

A study on bronchial epithelial alterations in severe equine asthma and their reversibility

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Severe equine asthma Clinics



Bronchospasm, mucous secretions, cough

Pathophysiology

Inflammation

Remodeling



Innate immune response

Research paper

Systemic inflammation and priming of peripheral blood leukocytes persist during clinical remission in horses with heaves $^{\, 1, \, 1, \, 1}$

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Bronchial epithelium & innate immune response

- Bronchial epithelial cells (BEC) participate in innate immunity
- BEC activation is thought to play a central role in bronchial response to various external triggers (both chemical and mechanical) and in orchestrating the inflammatory cascade locally (Th2-type)
- Alterations in their structure and function might influence or being associated with disease development, evolution and/or severity



Hypotheses and objectives

<u>HYPOTHESES</u>

Structural alterations of the airway epithelium occur in conducting bronchi of asthmatic horse, are related to IL-13, a Th-2 type cytokine with a central role in asthma pathophysiology, and are reversible by antinflammatory treatment.

OBJECTIVES

- 1. To assess and compare the structure of bronchial epithelium of healthy and asthmatic
- 2. To assess and comapre the effect of inhaled corticosteroid and bronchodilator on the gene expression

horses kept in the same conditions and their bronchial inflammatory gene expression

structure of bronchial epithelial cells of asthmatic horses and their bronchial inflammatory





Study design — disease effect

	Pasture (3 months) N=11 horses		N=5 control horses on antigen challenge		
			N=6 asthmatic horses on antigen challenge		
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		ТО		Т4	
	Lung mechanics (PFT)	X		x	
	Broncho-alveolar lavage (BAL)	X		X	
	Endobronchial biopsies (EBB)	X		x	
	Pulmonary biopsy (PB)	Х		Х	



From Leclère et al., 2011







Study design — drug effect

	Antigen challenge (5 weeks) N=12 horses T Lung mechanics (IOS) x Broncho-alveolar lavage (BAL) x		N	=6 <i>A</i> N=6
	Endobronchial biopsies (EBB)	х		
	Pulmonary biopsy (PB)	Х		



From Bullone et al., 2017







Methods

- Ethical approval obtained from UdeM (protocol Rech-1324)

-Lung function: PFT (Leclère 2011), IOS (Bullone, 2014)

-BAL and EBB (Bullone, 2014; Jean, 1999)



-RT-qPCR: IL-8, IL-6, TSLP, IL-13, IL-17A, RPL9 (housekeeping)









Methods - morphometry

- Epithelial thickness (µm)
- Epithelial cell density (nuclei/µm BM)
- Goblet cell density (GC/µm BM)

Pentachrome Russel Movat staining 40x magnification 2-6 images/horse/time blinded analysis

- Epithelial proliferation (PCNA⁺ BEC/µm BM)

-Epithelial and submucosal inflammation



Results – asthma vs. control



horses after 30 days.

BAL fluid cytology revealed a neutrophilic response in both group, which was more challenge.

From Leclère et al., 2011



- As previously reported, antigen challenge evoqued an altered lung function only in asthmatic
- accentuated in asthmatic horses. Mast cells also increased in asthmatic horses with antigen





Results – asthma vs. control

During exposure to environmental antigens, asthmatic horses have an increased number of epithelial cells per um of BM compared to control horses.

This is accompanied by a similar trend towards increased epithelial proliferation and epithelial thickness.



Inexpectedly, GC number was not increased in asthmatic horses, possibly due to

- Insensitivity of the method used for counting them
- Rapid/increased rate of mucus secretion (less storage into the cell)





Results – asthma vs. control









-Th2-type cytokines, and especially IL-13, induce mucus hyper/metaplasia in BEC

horses in our first study



-Correlation coefficient between IL-13 expression and goblet cell number was positive in control







Atherton et al., 2003, AJP Lung







Results – drug effect



Both treatments normalized lung function but had no effect on BALF inflammation after 4 weeks





Results – drug effects



However, fluticasone but not salmeterol significantly reduced the number of inflammatory cells in EBB epithelium and submucosa







Salmeterol Δ



STATISTICAL ANALYSIS: Significant effect of time (p=0.008) Significant interaction between treatment and time (p=0.025) Significant subject matching (p=0.003).







STATISTICAL ANALYSIS: Significant subject matching (p<0.01). Concerning epithelial remodeling, data observed in this second project were much more severe compared to the first one

Fluticasone had a positive effect but not salmeterol

Epithelial cell density was not affected by treatment



STATISTICAL ANALYSIS: Significant effect of time (p=0.002) Significant interaction between treatment and time (p=0.03)





Results – drug effects



On the other hand, no significant changes were obaserved concerning mRNA expression of pro-inflamatory genes in EBB









Discussion and conclusions

-Epithelial remodeling characterized by great variability in asthma, likely linked to environmental exposure

- Unpredictable; important information for future studies and for external validity
- proliferation, which seems irreversible by currently available treatments.
- -Epithelial changes are not affected by reduced mechanical stress, at least not in the short time
- epithelial remodeling, probably not mediated by the cytokines studied
- -Mucus production/secretion is linked to IL-13 with a complex regulatory mechanism

-Equine asthma is characterized by **epithelial hyperplasia** (increased cell density) possibly due to cell

-Epithelial changes are partly affected by inflammation as fluticasone treatment had some positive effects on







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