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Short communication

Antitussive activity of the tachykinin NK_1 receptor antagonist, CP-99994, in dogs

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Abstract

CP-99994 [(+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine] is a selective tachykinin NK_1 receptor antagonist that inhibits cough in guinea pigs and cats. This study examined the antitussive effects of CP-99994 in dogs produced by mechanical stimulation of the intrathoracic trachea. CP-99994 (10 mg/kg, p.o.) inhibited cough frequency by 52% at 2 h, 31% at 6 h and by 21% at 24 h. Cough amplitude was inhibited by 45% at 6 h but unchanged at 2 and 24 h after CP-99994. Plasma levels of CP-99994 were highest at 2 h (75 ± 26 ng/ml) and fell to 22 ± 6 ng/ml at 6 h. These results demonstrate antitussive activity of CP-99994 in dogs at a dose proven to antagonize tachykinin NK_1 receptors in this species.

Keywords: Cough; Tachykinin NK_1 receptor; CP-99994; (Dog)

1. Introduction

Tachykinins are important neuromodulators of the cough reflex and have effects in several species including guinea pigs (Kohrogi et al., 1988; Advenier et al., 1993; Takahama et al., 1993; Ujie et al., 1993; Sekizawa et al., 1995; Bolser et al., 1997) and cats (Bolser et al., 1997). In guinea pigs, a tussive effect of inhaled substance P and neurokinin A has been demonstrated (Kohrogi et al., 1988; Takahama et al., 1993; Sekizawa et al., 1995), and in both these species, antagonists for the tachykinin NK_1 (Ujie et al., 1993; Bolser et al., 1997) and the tachykinin NK_2 (Advenier et al., 1993; Ujie et al., 1993) receptors inhibit cough. Furthermore, in guinea pigs, inhibitors for the tachykinin NK_3 receptor also inhibit cough (Daoui et al., 1998). In a recent study, a technique was described that measures the cough reflex in propofol-anesthetized dogs (Chapman et al., 2001). This method involves the measurement of cough after stimulation of the carina with either intratracheal instillation of distilled water (Chapman et al., 2001) or by mechanical activation of airway sensory nerves by probing the region of the carina (Chapman et al., 2003).

In this experiment, the antitussive activity of CP-99994 [(+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine], a tachykinin NK_1 receptor antagonist that inhibits cough in both guinea pigs and cats (Bolser et al., 1997), was measured in mechanically induced cough in dogs.

2. Materials and methods

Male beagle dogs (weight 10–18 kg) were fasted overnight but given water ad libitum. The experiments were performed with the prior approval from the Animal Care and Use Committee of Schering-Plough Research Institute (SPRI) which is a facility accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

2.1. Cardiopulmonary measurements

Details of the methods used to measure cardiopulmonary functions in dogs are described elsewhere (Chapman et al., 2001). Briefly, the dogs were anesthetized with bolus injection of propofol (8 mg/kg, i.v.) that was continuously infused (rate = 0.3–0.8 mg/kg, i.v.) throughout the studies. The amount of propofol (both bolus injection and infusion rate) was optimized for individual dogs such that cough was

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produced upon deflation and reinflation of the cuff on the endotracheal tube.

The dogs were placed in the supine position and a cuffed endotracheal tube was inserted into the trachea and positioned at a location just above the carina. The endotracheal tube was connected to a pneumotachograph/differential pressure transducer system for the measurement of pulmonary airflow and tidal volume. A balloon-tipped polyethylene catheter (i.d. = 2 mm) was inserted through the mouth into the esophagus and positioned at a location in the mid-thoracic region. Transpulmonary pressure was measured as the difference in pressure between the esophageal catheter and the endotracheal tube. The pulmonary airflow, tidal volume and transpulmonary pressure signals were processed by a pulmonary function computer (Buxco Electronics, Sharon, CT, USA). Additionally, these signals were visually displayed on a chart recorder.

Mean arterial blood pressure was measured with a veterinary blood pressure monitor that derives blood pressure from an inflatable cuff placed around the hind paw. Arterial oxygen saturation and heart rate were measured with a pulse oximeter that was clipped onto the tongue. End-tidal CO_2 was measured directly at the tip of the endotracheal tube using a Cardiocap™ monitor.

2.2. Experimental protocol

The baseline measurement of tidal volume, respiratory rate, minute volume, mean arterial blood pressure, heart rate, arterial oxygen saturation, end-tidal CO_2 and the amount of propofol (both bolus injection and infusion rate) were recorded in each dog to assess the side effect profile of the drugs as previously described for torbutrol (Chapman et al., 2001). The endotracheal tube was then carefully disconnected from the pneumotachograph and a predetermined length of fishing line was inserted through the endotracheal tube to mechanically stimulate the region of the carina (Chapman et al., 2003). The fishing line (50-lb test, Berkley, Spring Lake, IA, USA) was inserted and withdrawn five times over a 5-s period and cough was measured during and after this stimulation. Measurements of the cough frequency (no. of coughs) and cough amplitude (cm H_2O increase in expiratory pressure over baseline) were made from chart recordings of transpulmonary pressure as previously described (Chapman et al., 2001, 2003). This procedure was repeated three times, and from these three trials, the average cough response was reported. When the cough response to the final stimulation had finished and the animals had returned to normal breathing, the endotracheal tube was reconnected to the pneumotachograph and cardiopulmonary functions were measured for up to 3 min after the challenge. At the end of the study, the propofol infusion was stopped and the dogs were allowed to recover.

The dogs were orally dosed with CP-99994 which was delivered in a gelatin capsule. The placebo trial involved oral dosing with an empty capsule. CP-99994 was evaluated at a dose of 10 mg/kg, p.o., which is a dose that demonstrates tachykinin NK_1 receptor antagonist activity in dogs (Sherwood et al., 1998). Experiments with CP-99994 or vehicle control were performed at 2, 6 and 24 h after dosing with the drug. Each dog was studied at each of the three time points. A venous blood sample was obtained in each dog dosed with CP-99994 at each time point. The blood was placed in heparinized tubes, and the plasma was separated by centrifugation. Plasma levels of the drug were measured by liquid chromatography-mass spectrometry/mass spectrometry with an atmospheric pressure chemical ionization source (LC-APCI/MS/MS) as described previously (Chapman et al., 1999).

2.3. Statistics

The drug evaluations were performed with a crossover experimental design. Statistically significant effects of CP-99994 were assessed using a paired *t*-test comparing responses at each time point in drug treatment to vehicle treatment. A $P < 0.05$ was considered a statistically significant effect.

3. Results

Treatment of dogs with CP-99994 (10 mg/kg, p.o.) inhibited the cough frequency response to mechanical stimulation of the carina (Fig. 1). CP-99994 inhibited the cough frequency by 52% at 2 h ($P = 0.009$), 31% at 6 h ($P = 0.048$) and 21% at 24 h ($P = 0.177$). There was no significant effect of CP-99994 on cough amplitude at 2 h, but CP-99994 inhibited cough amplitude by 45% at 6 h ($P = 0.038$). By 24 h, the effect of CP-99994 on cough amplitude had dissipated (Fig. 2). The plasma levels of CP-99994 were highest at 2 h (75 ± 26 ng/ml) and fell to 22 ± 6 ng/ml at 6 h and to 1 ± 0 ng/ml at 24 h after dosing with 10 mg/kg of the drug. CP-99994 (10 mg/kg, p.o.) had no significant effect on ventilation, end-tidal CO_2, arterial

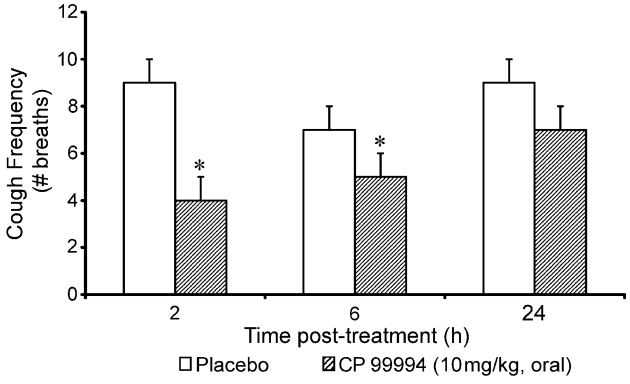

Fig. 1. Effect of CP-99994 on cough frequency. Values are mean ± S.E.M. ($n = 9$ per treatment). *$P < 0.05$ compared to placebo.

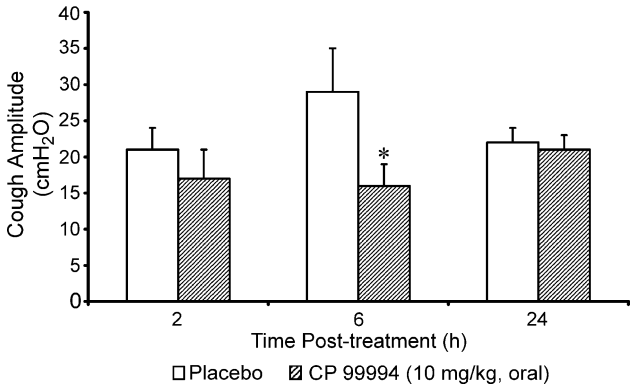

Fig. 2. Effect of CP-99994 on cough amplitude. Values are mean ± S.E.M. ($n = 9$ per treatment). *$P < 0.05$ compared to placebo.

oxygen saturation, mean arterial blood pressure, heart rate or on the amount of anesthesia used (data not shown).

4. Discussion

The results in this study demonstrate the antitussive effects of CP-99994 against mechanically induced cough in dogs. Previous studies in guinea pigs (Ujie et al., 1993; Bolser et al., 1997) and cats (Bolser et al., 1997) demonstrate an inhibition of cough with tachykinin NK_1 receptor antagonists, including CP-99994, but this is the first report of tachykinin NK_1 receptor antagonists inhibiting cough in dogs. These results differ from the findings in human asthmatics where CP-99994 failed to inhibit cough induced by inhaled hypertonic saline (Fahy et al., 1995). Mechanistically, cough induced by mechanical stimulation of the intrathoracic trachea involves stimulation of rapidly adapting receptors in the region of the carina (Widdicombe, 1996), whereas cough induced by hypertonic saline involves activation of C-fiber afferents (Jonjegan et al., 1991), and there may be a contribution from sensory nerves in the larynx. Furthermore, cough induced by these two stimuli likely involves different neural pathways within the central nervous system (CNS) (Jordan, 1996). Therefore, CP-99994 may be an effective antitussive agent against mechanical cough but be less effective against cough induced by other stimuli such as inhaled hypertonic saline.

In a previous studies in dogs (Sherwood et al., 1998; Chapman, et al., 1999), CP-99994 at 10 mg/kg, p.o. given 2 h before intravenous substance P almost completely inhibited the increase in minute ventilation and decrease in blood pressure due to substance P. Plasma levels of CP-99994 that were associated with this pharmacological effect averaged 55 ± 9 ng/ml (Chapman et al., 1999). In the present study, plasma levels of CP-99994 2 h after oral dosing with 10 mg/kg of the drug averaged 75 ± 26 ng/ml. At this time point, CP-99994 significantly inhibited the cough frequency response to mechanical stimulation of the carina with no effect on cough amplitude. By 6 h after oral dosing with CP-99994 at 10 mg/kg, the plasma levels of the drug averaged 22 ± 6 ng/ml and there was significant inhibition of both cough frequency and amplitude. By 24 h after CP-99994, the plasma levels of CP-99994 were barely above the level of quantification and its antitussive activity had dissipated.

It is important to note that CP-99994 rapidly penetrates into the CNS (Ward et al., 1995) and blocks the emetic reflex in dogs by acting at the level of the brainstem (Andrews et al., 2001) which is also the region of the CNS that integrates the cough reflex. CP-99994 is a centrally acting antitussive drug (Bolser et al., 1997) and may act at two distinct CNS sites with one controlling input from sensory afferent nerves, thereby inhibiting cough frequency, and another acting on expiratory motor neurons, thereby inhibiting expiratory cough amplitudes (Bolser et al., 1999). Therefore, CP-99994 may temporally act at these two sites blocking only cough frequency at 2 h but blocking both cough frequency and amplitude at 6 h. There were no adverse side effects of CP-99994 on ventilation or cardiovascular function at the doses studied. Standard agents like torbutrol, an opioid antitussive drug, produce mild respiratory depression in anesthetized dogs that is usually manifest in propofol-anesthetized dogs as an increase in end-tidal CO_2 (Chapman et al., 2001, 2003).

In summary, the results of this study show an inhibition of cough by CP-99994 in dogs and this effect was seen for up to 6 h after oral administration of the drug. These results demonstrate antitussive activity of CP-99994 in dogs at a dose of the drug proven to antagonize tachykinin NK_1 receptors in this species.

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