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Mucolytic and Antitussive Effects of Erdosteine

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Abstract

To investigate the influence of erdosteine, a new homocysteine-derived expectorant, on airway clearance we studied the effects of the drug on the viscosity of mucin, on the mucociliary transport rate in quails, on airway secretion in rats and on the cough reflex in guinea-pigs.

The active metabolite of erdosteine, M1 (10 μM to 1 mM), significantly reduced the viscosity of porcine stomach mucin. Erdosteine by itself did not reduce viscosity. Erdosteine significantly promoted mucociliary transport in quails and increased airway secretion in rats. The effect was still apparent 24 h after administration. Erdosteine significantly suppressed citric acid-induced cough reflexes in guinea-pigs but did not suppress mechanical stimuli-induced cough reflexes. Erdosteine suppressed the reduction of the recovery volume of bronchoalveolar lavage fluid and albumin leakage into the fluid in citric acid-exposed guinea-pigs.

These results indicate that erdosteine removes sputum by reducing its viscosity, and by promoting mucociliary transport and sustained enhancement of airway secretion. It also suppressed the chemical stimulation-induced cough reflex and plasma leakage into the airway. These results suggest that erdosteine is an excellent expectorant with several modes of action.

The cough reflex is an important respiratory defence system and is one of the most important symptoms of respiratory disease (Banner 1986). Although the cough reflex expels sputum and inhaled materials from the airways and plays an important role in pulmonary clearance, a persistent and intolerable dry cough (non-productive cough) is also induced by chemical mediators released from inflammatory cells (neutrophils, eosinophils, macrophages) which accumulate with airway inflammation (bronchitis, asthma) or airway infection. Thus, attenuation of cough is generally important in clinical therapy. Antitussive drugs, for example codeine phosphate, are used for this purpose. These drugs substantially suppress the cough reflex. However, for patients with sputum, significant suppression seems to cause prevention of sputum expulsion and sometimes leads to airway obstruction, requiring the use of expectorants.

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Expectorant drugs remove sputum by several mechanisms in patients with chronic bronchitis, asthma and airway infections. Ambroxol promotes normal mucous and surfactant secretion (Post et al 1983). *N*-acetylcysteine reduces the viscosity of sputum through the proteolytic activity of its thiol group (Carina et al 1990). *S*-Carboxymethylcysteine restores abnormal mucous secretion (Quevauviller et al 1976).

Erdosteine (KW-9144; (±)-({[(Tetrahydro-2-oxo-3-thienyl)carbamoyl]methyl}thio)acetic acid; Figure 1), a new homocysteine-derived expectorant developed by Refamed, promotes mucociliary transport (MCT) in pigeons and increases airway secretions in mice and rabbits (Scuri et al 1988); it also suppresses the inactivation of α-1-antitripsin by cigarette smoke (Gazzani et al 1989). Orally

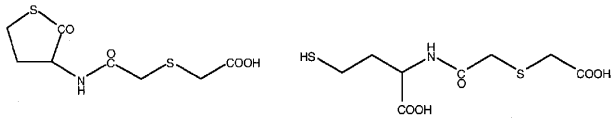

Figure 1. The chemical structures of erdosteine and its metabolite M1.

administered erdosteine is immediately converted to the active metabolite (M1, *N*-thioglycolylhomocysteine) which has mucokinetic and mucolytic properties (Scuri et al 1988). The antitussive effects of erdosteine have not been reported. To study the systemic airway cleaning effects of erdosteine we investigated the influence of erdosteine on mucin solution in-vitro, on mucociliary transport in quails, on airway secretion in rats and on cough reflexes in guinea-pigs.

Materials and Methods

Animals

Quails of both sexes, 100–130 g, were purchased from Kyudo (Fukuoka, Japan). Quails were chosen because a previous study showed that the MCT rate can be measured precisely in-situ by use of these birds (Miyata et al 1998). Male Hartley guinea-pigs, 300–500 g, and male Wistar rats, 180–220 g, were purchased from Japan SLC (Hamamatu, Japan). We used guinea-pigs to evaluate antitussive effects, because guinea-pigs are widely used in such experiments and the mechanisms of cough reflexes have also been investigated in these animals. We used rats to evaluate airway secretion, because the effects of several drugs have been investigated with these animals. These animal experiments were approved by the Animal Ethical Committee of Kyowa Hakko Kogyo (Shizuoka, Japan).

Drugs

Erdosteine and its metabolite M1 were obtained from Edmond Pharma (Milan, Italy). Ambroxol, *S*-carboxymethylcysteine, *N*-acetylcysteine and porcine stomach mucin were purchased from Sigma (St Louis, MO). Codeine phosphate was purchased from Takeda Chemical Industries (Osaka, Japan). For treatment of quails, erdostiene and *S*-carboxymethylcysteine were suspended in distilled water. For treatment of rats or guinea-pigs, erdosteine was dissolved in equimolar amounts of $NaHCO_3$ in distilled water. For treatment of rats or guinea-pigs, *S*-carboxymethylcysteine was dissolved in 1 M NaOH and neutralized with 1 M HCl before dilution with distilled water. Ambroxol and codeine phosphate were dissolved in distilled water. Administration volumes were 5 mL kg^{-1} for quails and guinea-pigs and 10 mL kg^{-1} for rats. In the in-vitro study all drugs were dissolved in Tris buffer.

Viscosity of porcine stomach mucin

A solution (15%) of mucin was prepared in Tris buffer (30 mM), and the pH was adjusted to 7·4 with 1 M NaOH. One-tenth the volume of the drug preparation in Tris buffer (30 mM, pH 7·0) was added and the solution was gently mixed. The reaction mixture was incubated at 37°C for 60 min. The rheological characteristics of the reaction mixture were then estimated with a cone and plate microrheometer (type R500; Toki, Tokyo). To measure the apparent viscosity the shear stress and measuring time were set at 0·015 s^{-1} and 90 s, respectively.

Mucociliary transport rate (MCT rate) in quails

The experimental protocol was based on a previous report (Tai et al 1996). Quails (five animals per group) were anaesthetized by intraperitoneal administration of urethane (1·6 g kg^{-1}). The trachea was carefully denuded and exposed. An incision was made in the trachea to enable observation of the interior. The observed site was placed in a box thermoregulated at 37°C and 100% humidity. Fine ash powders were placed on the mucus layer and the time for 2 mm movement was measured. The velocity was calculated and used as the MCT rate. The drug was given intragastrically. The MCT rate was measured at 10-min intervals during the next 120 min. The change in the MCT rate at each time was expressed as a percentage of the value before pretreatment.

Airway secretion in rats

Airway secretion was measured by use of a dye as detailed elsewhere (Koda et al 1978) with minor modifications. Between 5 and 20 animals per group were used. After oral administration of the tested drugs, phenolsulphophthaleine (6 mg kg^{-1}) was injected intravenously and the animals were exsanguinated 30 min later. Bronchoalveolar lavage was performed with $NaHCO_3$ solution (5%, 5 mL). The bronchoalveolar lavage fluid was gently aspirated, and centrifuged at 3000 rev min^{-1} for 30 min. The pH of the supernatant was adjusted to 8·0 with 1 M HCl. The absorbance at 558 nm was measured and the dye concentration was calculated.

Citric acid-induced cough reflex

The experimental protocol was a slightly modified version of the method of Miyata et al (1993).

Conscious guinea-pigs (7–10 animals per group) were placed in a whole-body plethysmograph, exposed to 5% citric acid (in distilled water) aerosols for 1 min, and the number of cough reflexes was counted for the next 13 min. Guinea-pigs that experienced cough reflexes from 4 to 10 times were chosen. After a 4-h interval, the cough reflex was induced again after administration of drug. Bronchoalveolar lavage with physiological saline (10 mL) was performed with some animals (six animals per group) before and after citric acid exposure and albumin concentrations in the bronchoalveolar lavage fluid were measured (Doumas & Biggs 1972). All drugs were administered 1 h before exposure to citric acid.

Mechanical stimuli-induced cough reflex

The experimental protocol was that of Miyata et al (1989). Guinea-pigs (seven animals per group) were anaesthetized with pentobarbital ($30\,mg\,kg^{-1}$, i.p.) and fixed. The trachea was denuded, a cannula was inserted, and the expiration flow was monitored. Sixty minutes after surgery tracheal mucus was stimulated mechanically to induce a cough reflex. The expiration flow speed of the cough reflex was measured. Guinea-pigs for which the flow speed was $< 75\,mL\,s^{-1}$ were chosen. Drugs were administered intragastrically and the cough reflex was induced 60 min after drug administration.

Statistical analysis

Results of in-vivo and in-vitro studies are given as means ± s.e.m. and means ± s.d., respectively. The Steel test was used for multiple comparisons. The Wilcoxon test and the Sigh–Wilcoxon test were used for non-paired and paired comparison, respectively. $P < 0{\cdot}05$ was regarded as statistically significant.

Results

Viscosity of porcine stomach mucin

Although M1 at 10 μM to 1 mM significantly reduced mucin viscosity, erdosteine did not (Figures 2A, B). The mucolytic drug *N*-acetylcysteine at 1 μM to 1 mM also reduced the viscosity; the effect was significant for concentrations $> 0{\cdot}1$ mM (Figure 2C). A sulphurous smell and a change in solution colour were observed for mucin treated with 1 mM *N*-acetylcysteine. *S*-Carboxy-methylcysteine had no effect (Figure 2D).

Mucociliary transport (MCT) rate in quails

The MCT rate of the control group was time-dependently reduced after 60 min and significantly reduced between 90 and 120 min compared with the pretreatment value (Figure 3A). Although the MCT rate of the group treated with $300\,mg\,kg^{-1}$ erdosteine did not increase, it did not decrease after 90 min, when a decrease was observed in the control group (Figure 3A). The MCT rate of the group treated with $600\,mg\,kg^{-1}$ erdosteine increased from 50 min after administration and significantly increased between 90 and 110 min (Figure 3B). Changes in the MCT rates of the group treated with $600\,mg\,kg^{-1}$ *S*-carboxymethylcysteine were similar to those of the control group (Figure 3C).

Airway secretion in rats

The colour of the bronchoalveolar lavage fluid collected was weak red and no blood contamination was observed, suggesting that the phenol red dye in the fluid was not derived from blood. The absorbance of the fluid in the group treated with $600\,mg\,kg^{-1}$ erdosteine was significantly higher 1 and 24 h after drug administration (Figures 4A, B). The absorbance of bronchoalveolar lavage fluid from animals treated with *S*-carboxymethylcysteine ($600\,mg\,kg^{-1}$) or ambroxol ($30\,mg\,kg^{-1}$) groups did not differ from that of the control group (Figure 5A, B).

Citric acid-induced cough reflex

After citric acid exposure the respiratory sounds became wet and cough reflexes were induced. In the control group the number of cough reflexes was slightly reduced, but not significantly compared with the value before treatment. The cough reflexes of the groups treated with 30, 100 and $600\,mg\,kg^{-1}$ erdosteine decreased significantly compared with each respective pretreated value (Table 1). In the group treated with $300\,mg\,kg^{-1}$ erdosteine the cough reflex tended to decrease. Although the cough reflex of the group treated with $600\,mg\,kg^{-1}$ *S*-carboxymethylcysteine decreased significantly, no obvious reduction was observed in the group treated with $300\,mg\,kg^{-1}$ *S*-carboxymethylcysteine. The cough reflex of the group treated with $20\,mg\,kg^{-1}$ ambroxol decreased, but this effect was not significant. The cough reflex of the group treated with $20\,mg\,kg^{-1}$ codeine phosphate decreased significantly. The recovery volume and albumin content of the bronchoalveolar lavage fluid of the erdosteine-treated group were not different from those of the fluid from the group not exposed to citric acid (Table 2). Citric acid exposure induced a significant reduction of the recovery volume and an increase in albumin concentration.

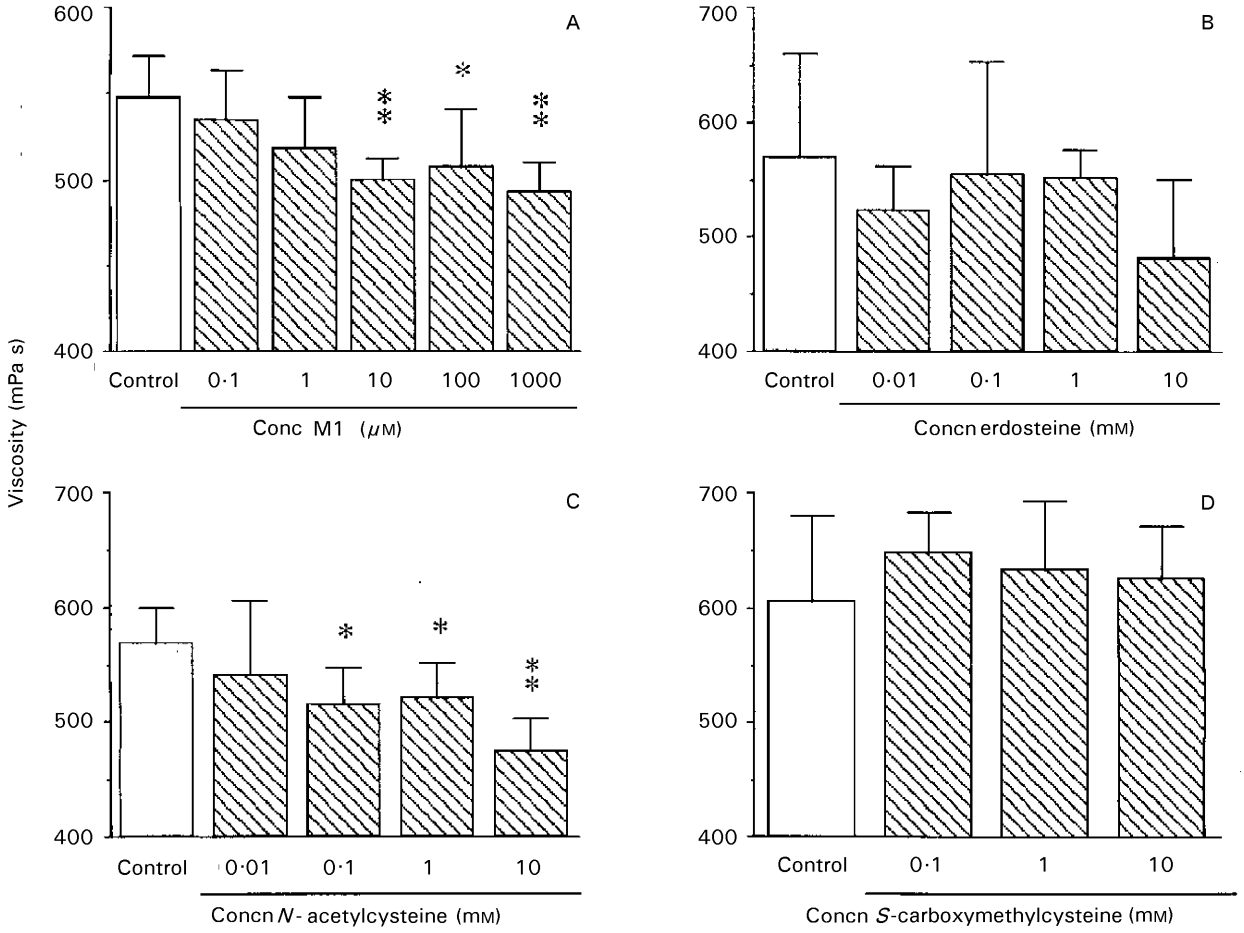

Figure 2. Effects of M1 (A), erdosteine (B), *N*-acetylcysteine (C) and *S*-carboxymethylcysteine (D) on the viscosity of porcine stomach mucin. Drugs were mixed with 15% mucin and viscosity was measured after incubation for 1 h. Results shown are means ± s.d. from 5–13 experiments. Dunnett's test was used for statistical analysis. $*P < 0.05$, $**P < 0.01$ compared with control.

In the group treated with 600 mg kg^{-1} erdosteine these effects were significantly inhibited.

Mechanical stimuli-induced cough reflex

In normal respiration, inspiration could not be recorded. After mechanical stimulation, fast inspiration was recorded and a strong expiration followed. This respiratory change seemed to be a cough reflex. The speeds of expiratory flow of the cough reflex before treatment were not significantly different among all groups. In the control group the expiratory flow of the cough reflex was slightly higher after 60 min (Table 3). In the group treated with 600 mg kg^{-1} erdosteine the cough reflex was slightly attenuated, but not significantly. In the groups treated with 600 mg kg^{-1} *S*-carboxymethylcysteine or 20 mg kg^{-1} ambroxol the flow rates of the cough reflexes were increased similarly to those of the control group, but not significantly. The cough reflex was significantly suppressed in the group treated with 20 mg kg^{-1} codeine phosphate.

Discussion

There are several closely related mechanisms of expectoration. Expectorants act by reducing the viscosity of sputa, promoting the secretion of respiratory tract fluid and surfactant, and normalizing tracheal mucus (Quevauviller et al 1976; Post et al 1983; Carina et al 1990). In this study we investigated the various effects of erdosteine and its active metabolite, M1, on expectoration.

Viscosity of porcine stomach mucin

The rheological characteristics of sputum are important determinants of the therapeutic effects of an expectorant. The free thiol group of *N*-acetylcysteine cleaves the disulphide binding between mucoproteins and destroys the structure of sputum

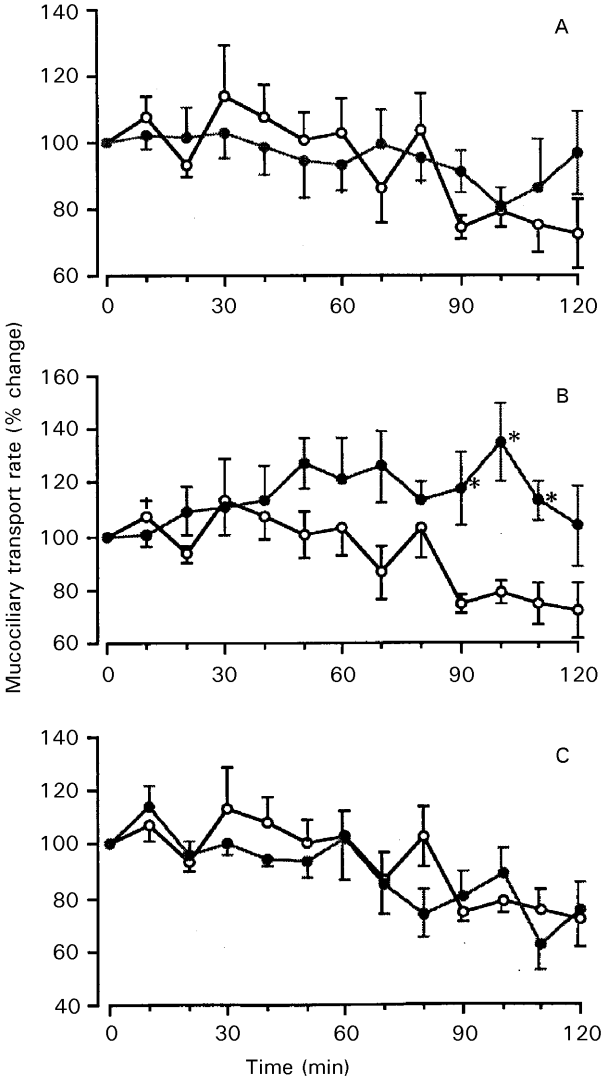

Figure 3. Effects of 300 mg kg^{-1} (A) and 600 mg kg^{-1} (B) erdosteine and 600 mg kg^{-1} *S*-carboxymethylcysteine (C) (●), administered intragastrically, on the mucociliary transport rate in quails. The mucociliary transport rate was measured for 120 min after administration and is indicated as a percentage of the value before treatment. Results shown are means ± s.e.m. from five animals. The Steel test was used for statistical analysis. *$P < 0.05$ compared with control (○).

(Sheffner 1963; Aylward 1975). In the current study, drugs which do not have a thiol group, erdosteine and *S*-carboxymethylcysteine, failed to change the viscosity whereas M1 and *N*-acetylcysteine, which have a free SH group, had mucolytic effects. Although our results correlate well with those of previous reports (Degand 1973; Livingstone et al 1990), a high concentration of *N*-acetylcysteine seemed to denature mucin solution and the mucolytic effects of M1 and *N*-acetylcysteine were detected at low concentrations. Marchioni et al (1990) reported the mucolytic effects of erdosteine on sputum from patients with chronic bronchitis. This activity seems to be

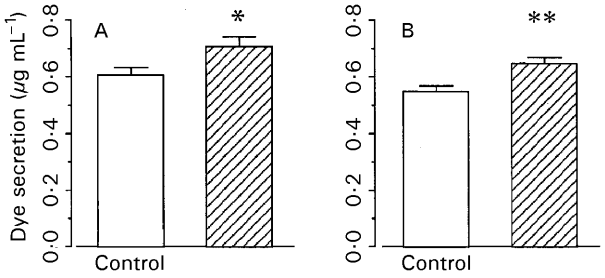

Figure 4. Effect of erdosteine at a dose of 600 mg kg^{-1} on dye secretion into the airway tract in rats. Erdosteine was administered orally 1 h (A) or 24 h (B) before dye injection. Bronchoalveolar lavage was performed 30 min after dye injection and the concentration of dye in the bronchoalveolar lavage fluid was measured. Results shown are means ± s.e.m. from 5–20 animals. The Wilcoxon test was used for statistical analysis. *$P < 0.05$, **$P < 0.01$ compared with control.

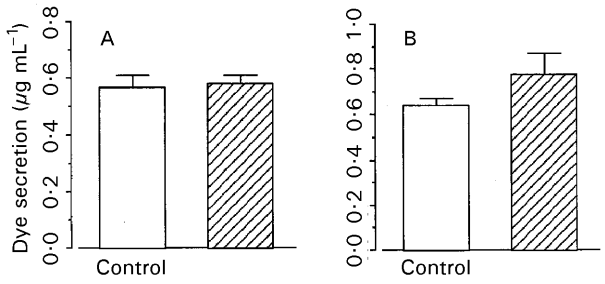

Figure 5. Effects of *S*-carboxymethylcysteine (A) and ambroxol (B) on dye secretion into the airway tract in rats. Drugs were administered orally 1 h before dye injection. Bronchoalveolar lavage was performed 30 min after dye injection and the dye concentration in the bronchoalveolar lavage fluid was measured. Results shown are means ± s.e.m. from 7–12 animals. The Wilcoxon test was used for statistical analysis.

Table 1. Effect of erdosteine on the citric acid-induced cough reflex in guinea-pigs.

Treatment	Number of cough reflexes	
	Pretreatment	Post-treatment
Control (n = 10)	5·6 ± 0·5	4·7 ± 0·6
Erdosteine		
30 mg kg^{-1} (n = 7)	6·0 ± 0·7	3·7 ± 0·7*
100 mg kg^{-1} (n = 9)	7·1 ± 0·6	3·8 ± 0·9
300 mg kg^{-1} (n = 8)	6·3 ± 0·5	4·3 ± 0·9
600 mg kg^{-1} (n = 7)	5·9 ± 0·6	3·3 ± 0·4*
S-carboxymethylcysteine		
300 mg kg^{-1} (n = 7)	7·0 ± 0·8	5·4 ± 1·0
600 mg kg^{-1} (n = 8)	6·9 ± 0·6	4·1 ± 0·8*
Ambroxol		
20 mg kg^{-1} (n = 9)	6·3 ± 0·7	4·3 ± 1·5
Codeine phosphate		
20 mg kg^{-1} (n = 8)	5·9 ± 0·7	3·3 ± 0·5

Results are means ± s.e.m. *$P < 0.05$ compared with pretreatment value.

Table 2. Effect of erdosteine on the citric acid-induced change in bronchoalveolar lavage fluid in guinea-pigs.

Group	Bronchoalveolar lavage fluid volume (mL)	Albumin ($mg\,mL^{-1}$)
Non-exposed		
Vehicle	8·6 ± 0·1	0·133 ± 0·008
Erdosteine, $600\,mg\,kg^{-1}$	8·7 ± 0·1	0·144 ± 0·008
Citric acid-exposed		
Vehicle	6·0 ± 0·7*	0·209 ± 0·016**
Erdosteine, $600\,mg\,kg^{-1}$	8·2 ± 0·2†	0·155 ± 0·016†

Results are means ± s.e.m. from six animals. $*P < 0·05$, $**P < 0·01$, compared with the non-exposed, vehicle-treated group. $†P < 0·05$ compared with the citric acid-exposed, vehicle-treated group.

Table 3. Effect of erdosteine on the mechanical stimuli-induced cough reflex in guinea-pigs.

Treatment	Expiratory flow ($mL\,s^{-1}$)	
	Pre-treatment	Post-treatment
Control	53·4 ± 3·6	66·6 ± 10·3
Erdosteine, $600\,mg\,kg^{-1}$	45·3 ± 6·0	41·3 ± 9·1
S-Carboxymethylcysteine, $600\,mg\,kg^{-1}$	60·0 ± 5·2	75·7 ± 10·4
Ambroxol, $20\,mg\,kg^{-1}$	46·9 ± 8·2	79·2 ± 12·9
Codeine phosphate, $20\,mg\,kg^{-1}$	78·3 ± 3·3	61·9 ± 9·9*

Results are means ± s.e.m. from seven animals. $*P < 0·05$ compared with control group.

because of the mucolytic effects of the active metabolite, M1.

If a secretion is too viscous or too serous, it is poorly cleared by the mucociliary system. Optimum viscosity and elasticity are necessary for good mucociliary system function (Puchelle et al 1980). Thus, the mucolytic effect of erdosteine might be an advantage in the patients with highly viscid or purulent sputum. Indeed, erdosteine improved the viscosity and purulence of the sputum in the patients with an infective exacerbation of chronic bronchitis (Ricevuti et al 1988).

Solutions of purified mucin and sputum are non-Newtonian fluids. Thus, some rheological parameters (yield value, apparent viscosity and elasticity) are important in drug evaluation. Some studies have reported that *N*-acetylcysteine reduces the elasticity much more than the viscosity (Takishima et al 1980; Marriott et al 1983). It was reported that *N*-acetylcysteine reduces yield value and apparent viscosity in patients with chronic bronchitis (Dippy & Davis 1969). Further investigation is necessary to evaluate the activity of M1 on the basis of these parameters.

Mucolytic effects possibly induce adverse effects in the stomach. Koo et al (1986) reported the adverse gastric effects of *N*-acetylcysteine. However, erdosteine has no mucolytic effects and is converted to M1 after hepatic metabolism. The kinetics indicate the low risk of inducing adverse gastric effects compared with other mucolytic drugs. Indeed, De Giovanni et al (1991) reported the absence of adverse gastric effects of erdosteine in rats and man. This prodrug system is a unique property of erdosteine.

Mucociliary transport rate

Mucociliary transport plays an important role in respiratory clearance. In patients with chronic bronchitis the MCT rate is impaired because of the reduction in the number of ciliary cells and the change in the composition and balance of the mucus layer (Wanner 1977). Enhancement of the MCT rate is an extremely important action for expectorants.

The mucociliary transport rate was reduced in a time-dependent manner in the control group. This reduction might have been caused by denudation, drying out or injury of the respiratory mucous. Erdosteine ($600\,mg\,kg^{-1}$) induced a 10–20% increase in the MCT rate within 50–120 min of administration. A significant increase was induced between 90 and 110 min. These results indicate that enhancement of the MCT response by erdosteine had a slow onset but long duration.

The MCT rate depends on both the integrity and function of ciliated cells and the proper consistency and amount of mucus. The mucus layer consists of an upper gel-phase and lower sol-phase. The upper phase is escalated by the ciliary activity. The enhancing effect of ciliary activity induces the increase in the MCT rate. The effects of several drugs have been reported (Wanner 1986; Disse & Ziegler 1987). Although the mechanism of action of erdosteine remains unclear, it might affect ciliary activity through possible anti-inflammatory effects against inflammatory inhibitory factors (Miyata et al 1998).

Airway secretion

Secretion of airway tract fluid and pulmonary surfactant plays an important role in expectoration, because the secretions are closely related to the MCT response. The MCT response in a respiratory tract is the result of movement of the upper gel-type layer above the lower sol-type layer of mucus. Surfactant and tract fluid secretion control the thickness-balance and property of the two mucus

layers. Erdosteine has been reported to increase airway secretion (Scuri et al 1988). In this study, erdosteine consistently promoted airway secretion in rats, enhancement which was detected 24 h after drug administration. This long-acting effect of erdosteine might be an advantage in clinical therapy, because sputum might still be expelled efficiently in the middle of the night or early morning.

Cough reflex

Cough reflexes are induced by stimulation of the sensory nerve endings and transmission of cough signals from the airways to the central nervous system via the vagus nerves (Forsberg & Karlsson 1986). Two kinds of sensory nerve are reported to induce cough reflexes (Widdicombe 1954; Kawakami et al 1973; Coleridge et al 1976). The rapidly adapting stretch receptors are well-known as irritant receptors (Widdicombe 1954). Nadel & Widdicombe (1963) reported that mechanical stimuli induced cough reflexes and bronchoconstriction via this sensory nerve. Bronchial C fibres also induce this reflex and are stimulated by capsaicin, prostaglandins E_1 and E_2, and bradykinin (Coleridge & Coleridge 1977; Kaufman et al 1980; Roberts et al 1985). Inhalation of these mediators causes dramatic cough reflexes in man (Smith et al 1975; Fuller et al 1985).

In the current study erdosteine suppressed citric acid-induced cough reflexes. The effect of erdosteine was manifested by a reduced number of cough reflexes. The exact expiration flow speed could not be measured, but the cough strength did not seem to be changed.

Coughs in patients seem to be caused by two types of stimulation—chemical mediators which are released during airway inflammation or physical stimulation such as adhesion of sputum to the epithelium. Coughs to expel sputum seem to be caused by physical stimulation. We found that erdosteine reduced the chemical stimulation-induced cough reflex only, i.e. it did not inhibit physical stimulation-induced cough reflexes. This suggests that erdosteine reduces only coughs induced by airway inflammation without prevention of sputum expulsion. Indeed, erdosteine improved the sputum and cough in clinical therapy (Marchioni et al 1990).

The mechanism of the antitussive action of erdosteine is not clear. Erdosteine might not suppress the central nervous system because mechanical stimuli-induced cough reflex could not be reduced. Chausow & Banner (1983) reported that a histamine-induced cough is reduced by a β-adrenergic agonist, which suggests that cough responses are in part mediated via bronchoconstriction. Our experimental discovery that the volume of bronchoalveolar lavage fluid was reduced in the citric acid-exposed group indicated the occurrence of bronchoconstriction. That this volume was increased by erdosteine treatment indicates that erdosteine significantly inhibits bronchoconstriction. This effect might be one of the antitussive mechanisms of the drug.

Another explanation is the influence on neutral endopeptidase, a membrane-bound endopeptidase distributed in the epithelial cells, airway smooth muscle, and pulmonary nerves. A neutral endopeptidase inhibitor enhances substance P- or capsaicin-induced cough reflexes, and recombinant inhalation of neutral endopeptidase reduced these responses (Kohrogi et al 1988, 1989). These reports indicate that this endopeptidase might regulate cough responses by proteolysis of tachykinins. Increasing the distribution or activity, or both, of the endopeptidase might reduce coughing. Thus, the effect of erdosteine on neutral endopeptidase activity remains to be established.

The current results indicate that erdosteine has both mucolytic and antitussive activity.

Acknowledgements

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