The Veterinary Journal 187 (2011) 54–59

Contents lists available at ScienceDirect

The Veterinary Journal

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

Airway hyperresponsiveness to adenosine 5'-monophosphate in feline chronic inflammatory lower airway disease

Reinhard A. Hirt *, Alexandra Galler, Sarina Shibly, Andrea Bilek

Department for Small Animals and Horses, Clinic of Internal Medicine and Infectious Diseases, Veterinary University of Vienna, A-1210 Vienna, Austria

ARTICLE INFO

Article history:
Accepted 10 October 2009

Keywords:
Feline
Asthma
Chronic bronchitis
Airway hyperresponsiveness
Barometric whole body plethysmography

ABSTRACT

Airway hyperresponsiveness is a key feature of human asthma and chronic bronchitis and response to the indirectly acting agonist adenosine 5′-monophosphate (AMP) is thought to reflect underlying airway inflammation. To examine whether airway responsiveness testing (ART) with AMP may be used to differentiate healthy cats from those with asthma (FA) and chronic bronchitis (CB), 24 cats (9 FA, 6 CB, 9 controls) underwent ART with AMP at concentrations of 0.1, 1, 10, 100 and 500 mg/mL using barometric whole body plethysmography.

The defined endpoint of ART, an increase in enhanced pause (Penh) exceeding 300% of the post-saline value (baseline), was reached in 9/15 patients (7 FA, 2 CB), but in none of the controls. Mean Penh (±SD) at baseline (BL) was 0.49 ± 0.16 for cases, and 0.54 ± 0.16 for controls, and was significantly increased after AMP challenge in clinical cases (2.62 ± 2.20), but not in controls (0.63 ± 0.30, $P < 0.05$). After separating responder (R) and non-responder (NR) cases, a more pronounced difference after challenge was found (R: 3.96 ± 1.84, NR: 0.6 ± 0.21, $P < 0.001$). The provocative concentration of the agonist that increased Penh to 300% of BL (PC Penh 300) in R cases was 52.98 ± 48.04 mg/mL AMP. Age had no influence on the responder status or PC Penh 300. It was concluded that AMP challenge may offer a new method for the identification of cats with lower inflammatory airway disease, and possibly for monitoring disease progression or response to therapy.

Introduction

Feline asthma (FA) is a chronic inflammatory disease affecting the lower airways of cats and shares multiple similarities with human asthma (Hirt, 2005; Reinero et al., 2009). The disease is characterised by chronic coughing, wheezing and intermittent respiratory distress due to bronchoconstriction (Dye, 1992). In addition, airway hyperresponsiveness to a variety of stimuli (a key feature in human asthma) is believed to occur in cats (Hoffman et al., 1999). Diagnosis is based on history, clinical signs, radiographic changes, the presence of eosinophils in airway lining fluids and exclusion of other diseases causing similar clinical signs. One major differential diagnosis is chronic non-allergic bronchitis (CB), which is not associated with spontaneous bronchoconstriction, and where neutrophils predominate (Padrid, 2009).

In humans, airway responsiveness testing (ART) with non-specific agonists of airflow limitation is a diagnostic tool to detect airway hyperresponsiveness, an important characteristic of lower airway inflammatory disease (Van Schoor et al., 2000), such as asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (Van Asperen et al., 1981; Sears et al., 1986; Pauwels et al., 1988; Oosterhoff et al., 1993; Rutgers et al., 2000). This phenomenon has also been observed in several animal species, including rodents (Chand et al., 1993; Hamelmann et al., 1997; Theodorou et al., 1997; Hannon et al., 2001), pigs (Halloy et al., 2004), and dogs (Hirt et al., 2007).

ART is commonly performed in humans by administering histamine and cholinergic agonists (methacholine or carbachol), which cause airflow limitation by a direct action on effector cells (smooth muscle cells, bronchial endothelial cells, mucus producing cells). Whereas histamine has unpredictable effects on feline airways (Austin and Humphrey, 1963; Lulich et al., 1976), carbachol has been shown to consistently induce airflow limitation in cats in a dose-dependent manner (Hoffman et al., 1999). Unfortunately, airway responsiveness to carbachol decreases significantly in cats with increasing age (Hirt et al., 2003), and much higher doses are required in old cats to elicit airflow limitation. Consequently, age-matched cut-off values are required to differentiate normal and hyperresponsive airways.

Indirect agonists, such as adenosine 5′-monophosphate (AMP), elicit their effects on intermediaries that subsequently interact with effector cells (Cushley et al., 1983). Such intermediaries are thought to be inflammatory cells (e.g. mast cells) and activated airway neural pathways (Polosa and Holgate, 1997; Van Schoor et al., 2000; Currie et al., 2003). Thus, airway hyperresponsiveness to

* Corresponding author. Tel.: +43 1 25077 5137; fax: +43 1 25077 5190.
E-mail address: Reinhard.Hirt@vu-wien.ac.at (R.A. Hirt).

1090-0233/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2009.10.007

indirect stimuli may more closely correlate with an underlying inflammation in airway diseases (Pauwels et al., 1988). In contrast to directly acting agents, no or very weak responses are expected in healthy subjects with indirect agonists because of the lack of intermediate effector cells (Polosa and Holgate, 1997; Van Den Berge et al., 2001).

Considering the unpredictable effects of histamine and the age dependency in airway responsiveness to carbachol in cats (Hirt et al., 2003), we sought other agonists of airflow limitation. The purpose of the present study was to investigate the airway responsiveness to AMP using barometric whole body plethysmography (BWBP) to identify cats with inflammatory airway disease. We hypothesised that AMP would cause airflow limitation in cats with inflammatory lower airway disease, but not in healthy cats.

Materials and methods

Animals

The study used 24 animals. The clinical case group consisted of 15 client owned cats presented for respiratory problems (Table 1). Owners gave their consent to include the cats in the study. Diagnostic work-up included physical examination, haematology and biochemical blood analysis, exclusion of endoparasitism, and lateral and ventrodorsal radiographs. Diagnosis was based on the results of these examinations and on cytological examination of endoscopically obtained bronchoalveolar fluid (BALF) in all but two cats, for which the owners refused bronchoscopy. Cats with eosinophils as the predominant inflammatory cell type (aside from alveolar macrophages) in BALF were classified as FA patients, whereas cats with predominantly neutrophils were classified as CB patients. In the two patients without bronchoscopy, a presumptive diagnosis was established based on history and clinical presentation, laboratory results and thoracic radiographs.

Nine healthy Domestic shorthair cats from a colony of experimental animals at the Veterinary University Vienna, Austria (4–6 years old, 2 castrated males, 7 neutered females) without history or clinical signs of respiratory disease in the previous 12 months served as controls. All of the animals had been regularly vaccinated and dewormed. Physical examination, haematology and biochemical blood analysis, lateral and ventrodorsal thoracic radiographs revealed no abnormalities. All procedures were approved by the National Animal Health Care Authorities to fulfil the criteria concerning use of animals for experimental studies.

Procedure

ART, bronchoscopy and BAL were performed on the same day with ART preceding bronchoscopy. Data acquisition of respiratory parameters with a focus on detection of airflow limitation was performed using BWBP as described elsewhere (Hirt et al., 2003). Analysis of the waveforms was performed using commercial software (Buxco XA Biosystem, Non-invasive Mechanics) to obtain values for different respiratory variables, especially enhanced pause (Penh), a surrogate for classical measurements of airway obstruction (Chand et al., 1993; Hamelmann et al., 1997).

For ART, stock solutions of AMP (Fluka Chemie GmbH) were freshly prepared by dissolving the dry powder in 0.9% sodium chloride. Serial dilutions were prepared in 0.9% sodium chloride and stored at 4 °C during the whole testing period.

Each animal was allowed to acclimate to the plethysmography box for several minutes before ART. Respiratory variables were first measured at baseline (BL, i.e. after nebulisation of 0.9% sodium chloride), and after subsequent nebulisation of AMP at concentrations of 0.1, 1.0, 10, 100, and 500 mg/mL through a valve in the chamber via a compressor driven jet nebuliser (Pari Master, PARI GmbH; average particle diameter 2–3 μm). Nebulisation of saline and AMP was performed for 60 s each, followed by a 7-min observation and data acquisition period.

Penh was examined as a function of increasing concentrations of AMP to achieve concentration–response curves for determination of airflow limitation. The peak value for Penh after each concentration was the highest mean value for 10 consecutive breaths during the 7-min observation period. The decision as to which consecutive breaths were to be used to generate a data set was based on visual observation of the BWBP-derived pressure curve. The chosen endpoint was defined as the peak value for Penh exceeding 300% of its post-saline value for $\geqslant$10 consecutive breaths in association with recognisable changes in respiratory pattern (e.g. increased expiratory effort). If the endpoint was reached, a bronchodilatator (0.01 mg/kg terbutaline) was administered intramuscularly. The provocative con-

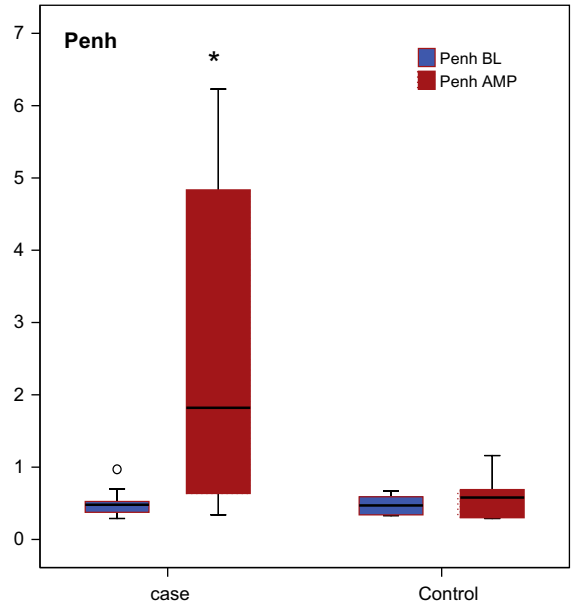

Fig. 1. Penh in cases and in control cats at baseline (i.e. after nebulisation of 0.9% isotonic sodium chloride, Penh BL) and after nebulisation of the final AMP concentration (Penh AMP). Penh significantly increased after AMP in cases, when compared to baseline, as well as pre- and post-AMP in controls (∗, $P < 0.05$).

Table 1
Details on individual cats.

Case number	Breed	Gender[a] age (years)	Presenting complaints	Radiographic lung pattern	Diagnosis
1	DSH	m, 1	Chronic cough	BI, ALV	FA
2	DSH	mn, 2	Chronic cough	BI	FA
3	DSH	mn, 3	Chronic cough, acute respiratory distress	Normal	FA
4	Siamese	fs, 3	Chronic cough, recurrent respiratory distress	B, PB	FA
5	DSH	mn, 3	Chronic cough, recurrent respiratory distress	BI	FA
6	DSH	mn, 5	Chronic cough, recurrent respiratory distress	BI, PB	FA
7	DSH	fs, 9	Coughing, sneezing for years	BI	FA
8	DSH	fs, 9	Chronic cough, acute respiratory distress	BA	FA
9	DSH	mn, 13	Diagnosed asthmatic (PV), recurrent respiratory distress	B	FA
10	DSH	mn, 4	Chronic cough	BI, dense rML	CB
11	DSH	fs, 9	Chronic cough	NA	CB
12	Siamese	fs, 10	Chronic cough	BI	CB
13	DSH	fs, 10	Chronic cough	BI, PB	CB
14	DSH	mn, 13	Chronic cough, recurrent respiratory distress	BI, PB, AT	CB
15	DSH	fs, 13	Chronic cough	B	CB

DSH, domestic short hair; BI, bronchointerstitial lung pattern; ALV, patchy alveolar lung pattern; PB, peribronchial lung pattern; AT, air trapping; B, bronchial lung pattern; rML, right middle lobe; FA, feline asthma; CB, chronic bronchitis; PV, primary veterinarian; NA, not available or applicable.
[a] m, male; mn, male neutered; fs, female spayed.