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Increased adenosine concentration in bronchoalveolar lavage fluid of horses with lower airway inflammation

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Abstract

Several reports have suggested a role for adenosine in the pathogenesis of chronic airway conditions and this has led to new therapeutic strategies to limit airway inflammation. In this study, detectable levels of adenosine in bronchoalveolar lavage (BAL) samples from 11 horses with non-infectious lower-airway inflammation and 14 healthy controls are reported, with significantly higher values in horses with airway inflammation. Although these increased levels did not correlate with changes in neutrophil percentage in BAL, a positive association between adenosine levels and signs of lower airway inflammation (clinical score) was observed. These novel findings support the hypothesis that adenosine may contribute to bronchoconstriction and also act as a pro-inflammatory mediator in the bronchoalveolar milieu of horses with airway inflammation. Further investigation of this axis could lead to new approaches for the treatment of highly prevalent lower airway inflammatory conditions in the horse.

Keywords

Horse; Airway inflammation; Adenosine; Bronchoalveolar lavage

There is an increasing amount of information available about the role of adenosine signalling in airway inflammation. This endogenous nucleoside is a metabolic by-product that accumulates in bronchoalveolar lavage (BAL) in patients with asthma (Driver et al., 1993). Administration of adenosine by inhalation induces bronchoconstriction of asthmatic (but not of normal) airways. Recently, adenosine 5'-monophosphate has been shown to have a similar effect on cats affected with chronic airway disease (Hirt et al., 2011). In addition, adenosine modulates the course of inflammation through the release of cytokines and chemokines from various cell types in the airways (Caruso et al., 2009). Therefore,

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Conflict of interest statement

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strategies based on adenosine receptor ligands are currently being developed aimed at controlling adenosine-mediated inflammatory signalling (Dip, 2009).

Persistent inflammation is a central feature of chronic non-infectious airway conditions, such as recurrent airway obstruction (RAO) and inflammatory airway disease (IAD). RAO is characterised by bronchospasm, mucus secretion, airway wall thickening and deterioration of lung function, and affects older individuals. IAD presents clinical and cytological similarities with RAO, but usually affects younger horses and clinical signs are mild (Couetil et al., 2007).

In the current study, we wanted to determine whether increased adenosine levels are associated with lower airway inflammation in the horses. A total of 11 adult horses (three mares and eight geldings; age range 7–17 years, median 9.5 years) with mild to moderate signs of airway inflammation were included in the study. Lower airway inflammatory disease (either IAD or RAO) was diagnosed based on clinical signs, history, mucus (amount, quality and cellularity) and cytological examination of BAL samples. Horses medicated within 28 days prior to the examination and those with concomitant signs of infectious or systemic disease were excluded from the study. In addition, 14 healthy controls (four mares and 10 geldings; age range 5–22 years, median 11 years) with no history of airway inflammation or clinical signs of respiratory disease and normal BAL cytology were employed as a control group.

Each horse was clinically examined and given a weighted score, using a scale with range 0 to 25 as described by Tesarowski et al. (1996; see Appendix 1, Supplementary data). Two individuals performed all clinical observations. All healthy controls had a clinical scores of 2 or lower (range 0–2, median 0.5) while horses with airway inflammation had higher scores (range 0–8, median 4), corresponding to moderate clinical signs (horses with experimentally induced RAO had mean scores of approximately 12; Tesarowski et al., 1996).

The BAL and cytological analysis was performed as previously described (Zhang et al., 2011). In addition, BAL-samples were collected in vials containing 0.1 μ M iodotubercidin and 10 μ M of erythro-9-(2-hydroxy-3-nonyl)adenine (Sigma), centrifuged, and cell-free fractions immediately frozen. All samples were analysed by a specific spectrometry-based assay at a later time point (Ren et al., 2008). Briefly, a Zorbax eclipse XBD-C-18 column (Agilent) was used with a TSQ Quantum-Ultra system and the HSE source (Thermo Electron). The mobile phase consisted of linear gradient changes of 0.1% formic acid in water and 0.1% formic acid in methanol. The use of dilutional markers has been investigated in the horse, but remains a matter of controversy (Kirschvink et al., 2001). To avoid dilutional artefact, urea concentrations were determined (QuantiChrom assay, BioAssay Systems) in both in BAL and serum samples that had been collected simultaneously. The ratio between urea concentrations served as an indicator of epithelial lining fluid dilution and was employed for normalisation (corrected adenosine concentration).

The BAL cytology, clinical score, and uncorrected and corrected adenosine concentrations are summarised in Table 1. Interestingly, these data suggested a limited dilution effect on BAL samples when considered collectively, despite dilution differences of individual samples. Uncorrected adenosine values were employed for further analysis, following the recommendation of the European Respiratory Society that acellular BAL components should be expressed as concentration per volume recovered¹.

¹See: <http://www.ers-education.org/pages/default.aspx?id=335&idBrowse=37508&det=1>

Each BAL sample revealed adenosine levels above the limit of detection (LOD: 0.02 pg/ μ L BAL). Furthermore, adenosine concentration was significantly higher in horses with airway conditions (range 26.4–377.3 pg/ μ L, median 112.8 pg/ μ L), compared to healthy controls (range 17–191 pg/ μ L, median 43.6 pg/ μ L), which suggested a link between lower airway inflammation and adenosine in this species (Fig. 1).

The association between adenosine concentration in BAL and clinical findings was then assessed quantitatively, independently of disease state. Fig. 2A shows the significant correlation (Spearman) between adenosine concentration and clinical score. A similar correlation was observed when only horses with airway inflammation were considered.

Since the assessment of BAL cytological alterations (neutrophils relative counts) remains a major diagnostic tool for lower airway disease, the association between neutrophil percentage and adenosine concentration was investigated. In contrast to clinical score, a correlation between these two variables could not be established (Fig. 2B). Yet this observation should be interpreted with care because only three samples with neutrophil count indicative of moderate to severe airway inflammation (> 20%) were tested. Analysis of a larger set of samples with high percentage of neutrophils (i.e. acute RAO phase) would be required to clarify the relationship between these two parameters.

The presence of increased adenosine concentrations in horses with airway inflammation suggested that adenosine could represent a marker of inflammation. However, in view of the partial overlap in the values of both groups of horses analysed, assessment of adenosine levels in BAL did not seem to be suitable as the sole indicator of disease, but should be interpreted in combination with other diagnostic parameters (i.e. clinical signs).

Two initial reports suggested the presence of an adenosine-signalling axis in equine airways. One early study indicated that adenosine influences contractility in equine tracheal smooth muscle (Norris and Eyre, 1982). Our own research group then recently showed enhanced interleukin (IL)-6 transcriptional responses to adenosine receptor ligands in BAL cells from horses with lower airway inflammation (Zhang et al., 2011). The increased adenosine levels reported in the current study therefore supported the hypothesis that this nucleoside both contributes to bronchoconstriction and acts as a pro-inflammatory mediator in the bronchoalveolar milieu of horses with airway inflammation. Further characterisation of this inflammatory signalling axis could potentially lead to new approaches for the treatment of highly prevalent lower airway inflammatory conditions, such as RAO and IAD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix 1, Supplementary data

Supplementary data available in the on-line version of this article. See: Setters insert doi number here

Fig. 1. Uncorrected adenosine concentration in BAL fluid samples in horses with airway inflammation and controls. *: $P < 0.05$, compared to the control group (Mann-Whitney U -test).

Fig. 2.

(A) Positive correlation between adenosine concentration and clinical score for all 25 horses included in the study (Spearman r correlation coefficient = 0.36, P 0.05; slope: 0.018, P 0.05). A similar correlation is observed when considering only horses with airway inflammation (Spearman r correlation coefficient = 0.53, P 0.05). (B) Association between adenosine concentration and neutrophil count. No significant correlation was observed; (○), horses diagnosed with lower airway inflammation; (●), healthy controls.

Table 1

BAL cytology, clinical score and adenosine concentration from horses with airway inflammation (15–25) and healthy controls (1 – 14)

Horse	Sex	Age	Neutrophils (%) [RV 5%] <i>I</i>	Lymphocytes (%) [RV 30–50%]	Macrophages (%) [RV 40–60%]	Eosinophils (%) [RV 0.5%]	Basophils (%) [RV 2%]	Clinical score	Uncorrected adenosine concentration (pg/ μL)	Corrected adenosine concentration (pg/ μL)
1	M	10	1.3	46.6	52.1	0.0	0.0	0	40.5	51.1
2	F	10	1.3	45.9	52.1	0.7	0.0	1	43.1	67.9
3	M	17	1.9	52.2	45.4	0.0	0.5	0	46.9	25
4	M	5	5.7	56.3	36.8	0.0	1.2	1	108.6	140.1
5	F	15	6.1	69.3	24.2	0.0	0.4	1	52.9	32.8
6	M	5	0.8	48.9	46.8	0.0	3.5	0	38.7	64
7	F	8	2.1	41.6	55.2	0.0	1.1	0	44.1	41
8	M	16	3.4	62.4	33.3	0.0	0.9	1	29.0	20.9
9	F	9	1.9	46.9	48.3	0.5	2.4	0	127.7	237.5
10	M	5	3.5	57.9	38.2	0.0	0.4	0	31.2	27.1
11	M	5	1.2	36.0	62.2	0.0	0.6	0	101.1	174.3
12	M	10	6.0	39.0	53.4	0.0	1.6	2	17.0	20.6
13	M	13	3.5	32.2	60.4	0.0	0.0	2	17.2	23.8
14	M	9	0.5	60.5	37.0	0.5	1.5	1	191.8	180.1
Average		9.8	2.8	49.6	46	0.1	1	0.6	63.57	79.02
Range		5–16	0.5–6.1	32.2–69.3	24.2–62.2	0–0.7	0–3.5	0–2	17.0–191.8	20.6–237.5
15 ²	M	6	5.7	61.9	31.5	0.0	1.0	1	85.4	71.7
16	M	12	5.6	38.1	53.8	0.2	2.5	8	215.8	231.4
17	M	7	10.4	53.9	35.7	0.0	0.7	4	26.4	21.5
18	M	7	7.3	61.4	31.3	0.1	1.0	0	57.8	64.9
19	F	15	4.1	25.2	67.4	0.0	0.5	5	34.7	50
20	M	5	4.5	46.8	47.8	0.0	0.2	4	377.3	315.1
21	F	11	31.9	20.2	43.7	0.5	0.0	3	112.8	101
22	F	13	12.4	59.7	27.2	0.0	0.7	8	316.3	367.7
23	M	8	16.0	48.1	31.4	0.0	4.5	8	201.7	217.1

Horse	Sex	Age	Neutrophils (%) [RV 5%] ¹	Lymphocytes (%) [RV 30–50%]	Macrophages (%) [RV 40–60%]	Eosinophils (%) [RV 0.5%]	Basophils (%) [RV 2%]	Clinical score	Uncorrected adenosine concentration (pg/ μL)	Corrected adenosine concentration (pg/ μL)
24	M	22	76.3	11.8	11.4	0.1	0.5	8	352.0	228.9
25	M	13	50.0	23.9	25.7	0.0	0.4	2	103.5	131.1
Average		10.8	20.7	41.1	37.1	0.1	1.1	4.6	171.23	163.68
Range		5–22	4.1–76.3	11.8–61.9	11.4–67.4	0–0.5	0–4.5	0–8	26.4–377.3	21.5–367.7

RV, reference value.

¹ Neutrophil counts higher than 5% were considered indicative of airway inflammation. However, values between 4% and 6% were interpreted in relation to other diagnostic criteria.

² Lower airway inflammation was diagnosed on the basis of medical history and decreased exercise tolerance.