jfms

Endotracheal nebulization of N-acetylcysteine increases airway resistance in cats with experimental asthma

Carol R Reinero DVM, DACVIM (Small Animal Internal Medicine), PhD [1]*, **Tekla M Lee-Fowler** DVM, DACVIM (Small Animal Internal Medicine), MS [1], **John R Dodam** DVM, DACVA, MS, PhD [1], **Leah A Cohn** DVM, DACVIM (Small Animal Internal Medicine), PhD [1], **Amy E DeClue** DVM, DACVIM (Small Animal Internal Medicine), MS [1], **Vamsi P Guntur** MD, MSc [2]

[1]*Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, 900 East Campus Drive, Columbia, MO 65211, USA*
[2]*Division of Pulmonary and Critical Care Medicine, School of Medicine, University of Missouri, 5 Hospital Drive, Columbia, MO 65212, USA*

N-acetylcysteine (NAC), a mucolytic and antioxidant, is speculated to cause bronchoconstriction in cats when delivered via aerosol. We hypothesized that in cats with experimental asthma, aerosol delivery of NAC (400 mg cumulative dose) via an endotracheal tube would increase airflow limitation as measured by ventilator-acquired mechanics. After endotracheal drug delivery, airway resistance and inspiratory plateau pressure (Pplat) measurements were obtained in six mechanically ventilated asthmatic cats. Results demonstrated significantly increased airway resistance ($P = 0.0007$) compared with aerosolized saline control; Pplats were not significantly different ($P = 0.059$). All cats exhibited at least one adverse effect: excessive airway secretions ($n = 3$), spontaneous cough ($n = 2$), unilateral strabismus ($n = 1$) and post-anesthetic death ($n = 1$). No adverse reactions were noted with saline aerosol; cough was noted in one cat with methacholine challenge. In conclusion, airway resistance and adverse reactions were documented in all cats after NAC aerosol delivery. Further studies must be performed to evaluate if it is an effective mucolytic and/or antioxidant in cats and to determine if bronchodilator pre-treatment will negate NAC-induced bronchoconstriction.

Feline asthma and chronic bronchitis are disorders of the lower airways associated with airway inflammation, hyperreactivity and remodeling. One component of remodeling includes hypertrophy and hyperplasia of goblet cells and submucosal glands leading to increased mucus secretions. Accumulation of mucus and bronchoconstriction affect pulmonary function by impairing airflow. Furthermore, mucus hypersecretion can result in occlusion of the bronchi and atelectasis. This finding is fairly common (especially in the right middle lung lobe) in asthmatic cats.[1] N-acetylcysteine (NAC) is both a mucolytic and antioxidant which might have benefit in inflammatory airway diseases associated with mucus overproduction; this has been shown in humans.[2] It is available in injectable, oral and aerosol formulations, none of which have been adequately studied in cats with asthma or chronic bronchitis. In humans bronchospasm is a recognized

complication of inhaled NAC.[3] Given this adverse effect in humans, there is concern of a similar problem in cats and despite mucus hypersecretion being a recognized component of feline asthma, aerosol delivery of NAC has not been recommended as therapy for asthmatic cats.

Airway resistance can be measured indirectly or directly. Indirect measures are non-invasive and in cats include the techniques of tidal breathing flow—volume loops using a tight-fitting face mask, forced expiratory flow—volume curves using thoracic compression, or barometric whole body plethysmography (BWBP).[4—7] Direct measures of pulmonary mechanics are the gold standard, and are more accurate but require anesthesia, dedicated equipment, and are minimally to highly invasive depending upon technique.[8—10] More recently, direct calculation of airway resistance has been performed with ventilator-acquired pulmonary mechanics.[11,12]

Recently, a pilot study in 27 pet cats delivered 150 mg of NAC into a BWBP chamber reported no adverse

*Corresponding author. E-mail: reineroc@missouri.edu

effects or alterations in enhanced pause (Penh), a surrogate measure of airway resistance.[13] However, delivery of NAC by nebulizing into a 38 L chamber likely resulted in minimal delivery to the lower airways. Moreover, Penh is not a true measure of airway resistance and may be insensitive to some changes in airway function; in some cases it does not correlate to airway resistance at all.[14] In addition, only two asthmatic cats were studied; the remaining cats were healthy or had other respiratory or non-respiratory illnesses.

The purpose of the study was to determine if NAC could be aerosolized to experimentally asthmatic cats without inducing measurable changes in airflow limitation or inducing clinical adverse reactions. We hypothesized that in experimentally asthmatic cats, aerosolized NAC would increase airway resistance as measured by direct ventilator-acquired mechanics. To do so, we compared differences in the maximum values of ventilator calculated airway resistance and plateau pressures (Pplats) after aerosolized NAC versus their respective maximum values after aerosolized saline. Additionally, adverse events were recorded during and after NAC treatment.

Materials and methods

Cats

Six mixed breed cats (three intact females and three intact males) were either bred from a high-responder asthmatic cat research colony (University of Missouri, Columbia, MO) or were obtained from an outside vendor (Liberty Research, Waverly, NY). Cats were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals. This study was approved by the University of Missouri Animal Care and Use Committee.

Induction of experimental asthma

Prior to induction of an asthmatic phenotype, all cats were tested at baseline to confirm they had not previously been sensitized to Bermuda grass allergen (BGA) by performance of intradermal skin testing (IDST). They were also screened to ensure they did not have pre-existing eosinophilic airway inflammation by analysis of bronchoalveolar lavage fluid (BALF). Cats were sensitized and challenged as previously described, with slight modification.[15] Briefly, on day 0, cats received a subcutaneous (SC) injection of 12 μg of BGA in 10 mg of alum and a SC injection of 100 ng *Bordetella pertussis* toxin (Sigma–Aldrich, St Louis, MO); on day 14, 75 μg BGA in 0.2 ml of phosphate buffered saline (PBS) intranasally; and on day 21, another SC injection of 12 μg of BGA in 10 mg of alum. On day 28, IDST was repeated to confirm sensitization to BGA. After parenteral sensitization, aerosol challenges of BGA (100 μg of BGA dissolved in PBS) were administered to awake, spontaneously breathing cats in a sealed chamber. Seven challenges were administered within 2 weeks following parenteral sensitization (at which time eosinophilic airway inflammation was confirmed by BALF collection), and a single challenge was given once weekly thereafter. Cats were enrolled in this study after an average of 3 months of an asthmatic phenotype. The allergen aerosol challenge prior to pulmonary mechanics measurement and BALF collection was timed so that it was administered 48 h before sample collection (see below).

Collection and analysis of BALF

The BALF was collected in a blind fashion. Briefly, an 8 FR red rubber catheter was advanced through the endotracheal tube (ETT) until gentle resistance as met as it wedged in a lower airway. A 20 ml aliquot of sterile saline was instilled from an attached syringe and was followed by manual suction. All samples were placed on ice during the time between collection and analysis (within 2 h). Cytological evaluation and differential cell counts were performed on samples prepared by cytocentrifugation (Shandon Cytospin 4, ThermoElectron Corporation, Waltham, MA). Differential cell counts were performed by evaluating 200 nucleated cells per slide and reporting cell percentages. Collection of BALF followed pulmonary mechanics measurement.

Pulmonary mechanics measurement

Airway obstruction patterns were measured using the neonatal mode on the Engstrom Carestation (GE Healthcare, Fairfield, CT) ventilator. Methacholine (MCh) challenges and delivery of NAC with mechanics measurements were performed >1 week apart. Cats were sedated with 30 mg ketamine to facilitate intravenous catheter placement. Anesthesia was induced with 6 mg/kg propofol and maintained with a continuous infusion of the same (0.2 mg/kg/min). Intermittent boluses were given (0.5–1 mg/kg) as needed to maintain an appropriate anesthetic plane. To standardize resistance contributions from ETTs, all cats were intubated with 4 mm internal diameter, 14 cm long cuffed ETT. The neonatal flow sensor of the ventilator (heated pneumotachograph) was placed in line at the oral end of the ETT. Animals were mechanically ventilated using a volume-controlled ventilation mode with a tidal volume of 10 ml/kg, at 10 breaths/min, and an inspiratory-to-expiratory ratio of 1:3. Fractional inspired oxygen was supplied at 0.4 through the ETT. Cats were ventilated for 5 min prior to baseline data collection. Sterile saline was delivered initially for 30 s through an Aeroneb solo in-line nebulizer (Aeroneb Pro; Aerogen, Mountain View, CA) followed by 4 min of baseline data collection. The nebulizer generates particles with a mass median aerodynamic diameter of 2.1 μm and was used both for bronchoprovocation and NAC delivery (see below). Airway resistance (Raw) was continuously calculated by the ventilator. Airway resistance was calculated during ventilation as the average linear resistance measured during inspiratory and expiratory flows reported at the average flow rate. End-inspiratory pressure (Pplat), was measured by the ventilator after an

end-inspiratory breath hold during minutes 2 and 4 of data collection.

Bronchoprovocation challenge

A bronchoprovocation challenge test, using MCh, was performed by delivering doubling doses of the drug (0.0625—32 mg/ml) through the in-line nebulizer for 30 s followed by 4 min of data collection. MCh is a direct agonist of smooth muscle receptors that causes constriction that has been previously used in feline asthma models and clinically in humans to measure bronchial hyperreactivity.[9,16] Bronchoprovocation was terminated when displayed Raw was increased 200% above baseline. Data were expressed as the concentration of MCh that increased Raw by 200% (EC200Raw); this was determined by linear interpolation of the log—log plot of the dose response curve, with the response expressed as the percent of baseline Raw.

Response to NAC

Delivery of NAC 10% solution for inhalation (American Regent, Shirley, NY), through the in-line nebulizer was followed by 4 min of data collection after each dose. The dosing scheme was 50 mg, 50 mg, 100 mg and 200 mg for a cumulative dose of 400 mg/cat. The dose escalation was based on ensuring safety of the cats first (ie, conservative doses in case of severe bronchoconstriction) and efficacy second (ie, reaching the high end of the dose recommended for pediatric human patients). Data collection was performed for a minimum of 30 min past aerosol delivery of the first dose of NAC. Data were expressed as the percent of increase in baseline Raw (ie, maximum Raw after NAC minus maximum Raw after saline multiplied by 100). Additionally, the maximum Pplat during aerosolized saline delivery and over the course of all doses of NAC were measured.

Statistical analysis

Differences between the maximum values of Raw and Pplat after delivery of aerosolized NAC compared with their respective maximum values after aerosolized saline were analyzed using a one-sided paired t-test analysis. Data were considered to be statistically significant when the P-values were ≤ 0.05 and marginally statistically significant when $0.05 < P < 0.1$.

Results

Airway eosinophilia and airway hyperresponsiveness

All cats had increases in their BALF eosinophils after allergen sensitization and challenge, and the increases were consistent with an asthmatic phenotype. The mean $\pm$ SD BALF % eosinophils was $55 \pm 22\%$ (Table 1). Response to bronchoprovocation with MCh in five of the cats is also listed in Table 1. One of these cats coughed during the MCh challenge. No other adverse reactions were noted.

Response to aerosol delivery of NAC

Airflow limitation induced by aerosol delivery of NAC was noted in all cats with a mean $\pm$ SD percentage increase over baseline (ie, post-saline) Raw of $150 \pm 18\%$ ($P = 0.007$; Table 1). The time to peak increase in Raw after aerosol delivery of NAC was variable between cats, occurring at a mean $\pm$ SD of 26 ± 10 min after delivery of the first 50 mg dose. The mean $\pm$ SD maximum Pplat after saline aerosol was 9 ± 1 cmH$_2$O (range, 8—10) and the maximum Pplat after NAC aerosol was 14 ± 6 cmH$_2$O (range, 7—24). The Pplat values were marginally significant ($P = 0.059$); however, the power of the test with $\alpha = 0.05$ was only 0.247 (below the desired power of 0.8).

Adverse effects after inhalation of NAC were noted in all cats studied. The most common side effect was increased upper ($n = 1$) or lower ($n = 2$) airway secretions. Lower airway secretions were noted as a rattling noise without a stethoscope or as crackles with a stethoscope; additionally, when touching external thoracic cavity, vibrations could be easily palpated. Two cats had spontaneous cough under anesthesia during administration of NAC (but not saline). One cat had self-limiting

Table 1. Airway inflammation, airway response to MCh and changes in airway resistance to NAC administration in six cats.

Cat	%BALF eosinophils	ED200RL	%ΔRaw (NAC)	Saline Pplat max	NAC Pplat max
1	38	1	159	10	13
2	77	12.8	169	8	24
3	49	3.7	123	8	7
4	80	N/A	158	9	17
5	61	2.7	156	10	10
6	25	3.3	133	10	13

ED200RL = effective dose of MCh required to increase airway resistance to 200% over baseline resistance (mg/ml); %ΔRaw (NAC) = percentage change in airway wall resistance from baseline induced by NAC; Pplat max = maximum plateau pressure in cmH$_2$O recorded after aerosolized saline or NAC; N/A = not available.

unilateral strabismus noted during anesthetic recovery. One cat (who had the most severe asthmatic phenotype of the group) died during anesthetic recovery. Necropsy did not reveal a specific cause of death although remodeling changes consistent with asthma were moderate to severe. Adverse effects were not noted after inhalation of saline (given at baseline for both the NAC administration as well as the MCh challenge); one cat given MCh spontaneously coughed under anesthesia, but no other adverse effects to MCh were observed.

Discussion

NAC, a drug with mucolytic and antioxidant properties, led to airflow limitation and adverse clinical effects in experimentally asthmatic cats when delivered by an aerosol route. This is the first study in the cat to report that aerosolized NAC has the potential to increase airway resistance, a finding which has important clinical implications in cats with lower airway disease that are already predisposed to have airway hyperreactivity and/or airway narrowing due to inflammation, edema or intraluminal secretions.

The results of this study are in contrast to an earlier preliminary report which concluded aerosol delivery of 150 mg of NAC to a heterogeneous population of client owned cats into a 38 L chamber for BWBP was safe and did not lead to airflow limitation (as determined by BWBP-detectable changes).[13] There are several reasons for the discrepancies between these studies. First, the cats in our study were anesthetized for direct measurement of pulmonary mechanics in which a heated pneumotachograph (neonatal sensor) was placed in line with the ETT; this is in contrast to BWBP which measures pressure changes induced by respiratory movements in unrestrained, unsedated cats in a box.[17] The latter technique is primarily related to the respiratory cycle and has been criticized for not measuring airway resistance.[14] Direct, confirmatory methods are preferred for functional measurements[14] and ventilator-acquired mechanics have been demonstrated to be consistent and reliable in cats in a pilot study.[11] Second, the cats in our study were administered NAC directly to the lower airways through the ETT via the in-line nebulizer for the ventilator. Aerosol delivery of medications into a large (38 L) chamber will dramatically affect the amount of drug which reaches the lower airways. Using scintigraphy to quantify aerosol deposition, it was determined in cats using a spacer and tight-fitting face mask that only 1% of the aerosol reached the lungs (Brendan McKiernan, personal communication). Therefore, it would be expected that substantially less NAC would reach the lower airways when delivered into a 38 L box. Third, the dose of NAC used in our study was 2.7 times higher than in the other report. Fourth, data collection was performed for a minimum of 30 min past aerosol delivery of the first dose of NAC in our study compared with 5 min in the other study. There may be delayed effects of NAC on airway resistance which may not have been detected in the shorter sampling period of the latter study. Finally, all the cats in the current study were more homogenous in terms of their disease, having all undergone the same experimental protocol for allergen sensitization and challenge and induction of asthma. In comparison, the previous study only included two asthmatic cats, and the majority of cats evaluated were either healthy or had non-respiratory diseases. Therefore, it would be expected that alterations in airflow limitation would be more pronounced in our study population overall.

Ventilator-acquired mechanics were useful to document that cats developed an increase in Raw to a traditional bronchoprovocant, MCh. All cats reached the endpoint of the MCh challenge (ie, the ED200RL); lower values of ED200RL imply more hyperresponsive airways to this direct acting bronchoprovocant. In this study, cats had a significant increase in Raw in response to NAC and a marginally significant increase in Pplat compared with values obtained after aerosolized saline. The power of the Pplat test was determined to be 0.247 (below the desired power of 0.8) meaning that we were less likely to detect a difference when one actually exists. Pplat is a measure of airway resistive pressures which correlate with airway resistance changes from increased intraluminal secretions, inflammation or bronchoconstriction. Previously, ventilator-measured Pplat was shown to significantly increase after tracheal lavage in healthy kittens.[12]

The dose of NAC used in the current study is based on recommendations for human pediatric patients in which 2–4 ml of a 10% solution of NAC is nebulized three to four times daily.[18] In humans, the onset of action by inhalation is 5–10 min with a duration of >1 h. Interestingly, in our cats, peak changes in airway resistance occurred an average of 26 ± 10 min after delivery of the first 50 mg dose. Pharmacokinetic studies would need to be performed to determine the best dose in cats, as the dose may differ from humans. Interestingly, after oral dosing in humans, NAC has low bioavailability[19] and is not found in airway secretions.[20]

The indications for use of NAC include acute and chronic bronchopulmonary diseases in which there are viscid mucous secretions. In cats, asthma and chronic bronchitis are two common examples of pulmonary disorders with increased mucus, although there are others. Respiratory adverse reactions to NAC in humans include increases in bronchial secretions and rhinorrhea.[18] Compatible with that, in half the cats of this report, increases in upper and lower airway secretions were noted. In humans, bronchospasm is also recognized as a complication of inhaled NAC likely due to spontaneous and IgE-induced histamine release.[3] Administration of an aerosolized bronchodilator prior to NAC is thus recommended. This was not performed in the current study as it would have impacted our ability to document airflow limitation. Thus, it is possible that pre-treatment with a bronchodilator could negate the adverse effects on airflow limitation. In humans, asthma is a known risk factor for adverse reactions with NAC and while

caution is recommended, asthma is not considered a contraindication to use of this medication.[21]

In addition to documenting the safety of aerosolized NAC, studies must also be performed to assess its efficacy in cats by determining its mucolytic and/or anti-inflammatory properties. Inhaled NAC locally dissociates the disulphide bonds of mucins to reduce the viscosity of airway secretions.[22] Because it has free thiol groups, it is postulated to have antioxidant properties. Reasons that NAC are appealing for the treatment of asthma is that accumulation of viscid mucus may obstruct the airway lumen, exacerbate airway hyperreactivity, and/or contribute to clinical signs.[2,22] Thus having an additional medication to glucocorticoids and bronchodilators might be valuable, especially in cats that appear to be refractory to these traditional therapies.

In conclusion, inhaled NAC in experimentally asthmatic cats does induce airflow limitation and is not without the potential for adverse effects. To further explore the use of this drug in cats with asthma or chronic bronchitis, future studies should assess if:

1. NAC acts as a mucolytic and/or antioxidant when inhaled by an aerosol route and by what mechanism.
2. Nebulized NAC is associated with clinical benefits.
3. Pre-treatment with a bronchodilator reduces NAC-induced increases in airway resistance.
4. Adequate drug is delivered to the lower airways when nebulized by other means besides through an ETT (eg, a tight-fitting face mask).

Acknowledgements

References

1. Mardell E. Investigation and treatment of feline chronic bronchial disease. *In Practice* 2007; **29**: 138−46.
2. Rogers D. Airway mucus hypersecretion in asthma: an undervalued pathology? *Curr Opin Pharmacol* 2004; **4**: 241−50.
3. Dorsch W, Auch E, Powerlowicz P. Adverse effects of acetylcysteine on human and guinea pig bronchial asthma in vivo and on human fibroblasts and leukocytes in vitro. *Int Arch Allergy Appl Immunol* 1987; **82**: 33−9.
4. Bark H, Epstein A, Bar-Yishay E, Putilov A, Godfrey S. Non-invasive forced expiratory flow−volume curves to measure lung function in cats. *Respir Physiol Neurobiol* 2007; **155**: 49−54.
5. Hoffman A, Dhupa N, Cimetti L. Airway reactivity measured by barometric whole-body plethysmography in healthy cats. *Am J Vet Res* 1999; **60**: 1487−92.
6. Kirschvink N, Leemans J, Delvaux F, Snaps F, Clercx C, Gustin P. Non-invasive assessment of airway responsiveness in healthy and allergen-sensitized cats by use of barometric whole body plethysmography. *Vet J* 2007; **173**: 343−52.
7. McKiernan B, Dye J, Rozanski E. Tidal breathing flow−volume loops in healthy and bronchitic cats. *J Vet Intern Med* 1993; **7**: 388−93.
8. Dye J, McKiernan B, Rozanski E, Hoffmann W. Bronchopulmonary disease in the cat: historical, physical, radiographic, clinicopathologic, and pulmonary functional evaluation of 24 affected and 15 healthy cats. *J Vet Intern Med* 1996; **10**: 385−400.
9. Norris Reinero CR, Decile KC, Berghaus RD, Williams KJ, Leutenegger CM, Walby WF, et al. An experimental model of allergic asthma in cats sensitized to house dust mite or Bermuda grass allergen. *Int Arch Allergy Immunol* 2004; **135**: 117−31.
10. Padrid P, Snook S, Finucane T. Persistent airway hyperresponsiveness and histologic alterations after chronic antigen challenge in cats. *Am J Respir Crit Care Med* 1995; **151**: 184−93.
11. Lee-Fowler T, Dodam J, Guntur V, Reinero C. Ventilator-acquired pulmonary mechanics using methacholine challenge in healthy and experimentally asthmatic cats. 27th Symposium of the Veterinary Comparative Respiratory Society; Plymouth, MA, 2009.
12. Rozanski EA, Buckley G, Mazan MR, Bedenice D, Hoffman AH. The effect of tracheal lavage administration on lung function in healthy kittens. *J Vet Intern Med* 2009; **23**: 727.
13. Shibly S, Galler A, Hirt R. Nebulised N-acetylcysteine does not cause plethysmography-detectable bronchoconstriction in cats. 27th Symposium of the Veterinary Comparative Respiratory Society; Plymouth, MA, 2009.
14. Bates J, Irvin C, Brusasco V, Drazen J, Fredberg J, Loring S, et al. The use and misuse of Penh in animal models of lung disease. *Am J Respir Cell Mol Biol* 2004; **31**: 373−4.
15. Reinero C, Cohn L, Delgado C, Spinka C, Schooley E, DeClue A. Adjuvanted rush immunotherapy using CpG oligodeoxynucleotides in experimental feline allergic asthma. *Vet Immunol Immunopathol* 2008; **121**: 241−50.
16. Brannan J. Bronchial hyperresponsiveness in the assessment of asthma control. *Chest* 2010; **138**: 11S−7S.
17. Kirschvink K. Barometric whole body plethysmography and enhanced pause (PENH): how relevant are they? *Vet J* 2008; **176**: 12−6.
18. Acetylcysteine. Drug monographs. New York: McGraw-Hill Companies, 2010.
19. Borgstrom L, Kagedal B, Paulsen O. Pharmacokinetics of N-acetylcysteine in man. *Eur J Clin Pharmacol* 1986; **31**: 217−22.
20. Bridgeman M, Mardsen M, MacNee W, LFlenley D, Ryle A. Cysteine and glutathione concentrations in plasma and bronchoalveolar lavage fluid after treatment with N-acetylcysteine. *Thorax* 1991; **46**: 39−42.
21. Schmidt L, Dalhoff K. Risk factors in the development of adverse reactions to N-acetylcysteine in patients with paracetamol poisoning. *Br J Clin Pharmacol* 2001; **51**: 87−91.
22. Rogers D. Mucoactive agents for airway mucus hypersecretory diseases. *Respir Care* 2007; **52**: 1176−97.