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Research article

Regulatory respiratory data refinement with reduced animal usage

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ABSTRACT

Introduction: Assessment of effects of potential drug candidates on the respiratory system is part of the regulatory preclinical safety assessment conducted prior to first in human trials (FTIH). Commonly, this is carried out utilizing head out plethysmography (HOP) or whole body plethysmography (WBP) which record only ventilatory parameters. When dosing via the inhaled route a more thorough respiratory assessment, including a direct measure of airway mechanics, is desirable. The aim of the present work was to improve the strategy for respiratory safety testing by a) evaluating a telemetered pleural pressure – HOP (PP-HOP) model and b) evaluating a crossover study design protocol in the WBP model to reduce variability and animal usage.

Methods: For the PP- HOP model, rats were surgically implanted with a telemetry device for measurement of pleural pressure. Animals were placed in HOP tubes and respiratory function assessed when exposed to methacholine at doses of 0 (saline only), 0.42, 1.6 and 3.8 mg/kg. WBP assessment was performed in rats in a crossover study design when treated with theophylline at doses of 0 (saline only), 3, 10 and 30 mg/kg.

Results: Data from the PP-HOP study confirmed the expected changes in ventilatory parameters and airway mechanics in response to inhaled methacholine, including an increase in pulmonary resistance and decrease in tidal volume.

Data from the WBP crossover study demonstrated similar sensitivity and statistical power to detect changes in respiratory rate and tidal volume to a standard parallel group design.

Conclusion: Measurement of PP-HOP in a stand-alone safety pharmacology study in conjunction with HOP assessment conducted as part of a toxicology study, represents an improved respiratory testing strategy for inhaled drugs. For compounds administered by other routes, we conclude that use of WBP using a crossover dosing design is a suitable alternative to parallel dosing groups, with a significant reduction in animal numbers and no loss of statistical power.

1. Introduction

Assessment of respiratory function is part of the core battery of safety pharmacology studies defined by ICH S7A (ICH S7A, 2000). Rodent whole body plethysmography (WBP) or head out plethysmography (HOP) are accepted methods of respiratory assessment and are used routinely in stand-alone safety pharmacology studies or, as an add-on to toxicology studies (Authier, Legaspi, Gauvin, & Troncy, 2009). The standard design for a stand-alone study utilises 4 separate treatment groups of 8 male rats (Ewart et al., 2013).

For inhaled compounds, in order to measure respiratory parameters during dosing, the “gold” standard for respiratory assessment (or dosimetry) is plethysmography, which includes both head-out and nose-only exposures in parallel groups of 8 male rats (n = 32 in total). Plethysmography, whether WBP or HOP, provides data solely for ventilatory parameters including tidal volume, respiratory rate and breathing patterns with no assessment of lung mechanics.

Inhaled compounds, regardless of mechanism, carry a risk of paradoxical bronchoconstriction upon inhalation dosing. It is therefore essential to have a direct measure of airway mechanics, in conjunction with ventilatory parameters, to adequately de-risk this potential issue. Standard HOP recordings do not encompass an assessment of airway mechanics which can therefore result in production of ventilatory data that are potentially inadequate and difficult to interpret. As a

Abbreviations: FTIH, first time in human; PP-HOP, pleural pressure-head out plethysmography; WBP, whole body plethysmography; i.t., intra tracheal; V_T, tidal volume; Bpm, breaths per minute; MV, minute volume; ICH, The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; GLP, good laboratory practice

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consequence, it is advisable to conduct a follow up assessment of lung mechanics, most commonly, in an assay using anaesthetised rats. We have evaluated the utility of the pleural pressure-HOP (PP-HOP) model, originally described by Murphy et al. (Murphy, Renninger, & Gossett, 1998), to allow a full respiratory assessment during inhalation dosing in conscious rats, in order to provide a thorough assessment of the whole respiratory system to enable interpretation of the standard HOP findings.

PP-HOP incorporates a measure of pleural pressure (pulmonary mechanics) via surgical implantation of a pressure sensitive telemetry device, in combination with HOP to collect ventilatory parameters. This approach allows for the assessment of both ventilatory (flow and volume) and mechanical (pressure) properties of the respiratory system and as a consequence of the PP-HOP study design, we have also reduced animal numbers per study from a total of 32 to 8. By using PP-HOP we aim to increase the robustness of respiratory evaluation and negate the need for a follow-up anaesthetised lung mechanics study should an effect on ventilatory parameters be observed.

As a second objective, separate from the PP-HOP study, we have also assessed the suitability of a crossover study design in conscious rodent WBP studies in order to reduce variability in the data (all animals serve as their own control) and consequently reduce the number of animals used per study from 32 to 8.

Therefore, by utilizing PP-HOP with inhaled dosing and adopting a crossover design for standard respiratory studies utilizing WBP, it has been possible to significantly reduce our animal usage whilst increasing the robustness of the respiratory data, overall providing a more thorough respiratory risk assessment of novel compounds prior to clinical trials commencing.

2. Materials and methods

2.1. Ethics

All work involving the use of animals was conducted in compliance with regional legislation, following thorough ethical review with application of the 3Rs where possible and reported in compliance with the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010; McGrath & Lilley, 2015).

2.2. Study designs

In all of the studies carried out, the group sizes reflect assessment of the power of each of the studies to detect a reliable, biologically relevant change from control (data not shown). Use of this analysis allows the studies to be designed to use the minimum number of animals required to detect this change. For safety assessment, it is desirable to use the same strain of rats throughout the package of studies. Wistar rats are often selected as they are more suitable for longer duration studies (e.g. carcinogenicity).

2.3. Evaluation of respiratory effects of methacholine using PP-HOP

Respiratory parameters (respiratory rate, tidal volume, minute volume, pulmonary resistance) were assessed by HOP in telemetered animals before, during and after inhalation exposure to methacholine. Naïve male Han Wistar (Crl:WI (Han)) rats (Charles River Raleigh, NC, USA, 333 to 358 g at start of dosing, approx. 14 weeks (n = 8)) were surgically instrumented (based on the novel surgical procedures developed by Murphy et al., 1998) with a telemetry transmitter implant (model HD-S11, Data Sciences International, St. Paul, MN, USA) for acquisition of pleural pressure. Briefly, an abdominal incision was made on the anaesthetised rat through the skin and musculature approximately 1 cm below the xiphoid process, continuing caudally approximately 5 cm along the linea alba. The lobes of the liver were retracted, and the oesophagus isolated. A 22 gauge blunt needle was inserted between the serosal and muscularis layers of the oesophagus. The needle was tunnelled cranially past the juncture with the diaphragm and approximately 2 cm into the thoracic cavity. The needle was then removed and the transmitter catheter (15 cm in length) threaded through the channel. The pressure was monitored to confirm correct catheter placement. After catheter placement was confirmed, a suture was tied around the catheter and the catheter was fixed to the oesophagus using medical grade tissue adhesive. The body of the transmitter was placed into the abdominal cavity and secured to the abdominal wall during closure of the abdominal musculature. The antibiotic, anti-inflammatory and analgesic agents used were Benzathine Penicillin G and Procaine Penicillin G, Carprofen, and Buprenorphine. In combination with flow and volume measurements total pulmonary resistance measurements can then be derived. There was a recovery period of 18 days from surgery to the first restraint tube acclimation occasion.

One group comprising of 8 male rats, implanted with DSI HD-S11 transmitters, were restrained in HOP tubes with the snout of the animal protruding from the front of the nose cone adaptor into the exposure chamber. Despite the restraint procedures involved, HOP has previously been shown to be an acceptable model for assessing respiratory parameters, when appropriate acclimation procedures are conducted (Harris, Graham, et al., 2005). Therefore, prior to the start of the dosing phase, rats were acclimatized to the restraint tubes for increasing time periods up to 225 min, over 7 days. During the final acclimation session, prestudy respiratory assessments were conducted and only animals displaying stable parameters were included on study.

Cylindrical flow-past, nose-only inhalation chambers were used for test material administration. Test atmospheres were generated using a Pari LC Plus nebulizer. A dose formulation solution was added to the nebulizer, which was then supplied with pre-dried compressed air to nebulize the test atmosphere into the inhalation chamber. The high dose for this study was selected in order to induce bronchoconstriction in a conscious rat without producing excessive morbidity that would prevent meaningful evaluation. The low and mid doses were selected in an attempt to produce graded responses to methacholine and to evaluate potential dose response relationships. Each animal received escalating inhaled nebulised doses (10 min) of the control item (saline), and methacholine at estimated achieved doses of 0.42, 1.6, and 3.8 mg/kg (solution concentrations of 3, 10 and 30 mg/mL, respectively), and inhalation chamber aerosol concentrations of 0, 0.0550, 0.2500 and 0.6825 mg/mL, respectively, with 7 days between each methacholine dose. The particle size distribution (mass median aerodynamic diameter (μm) ± geometric standard deviation) in the saline control, low, mid and high dose groups were 1.3 μm ± 1.8, 1.5 μm ± 2.2, 1.5 μm ± 2.2 and 1.8 μm ± 2.1, respectively. Respiratory function measurements were collected continuously whilst on the inhalation chamber, for 30 min predose, and continuously for 3 h commencing at the start of the inhalation dosing, via Ponemah acquisition software system (version 5.1). The peak effects of methacholine were expected to occur within the 10 min inhalation exposure or shortly thereafter, therefore 3 h recording duration allows demonstration of any reversibility of effect over time. The telemetry receiver was placed in the proximity of the animal's plethysmograph chamber to obtain pleural pressure measurements. On a given dosing day the procedure was carried out in all animals simultaneously using a flow-past exposure chamber. The exposure chambers were operated to sustain a dynamic airflow sufficient to ensure adequate oxygen content and an evenly distributed exposure atmosphere, temperature and humidity within the chamber were monitored throughout the study. Respiratory variables were continuously recorded at a sampling frequency of 500 Hz and with an output of raw data recorded as breath-by-breath (1 epoch). Data were then expressed as 5-minute mean values for the first 30 min post dose start, and then as 30-minute mean values thereafter.