

PULMONARY ASSESSMENT OF D-AMPHETAMINE SULFATE IN RATS USING HEAD-OUT PLETHYSMOGRAPHY

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ABSTRACT

This study was designed to evaluate the pulmonary effects of an escalating dose of d-amphetamine sulfate (d-AMP) in rats. The head-out plethysmographic method of assessment was employed for this analysis. Eight rats were placed on study and all animals received vehicle and each dose of d-AMP as defined by an escalating dose design. Animals were placed in the head-out plethysmograph (Buxco Electronics, Inc.) and provided approximately 30 minutes of acclimation time with the last five minutes used to define the baseline tidal volume, frequency, and minute volume. The Gould/PONEMAH Physiology Platform software was used to sample data at 1000 Hz at a span of ± 5.00 V, logged every 5 seconds, and collapsed to 20-second intervals. All doses of d-AMP elicited significant pulmonary changes in frequency, tidal volume, and minute volume. Frequency was dose-dependently elevated (0-1.5 mg/kg; 178-307 breaths/minute, respectively) at 1 hour post-dose. Frequency remained elevated for 2 hours following all doses of d-AMP. The 1.0 and 1.5 mg/kg doses elicited observable increases in tidal volume for approximately 40 minutes with a peak effect occurring at 8 and 6 minutes, respectively. All doses of d-AMP dose-dependently elevated minute volume for at least 40 minutes. After 40 minutes, minute volume began to decrease. The minute volume for the 0.5 mg/kg treated rats returned to baseline by 100 minutes post-dose.

INTRODUCTION

In July 2001, the FDA adopted the Center for Biologics Evaluation and Research (CBER) International Conference on Harmonization (ICH) Draft Guidance, Safety Pharmacology Studies for Human Pharmaceuticals, known as Topic ICH S7A. The guidelines outline the requirements for the three core battery evaluations: cardiovascular, central nervous system, and respiratory system. In order to meet the core battery requirements for the respiratory system, WIL Research Laboratories, Inc. has recently installed state-of-the-art equipment for the purpose of evaluating dynamic pulmonary parameters in conscious rats. This study was part

of the in-house Biophase validation of the system that also included simultaneous radiotelemetry cardiovascular assessment.

EXPERIMENTAL DESIGN

Eight plethysmographic-acclimated and radiotelemetry-implanted male Sprague-Dawley rats received a single administration of vehicle prior to initiation of the first test article dose. Over the duration of the study, each animal received each dose as defined by an escalating dose design (Table 1).

Table 1. Escalating Dose Study Design

Treatment	Dosage Level (mg/kg)	Concentration (mg/ml)	Dosing Volume (ml/kg)	Study Day
Vehicle	0	0	1.0	1
Low-dose	0.5	0.5	1.0	3
Mid-dose	1.0	1.0	1.0	6
High-dose	1.5	1.5	1.0	8

On the day of dosing, each animal was placed in a head-out plethysmograph similar to that shown in Figure 1. Thirty minutes of acclimation time was provided before 5 minutes worth of data were averaged to define the pulmonary baseline values for tidal volume, frequency, and minute volume. Following baseline data collection, all animals were removed from the plethysmographs and returned to their home cages. The first animal was then dosed, returned to the appropriate plethysmograph and post-dose data collection commenced. This procedure was repeated for the remaining animals and post-dose data were collected for at least 90 minutes. The 90 minutes of post-dose data were divided into six, 15-minute subphases for statistical analysis. A repeated measure analysis of variance (RANOVA) was performed using the calculated percent change from baseline as the dependent variable with $p < 0.05$ indicating significance.

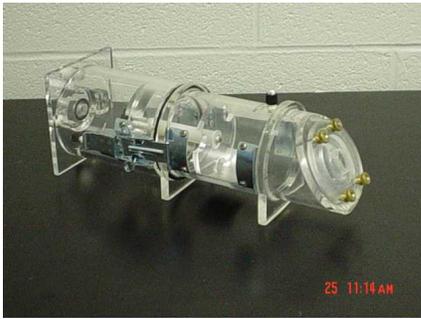


Figure 1. Head-Out Plethysmograph

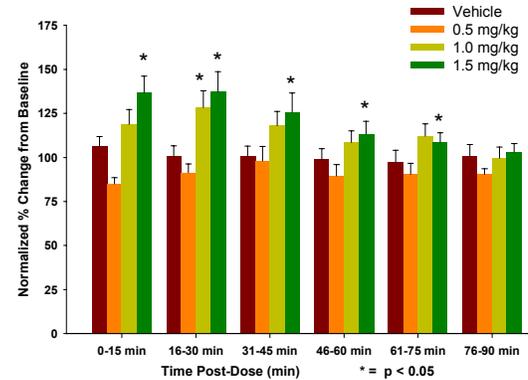


Figure 3. Tidal Volume (ml)

RESULTS

There was no significant difference in frequency (breaths/min) between treatment groups at baseline. Mean baseline frequencies were 190, 172, 192, and 190 breaths/min for the vehicle, 0.5, 1.0, and 1.5 mg/kg doses, respectively. There was a significant increase in frequency following the administration of 0.5, 1.0, and 1.5 mg/kg d-AMP at each individual time point and for the phase overall (Figure 2).

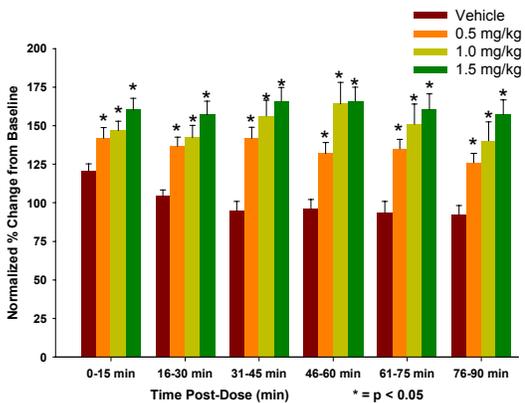


Figure 2. Frequency (breaths/minute)

There was no significant difference in tidal volume between treatment groups at baseline. Mean baseline tidal volumes were 1.9, 2.1, 2.0, and 1.8 ml for the vehicle, 0.5, 1.0, and 1.5 mg/kg doses, respectively. Tidal volume was significantly increased following administration of 1.5 mg/kg d-AMP for each individual time point with the exception of the 76-90 minute interval. Additionally, tidal volume was significantly increased at the 16-30 minute interval following administration of 1.0 mg/kg d-AMP (Figure 3).

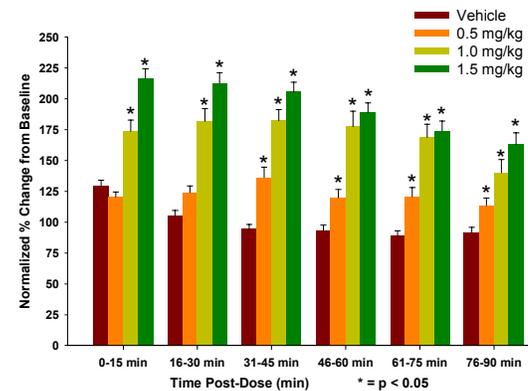


Figure 4. Minute Volume (ml/minute)

SUMMARY

- The head-out plethysmograph model provided a stable environment to allow for assessment of the acute pulmonary response following intraperitoneal administration of increasing doses of d-AMP
- The Gould/Ponemah/Buxco system is Part 11 compliant and GLP-validated for use in detecting test-article related alterations in pulmonary function
- Plethysmography is a useful method for measuring and understanding drug effects on physiological end points critical to safety evaluation