

Endothelin-1 Concentrations in Bronchoalveolar Lavage Fluid of Cats with Experimentally Induced Asthma

C.R. Sharp, T.M. Lee-Fowler, and C.R. Reinero

Background: There is a need for biomarkers for diagnosis, therapeutic monitoring, and prognosis for asthma in cats. Endothelin-1 (ET-1) is implicated in the pathogenesis of inflammatory airway diseases in other species but not the cat.

Objective: To conduct a prospective experimental study to show that experimentally asthmatic cats, but not control cats without airway inflammation, would have increased concentrations of ET in BALF.

Animals: Eleven healthy, adult research cats.

Methods: Prospective experimental study. Six healthy cats without airway inflammation were used as controls. Asthma was induced using Bermuda grass allergen (BGA) in 5 cats. Collection of BALF for total nucleated cell and differential counts was performed. The concentration of ET-1 in cell-free BALF samples was determined. Data were analyzed using a Mann-Whitney *U*-test with *P* < .05 considered significant.

Results: The median [range] BALF total cell numbers, eosinophil numbers, and eosinophil percentages were significantly higher in the cats following experimental induction of asthma (1,870 cells/ μ L [1,450–3,440], 711 cells/ μ L [356–1,686] and 38% [20–49]) compared to baseline control parameters (462 cells/ μ L [239–780], 18 cells/ μ L [18–62] and 3.5% [0–8]) (*P* < .01). The median [range] BALF ET concentration was also significantly higher after induction of asthma (1.393 fmol/mL [0.977–2.247]) compared to healthy control cats (0.83250 fmol/mL [0.625–1.038]) (*P* = .012).

Conclusions and Clinical Importance: This study suggests that BAL ET-1 concentration can be used to differentiate normal cats from those with experimentally induced asthma. If the same holds true for cats with naturally developing asthma, BAL ET-1 may prove a useful diagnostic biomarker for asthma.

Key words: Airway inflammation; Allergy; Animal model; Immunology.

Asthma is one of the more common respiratory diseases in cats, affecting 1–5% of the pet cat population.¹ The clinical spectrum of disease ranges from mild, intermittent coughing, wheezing or both, to acute, severe, and life-threatening respiratory distress. Activation of inflammatory pathways, proinflammatory cytokine release, and pulmonary recruitment of inflammatory cells in allergic asthma results in the classic findings of eosinophilic airway inflammation, hyperreactivity and remodeling.² Endothelins (ET), in particular ET-1, are implicated in airway inflammation, bronchoconstriction, and structural remodeling of the airways in humans.^{3,4} Their role in asthma in cats has not been previously investigated.

A greater understanding of the pathophysiology of asthma in cats is needed to allow development of novel therapeutic targets, and to discriminate asthma from

Abbreviations:

BALF	bronchoalveolar lavage fluid
BGA	Bermuda grass allergen
ET	endothelin
ET-1	endothelin-1
IDST	intradermal skin testing
RAO	recurrent airway obstruction

other airway disorders, monitor efficacy of treatment, and provide prognostic information. Thus, the objective of this study was to evaluate bronchoalveolar lavage fluid (BALF) of healthy and experimentally asthmatic cats for the presence of immunoreactive endothelin using a commercially available ELISA. We hypothesized that experimentally asthmatic cats, but not control cats without airway inflammation, would have increased concentrations of ET-1 in BALF.

Materials and Methods

Eleven healthy, SPF, research cats belonging to a colony maintained at the University of Missouri were used for this study. The study was approved by the Animal Care and Use Committee at the University of Missouri. Cats were cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals. The cats were group housed and maintained on commercial adult feline food and water *ad libitum*.

Healthy research cats (*n* = 6) with no clinical respiratory signs and no abnormalities detected by physical examination had BALF collected in a blind fashion to confirm the absence of airway inflammation. Asthmatic cats (*n* = 5) had induction of asthma by sensitization and challenge with Bermuda grass allergen (BGA) according to a protocol that has been well described and previously used by our laboratory.² Briefly, cats were tested at baseline to confirm they had not previously been sensitized to

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BGA by performing intradermal skin testing (IDST) and by collection of BAL to ensure they did not have pre-existing eosinophilic airway inflammation. Subsequently, all cats received a SC injection of BGA (12 μ g in 10 mg alum) and a SC injection of 100 ng pertussis toxin to induce IgE antibody isotype switching. On day 14, the cats received another 75 μ g of BGA intranasally (in 0.2 mL phosphate buffered saline). A final injection of 12 μ g of BGA in 10 mg of alum SC was given on day 21. After parenteral sensitization, aerosol allergen challenge was conducted on awake, spontaneously breathing cats by aerosol delivery of BGA into a sealed chamber. Allergen aerosol challenges were performed 7 times over an initial 2-week period. The asthmatic phenotype was then confirmed by repeated IDST (the presence of dermal wheals was used to confirm sensitization to BGA) and demonstration of eosinophilic inflammation in the BALF. We defined an asthmatic phenotype as >17% eosinophils. Cats with a confirmed asthmatic phenotype had weekly delivery of BGA aerosols to maintain allergic sensitization.

To mimic immunologic changes that occur after an acute exacerbation of asthma, collection of BALF was performed 48 hours after BGA challenge by aerosol for the current study. Samples of BALF were placed immediately on ice and processed for total and differential cell counts within 2 hours. The remaining BALF was centrifuged at $300\times g$ for 10 minutes and the cell-free supernatant stored in aliquots at -20°C until batch analysis. The concentration of immunoreactive ET-1 in cell-free BALF samples was determined using a commercially available Endothelin (1–21) enzyme immunoassay (Biomedica Gruppe, Austria, distributed by Alpco Diagnostics, NH), according to manufacturer's instructions. Undiluted samples were analyzed in triplicate. The lower limit of detection is 0.625 fmol/mL. This kit has been validated for use in feline plasma,⁵ but not with bronchoalveolar lavage fluid.

For samples in which BALF ET-1 concentrations were below the lower limit of detection of the assay, the ET-1 concentration was reported as 0.625 fmol/mL for statistical analysis. Continuous variables were evaluated for normality by visual inspection of histograms, P-P plots, Q-Q plots, and the Shapiro-Wilk test of normality. All data were assessed as nonparametric. Data were analyzed using a Mann-Whitney *U*-test with $P < .05$ considered significant. A Pearson product moment correlation was used to evaluate for correlation between BALF ET-1 concentration and BALF total cell numbers and eosinophil numbers.

Results

All allergen-challenged cats developed an asthmatic phenotype including respiratory signs associated with aerosol challenge. The median [range] BALF total cell numbers, eosinophil numbers, and eosinophil percentages were significantly higher in the cats after experimental induction of asthma (1,870 cells/ μ L [1,450–3,440], 711 cells/ μ L [356–1,686], and 38% [20–49]) compared to baseline control parameters (462 cells/ μ L [239–780], 18 cells/ μ L [18–62], and 3.5% [0–8]) ($P < .01$) (Fig 1a–c). The median [range] BALF ET concentration was also significantly higher after asthma induction (1.393 fmol/mL [0.977–2.247]) compared to healthy control cats (0.83250 fmol/mL [0.625–1.038]) ($P = .012$) (Fig 2). Two cats in the control group had a BALF ET concentration below the lower limit of detection; in all other cats, ET was detected. BALF ET concentration was strongly correlated with both total nucleated cell count ($R = 0.802$) and eosinophil numbers ($R = 0.793$) ($P = .01$).

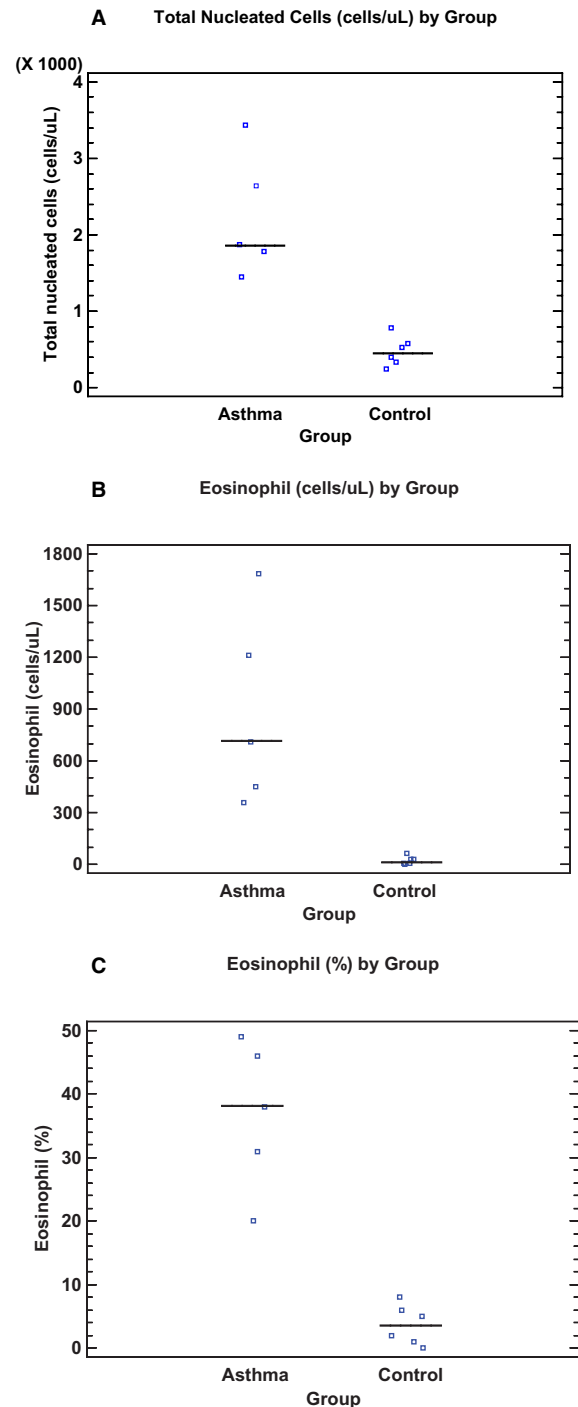


Fig 1. (A) Comparison of bronchoalveolar lavage fluid (BALF) total nucleated cell count in cats with experimental asthma versus healthy control cats. The dots represent individual cat results, and the horizontal bar denotes the group median. There was a significant difference ($P < .05$) between groups. (B) Comparison of BALF eosinophil numbers in cats with experimental asthma versus healthy control cats. The dots represent individual cat results, and the horizontal bar denotes the group median. There was a significant difference ($P < .05$) between groups. (C) Comparison of BALF eosinophil percentage (%) in cats with experimental asthma versus control cats. The dots represent individual cat results, and the horizontal bar denotes the group median. There was a significant difference ($P < .05$) between groups.

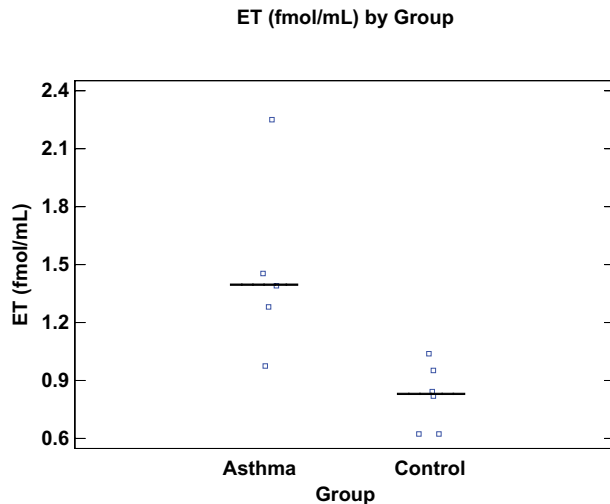


Fig 2. Comparison of bronchoalveolar lavage fluid endothelin concentrations in cats with experimental asthma versus healthy controls. The dots represent individual cat results, and the horizontal bar denotes the group median. There was a significant difference ($P = .012$) between groups.

Discussion

This study documented increased concentrations of BALF ET-1 in cats with experimentally induced allergic asthma compared with controls making ET an appealing biomarker for further study in pet cats with naturally developing asthma. Our findings of increased concentrations of ET in BALF in experimentally asthmatic cats are consistent with findings in people with asthma⁶ as well as horses with season recurrent airway obstruction (RAO),⁷ a disease with many similarities to allergic asthma. Previous attempts to identify diagnostic biomarkers for asthma in cats have failed to identify a significant difference in BALF concentrations of IL-4, IFN- γ , TNF- α , and NO metabolites between cats with asthma and healthy control cats.⁸

Endothelins are potent proinflammatory mediators, bronchoconstrictors, and secretagogues. Endothelin-1 (ET-1), the most studied of the 3 ET isoforms, is generated in and released from airway epithelial and endothelial cells, vascular endothelial cells, smooth muscle, and inflammatory cells, and has been implicated in the pathogenesis of inflammatory airway diseases.^{3,4} In particular, there is evidence that ET-1 has several properties important in the pathogenesis of asthma.⁹ Notably ET-1 has been found to increase mucus secretion, contract bronchial smooth muscle, enhance proinflammatory mediator release, and stimulate collagen production by pulmonary fibroblasts.^{3,4} Various studies have also demonstrated association between polymorphisms in ET-1 and asthma in people,¹⁰ and human asthma patients have increased concentrations of ET-1 in BALF, exhaled breath condensate and plasma.^{6,11} Similar findings are evident in horses with seasonal RAO.⁷ Documenting increased BALF concentrations of ET-1 in experimentally asthmatic cats is the first step in demonstrating the involvement of this mediator in feline asthma.

The experimental feline model of asthma used in this study is useful for preliminary investigations such as this, but ultimately findings from this model are intended to be translated to pet cats with naturally occurring disease. Although this study provides promising pilot data, future studies with larger numbers of cats and cats with naturally developing asthma are needed. In addition, in order for ET to be a clinically useful diagnostic biomarker for asthma, it is important to discern whether its concentrations can differentiate cats with asthma from those with chronic bronchitis. Also, because collection of BALF is invasive, analysis of ET in blood and exhaled breath condensate should also be investigated. It will also be prudent to evaluate whether BALF ET concentrations are correlated with disease severity and lung function indices, as is the case in human medicine.⁶ And finally given the likelihood that ET is a biologic mediator, not just as biomarker, in asthma, the potential role of endothelin receptor antagonist drugs requires further investigation.

In conclusion, the finding of increased concentrations of BALF ET in experimentally induced asthmatic cats holds promise not only for its diagnostic utility but also for investigations into ET blockade as a novel therapeutic approach in cats with asthma.

Acknowledgment

Conflict of Interest: Authors disclose no conflict of interest.

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