Journal of Pharmacological and Toxicological Methods 64 (2011) 187–195

Contents lists available at SciVerse ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Airway responsiveness in an allergic rabbit model

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ARTICLE INFO

Article history:
Received 14 December 2010
Accepted 3 August 2011

Keywords:
Airway responsiveness
Allergic
Methods
Rabbit
Skin test

ABSTRACT

Background: Animal models of allergy and bronchial hyperresponsiveness (BHR) are useful in researching pulmonary diseases and evaluating drug effects on the airways. Neonatally immunised rabbits exhibit several features of asthma as adults, including early and late airway responses following antigen challenge, oedema and inflammatory cell infiltration into the lung, BHR to inhaled histamine and methacholine (compared with naïve rabbits) and exacerbations of BHR following antigen challenge. Therefore this model can be used to investigate the underlying mechanisms of BHR and for the preclinical evaluation of new drugs for the treatment of asthma. **Aim:** To describe the characteristics of airway responses in a rabbit model of allergic inflammation and to evaluate the relationship between skin test reactivity to antigen, airway inflammation and BHR. **Methods:** New Zealand White rabbits were neonatally immunised against *Alternaria tenius*. At 3 months of age, airway responsiveness was measured to aerosolised histamine, methacholine or allergen. Bronchoalveolar lavage (BAL) was performed and cell counts recorded. Direct skin tests were performed to assess skin reactivity to allergen and passive cutaneous anaphylaxis (PCA) tests were performed to determine titres of circulating IgE. **Results:** In a population of allergic rabbits, allergen challenge induced a significant bronchoconstriction, airway inflammation and BHR. Skin test responsiveness to allergen did not correlate with various indices of pulmonary mechanics e.g. baseline sensitivity to methacholine and histamine, or allergen-induced BHR. In contrast, skin test responsiveness did predict airway inflammation as assessed by measurements of eosinophil recruitment to the lung. **Conclusion:** The allergic rabbit is a useful model to further our understanding of allergic diseases. Recording lung function using a minimally invasive procedure allows repeated measurements to be made in rabbits longitudinally, and each animal may thus be used as its own control. Our observations do not support the use of skin responsiveness to allergen as a predictor of airway sensitivity as we observed no correlation between skin sensitivity and airway responsiveness to inhaled histamine, methacholine or allergen. However, skin reactivity did predict airway inflammation as assessed by measurements of eosinophil recruitment to the lung. Our results also further highlight the likely dissociation between airway inflammation and BHR.

1. Introduction

Asthma is a chronic disease characterised by a variety of features, including increased airways responsiveness, airway inflammation and reversible airways obstruction. Various animal models have been established to try and better understand specific aspects of the human disease. Whilst no single animal model is able to reproduce all the features of human asthma, animal models remain useful.

Guinea pigs have traditionally been the most commonly used small animal species for studying airway diseases, as receptor pharmacology in the guinea pig closely matches that of human receptors compared with other small species and many of the existing drugs currently used in the treatment of airway diseases have been extensively studied in guinea pigs (Bos et al., 2007; Harrison et al., 2008; Keir, Spina, & Page, 2002). However, disadvantages of the guinea pig model are a lack of transgenic models and a limited number of guinea pig strains for comparative work. Mice are increasingly being used to study genetic factors involved in disease pathophysiology and are also being used in the investigation of airway remodelling and inflammation (Pitchford et al., 2004). Genetic manipulation and short breeding times are an advantage of the mouse, but there are some key differences in lung structure and function compared with human airways which limits their suitability to the study of human diseases, particularly airways diseases (Persson, 2002).

Rabbits immunised within 24 h of birth preferentially produce antigen specific immunoglobulin E (IgE) antibodies (Pinckard et al., 1977). Animals sensitised in this way exhibit several features of the asthma phenotype. They undergo both early and late airway responses following antigen challenge (Coyle et al., 1989; Murphy et al., 1986; Shampain, Behrens, Larsen, & Henson, 1982). This is associated with

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doi:10.1016/j.vascn.2011.08.003

oedema and inflammatory cell infiltration (Larsen, Wilson, Clark, & Behrens, 1987; Marsh, Irvin, Murphy, Behrens, & Larsen, 1985). Allergic rabbits also exhibit bronchial hyperresponsiveness (BHR) to inhaled histamine and methacholine compared with naïve rabbits (Bloom, Baumgartner-Folkerts, & Palmer, 1988) and also show exacerbations of airways responsiveness following challenge with antigen or inflammatory mediators such as platelet activating factor (PAF) (Coyle et al., 1989; Larsen et al., 1987; Marsh et al., 1985). Repeated measurements of lung function are readily made in the anaesthetised rabbit, and each animal may therefore be used as its own control (Minshall et al., 1993). We have studied the rabbit over many years and in doing so have evaluated the advantages and limitations of this model. We have collated and analysed our large data set to provide a detailed assessment and analysis of characteristics of this model. In addition we have used this model to investigate the relationship between skin test sensitivity to antigen and numerous other variables such as the magnitude of the early asthmatic response (EAR), late asthmatic response (LAR), extent of any airway eosinophilia and whether allergic skin reactivity can predict airway responses.

A major component of the asthma phenotype is atopy. Skin sensitivity to allergen as measured by allergen titration in the skin is often used to select subjects with asthma for entry into clinical trials to assess the clinical effectiveness of novel agents for the treatment of asthma (DuBuske, 2006; Finnerty, Holgate, & Rihoux, 1990), to select asthmatic subjects for genotyping studies (Knudsen, Thomsen, Nolte, & Backer, 2009) and to assess asthma severity (Harmanci, Bakirtas, & Turktas, 2008).

Several studies have pointed to a possible relationship between the degree of allergen sensitivity in the skin and airway hyperresponsiveness to the spasmogens histamine or methacholine and inhaled allergen (Bryant, Burns, & Lazarus, 1975; Cockcroft et al., 1979). BHR to both histamine (Cockcroft, Murdock, Kirby, & Hargreave, 1987) and methacholine (Fowler & Lipworth, 2003) has been associated with the presence of atopy and an understanding of the relationship between BHR and atopy has been identified as fundamental to further our understanding of asthma (Woolcock & Peat, 2000). Furthermore, clinically it has been reported that there is an excellent correlation between the PC_{20} for histamine and methacholine, and consequently these agents have been used interchangeably, and have been suggested to be useful markers for disease severity or for assessing the effects of drugs on BHR (Cockcroft et al., 2005).

It has also been reported that both the severity of the early asthmatic response to allergen and airway responsiveness to histamine or methacholine can be predicted from the skin-sensitivity to that allergen, as can airway responsiveness to histamine (Cockcroft et al., 1987) or methacholine (Cockcroft et al., 2005). Furthermore, a relationship between skin test sensitivity to allergen and allergen-induced airway inflammation assessed by sputum eosinophilia has also been suggested (Boulay, Lemieux, Deschesnes, & Boulet, 2003).

We have collected a considerable body of data on airway responsiveness to inhaled histamine and methacholine and airways responses to inhaled allergen in allergic rabbits. Here, we report how the airway responsiveness and allergen-induced airway inflammation relate to skin reactivity to allergen and serum IgE titres as markers of allergic status, to determine whether the variability of airway responsiveness between individuals is related to allergic status.

2. Materials and methods

2.1. Animals

New Zealand white (NZW) rabbits (2–3 kg) of either sex were obtained from Froxfield farms UK (Petersfield, Hampshire, UK) or Highgate Farm (Lincolnshire, UK). All procedures described in this paper were subject to Home Office approval and were performed under the Animal (Scientific Procedures) Act, 1986.

2.2. Immunisation protocol

Rabbits were immunised with an extract of *Alternaria tenius* in aluminium ($Al(OH)_3$) gel (Minshall et al., 1993), and which was first established as a model of allergic pulmonary inflammation in this species (Shampain et al., 1982). *A. tenius* extract (0.5 ml of a solution containing 40,000 protein nitrogen units (PNU)/ml) was mixed with sterile $Al(OH)_3$ moist gel (0.5 ml) and saline (1 ml). Each rabbit received 0.5 ml of this allergen–adjuvant mixture within 24 h of birth by the intraperitoneal route. The injection was repeated weekly for the first month of life and then bi-weekly until 13 weeks of age. Sham immunised littermates received an i.p. injection of 0.5 ml $Al(OH)_3$ and saline in the ratio of 1:3. A third group of animals received an i.p. injection of 0.5 ml saline only (saline group). This was repeated as described above. Allergic rabbits used in this study were 3 months of age when they were transferred from the breeding unit to our laboratory.

2.3. Measurement of pulmonary function

Measurements of pulmonary function and BHR were made in spontaneously breathing rabbits under neuroleptanalgesia induced by one of 2 methods. Either the animals were sedated with diazepam (2.5 mg/kg, i.p.) and then anaesthetised with an intramuscular (i.m.) injection of Hypnorm™ (0.4 ml/kg), anaesthesia was then maintained throughout the course of the experiment by further administration of Hypnorm™ every 20 min. Alternatively, they were administered ketamine hydrochloride (0.35 mg/kg) and xylazine (0.25 mg/kg) intramuscularly (i.m.) and anaesthesia was maintained with an i.m. injection of a 3:2 mixture of ketamine and xylazine every 30 min. The rabbits were then placed supine and were intubated with a 3.0 mm internal diameter cuffed endotracheal tube which was attached to a heated (37.5 °C) Fleish pneumotachograph (size 00) connected to a Validyne differential pressure transducer (model MP 45-14-87; Validyne Engineering Corp; CA, USA) from which measurements of flow and tidal volume were obtained. A latex oesophageal balloon attached to a polyethylene cannula (internal diameter 1.67 mm; outer diameter 2.42 mm) was inserted into the lower third of the oesophagus and attached to a second differential pressure transducer, connected between the balloon and atmospheric air, from which values of transpulmonary pressure (TPP), the difference between atmospheric and pleural pressure, were derived. The position of the balloon was adjusted in order to maximise the TPP recorded and it then remained in this position throughout the experiment. The flow was integrated to give continuous recording of tidal volume. Values for total lung resistance (R_L; $cmH_2O/L/s$) and dynamic lung compliance (Cdyn; ml/cmH_2O) were calculated using an on-line respiratory analyser (Pulmonary Monitoring System (PMS) Version 5.1; Mumed Ltd, London, UK) on a breath by breath basis.

2.4. Measurement of airway responsiveness to spasmogens

Airway responsiveness was measured in response to inhaled methacholine hydrochloride or histamine diphosphate. The spasmogens were aerosolised in an ultrasonic nebuliser (deVilbiss Ultraneb 99; DeVilbiss Healthcare Ltd, Heston, UK) and delivered directly into the lungs via the endotracheal tube. Initially, baseline lung function parameters were measured, followed by the administration of aerosolised 0.9% physiological saline for 2 min. Following the saline administration, further lung function parameters were recorded and these served as the baseline measurement with which to compare responses to inhaled spasmogens, which were also administered for a 2-min period at each concentration. Doubling concentrations of the spasmogens were cumulatively administered. Histamine diphosphate was administered at doses of 1.25–80 mg/ml and methacholine hydrochloride 0.31–20 mg/ml. The provocation concentration (PC) that produced a 50% increase