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Effect of antigen challenge on dynamics of CD62P and CD41/61 expression on platelets in horses with recurrent airway obstruction (RAO)

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ABSTRACT

Recurrent airway obstruction (RAO), also known as heaves, is an allergic respiratory condition that develops in horses following an exposure to aeroallergens in hay and straw. This is manifested by airway hyperreactivity, inflammation, bronchoconstriction, as well as a leukocyte and platelet infiltration into the airways. Platelet activation and an increase in circulating platelet-leukocyte aggregates may lead to airway remodeling. The aim of this study was to explore the effect of seven-day antigen challenge on dynamics of platelet indices and CD41/61 and CD62 P expression on platelets in horses with RAO. Ten RAO-affected horses and ten healthy horses were included in this study. All horses were exposed to 7 days hay and straw challenge. Blood samples were collected prior to the challenge (Pre-challenge) and 1, 2, 3, 7 and 14 days after the initiating the antigen challenge. Blood samples were obtained to determine the platelet count (PLT), mean platelet volume (MPV) and platelet large cell ratio (P-LCR). Expression of CD62 P and CD41/61 was detected by flow cytometry on activated platelets. Antigen challenge resulted in a significant gradual decrease of PLT in RAO horses, but not in controls. MPV and P-LCR in control and RAO-affected horses remained unchanged after antigen challenge. The expression of CD62 P and CD41/61 in RAO horses was significantly higher compared to control horses. The antigen challenge resulted in an increase expression of CD62 P and CD41/61 on the platelets of RAO-affected horses, while did not lead to significant changes in the control group. An increased expression of CD62 P and CD41/61 indicates platelet activation what may contribute to the formation of platelet aggregates in their respiratory system.

1. Introduction

Recurrent airway obstruction (RAO), also known as heaves, is an allergic respiratory condition that develops in horses following an exposure to aeroallergens in hay and straw (Leguillette, 2003; Moran and Folch, 2011). This is manifested by airway hyperreactivity, inflammation, bronchoconstriction, as well as a polymorphonuclear neutrophil (PMN) and platelet infiltration into the airways (Moran and Folch, 2011). Recruitment of PMNs and platelets into the lung is a key event in the development of the chronic inflammatory airway disease, which may lead to the remodeling of the airway wall (Cunningham and Dunkel, 2008; Dunkel et al., 2007; Lavoie-Lamoureux et al., 2014).

Although platelets lack a nucleus, they are fully functional cells. They have many important functions apart from their well-known role in haemostasis (Herd and Page, 1994). Platelets behave as classical inflammatory cells, contain and release a number of growth factors which can play an important role in airway remodeling, express adhesion and aggregation molecules on their surface, and become activated in response to mediators released by other cells (Herd and Page, 1994; Kornerup and Page, 2007; Pitchford et al., 2004). Platelet activation is associated with an increased expression of cell surface markers such as CD41/61 and CD62 P (P-selectin). These receptors play an essential role in the adhesion, aggregation and platelet-neutrophil interactions. It has been shown that activated platelets contribute to the leukocyte recruitment into the lungs (Brooks et al., 2007; Dunkel et al., 2009; Lalko et al., 2003; Pitchford et al., 2004, 2005, Weiss et al., 1999).

Zarbock et al. (2006) have demonstrated that CD41/61 receptor play important role in platelet-endothelial interactions and P-selectin is responsible for the platelet-neutrophil aggregate formation. Platelet

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activation, an increased amount of circulating platelet-leukocyte aggregates and their infiltration in the alveolar compartments have been detected in patients with asthma and in animal models of allergic airway disease (Dunkel et al., 2007, 2009, Lellouch-Tubiana et al., 1985; Pitchford et al., 2003, 2004, 2005, 2008, Yoshida et al., 2002; Zarbock et al., 2006). The presence of activated platelets in the circulation of horses with symptomatic RAO has also been reported (Ablett et al., 1997; Iwaszko-Simonik et al., 2015). Furthermore, an increased amount of neutrophil and platelet aggregates in the bronchoalveolar lavage fluid (BALF) from RAO-affected horses has been demonstrated (Fairbairn et al., 1993a,b). Clinical remission of this disease is induced by reducing the exposure of the animal to the antigen or by administering corticosteroids (Dauvillier et al., 2011; Munoz et al., 2015). Some authors have shown that blood and airway neutrophils are activated in horses with RAO even during a remission (Tremblay et al., 1993; Marr et al., 1997). Those findings suggest that platelets may also contribute to the pathogenesis of allergic respiratory diseases, but their role, function and molecular mechanisms are poorly understood. In view of the aforementioned facts, we hypothesized that an increased platelet expression of CD62 P and CD41/61 may play a role in platelet-leukocyte interactions and development of inflammatory events in the lungs.

The activation of neutrophils and their contribution to the pathogenesis of RAO has been extensively investigated (Dunkel et al., 2007, 2009, 2010, Fairbairn et al., 1993a,b, Niedzwiedz et al., 2014). The activation of circulating platelets in RAO-affected horses shortly after an antigen challenge has also been analyzed (Iwaszko-Simonik et al., 2015). To date, the function of platelets in horses with RAO following long time exposure to moldy hay and straw has not been investigated. Thus, the aim of this study was to explore the effect of an antigen challenge on the dynamics of the platelet indices and the CD41/61 and CD62 P expression on platelets in horses with RAO.

2. Materials and methods

2.1. Animals

The study group included ten warmblood, mixed breed horses of both sexes (geldings n = 4, stallions n = 3 and mares n = 3) with a history of RAO. The horses were from 5 to 15 years old (mean 8.5 ± 2.9 years) and had a mean body weight of 523 ± 37 kg.

Ten healthy Polish half-bred horses (geldings n = 4, stallions n = 3 and mares n = 3), with a mean age of 7.5 ± 2.1 years (range 5–12 years) and a mean body weight of 489 ± 32 kg were used as controls (C). The animals were pasture-kept and had access to water ad libitum. All the horses were regularly dewormed and vaccinated against influenza and tetanus. The control horses were included in the study with the owner's consent.

All the horses were allocated to the groups based on their history and a thorough clinical examination, including a blood gas analysis, hematology, screening biochemistry, endoscopy of the airways and the bronchoalveolar lavage fluid (BALF) cytology and a clinical RAO score. All RAO horses were judged to be asymptomatic prior to the start of the challenge. All RAO horses showed no clinical signs of RAO prior to the start of the challenge. None of the horses received any medical treatment during the 8 weeks preceding the study and during its course.

2.2. Experimental protocol

The timeline of the study is outlined in Fig. 1. All horses were considered by their owner to be in remission and had been housed on pasture or in a dust-free environment prior to the start of the study. An acute crisis of RAO in susceptible animals, was induced by placing the horses in a poorly ventilated stable, bedding them on straw and feeding them hay with a visible mold growth. The bedding was shaken right in front of the horses twice daily to encourage the dispersal of antigens. All the horses in both, control and study groups were exposed to the same environmental protocol, to achieve similar dusty condition. The response to the environmental exposure was confirmed based on a clinical examination and additional testing, including an endoscopic examination, BALF cytology, arterial blood gas analysis and venous blood sampling. The clinical RAO score, assessing the condition of the respiratory system based on a cough score, nostril flare and abdominal lift (Robinson, 2001, S1 Table]. To be classified as healthy control, the horses needed to have less than 10% of neutrophils in the BALF. RAO-affected horses were classified as being in crisis if they had > 50% neutrophils in a differential cell count following the environmental exposure.

Following the exposure, all horses were kept in uniform environmental conditions, optimal for horses with respiratory disease. At that time, they were pasture-kept or remained in the stable, and wood shavings were used as bedding (Allspan, Allspan GmbH, Karlsruhe, Germany). All the horses were fed a complete horse feed (EMH Heu Cubes, Eggersmann, Rinteln, Germany) with an addition of muesli (EMH Kräuter Müsli, Eggersmann, Rinteln, Germany). All the animals were evaluated using identical diagnostic procedures at baseline and on the seventh day of the antigen challenge (Fig. 1).

Blood samples were obtained in the morning at intervals prior to the challenge (Pre-challenge), and on days 1, 2, 3 and 7 of the antigen challenge (Fig. 1). Further samples were taken 14 days after initiating the challenge.

2.3. Endoscopic examination and bronchoalveolar lavage fluid collection and cytology

Endoscopy of the airways and the bronchoalveolar lavage were performed in horses sedated with 0.01 mg/kg of detomidine (Domosedan, Orion Corporation, Espoo, Finland) and 0.01 mg/kg of butorphanol (Morphasol, aniMedica GmBH, Senden-Bösensell, Germany). A 1.8 m long endoscope was passed through the nasal passage into the trachea (Karl Storz GmbH, Tuttlingen, Germany). Changes in the airways were graded by two clinicians using a modified RAO staging scale, previously described by Tilley et al. (2012). The bronchoalveolar lavage was carried out by instilling 400 ml of sterile saline (0.9% NaCl) at body temperature through the endoscope working channel into the bronchus using successive 60 ml boluses. The BALF (bronchoalveolar lavage fluid) was then re-aspirated through gentle suction using a 60 ml syringe until no further fluid could be obtained. The amount of recovered fluid was recorded and the BALF for each individual horse was pooled in a sterile specimen cup, placed on ice and processed within 2 h after collection. In order to carry out the cytologic examination of the BALF, a 10 mL aliquot was centrifuged at 300 *g* for 10 min using a centrifuge (Beckman Coulter Allegra x-22; Beckman Coulter Inc., CA, Brea, USA), and the smear of the sediment was stained with Wright's stain. A 400-cell leukocyte differential count was performed. Epithelial cells were not included in the differential count.

2.4. Arterial blood gas analysis

Arterial blood was collected anaerobically into heparinized syringes through an arterial puncture of the facial artery using an 18 G butterfly needle. The blood was immediately analyzed for the partial pressure of oxygen and carbon dioxide (PaO_2 and $PaCO_2$), with the use of an OPTI CCA-TS (OPTI Medical Systems, Inc., Roswell, GA, USA) blood gas analyzer. Horses were identified as healthy if their $PaO_2 \geq 90$ mmHg, while those with a $PaO_2 \leq 85$ mmHg were considered to have a relapse of RAO (Stopyra, 2002; Stopyra et al., 2012).

2.5. Blood samples and platelet preparation

Blood samples were obtained from each horse by direct jugular venipuncture with a 19 G needle and vacuum tubes (S-Monovette; Sarstedt, Numbrecht, Germany). Samples were collected into a tube