Effects of Elemental Mercury Vapor Inhalation on Arterial Blood Gases, Lung Histology, and Interleukine-1 Expression in Pulmonary Tissues of Rats

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Abstract

We investigated the effects of elemental mercury vapor inhalation on arterial blood gases (ABG's), lung histology, and interleukin-1 (IL-1) expression in pulmonary tissues in rats. A total of 42 Sprague-Dowley rats were divided randomly into three groups. Rats in the first group were used as control (CG). A Short-Term Group (STG) and Long-Term Group (LTG) were exposed to 7.3 μl of elemental mercury vapor for 21 days and 65 days, respectively. After exposure periods were completed, arterial blood samples were obtained, and ABG's were measured. Lung tissue sections were prepared for histology evaluation and immune-stained to detect IL-1 expression. There was a significant decrease in body weight in both STG (15%) and LTG (22%) compared to the CG. In the LTG, six rats died (43%), while none of the rats in the STG died during the experiment. In both STG and LTG, a significant acid-base imbalance was characterized by a significant decrease in blood PH values and a significant increase in PCO₂ values. Both PO₂ and SpO₂ blood values were significantly decreased in the STG and LTG, while no changes were observed in HCO₃ values in all groups. Histological evaluation of lung tissues revealed severe lesions characterized by pulmonary emphysema and inflammatory cellular infiltrate. IL-1 expression in lung tissue was not significantly different between exposed rats and control subjects. These results indicate significant alterations in blood acid-base status characterized by severe respiratory acidosis with hypoxemia and no evidence of compensatory alkalosis in rats after short and long-term elementary mercury vapor exposure.

Introduction

Mercury is a highly toxic heavy metal with significant public health and safety implications worldwide (Ishitob et al. 2010; Azevedo et al. 2012; Pizzorno 2011; Bernhoft 2012; Chakraborty 2017). Mercury is commonly found in nature in many different forms (Zahir et al. 2005; Bernhoft 2012; Zhu et al. 2020). Inorganic mercury includes metallic mercury, mercury vapor (Hg^0), and mercurous (Hg^{+2}) or mercuric salts, while the organic form of mercury includes compounds containing carbon atoms such as methyl, ethyl, or phenyl groups (Costa et al. 2020). All forms of mercury compounds mentioned earlier can be found, and chemically interchangeable, in the environment (Graeme and Pollack 1998).

According to the World Health Organization (WHO), most human exposure to mercury occurs through the inhalation of elemental mercury vapor via occupational or dental amalgam exposure (WHO 1991; Zhu et al. 2020). The ingestion of seafood contaminated with organic mercury has been reported as another major route via which people are exposed to mercury (Zhu et al. 2020). Although mercury is present naturally in the environment, recent human industrial activities have resulted in the dangerous accumulation of more mercury in the land, water, and food supplies (Clarkson 2008; Zhu et al. 2020). This accumulation of mercury in the environment carries grave health risks and consequences. The most infamous case of mercury poisoning of the 20th century is the "Minamata disease" incident, with the first cases noted in 1956 (Harada 1995). Industrial waste containing methylmercury was being released into Minamata Bay in Japan by a Japanese chemical factory, which then reached the locals through contaminated fish as food. Thousands of locals have been affected since then, and even babies born in the 1960s-1970s to mothers that have been exposed to the contaminated fish were noted to have brain damage, mental retardation, and a variety of other diseases (Graeme and Pollack 1998). Industrial regulations have been put into action ever since recognizing the toxic nature of mercury and its ability to cross the blood-brain barrier. However, industrial activities such as coal combustion still produce high mercury levels disposed of in the atmosphere, land, and water (Streets et al. 2018).

The pathogenesis of mercury poisoning is often multifaceted as it manifests in many forms and can impact all body systems depending on the underlying pathways and enzymes affected. This vast pathogenic potential is due to the tendency of mercury to bind to sulfur groups (Graeme and Pollack 1998), which are an essential component of the chemical structure of cellular proteins, enzymes, channels, and pumps, thereby disrupting their physiological function and inducing pathological change. One molecular effect of mercury is the inhibition of vascular endothelial enzymes such as Na/K-ATPase and Ca²⁺-ATPase, leading to disrupted vascular reactivity (Vassallo et al. 2011). Another effect that mercury has on the body vasculature is mercury-induced nitric oxide (NO) inhibition, as a result of endothelial nitric oxide synthase (NOS) pathway inhibition (Omanwar et al. 2013). This leads to the disruption of normal vasodilation and vasoconstriction of the vasculature. Mercury has also been shown to increase the production of reactive oxygen species (ROS), which in turn led to the inactivation of numerous enzymes such as Paraoxonase, Glutathione peroxidase, Phospholipase D, and Mitogen-activated protein kinases (MAPKs) (Azevedo et al. 2012; Vassallo et al. 2011; Haase et al. 2010). MAPKs are extremely important to the functioning of the immune system, as they play an essential role in T-cell activation. Haase et al. (2010) demonstrated that mercury binding to MAPKs did not significantly result in dysfunction of MAPKs, but the overproduction of ROS triggered by the mercury was the cause of the MAPKs dysfunction. Mercury has also been associated with developing clinical manifestations of metabolic syndrome such as obesity, insulin resistance, and hypertension (Tinkov et al. 2015). Tinkov et al. (2015) proposed that mercury affects the renin-angiotensin-aldosterone system (RAAS), leading to hypertension and altering β -cell functionality leading to insulin resistance.

The IL-1 cytokines act to regulate pro-inflammatory mediators in tissue injury (Weber et al. 2010). The effect of mercury on IL-1 expression has been demonstrated in some studies, but the results have been inconclusive (Zdolsek et al., 1994; Batista-Duharte et al., 2018). IL-1 production has been shown to increase due to the presence of mercury in the tissue (Zdolsek et al., 1994), while in another study, mercury was shown to have the opposite effect and reduce IL-1 expression (Batista-Duharte et al., 2018).

Elemental mercury vapor inhalation has been shown to lead to direct lung tissue injury, capillary destruction, pulmonary edema, and eventually fibrosis (Asano et al. 2000). Acute severe exposure to elemental mercury vapor has been reported to lead to fatality as a result of pulmonary insufficiency and acute renal failure (Asano et al. 2000). Generally, mercury exposure is chronic at a low dosage resulting in subtle toxic manifestations characterized by loss of appetite, weakness, malaise, loss of weight, and gastrointestinal upset (Bernhoft 2012; Zhu et al. 2020). However, in the acute form, more severe manifestations related to the immune system, gastrointestinal tract, renal, cardiopulmonary, and nervous systems have been reported (Houston 2011; Bernhoft 2012). Rapid recognition of mercury poisoning and its complications is critical in avoiding poor patient outcomes and lifesaving. The clinical management of mercury vapor poisoning revolves around maintaining ventilation, decontamination, chelation, and treating complications (Rafati-Rahimzadeh et al. 2014). In severe acute cases with high plasma concentrations of mercury, plasma exchange can be used as well (Russi and Marson 2011).

To our knowledge, no recent scientific reports are documenting the effects of exposure to elementary mercury vapor on various blood gas parameters and underlying pulmonary lesions that might explain possible acid-base alterations associated with inhalation of mercury vapor. Therefore, this study was designed to investigate the toxic effects of elemental mercury vapor on various arterial blood gas parameters and to determine the possible underlying pulmonary pathology that might lead to acid-base alterations using Sprague-Dawley rats.

Materials And Methods

Animals:

All experimental procedures performed in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Jordan University of Science and Technology (JUST). A total of 42 adult Sprague-Dawley rats weighing between 150–200 grams were used in the study. Rats were randomly divided into three equal groups; 14 rats for each group with seven males and seven females. The first group received no mercury vapor exposure and was defined as a control group (CG). In the second group (short-term group, STG) and the third group (long-term group, LTG), rats were exposed to elementary mercury vapor for 2 hours daily for 21 days and 65 days, respectively. During the experiment, rats were housed individually in cages and offered feed and fresh drinking water *ad libitum*. The room temperature was maintained at 22–25 degrees Celsius and 12/12 day/night cycles.

Experimental design:

In STG and LTG, rats were exposed to 7.3 μl elemental mercury (Shijiazhuang Shuliang Commerce Trade CO., China) using stainless steel exposure chamber (50 cm x 50 cm x 80 cm) and with plastic top cover and rubber sealing as described previously (Ishitob et al. 2010). The chamber on its top was connected by a tube to an oxygen cylinder to provide pure oxygen at a rate of 10 L/min during the mercury vapor exposure. The chamber was also connected to a vacuum pump on its lower part to suck out the air saturated with mercury vapor at the end of exposure and provide the exposure chamber with fresh air. Mercury was injected into the chamber at 7.3 μl (equal to 500 mg/ m^3) by micropipette for 2 hours per day for 21 days (STG) and 65 days (LTG) using small heated glass bottles. A whole-body exposure technique was used to expose the rats to mercury vapor. All personnel and researchers are responsible for strictly performing the experiments followed the safety protocols and procedures, and adhered to university laboratory policies to ensure their safety. The research team wore personal protective equipment (PPE) during the experiment, including gloves, head cap, shoe cover, gown, and N95 face mask. Micropipette cover, mercury residuals, and PPE used in the experiment were kept in plastic zipper bags inside a biohazard box and then handed over to the safety, occupational, and environmental health department at JUST.

Clinical monitoring:

During the exposure and afterward, rats were closely monitored for any abnormal signs such as respiratory distress, weakness, or diarrhea.

Arterial blood sample collection:

After completing the exposure periods (21 days for the STG and 65 days for the LTG), arterial blood was collected from each rat under light sedation using ether in a glass chamber. Arterial blood was collected via cardiac puncture of the left ventricle using heparinized syringes attached to 22 gauge needles (Becton, Dickinson, and Company; USA). Arterial blood gases (ABG's) were measured immediately using a blood gas analyzer (Cobas; Roche Diagnostics, Switzerland).

Necropsy and histopathology:

After arterial blood was collected, rats were humanely euthanized using ether overdose in a glass chamber. A thorough necropsy was performed on all rats, and any abnormal gross findings were recorded. Tissue samples from both lungs were collected and placed immediately in 10% neutral buffered formalin. A portion of the tissue

samples was processed and stained with H&E for routine histology examination as previously described (Alturkistani et al. 2016).

Immunohistochemistry:

Another portion of the tissue samples was subjected to immunohistochemistry staining to evaluate IL-1 expression (Suker et al. 2017). Briefly, 4µm thick paraffin-embedded sections were dewaxed twice using xylene and then hydrated in descending grades of ethyl alcohol. Antigen retrieval was carried out using a microwave instrument to heat the slides in citrate buffer for 3 minutes. Sections were left to cool down and then treated with 2.5% hydrogen peroxide to block endogenous peroxidase activity. Nonspecific binding was prevented by incubating slides with nonspecific serum for 15 minutes. Slides were covered by the IL-1 antibody (diluted 1:100) for 60 minutes, then washed twice with phosphate-buffered saline (PBS). Slides were then covered with the secondary antibody for 30 minutes. Again, slides were washed twice with PBS, and the signal was detected by color development using a DAB chromogen kit (Biocare Medical, USA). Finally, slides were counterstained with Mayers hematoxylin and mounted with DPX. The primary antibody (IL-1) was omitted in the control slides. Sections were viewed under light microscopy. Analysis of the immunohistochemistry images was performed using Image J software (<https://imagej.nih.gov/ij/download.html>) according to previously published methods (Jensen 2013).

Statistical analysis:

The ABG's results were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Post Hock Test (Bonferroni). Independent sample T-test was used to compare subjects within each group by sex. A *p*-value of less than or equal to 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 23 statistical software package (IBM Statistics, USA).

Results

Results were obtained for ABG's, a histological study of rats' lung tissues, an immunohistochemical study of rats' lung tissues, and an animal wellbeing assessment based on animal weight and mortality.

Animal Well-Being:

The exposure of the STG and LTG to 7.3 µl of elemental mercury vapor led to a statistically significant reduction in weight compared to the CG, which in contrast, gained weight during the experiment. Results are reported in Fig. 1. The percentages of body weight loss were 15% and 22% in the STG and LTG, respectively. The CG and STG subjects all survived the course of the study. The LTG had 6 out of the 14 (42.8%) subjects die during the study.

Arterial Blood Gas Parameters:

Five parameters in the ABG's were compared between groups, and those parameters were: PH, PCO₂, PO₂, SP0₂, and HCO₃. The results for ABG's findings in all groups after short- and long-term exposure to elemental mercury vapor inhalation, as well as, CG are reported in Table 1. The ABG's testing showed a significant decrease in PH values for STG and LTG (*p* < 0.05) throughout the study, with no significant change in the control PH values being observed. PH values within the exposed groups were lower than the CG (*p* < 0.05). PCO₂ values were significantly higher in the exposed groups compared to the CG (*p* < 0.001). PO₂ values were significantly lower in the STG than the CG (*p* = 0.03), while no significant difference was found between the LTG and the CG PO₂ values (*p* = 0.21).

SPO₂ values in the STG and LTG were significantly lower than the SpO₂ in the CG ($p = 0.001$). No significant difference was found in the HCO₃ values between the exposed groups and the CG ($p = 0.506$). No significant difference in the ABG's parameters examined was found between male and female subjects within the same group in the STG or LTG ($p > 0.05$).

Table 1

The arterial blood gas parameters in Sprague-Dowley rats after short- and long-term elemental mercury vapor exposure and in those with no mercury vapor exposure.

Arterial Blood Gas Parameters	Groups								
	Short-term exposure			Long-term exposure			Control		
	All animals	Males	Females	All animals	Males	Females	All animals	Males	Females
PH	7.23 ± 0.05*	7.22 ± 0.03	7.24 ± 0.07	7.18 ± 0.02*	7.18 ± 0.02	7.19 ± 0.0	7.36 ± 0.09	7.32 ± 0.02	7.4 ± 0.06
PCO ₂	62 ± 10*	65 ± 6	60 ± 13	68 ± 4*	69 ± 4	66 ± 4	44 ± 8	50 ± 10	37 ± 4
P0 ₂	49 ± 21*	42 ± 10	55 ± 18	52 ± 14*	55 ± 15	44 ± 5	67 ± 16	57 ± 13	77 ± 11
SpO ₂	66 ± 19*	63 ± 17	69 ± 20	71 ± 16*	73 ± 18	65 ± 7	89 ± 9	84 ± 10	94 ± 3
HCO ₃	25 ± 3	26 ± 2	24 ± 3	25 ± 2	25 ± 15	25 ± 5	24 ± 3	25 ± 2	23 ± 2
* statistically significant in comparison to the control group ($p \leq 0.05$)									

Lung Histopathology:

Histological study under light microscopy was performed for dissected and prepared lung tissues for all groups. Lung tissue sections obtained from rats after short-term and long-term exposure are presented under light microscopy in Fig. 2. The histological study of lung tissue from the CG revealed mild inflammation. The examination of lung tissue sections from the STG and the LTG showed marked inflammatory cellular infiltration, emphysema, and dilatation of the alveoli with destruction and obstruction of intra-alveolar septae. The LTG tissue sections showed a higher degree of tissue injury and more severe inflammation than the STG.

Immunohistochemical Study of Rats' Lung Tissues:

Immunohistochemistry staining to detect IL-1 expression in lung tissues was performed and is presented in Fig. 3. No signs of significant IL-1 expression were noted in any of the groups.

Discussion

This study is one of the first scientific studies that evaluated the toxic effects of elementary mercury vapor inhalation on various arterial blood gas parameters in rats and attempted to evaluate possible underlying lung pathology as a direct causal effect of significant alterations in blood acid-base balance. Several side effects related to various body organs and systems have been reported after acute or chronic exposure to elemental mercury (Clarkson and Magos 2006; Thomas and Clarkson 2008; Houston 2011; Park and Zheng 2012;

Chakraborty 2017). In human beings, exposure to mercury inhalation was reported to cause flu-like symptoms, including fever, cough, dyspnea, and chest pain (Cortes et al. 2018). In this study, rats in both exposure groups lost weight significantly ($p \leq 0.05$) during the experiment, while rats in the CG gained weight. A total of 6 rats out of 14 died (42.8%), all belonging to the LTG, while none of the rats in the short-term or control groups died during the experiment. The high mortality rates in the LTG can be explained by severe lung injury, hypoxia, acidemia, and weight loss, observed in such a group, due to the long duration of exposure to elemental mercury vapor.

In both the STG and LTG, there was a significant decrease in blood PH values compared to the CG. This state of severe acidemia was accompanied by a significant increase in blood concentrations of CO₂ and a significant decrease in blood concentration and saturation of O₂ regardless of sex. Simultaneously, no significant changes were observed in blood concentrations of HCO₃, indicating a lack of renal compensation. However, the PO₂ and SPO₂ levels were found to be higher at the end of the study in the LTG than in the STG, indicating some form of adaptive response to the long-term exposure to elemental mercury vapor, leading to improved oxygen saturation of the blood. The ABG's results indicate that rats exposed to elemental vapor inhalation suffered a significant degree of respiratory acidosis, in contrast to previously established evidence in the literature leaning predominantly towards metabolic acidosis with mercury poisoning in both animals (Pathak and Bhowmik 1998) and humans, predominantly children (Husband and McKellar 1970; Counter and Buchanan 2004). The higher incidence of acidosis in the pediatric age group than in adults exposed to elemental mercury in the literature can be explained by the difference in body weight and physiological maturity, which results in a much lower degree of exposure being necessary to bring mercury to highly toxic and potentially fatal concentrations in the blood and tissues. This carries clinically significant implications in managing pediatric mercury poisoning cases, where healthcare providers should critically consider both metabolic and respiratory acidosis.

Histopathological examination of lung tissues from rats exposed to elementary mercury vapor inhalation revealed substantial pulmonary inflammation characterized by inflammatory cellular infiltration, emphysema, and dilatation of the alveoli with thickening of the intra-alveolar septae. These inflammatory lesions appeared more pronounced in the rats in the LTG than those in the STG. Interestingly, pulmonary lesions were found to be most severe in female rats compared to males. The severe inflammatory lesions of the pulmonary tissues are presumably the underlying cause of the changes noted in the acid-base status of exposed rats. This also is evident by the observed clinical signs of hyperventilation, hypoxia, and weakness. These findings are congruent with previously reported pulmonary lesions in human beings after acute exposure to mercury vapor (Smiechowicz et al. 2017). Diffuse inflammatory cellular infiltrates, acute pulmonary edema and emphysema, chemical pneumonitis, bronchiolitis, and pneumothorax, and death have been reported in humans after acute mercury vapor inhalation (Smiechowicz et al. 2017). These findings emphasize the importance of early treatment of lung tissue injury with medications, supplemental oxygen, endotracheal intubation, and mechanical ventilation. These early interventions are crucial to maintaining adequate gas exchange in elemental mercury vapor poisoning to prevent well-known respiratory complications such as hypoxemia and lesser-known significant complications that may arise, such as respiratory acidemia.

In this study, immunohistochemistry results to detect IL-1 expression in lung tissues revealed no significant differences between all groups. These results disagree with previous findings where mercury vapor inhalation induced increased secretion of IL-1 (Zdolsek et al. 1994). The effects of mercury on the IL-1 have been demonstrated in several laboratory animal models (Gardner et al. 2009). It has been suggested that exposure of mice to methylmercury induced expression of IL-1 in the brain tissue, causing central nervous cytotoxicity

(Takahashi et al. 2015). In contrast, one study found that macrophages from mercury-treated mice exhibited a reduced capacity to produce IL-1 (Batista-Duharte et al. 2018). Therefore, the exact effects of mercury molecules on the production and expression of IL-1 in different body tissues remain controversial, and further clinical trials are warranted to determine the exact pathophysiological effects of mercury on pro-inflammatory cytokines production and function.

Conclusions

The findings of this study indicate that exposure to elementary mercury vapor induces significant pulmonary injury resulting in severe clinical manifestations, severe respiratory acidosis with hypoxemia, and no evidence of compensatory alkalosis in Sprague-Dowley rats. These manifestations are correlated with the duration of mercury vapor exposure regardless of gender. Health care professionals should consider respiratory acidosis in their treatment plans for patients affected with mercury exposure.

Declarations

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Conflicts of Interest

There is no conflict of interest.

Availability of Data and Material

The datasets generated and analyzed during the current study are available with the corresponding authors on reasonable request.

Code Availability

Not Applicable.

Authors contributions

All authors have seen and approved the content, fulfilled the authorship criteria, and have contributed significantly to this work. All authors presented substantial contributions to the conception and design of the study and/or to

the acquisition, analysis, and interpretation of data, and they drafted the manuscript and revised it critically for content. All authors read and approved the final manuscript version submitted for publication.

Ethics Approval

All experimental procedures performed in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Jordan University of Science and Technology (JUST).

Consent to Participate

Not applicable.

Consent for Publication

This study was approved for publication by the Institutional Animal Care and Use Committee (IACUC) of Jordan University of Science and Technology (JUST).

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Figures

Figure 1

The effects of elemental mercury vapor exposure for short-term (21 days) and long-term (65 days) on body weight in rats (N=14).

Figure 2

H&E stained lung tissue sections obtained from rats after short-term (left) and long-term (right) exposure to elemental mercury vapor inhalation. Severe inflammatory cellular infiltration, emphysema, intra-alveolar edema, and intra-alveolar septal thickening, especially in rats with long-term exposure.

Figure 3

Immunohistochemistry staining to detect IL-1 expression in lung tissues of rats after short-term (left) and long-term (right) exposure to elemental mercury vapor inhalation. No significant differences were observed in IL-1 expression in any of the groups.