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Short communication

Antitussive effect of moguisteine on the enhanced coughing associated with enalapril in guinea-pig

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Abstract

The effect of moguisteine, a novel peripherally acting non-narcotic antitussive drug, on coughs associated with enalapril was examined in guinea-pigs. Chronic treatment with enalapril markedly enhanced the number of capsaicin-induced coughs. Moguisteine dose-dependently suppressed the number of coughs at doses between 3–30 mg/kg p.o., in both vehicle-treated and enalapril-treated animals. There was no significant difference in the antitussive ED_{50} (95% confidence limit) value of moguisteine between vehicle-treated (16.4 (13.7–19.7) mg/kg) and enalapril-treated (13.7 (3.9–47.6) mg/kg) animals. On the other hand, dihydrocodeine also dose-dependently suppressed the number of coughs in the same dose range as moguisteine in both vehicle-treated and enalapril-treated animals. There was no significant difference in the antitussive ED_{50} (95% confidence limit) of dihydrocodeine between vehicle-treated (11.7 (4.9–28.3) mg/kg) and enalapril-treated (11.2 (9.4–13.3) mg/kg) animals. Furthermore, the antitussive effect of moguisteine was identical to that of dihydrocodeine in both vehicle-treated and enalapril-treated animals. On the other hand, while chronic co-treatment with moguisteine significantly reduced the number of enhanced coughs associated with enalapril, chronic co-treatment with dihydrocodeine had no significant effect on the number of enhanced coughs associated with enalapril treatment. These results suggest that moguisteine may have a therapeutical benefit in reducing the coughing associated with treatment with inhibitors of angiotensin-converting enzyme.

Keywords: Cough reflex; Angiotensin-converting enzyme inhibitor; Enalapril; Moguisteine; Non-narcotic antitussive drug

1. Introduction

Moguisteine ((*R,S*)-2-(2-methoxyphenoxy)-methyl-3-ethoxycarbonyl-acetyl-1,3-thiazolidine), a non-opioid compound, was first synthesized by Gallico et al. (1994), who reported that it was an effective antitussive agent under several common experimental cough conditions. Moguisteine proved to be as active as codeine in reducing coughs induced in guinea-pigs by chemical irritants, such as citric acid and capsaicin, or by mechanical or electrical stimulation of the trachea. Naloxone, an opioid antagonist, abolished the antitussive effect of codeine, but not that of moguisteine. Furthermore, while intracerebral administration (i.c.v.) of moguisteine had no effect on electrically induced coughs, i.c.v. codeine and dextromethorphan were dose-dependently effective under the same experimental conditions. Based on these results, Gallico et al. (1994) suggested that the site of action of moguisteine is at a peripheral level.

Several reports have linked the use of angiotensin-converting enzyme inhibitors in the treatment of hypertension with a dry and non-productive coughs (Saseko and Kaneko, 1985; Webb et al., 1986; Nash, 1986; Fuller and Choudry, 1987; Hood et al., 1987; Bucknall et al., 1988). Bucknall et al. (1988) and Subissi et al. (1990) suggested that coughs induced by angiotensin-converting enzyme inhibitors may be associated with bronchial hyperreactivity. Previously, we suggested that the airway hyperreactivity associated with angiotensin-converting enzyme inhibitors may be caused by airway inflammation (Kamei and Kasuya, 1992). Recently, Gallico et al. (1996) reported that moguisteine reduced tobacco smoke-induced bronchial hyperreactivity. Furthermore, moguisteine abolished eosinophil recruitment in bronchoalveolar lavage, prevented the sloughing of the epithelium and significantly reduced airway microvascular leakage. Based on these results, they

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suggested that moguisteine has interesting inhibitory effects on the airway-inflammation process, in addition to its antitussive properties.

An antitussive drug with airway anti-inflammatory properties could be a valuable tool for the treatment of coughing associated with angiotensin-converting enzyme inhibitors. To test this hypothesis, we investigated the effect of moguisteine on coughs associated with chronic treatment with enalapril.

2. Materials and methods

2.1. Animals

Male Hartley guinea-pigs (Tokyo Animal Laboratory, Tokyo, Japan) weighing about 300 g at the beginning of the experiments were used. The animals were housed in groups of four per cage under a 12-h light-dark cycle with food and water continuously available. The studies were carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on care and use of laboratory animals of Hoshi University which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Drug treatment

2.2.1. Effect of single treatment with moguisteine

Guinea-pigs received oral doses of 3 mg/kg of enalapril daily at 13:00 h for 14 days. On day 15, the effects of moguisteine and dihydrocodeine on the number of capsaicin-induced coughs were determined. Control animals received similar oral doses of the vehicle alone.

2.2.2. Effect of chronic treatment with moguisteine

Guinea-pigs received oral doses of 3 mg/kg of enalapril in combination with either dihydrocodeine (3 mg/kg), moguisteine (3 mg/kg) or vehicle (purified water) daily at 13:00 h for 14 days. On day 15, the effects of these drugs on the number of capsaicin-induced coughs were determined.

2.3. Antitussive assay

The cough reflex was induced as previously described (Kamei et al., 1989; Kamei and Kasuya, 1992). Briefly, animals were exposed to a nebulized solution of capsaicin (30 μM) under conscious and identical conditions using a body plethysmograph. Capsaicin was dissolved to a concentration of 30 mg/ml in a 10% ethanol and 10% Tween 80 saline solution. The solution was diluted with saline. The animals were exposed for 10 min to capsaicin 60 min before injection of antitussive drugs, to determine the frequency of control coughs (Cc). The animals were also exposed for 10 min to capsaicin 60 min after administration of the drugs. The number of coughs produced after antitussive drug injection (Ct) was compared with the number of control coughs (Cc). The antitussive effect was expressed as the % inhibition of the number of control coughs $((Cc - Ct)/Cc \times 100)$.

2.4. Drugs

Moguisteine ((*R,S*)-2-(2-methoxyphenoxy)-methyl-3-ethoxycarbonyl-acetyl-1,3-thiazolidine) was generously supplied by Boehringer Mannheim Italia. Morphine hydrochloride and enalapril maleate were purchased from Sankyo (Tokyo, Japan) and Sigma Chemical Co. (St. Louis, MO, USA), respectively. Moguisteine was suspended in 0.5% sodium carboxymethyl cellulose. All other drugs were dissolved in saline.

2.5. Statistics

Data are expressed as the means ± S.E. The statistical significance of differences was assessed by the Mann-Whitney *U*-test to evaluate the antitussive effect. A level of probability of 0.05 or less was considered significant. ED_{50} values for the antitussive effect and 95% confidence limits (95% CL) were determined using linear regression techniques.

3. Results

3.1. Antitussive effect of moguisteine in naive animals

Moguisteine dose-dependently inhibited the number of capsaicin-induced coughs when the antitussive effect was examined 60 min after administration (Fig. 1A). The inhibition of capsaicin-induced coughs by dihydrocodeine was very similar to that obtained with moguisteine, with ED_{50} (95% CL) values of 12.3 (8.2–18.5) and 10.3 (5.6–19.1) mg/kg p.o., respectively (Fig. 1A).

3.2. Antitussive effect of moguisteine in enalapril-treated animals

Exposure of control (vehicle-treated) animals to a nebulized solution of capsaicin induced 26.9 ± 3.6 coughs/10 min ($n = 45$). After administration for 14 days, enalapril significantly ($P < 0.05$) increased the number of capsaicin-induced coughs (45.6 ± 4.6 coughs/10 min, $n = 45$).

Moguisteine dose-dependently inhibited the enhancement of capsaicin-induced coughs associated with enalapril at the same doses as in vehicle-treated guinea-pigs (Fig. 1B). Indeed, there was no significant difference in the ED_{50} (95% CL) of moguisteine between enalapril-treated

(13.7 (3.9–47.6) mg/kg p.o.) and vehicle-treated (16.4 (13.7–19.7) mg/kg p.o.) animals. Dihydrocodeine also effectively reduced the enhancement of capsaicin-induced coughs associated with enalapril at the same doses as in vehicle-treated guinea-pigs. The antitussive effect of dihydrocodeine in enalapril-treated guinea-pigs was very similar to that in vehicle-treated guinea-pigs, with ED_{50} (95% CL) values of 11.2 (9.4–13.3) and 11.7 (4.9–28.3) mg/kg p.o., respectively (Fig. 1C).

3.3. *Effect of chronic treatment with moguisteine on the enhancement of capsaicin-induced coughs associated with enalapril*

Chronic treatment with moguisteine (3 mg/kg p.o.) in combination with 3 mg/kg of enalapril significantly reduced the enalapril-induced increase in the number of capsaicin-induced coughs (Fig. 2). On the other hand, chronic treatment with dihydrocodeine (3 mg/kg p.o.) in combination with 3 mg/kg of enalapril had no significant effect on the enalapril-induced increase in the number of capsaicin-induced coughs (Fig. 2). Furthermore, chronic treatment with either moguisteine or dihydrocodeine, by themselves, had no effect on the number of capsaicin-induced coughs.

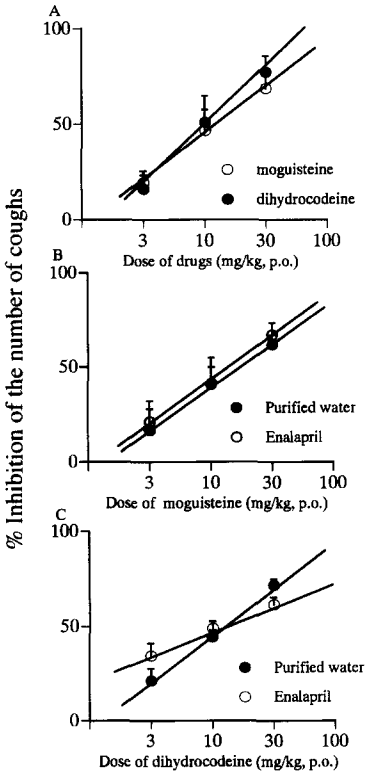

Fig. 1. Dose-response curves for the antitussive effects of moguisteine and dihydrocodeine in guinea-pigs. (A) Antitussive effects of moguisteine and dihydrocodeine in naive guinea-pigs, (B) antitussive effects of moguisteine in enalapril-treated guinea-pigs, and (C) antitussive effects of dihydrocodeine in enalapril-treated guinea-pigs. The antitussive effects of moguisteine and dihydrocodeine were assessed 60 min after p.o. administration of each drug. The guinea-pigs received oral doses of 3 mg/kg of enalapril daily at 13:00 h for 14 days. On day 15, the effects of moguisteine and dihydrocodeine on the number of capsaicin-induced coughs were determined. Control animals received similar oral doses of purified water alone. Each point represents the mean with S.E. ($n = 6$).

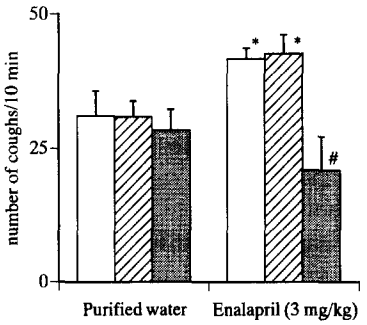

Fig. 2. Effects of moguisteine and dihydrocodeine on the coughs associated with chronic treatment with enalapril. The guinea-pigs received 3 mg/kg of enalapril in combination with moguisteine (3 mg/kg), dihydrocodeine (3 mg/kg) or vehicle (purified water) orally, daily for 14 days. On day 15, the effects of these drugs on the number of capsaicin-induced coughs were determined. Control animals received similar oral doses of purified water alone. Each column represents the mean with S.E. ($n = 6$) of the number of coughs produced during a 10-min exposure of capsaicin. * $P < 0.05$ vs. the respective purified water-treated group. $^{\#}$ $P < 0.05$ vs. the vehicle-treated value (open column).

4. Discussion

In the present study, we found that: (1) moguisteine is an effective antitussive agent that is as active as dihydrocodeine in reducing capsaicin-induced coughs in guinea-pigs; (2) the antitussive efficacy of both moguisteine and dihydrocodeine is also clearly reflected by their prevention of enhanced coughing associated with chronic treatment with enalapril; and (3) chronic treatment with moguisteine, but not dihydrocodeine, in combination with enalapril significantly reduced the number of coughs associated with enalapril. The first finding is consistent with the result of Gallico et al. (1994). They demonstrated that the antitussive effect of moguisteine on capsaicin-induced coughs were very similar to those of codeine, with ED_{50} (95% CL) values of 19.3 (12.1–26.3) and 15.2 (6.2–35.8) mg/kg p.o., respectively (Gallico et al., 1994). Furthermore, the second finding that moguisteine, as well as dihydrocodeine, effectively reduced the enhanced coughing associated with chronic treatment with enalapril with ED_{50} values similar to those in naive animals indicates that moguisteine may be effective in treating the coughing associated with angiotensin-converting enzyme inhibitors.

We were surprised to find that treatment with a low dose (3 mg/kg p.o.) of moguisteine, which had no significant effect on the number of coughs by itself, in combination with enalapril significantly reduced the enhanced coughing associated with enalapril. On the other hand,

treatment with a low dose (3 mg/kg p.o.) of dihydrocodeine in combination with enalapril had no significant effect on the enhanced coughing associated with enalapril. We previously obtained a similar result using hydrochlorothiazide, a diuretic (Kamei and Kasuya, 1992). Since angiotensin-converting enzyme degrades bradykinin, it has been shown that angiotensin-converting enzyme inhibitors increase bradykinin levels. Lötvall et al. (1990) found that captopril, an angiotensin-converting enzyme inhibitor, potentiated bradykinin-induced microvascular leakage in the airway. Therefore, it is possible that the higher levels of bradykinin in the airway that are induced by angiotensin-converting enzyme inhibitors may lead to pulmonary edema. Furthermore, it is also possible that angiotensin-converting enzyme inhibitors induce coughing in part via the pulmonary edema due to elevated levels of bradykinin. Based on these results, we previously proposed that the inhibitory effect of hydrochlorothiazide on the coughing associated with enalapril may be due to inhibition of the development of pulmonary edema. In this regard, Gallico et al. (1996) reported that moguisteine reduced airway inflammation, which is characterized by bronchial hyperreactivity, induced by a variety of stimuli, such as exposure to cigarette smoke, infusion of platelet activating factor and inhalation of an allergen. The anti-inflammatory effect of moguisteine was observed at doses within the range of those that are effective against experimental coughs (Gallico et al., 1994). In humans, although there is no report which obviously indicates the interaction between angiotensin-converting enzyme inhibitor-induced coughs and airway inflammation, several clinical investigations suggested that increased inflammatory substances, such as bradykinin and prostaglandin, have an important role in the angiotensin-converting enzyme inhibitor-induced coughs (Bucknall et al., 1988; Fuller and Choudry, 1987; Saseko and Kaneko, 1985; Subissi et al., 1990). In this regard, we recently demonstrated that the cough-enhancing effect of angiotensin-converting enzyme inhibitors was reduced by pretreatment with indomethacin, one of potent anti-inflammatory drugs (Itoh et al., 1995). Therefore, it seems likely that the inhibitory effect of moguisteine on the enhanced coughing associated with enalapril and the anti-inflammatory effect of moguisteine may somehow be related.

On the other hand, it has been suggested that substance P released from sensory nerves in the airways may be an endogenous substance that causes coughs. Since (1) moguisteine dose-dependently reduces capsaicin-induced coughing, which is provoked by the release of substance P, (2) coughing during treatment with angiotensin-converting enzyme inhibitors is caused by increased sensitivity to capsaicin (Fuller and Choudry, 1987), and (3) angiotensin-converting enzyme inhibitors potentiate the bronchoconstriction induced by substance P (Subissi et al., 1990), it is possible that the inhibitory effect of moguisteine on the enhanced coughing associated with enalapril might be due to reduction of the sensitivity to substance P in the airway. However, this possibility can be eliminated by the findings of Gallico et al. (1996). They suggested that the anti-inflammatory effect of moguisteine is not mediated by substance P-antagonistic activity, since moguisteine did not inhibit capsaicin-induced microvascular leakage (Gallico et al., 1996). Furthermore, moguisteine is an effective antitussive agent in a number of commonly used experimental cough models, such as chemical irritant (capsaicin or citric acid), mechanical stimulation and tracheal electrical stimulation (Gallico et al., 1994). Moreover, it has been reported that moguisteine is highly effective in treating the dry-cough in humans (Morrone et al., 1993). Although we examined the effect of moguisteine on the enhanced capsaicin-induced coughing associated with angiotensin-converting enzyme inhibitor, it is supposed that moguisteine may be effective not only in capsaicin-induced but also other cough stimuli-induced enhanced coughing associated with angiotensin-converting enzyme inhibitors.

In summary, the present study provides a basis for the potential use of moguisteine for reducing the coughing associated with angiotensin-converting enzyme inhibitors.

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