Article

Failure of nebulized irritant, acidic, or hypotonic solutions or external mechanical stimulation of the trachea to consistently induce coughing in healthy, awake dogs

Tonya E. Boyle, Eleanor C. Hawkins, Jennifer L. Davis, Ian D. Robertson

Abstract

A useful approach for evaluating antitussive drugs in humans is to determine the sensitivity of the cough reflex to a standard challenge. The purpose of this study was to determine if methods used to induce coughing in humans would be effective when used on awake, untrained, healthy dogs for future application in therapeutic trials involving dogs with spontaneous disease. Methods tested were: mechanically stimulating the trachea by digital compression as well as by vibration from an electric shaver, neck massager, and palm sander (11 dogs), and administering nebulized irritant (3000 μM capsaicin), acidic (1 M citric acid), and hypotonic (deionized water) solutions using face masks (4 dogs). The threshold for success was defined as induction of at least 2 moderate or strong coughs in at least 75% of the dogs. None of the methods tested was successful. Digital compression induced soft ($n = 2$) or moderate ($n = 1$) coughing in 3 of 11 dogs tested. Nebulization of citric acid induced 1 soft cough in 1 of 4 dogs. It was concluded that coughing cannot be successfully induced in awake, healthy dogs using methods that are successful in humans. Other strategies must be developed so that cough sensitivity can be objectively and non-invasively measured in dogs for clinical research purposes.

Résumé

Une approche utile pour évaluer les médicaments anti-toussifs chez l'humain est de déterminer la sensibilité du réflexe de la toux à un challenge standard. L'objectif de la présente étude était de déterminer si les méthodes utilisées pour induire la toux chez l'humain seraient efficaces lorsqu'utilisées chez des chiens éveillés, non-entraînés, et en santé pour applications futures lors d'essais thérapeutiques impliquant des chiens avec des maladies spontanées. Les méthodes testées étaient : stimulation mécanique de la trachée par compression digitale ainsi que par vibration à l'aide d'un rasoir électrique, massage du cou et sableur palmaire (11 chiens), et administration d'un irritant par nébulisation (3000 μM de capsaicine), et de solutions acide (1 M acide citrique) et hypotonique (eau déinonisée) par masque facial (4 chiens). Le seuil de succès était défini par l'induction d'au moins 2 épisodes de toux modérée ou forte chez au moins 75 % des chiens. Aucune des méthodes testées ne s'est avérée efficace. La compression digitale a induit une toux légère (n = 2) ou modérée (n = 1) chez 3 des 11 chiens éprouvés. La nébulisation d'acide citrique a induit 1 toux légère chez 1 des 4 chiens. Il a été conclu que la toux ne peut être induite avec succès chez des chiens éveillés, en santé en utilisant les méthodes qui ont du succès chez l'humain. D'autres stratégies doivent être développées afin que la sensibilité à la toux puisse être mesurée de manière objective et non-invasive chez des chiens aux fins de recherche clinique.

(Traduit par Docteur Serge Messier)

Introduction

Chronic bronchitis is a common progressive disease that affects primarily older dogs. Coughing can be so frequent or severe that it interferes with normal daily activities and sleep, and in some cases, necessitates euthanasia. Effective treatments are desperately needed. The ability to stimulate coughing is a valuable tool for studying the pathophysiology of cough and the effectiveness of antitussive therapy used in humans. Inhaling nebulized capsaicin is a safe and reliable way to induce coughing in both healthy people and those with respiratory disease. Accepted protocols include incremental increases in the inhaled concentration of capsaicin until a predetermined threshold of 2 or 5 coughs is produced. The relative sensitivity of the cough reflex can then be compared in individuals before and after treatments or between study groups. Nebulized capsaicin has also been shown to induce cough in awake guinea pigs (1–3) and in cats (4). Other methods for inducing cough in humans include: inhalation of acids, usually nebulized citric or tartaric acid; inhalation of nebulized hypotonic solutions; and external vibration of the trachea.

Published methods for stimulating cough in dogs are not suitable for routine application to patient populations. One of the first methods described involved suspending an iron slug between rubber bands inside the trachea (5). An electromagnet was used to vibrate

Department of Clinical Sciences (Boyle, Hawkins, Davis) and Department of Molecular Biomedical Sciences (Robertson), College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606, USA.

Address all correspondence to Dr. Eleanor Hawkins; telephone: (919) 513-7727; fax: (919) 513-6336; e-mail: eleanor_hawkins@ncsu.edu

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the slug to induce coughing. In later studies, coughing was induced in anesthetized dogs by mechanically stimulating the laryngeal or tracheal mucosa using a stiff, small-gauge catheter or similar device (6–11). Coughing has also been induced by introducing liquid or nebulized irritants or acids directly into the trachea through an intratracheal catheter, endotracheal tube, or tracheostomy tube (12–14).

The objective of this study was to determine if methods used to induce coughing in humans would consistently induce coughing in dogs. The success of such a methodology would allow the sensitivity of the cough reflex to be objectively measured in canine patients, without requiring anesthesia, invasive procedures, or training of subjects (such as adaptation to specialized masks). This would decrease costs and make it easier to recruit client-owned dogs with spontaneous cough for future studies of existing and novel therapies. To mechanically stimulate the trachea, digital compression and external stimulation using vibration from an electric shaver (15) and 2 additional devices were tested. Three solutions were administered by nebulization: capsaicin, citric acid, and deionized water. Additionally, a moderately invasive technique of internal stimulation under anesthesia was used as a positive control.

Materials and methods

Eleven dogs were included in this study. Nine dogs were obtained from Laboratory Animal Resources of North Carolina State University and 2 dogs were pets belonging to 1 of the investigators (Boyle). The research dogs consisted of 2 beagles and 7 mesocephalic mixed-breed dogs. There were 2 female and 7 male dogs with a median age of 9.5 mo (mean, 20.2 mo; range: 9.5 to 58 mo) and median weight of 25.6 kg (mean, 22.3 kg; range: 14 to 27 kg). The pet dogs were a 4-year-old female English cocker spaniel weighing 9 kg and a 3-year-old female golden retriever weighing 26 kg.

It was determined that all dogs taking part in the study were healthy based on physical examination, heartworm antigen test, arterial blood gas analysis, complete blood (cell) count (CBC), and serum biochemical panel. In addition, the dogs had unremarkable thoracic radiographs and no fluoroscopic evidence of airway collapse during tidal breathing. All dogs had been observed for a minimum of 6 mo before the study and none had a history of coughing. All procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

The dogs were awake and minimally restrained for all procedures. One investigator (Boyle) performed all attempts at cough induction and assessed responses in collaboration with 1 or more assisting investigators. The dogs were observed for coughing during stimulation attempts and continuously for a minimum of 1 h afterwards. The number of coughs and the time of coughs relative to stimulation attempts were noted. In addition, the intensity of cough was subjectively assessed as either soft (minimal effort and of low volume, as with throat clearing), moderate (between soft and strong in volume and effort), or strong (loud, harsh cough with abdominal push). Any resistance to procedures was noted.

Dogs were observed for adverse effects such as rhinitis, conjunctivitis, delayed cough, respiratory distress, or depression throughout the procedures, the post-stimulation observation period, and every 8 h for 24 h. In order for a method to be considered successful for clinical use, a threshold was set for induction of at least 2 coughs of moderate or strong intensity in at least 75% of the dogs.

Four methods of external stimulation of the trachea were tested: digital compression; vibration with a bladeless electric shaver (Norelco Electric Razor 7310XL; Philips, Stamford, Connecticut, USA); a vibrator removed from a neck massager (n.a.p. Massaging Wrap; Brookstone, Merrimack, North Carolina, USA); and a palm sander without sand paper (Corner Cat Finish Sander #CFS1503K; Ryobi, Hiroshima, Japan). The trachea of each dog was externally stimulated both manually and mechanically (N = 11). Digital compression was attempted first, which involved manually compressing the trachea between the fingertips and thumb up and down the length of the extra-thoracic trachea, multiple times, for at least 2 min or until a cough was produced. Different aspects of the trachea were compressed in each dog, with the fingertips and thumb approximately 180° apart on the either side of the trachea for some attempts, and with the fingertips near the dorsal tracheal membrane for other attempts. While it was not measured, the force of compression was aggressive and the same as would be used in routine clinical practice to evaluate tracheal collapse or sensitivity.

Each of the 3 devices for producing vibration was then tested in order of gentlest to strongest vibration (razor, massager, and sander). Each device was placed firmly against the trachea and incrementally moved along the length of the extra-thoracic trachea, maintaining each position for a minimum of 5 s. This action was continued for at least 2 min for each device.

Nebulization of irritant, acidic, and hypotonic solutions was tested. A 3000 μM solution of capsaicin was used as the irritant solution. It was prepared by dissolving 30.5 mg of capsaicin powder in 1 mL of ethanol and 1 mL of Tween 80, followed by dilution in 0.9% sodium chloride, as previously described (2). Citric acid, the acidic solution, was dissolved in deionized water to a concentration of 1 M. Deionized water was used for the hypotonic solution. Stock solutions were stored at 4°C and used within 1 mo of preparation. A jet nebulizer (Micro Mist nebulizer; Devilbiss Healthcare, Somerset, Pennsylvania, USA) and compressed oxygen were used to nebulize the solutions, with an approximate rate of output of 1 mL/min. The solutions were administered to the dogs using an anesthetic face mask with the rubber gasket placed completely over the dog's muzzle. Each nebulized solution was administered continuously for 5 min or until repeated coughing was induced.

One or more solutions were tested in 8 different dogs. Tests of more than 1 solution in the same dog were separated by at least 3 h and each solution was administered to at least 2 dogs that had not been previously exposed to nebulized solutions.

Because of our failure to produce a cough using non-invasive methods, attempts were made to induce cough in 4 dogs by directly stimulating the larynx and carina under anesthesia. Cough induction had been attempted in all 4 dogs using external stimulation and in 1 dog using nebulized solutions. Each dog was anesthetized with ketamine (Ketaset; Fort Dodge Animal Health, Ames, Iowa, USA), 5 to 7.5 mg/kg bodyweight (BW), IV and midazolam (Hospira; Wake Forest, Illinois, USA), 0.3 mg/kg BW. A polypropylene urinary catheter (3.5 to 5 mm in diameter) was used to mechanically stimulate the larynx and the distal trachea at the level of the carina. Liquid barium was placed within the catheter and fluoroscopic guidance was used to assess the position of the catheter within the trachea. Each dog's larynx was stimulated for 30 s, followed by stimulating the distal trachea for an additional 30 s. Each site was then stimulated a second time. The timing, number, and subjective intensity of coughs were noted.

Results

None of the non-invasive methods for inducing cough was successful. Digital compression was the only method of mechanical stimulation that induced a cough in any of the 11 dogs tested. A soft cough was repeatedly induced in 1 dog, a single soft cough was induced in 1 dog, and a single soft cough and a single cough of moderate intensity were induced in 1 dog. Coughs occurred only during the actual digital compression procedure. Digital compression was the only method during which the dogs appeared to be slightly uncomfortable, based on their weak attempts to avoid further manipulation.

Capsaicin and deionized water did not induce a cough in any dog during nebulization. One dog administered capsaicin had a single moderate intensity cough 5 min after nebulization when pulling against his collar on the way back to his cage. Citric acid induced 1 soft cough in 1 dog. This dog also exhibited licking behavior during nebulization. One dog administered water also exhibited licking behavior during nebulization. All dogs were at ease throughout nebulization and no adverse signs were noted as a result of nebulization using a face mask. Although not an intentional positive control, the citric acid and capsaicin induced coughing in the investigators before the mask was placed on the dogs. Remarkably, the relatively small concentration of capsaicin that escaped through the closed door of the room where testing was performed also induced coughing in several people passing through the hallway while the dogs were being nebulized.

Mechanical stimulation of the larynx and distal carina during anesthesia was effective for inducing multiple coughs of moderate intensity in all 4 dogs tested and several strong coughs in 1 of the dogs. The subjectively strong coughs induced in 1 dog were a result of laryngeal stimulation. Coughs occurred singly or in pairs during the time of stimulation; no paroxysms of cough were induced.

Discussion

To the authors' knowledge, there are no safe, reliable methods for inducing cough that are suitable for studying naturally occurring disease in client-owned dogs. No previous studies in dogs could be found that have investigated the non-invasive methods for cough induction used successfully in humans. The failure of these methods to induce cough in dogs is an important addition to the available literature for investigators planning future studies of cough, as their study design must either include development of a novel approach to cough induction or allow for subjects to undergo anesthesia or invasive procedures. Indeed, this study was intended to be preliminary to a more extensive study of spontaneous coughing in dogs. Although nebulized solutions were tested at a single concentration and in only 4 dogs each, the lack of success in any dog suggests that the protocols used will not induce cough in a sufficient number of subjects to be useful in the study of naturally occurring disease populations.

External mechanical stimulation of the trachea is a less reliable method for inducing cough in humans than nebulization of capsaicin or acidic solutions. Vibration created by an electric shaver placed over the jugular notch induced cough in most humans with acute upper respiratory tract infection (28 out of 30), but caused little or no cough in healthy subjects (15). Methods of external mechanical stimulation were included for evaluation in dogs in this study because of their great safety, ease of use, and affordability. It is possible that the relatively thicker skin and hair coat over the trachea in the dog prevented vibration from even a palm sander from stimulating cough receptors.

Capsaicin has become the favored solution for inducing cough in humans (16). It is safe, consistently induces cough, and has a high degree of long-term reproducibility for individuals (17,18). The European Respiratory Society recommends using serial doubling concentrations of capsaicin ranging from 0.49 to 1000 μM (18). The fixed time periods of administration during tidal breathing range from 15 to 60 s (18). In studies by Laude et al (1) and Fujimora et al (19,20), mean concentrations of capsaicin that induced 5 coughs in healthy humans were as low as 2.8 to 19.8 μM. The protocol used by Laude and co-workers was to administer each concentration of capsaicin in 4 single inhalations of 1 s each (1). The protocol used by Fujimora et al (19,20) was to administer capsaicin for 15 s, with a 60-s break between concentrations.

Ultrasonic nebulization of capsaicin delivered by face mask was successful in inducing cough in cats. Capsaicin was delivered at increasing concentrations every minute, with 1 min in between. Five or more coughs were induced in 8 of 9 cats at a mean concentration of 13.8 μM, with a maximum delivered concentration of 500 μM (4). In guinea pigs, a 2-min challenge induced 5 or more coughs at a mean concentration of 6.0 μM (1).

We chose a single, relatively high concentration of capsaicin (3000 μM) with the original intention of then lowering the concentration to determine an average minimum concentration for cough induction. While the concentration tested is only 3 times the maximum recommended concentration for routine testing in humans, it is over 100 times greater than some of the reported mean concentrations resulting in cough in humans, guinea pigs, and cats. Exposure times were also prolonged to increase the total quantity of inspired capsaicin. The relative insensitivity of dogs compared with humans was confirmed in this study by the induction of cough in humans with incidental exposure.

Citric acid nebulization is also considered to be safe, consistent, and reproducible (18). The European Respiratory Society recommends using serial doubling concentrations of citric acid ranging from 1.95 to 3000 mM (18). As with administering capsaicin, fixed time periods during tidal breathing range from 15 to 60 s (18). Cough was consistently induced in dogs by nebulization of 0.05 M (13) and 0.5 M of citric acid (14) through a tracheal tube. Citric acid has been administered by nebulization to guinea pigs inside a whole body plethysmograph at the single concentration of 0.6 M delivered for 3 min and successfully induced cough (21). Reported mean concentrations required to induce coughs in humans include 0.14 M (5 coughs) (1) and 0.15 M (2 coughs) (22). The concentration of citric acid tested in the current study (1 M) was equal to the maximum concentration recommended for routine testing, but over 6 times the reported mean concentrations resulting in cough in some humans.

When nebulizing hypotonic solutions to induce cough, the European Respiratory Society recommends that high volumes of deionized water be administered using an ultrasonic nebulizer (18). Deionized water was included in the testing protocol because it was readily available and inexpensive. It was administered at the maximum rate achievable with the system used. Even with ultrasonic delivery, nebulized deionized water is not as reliable for inducing cough as capsaicin and citric acid are in humans (16).

Cough was not successfully induced in dogs despite higher concentrations of capsaicin and citric acid than those used successfully in humans, guinea pigs, and cats. It is likely that the filtering capacity of the canine nasal cavity decreased the amount of capsaicin or acid reaching the lower airways. Citric acid successfully induced cough in dogs at a relatively dilute concentration (0.05 M) when nebulized through a tracheostomy tube (13). However, 0.5 M of citric acid produced only a weak cough in 2 of 6 dogs when nebulized through the larynx, compared with successful induction of cough in all 6 dogs when administered through a tracheal tube (14). The concentration of nebulized solutions that reaches the airways of dogs when delivered by face mask has not been well studied, but saline nebulization is commonly recommended as therapy for dogs with severe pneumonia and delivering steroid by metered dose inhaler and face mask can be effective in treating bronchitis (23).

The location, concentration, and sensitivity of cough receptors in dogs, compared with humans, may also have contributed to the failure of nebulized solutions to induce cough. Cough receptors may be more sensitive in dogs with airway inflammation and it is possible that the protocols tested would be successful in inducing cough in studies of diseased animals. However, the inability to induce cough in healthy dogs precludes their inclusion as control dogs in such studies.

Adaptation of cough receptors to stimuli (tachyphylaxis) has been reported and the European Respiratory Society recommends no less than 1 h, and ideally 2 h, between challenges with capsaicin or citric acid (18). In the current study, at least 3 h elapsed between test solutions, and each solution was administered to at least 2 dogs before the testing of other solutions.

Considerations for future tests of nebulized solutions include the use of higher concentrations of capsaicin or citric acid. Higher concentrations increase the risk of inducing local inflammation of the oral or nasal mucosa or skin and could require that testing be done in controlled environments that minimize exposure to people. Another strategy would be to develop a system of administration that would noninvasively bypass the nasal cavity, perhaps through a specially designed mouthpiece in conjunction with obstruction of the nares. Such methods are likely to require training the dogs to accept the device, as a relaxed patient and exposure to numerous concentrations would ultimately be required to obtain reliable data.

In conclusion, methods that are successful in inducing cough in humans, guinea pigs, and cats were not successful in inducing cough in dogs. Other strategies must be developed so that cough sensitivity can be objectively and non-invasively measured in awake, untrained dogs. A successful strategy for inducing cough in dogs would allow studies to be conducted of dogs with naturally occurring disease and thus allow treatments to be objectively evaluated.

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