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The anti-inflammatory effects of IV administered clenbuterol in horses with recurrent airway obstruction

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Abstract

Cyclic AMP elevating agents have been shown to exhibit anti-inflammatory properties in addition to functions such as bronchodilation. The aim of this study was to investigate this dual action of clenbuterol (CB; Ventipulmin) on horses affected with recurrent airway obstruction (RAO). Seven RAO susceptible horses received inhalation challenges with aerosolised lipopolysaccharide (LPS), hay dust suspension (HDS) and *Aspergillus fumigatus* antigen (AF) with and without prior treatment with intravenous CB. Data showed that CB exerted significant beneficial effects on lung function, total cell count (TCC) and bronchoalveolar lavage neutrophil influx. In addition, CB significantly decreased the expression of several pro-inflammatory cytokines and chemokines in the alveolar macrophages of RAO-susceptible horses after challenge with LPS and HDS, and increased the expression of interleukin-6, known to act as a pro-and anti-inflammatory cytokine, following different challenges. This anti-inflammatory activity of CB is of additive value to its currently recognised use in equine RAO.

Keywords: RAO; Horse; Clenbuterol; Anti-inflammatory; Alveolar macrophage

1. Introduction

Recurrent airway obstruction (RAO) is a commonly occurring respiratory disease in mature horses and is predominantly observed in the northern hemisphere (Robinson et al., 1996). Affected horses are susceptible to inhaled antigens and other pro-inflammatory agents, including fungal spores (McGorum et al., 1993a; McPherson et al., 1979) and dust-derived endotoxin (Pirie et al., 2001, 2003).

The inflammation that develops during exacerbation of the disease has been linked to a Th cell 2 pathway (Lavoie et al., 2001), as well as to a more primary inflammatory response involving, amongst others, alveolar macrophages (AM) (Franchini et al., 1998) and is typically characterised by an influx of neutrophils into the airways (Dixon et al., 1995; Robinson et al., 1996).

Clenbuterol (CB; Ventipulmin), an adrenergic receptor agonist with high β_2-receptor selectivity, is frequently used in equine practice both for its bronchodilating effects and for the stimulation of the mucociliary transport system. Optimal efficacy is achieved by removing horses from dusty environments during treatment and, in some cases, using a combination therapy with corticosteroids (Lavoie, 2003).

β_2-Adrenergic receptors are not only restricted to airway smooth muscles cells but are also found on other

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resident and infiltrating cells (Johnson, 2002). Evidence has emerged from both in vitro and in vivo studies that stimulation of AR's by catecholamines not only leads to relaxation of smooth muscle tissue but will also modulate the inflammatory reaction (Madden et al., 1995). Due to this dual action of adrenergic agonists, interest has increased regarding the potential anti-inflammatory properties of β-agonists frequently used in the treatment of respiratory disease. The ability to modulate the inflammatory reaction has been associated with an inhibition of the phosphorylation and subsequently delayed release of inhibitor κB (IκB) from the transcription factor nuclear factor (NF)κB, (Farmer and Pugin, 2000; Ye, 2000) thus preventing its translocation to the nucleus. Subsequently the transcription of cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-8 and IL-10 is reduced or delayed as well.

Alveolar macrophages (AM) not only play an important role in recognizing and eliminating foreign particles from the lung but will also initiate an inflammatory response, and also play a part in the initiation of the adaptive immunity (Strieter et al., 2003). It has even been suggested that AM will drive the allergic phenotype in the occurrence of human asthma (Alexis et al., 2001). Although only a few reports focus on the role of the alveolar macrophage in equine RAO they are implicated in the initial stage of the disease (Franchini et al., 1998). Therefore, modulating this macrophage driven pro-inflammatory response may be beneficial in the therapeutic management of equine RAO. Hence, the aim of this study was to investigate the effects of intravenously (IV) administered pre-treatment with CB, given prior to a challenge, on bronchoalveolar lavage (BAL) fluid cytology, total cell counts (TCC) and cytokine production by AM isolated from RAO susceptible horses. The challenges consisted of lipopolysaccharide (LPS), hay dust suspension (HDS) and *Aspergillus fumigatus* antigen (AF), all known to be involved in the aetiopathogenesis of RAO.

2. Materials and methods

2.1. Statement of animal care

The Committee on Ethical Considerations in Animal Experiments of the Faculty of Veterinary Science of the University of Utrecht, The Netherlands, approved all experiments under project number 0301.0602.

2.2. Animals

Seven adult Dutch warm blood horses, six mares and one gelding (mean body weight 629 kg, range 603–700 kg, age 7–10 years) were used in this study. Classification of the horses was based on anamnesis, complete clinical examination, and a natural challenge (NC) experiment. Horses were stabled for 24 h in a dusty environment and fed mouldy hay following which the neutrophil ratio in the BAL fluid (BALF) was ascertained. Horses were classified as RAO-susceptible when the BALF neutrophil ratio exceeded 20%. These criteria used for the classification of horses were in accordance with the consensus-report for experimental studies in heaves by Robinson (2001).

Compared with healthy controls, the RAO susceptible horses used in this study previously showed significant differences in pleural pressure, BALF-cytology and expression of AM-derived cytokine mRNA after inhalation challenges with hay dust solution and endotoxin as described elsewhere (Laan et al., submitted). Prior to the initiation of the experiments, horses were stabled on shavings and fed silage and pelleted feed, were vaccinated, received routine anthelmintic treatment and were accustomed to wearing the facemask subsequently used for the challenge procedures. Throughout the study, the horses were individually stabled but shared a common airspace. Horses were evaluated clinically and, when necessary, endoscopically for upper airway disease prior to each challenge. The minimal time between separate challenges was at least two weeks.

2.3. Challenge solutions

Saline (0.9%): 2.5 mL served as control.

Lipopolysaccharide: 2000 μg *Escherichia coli* derived LPS (O111:B4, Sigma–Aldrich) as earlier described by Pirie et al. (2001).

Hay dust suspension: The HDS used in this study was a mixture of the hay dust suspensions 1 and 3 (ratio 1:1), previously described by Pirie et al. (2002a,b,c). One millilitre of the challenge substance was delivered to the inhalation mask.

Aspergillus fumigatus: The AF antigen, 200 μg, was supplied by Greer Laboratories (Lot number My3-79). The applied dose was derived from experiments conducted by Pirie et al. (2001) assessing the effect of inhalation challenges with different mould antigens in RAO susceptible horses.

All challenge solutions were diluted to a final volume of 2.5 mL with sterile saline prior the application.

2.4. Challenges

All horses received an inhalation challenge with saline, LPS, HDS or AF antigen following three days pre-treatment with either CB (Ventipulmin, Boehringer Ingelheim, 0.75 μg/kg IV, b.i.d.) or with isotonic saline (10 mL NaCl IV b.i.d.) as a placebo in a randomised crossover design. With the last treatment administered 1–2.5 h before the challenge. The aerosol challenge was