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Characterisation of the acute and reversible airway inflammation induced by cadmium chloride inhalation in healthy dogs and evaluation of the effects of salbutamol and prednisolone

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Abstract

The aims of this study were firstly to characterise a model of subclinical and reversible bronchial inflammation induced by cadmium chloride inhalation in healthy dogs and then to examine the effect of prednisolone or salbutamol treatment on the resulting bronchitis. The model characterisation and the effects of treatment were studied using clinical symptoms, haematology, thoracic radiography, bronchoscopy and bronchoalveolar lavage, barometric whole-body plethysmography and histamine broncho-provocation tests. In addition, the activity of matrix metalloproteinases (MMP)-2 and -9 were determined in serum and bronchoalveolar lavage fluid (BALF). Cadmium inhalation induced: (1) a transient bronchial inflammation, dominated by neutrophils; (2) a neutrophilia of the blood that persisted for up to 4 weeks; (3) a transient increased bronchial reactivity, and (4) a significant increase in MMP-9 activity in the BALF. Prednisolone treatment reduced the influx of inflammatory cells into the BALF, but not significantly, had no effect on pulmonary function, and did not reduce of airway hypersensitivity. Salbutamol had almost no effect on any of the parameters investigated.

Keywords: Dog; Barometric whole-body plethysmography; Cadmium chloride; Salbutamol; Prednisolone

Introduction

Although both acute and chronic forms of bronchitis are frequently encountered in dogs, little is known about the role of inflammation in canine airway hypersensitivity. The treatment of these pathologies is currently based on empirical results (Padrid, 1995; McKiernan, 2000) as there is little in the scientific literature relating the biochemical and cellular effects of controlled therapeutic trials in canine bronchitis. There is a need to develop dog models of airway inflammation, allowing a better investigation of inflammation mechanisms and airway reactivity, as well as to document the influence of therapeutic agents.

Many agents, such as ozone (Lu et al., 2006), sulfur dioxide (Heyder and Takenaka, 1996), oleic acid (Li et al., 2006), paraquat (Whitehead et al., 2003), cold air (Davis et al., 2005), lipopolysaccharides (LPS) (Numata et al., 1998) and cadmium chloride (Cd) (Oberdörster et al., 1994), have been used to induce experimental airway inflammation. Cadmium chloride, a toxic ambient pollutant, has been recently used by inhalation to induce reversible airway inflammation in dogs (Hirt et al., 2007) and was therefore selected for the present study to induce experimental airway inflammation.

In both clinical and experimental settings, methods used to evaluate bronchial inflammation include blood

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testing, thoracic radiography (Rx), bronchoscopy and analysis of bronchoalveolar lavage fluid (BALF). Differential cell counts in BALF, as measured in the course of experimentally induced or spontaneous respiratory disorders, are a good indicator of the level of the local inflammation and can be helpful in monitoring the response to the treatment, as well as in correlating with other clinical parameters (Clercx et al., 2002; Kirschvink et al., 2007a).

Pulmonary function tests are not routinely used in veterinary practice due to their invasive nature. Additionally, most of them need sophisticated equipment and experienced investigators. Barometric whole-body plethysmography (BWBP) is a non-invasive pulmonary function test first used in human medicine to test the pulmonary function of new born infants. The technique was then adapted for veterinary medical applications (Rozanski and Hoffman, 2004) and validated in mice (Hamelmann et al., 1997), rats (Kirschvink et al., 2005a), guinea pigs (Bergren, 2001), pigs (Halloy et al., 2004), cats (Hoffman et al., 1999) and, recently, in dogs (Talavera et al., 2006a). In the current study, BWBP was used to assess pulmonary function and airway reactivity following a broncho-provocative test (BT) using inhalation of increasing concentrations of histamine.

In human respiratory medicine, important efforts are being made to monitor persistent airway inflammation as well as to determinate the level of biomarkers in BALF and serum. Recent studies have investigated the pulmonary proteases–antiproteases equilibrium, which is the balance between proteases produced by inflammatory and other cells in the lower respiratory tract and the antiproteolytic defences of the lung (Van den Steen et al., 2002; Barnes et al., 2003; Cataldo et al., 2003). Matrix metalloproteinases (MMPs) are extracellular and cell-associated endopeptidases which are produced and secreted in a latent proform and activated thereafter by other proteinases (Rajamaki et al., 2002). Matrix metalloproteinases are involved in numerous physiological and pathological processes (Van den Steen et al., 2002). When acute inflammation occurs, polymorphonuclear cells are recruited to the inflammation site and help tissue repair by modulating MMPs secretion (Corbel et al., 2000). Among the MMPs, gelatinases A (MMP-2) and B (MMP-9) are believed to play a predominant role in lung tissue remodelling and repair (Atkinson and Senior, 2003; Cataldo et al., 2003). An increased secretion and/or activity of MMP-9 has been demonstrated in BALF collected from humans with asthma (Atkinson and Senior, 2003), from cats with allergen-induced asthma (Kirschvink et al., 2007a), and from dogs with eosinophilic bronchopneumopathy (Rajamaki et al., 2002).

We investigated our Cd model (1) on airway morphology using Rx and BAL; (2) on pulmonary function using BWBP and a bronchoprovocation test; (3) on the local inflammatory response by analysing the BALF and determining the concentrations of MMP-2 and -9, and (4) on the systemic inflammatory response using haematology.

β_2-adrenergic receptors play an important role in the pulmonary function. Indeed, they have an important part in the regulation of the tonicity of the airways in association with the muscarinic cholinergic system (Johnson, 2006) and the non-adrenergic non-cholinergic system (Widdicombe, 1998). In veterinary medicine, β_2-adrenergic agonists are used to treat numerous airway diseases, such as asthma in cats (Kirschvink et al., 2005b), chronic bronchitis (McKiernan, 2000) or idiopathic eosinophilic bronchopneumopathy in dogs (Clercx et al., 2000) and have proven to be beneficial. Glucocorticoid administration, orally or by nebulisation, has been recommended to treat inflammatory bronchitis in dogs (Corcoran et al., 1999; Bexfield et al., 2006), although their use is still largely empirical (Schimmer and Parker, 2006).

The current study investigated the effects of administration of a β_2-adrenergic agonist (salbutamol) or a glucocorticoid (prednisolone), both of which were evaluated by the clinical, functional and morphological parameters measured in the present model of airway inflammation.

Materials and methods

Animals

Seven Beagle dogs (6 males, 1 female), with no history of tobacco exposure or respiratory disease, weighing 17.4 ± 3.2 kg (mean ± SEM), aged 2–12 years, were used in this study. The experiment protocol was approved by the Ethics Committee for Animal Well Being of the University of Liège.

Study design

The study was divided in two major parts. In the first part, we characterised our model of subclinical and reversible bronchial inflammation induced by Cd inhalation. All of the tests were therefore performed on healthy and non-exposed animals (protocol H). Then we evaluated the effect of Cd inhalation during a 4 week period (protocol Cd), including measurements performed 1 day and 1, 2, 3 and 4 weeks after Cd inhalation (Cd-D1, Cd-W1, Cd-W2, Cd-W3 and Cd-W4, respectively). In the second part of the study, we examined the effect of salbutamol (protocol S) or prednisolone (protocol P) on the bronchitis induced by the previous protocol. Salbutamol (0.1 mg/kg) (Ventolin injectable, GlaxoSmithKline) or prednisolone (0.5 mg/kg) (Prednisolone 2.5%, VMD) was injected intramuscularly (IM) 6 h after Cd inhalation. On the following day, salbutamol (0.1 mg/kg) or prednisolone succinate (10 mg/kg) (Solu-Delta-Cortef 50 mg/mL, Pharmacia & Upjohn) was injected intravenously (IV) into sedated dogs after basal recording of the different BWBP parameters and before the start the bronchoprovocative test. All these protocols were performed at 4 week intervals.

Tests that were performed at different stages during the different protocols are summarised in Table 1 and are described below.

Cadmium exposure

For Cd administration, dogs were sedated with acepromazine (0.03 mg/kg) and buprenorphine 0.015 mg/kg (Temgesic, Schering-Plough) and placed in the main chamber of the BWBP box. A 0.2% Cd solution was nebulised in this chamber by an ultrasonic nebuliser (Aerosol Micron Sonic Home, International Medical Pharmaceutical Equipment Company) for 15 min.