Paraquat Model of Lung Injury in the Vium Digital Vivarium

INTRODUCTION

Animal models of lung injury play an important role in understanding the pathogenic mechanisms of respiratory diseases, including acute lung injury (ACI) and pulmonary fibrosis (PF), and in developing novel therapies (1). The paraquat (PQ)-induced model is a commonly-used and well-described model of lung injury due to its rapid disease onset and clinical translation (2). Since PQ selectively accumulates in the lungs, exposure, either through physical contact, inhalation, or ingestion, can lead to severe lung injury and even death. Many of the physiological and pathological features of human disease are present in rodents exposed to PQ. These features include respiratory dysfunction, as well as lung edema, interstitial inflammation, and progressive fibrosis (2). A number of potential therapies to treat lung disease use this model to evaluate drug safety and efficacy (3,4).

In the PQ-induced rodent model of lung injury, disease symptoms, such as body weight and body temperature loss are measured manually; pulmonary function is commonly measured using a plethysmograph; lung edema is quantified by ex vivo tissue analysis (4-6). These techniques, with emphasis on ex vivo tissue analysis and pulmonary function measurements, present with their own technical challenges; ex vivo tissue collection is terminal and may require histopathological analysis, while pulmonary function measurements can be laborious, invasive, and time-consuming (7,8). In addition to these technical challenges, most standard in-life methods recover over time in PQ-induced lung injury (<72 hours post-induction) despite the persisting effects of underlying pathology (>7 days post-induction) (6). Therefore, there is also a need for in-life metrics that can track disease symptoms for a longer period of time.

These current challenges provide opportunities to develop low-touch, automated, in-life methods to assess pulmonary function and lung edema in rodents. We hypothesize that Vium’s automated metrics, specifically its Breathing Rate metric, will provide physiologically relevant data to assess respiratory disease progression in a PQ-induced rat model of lung injury.

METHODS

Animals

Male Lewis rats (aged 11-14 weeks and weighing between 290 and 350g) (Charles River Laboratories, Hollister, CA) singly-housed in Vium Digital Smart Houses were anesthetized with isoflurane and administered 0.3 mL intratracheal doses of 0.02 mg paraquat (0.667 mg/mL) (Sigma; St. Louis, MO) in sterile saline (VetOne, Boise, ID). Control rats were injected with saline only. Experiments were conducted in Vium’s AAALAC-accredited facility in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Vium.
**Body Weight and Body Temperature Measurements**
Throughout the study, body weight and body temperature were assessed in individual animals to monitor disease progression. Body temperature was measured rectally using a thermometer.

**Tissue Collection**
Animals were euthanized by isoflurane inhalation two or six days post-induction. Lung tissues were harvested and weighed.

**Motion and Breathing Rate**
Subjects were housed within the Vium Digital Vivarium, where intelligent sensors and HD cameras allow for continuous and minimally invasive monitoring of animals, as well as collection of automated metrics including motion and breathing rate, in the home cage. All study data is available in real-time and accessible via the online Research Suite.

**Statistical Analysis**
Two-way ANOVAs with Tukey’s multiple comparison tests were used to compare groups across time. Unpaired t-tests were used to assess tissue weights. Pearson tests were performed for correlation analyses. P values less than 0.05 were considered statistically different. Retrospective power analysis was calculated using G*Power (Heinrich-Heine-University Düsseldorf, Germany).

**RESULTS**
Post-induction, body weight and body temperature both significantly decreased in Paraquat (PQ) rats (Figs. 1A and 1B). These changes were observed as early as two days post-injury. Reductions in body weight persisted until study end (six days post-injury), while body temperature recovered to baseline values by Day 5 post-injury. When assessed using Vium’s metrics, elevated breathing rates and reduced motion were detected in PQ rats (Figs. 1C and 1D). In contrast to body weight and body temperature, these changes were observed as early as 12 and 16 hours post-injury, respectively. Elevations in breathing rate persisted until study end, while night-time motion recovered to baseline values by Night 5 post-injury.

**Figure 1.** In a rat model of lung injury, paraquat-induced rats demonstrated physiological and behavioral symptoms. Compared to controls:

- **(A)** Body weight was significantly decreased in paraquat (PQ) rats from days 2-6 post-induction ($F_{(6,80)} = 56.93; P \leq 0.0001$, $*P \leq 0.0001$ vs. Control).

- **(B)** Body temperature was significantly decreased in PQ rats from days 2-4 post-induction ($F_{(6,77)} = 5.41; P \leq 0.0001$, $*P \leq 0.01$ vs. Control).

- **(C)** Breathing rate was elevated as early as 12 hours post-induction until study end (day 6 post-induction) ($F_{(36,377)} = 17.06; P \leq 0.0001$, $*P \leq 0.0001$ vs. Control).

- **(D)** Night-time motion was significantly decreased as early as 16 hours until 108 hrs post-induction ($F_{(56,393)} = 19.60; P \leq 0.0001$, $*P \leq 0.01$ vs. Control). $*P \leq 0.05$ vs. Control.

Error bars are +/- SEM. n=10/group.
At study end, lung tissue from PQ-induced rats weighed heavier than controls (Fig. 2A). Correlation analyses revealed strong correlation between breathing rate and lung weights ($R=0.9217$, $P<0.0001$). Additional linear regression analyses corroborated that breathing rate is a positive predictor of lung weights (Fig. 2B).

The large changes in breathing rate led us to perform retrospective power analysis on all in-life metrics and lung weights post-induction (Table 1). To compute sample size, we first determined effect size empirically using the following post-induction measurements from PQ and Control rats: maximum breathing rate (bpm), minimum night-time motion (mm/sec), minimum body temperature (degrees C), and minimum body weight (g). The Vium Breathing Rate metric revealed the largest effect size (9.48) compared to other disease measurements (range: 1.40-5.33). Power analysis determined a smaller sample size requirement to detect the effect size of breathing rate (n=2) compared with other disease measurements (range: n=3-15) as significant at the 5% level and with 95% chance.

**Table 1. Retrospective power analysis for Paraquat-induced vs. control rats**

<table>
<thead>
<tr>
<th></th>
<th>Max Breathing Rate Post-Induction</th>
<th>Min Motion Post-Induction</th>
<th>Min Body Temperature Post-Induction</th>
<th>Min Body Weight Post-Induction</th>
<th>Lung Weights</th>
</tr>
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<tbody>
<tr>
<td>Effect Size</td>
<td>9.48</td>
<td>5.33</td>
<td>2.23</td>
<td>140</td>
<td>394</td>
</tr>
<tr>
<td>Sample Size (N)</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>15</td>
<td>4</td>
</tr>
</tbody>
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N=5-6 rats per group. To compute sample size, the following parameters were used: two-tailed t-test, an alpha error probability of 0.05, and power of 0.95. Effect sizes were determined using experiment data.

**DISCUSSION**

Here we demonstrate how Vium’s platform can be used to monitor respiratory disease progression in a Paraquat (PQ)-induced rat model of lung injury. Vium’s metrics, specifically its automated Breathing Rate metric, showed changes as early as 12 hrs post-induction, and these changes persisted until study end. Increased breathing rate may be indicative of dyspnea commonly observed in patients with lung disease (2,6). In previous studies, plethysmograph data revealed either transient or inconsistent responses to PQ (6), while our data showed a consistent response across several days. The advantage of using a continuous monitoring platform to evaluate breathing rates in the home cage is the ability to assess disease progression in a low-touch and automated manner that does not require animals to be removed from their home cage. This approach may lead to more sensitive and consistent data collection.
CASE STUDY
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Lung disease progression in individuals exposed to PQ can be divided into two phases: 1) an acute or early phase of disease characterized by pulmonary edema, and infiltration of inflammatory cells, and a 2) chronic or later phase of disease characterized by infiltration of myofibroblasts in the alveolar space and septa, differentiation of fibroblasts, and lung fibrosis (9,10). Individuals who survive the acute phase of disease show a period of improvement although irreversible lung damage may still ensue (9). A similar pattern of disease has been observed in rodents administered PQ (2). Vium’s Motion and Breathing Rate metrics track acute and longer-term phases of disease, respectively. The advantage of Vium’s metrics is its ability to detect respective phases of disease earlier and for longer periods of time.

In PQ rats, breathing rate was also positively correlated with lung weights, which is indicative of lung edema (10). Linear regression analysis corroborated these results, suggesting that breathing rate can be used to potentially predict endpoint lung edema in this model.

The Vium Breathing Rate also showed a larger effect size compared to standard in-life metrics, which can subsequently lead to smaller sample sizes in future experiments. For example, at its peak, the breathing rate of PQ-induced rats was approximately 2x baseline or control values, while body weight showed a 10-15% loss. These results directly contribute to a growing movement in preclinical research to both optimize scientific research and improve animal welfare with the adoption of the “Three Rs:” Replacement, Reduction, and Refinement.

In summary, Vium’s Breathing Rate metric refines respiratory disease preclinical research by allowing researchers to non-invasively track disease earlier and for longer periods of time using smaller sample sizes. Furthermore, our data insights reveal that Vium’s Breathing Rate metric complements standard disease measurements, including endpoint tissue analysis.

REFERENCES