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Provision of food and water in rodent whole body plethysmography safety pharmacology respiratory studies – Impact on animal welfare and data quality

Claire Grant[a,*], Louise Marks[a], Helen Prior[b]

[a] Drug Safety & Metabolism, AstraZeneca UK Ltd, Mereside, Alderley Park, Macclesfield, Cheshire, England, UK
[b] National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, UK

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ABSTRACT

Introduction: We evaluated the feasibility of providing food and water to rodents during whole body plethysmography (WBP) studies as a welfare improvement to standard conditions.

Methods: Male Han Wistar rats or CD1 mice (n = 8) were placed in WBP chambers and respiratory parameters recorded for approximately 6 h on four separate occasions. On each occasion the animals were exposed to a different plethysmography chamber environment using a randomised design: no food/water (the standard conditions), water bottle, hydrating gel and wet food. In a further session, rats (n = 8) were administered theophylline, or vehicle and respiratory parameters measured in the plethysmography chamber containing wet food.

Results: Respiratory parameters of rats were not significantly altered by the provision of water or food. Providing wet food resulted in reduced body weight loss. Administration of theophylline caused the expected increase in respiratory rate. When mice were given access to hydrating gel or wet food the respiratory parameters were significantly affected; respiratory rate and tidal volume were increased. Providing wet food resulted in reduced bodyweight loss.

Discussion: The provision of food and water did not impact on respiratory parameters in rats placed in WBP chambers. When provided with wet food, rats lost less bodyweight. Therefore, to improve welfare conditions for rats during WBP respiratory studies wet food should be provided when appropriate to the study design. In mice, provision of food and water led to changes in respiratory parameters, therefore these improvements in welfare conditions are not suitable for mice.

1. Introduction

Animal models are used extensively in all stages of the drug discovery and development process. Respiratory function is routinely assessed in preclinical in vivo studies. The results from these studies can provide clinicians with valuable information on the potential for respiratory side-effects and may contribute to the overall 'marketability' of the drug. Whilst some effects on respiratory function in the short-term might be considered to be life-threatening (e.g. decreased respiratory rate), they may also affect patient compliance and quality of life in the long-term, contribute to undesirable interactions with other drugs or necessitate the co-administration of other medications. There is therefore the potential for respiratory findings from preclinical studies to drive decision making during the development of a compound and to contribute to the overall risk package.

Respiratory function may be assessed as part of a pharmacology/efficacy study (if the drug being developed is for treatment of respiratory related diseases) and/or as part of a toxicology study (particularly if the airways are a key safety concern), but they are most routinely performed within safety pharmacology studies (Hoymann, 2012). Drugs of various pharmacological classes are known to have deleterious effects on respiratory function (Murphy, 2002). Therefore, as described in ICHS7A guidelines, the potential adverse reactions of new chemical entities requires evaluation of respiratory function as part of the core battery of studies involving the vital organ systems (Anon, 2001). The guidelines describe two tiers of respiratory studies – core battery assessments and follow-up investigations, if necessary. Core studies require the assessment of respiratory rate, tidal volume and/or

Abbreviations: bpm, breaths per minute; ICH, International Conference on Harmonisation; p.o., oral gavage administration; MV, minute volume; f, respiratory rate; S.E.M., standard error of the mean; TV, tidal volume; WBP, whole body plethysmography

* Corresponding author at: Drug Safety & Metabolism, AstraZeneca UK Ltd, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, England, UK.
E-mail address: claire.draper@hotmail.co.uk (C. Grant).

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haemoglobin oxygen saturation (Murphy, 2014). Respiratory function can be separated into assessment of pumping efficiency and gaseous exchange. One of the most common methods to measure respiratory function is to use plethysmography in rodents (Baldrick, 2008; Lindgren et al., 2008). Plethysmography allows drug-induced changes in respiratory patterns to be assessed in conscious animals. The technique is non-invasive and measures a number of respiratory parameters, including respiratory rate, minute volume and tidal volume. There are different types of chamber available including whole body, head out and dual chamber. A study comparing the different chamber models in rodents has shown that each system was equally sensitive (Hamdam et al., 2013).

Whole body plethysmography (WBP) has been used for many years to evaluate the potential of a new chemical entity to cause an adverse effect on the respiratory system. The model has been validated with multiple compounds that have shown respiratory stimulation (Ewart et al., 2013) and respiratory depression (Valentin et al., 2009). The examples cited illustrate good concordance of effects across laboratories and species. Non-invasive WBP is one of the most commonly used systems for the evaluation of respiratory function in conscious animals. The technique provides reliable pulmonary mechanics data, with no restraint stress to the animal and no requirement for training to the chamber. The data generated is reliable, simple to handle, the breathing pattern is natural and it allows high-throughput screening of compounds. An investigation has been conducted to determine the concordance of WBP in the rodent with data from phase I clinical trials. A large number of compounds (82) were assessed, 19 of which had been shown to have an effect on respiratory parameters pre-clinically. WBP model predictivity was calculated as 70% (Ewart et al., 2010). This data shows that WBP offers significant value preclinically for the detection of adverse drug effects in the respiratory system.

ICHS7A guidelines state that the time course (e.g., onset and duration of response) of the adverse effect should be investigated. Routinely the effects of compounds on respiratory parameters using whole body plethysmography are assessed for up to 4 h post-dose, as this period is considered a sufficient duration to observe the onset and offset of most effects caused by orally administered drugs. However, for certain compounds with extended Tmax there may be a need to record respiratory parameters for longer, up to 6 h post-dose (animals would be in chambers for 450 min, 90 min hours pre-dose baseline recording and 360 min post-dose). Due to the additional period of time the animals would be in the chambers in these circumstances, the present study investigated the feasibility of food and water provision.

Food and water is not routinely provided to animals (Ewart et al., 2013) whilst in the chambers due to the short time period that animals were exposed to these conditions and concerns that it might impact on the parameters being recorded. Movement will alter the animal's basal respiratory physiology and provide poor results and there was a concern that providing stimuli such as food or water would increase movement. Provision of food or water may also increase the humidity in the chambers, which may affect tidal volume calculations. During inspiration there is a slight net pressure rise within the chamber due to increased temperature and humidity of the air as it enters the subject's lungs (gas conditioning). This change in temperature and humidity is compensated for using Epstein-Epstein correction factors when calculating tidal volume (Epstein, Epstein, Hadda, & Mellins, 1980). Changing the humidity in the chamber by providing food and water may affect the ability of the correction factor to compensate for these changes in temperature and humility leading to inaccurate data. This study was conducted to investigate these concerns.

Fasting and water deprivation may be necessary for animals undergoing experiments, and may be performed prior to surgery, before test compound dosing and during behavioural tests. However, this should be minimised whenever possible as a common result of food and water deprivation is a significant decrease in body weight. Prolonged fasting has been reported to cause a wide range of adverse effects in laboratory rodents. These include: a loss in body and liver weights and an increase in locomotor activity and grooming in rats fasted for 18 h (Vermeulen, de Vries, Schlingmann, & Remie, 1997); changes in brain neurochemistry in rats fasted for 24 h (Fuenmayor & Diaz, 1984); and episodes of bradycardia, hypotension and decreased body temperature (suggestive of reduced metabolic rate) in mice following an overnight fast (Williams, Chambers, Henderson, Rashotte, & Overton, 2002; Swoap & Gutilla, 2009). It has been demonstrated that rats without food and water for over 40 h, lose on average, 12% of their bodyweight, compared to controls (Kiss, Jeova, & Aguilera, 1994). Mice have been shown to lose approximately 6% bodyweight after a 6 h fast (Prior, Ewart, Bright, & Valentin, 2012). Fasting also causes other adverse effects in laboratory rodents, including stress and a lowering of plasma glucose after fasts of 6 h (Nowland, Hugunin, & Rogers, 2011).

A lack of water can also have serious adverse effects. Water deprivation depletes the intracellular and extracellular compartments of water and consequently increases plasma osmolality and decreases intravascular volume (Stocker, Keith, & Toney, 2003). A clinical formula for calculating fluid maintenance requirements is estimated as 50 mL/kg/day (Toth & Gardiner, 2000). Therefore a 250 g rat would be expected to drink approximately 3 mL water over a 6 h period. A 30 g mouse would be expected to drink approximately 0.38 mL over the same period.

The data above shows that fasting and water deprivation is considered stressful and may have a number of adverse effects on the physiological workings of the body (Toth & Gardiner, 2000). The replacement, refinement and reduction of animals in research (the 3Rs) is a well-established concept, originally described in the 1950s (Russell & Burch, 1959). As scientists working with animals, we are obliged to identify opportunities to review models and implement welfare improvements. Even when restricting food may be scientifically justified, it should be minimised to 'that required to achieve the scientific objectives.' (Anon, Institute for Laboratory Animal Research). As a refinement to current practices, the present study aimed to investigate whether food and water could be provided to rodents during WBP studies without impacting on the quality of the data generated.

The objective of this study was to assess the effect of different plethysmograph environments (no access to food or water, access to water, access to hydrating gel or access to wet food) on respiratory parameters measured using WBP in rats and mice. Theophylline is known to stimulate respiratory rate in both rodents and humans and was included in this study as a standard reference compound (Murphy, 2002; Murphy, 2014).

2. Methods

2.1. Animal care and procedures

All protocols and experiments were performed under the authority of a valid Home Office Project Licence and conformed to UK Governmental regulations regarding laboratory animal use and care (United Kingdom [UK] Scientific Procedures Act, 1986) and approved by institutional ethical review committees.

Assessments were conducted in male Han Wistar rats (227 to 259 g body weight and approximately 8 weeks old on experimental Day 1) obtained from Harlan Laboratories UK Ltd., Bicester, Oxon, UK or male CD1 mice (35.0 to 40.9 g body weight and approximately 6–9 weeks old on experimental Day 1) obtained from Charles River Laboratories, Margate, UK. Rats and mice were housed in groups of 4 in perspex cages containing aspen chip bedding, sizzle nest and environmental enrichment. Animals were permitted at least 6 days acclimatisation prior to first use and during this time had free access to pelleted food and to water from the site drinking water supply. Room temperature and relative humidity were monitored and maintained between 19 and 23 °C and 40 to 70%, respectively and animal rooms were illuminated by artificial light on a 12-h light/dark cycle.