Effect of inhaled opioids on the heart and lungs of ferrets

Caroline Kragh
Mentored by Dr. Bryan McCranor

Introduction

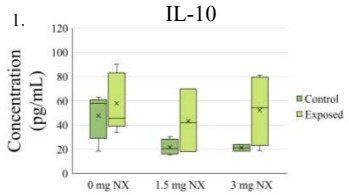
Opioids are commonly prescribed as a pain reliever, and have produced a threat to the public due to physical dependence and addiction. Opioids have previously been used as a weapon by the Russian Military in 2002 to rescue a group of hostages in a theater (Riches, Read, Black, Cooper, & Timperly, 2012). Apnea and cardiac issues have been observed as a side effect in animals during exposure to opioids. The known effects of opioid usage include sedation, dizziness, nausea, constipation, and respiratory depression (Benyamin et al., 2008). Although apnea and irregular echocardiogram signals appear to resolve after opioid exposure is treated with naloxone (Narcan®), underlying damage to the lungs or heart could occur during exposure. The purpose of this experiment was to determine whether the inhalation of opioids causes injury to the lungs and heart. It is hypothesized that the inhalation of this substance will cause injury to the lungs, evident through protein levels and the presence of blood in lung lavage fluid, and the heart, evident through presence of troponin or myoglobin in the serum. Studying the effects of opioids on the heart and lungs will be beneficial to clinical diagnostics of exposure and development of long term treatment.

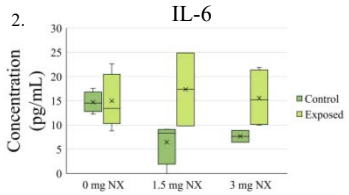
Materials and Methods

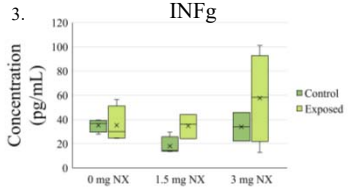
Experimental ferrets were exposed to opioid inhalants and treated with varying doses of naloxone (NX). Control ferrets were exposed to water vapor and treated with naloxone. At 24 hours following exposure, animals were euthanized and a bronchoalveolar lavage was performed with phosphate buffered saline (PBS). Bronchoalveolar lavage fluid (BALF) samples were centrifuged and frozen at −80 °C. Samples were thawed to perform the ProcartaPlex Luminex multiplex immunoassay procedure. Samples were assayed in duplicate with an 18-hour incubation, then added into the 96-well plate containing the antibody coupled beads. The plate was and analyzed with Bio-Plex manager software. The protein content of BALF was determined with a Pierce 660 nm protein assay reagent kit according to the manufacturer's protocol using a microplate reader and SoftMax Pro software. Heme content was determined by measuring the absorbance of the BALF at 540 nm with the microplate reader. Western blotting technique was used to determine the presence of proteins in serum that indicate injury to the heart and lungs. Serum was run on a 4–15% precast gel in a 1:1 dilution with sample buffer and transferred onto a membrane using the iBlot system. The membrane was blocked in iBlock buffer and incubated at 4 °C with anti-troponin and fluorescent tagged secondary antibody for 24 hours each with PBS washing in between. The membranes were imaged on the LiCor imager.

Results

Concentrations of inflammatory cytokines such as IL-10, IL-6, and INFg were determined to assess trauma to the lungs (Graphs 1-3). As seen in Table 1, all *p*-values generated by the Student's *t*-Tests were greater than the alpha level of 0.05, indicating no statistical significance in the differences between mean concentrations of inflammatory cytokines in the BALF of exposed and control ferrets given Naloxone treatments of any dosage.



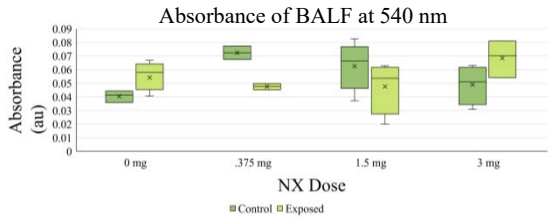




Cytokine	NX Dose (mg/pg)	*p*-value
IL-10	0	0.801
	1.5	0.284
	3	0.130
IL-6	0	0.926
	1.5	0.371
	3	0.081
INFg	0	0.994
	1.5	0.079
	3	0.342

Graphs 1-3 (above): The concentration of inflammatory cytokines indicative of lung trauma are graphed for control and experimental ferrets given varying doses of Naloxone treatment ($N = 30$). Table 1 (bottom right): Displayed are the *p*-values generated from running Student's *t*-Tests with $\alpha = 0.05$ comparing the mean concentrations of inflammatory cytokines of control and experimental ferrets within their respective Naloxone dose groups.

Heme analysis showed higher mean levels of absorbance in experimental ferrets for the high and low Naloxone dosages. Alternatively, the control ferrets had higher mean absorbances for the middle dosages. For each treatment group, a Student's *t*-Test was run to compare the mean absorbance of the BALF from control and experimental ferrets. This test yielded *p*-values of 0.016, 0.011, 0.272, and 0.131 for the 0 mg, 0.375 mg, 1.5 mg, and 3 mg treatment groups, respectively. Statistical significance was found only for the 0 mg and 0.375 mg Naloxone treatments, although the experimental BALF absorbance was higher only in the 0 mg treatment out of these two groups (Graph 4).

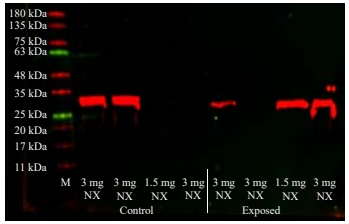


Graph 4 (left): The absorbance of BALF at 540 nm is displayed ($N = 30$). A higher absorbance of the BALF indicates a larger presence of heme in the lungs, a sign of trauma.

Results (continued)

Six out of eight control and seven out of eight experimental ferrets tested positive for presence of troponin in the serum, a marker of cardiac injury (Figure 1).

Figure 1 (right): This western blot highlights subjects that have troponin — a protein released during cardiac trauma — in the blood. Troponin has a molecular weight of 37 kDa, appearing between the two green lines of the ladder to the left of the samples.



Conclusions

Inflammatory cytokine analysis did not indicate significant trauma in the experimental ferrets compared to controls. Western blots consistently showed the presence of troponin in serum in both control and experimental ferrets. Serum samples were taken with a heart stick, where the heart is punctured and may have caused the release of troponin into the blood. Elevated heme levels in the control ferret BALF may have been caused by the treatment of control ferrets with Naloxone. Literature suggests that the administration of Naloxone can cause pulmonary edema (PE), among other cardiovascular issues (Horng, Huang, Yeh, & Cherng, 2010). Sample collection techniques must be refined to eliminate extraneous causes of trauma to the heart and lungs before sound conclusions can be drawn about the injury caused by inhaled opioids. Data was collected using kits intended for rats. This shows the possibility of collecting samples from ferrets when there has not been a protocol previously developed for them specifically.

This research shows the possible dangers of inhaled opioids and provides a model applicable to humans. Understanding the damage inhaled opioids may cause is important in the development of therapies to combat the effects of opioid exposure in people.

References

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