>
<td>7.50 (3.22)</td>
<td>1.85–11.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Workers exposed on the job (n = 24)</td>
<td>60.8 (70.2)</td>
<td>5.7–210.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nonexposed group (n = 35)</td>
<td>0.91 (0.28)</td>
<td>0.41–1.20</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>Mercury-exposed group</td>
<td>86.8 (65.2)</td>
<td>11.0–205.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total (n = 33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local residents (n = 8)</td>
<td>23.3 (10.6)</td>
<td>11.0–38.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Workers exposed on the job (n = 25)</td>
<td>104.3 (63.3)</td>
<td>19.1–205.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Hg, ng/mg creatinine (n = 33)</td>
<td>76.5 (59.5)</td>
<td>8.2–293</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nonexposed group (n = 35)</td>
<td>0.95 (0.5)</td>
<td>0.3–2.4</td>
<td></td>
</tr>
</tbody>
</table>

*Data from Drasch et al. (22), used for comparison.

*Data from Iyengar (23), used for comparison.
are two-tailed. \( P \) values \(<0.05 \) were considered statistically significant. For the bivariate analyses, linear regression was performed.

**Results**

**MERCURY CONCENTRATIONS IN SERUM AND URINE**

The mercury concentrations in participants from the geographic areas where mercury exposure did or did not occur are shown in Table 1. The mean serum mercury concentration in the exposed group was almost 40-fold higher than that in the control group. The mercury concentrations in the persons with ongoing or recent occupational exposure were also higher (approximately eightfold) than in the local residents with environmental exposure. As shown in Fig. 1, mercury concentrations in the urine and serum of the mercury-exposed individuals were positively correlated \( (r = 0.746; \ P <0.001) \).

The mean urinary mercury concentration (U-Hg) in the mercury-exposed group was significantly higher than previously reported data in the literature \( (22, 23) \). The U-Hg concentrations in 10 of the exposed individuals were very high \( (150–205 \ \mu g/L) \). If normalized to the creatinine concentration, U-Hg was also >100-fold higher than reported data from Germany \( (\text{mean}, 0.71 \ \text{ng Hg/mg of creatinine}) \), and almost 9-fold higher than the concentration found in people from Mt. Diwata, Philippines, a small-scale gold mining area \( (\text{mean}, 8.40 \ \text{ng Hg/mg of creatinine}) \) \( (22) \).

**Urinary 8-OHdG**

The analytical run time for the HPLC-ECD method was 20 min, and 8-OHdG eluted at \(~13.8 \ \text{min} \) \( (11, 12) \). 8-OHdG can be monitored by use of applied voltages of 0, 100, 300, 400, or 500 mV. In this study, the maximum sensitivity and selectivity for 8-OHdG were obtained by use of 300 mV. Kasai \( (13) \) reported that results obtained at 280 and 350 mV were better than those obtained at 400 mV. Representative HPLC chromatograms for 8-OHdG in urine samples and the calibrator are shown in Fig. 1 of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol51/issue4/. The calibration curves were drawn from the peak heights after injection of the same aliquots to which 0, 10, 25, 50, 100, and 200 \( \mu g/L \) of the commercial 8-OHdG had been added. The calibration curves were linear within this working range \( \) (data not shown). The value for the reagent blank was used to correct 8-OHdG concentrations in the samples. Some urine samples that had very high 8-OHdG concentrations were diluted. The recovery of 8-OHdG added to urine samples was 90–110%.

The raw and corrected data for urinary 8-OHdG are listed in Table 2. The raw values \( (\mu g/L) \) were normalized to creatinine \( (\text{mg/L}) \) for proper comparisons with each other and with data in the literature. Urinary 8-OHdG was increased in the mercury-exposed individuals. Compared with the data from previous studies of a healthy population \( (10, 12, 13) \) and the control group in our study, the mean urinary 8-OHdG concentration in the mercury-exposed persons was 60- to 120-fold higher. This supports the idea that urinary concentrations of 8-OHdG can serve as a reliable biomarker for evaluating oxidative DNA damage caused by mercury.

As shown in Fig. 2, urinary 8-OHdG and U-Hg concentrations were positively correlated \( (r = 0.62; \ P <0.001) \), as were urinary 8-OHdG and serum mercury concentrations \( (r = 0.71; \ P <0.001) \).

**SERUM REDOX STATUS**

The GSH and total protein thiol group concentrations and GSH-Px and SOD activities in sera are listed in Table 3. The concentrations of GSH and total protein thiols were much higher in the mercury-exposed group than in the control group, and the GSH-Px and SOD activities were also increased. The total protein concentration in serum was slightly higher in the control group than in the mercury-exposed group, but the difference was not sta-

![Fig. 1. Linear regression analysis of mercury concentrations in urine and serum of the mercury-exposed individuals.](image)

**Table 2. Urinary 8-OHdG concentrations.**

<table>
<thead>
<tr>
<th>Group</th>
<th>8-OHdG, ng/mg creatinine</th>
<th>Range</th>
<th>8-OHdG, µg/L</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury-exposed persons ( n = 33 )</td>
<td>242.9 (423.6)</td>
<td>9.2–1400</td>
<td>287.8 (501.9)</td>
<td>9.3–2003.7</td>
</tr>
<tr>
<td>Nonexposed group ( n = 15 )</td>
<td>2.08 (1.23)</td>
<td>0.95–4.7</td>
<td>2.28 (1.37)</td>
<td>1.05–5.33</td>
</tr>
<tr>
<td>( P ), \text{t-test}</td>
<td>(&lt;0.01</td>
<td></td>
<td>(&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>
tistically significant. SOD activity was more than twofold higher in the mercury-exposed group than in the controls.

**SERUM MERCURY AND REDOX STATUS**

In the sera of mercury-exposed individuals, mercury concentrations were strongly positively correlated with the protein-bound thiol concentration \((r = 0.803; P < 0.001)\), SOD activity \((r = 0.890; P < 0.001)\), and GSH-Px activity \((r = 0.795; P < 0.05)\), but serum GSH was only weakly correlated \((r = 0.480; P < 0.05)\) with serum mercury concentrations (Fig. 3).

**URINALYSIS**

The qualitative protein, qualitative glucose, urobilinogen, bilirubin, ketone body, nitrite, leukocyte esterase, and occult blood tests results were negative for specimens from all groups. The mean (SD) concentrations of the other studied markers of kidney damage, such as urea nitrogen \([117.0 (14.4) \text{ mmol/L}]\), pH, specific gravity, and calcium \([114 (83) \text{ mg/L}]\); see Table 1 of the online Data Supplement], were within the appropriate reference intervals and were not increased in the persons exposed to mercury. Although the mean creatinine concentration \([10.57 (4.04) \text{ mmol/L}]\) was within the reference interval, urinary creatinine concentrations in 12 mercury-exposed individuals who had high U-Hg \((100–205 \mu \text{g/L})\) were highly increased. The maximum in one individual was 19.8 mmol/L creatinine. Urinary creatinine concentrations showed an increasing relationship with the increase in U-Hg \((R = 0.471; P < 0.01; \text{Fig. 4})\), but not urea nitrogen and calcium \((R = 0.07 \text{ and } 0.08, \text{respectively}; P > 0.05)\). The range of calcium concentrations was wide (Table 1 of the online Data Supplement); the urinary creatinine concentration therefore seems to be a more sensitive indicator of a change in renal function. A higher mercury burden has been reported in the kidneys of mercury-exposed individuals, whereas proteinuria and a slight subclinical glomerular dysfunction may be associated with long-term mercury exposure \((24)\).

In general, the mercury exposure for most individuals in our study was not long or high enough to produce obvious nephrotoxicity.

**Discussion**

Blood, urine, and hair samples have been used to evaluate mercury burden and its impact on human health \((1, 25)\). Previous studies \((26–28)\) have reported that total urinary mercury for a non-mercury-exposed population is \(<10 \mu \text{g/L}\) \((\text{reference interval, } 1–5 \mu \text{g/L}; \text{toxic concentrations, } >50 \mu \text{g/L})\), whereas serum mercury is \(<5 \mu \text{g/L}\) \((\text{reference interval, } 1–8 \mu \text{g/L}; \text{toxic concentrations, } >200 \mu \text{g/L})\). Although the selected indicator of mercury exposure was the concentration of mercury in urine (U-Hg), the concentrations were significantly higher in workers with

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**Table 3. Comparison of redox status in sera from local residents in the mercury-contaminated and noncontaminated areas.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mercury-exposed group ((n = 13))</th>
<th>Nonexposed group ((n = 15))</th>
<th>(P) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g/L</td>
<td>69.9 (8.0)</td>
<td>72.3 (9.6)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GPx, (^a) U · (mg protein(^{-1}) · min(^{-1})</td>
<td>1.21 (0.17)</td>
<td>1.07 (0.20)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GSH, mg/L</td>
<td>164.6 (23.1)</td>
<td>148.7 (18.6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Thiols, mmol/L</td>
<td>0.343 (0.056)</td>
<td>0.243 (0.100)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SOD, (^b) mU · (mg protein(^{-1}) · min(^{-1})</td>
<td>66.7 (19.7)</td>
<td>22.2 (13.4)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

\(^a\) One unit of enzyme activity is defined as a decrease in the log(GSH) concentration of 1 \(\mu \text{mol} · \text{L}^{-1} · \text{min}^{-1}\) at 37 °C.

\(^b\) One unit of enzyme activity is the enzyme concentration that causes a 50% inhibition in the autoxidation of pyrogallol in alkaline solution after 10 min at 25 °C.
prolonged exposure to low doses of inorganic mercury than in controls [9.7 (5.5) vs 2.4 (1.2) μg/L] (29, 30).

Compared with previous studies (22, 23), in our study, the nonexposed group from an uncontaminated area of Beijing had serum mercury concentrations within the reference interval (Table 1). Among the persons from Wanshan, both urine and blood samples had mercury concentrations that exceeded the upper limits of the reference intervals (10 μg/L for urine and 5 μg/L for blood, respectively). More importantly, workers with long-term occupational exposure had a much higher mercury body burden than the local residents [in serum, 60.8 (70.2) vs 7.50 (3.22) μg/L; in urine, 104.3 (63.3) vs 23.3 (10.6) μg/L, respectively]. These occupationally exposed individuals have shown signs of problems with sleeping, walking, hearing, and vision (constricted visual fields). The U-Hg values for these workers are higher than the values reported in the study of residents of Mt. Diwata in the Philippines (22), where exposure of the population to mercury resulted from small-scale gold mining. Therefore, in addition to serum, evaluation of mercury in urine can be a good and reliable indicator of the extent of exposure, whereas human hair is susceptible to environmental contamination.

Although the oxidative properties of mercury have been studied and accepted, the actual process of ROS generation is still unclear. In most studies, lipid peroxidation, DNA damage, and GSH imbalances caused by mercury have been assessed and have suggested an oxidative stress-like mechanism for mercury cytotoxicity (6, 31–33).

In the present study, the results for the redox enzymes
in the exposed individuals suggest that mercury overload induces an imbalance in the redox cycle. Enzymatic activities and thiol concentrations were strongly positively correlated with the mercury concentrations (Fig. 3). The mercury-exposed patients had significantly higher serum concentrations of GSH and protein-bound thiols than did the control groups. However, the role of GSH in the detoxification of mercury is not yet clear. GSH and protein-bound thiols could directly bind mercury through the sulfhydryl group. It has been reported that mercury-protein-bound thiols could directly bind mercury through the sulfhydryl group. Thus, glutathione is also used to prevent oxidative stress and toxic agents. In addition, GSH can also be a substrate for GSH-Px, thioredoxin reductase, and glutathione S-transferase (5), and is involved in the enzymatic detoxification reactions for ROS. Thus, glutathione is also used to prevent oxidative stress in most cells and helps to trap free radicals that can damage DNA and RNA. The effect on total GSH concentrations in cultured cells has been studied at different concentrations of mercury. Dose-dependent increases in GSH concentrations and γ-glutamyl cysteine synthetase activity were observed after exposure to 1 and 2 μmol/L mercury for 20 h (32, 34). The intracellular concentrations of GSH and metallothionein were increased significantly in NIH 3T3 cells at a Hg^{2+} concentration of 0.5 μmol/L (6).

We also observed significant increases in serum GSH-Px and SOD activities in the exposed individuals studied. The main functions of these two enzymes involve their antioxidant properties. Our findings are consistent with those of Girardi and Elias (35), who reported a positive correlation between GSH-Px activities and kidney mercury content in mercury-treated rats. SOD, GSH-Px, and lipid peroxidation were significantly increased in the livers, kidneys, and brains of Atlantic salmon fed fish-meal-based diets supplemented with mercuric chloride or methylmercury chloride for 4 months (3). Thus, the present findings further confirm that oxidative stress could be involved in the cytotoxicity of mercury in vivo. Antioxidative functions in the mercury-exposed individuals could be activated to detoxify mercury to protect the functions of the human body.

In a previous study we found that, in mercury-exposed pigs, mercury appeared to be concentrated in the nuclear and mitochondrial fractions of the liver, kidney, lung, and spleen compared with other subcellular fractions (16). This suggests that mercury being accumulated in nuclei could directly act on DNA, which is related to mercury's genotoxicity. The higher concentration of mercury in mitochondria may have a relationship with previous reports that mercury-induced apoptosis may occur via a mitochondria-dependent pathway in several cell lines (36).

Earlier studies have suggested that mercury is genotoxic, whereas ROS generation is associated with the effect of specific oxidative DNA damage. One reason is the ability of mercury compounds to bind directly with tubulin sulfhydryl, which impairs spindle function and causes chromosome aberrations. In humans, structural chromosomal aberrations, micronuclei, or sister chromatid exchanges have been induced in cultured human blood lymphocytes (9, 37, 38). On the other hand, DNA lesions are sensitive to free radicals induced by mercury. For example, the damage to DNA by ‘OH includes single-strand breaks, base modifications, and conformational changes. The damaged DNA can be repaired in vivo by endonucleases or by a base-specific glycosylase. The DNA repair products are transported through the blood and excreted into urine without further metabolism (39). 8-OHdG is a direct adduct of hydroxylation of DNA bases formed when ‘OH reacts with DNA. Our results indicate that urinary 8-OHdG is as sensitive as chromosome aberration analysis and micronucleus techniques for the assessment of the genotoxicity of mercury compounds. Moreover, urine samples are much easier to collect and handle.

Previous data on the urinary excretion of 8-OHdG have been summarized by Renner et al. (10). The mean 8-OHdG concentration ranged from 2.1 to 4.0 ng/mg of creatinine in healthy individuals, as measured by SPE-HPLC-ECD or liquid chromatography–liquid chromatography–ECD, whereas the mean 8-OHdG concentrations in smokers (3.6–7.8 ng/mg of creatinine) were almost two-fold higher than in nonsmokers. Lifestyle characteristics such as exercise, working conditions, meat intake, body mass index, and smoking could modestly affect urinary 8-OHdG excretion (40). Increased urinary concentrations of 8-OHdG were found in occupationally exposed individuals by Pilger et al. (12) and Tagesson and coworkers (41, 42), who reported 8-OHdG excretion rates of 3.5 (1.4),
3.7 (1.4), 4.8 (2.1), and 4.3 (1.9) ng/mg of creatinine, respectively, for workers in the asbestos, rubber, azo-dye, and art glass industries and those exposed to indoor background radiation. In contrast to earlier published data, the concentrations in the mercury-exposed individuals in the present study are 60- to 120-fold higher. In the present study, we also noted interindividual variations in 8-OHdG excretion. The correlation between 8-OHdG excretion and the mercury-exposure concentration was significant (see Fig. 2). Some persons had extremely high concentrations of urinary 8-OHdG (238–1400 ng/mg of creatinine). To our knowledge, this is the first study to demonstrate a relationship between the concentrations of mercury and 8-OHdG in urine. The significant increase in urinary 8-OHdG concentrations, which is used as a biomarker of oxidative DNA damage in mercury-exposed individuals, indicates that mercury-induced oxidative damage to DNA occurs in vivo. Thus, free radicals play an important role in mercury toxicity.

In conclusion, DNA breakage may be associated with the production of free radicals caused by mercury in vivo. The DNA repair product 8-OHdG directly indicates the hydroxylation of DNA bases by the reaction of -OH with DNA. We also speculate that the corresponding increases in serum GSH concentrations and SOD and GSH-Px activities could be an indirect compensatory response of these cells to increased oxidative challenge attributable to mercury stress. This could be an autoproective mechanism in organisms.

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