CASE STUDY

Mouse Model of Liver Toxicity in the Vium Digital Vivarium

INTRODUCTION

Liver disease, including hepatocellular carcinoma (HCC), hepatitis, alcoholic liver disease (ALD), autoimmune or drug-induced liver disease, and non-alcoholic steatohepatitis (NASH), represents a considerable public health burden. End-stage liver disease or cirrhosis accounts for 2% of deaths worldwide (1). Regardless of the type of liver disease, a significant driver of fibrosis in the liver is inflammation (2,3).

Intravenous injection of the plant lectin Concanavalin A (Con A) in mice is a commonly used model of acute immune-mediated hepatitis and immune-mediated drug hepatotoxicity due to its rapid disease onset and clinically relevant disease mechanisms. Con A-induced toxicity is driven by the recruitment and activation of T lymphocytes into liver tissue, thereby allowing researchers to address the involvement of immune cell activation and inflammation in liver disease (2,3).

Similar to clinical measurements, standard endpoints in this mouse model include quantification of serum liver transaminases, which are indicative of hepatocellular damage (3,4). In a typical experiment, mice intended for analysis 12 or 24 hrs after Con A administration must be monitored closely by trained technicians for signs of side-effects associated with liver failure, including inactivity, cardiorespiratory failure, and hypothermia. Mice with severe signs of liver failure are euthanized prematurely to ensure tissue/data collection and humane treatment of animals (3). These "hands-on" approaches may limit the number and frequency of observation periods, as well as present unavoidable sources of experimenter and environmental variability that can confound results. Furthermore, time-consuming and laborious standard endpoints may impede the rapid screening of disease-modifying compounds.

We hypothesize that continuous monitoring of behavioral and physiological parameters will provide clinically relevant data to assess disease in induction rodent models, including the Con A-induced mouse model of liver disease. The objective of this study was to evaluate behavioral and physiological characteristics of Con A-induced mice using different doses of Con A.

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METHODS

Animals

Singly-housed, six-week old, male BALB/c mice (Charles River Laboratories, Hollister, CA) housed in Vium Digital Smart Housing™ were injected with Con A (15 mg/kg or 25 mg/kg) (Sigma Aldrich, St. Louis, MO) or PBS intravenously (IV). Using the online Research Suite, remote clinical observations were performed hourly from 5-8 hrs post-dose and every 30 mins from 8 hrs post-dose until endpoint. If remote clinical observations suggested an animal was nearing humane endpoint or in distress, a cage-side observation was conducted. Mice found to be at humane endpoint (i.e. moribund or presenting with cardio-respiratory distress, ascites/edema, or hemorrhage) were euthanized. 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.

Blood Collection

At 8 hrs post-dose or endpoint (humane endpoint or 24 hrs post-Con A challenge), blood was collected via the submandibular vein, stored, and assayed for liver transaminases, including alanine liver transaminase (ALT) and aspartate liver transaminase (AST).

Breathing Rate and Motion

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. Kruskal-Wallis tests were used to compare liver transaminase activity levels among groups. P values less than 0.05 were considered statistically different. Retrospective power analysis was calculated using G*Power (Heinrich-Heine-University Düsseldorf, Germany).

RESULTS

Liver Transaminase Activity (ALT and AST)

As early as 8 hrs post-dose, Con A-induced mice showed elevations in liver enzymes ALT and AST, which persisted until study end (24 hrs post-induction) (Fig 1A and 1B).

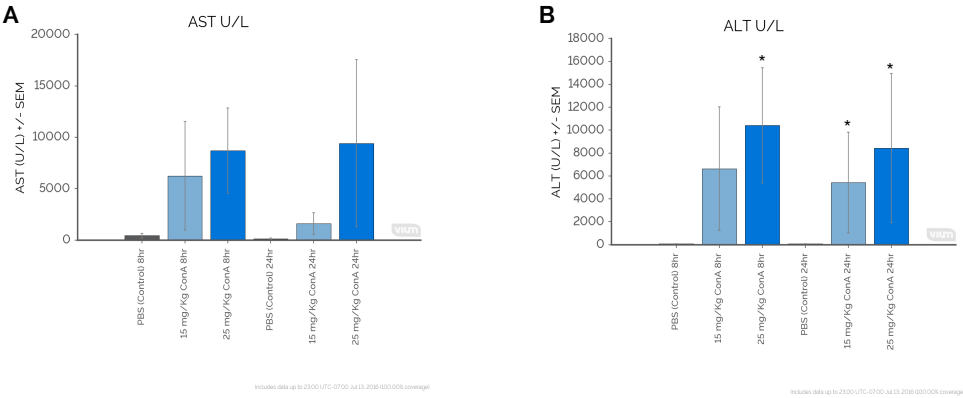

Figure 1. Liver transaminases confirm liver injury.
(A) Con A-induced mice showed elevated AST levels. There was a significant main effect of dose only at 24 hrs post-induction ($P<0.01$).
(B) Con A-induced mice showed elevated ALT levels at 8 and 24 hrs post-induction ($P<0.05$ and $P<0.01$, respectively). N=3-5 per group. Values represent Means ± SEM. * $P<0.05$ vs. PBS Control.

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Vium Metrics

When assessed using Vium's metrics, Con A-induced mice showed a dose-dependent decrease in activity levels as early as three hrs post-dose and increase in breathing rate levels, both of which persisted until study end (Fig. 2A and 2B).

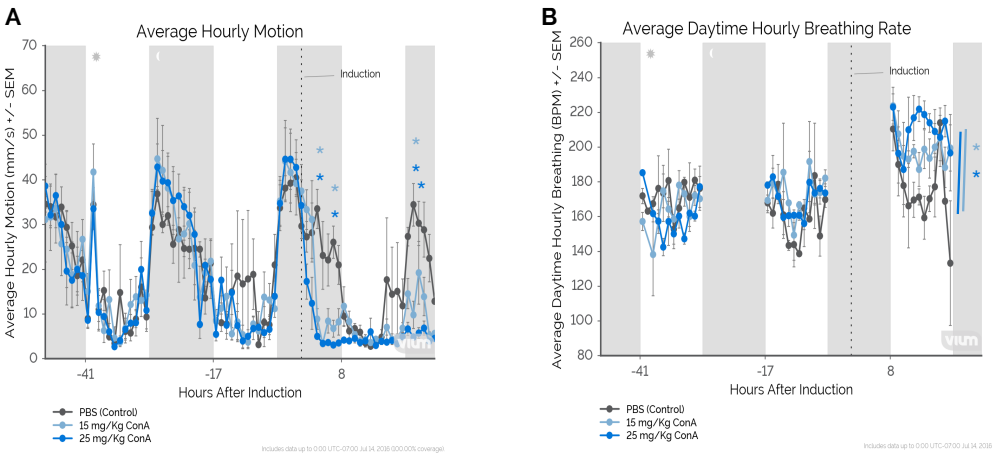

Figure 2. Motion and breathing rate track disease progression and severity.
(A) Con A-induced mice showed reduced activity levels as early as 3 hrs post-dose. On average, mice induced with 25 mg/kg Con A showed lower activity levels compared with mice induced with 15 mg/kg Con A ($P<0.05$). **(B)** On average, Con A-induced mice showed increased breathing rate ($P<0.01$, 25 mg/kg Con A or 15 mg/kg Con A vs. PBS Control). N=5 per group. Values represent Means ± SEM. * $P<0.05$ vs. PBS Control.

Sample Size Determination

Effect sizes for motion and breathing rate were larger compared with liver enzyme measurements (Table 1). Retrospective power analysis indicated smaller sample size requirements to detect these effect sizes with 95% power and an alpha at 0.05.

Table 1. Retrospective power analysis for Con A (25 mg/ kg) vs. PBS

	Night-Time Motion Night 0 Post-Induction	Breathing Rate Day 1 Post-Induction	AST 8-hr Post-Induction	ALT 8-hr Post-Induction
Effect Size	4.01	1.67	1.31	1.40
Sample Size (N)	4	11	17	15

N=5 per group. To compute sample size, the following parameters were used: two-tailed t-test (Motion and Breathing Rate) or two-tailed Mann-Whitney (ALT and AST), an alpha error probability of 0.5, and power of 0.95. Effect sizes were determined using experiment data from average night-time motion, breathing rate, and liver enzymes at specified times post-induction.

Remote Clinical Observations

Subjects were continuously monitored throughout the experiment: 439 remote observations were performed using the online Research Suite and an additional 57 cage-side observations were performed to clinically observe animals that remotely showed inactivity or some form of distress. Two mice from the 25 mg/kg Con A group reached humane endpoint before study end. These mice were identified as inactive using remote observations, followed-up with in-person clinical observations, then humanely euthanized due to moribundity.

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Mouse Model of Liver Toxicity in the Vium Digital Vivarium

DISCUSSION

We demonstrate that continuous and automated detection of motion and breathing rate in the home cage provides physiologically relevant information for evaluating disease progression in the Concanavalin A (Con A)-induced mouse model of liver disease. More specifically, motion and breathing rate showed changes in disease severity as early as three hrs post-induction. Elevated liver enzymes performed at 8 and 24 hrs confirmed liver injury.

In liver toxicity studies, animals must be closely and frequently monitored in order to restrict pain and distress. Furthermore, standard measurements involve ex-vivo analysis through histopathology and the measurement of liver transaminase activity levels, which peak ~8 hrs post-induction (3,4). We demonstrate that the Vium Digital Platform provides automated behavioral and physiological measurements, which track disease at earlier time points and persist until study end. Significant and persistent changes in metrics (i.e. ~60-80% motion reductions post-induction) also improved statistical power to subsequently reduce sample size requirements. Decreased motion and increased breathing rate may be associated with underlying symptoms, such as, fever, fatigue, or dyspnea related to pulmonary complications observed in patients with liver disease (5,6). Acute inflammation of the liver produces a systemic release of cytokines, which may result in such symptoms (2-4).

In addition to providing more sensitive and physiologically relevant metrics, a low-touch, continuous monitoring platform can be used to perform more frequent and remote observations of subjects. For screening purposes, the ability to continuously monitor motion and breathing rate allows researchers the option to omit blood collection at the 8-hr timepoint. These behavioral endpoints can be used to rapidly screen compounds that suppress inflammation in conjunction with traditional biochemical and histopathology analyses.

This platform refines animal research by decreasing animal stress, sample size requirements, and invasive procedures with unintended human impact. This approach also promotes scientific research by evaluating clinically relevant behavioral endpoints to make more informed and rapid decisions during compound screening.

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