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Original article

Robustness of arterial blood gas analysis for assessment of respiratory safety pharmacology in rats

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ABSTRACT

Whole body plethysmography using unrestrained animals is a common technique for assessing the respiratory risk of new drugs in safety pharmacology studies in rats. However, wide variations in experimental technique make cross laboratory comparison of data difficult and raise concerns that non-appropriate conditions may mask the deleterious effects of test compounds — in particular with suspected respiratory depressants. Therefore, the objective of this study was to evaluate the robustness of arterial blood gas analysis as an alternative to plethysmography in rats. We sought to do this by assessing the effect of different vehicles and times post-surgical catheterization on blood gas measurements, in addition to determining sensitivity to multiple opioids. Furthermore, we determined intra-lab variability from multiple datasets utilizing morphine and generated within a single lab and lastly, inter-lab variability was measured by comparing datasets generated in two separate labs. Overall, our data show that arterial blood gas analysis is a measure that is both flexible in terms of experimental conditions and highly sensitive to respiratory depressants, two key limitations when using plethysmography. As such, our data strongly advocate the adoption of arterial blood gas analysis as an investigative approach to reliably examine the respiratory depressant effects of opioids.

1. Introduction

The sponsor of an investigational new drug application to the food and drug agency (FDA) in the United States is required to investigate effects of a test agent on the respiratory system. This is normally conducted as part of the overall battery of safety pharmacology studies. A guidance for industry document regarding safety pharmacology studies (S7A) (ICH, 2001) is available from the FDA and it states that "*respiratory function* (*e.g., tidal volume or hemoglobin oxygen saturation*) *should be evaluated*" and that "*clinical observation alone is insufficient*". As such, either whole body plethysmography or arterial blood gas analysis (ABG) is required to satisfy the agency's requirements.

Historically, the most common method utilized for assessing respiratory safety pharmacology in rats is plethysmography (Goineau, Rompion, Guillaume, & Picard, 2010). Furthermore, a survey of drug developers indicated that 97% of respondents used plethysmography in their regulatory filings; however, only 9% utilized other techniques (Lindgren et al., 2008). A known issue with plethysmography is limited sensitivity, which can be improved by artificially raising the concentration of carbon dioxide (CO_2) in the plethysmography chamber (Goineau et al., 2010; van den Hoogen & Colpaert, 1986). While increasing sensitivity this also leads to a wide variation in experimental parameters employed across different laboratories. Therefore, data generated from different laboratories cannot be directly compared. Cited CO_2 concentrations range from 3 to 10% and the duration of exposure varies throughout the entire duration of the experiment, to a single early challenge, to repeated short challenges of 5–15 min in a pulsatile fashion (De Sanctis, Green, & Remmers, 1991; Nettleton, Ransom, Abraham, Nelson, & Olsen, 2007; Romberg et al., 2003; Shimoyama et al., 2005; Strohl et al., 1997;

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Tankersley, Fitzgerald, & Kleeberger, 1994; Thorne & Karol, 1988; van den Hoogen & Colpaert, 1986). While head out plethysmography provides more direct measurements of ventilation as compared to whole body plethysmography (Murphy, 2005), varying the concentrations of CO_2 become more technically demanding, particularly in the rat.

Beyond cross-lab comparisons, it has been noted that plethysmography could potentially lead to a masking of deleterious results produced by some substances if non-appropriate conditions are utilized (Goineau et al., 2010). In particular, respiratory depressants remain more difficult to detect under normocapnic conditions (Goineau et al., 2010; van den Hoogen & Colpaert, 1986). This is evidenced by a study using the opioid remifentanil (Authier et al., 2008). Similarly, when very high doses of the opioid morphine (40 mg/kg) were administered to Wistar, plethysmography failed to show a ventilatory depressant effect (van den Hoogen, Bervoets, & Colpaert, 1988). This lack of sensitivity and wide variability is concerning considering that morphine is used frequently as a positive control or comparator when studying the pharmacological profile of respiratory depressants.

Also relevant to this study is the scrutiny to which preclinical research has recently been subjected. Some reports have cited "reproducibility" as an issue that is both commonplace and has impacted the viability of a number of drug discovery programs (Begley & Ellis, 2012; Prinz, Schlange, & Asadullah, 2011). As such, the use of techniques that have increased flexibility in terms of experimental parameters as well as higher reproducibility, sensitivity and reliability are warranted. Cross laboratory validation is also being considered as a way to reduce the occurrence of spurious, non-confirmatory results and to increase confidence in published findings (Andrews et al., 2015).

With the above noted limitations of whole body plethysmography and in the context of increased skepticism towards preclinical data, the objective of the current study was to evaluate the flexibility and reproducibility of ABG as a reliable alternative to plethysmography. We sought to do this by assessing the effect of different vehicles and times post-surgical catheterization on blood gas measurements, in addition to showing sensitivity to multiple opioids. Furthermore, we determined intra-lab variability from multiple datasets utilizing morphine and conducted within a single lab and lastly, inter-lab variability was measured by comparing datasets generated in two separate labs.

2. Materials and methods

2.1. General experimental conduct

Studies were conducted at two sites (Product Safety Labs; PSL and Purdue Pharma). Dose–response data were collected in individual studies. Single-dose morphine and vehicle data were accrued from control groups (run as part of separate studies) in a series of 12 studies conducted over approximately 19 months.

2.2. Animals

All animal care and experimental protocols were in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals, and were fully approved by either the Purdue or PSL Institutional Animal Care and Use Committees, respectively. Male Sprague–Dawley rats with indwelling catheters in the femoral (PSL) or carotid (Purdue) artery were used (Harlan Laboratories; Dublin, VA); rats were 6–7 weeks of age, weighing 175–275 g (Purdue) or 8–9 weeks of age, weighing 275–325 g (PSL). 5–11 rats were used per group (50–60 animals per study). Each animal was used once at PSL; at Purdue, a crossover design was implemented. Each study included a vehicle control group and most included a positive control group (morphine). Rats were individually housed in solid-bottom caging with contact bedding (cob or absorbent paper). Rats had access to food and water ad libitum and were maintained under artificial lighting (12 h) between 7:00 a.m. and 7:00 p.m. at a controlled ambient temperature of 21 ± 3 °C and relative humidity of 30–80%. Studies were conducted 4–14 days after arterial cannulation and were performed in an AAALAC-accredited facility, with randomization and assessed without knowledge of drug treatments.

2.3. Measurement of arterial blood gases

On testing days, baseline samples were collected from the arterial cannula. All samples were approximately 200 μL (Purdue) or 250 μL (PSL) in volume; following sample collection, the cannula was flushed with heparinized saline. Compounds were administered either orally (PO) or subcutaneously (SC), and samples were collected 1, 3, 5 and 24 h post-dose. The 24 hour time point was omitted if the 5 hour time point approached baseline levels. Samples were analyzed using an IDEXX VetStat analyzer (IDEXX Laboratories; Westbrook, ME) with Respiratory/Blood Gas test cartridges (PSL) or using an ABL 800 Flex (Radiometer America, Westlake, OH) (Purdue).

2.4. Materials and compounds

All doses are expressed as the free base. Morphine sulfate (salt factor, 0.752) was prepared in saline and administered either PO or SC. Buprenorphine (free base) was prepared in water and administered PO or alternatively prepared in 25% hydroxypropyl-beta-cyclodextrin and administered subcutaneously. Fentanyl citrate (salt factor, 0.636), oxycodone hydrochloride (salt factor, 0.896) and hydrocodone bitartrate (salt factor, 0.6) were prepared in saline and administered SC. Doses were selected to cover and exceed the analgesic range (see Table 1). All drugs were administered in a volume of 5 mL/kg SC or 10 mL/kg PO (PSL) and 2 mL/kg SC or 5 mL/kg PO (Purdue) unless otherwise stated. All compounds and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) except oxycodone which was purchased from Mallinckrodt (St. Louis, MO USA).

2.5. Statistical analyses

Dose–response data were analyzed by two-way repeated measures analysis of variance, with Bonferroni-corrected multiple comparison tests used to follow up any findings of treatment effect or significant treatment X time interaction. In the case of buprenorphine dose–response data, the results of two studies were pooled for analysis. Single-dose data were selected from individual studies and are presented to describe individual variation; accordingly, these data are presented as medians with interquartile distances without significance testing.

3. Results

3.1. Effects of different vehicles

The effect of vehicle administration on blood gas parameters are represented as pooled datasets from 12 experiments from one lab (PSL) (Fig. 1). Water or carboxymethylcellulose were administered PO; saline was given SC (n = 20–60 per treatment). Each vehicle produced in a similar ABG profile for oxygen tension, oxygen saturation,

Table 1
Compounds and doses

Compound, route	Doses tested (mg/kg)	Analgesic MED (mg/kg)*
Morphine SC	1, 5, 10, 30	5
Morphine PO	10, 30, 100	30
Buprenorphine SC	0.005, 0.05, 0.5, 3	0.1
Buprenorphine PO	1, 3, 10, 30, 100, 300	3
Oxycodone SC	1, 3, 8	3
Hydrocodone SC	5, 10, 15	10
Fentanyl SC	0.1, 0.3, 1	0.3

* MED: minimum effective dose in rat hot plate assay; Purdue data not shown.