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Original article

Comparison of whole body and head out plethysmography using respiratory stimulant and depressant in conscious rats

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ABSTRACT

Introduction: Assessment of respiratory safety is one of the most important requirements for new chemical entity (ICH Guideline S7A). The aim of the present study was to compare and validate respiratory safety pharmacology models in conscious rats, to find out the most appropriate method for detection of drug-induced adverse effects on respiratory function in preclinical safety studies. **Methods:** Head out plethysmography and whole body plethysmography methods were used to monitor typical parameters of ventilatory function like respiratory rate (RR), tidal volume (TV), minute volume (MV) and mid expiratory flow (EF50). The effects of respiratory stimulant theophylline (100 mg/kg) and respiratory depressant chlordiazepoxide (100 mg/kg) were evaluated in both models. Propranolol (60 mg/kg) was also used to compare head out and whole body plethysmography because of its bronchoconstrictor effects on airway function. **Results:** Theophylline caused a significant increase in TV, EF50 and MV in both whole body and head out plethysmography. In whole body plethysmography, theophylline significantly increased RR, but this increase was not observed in head out plethysmography. Chlordiazepoxide significantly decreased RR, TV, EF50 and MV in head out plethysmography, but it significantly reduced only TV in whole body plethysmography. A significant reduction in TV was observed with propranolol in both whole body and head out plethysmography. **Discussion:** We conclude that ventilatory function can be accurately assessed using head out plethysmography compared to whole body plethysmography. Our experimental results of EF50 from non-invasive methods suggest that reliable assessment of airway function demand additional invasive methods.

1. Introduction

Safety pharmacological testing of developing compound prior to first use in humans is to rule out the possibility of adverse, life threatening events occurring in volunteers receiving the drug such that the focus is on vital functions including cardiovascular, respiratory and central nervous system activity. The international Conference of Harmonization (ICH) established guidelines for safety testing and assessment of respiratory function is currently a component in the core battery of safety pharmacology studies (Harris et al., 2005). The ICH S7 document defines the respiratory system as a "vital organ system" that is considered to be one of the most critical ones to assess in safety pharmacology studies. As such, the respiratory system should be assessed with the same scientific rigor as the other vital organ systems (i.e., CNS and cardiovascular). Many pharmacological agents are known to adversely affect the respiratory function. Drug-induced respiratory disease has a low incidence but is associated with a high mortality. Only 0.5%–1.2% of total adverse drug events are related to respiratory system but account for an astonishing 12.3% of life-threatening drug-induced disease and 25%–30% of drug-induced deaths (Raissy, Marshik & Kelly, 1993). This may be attributable to the high blood flow received by the lung.

Respiratory safety studies are further supported by the fact that many patients are compromised in respiratory function while involving in clinical testing of drug or undergoing medication. Thus, administration of drugs to patients with pulmonary disorder is a safety concern because drugs producing non significant effect in the healthy subjects may be life threatening in this individual e.g. β- blockers in asthmatic patients and respiratory depressants in sleep apnea (Murphy, 2002). These acute life-threatening changes are the primary reason for evaluating new drugs on respiratory function in animal models prior to initiation of human clinical studies (Monro & Mehta, 1996).

Lung function measurements in preclinical research include invasive and non-invasive technologies and they have their own advantages and disadvantages. Non-invasive whole body and head out plethysmography are the most commonly used methods to assess the ventilatory function in conscious animals in the laboratory. Plethysmograph

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chambers are commonly used to assess the ventilatory function, which directly monitor lung volume changes or airflows generated by thoracic movements (Murphy, 2005). In head out plethysmography, the thoracic cavity movement is converted into a digital signal by pressure transducers, whereas in whole body plethysmography, the signal is created by changes in the chamber pressure due to both conditioning (heating and humidification of respired air) and resistance acting on the respired air stream. There are many drugs known to influence ventilatory control by selective interaction with the central nervous system or peripheral chemoreceptors. They include opiates, benzodiazepines, and adenosine analogs that depress the respiratory system while xanthine analogs, alkaloids and GABA antagonists act as stimulants (McHugh, Miyamoto, Kelly, Karantabias, & Gosselin, 2006; Murphy, 2005).

Respiratory safety investigations rely on pulmonary endpoints such as changes in respiratory rate (RR), tidal volume (TV) and minute volume (MV) resulting from xenobiotics. The aim of the present study was to compare and validate respiratory safety pharmacology models in conscious rats, to find out the most appropriate method for detection of drug-induced respiratory disorders in preclinical safety studies using respiratory stimulant theophylline and depressant chlordiazepoxide. Propranolol, a non-selective β-adrenergic blocker known for its respiratory side effects on the airway function (Kumana & Ruffin, 1978, Maclagan & Ney, 1979) was also used to compare head out and whole body plethysmography.

2. Methods

2.1. Animals

Male Wistar rats of body weight 250–300 g were procured from National Institute of Nutrition, Hyderabad, India for use in the experiments. Animals were housed in a group of four in polycarbonate cages with stainless steel grill tops, facilities for food and water bottle and bedding of clean paddy husk. The cages were suspended on stainless steel racks. Animals were introduced to the experimental holding rooms at least 7 days prior to the commencement of the study and maintained at 21 ± 3 °C relative humidity 30–70% and illumination cycle set to 12 h light and 12 h dark. 'Rayons' brand extruded pelleted rat/mouse feed manufactured by Rayons Hyderabad Pvt. Ltd., were provided *ad libitum*. Potable water passed through water filtration system was provided in polycarbonate bottles with stainless steel sipper tubes. All procedures were performed in accordance with the Institutional Animal Ethics Committee of Suven Life Sciences Ltd. constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

2.2. Respiratory equipment

Rats can move freely in the whole body plethysmograph chambers, whereas in head out plethysmograph chamber they were restrained. Respiratory parameters were monitored in conscious rats by using Buxco Biosystem XA software (version 2.10). The ventilatory parameters were measured in unrestrained whole body and restrained head out plethysmography chambers (PLY 4215 and PLY 3013 respectively, Buxco, UK). A quiet, constant, smooth flow of fresh air at a rate of 2.5 liters per minute was maintained through the animal chamber to avoid an increase in humidity, temperature and CO_2 levels using bias flow supply (BFL 0250, Buxco, UK). The system was validated for flow derived parameters like respiratory rate (RR), tidal volume (TV), mid expiratory flow (EF50), inspiratory time (Ti), expiratory time (Te) peak inspiratory flow (PIF) and peak expiratory flow (PEF). Both systems were calibrated for airflow, following Into-Zero method by evenly injecting 10 mL of air into the plethysmograph using 10 mL syringe that can be attached to a port. Basic two-point calibration method was followed for humidity and temperature of whole body plethysmograph chamber. The volume changes within the chambers were measured by a differential pressure transducer (TRD-5715, Buxco, UK) and were amplified using amplifier unit (MAX 1420, Buxco, UK).

2.3. Experimental procedure

Animals were acclimatized to the plethysmograph chambers for the duration of 2 h twice a day for 3 days prior to the experiment. The experimental conditions during acclimatization and recording sessions were maintained similarly to avoid variation in the results. Respiratory parameters were not recorded during the acclimatization sessions.

Rats were segregated into four main groups for pharmacological treatment according to their body weight. They were divided again into two subgroups (n = 8) for head out and whole body plethysmography. On the day of experiment, rats were administered with corresponding vehicle after 30 min of habituation in the plethysmograph chamber and basal readings were recorded for 30 min. Rats received either vehicle (10% PEG400, 2 ml/kg, p.o.) or theophylline (100 mg/kg, p.o.) or chlordiazepoxide (100 mg/kg, i.p.) or propranolol (60 mg/kg, i.p.) treatment after basal recordings, and they were placed immediately in the plethysmograph chambers. For oral administration, the dose volume was 2 mL/kg and 1 mL/kg for intraperitoneal administration. Experiment was designed in such a way equal number of animals received all treatments in a day. This methodology was followed to minimize day-to-day variation.

Response was recorded after a post dose interval of 30 min following drug treatment. Respiratory parameters viz. RR (bpm), TV (mL), MV (mL/min), EF50 (mL/s), Ti (s), Te (s), PIF (mL/s) and PEF (mL/s) were recorded for a period of 1.5 h at an interval of 15 min. At each time interval, respiratory parameters were recorded for a period of 5 min. From the flow signal RR, TV and EF50 were calculated for each breath and all these parameters were averaged in 10 sec segments with the use of Biosystem XA software. Raw data were integrated using data analyzing software (Finepointe, Buxco, UK). Movement of the animals can cause changes in RR, so values above 250 bpm were excluded from the data analysis. Vital ventilatory parameters like TV, RR, MV and EF50 were only presented in the results section.

2.4. Drugs

Theophylline and propranolol were purchased from Sigma Chemicals, Germany and chlordiazepoxide (Libirium, Lot no-LBA 9006, Nicholas Piramal, Mumbai, India) was extracted from commercially available tablets. Chlordiazepoxide was dissolved in saline whereas theophylline and propranolol were suspended in deionised water containing 10% PEG-400. Normal saline (0.9% Nacl) and deionised water containing 10% PEG-400 were used as a vehicle for oral and intraperitoneal administration. Drug solutions or suspensions were freshly prepared throughout all experimental days. The doses of theophylline, chlordiazepoxide and propranolol were selected on the basis of previous preliminary studies (unpublished results).

2.5. Statistical analysis

Data were continuously monitored for all treated groups, and ventilatory parameters recorded from the whole body and head out plethysmography were expressed as mean ± SEM. For each respiratory parameter, 15 min average was analyzed and compared to the basal values using One-Way ANOVA with repeated measures followed by Dunnett's post hoc analysis. The threshold for statistical significance was set at 0.05.