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Abstract

Objectives Airway hyper-responsiveness (AHR), a key feature of feline asthma, can be measured using bronchoprovocation testing. Limitations of both direct and indirect bronchoprovocants evaluated to date in experimental feline asthma have led to a search for a more specific indirect bronchoprovocant (ie, one which relies on existing inflammatory cells or activated neural pathways in diseased but not healthy airways). We hypothesized that capsaicin, a transient receptor potential cation channel subfamily V member 1 agonist, would lead to dose-responsive increases in airway resistance as measured by ventilator-acquired pulmonary mechanics in experimentally asthmatic cats.

Methods Five cats induced to have asthma using Bermuda grass allergen (BGA) were studied. Twenty-four hours after aerosol challenge of BGA, cats were anesthetized and underwent neuromuscular blockade for ventilator-acquired pulmonary mechanics. Cats were monitored with pulse oximetry for hemoglobin desaturation. Parameters recorded on a breath-by-breath basis on the ventilator included airway resistance (Raw) and compliance. Saline at baseline and 10-fold increasing concentrations of capsaicin (0.4–4000.0 μ M) were aerosolized for 30 s and data collected for 4 mins between doses. The intended endpoint of the study was a doubling in baseline airway resistance, halving of compliance or oxygen desaturation $<75\%$.

Results All cats completed the trial, reaching the highest dose of capsaicin without reaching any of the aforementioned endpoints. No biologically significant alteration in any other pulmonary mechanics parameter was noted.

Conclusions and relevance Capsaicin does not appear to be an effective bronchoprovocant in a feline asthma model.

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Feline asthma has three major features: airway inflammation, airway remodeling and airway hyper-responsiveness (AHR).^{1–3} AHR can be defined as narrowing of the airways excessively in response to a given stimulus, resulting in increases in airway resistance. As such, measuring AHR has been used in human asthmatics for disease screening and to assess response to therapy.^{2,3}

The current reference standard for inducing measurable changes in airway resistance involves direct bronchoprovocation using methacholine; bronchoconstriction occurs in both asthmatic and non-asthmatic humans and cats.^{4,5} Methacholine is a sensitive, though non-specific, means of inducing bronchoconstriction. Indirect bronchoprovocants, which rely on pre-existing airway inflammation and activated neural pathways, are

intended to allow improved discrimination between healthy and inflamed airways, as well as serving as points of potential intervention.⁶ However, in a feline asthma model the indirect bronchoprovocants adenosine-5-monophosphate and mannitol did not reliably induce bronchoconstriction in either asthmatic or healthy cats.⁵ This has led to interest in identifying a more

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Table 1 Five experimentally asthmatic cats had ventilator-acquired pulmonary mechanics using capsaicin as a bronchoprovocant. Results are displayed as average $\pm$ SD of the maximum airway resistance (Raw) and minimum compliance. No cat reached any of the pulmonary mechanics endpoints (doubling of baseline resistance or halving of baseline compliance)

Max Raw (cmH ₂ O/l/s)	Average $\pm$ SD
Baseline	45.80 $\pm$ 1.92
0.4 μ M capsaicin	46.60 $\pm$ 1.67
4 μ M capsaicin	45.40 $\pm$ 3.05
40 μ M capsaicin	45.20 $\pm$ 3.27
400 μ M capsaicin	48.20 $\pm$ 3.56
4000 μ M capsaicin	46.80 $\pm$ 2.39
Minimum compliance (ml/cmH ₂ O)	
Baseline	7.48 $\pm$ 1.72
0.4 μ M capsaicin	6.60 $\pm$ 1.14
4 μ M capsaicin	7.00 $\pm$ 1.41
40 μ M capsaicin	7.30 $\pm$ 1.91
400 μ M capsaicin	7.20 $\pm$ 2.58
4000 μ M capsaicin	7.44 $\pm$ 2.88

suitable indirect bronchoprovocant for both research and clinical monitoring purposes.

The transient receptor potential cation channel subfamily V member 1 (TRPV1) is considered the hub of most neuronal inflammatory signaling pathways, thus playing a key role in neurogenic airway inflammation.⁷ The TRPV1 channel responds to various physical and chemical stimuli activating airway C-fibers and parasympathetic nerves. Stimulation of the TRPV1 contributes to airway smooth muscle constriction, vascular permeability and inflammatory cells through the release of the local peptides substance P and neurokinin A.⁸ In human studies, expression of TRPV1 has been shown to be upregulated in patients with severe asthma compared with those with controlled asthma and healthy subjects, suggesting a role for this receptor in refractory asthma.⁹ While studies of TRPV1 expression have not been performed to date in asthmatic cats, the similarities in non-adrenergic, non-cholinergic inhibitory nervous control of airway caliber in humans and cats would suggest that TRPV1 may also be involved in feline neurogenic airway inflammation.¹⁰ A study evaluating nebulized capsaicin in apparently healthy research cats showed increases in airway resistance and decreases in compliance at doses of 0.1 mg/ml and 1 mg/ml, supporting its effect in inducing changes in pulmonary mechanics in normal feline lungs.¹¹ Likewise, capsaicin has been used as a TRPV1 agonist in human asthmatics to study cough and sensory airway reactivity,^{7,12–14} although it is not considered a reliable bronchoprovocant.¹⁴ No studies to date have been performed using capsaicin as a bronchoprovocant in

asthmatic cats. We hypothesized that in experimentally asthmatic cats aerosolized capsaicin would lead to dose-responsive increases in airway resistance measured by ventilator-acquired pulmonary mechanics, making it suitable for bronchoprovocation testing.

Five research cats were enrolled in the study having been previously induced to have asthma using Bermuda grass allergen (BGA). The asthmatic phenotype was confirmed after initial sensitization and challenge with positive skin reactivity to BGA and bronchoalveolar lavage fluid eosinophil percentage of $>17\%$. Our laboratory had documented persistent airway eosinophilia post-allergen challenge for 1 year.¹ Twenty-four hours prior to bronchoprovocation testing, cats were challenged with an aerosol of BGA. Ventilator-acquired pulmonary mechanics was performed on anesthetized and mechanically ventilated cats as previously described, with minor modification.¹⁵ Briefly, ketamine (30 mg IV) was used as a premedicant, propofol was used for induction (6 mg/kg) and maintenance (0.3 mg/kg/min), and Cis-atracurium (0.1 mg/kg IV with additional doses of 0.01–0.02 mg/kg titrated to effect) was administered for neuromuscular blockade. Neuromuscular blockade was monitored with a peripheral nerve stimulator and edrophonium (0.5 mg/kg IV) was administered to reverse neuromuscular blockade after completion of data collection and when indicated.

Pulse oximetry was used to monitor oxygen saturation (SpO₂). Initial ventilator settings were as follows: tidal volume 10 ml/kg, respiratory rate 10 breaths/min, inspired oxygen fraction 0.4, inspiratory to expiratory ratio 1:3, and a positive end expiratory pressure 2 cm H₂O. Parameters recorded on a breath-by-breath basis on the ventilator included airway resistance (Raw), compliance and peak pressure (Ppeak); after inspiratory and expiratory breathholds plateau pressure (Pplat) and endogenous positive end expiratory pressure (PEEPe) were measured. Prior to data collection, cats were ventilated for 5 mins to allow for steady state conditions. Saline was nebulized for 30 s followed by 4 mins of data collection to determine baseline data. Capsaicin was aerosolized for 30 s with 4 mins of data collection between each dose. The intended endpoint of the study was a doubling in baseline airway resistance, halving of compliance, or oxygen desaturation $<75\%$. Ten-fold increases of capsaicin from 0.4 to 4000.0 μ M (0.0012–1.2200 mg/ml) were administered. Data were expressed as the mean $\pm$ SD for all cats over each 4 min data collection period throughout the bronchoprovocation trial.

Values for mean $\pm$ SD Raw and compliance as well as maximum Raw and minimum compliance for saline (ie, baseline data) and each dose of capsaicin are shown in Table 1. No cat reached their EC200Raw, nor was compliance halved at any nebulized capsaicin concentration. Oxygen desaturation $<75\%$ did not occur in any

Table 2 A variety of other parameters relevant to lung function were determined on experimentally asthmatic cats undergoing capsaicin bronchoprovocation including minimum oxygen saturation (SpO_2); and peak pressure (Ppeak), plateau pressure (Pplat) and positive end expiratory pressure measured on end expiration (PEEPe), all measured at their maximum value recorded over each dose of saline or bronchoprovocant. Calculations were subsequently performed for Ppeak - Pplat (maximum Ppeak minus maximum Pplat, each recorded over all doses of capsaicin), Pplat - PEEPe (maximum Pplat minus maximum PEEPe, each recorded at min 4 of data collection over all doses of capsaicin) and change in Pplat (maximum Pplat at 4000 μM capsaicin minus maximum Pplat at baseline). Increases in Ppeak - Pplat are proportional to increases in airway resistance. Increased Pplat-PEEPe values indicate decreased respiratory compliance. Increases in Pplat (change in Pplat) imply enhanced airflow limitation. Bronchoprovocation with capsaicin failed to alter any of these parameters in a dose-dependent fashion. Values given as mean $\pm$ SD

	SpO_2	Ppeak - Pplat	Pplat - PEEPe	Change in Pplat (cmH ₂ O)
Baseline	99.00 $\pm$ 1.41	2.40 $\pm$ 0.89	4.80 $\pm$ 1.30	0.00
0.4 μM capsaicin	96.40 $\pm$ 3.85	2.80 $\pm$ 0.45	4.80 $\pm$ 0.45	-0.20 $\pm$ 0.84
4 μM capsaicin	98.80 $\pm$ 1.10	2.40 $\pm$ 0.55	4.80 $\pm$ 0.45	-0.20 $\pm$ 0.84
40 μM capsaicin	98.80 $\pm$ 1.30	2.40 $\pm$ 0.55	4.80 $\pm$ 0.45	-0.20 $\pm$ 0.84
400 μM capsaicin	98.40 $\pm$ 1.14	3.20 $\pm$ 1.64	4.40 $\pm$ 1.34	0.60 $\pm$ 1.52
4000 μM capsaicin	98.80 $\pm$ 1.30	3.20 $\pm$ 1.10	4.40 $\pm$ 1.34	0.40 $\pm$ 1.14

cat; the mean SpO_2 in all cats over all time points was $98 \pm 1\%$. No biologically significant alteration in any other pulmonary mechanics parameter was noted (Table 2).

Unexpectedly and in contrast to our hypothesis and previously published data,¹¹ capsaicin did not result in concentration-dependent increases in airway resistance in a feline asthma model. Although methacholine reliably induces bronchoconstriction in the same model, it is unclear why even high concentrations of capsaicin failed to produce a measurable increase in airway resistance in cats with experimental asthma, a condition in which airway inflammatory cells are prevalent and neurogenic pathways should be activated. To our knowledge this is the first study to evaluate the utility of capsaicin as a bronchoprovocant in an experimental feline asthma model.

The doses of capsaicin used in the present investigation were extrapolated from human and animal studies. Moreover, the dose range in the present investigation exceeded that used in a previous study in healthy cats where increased airway resistance and decreased compliance were observed in response to capsaicin.¹¹ Thus, it is unclear why capsaicin failed to elicit changes in any pulmonary mechanics measurement in our asthmatic cats. Possible explanations could include differences in equipment used for ventilation or capsaicin delivery, which may have decreased the amount delivered to the lower airways. It is important to note that the same experimental system used in this study produces reliable bronchoconstriction in response to methacholine.

Responses to capsaicin in earlier feline studies have occurred within 2–3 mins;¹¹ the entire dose-response curve took roughly 25 mins to generate in our cats (from start of the 0.4 μM aerosol to 4 mins after the 4000 μM

aerosol). While a longer collection period may have helped to evaluate a delayed response to capsaicin nebulization, we thought this was unlikely as cats were monitored on the ventilator for an additional 5–15 mins, with no marked changes in pulmonary mechanics parameters, and for 1–4 h during/after anesthetic recovery, with no evidence of an elevated respiratory rate or effort. Based on previously documented protocols showing stability up to 30 days, instability of the nebulized capsaicin solution was unlikely to be a contributor. Finally, as the effect of TRPV1 activity is dependent on its ability to release adequate substance P and neurokinin A for a bronchomotor response,^{16,17} it could be hypothesized that in contrast to healthy cats that responded to capsaicin, perhaps experimentally asthmatic cats have depletion of substance P and/or neurokinin A, and are, paradoxically, less responsive to bronchoprovocation with capsaicin. This may be supported by previous studies that showed a decreased response with increasing concentrations of nebulized capsaicin in healthy cats, as well as a study demonstrating diminished AHR after capsaicin pretreatment in rabbits undergoing histamine challenge.^{11,18} Additionally, vasoactive intestinal polypeptide depletion has been demonstrated in asthmatic, compared with healthy, humans.¹⁹

Weaknesses of this study include small sample size and lack of airway sampling prior to challenge. Evaluation of a control, non-asthmatic population was not performed as a part of this study but would have been interesting, particularly as a comparison to previous studies.¹¹ However, given the objective of this study to examine the utility of capsaicin as a bronchoprovocant in experimentally asthmatic cats it is not considered a significant weakness in the study design.

Conclusions

In summary, the present study suggests that capsaicin is an unacceptable indirect bronchoprovocant to assess AHR in experimentally asthmatic cats. As AHR is a key feature of asthma pathogenesis and its measurement is valuable for diagnosis/discrimination from other respiratory disorders, stratification of disease severity and monitoring therapeutic efficacy, additional research into a suitable indirect bronchoprovocant in the asthmatic cat is still needed. Further study is needed to determine if chronic airway allergic inflammation depletes neurogenic inflammatory mediators, thus potentially limiting the efficacy of downstream receptor antagonism as a means of therapy.

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Conflict of interest The authors do not have any potential conflicts of interest to declare.

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