Evaluating the effects of milrinone on the heart, liver, and kidneys of phosphine-exposed rats

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Introduction

Commonly used as a grain fumigant and pesticide, phosphine (PH_3) is integral in increasing global food production (Wong et al., 2017). The availability and affordability of phosphine led to its widespread use in undeveloped countries; however, exposure to phosphine is fatal. The functions of three major organs—heart, liver, and kidneys—are degraded by phosphine, which leads to multi-organ dysfunction and death.

The cellular mechanism of phosphine is presumed to be the inhibition of the cytochrome c oxidase, the last complex of the mitochondrial electron transport chain (ETC) (Aminjan et al., 2019). ETC inhibition and the release of free radicals extensively heightened inflammation and apoptosis within the cell (Figure 1). This is currently irreversible and incurable due to the lack of treatment options.

Milrinone is being tested as a potential medication to treat phosphine poisoning. The purpose of this project was to assess the effectiveness of milrinone in mediating the damages done by phosphine on the heart, liver, and kidneys.

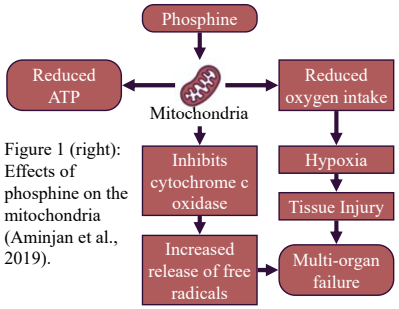


Figure 1 (right): Effects of phosphine on the mitochondria (Aminjan et al., 2019).

Materials and Methods

Blood serum samples (Figure 2) were used in the kidney and liver assays and heart homogenate samples were used in the heart assay. These samples were obtained at the end of previously conducted studies on female Sprague-Dawley rats, whose primary focus was to observe the physiological effects of phosphine. Blood, tissue, and organ samples are then extracted and frozen for future analysis.

Enzyme-linked immunosorbent assays (ELISA) and a Luminex assay were used to measure the antigen concentrations that revealed the extent of the damages in the samples. The antigens being tested were ELISA KIM-1 for kidney damage, ELISA troponin for cardiac damage, and Luminex glutamic-oxaloacetic transaminase 1 (GOT1) for liver damage.

To have a reference point when evaluating the results of the assays, standards are required. The provided standards were serial diluted as indicated in the manufacturer's protocols. These were predetermined to have a certain antibody concentration dependent on the assay performed.

Manufacturer protocols were followed throughout both the ELISA and the Luminex assays. The samples and standards were each triplicated to ensure validity of the results. The ELISA assays required

Materials and Methods (continued)

an antibody cocktail, a detection antibody, a development solution, and an assay plate which were provided in the specific assay kits. Magnetic microbeads which were coated with capture antibodies were present in each well of the Luminex Assay. These microbeads measure multiple antibodies at once. A microparticle cocktail, antibody cocktail, and Steptavidin-PE (reagent) were provided and utilized in the Luminex assay. The ELISA and Luminex assays were performed in a SpectraMax M5 and a BioPlex 200 plate reader, respectively, to collect the wavelength results.

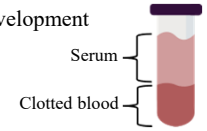
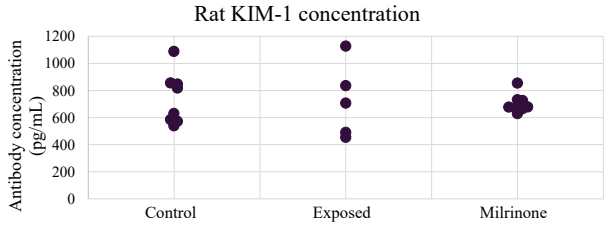


Figure 2 (above): After centrifuging blood without the addition of anticoagulants, the lighter pigmented liquid suspended above the clotted blood is the serum.

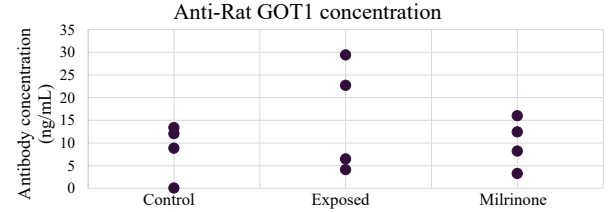
Results

Once the readings were obtained from the plate reader, the readings were averaged and normalized. To normalize, the average of the blank standard was subtracted from the standard and sample averages.

The standard concentrations and wavelengths were then graphed as a four-parameter logistics curve (4PL) (AAT Bioquest, Inc., 2023). This curve was used to interpolate the antigen concentration of the samples based on the readings from the plate reader and the dilution factor. A one-way analysis of variance (ANOVA) test was used for analysis.

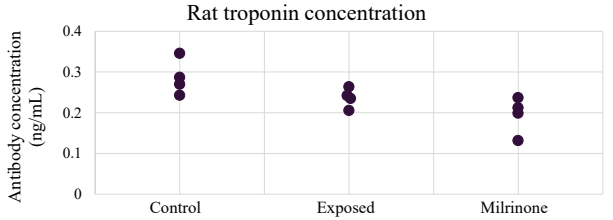


Graph 1 (above): Individual value plot of the KIM-1 antibody concentration (n = 18). An ANOVA test failed to reject the null hypothesis with $F(2, 18) = 0.10$ and $p = .909$.



Graph 2 (above): Individual value plot of the GOT1 analyte concentration (n = 12). An ANOVA test failed to reject the null hypothesis with $F(2, 9) = 0.77$ and $p = .490$.

Results (continued)



Graph 3 (above): Individual value plot of the troponin antibody concentration (n = 12). An ANOVA test revealed a significant difference in the means of the control ($M = 0.286$, $SD = 0.044$), exposed ($M = 0.236$, $SD = 0.024$), and milrinone-treated ($M = 0.195$, $SD = 0.045$) groups with $F(2, 9) = 5.59$ and $p = .026$. A post-hoc Tukey test revealed a significant difference between the control and the milrinone-treated group with a 95% CI [−0.168, −0.015] and $p = .021$.

Conclusions

The purpose of the project to assess the effects of milrinone was met. Both kidney and liver assays showed insignificant differences within the groups tested. Despite a significant ANOVA test within the cardiac assay, post-hoc analysis revealed a statistically significant difference between only the control and milrinone-treated groups. This indicated milrinone exhibited no cardioprotective effects. Antibody concentrations in the control groups of kidney and cardiac assays were higher than experimental groups. Potential causes include ketamine and xylazine use when euthanizing rats or unidentified increased troponin levels in untreated rats. Further studies should work to determine the cause of elevated antibody levels found within control groups.

References

AAT Bioquest, Inc. (2023, February 13). *Quest Graph™ Four Parameter Logistic (4PL) Curve Calculator*. AAT Bioquest. https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

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