Genomic Analysis of the Rat Lung following Elemental Mercury Vapor Exposure

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Elemental mercury (Hg\(^{0}\)) is a highly toxic chemical with increasing public health concern. Although the lung receives the highest exposure to Hg\(^{0}\) vapor, it is resistant to Hg\(^{0}\) toxicity relative to the kidney and brain. In an earlier study, exposure of rats to 4 mg Hg\(^{0}\) vapor/m\(^3\), 2 h per day for 10 days, did not produce pathological alterations in the lung but increased metallothionein and glutathione S-transferase in the kidney. This study was undertaken to examine pulmonary gene expression associated with Hg\(^{0}\) vapor inhalation. Total RNA was extracted from lung tissues of rats, previously exposed to air or Hg\(^{0}\) vapor, and subjected to microarray analysis. Hg\(^{0}\) vapor exposure increased the expression of genes encoding inflammatory responses, such as chemokines, tumor necrosis factor-\(\alpha\) (TNF\(\alpha\)), TNF-receptor-1, interleukin-2 (IL-2), IL-7, prostaglandin E2 receptor, and heat-shock proteins. As adaptive responses, glutathione S-transferases (GST-pi, mGST1), metallothionein, and thioredoxin peroxidase were all increased in response to Hg exposure. Some transporters, such as multidrug resistance-associated protein (MRP), P-glycoprotein, and zinc transporter ZnT1, were also increased in an attempt to reduce pulmonary Hg load. The expression of transcription factor c-jun/AP-1 and PI3-kinases was suppressed, while the expression of protein kinase-C was increased. Expression of epidermal fatty acid-binding protein was also enhanced. Real-time RT-PCR and Western blot analyses confirmed the microarray results. In summary, genomic analysis revealed an array of gene alterations in response to Hg\(^{0}\) vapor exposure, which could be important for the development of pulmonary adaptation to Hg during Hg\(^{0}\) vapor inhalation.

Key Words: mercury vapor inhalation; lung; microarray; gene expression; adaptation.

Elemental mercury (Hg\(^{0}\)) is a ubiquitous metal with significant human exposure. At room temperature, Hg\(^{0}\) exists as a heavy, silver liquid; however, because it has a relatively high vapor pressure, inhalation of Hg\(^{0}\) vapor is the primary route of human exposure (IPCS, 1991). Elemental mercury vapor released from dental amalgams is the primary source of non-occupational exposure (Clarkson, 1997; Goering et al., 1992; Mackert and Berglund, 1997). Dental silver amalgam for tooth filling contains up to 70% Hg\(^{0}\), and its use can result in exposure of dentists, dental assistants, and patients (IPCS, 1991). Inhalation exposure to significantly higher concentrations can occur during accidental or occupational exposure in chloralkali plants, metal smelting and refining, and in mining industries (ATSDR, 1997; IPCS, 1991; Satoh, 2000).

During Hg\(^{0}\) vapor exposure, Hg\(^{0}\) is rapidly absorbed from the inhaled air into the lung tissue. Approximately 80% of the inhaled Hg is retained in the body (Hurst et al., 1976). Because Hg\(^{0}\) is a small, highly lipophilic element, it readily crosses the alveolar membranes into the pulmonary blood stream. Once in the blood, Hg\(^{0}\) can diffuse through erythrocyte cell membranes, where it is rapidly oxidized by cytosolic catalase to mercuric mercury (Hg\(^{2+}\)), the reactive species for most mercury compounds (Clarkson, 1997). Some Hg\(^{0}\) is distributed to distant organs before it is oxidized to Hg\(^{2+}\). Unlike Hg\(^{0}\), the charged Hg\(^{2+}\) does not easily cross cell membranes and accumulates within the cell. Mercuric Hg is highly reactive and rapidly combines with intracellular ligands such as sulfhydrys, lipids, enzymes, proteins, and macromolecules critical for normal function.

Although the lungs receive the highest concentrations of Hg\(^{0}\) vapor, the kidney and the central nervous system (CNS) are the major target organs of Hg\(^{0}\) toxicity (ATSDR, 1997; IPCS, 1991). The lung appears to be inherently less susceptible to Hg\(^{0}\) vapor toxicity than other organs, with pulmonary toxicity reported in humans only after acute occupational or accidental exposure to high concentrations of Hg\(^{0}\) vapor. The results of severe cases include respiratory distress, pulmonary edema, bronchiolar obstruction, pneumothorax, and death (Asano et al., 2000; ATSDR, 1997; Solis et al., 2000). Dyspnea, reduced vital capacity, diffuse pulmonary infiltrates, and evidence of interstitial pneumonitis have been reported in workers accidentally exposed to an estimated concentration up to 44 mg/m\(^3\) Hg\(^{0}\) vapor for 4 to 8 hours (McFarland and Reigel, 1978). In laboratory studies, rats exposed to 27 mg/m\(^3\) for 2 h developed pulmonary edema, necrosis of the alveolar epithelium with

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hyaline membranes, and fibrosis (Livardjani et al., 1991). In our earlier studies (Brambila et al., 2002; Morgan et al., 2002), it was shown that in pregnant and non-pregnant rats exposed to 4 mg/m³ Hg⁰ vapor exposure 2 h/day for 10 days, the kidney and the lung accumulated the highest concentrations of Hg. Histopathological evidence of pulmonary injury was not evident despite the significant accumulation of Hg in the lung (Morgan et al., 2002). In the kidneys of these exposed animals, a significant induction of metallothionein (a sulfhydryl-rich metal-binding protein) and glutathione S-transferase-pi (an important detoxification component of the glutathione system) was postulated to be an adaptive mechanism to overcome Hg-induced oxidative stress and cell damage (Brambila et al., 2002).

We hypothesized that Hg⁰ vapor exposure could also produce an array of gene expressions in the lung, based upon the genetic adaptations observed in the kidney and the resistance of the lung to high tissue levels of Hg. The current study was therefore designed to utilize microarray technology to examine the genomic events in the lung that are associated with Hg⁰ vapor exposure. Real-time RT-PCR and Western blot were followed to confirm microarray results on selected genes. Our results clearly showed that Hg⁰ vapor exposure induces inflammation-related genes, oxidative stress-related genes, and genes encoding for various antioxidants in the lung. It also revealed that several transporters, such as multidrug resistance-associated proteins and P-glycoproteins, were also increased in an attempt to reduce Hg load in pulmonary cells. These molecular events add to our understanding of toxicological significance for the development of adaptation to Hg⁰ vapor exposure.

MATERIALS AND METHODS

**Chemicals.** Elemental (metallic, Hg⁰) mercury (CAS# 7439-97-6) was obtained from Aldrich Chemical Co. (Milwaukee, WI). The rat cDNA expression arrays were purchased from Clontech (Palo Alto, CA). The oligo primers for real-time PCR were synthesized by Sigma Genosys (The Woodlands, TX). Antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). [α-³²P]dATP was obtained from Perkin Elmer Life Sciences (Boston, MA). All other chemicals were commercially available and of reagent grade.

**Animals.** Time-pregnant female Long-Evans rats (200 to 250 g, Charles River Laboratories, Portage, MI) were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care at the National Institute of Environmental Health Sciences. Animals had free access to Rodent Laboratory Chow (Ralston Purina Co., St. Louis, MO) and tap water except during exposure. The Hg⁰ vapor exposure protocol was previously reported (Brambila et al., 2002; Morgan et al., 2002), i.e., rats were exposed by nose-only to 4 mg/m³ Hg⁰ vapor, 2 h per day for 10 consecutive days (from gestation day 6 to gestation day 15). Twenty-four h after the last exposure, tissues were removed and quick frozen for later genomic analysis. All procedures involving the use of laboratory animals were in accordance with National Institutes of Health guidelines.

**Microarray analysis.** Total RNA was isolated from maternal lung (n = 5) with RNeasy columns (Qiagen, Valencia, CA). The pooled RNA samples were used to run array in triplicates. Approximately 5 µg of total RNA was converted to [α-³²P]-dATP-labeled cDNA probe, using Moloney murine leukemia virus (MuLV) reverse transcriptase and the rat cDNA expression array-specific cDNA synthesis primer mix (588 genes) and purified with NucleoSpin columns (Clontech, Palo Alto, CA). The membrane was prehybridized with Expresshyb from Clontech for 60 minutes at 68°C, followed by hybridization with the [³²P]-labeled cDNA probe in similar amount overnight at 68°C. The membranes were then washed four times in 2 × standard saline citrate/1% SDS, 30 minutes each, and two times in 0.1 × standard saline citrate/0.5% SDS. The membranes were then wrapped and exposed to a Molecular Dynamics Phosphoimage Screen (Sunnyvale, CA). The images were quantified densitometrically using AtlasImage (version 2.01) software. The gene expression intensities were first corrected with background and then normalized with the sum of all nine housekeeping genes on the array.

**Real-time RT-PCR analysis.** Expression of the selected genes was quantified using real-time RT-PCR analysis as described by Walker (2001). Briefly, total RNA was reverse transcribed with MuLV reverse transcriptase and oligo-dT primers. The forward and reverse primers for selected genes were designed using Primer Express software (Applied Biosystems, Foster City, CA), and listed in Table 1. The SYBR green DNA PCR kit (Applied Biosystems, Foster City, CA) was used for real-time PCR analysis. The relative differences in expression between groups were expressed using cycle time (Ct) values, and Ct values for interested genes were first normalized with that of β-actin in the same sample, and then relative differences between groups were

<table>
<thead>
<tr>
<th>Gene/protein</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
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<tbody>
<tr>
<td>CXC chemokine LIX</td>
<td>CACCCCTGCTGGCATTTCCTG</td>
<td>AACCATGGCGGAGAAAGGA</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TCGTACGAAACACCAACAGCA</td>
<td>CCTTTGAAGAGAACCTGGGAGTA</td>
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<tr>
<td>TNFRI</td>
<td>GAAACATTTCCAGGCTACCT</td>
<td>GGTGCACTTTCCGCTTAG</td>
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<td>MIP-1</td>
<td>GCTGAGCCGAGAACATTC</td>
<td>GATGTGGCTACTTGGCAGCAA</td>
</tr>
<tr>
<td>GST-pi</td>
<td>GGGTGCGCTTCTTGGCCTT</td>
<td>GCAGGGCCTTCACATAGC</td>
</tr>
<tr>
<td>Heat shock protein-60</td>
<td>TACGTCCTATGCTACGCTTTG</td>
<td>CTTTTAGCCTGTACAAACATGTG</td>
</tr>
<tr>
<td>Heme oxygenase-1</td>
<td>TACGTCCTATGCTACGCTTTG</td>
<td>CACGGATGTCGACCTCTCTT</td>
</tr>
<tr>
<td>Metallothionein-1</td>
<td>TGTGCTGCTAGATGACGACAG</td>
<td>TACATGCCTGCTAGAAGAAAAGG</td>
</tr>
<tr>
<td>P-glycoprotein-1</td>
<td>GAAACAATTTCCAGGCCACCT</td>
<td>GGTGCACCTTACCCGCTTAG</td>
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<td>MRPI</td>
<td>GCCGCTCAAGATCACCATCA</td>
<td>GCCATGGACTACCTTGCTTCA</td>
</tr>
<tr>
<td>MRPI2</td>
<td>GCTTCTGCCACCTGTGGA</td>
<td>TCTGCGTCTGCTCTTAAAGTG</td>
</tr>
<tr>
<td>ZnT-1</td>
<td>AGAGTTGAGGCCATCCGCTA</td>
<td>GGCACCTGCTAGTGAAGGCT</td>
</tr>
<tr>
<td>c-jun/AP-1</td>
<td>GAGAGGGAAGGCCATGAGAA</td>
<td>CACGTGTTCCCTGaGACTTGT</td>
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<tr>
<td>β-actin</td>
<td>TCTCCTGAGGGCAACTCTC</td>
<td>GCATGATACACGGGCTCAGAAA</td>
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Microarray Analysis of Gene Expression following Hg\textsuperscript{v} Vapor Exposure

Microarray analysis was used to profile gene expression in the lung associated with Hg\textsuperscript{v} vapor exposure. Representative microarray images are shown in Figure 1. There was no visible difference in housekeeping gene expression between air and Hg\textsuperscript{v} vapor exposure groups (image not included). After Hg\textsuperscript{v} vapor exposure, alterations in certain gene expressions were readily visualized. For example, expression of the genes encoding tumor necrosis factor receptor-1 (TNFR1), microsomal glutathione S-transferase-1 (mGST1), and glutathione S-transferase pi (GST-pi) was markedly increased by Hg\textsuperscript{v} vapor inhalation.

Various gene categories were our primary focus, and the genes of interest are listed in Table 2. Mean and SEM of the hybridization intensity from three hybridizations were calculated for statistical analysis as described previously (Liu et al., 2001). The first category of selected genes was related to inflammation in the lung. There was a tremendous (14-fold) induction of CXC chemokine LIX and significant (two- to threefold) induction of tumor necrosis factor-\(\alpha\), TNF receptor 1 (TNFR1), macrophage inflammatory protein-1, interleukin-2 (IL-2), and prostaglandin E2 receptor. IL-7 was also increased 1.7-fold but was not statistically significant. These gene alterations indicated that Hg\textsuperscript{v} vapor exposure induced inflammatory response in the lung.

The second gene category involves genes encoding cellular adaptive mechanisms. The expression of GST-pi was increased 2.7-fold, and the expression of mGST1 was also increased, but to a lesser extent (1.5-fold). The expression of glutathione reductase tended to increase (1.7-fold), but was not statistically significant. The enhanced expression of genes encoding components of the glutathione (GSH) system indicates that GSH is important in the response to inhaled Hg\textsuperscript{v}-induced stress/oxidative stress, which is evidenced by enhanced expression of mitochondria heat shock protein-60 (HSP60). In addition to GSH system, the expression of genes encoding thioredoxin peroxidase and Cu,Zn-superoxide dismutase was also marginally enhanced.
The expression of transporter genes was also increased following Hg\(^\text{v}\) vapor inhalation. The enhanced GST expression may imply the increased formation of mercury-glutathione conjugate for cellular efflux via multidrug resistance-associated proteins (MRP). The enhanced expression of MRP2 supports this assumption. In addition, the threefold increase in multidrug resistance gene (MDR1) encoded P-glycoprotein indicates that cellular mercury could also be pumped out of cells by the P-glycoprotein pump. In addition, the expression of genes encoding the zinc transporter ZnT1 and the organic cation transporter OCT1 was also increased.

Alterations were also detected in signal transduction-related genes. The expression of genes encoding for acute-phase responses, such as c-jun/AP-1 complex, Nur 77 early response protein, and phosphatidylinostiol-3-kinase (PI3-p85) was depressed. On the other hand, the expression of genes encoding the protein kinase C alpha and protein kinase C gamma was enhanced.

Mercury has a high affinity to lipid, and the expression of epidermal fatty acid-binding protein was significantly increased. Epidermal fatty acid-binding protein has been shown to increase following spinal cord injury in rats, using microarray (Tachibana et al., 2002), and it has been proposed to function as an antioxidant protein by scavenging reactive lipids through covalent modifications (Bennaars-Eiden et al., 2002).

**Confirmation of Microarray Analysis Via Real-Time RT-PCR and Western Blot Analysis**

To confirm the altered gene expression observed in microarray, a real-time RT-PCR analysis was performed on selected genes. As shown in Table 3, the selected 10 gene expressions were in good agreement with microarray analysis, including three genes (heme oxygenase-1, metallothionein, and MRPl) not included in commercially available membranes. In general, real-time RT-PCR seems to be more sensitive to gene expression changes, and the results obtained are in full agreement with microarray analysis.
To confirm microarray results at the translation product level, Western blot analysis was performed on selected proteins. As shown in Figure 2, expression of GST-pi (32 kDa) and multi-drug resistance-associated protein-1 (MRP1, 190 kDa) and the expression of P-glycoprotein (170 kDa) were increased. Expression of TNFR1 (47 kDa) and tumor necrosis factor receptor associated death domain (TRADD) (20 kDa) are in agreement with microarray and RT-PCR analysis. The expression of β-actin (43 kDa) was similar between groups.

DISCUSSION

The current study demonstrated that inhalation exposure to Hg vapor produced significant alterations in gene expression in the lung without causing histopathological changes. Alterations in the genes encoding inflammatory responses, adaptive mechanisms, transporters, and signal transduction were probably an attempt to cope with mercury-induced stress to the lung. Among 588 genes examined, the expression of 36 genes was increased more than twofold with \( p < 0.05 \); while the expression of 25 genes was depressed to below 65% of air controls with \( p < 0.05 \). The selected genes/proteins from microarray analysis were further confirmed by real-time RT-PCR and Western blot analysis. Although the study was performed using pregnant females, similar response could be expected to occur in nonpregnant females (Brambila et al., 2002), as well as to males.

In response to inhalation of foreign or toxic agents, the lung typically mounts an acute inflammatory response to facilitate removal of the agent and to repair any tissue damage. Human exposure to more than 2 mg/m\(^3\) of Hg vapor for several hours can cause acute chemical bronchiolitis and pneumonitis (Asano et al., 2000). In the current study, histopathological evidence of pulmonary inflammation was not present in rats after ten days of exposure, suggesting that Hg-induced inflammation may have been an early transient event and resolved without tissue damage. In an earlier study a mild transient influx of polymorphonuclear leukocytes (PMNs) was observed in bronchoalveolar lavage fluid (BALF) one day after exposure of rats to 4 mg/m\(^3\) Hg vapor; however, by 3 days after exposure, BALF cell differentials returned to normal (unpublished data).

Alteration of a number of genes involved in inflammation indicated that Hg vapor exposure induced an inflammatory response in the lung. The lipopolysaccharide-inducible CXC chemokine LIX was increased 14-fold in lungs of Hg-exposed rats. This chemokine has potent chemoattractant activity for neutrophils and is implicated in inflammatory reactions (Nadarajah et al., 1996). The increased expression of TNF-α, TNF-α receptor, macrophage inflammatory protein-1, interleu-

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**TABLE 3**

<table>
<thead>
<tr>
<th>GenBank</th>
<th>Gene/protein</th>
<th>Hg/Air</th>
<th>Hg/Air</th>
</tr>
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<tr>
<td>Inflammation-related genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U90448</td>
<td>CXC chemokine LIX</td>
<td>14.5</td>
<td>44.0</td>
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<tr>
<td>X66559</td>
<td>TNF-α</td>
<td>2.52</td>
<td>3.06</td>
</tr>
<tr>
<td>M63122</td>
<td>TNFR1</td>
<td>2.06</td>
<td>6.96</td>
</tr>
<tr>
<td>U94708</td>
<td>Macrophage inflammatory protein-1</td>
<td>2.88</td>
<td>4.47</td>
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<td>Glutathione system and antioxidants</td>
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<td>X02904</td>
<td>Glutathione S-transferase</td>
<td>2.74</td>
<td>8.28</td>
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<td>X54793</td>
<td>Heat shock protein-60</td>
<td>2.53</td>
<td>2.95</td>
</tr>
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<td>J02722</td>
<td>Heme oxygenase-1 (HO-1)</td>
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<td>NM138826</td>
<td>Metallothionein-1</td>
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<td>29.5</td>
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<td>Transporter and related genes</td>
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<td>M81855</td>
<td>P-glycoprotein-1</td>
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<td>3.72</td>
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<td>X96394</td>
<td>Multidrug resistance protein (MRP2)</td>
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<td>X90642</td>
<td>Multidrug resistance protein1 (MRP1)</td>
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<tr>
<td>U17133</td>
<td>Zinc transporter ZnT1</td>
<td>2.25</td>
<td>2.60</td>
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<tr>
<td>Signal transduction-related genes</td>
<td></td>
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</tr>
<tr>
<td>X17163</td>
<td>c-jun/AP-1</td>
<td>0.48</td>
<td>0.40</td>
</tr>
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</table>

*Note. Real-time PCR was performed in triplicates and the results were normalized with β-actin. n/d, the gene of interest is not on the commercial arrays.*
vapor exposure. It should be noted that alterations in inflammatory-related gene expression occurred without apparent histopathological evidence (Morgan et al., 2002), suggesting that gene expression is a much more sensitive index for adverse effects than histopathological analysis following a mild dose of Hg vapor exposure.

Exposure to Hg vapor also induced the expression of a number of genes involved in adaptive responses of the cell. The GSH redox system plays an important role in protecting against oxidative lung injury (Rahman et al., 1999). In the present study, the expression of genes encoding GST-pi and mGST1, glutathione reductase (GR), as well as thioredoxin peroxidase, was enhanced in Hg-exposed rat lung, indicating the activation of GSH redox system as an adaptive response to Hg-induced stress. The increased expression of GSTs was also observed in the kidney (Brambila et al., 2002) and brain (data not shown). The modest increases in the expression of heat-shock protein-60 and heme oxygenase-1, biomarkers for oxidative stress, support this notion. In response to Hg vapor, the activation of GSH redox system as an adaptive mechanism(s) must also be involved in response to Hg vapor exposure.

Metallothionein (MT), a cysteine-rich, metal-binding protein, plays an important role in Hg tolerance (Klaassen et al., 1999). In this study, the expression of MT-1 gene was enhanced more than tenfold following Hg vapor exposure, as evidenced by real-time RT-PCR analysis, along with a sixfold increase in MT protein (Morgan et al., 2002). The increased expression of metallothionein was also observed in the kidney (Brambila et al., 2002) and brain (Morgan et al., 2002). MT binds inhaled Hg in the cytosol (Yoshida et al., 1999b, 2002) and, thus, renders it relatively nontoxic. In fact, MT-null mice are highly sensitive to Hg-induced pulmonary toxicity and lethality (Yoshida et al., 1999a). Binding of Hg to MT also serves as a transplacental barrier to prevent the transfer of Hg from mother to fetus (Brambila et al., 2002; Yoshida et al., 2002).

The marked increase in GST also implies the involvement of transport systems in the development of Hg tolerance. GST catalyzes the formation of mercury-glutathione complex for cellular efflux via the MRP pump (Ballatori, 2002; Sugawara et al., 1998). Depletion of cellular GSH by buthionine sulfoximine (BSO) has been shown to increase Hg accumulation in the lung and liver following Hg vapor exposure (Kim et al., 1995), and MRP1-overexpressing lung tumor cells are resistant to Hg (Vernhet et al., 2000). In the present study, the lung accumulated much less Hg than kidney did (10 versus 150 mg/g) (Morgan et al., 2002), despite the fact that the lung was exposed to the highest concentrations of Hg vapor. The enhanced expression of genes encoding for MRP1 and MRP2, as evidenced by microarray and RT-PCR analysis, and the enhanced expression of MRP protein, via Western blot analysis, clearly indicate the involvement of GST and MRP system in the reduction of pulmonary Hg load.

In addition to the MRP transporter, the expression of other transporters was increased following Hg vapor exposure. The enhanced expression of multi-drug resistant gene (MDR)-encoded P-glycoprotein was detected at the both mRNA and protein levels. Little is known about the involvement of P-glycoprotein in metal transport, but the possibility exists, as P-glycoprotein has been recently implicated in arsenic transport (Liu et al., 2002). The role of P-glycoprotein in Hg transport warrants further investigation. The enhanced expression of zinc transporter ZnT1 and organic cation transporter OCT1 was also observed in the present study, but their role in Hg transport is unknown. The renal organic anion transport system has recently been implicated in the uptake of Hg and mercury-glutathione conjugates (Zalups and Barfuss, 2002). The metal transport system is an emerging issue of study in metal toxicology, including mercury (Ballatori, 2002).

In contrast to acute Hg exposure, the signal transduction pathways are differentially regulated following a threshold dose of Hg vapor exposure. The c-jun/AP-1 complex, which is activated following acute HgCl2 exposure (Turney et al., 1999), was suppressed at the mRNA levels, as evidenced by microarray and real-time RT-PCR analysis after 10 days of exposure to Hg vapor. However, this was not confirmed at the protein levels (data not shown). Whether this is due to the feedback regulation or altered protein degradation needs further verification. It has also been shown that 24-h exposure to mercuric mercury (Hg2+) inhibits NF-kB activity as a mechanism of Hg-induced nephrotoxicity (Woods et al., 2002). The expression of Nur77 early response protein and phosphatidylinositol-3 kinase (PI3-kinase) was also suppressed. In contrast, the expression of genes encoding protein kinase C (PKC) alpha and PKC gamma was enhanced. It has been shown that HgCl2 can activate PKC directly and induces a PKC-dependent Ca2+ influx as a molecular target for HgCl2 (Badou et al., 1997). The exact role of Hg vapor inhalation-induced alterations in these signal transduction components is not known and requires further investigation.

Another Hg vapor-induced gene expression of interest is...
epidermal fatty acid-binding protein, which was dramatically increased by Hg\(^6\) vapor exposure. Epidermal fatty acid-binding protein has been shown to increase following spinal cord injury in rats using microarray (Tachibana et al., 2002). The exact role of this protein is not known yet, but it has been suggested to play a role in the response to tissue damage or repair (Tachibana et al., 2002) or to function as an antioxidant protein by scavenging reactive lipids through covalent modifications (Bennaaars-Eiden et al., 2002).

In summary, Hg\(^6\) vapor exposure induced an array of altered gene expression in the lung at concentrations that do not result in pulmonary pathology. These alterations in gene expression include induction of genes involved with inflammation, induction of MT and GSH redox systems, enhancement of transporters, as well as alteration of cellular signal transduction. These altered gene/protein expressions could play integrated roles in developing an adaptive response to cope with Hg toxicity.

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REFERENCES


Badou, A., Savignac, M., Moreau, M., Leclerc, C., Philpot, S., Price, P., Rydell, and R. Wilson. Inhalation exposures were performed at the NIEHS inhalation facility under contract to ManTech Environmental Technology, Inc., Research Triangle Park, NC.


