



# Intrabronchial instillation of platelet-rich plasma in equines with inflammatory airway disease – preliminary report

*Instilação intrabronquial de plasma rico em plaquetas em equinos com doença inflamatória das vias aéreas – relato preliminar*

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## Resumo

Pulmonary inflammatory diseases are common among athletic horses. Treatments include environment control, corticosteroids and bronchodilators, and, in some cases, they may be palliative. Cell therapy plays a potential role in treating many horse injuries, but it has not yet been indicated for equine pulmonary inflammation. This study hypothesis was that intrabronchially instilled platelet-rich plasma (PRP) can benefit horses with pulmonary inflammation. 10 adult horses of different breeds and engaged in various activities, with spontaneous cough, were examined. Physical examination, endoscopic examination, and bronchoalveolar lavage (BAL) fluid cytology were conducted as study methods. A solution (20 mL) containing autologous PRP diluted in platelet-poor plasma (PPP) was instilled in the right and the left main bronchus through an endoscopically guided procedure. The animals were reevaluated seven days after the treatment. As a result, physical examination and BAL fluid cytology identified RAO, IAD and a normal BAL fluid cytological profile in two, five and three horses, respectively. However, none of the horses had cough, and tracheal mucus grade decreased ( $p = 0.051$ ) seven days after the treatment. The IAD horses showed significant improvement, indicated by a comparison of the mucus grade and BAL fluid neutrophil amount before and seven days after the treatment ( $2.4 \pm 1.1$  vs.  $1.4 \pm 0.5$ ,  $p = 0.034$  and  $13.0 \pm 5.0$  vs.  $5.0 \pm 3.3$ ,  $p = 0.014$ , respectively). Intrabronchial PRP instillation was beneficial for horses with IAD and may represent a novel therapy for athletic horses with pulmonary inflammation, further studies being necessary.

**Palavras-chave:** PRP. Pulmonary inflammation. Horse. Neutrophils.

## Abstract

As doenças pulmonares inflamatórias são comuns em cavalos desportistas. Os tratamentos incluem controle ambiental, corticosteroides e broncodilatadores, sendo, muitas vezes, paliativos. A terapia celular possui importante papel no tratamento de diversas afecções dos cavalos, mas, até o momento, não foi indicada para a inflamação pulmonar. Este estudo levantou a hipótese de que o plasma rico em plaquetas (PRP) instilado intrabronquialmente pode beneficiar cavalos com inflamação pulmonar. Examinou-se 10 cavalos adultos, de diferentes raças e envolvidos em atividades variadas, os quais foram avaliados por apresentarem tosse persistente. Foram realizados exame físico, avaliação endoscópica e citologia do lavado broncoalveolar (LBA). Instilou-se, nos brônquios principais direito e esquerdo, 20 mL de uma solução contendo PRP autólogo, diluído em plasma pobre em plaquetas, procedimento guiado por endoscopia. Os animais foram reavaliados sete dias após o tratamento. O exame físico e a citologia de LBA identificaram dois, cinco e três cavalos, com diagnósticos de obstrução recorrente (ORVA), doença inflamatória das vias aéreas (DIVA) e normal, respectivamente. Sete dias após o tratamento, a tosse havia cessado em todos os cavalos, bem como os graus de muco tinham diminuído ( $p = 0,051$ ). Os cavalos com DIVA tiveram melhora significativa, indicada pela redução nas quantidades de muco traqueal e de neutrófilos no LBA, sete dias após o tratamento ( $2,4 \pm 1,1$  vs.  $1,4 \pm 0,5$ ,  $p = 0,034$  e  $13,0 \pm 5,0$  vs.  $5,0 \pm 3,3$ ,  $p = 0,014$ , respectivamente). A instilação intrabronquial de PRP foi benéfica para cavalos com DIVA, representando uma nova possibilidade terapêutica para cavalos desportistas com inflamação pulmonar, sendo necessários novos estudos.

**Keywords:** PRP. Inflamação pulmonar. Cavalo. Neutrofilia.

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## Introduction

Airway diseases represent the second most common ailments at equine clinics, affecting the welfare of horses. Of these diseases, recurrent airway obstruction (RAO), characterized by marked lower airway inflammation in mature horses, and inflammatory airway disease (IAD), a nonseptic pulmonary inflammation affecting athletic horses, are the most commonly diagnosed (1). Racehorses can present IAD as early as the initial period of race training (2).

Cough is a common clinical sign in horses with pulmonary inflammation. Usually, corticosteroids and bronchodilators abate both clinical signs and pulmonary neutrophilia (1).

Stem cell therapy has been indicated for pulmonary inflammation in other species (3). However, anti-inflammatory properties of platelet-rich plasma (PRP) in the airways remain to be elucidated. Considering the anti-inflammatory and healing effects of autologous PRP in horses and humans with soft-tissue lesions, we assessed the hypothesis that intrabronchially instilled PRP could benefit horses with pulmonary inflammation.

## Materials and methods

### Subjects

This study included 10 horses (six horses and four mares; average age:  $9.4 \pm 4.7$  years) of different breeds – one Crioulo, three Quarter-horses, two Thoroughbreds and four of a mixed breed –, all of them examined in another study (data not shown), investigating spontaneous cough in horses in the region of Curitiba, PR - Brazil. The horses were maintained in a conventional stall, provided with pelleted food and grass hay, and regularly dewormed and vaccinated. The study occurred over a period of two months, during which the horses with spontaneous cough were identified, independently examined and promptly treated. Clinical and endoscopic evaluation and bronchoalveolar lavage (BAL) fluid collection were immediately followed by intrabronchial instillation of PRP. The same examinations were conducted seven days after the treatment.

This study was approved by the Committee on Animal Experimentation of the Pontifícia Universidade Católica of Paraná (PUCPR), in Curitiba,

PR - Brazil. This study was assigned under the registration number 614 and it was performed in accordance to the Guiding Principles for the Care and Use of Animals.

### BAL fluid collection and analysis

The animals were sedated with acepromazine (Acepran 1%, Univet Laboratory, São Paulo, Brazil: 0.03 mg/kg IM), followed by xylazine (Sedazine, Fort Dodge Laboratory, São Paulo, Brazil: 0.3 mg/kg IV) and butorphanol (Torbugesic, Fort Dodge Laboratory, São Paulo, Brazil: 0.05 mg/kg IV), for endoscopic evaluation (Olympus CF colonoscope, Center Valley, PA, USA) and BAL fluid collection. Tracheal mucus was scored from 0 to 5. BAL fluid was obtained using a bronchoalveolar lavage catheter (Cook Vet Products (V-PBAL-300), Hamburg, Germany), with the instillation of 300 mL of sterile and prewarmed saline. The recovered BAL fluid was processed and analyzed within 30 minutes, as previously described (2).

### PRP collection and instillation

Two hundred milliliters of blood was withdrawn from each animal by jugular puncture and immediately transferred into 50-mL centrifuge tubes containing a solution of citrate phosphate dextrose adenine (CPDA-1, JP 90 Pharmaceutical Industry SA, Brazil: 3.2%). Blood was centrifuged at 210 g for 10 min, at 22 °C. After centrifugation, 50% of the plasma near the buffy coat was separated and centrifuged at 300 g for 10 min, at 22 °C. Twenty milliliters of PRP, containing approximately five times the number of platelets in blood, was diluted in 20 mL of platelet-poor plasma (PPP). Thereafter, 20 mL of the final suspension, containing, on average, twice the concentration of blood platelets, was instilled in both the right and the left main bronchus through an endoscopically guided procedure.

### Statistical analysis

Data were analyzed using paired student's *t*-test (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA, USA) and all values presented as mean  $\pm$  SD.  $p < 0.05$  was considered significant.

## Results and discussion

The present study showed the possibility of a new therapeutic modality for equines with pulmonary inflammation, specifically IAD horses, by intrabronchial instillation of PRP.

Although the use of intrabronchial route for equine medication is not a common practice, in the present study, 20 mL of autologous plasma was instilled in both the right and the left main bronchus via an endoscopically guided procedure. In a previous study (4), 20 mL of concentrated equine serum was also administered to horses, through an invasive procedure of intratracheal injection into the airways; this procedure required elevation of the horses' head for 10 min to allow a gravity-dependent flow. Conversely, the method applied in the present study was noninvasive and well tolerated by the horses.

All the horses examined in this study received treatment with intrabronchial instillation of PRP after initial BAL fluid collection, regardless of the specific pulmonary condition diagnosed on the basis of BAL fluid cytology.

According to BAL fluid differential cytology, three horses showed a normal cytological profile (horses 5, 6, 7), two showed RAO (horses 9 and 10) and five showed IAD (horses 1, 2, 3, 4, 8) (Table 1).

Moreover, the tracheal mucus grade decreased seven days after PRP instillation ( $p = 0.051$ ).

However, while an increase in the percentual count of macrophages ( $35.7 \pm 12.3$  vs.  $43.4 \pm 13.0$ ,  $p = 0.11$ ) and a decrease in neutrophil count ( $19.1 \pm 25.4$  vs.  $14.3 \pm 23.1$ ,  $p = 0.93$ ) was observed in the differential cytology of BAL fluid, no significant benefit of the proposed treatment was observed when the data of all the horses were analyzed together. Therefore, to determine whether the intrabronchial PRP plays

**Table 1** - Endoscopic observation of tracheal mucus (graded 0-5) and bronchoalveolar lavage fluid cytology for 10 horses, before (b) and seven days after (a) the intrabronchial instillation of a platelet-rich plasma suspension containing approximately twice the blood platelet concentration.

(TNCC – total nucleated cell count; Macroph – macrophages; Lymph – lymphocytes; Eosinoph – eosinophils; Mast – Mast Cells; Neutroph – neutrophils; Hemosid – hemosiderophages.)

Horses	Tracheal mucus (0-5)	TNCC (cells- $\mu$ L)	Macroph %	Lymph %	Eosinoph %	Mast %	Neutroph %	Hemosid %
1b	4	87.5	46.4	41.6	4.0	0.0	8.0	0
1a	2	46.9	56.0	42.0	0.5	0.0	1.5	0
2b	2	47.1	32.6	47.6	0.3	0.0	19.5	0
2a	1	33.0	36.7	59.0	0.3	0.0	4.0	0
3b	1	45.5	9.0	7.0	0.0	0.0	8.3	75.5
3a	1	19.6	63.39	21.3	0.0	0.0	3.3	12.0
4b	3	180.0	32.0	36.7	0.3	0.0	15.7	15.3
4a	2	174.2	45.7	36.7	1.6	0.0	10.0	6.0
5b	2	92.8	30.0	66.7	0	0.0	0.3	3.0
5a	1	152.0	52.3	35.7	0.4	0.0	10.3	1.3
6b	1	95.0	41.3	50.4	0.3	0.0	0	8.0
6a	2	48.0	57.7	36.7	1.0	0.0	4.6	0
7b	3	33.5	41.7	57.7	0.3	0.3	0	0
7a	3	22.5	43.0	40.1	2.3	2.3	12.3	0
8b	2	100.0	50.0	36.7	0	0.0	13.3	0
8a	1	38.9	46.7	47.0	0	0.0	6.3	0
9b	4	241.1	12.3	14.7	0	0.0	73.0	0
9a	3	384.6	14.0	10.0	1.0	0.0	75.0	0
10b	5	318.3	23.0	19.0	1.0	0.0	57.0	0
10a	5	334.2	24.5	20.0	0.5	0.0	55.0	0

Source: Research data.

a beneficial role in inflammation in the airways of horses, we grouped the horses according to their specific respiratory condition.

Thus, RAO and normal horses did not benefit from PRP treatment in the present preliminary study, but the horses with IAD, in accordance to the American College of Veterinary Internal Medicine consensus statement (1), showed a significant improvement of the clinical and cytological parameters seven days after intrabronchial PRP instillation. These horses did not present cough, and their tracheal mucus grade was significantly reduced ( $2.4 \pm 1.1$  vs.  $1.4 \pm 0.5$ ,  $p = 0.034$ ). This decrease in the tracheal mucus grade possibly justifies a potential role of intrabronchial PRP in IAD, since an increased amount of tracheal mucus impairs pulmonary function and athletic capacity of racehorses (5), show jumpers and dressage horses (6).

Additionally, the relative neutrophil count was found to be reduced in the BAL fluid of IAD horses seven days after PRP intrabronchial instillation ( $13.0 \pm 5.0$  vs.  $5.0 \pm 3.3$ ,  $p = 0.014$ ). Therefore, these findings show an anti-inflammatory effect of intrabronchially instilled PRP in horses with IAD. However, this effect may be exerted via paracrine mechanism and not through cell contact mechanism, as described for the beneficial effect of the intrapulmonary-delivered bone marrow-derived mesenchymal stem cells, in acute lung injury in mice (3).

Additionally, although only two horses were found to have RAO in our study, an accurate diagnosis of IAD before the initiation of a treatment appears to be crucial, because the condition of these horses did not improve. Moreover, horses with normal BAL fluid cytology showed an increase in neutrophil count after the treatment (Table 1).

The reasons for the success of PRP treatment in the IAD horses and for the failure of improvement of pulmonary inflammation in the RAO horses, in the present study, may be the differences in the immunologic response underlying both the conditions. Neutrophilia in the BAL fluid is a common marker of both the diseases, although it is more pronounced in RAO. Neutrophilia in the BAL fluid of IAD horses was characterized by the overexpression of interleukin 1 beta (IL-1 $\beta$ ) in BAL cells (7). However, such an event was not important for the occurrence of RAO (8). Additionally, an increase in the gene expression of pro-inflammatory cytokines such as interleukin 1 beta (IL-1 $\beta$ ), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), IL-23, IL-4, and interferon  $\gamma$  (IFN- $\gamma$ ) in the cells of the BAL fluid

of IAD horses was observed. This finding suggests an up-regulation of the innate immunity in the airways of these horses, probably in response to inhaled antigens (7, 9). The beneficial effects of PRP on soft-tissue injuries are observed only if PRP is used during the acute phase of the lesion, because activation of the platelets normally occurs during the initial inflammatory phase, after which the activated platelets initiate the healing process. In a cell model study that used immortalized human articular chondrocytes, IL-1 $\beta$  and TNF- $\alpha$  induced chondrocyte degeneration and PRP inhibited this effect and rescued the chondrogenic-specific gene expressions (10). Thus, due to a lack of report on the role of PRP in airway inflammation, we assumed that the beneficial role of PRP in pulmonary inflammation in the IAD horses included IL-1 $\beta$  and TNF- $\alpha$  inhibition.

## Conclusion

In the present study, intrabronchial instillation of PRP proved to be benefic to pulmonary inflammation treatments in equines with IAD, showing the necessity of further investigations of PRP effects and the underlying mechanisms.

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