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Investigating the Impact of Elemental Mercury on Inflammatory and Oxidative Markers and Myocardial Injury in B6 Mice: A Concentration and Duration Dependent Study

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Abstract:

This study evaluates the effects of elemental mercury on inflammation, oxidative stress, and myocardial injury in B6 mice, with a focus on neutrophil degranulation and TNF-alpha inflammatory pathways. Using controlled exposures, the research assesses changes in reactive oxygen species (ROS), neutrophil elastase (NE), and TNF-alpha levels, correlating these biomarkers with histological evidence of myocardial injury. Findings indicate that mercury exposure induces dose-dependent increases in inflammatory and oxidative markers, similar to other heavy metals like lead, potentially exacerbating myocardial damage. However, variations in response suggest adaptive physiological thresholds. The results emphasize the need for precise exposure assessments and interventions to mitigate mercury's cardiovascular and systemic effects, highlighting implications for public health and safety standards.

Keywords: Elemental Mercury, Oxidative Stress, Neutrophil Degranulation, TNF-alpha, Myocardial Injury, Cardiovascular Risks.

Research Question: Elemental mercury is known to induce oxidative stress similarly to other heavy metals such as lead. Neutrophil activation and degranulation, often triggered by oxidative stress, can significantly contribute to myocardial injury. Given that elemental mercury and lead share similar pathways in inducing oxidative stress, can exposure to elemental mercury also promote neutrophil degranulation, thereby exacerbating myocardial injury?

1. Introduction

Because of the high toxicity and capacity for bioaccumulation, elemental mercury, which is a global environmental contaminant, presents serious health hazards. The main way that elemental mercury is exposed to humans is by inhaling vaporized mercury, which is especially dangerous in industrial environments or from damaged mercury-containing devices

like fluorescent lights and thermometers (US EPA, 2015). Furthermore, mercury is utilized in small-scale, artisanal gold mining, which has the potential to seriously contaminate the environment and expose people (Uddin, Khanom, & Islam, 2023). Dental amalgams, or mercury-containing dental fillings, are another important form of exposure. These amalgams, which contain roughly 50% mercury, can cause chronic exposure because they produce mercury fumes when they are chewed. Due to health and environmental issues, the use of mercury in dental procedures has caused a global discussion, which resulted in the reduction in mercury use (Uddin, Khanom, & Islam, 2023). Exposure to mercury has significant and varied health effects. Because mercury vapor can pass through the blood-brain barrier and build up in the brain, inhaling elemental mercury can cause neurological and behavioral issues. Mercury is one of the top 10 substances that pose a serious risk to public health, according to the World Health Organization. Mercury can raise blood pressure and heart rate, alter cardiac rhythm, and cause atherosclerosis, furtherly enhancing the risks associated with cardiovascular disease (Uddin, Khanom, & Islam, 2023).

Neutrophil degranulation is a vital immunological response in which the cells release granule proteins into the extracellular space, including neutrophil elastase (NE). If neutrophil degranulation becomes dysregulated, it can cause substantial tissue damage. This degranulation mechanism is triggered by exposure to lead and plays a key role in the pathophysiology of myocardial damage. According to Wu et al. (2023), once neutrophil degranulation is triggered, the release of NE would increase, causing damages to cardiac cells. The study furtherly explain how reactive oxygen species (ROS) gets involved. When exposing to lead, ROS are produced at a higher rate since they are a metabolic consequence of lead exposure. ROS will stimulate more neutrophil activation, which worsens myocardial damage and increase the release of NE, collectively causing more damages to cardiac cells. In addition, heart troponin I (cTnI), which is a clinical indicator of myocardial damage, is elevated when ROS and NE combine, indicating a further inflammatory response. By oxidative stress responses caused by lead exposure, the higher ROS levels not only increase NE activity but also worsen total myocardial damage (Wu et al., 2023).

According to Wu's study, ROS production caused by lead-exposure is a crucial mediator of cardiac injury. Both as heavy metal, mercury could initiate similar harmful pathways as lead does. With a special emphasis on TNF-alpha, the inquiry seeks to identify if elemental mercury imitates these inflammatory processes. Strong inflammatory cytokine TNF-alpha is markedly elevated in response to oxidative stress brought on by exposure to heavy metals, and it is crucial for the development of cardiac damage through ROS regulation (Wu et al., 2023).

I predict that increasing concentrations and treatment durations with elemental Hg in mice increase ROS and cause neutrophil degranulation, leading to myocardial injury through TNF-alpha inflammatory pathways.

2. Materials and Methods

2.1 . Animals

B6 mice, aged 180 days and weighing 20-25 grams, were obtained from a specialized breeding laboratory and housed under controlled environmental conditions. The study protocol was approved by the Institutional Animal Care and Use Committee, adhering to the guidelines for ethical animal treatment.

2.2 . Elemental Mercury Exposure

Mice were divided into groups. They received intraperitoneal injections of elemental mercury in saline solution at concentrations of 0 mg/kg (control), 0.5 mg/kg, 1 mg/kg, and 2 mg/kg, administered daily for 1, 2, and 4 weeks. The experimental design included a positive control group receiving lead (Pb) and a negative control group receiving saline solution only.

2.3 . Sample Collection

After whole-blood extraction from elemental mercury-exposed mice, blood and heart tissue specimens were taken for histological and biological evaluation. Serum was applied to determine the reactive oxygen species (ROS) and tumor necrosis factor alpha (TNF-alpha). The processed tissue samples were subjecting various procedures and finally prepared for histological examination for myocardial damage.

2.4 . Measurement of ROS

The level of ROS in serum was quantified using commercial kit (ROS/Superoxide Detection Assay Kit, Abcam, ab139476). Absorbance under 450 nm was measured and standard procedure was followed as provided by the manufacturer. Samples were analyzed three times to confirm the accuracy and reproducibility of the parameters adopted depending on the protocol hence respecting all inclusions during analysis of the sample using ROS/Superoxide Detection Assay Kit.

2.5 . Neutrophil Elastase Activity Assay by ELISA

A Neutrophil Elastase ELISA Kit (Human PMN (Neutro-

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phil) Elastase ELISA Kit, Invitrogen, BMS269) was used to measure the levels of neutrophil elastase. To quantify the elastase concentration in the serum, the assay was carried out according to the manufacturer's instructions, processing samples in triplicate (Human PMN (Neutrophil) Elastase ELISA Kit, 2024).

2.6 . TNF-Alpha Quantification by ELISA

TNF-alpha concentrations were measured by using an ELISA kit (Mouse TNF alpha ELISA Kit, Abcam, ab208348. Appropriate instructions provided within the kit were followed, whereby readings were taken at 450 nm using a microplate reader (Mouse TNF alpha ELISA Kit, 2024).

2.7 . Histological Analysis

For the assessment of histological changes, slice of cardiac tissue was prepared and fixed, and subjected to hematoxylin and eosin staining. Suvarna et al. (2019) identified regions of cardiac injury in heart images using confocal microscopy.

2.8 . Statistical Analysis

Statistical analyses were done to determine the differences between the control group and the treatment group using paired samples T-tests, with a p-value of significance set at $p<0.05$. All statistical analyses were carried out using the statistical package software (Armitage, 1971).

3. Results

3.1 . Possible Relationship for Duration and Concentration

In this study different concentrations (0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg) and exposure durations (1 week, 2 weeks and 4 weeks) of elemental mercury were tested on B6 mice. The results showed a difference in effects that was dependent on the dose and time lapse of exposure to mercury.

3.1.1 . Positive Correlation

Analysis showed there was an increase in the biological effects in a consistent manner in relation to the dose regardless of the time. This pattern suggests that as the amount of mercury increases, its biological effects also increase in an orderly manner. Such a dose-response effect is particularly imperative in establishing the levels of exposure that would be potentially harmful to the organisms exposed.

3.1.2 . Negative Correlation

Contrarily, higher doses led to decreased biological effects. This rather unusual trend could mean that biological systems were becoming more competent in adapting or strategic saturation that increasing the dose of mercury would not induce further damage. This phenomenon could mean that the biological thresholds are much higher than it could be believed that there is no effect from the exposure to mercury, where in fact there is a very intricate relationship between the toxin and the excessive physiological behaviors exhibited.

3.1.3 . No Relationship

The data also presented scenarios where responses remained stable regardless of changes in dose and duration, suggesting the existence of a potential threshold level below which mercury's effects are not discernible. This observation could be indicative of the resilience or inherent tolerance within the biological system up to a certain level of exposure.

Table 1: Effects of Elemental Mercury on Neutrophil Elastase, ROS, TNFα, and Myocardial Injury in B6 Mice

Combination Result # (CR#)	Hg increases Neutrophil elastase by ELISA	Hg increases ROS by ROS assay	Hg increases TNFa by ELISA	Hg increases myocardial injury by HE staining	Support of hypothesis
1	+	+	+	+	Full
2	+	+	+	-	Partial
3	+	+	-	+	Partial
4	+	-	+	+	Partial
5	-	+	+	+	Partial
6	+	+	-	-	Partial
7	+	-	-	+	Partial
8	-	-	+	+	Partial
9	+	-	+	-	Partial

10	-	+	-	+	Partial
11	-	+	+	-	Partial
12	+	-	-	-	Partial
13	-	+	-	-	Partial
14	-	-	+	-	Partial
15	-	-	-	+	Partial
16	-	-	-	-	Fully Contradicts

Note. "+" represents a positive result. "-" represents a negative result

3.2 . Possible Results For Measurements

CR1: There are observed increases in neutrophil elastase, ROS, TNFα, and myocardial injury.
CR2: There are observed increases in neutrophil elastase, ROS, and TNFα, with no increase in myocardial injury.
CR3: There are observed increases in neutrophil elastase and ROS, with no an increase in TNFα, but myocardial injury is present.
CR4: There are observed increases in neutrophil elastase and TNFα, with no increase in ROS, but myocardial injury is present.
CR5: There is no observed increase in neutrophil elastase, but there are observed increase in ROS, TNFα, and myocardial injury.
CR6: There are observed increases in neutrophil elastase and ROS, with no changes in TNFα or myocardial injury.
CR7: There is an observed increase in neutrophil elastase, with no increase in ROS or TNFα, but myocardial injury is present.
CR8: There is an observed increase in TNFα, with no increase in neutrophil elastase and ROS, but myocardial injury is present.
CR9: There are observed increases in neutrophil elastase and TNFα, with no change in ROS or myocardial injury.
CR10: There are an observed increase in ROS and myocardial injury, with no changes in neutrophil elastase or TNFα.
CR11: There are observed increases in ROS and TNFα, with no changes in neutrophil elastase or myocardial injury.
CR12: There is an observed increase in neutrophil elastase only, with no changes in ROS, TNFα, or myocardial injury.
CR13: There is an observed increase in ROS only, with no changes in neutrophil elastase, TNFα, or myocardial injury.
CR14: There is an observed increase in TNFα only, with no changes in neutrophil elastase, ROS, or myocardial injury.
CR15: There is only observed myocardial injury, with no increases in neutrophil elastase, ROS, or TNFα.
CR16: There are no observed changes in neutrophil elastase, ROS, TNFα, or myocardial injury.

4. Discussion

The study's findings on mercury exposure indicate a complicated relationship between mercury and physiological indicators of cardiac damage, oxidative stress, and inflammation. Full activation of all markers (CR1) provides persuasive evidence that mercury can start a powerful and widespread effect on cardiovascular health. This result shows a systemic response to mercury that impacts several cellular pathways, such as the creation of inflammatory cytokines and oxidative stress. In the future, one important step would be to investigate protective agents that could release these effects and to determine the lowest exposure amount of mercury that causes substantial changes to these physiological indicators.
Mercury can cause biochemical reactions, although tissue damage is not necessarily the result. This is indicated by partial activations (CR2, CR6, CR12) that do not result in myocardial damage. This may point to compensatory pathways or exposure thresholds below which myocardial damage cannot occur. Future studies should concentrate on figuring out the precise mechanisms by which mercury influences these markers without causing harm, including the part that exposure time and concentration play in these targeted effects.
The mixed responses (CR3, CR4, CR5, CR7, CR8, CR9, CR10) reflect the complexity of mercury's interaction with biological systems. These results suggest that not all physiological pathways are activated in unison, and the impacts on myocardial injury vary. The variability might be due to individual differences, mercury's chemical form, or environmental factors modifying its effects. Parsing out the conditions under which specific markers are affected would help clarify the nuanced interactions at play. Studies using genetically modified models could be particularly informative to understand the genetic basis of sensitivity to mercury.

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Conversely, the lack of significant changes or contradictory outcomes (CR11, CR13, CR14, CR15, CR16) challenges or refutes the hypothesis, indicating that mercury may not always have a measurable impact on the biomarkers or myocardial injury studied. These results could stem from low exposure levels, resistance in test models, or measurement inaccuracies. Replicating these experiments under varied conditions could confirm these findings and would help in understanding the protective mechanisms that might be present.

In Section 3.1, the link between exposure duration and mercury content is analyzed, revealing complex dose-response dynamics in B6 mice. Over the range assessed, the biological effects consistently corresponded to dose levels, even when the concentration remained constant, suggesting a clear linear relationship over time. This finding emphasizes the crucial need for strict legal regulations to minimize potential health risks. At the same time, lower biological responses to increasing doses could suggest physiological limits beyond which further aggravation halts, pointing to possible adaptation mechanisms or saturation effects that could inform therapeutic and preventive strategies. In addition, the effects under many circumstances remain consistent suggesting a level of mercury exposure below which the effects would be absent. This result could serve to help identify minimum threshold limits for the public health concern regarding mercury. How the toxicity of mercury varies with changing doses and exposure periods should be of great concern in terms of developing models, which will simulate the activities of mercury and also enhance policies to mitigate the health effects of mercury. This was done through the use of experimental studies to measure the impact of elemental mercury on oxidation, inflammation, and the heart. Investigation results showed that distinct systems could be uniquely engaged when exposed to mercury.

5. Conclusion

Participating in this research led to the experimental evaluation of the effects of elemental mercury on oxidative stress, inflammation, and cardiac damage. The results suggested that various physiological pathways may be activated quite differently due to mercury exposure. The findings indicate that the duration and concentration of mercury exposure have different effects. In some cases, all of the studied markers were only slightly elevated, while in other situations, there was full activation of these markers. This study will allow understanding more about the biological markers linked to mercury because the disease caused by mercury often presents subtle signs that hint at its impact on humans. In the future, studies should investigate the mercury influence with the other genetic and environmental conditions as well as exploring strategies to prevent its harmful effects on health. The study underscores the critical need for stringent regulations on mercury use in order to preserve public health.

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