Effects of milrinone on cAMP and cGMP following PH_3 inhalation exposure

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Introduction

Phosphine (PH_3) is a poisonous gas released mainly by metal phosphides used in agriculture. When these phosphides contact acid or water, PH_3 is released. Exposure is difficult to diagnose due to nonspecific symptoms, relating to multiorgan failure; specifically, weakened heart, lungs, and circulatory system (Proudfoot, 2009). Milrinone (MLR) is a drug that addresses cardiac strength and peripheral basal dilation, promoting cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP) respectively (Kokkonen & Kass, 2017). Past research at USMRICD has shown MLR to increase survival rates at low, medium, and high doses after inhalation exposure to PH_3. Since the mechanism of PH_3's toxicity is unknown, understanding why the treatment is effective is difficult.

The purpose of this project was to identify possible differences in the biochemical effects on cAMP and cGMP due to MLR treatment when there has been an acute inhalation exposure to PH_3. Respiratory data was collected to identify effects on the respiratory system with treatment and exposure, possibly leading to information to refine treatment regimens.

Materials and Methods

To determine biochemical effects of MLR and PH_3 on male Sprague Dawley rats, rats were divided into 4 groups (n = 4), receiving treatments of sham H_2O, or MLR (600 µg/kg), with exposure to filtered air (CNT) or PH_3 (10% in N2, 660 ppm) (EXP). The rats were passed through the exposure process (Figure 1) after being placed in inhalation chambers (Figure 2). Rats were then euthanized, and their hearts were removed to quantify cAMP and cGMP levels using cAMP Assay Kit (Competitive ELISA) (ab65355) and cGMP Assay Kit-Direct Immunoassay (ab65356). An ANOVA for difference in means between groups, with the null hypothesis assuming no differences in mean cAMP or cGMP levels exist between treatments (α = .05), was planned but unable to be performed due to insufficient sample size, as testing was halted due to COVID-19. During the exposure process, minute volume (MV) and duty cycle (DC) were recorded in 2 s intervals. After exposure, the data points were averaged first by minute, then by treatment group using Visual Basic. Averages were graphed in Excel. Due to data loss, two rats from the H_2O exposed group were omitted from the respiratory data analysis.

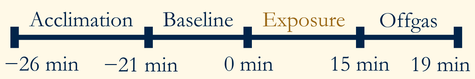
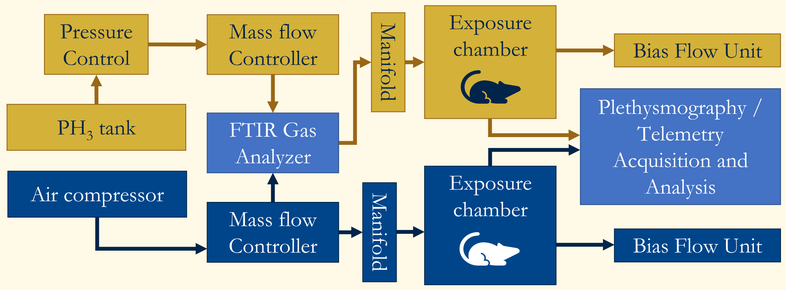


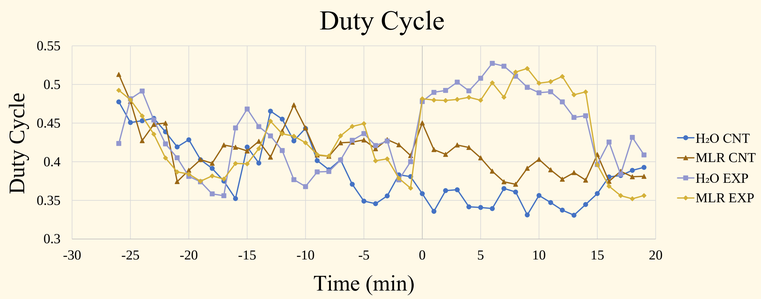
Figure 1 (left): The exposure process for the rats, with treatments given at t = −15 min.

Materials and Methods (continued)

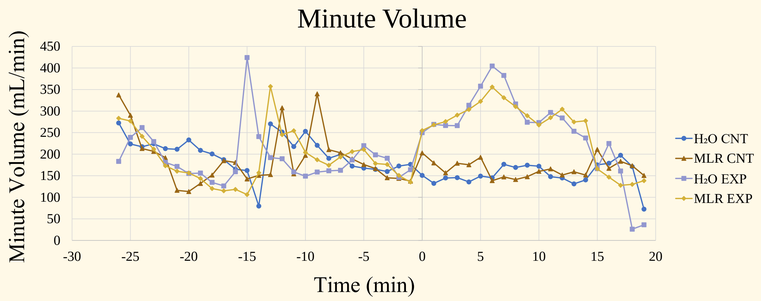
Figure 2 (below): A gas mixing and metering system consisting of PH_3 tank, air compressor (Gast Manufacturing, Inc.), mass flow and pressure controllers (Bronkhorst USA Inc.), a FTIR Gas Analyzer (Gasmet Technologies Inc.), and custom air manifolds, was hooked up to six exposure chambers with whole body plethysmography monitoring (Data Sciences International), which had air pulled through by bias flow units (Data Sciences International). Exiting gas was decontaminated by activated charcoal before release. The respiratory data was collected through a custom program (FinePointe Software version 2.3.1.16, Data Sciences International, St. Paul, MN).



Results

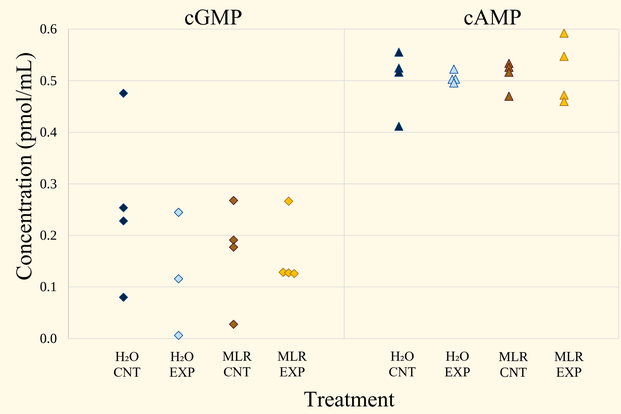


Graph 1 (above): Duty cycle shows the time spent inhaling during a breath cycle, throughout the exposure process. Increased values were mainly induced by PH_3 exposure, and slightly by MLR.



Graph 2 (above): The volume of air inhaled per minute by the rats. Application of treatments caused disturbance peaks around t = −15 min. Disparities in later timepoints are from PH3, originating from changes in breath frequency.

Results (continued)



Graph 3 (left): The net cAMP and cGMP concentration distributions in rat hearts after exposures. There was no pairing of exposure and treatment that altered cAMP or cGMP levels.

The difference in DC and MV results between exposed and control rats (Graphs 1 & 2) shows the effects of PH_3 on respiratory systems. The lack of evident difference in mean concentration in both cAMP and cGMP (Graph 3) suggests that neither net cardiac concentration was altered by either MLR or PH_3.

Conclusion

The purpose of this study was met by determining the physiological and biochemical effects resulting from exposure to PH_3 and MLR treatment. Physiological data of respiration did show PH_3 and MLR effect on heightened respiration. However, the effort to link this change in the cardiorespiratory system to net cardiac biochemicals cAMP and cGMP concentrations yielded no results. Thus, through this process, it cannot be determined that cAMP and cGMP regulation are the cause of heightened cardiorespiratory function seen in past studies that supports the increase in survivability with MLR treatment. However, the measurable biochemical effects may be localized inside the heart (possibly at subcellular levels), require higher measurement resolution, or originate further downstream. Thus, further studies may examine different quantification methods or new biomolecules in developing PH_3 identification or treatment regimens.

References

Kokkonen, K., & Kass, D. A. (2017). Nanodomain regulation of cardiac cyclic nucleotide signaling by phosphodiesterases. *Annual Review of Pharmacology and Toxicology, 57*, 455–479. https://doi.org/10.1146/annurev-pharmtox-010716-104756

Proudfoot, A. T. (2009). Aluminum and zinc phosphide poisoning. *Clinical Toxicology, 47*(2), 89–100. https://doi.org/10.1080/15563650802520675