J. Pharm. Pharmacol. 2000, 52: 1411–1416
Received March 30, 2000
Accepted June 26, 2000

Protective Effect of Erdosteine Against Hypochlorous Acid-induced Acute Lung Injury and Lipopolysaccharide-induced Neutrophilic Lung Inflammation in Mice

KEN-ICHI HAYASHI, HISASHI HOSOE, TOSHIHIKO KAISE AND KENJI OHMORI

Drug Development Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd, Japan

Abstract

The effect of erdosteine, a mucoactive drug, on hypochlorous acid (HOCl)-induced lung injury, and the lipopolysaccharide (LPS)-induced increase in tumour necrosis factor-α (TNF-α) production and neutrophil recruitment into the airway, was investigated.

Male BALB/c mice were orally administered erdosteine (3–100 mg kg^{-1}), ambroxol hydrochloride (ambroxol) (3–30 mg kg^{-1}), *S*-carboxymethyl-L-cysteine (S-CMC) (100–600 mg kg^{-1}) or prednisolone (10 mg kg^{-1}), 1 h before intratracheal injection of HOCl or LPS. In the HOCl-injected mice, erdosteine markedly suppressed increases in the ratios of lung wet weight to bodyweight and lung dry weight to bodyweight, whereas the other mucoactive drugs ambroxol and S-CMC had little effect. Erdosteine also inhibited the LPS-induced neutrophil influx, although it did not affect the increased level of TNF-α in the bronchoalveolar lavage fluid.

The results suggest that attenuation of reactive oxygen species and neutrophil recruitment is involved in the clinical efficacy of erdosteine in the treatment of chronic bronchitis.

During acute exacerbations in chronic bronchitis, large numbers of neutrophils accumulate in the airways, carrying a repository of potentially injurious substances including reactive oxygen species (ROS), arachidonic acid metabolites and proteinases (McCusker & Hoidal 1988). The secretory products of neutrophils increase mucus production by epithelial cells and impair cilial function (McCusker & Hoidal 1988; Repine et al 1997). These observations suggest that neutrophils and their secretions are closely related to abnormal mucus accumulation in the airway inflammation including chronic bronchitis. Drugs which can attenuate neutrophil recruitment and products such as ROS may be beneficial in the treatment of chronic bronchitis.

Erdosteine, (±)-((((tetrahydro-2-oxo-3-thienyl) carbamoyl)methyl)thio)acetic acid, a mucoactive drug which enhances respiratory ventilation, was developed for the treatment of chronic obstructive pulmonary disease including chronic bronchitis (Dechant & Noble 1996; Miyata et al 1998). Erdosteine facilitates sputum expectoration by reducing sputum viscosity and/or by increasing mucociliary clearance (Dechant & Noble 1996). In addition, erdosteine can protect against cigarette smoke-induced loss of α_1-antitrypsin activity in the lungs of rats and man (Dechant & Noble 1996). α_1-Antitrypsin is known to decrease the activity of neutrophil elastase and to prevent a variety of tissues from injury (Carp & Janoff 1980). Moreover, it has been reported that erdosteine attenuates paraquat toxicity in mice, characterized by progressive and irreversible respiratory failure, leading to death due to the formation of ROS (Kaise et al 1993; Inglesi et al 1994; Dechant & Noble 1996). We have previously shown that erdosteine suppresses the pulmonary oedema and the increase in the alveolar–arterial oxygen partial pressure difference induced by bleomycin in rats (Kaise et al 1993), suggesting that erdosteine exerts an anti-inflammatory action via an antioxidative effect in addition to its direct mucoregulatory activity. Clinically, both the antioxidative effect and the mucoregulatory effect of erdosteine may contribute

Correspondence: Ken-ichi Hayashi, Drug Development Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd, 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan.

to ameliorating the decrease in respiratory function due to the airway obstruction accompanying airway inflammation and abnormal mucus production.

In this study we assessed the effects of erdosteine on the acute lung injury induced by hypochlorous acid (HOCl), a ROS produced by neutrophils (Weiss et al 1982), and on lipopolysaccharide (LPS)-induced neutrophil recruitment into the airway.

Materials and Methods

Animals

Male BALB/c mice, 20–30 g, were purchased from Japan Charles Liver (Hino, Japan), and were allowed to acclimatize in an animal room maintained at a room temperature of 22–24°C and a relative humidity of 50–60%, with a 12-h light–dark cycle (light between 07 00 and 19 00 h) for 1 week. Healthy mice were chosen for use in the experiment which was approved by the Animal Ethical Committee of Kyowa Hakko Kogyo Co., Ltd (Shizuoka, Japan).

Drugs

On the day of the experiments, erdosteine (Edmond Pharma, Milan, Italy) was dissolved with an equivalent molar quantity of sodium bicarbonate in distilled water; ambroxol hydrochloride (ambroxol) (Sigma Chemical Co., St Louis, MO) was dissolved in distilled water; *S*-carboxymethyl-L-cysteine (S-CMC) (Sigma) was dissolved in 1 M NaOH and then neutralized with 1 M HCl (adjusted to pH 6–7); prednisolone (Sigma) was suspended in 0·5% methylcellulose. All drugs were prepared in the appropriate concentrations and administered in a volume of 10 mL kg^{-1}.

HOCl-induced lung injury

At 1 h after oral administration of either drug or distilled water, BALB/c mice were injected intratracheally with either 0·1 mL of the desired concentrations of NaOCl (Wako Pure Chemical Industries, Ltd, Osaka, Japan) in saline (buffered to pH 7), or with saline alone under anaesthesia with ether. At various intervals, mice were anaesthetized (pentobarbital sodium; 120 mg kg^{-1}, i.p.) and exsanguinated. The lungs were surgically removed, the extrapulmonary bronchi were cut away and the wet weight was recorded. The lungs were placed in a drying oven at 120°C for 24 h and the dry weight was determined. As an index of injury, the ratio of wet lung weight to body weight (lung wet wt/BW) and that of lung dry weight to body weight (lung dry wt/BW) were calculated.

LPS-induced airway inflammation

BALB/c mice were administered orally with either drug or distilled water and 1 h later, either 0·1 mL LPS (E. coli 055 : B5, Difco Laboratories, Detroit, MI) (10 ng mL^{-1} in saline), or saline alone was injected intratracheally under anaesthesia with ether. At the appropriate time, mice were killed by an intraperitoneal injection of pentobarbital sodium (120 mg kg^{-1}). The trachea was cannulated and then a total of 4 mL (four 1-mL portions) of saline was immediately injected into the lung and the intrapulmonary contents were aspirated. The bronchoalveolar lavage (BAL) fluid was centrifuged at 200 *g* for 10 min (4°C), and the supernatant was stored at −80°C until the determination of tumour necrosis factor-α (TNF-α), and the precipitate was suspended in 0·4 mL saline for cell counts.

The total cell count was determined on a cell counter (Celltac α MEK-6158; Nihon Kohden, Tokyo, Japan). For cell differentiation, a cytocentrifuge preparation was made by centrifuging the cells at 600 rev min^{-1} for 3 min in a Cytospin 3 (Shandon Inc., Pittsburgh, PA); the specimen was then stained with Giemsa stain, and differential cell counts were performed for 500 cells.

The level of TNF-α was measured with an enzyme-linked immunosolvent assay kit (mouse TNF-α ELISA kit; Endogen Inc., Woburn, MA). If a measurement value was lower than the quantitation limit of 50 pg mL^{-1}, the limit value was regarded as the level of TNF-α.

Statistical analysis

Statistical significance was analysed using the SAS system for Windows ver. 6.12 (SAS Institute Inc., Cary, NC). The Wilcoxon rank sum test was used for comparisons between the two groups. Multiple comparisons were made first by the Kruskal–Wallis test and, when appropriate, followed by the Steel test. All data were expressed as mean ± s.e.m. Differences were considered significant at $P < 0.05$.

Results

HOCl-induced lung injury

Intratracheal instillation of HOCl (0·3, 1, 3 and 10 mmol L^{-1}) produced concentration-dependent increases in lung wet wt/BW and lung dry wt/BW

1 h after the instillation. Both ratios in mice exposed to HOCl at a concentration of 1 mmol L^{-1} or higher, were significantly greater than those in the saline-treated control group. However, 10 mmol L^{-1} HOCl caused death in 8 out of 12 mice within 1 h of the instillation (Table 1).

The lung wet wt/BW at 0·5, 1 and 3 h, and the lung dry wt/BW at 1 and 3 h, after the instillation of 1 mmol L^{-1} HOCl were significantly elevated compared with the saline-treated control group. In particular, at 1 h later, these increases were highly significant. The lung wet wt/BW at 6 h, and the lung dry wt/BW at 0·5 and 6 h, after the instillation was not affected by HOCl (Table 2). Exposure to 1 mmol L^{-1} HOCl for 1 h, which was used in the subsequent experiments, produced sufficient injury to assess the effects of drugs.

Erdosteine significantly prevented HOCl-induced increases in lung wet wt/BW and lung dry wt/BW at concentrations of 10 mg kg^{-1} or higher, and 30 mg kg^{-1} or higher, respectively, and produced a maximum inhibition (61 and 100%, respectively) at 100 mg kg^{-1} (Table 3). Ambroxol tended to suppress the increase in lung wet wt/BW at 10 mg kg^{-1} and significantly inhibited it at 30 mg kg^{-1}, although the inhibition was just 29 and 23%, respectively. Ambroxol even at a concentration of 30 mg kg^{-1} failed to inhibit the increase in lung dry wt/BW (Table 3). S-CMC (100–600 mg kg^{-1}) did not alter the increases in lung wet wt/BW and lung dry wt/BW at any of the doses tested (Table 3).

Table 1. Effects of increasing concentrations of hypochlorous acid (HOCl) on the ratios of lung wet weight to bodyweight (lung wet wt/BW) and lung dry weight to bodyweight (lung dry wt/BW) 1 h after the intratracheal instillation.

Treatment	Concentration (mmol L^{-1})	Lung wet wt/BW ($\times 10^{-3}$)	Lung dry wt/BW ($\times 10^{-3}$)
Saline		6·09 ± 0·06	1·24 ± 0·01
HOCl	0·3	6·27 ± 0·13	1·26 ± 0·02
	1	7·05 ± 0·14***	1·32 ± 0·02**
	3	10·57 ± 0·17***	1·44 ± 0·02***
	10	13·43 ± 0·33**	1·72 ± 0·03**

Each value represents the mean ± s.e.m. (saline, 0·3, 1 and 3 mmol L^{-1} HOCl; n = 12, 10 mmol L^{-1} HOCl; n = 4). $^{**}P < 0·01$, $^{***}P < 0·001$ compared with the corresponding value in the saline-treated control group.

Table 2. Time-course of changes in the ratios of lung wet weight to bodyweight (lung wet wt/BW) and lung dry weight to bodyweight (lung dry wt/BW) after the intratracheal instillation of saline or 1 mmol L^{-1} hypochlorous acid (HOCl).

Treatment	Time after injection (h)	Lung wet wt/BW ($\times 10^{-3}$)	Lung dry wt/BW ($\times 10^{-3}$)
Untreated	–	5·73 ± 0·09	1·24 ± 0·02
Saline	0·5	7·37 ± 0·09	1·28 ± 0·02
	1	6·03 ± 0·07	1·20 ± 0·01
	3	5·55 ± 0·08	1·24 ± 0·02
	6	5·66 ± 0·12	1·25 ± 0·02
HOCl	0·5	7·76 ± 0·12*	1·29 ± 0·02
	1	7·23 ± 0·13***	1·33 ± 0·01***
	3	6·06 ± 0·06***	1·30 ± 0·01*
	6	5·74 ± 0·10	1·27 ± 0·02

Each value represents the mean ± s.e.m., n = 12. $^{*}P < 0·05$, $^{***}P < 0·001$ compared with the corresponding value in the saline-treated control group.

LPS-induced airway inflammation

At 3 h after intratracheal instillation of LPS (1 ng), the number of total cells in the BAL fluid did not differ from that in the saline-treated control group, however, neutrophil counts significantly increased and macrophage counts significantly decreased (Table 4). Under the same experimental conditions, the level of TNF-α in the BAL fluid was significantly increased by LPS (Table 4). Erdosteine (10–100 mg kg^{-1}) did not significantly alter these events, whereas prednisolone (10 mg kg^{-1}) inhibited the increase in neutrophil counts and TNF-α level (Table 4). At 24 h after the instillation of LPS, total cell counts and neutrophil counts in the BAL

Table 3. Effects of erdosteine, ambroxol hydrochloride (ambroxol) and *S*-carboxymethyl-L-cysteine (S-CMC) on hypochlorous acid (HOCl)-induced increases in the ratios of lung wet weight to bodyweight (lung wet wt/BW) and lung dry weight to bodyweight (lung dry wt/BW).

Drug	Dose (mg kg^{-1})	Lung wet wt/BW ($\times 10^{-3}$)	Lung dry wt/BW ($\times 10^{-3}$)
Negative control		5·94 ± 0·09	1·21 ± 0·01
Positive control		7·28 ± 0·14†††	1·32 ± 0·02††
Erdosteine	3	6·98 ± 0·12	1·27 ± 0·02
	10	6·73 ± 0·09*	1·27 ± 0·02
	30	6·79 ± 0·11*	1·25 ± 0·01*
	100	6·46 ± 0·09***	1·21 ± 0·02**
Negative control		5·73 ± 0·10	1·19 ± 0·02
Positive control		7·04 ± 0·09†††	1·28 ± 0·02††
Ambroxol	3	6·85 ± 0·10	1·25 ± 0·01
	10	6·66 ± 0·13	1·25 ± 0·02
	30	6·74 ± 0·10*	1·24 ± 0·01
Negative control		6·08 ± 0·09	1·24 ± 0·01
Positive control		7·31 ± 0·16†††	1·31 ± 0·02†
S-CMC	100	7·16 ± 0·12	1·29 ± 0·02
	300	7·07 ± 0·12	1·30 ± 0·02
	600	7·01 ± 0·14	1·27 ± 0·02

Each mouse was administered orally with distilled water (negative control and positive control), erdosteine, ambroxol or S-CMC 1 h before intratracheal instillation of saline or 1 mmol L^{-1} HOCl. Each value represents the mean ± s.e.m., n = 12. $^{\dagger}P < 0·05$, $^{\dagger\dagger}P < 0·01$, and $^{\dagger\dagger\dagger}P < 0·001$, compared with the corresponding value in the negative control group. $^{*}P < 0·05$, $^{**}P < 0·01$, and $^{***}P < 0·001$, compared with the corresponding value in the positive control group.

fluid significantly increased, whereas no changes in macrophage counts were observed compared with the saline-treated control group (Table 5). At this time, the release of TNF-α in the BAL fluid of LPS-injected mice was at a normal level (data not shown). Erdosteine significantly inhibited the increase in total cell counts and neutrophil counts at concentrations of $30\,mg\,kg^{-1}$ or higher and $100\,mg\,kg^{-1}$, respectively. Erdosteine did not affect the macrophage counts at any of the doses tested (Table 5).

Discussion

Activated neutrophils, which play a major role in chronic bronchitis (McCusker & Hoidal 1988; Repine et al 1997), produce HOCl (Weiss et al 1982). A previous report showed that intratracheal injection of HOCl evoked airway inflammation, as demonstrated by marked increases in protein concentration and the percentage of polymorphonuclear cells in the BAL fluid of rats (Ghio et al 1996). In this study, we found that instillation of HOCl into the airway increased lung wet wt/BW and lung dry wt/BW. In addition, significant increases in wet/dry and accumulation of Evans blue dye in the BAL fluid were observed after treatment with HOCl (data not shown). HOCl is known to cause disruption of various plasma membranes, including the epithelium and the endothelium, by oxidation (Schraufstätter et al 1990; Guo et al 1995; Ochoa et al 1997). We assume that, in this study, the increased permeability of the airway epithelium and blood vessels resulted in the plasma leakage into the bronchoalveolar space, leading to the formation of pulmonary oedema. Moreover, the increased lung dry wt/BW suggests that the increase in lung wet wt/BW resulted not only from excess water but also from the increased unknown factors in the lung, and that it may involve pulmonary congestion.

We assessed the effects of three mucoactive drugs, erdosteine, ambroxol and S-CMC, on the HOCl-induced lung injury. Erdosteine significantly inhibited the increase in lung wet wt/BW and lung dry wt/BW at a concentration of $10\,mg\,kg^{-1}$ or higher and $30\,mg\,kg^{-1}$ or higher, respectively. However, the increase in lung wet wt/lung dry wt was not significantly influenced by erdosteine as a result of inhibited increases in both lung wet wt/BW and lung dry wt/BW (data not shown). A previous study showed that erdosteine protected against cigarette smoke-induced loss of α_1-antitrypsin activity, suggesting that this drug scavenges ROS (Dechant & Noble 1996). Erdosteine contains two blocked sulphhydryl groups, which are unmasked after metabolic transformation in the

Table 4. Effects of erdosteine and prednisolone on changes in the number of leukocytes and the TNF-α level in BAL fluid 3 h after the intratracheal instillation of LPS.

Drug	Dose ($mg\,kg^{-1}$)	Total cells ($\times10^5$)	Neutrophils ($\times10^5$)	Macrophages ($\times10^5$)	TNF-α ($pg\,mL^{-1}$)
Negative control		1·5 ± 0·2	0·0 ± 0·0	1·5 ± 0·2	51·7 ± 1·7
Positive control		1·6 ± 0·2	0·8 ± 0·2††	0·7 ± 0·0††	1360 ± 104·2††
Erdosteine	10	1·4 ± 0·4	0·7 ± 0·2	0·7 ± 0·2	1782 ± 319·3
	30	1·5 ± 0·2	0·5 ± 0·2	0·9 ± 0·2	998·0 ± 292·9
	100	1·5 ± 0·2	0·3 ± 0·1	1·1 ± 0·2	880·0 ± 246·4
Prednisolone	10	1·3 ± 0·3	0·1 ± 0·0**	1·2 ± 0·3	441·2 ± 74·9**

Each mouse was administered orally with distilled water (negative control and positive control), erdosteine or prednisolone 1 h before intratracheal instillation of saline or 1 ng LPS. Each value represents the mean ± s.e.m., n = 5–6. ††$P < 0.01$ compared with the corresponding value in the negative control group. **$P < 0.01$ compared with the corresponding value in the positive control group.

Table 5. Effects of erdosteine on changes in the number of leukocytes in BAL fluid 24 h after the intratracheal instillation of LPS.

Drug	Dose ($mg\,kg^{-1}$)	Total cells ($\times10^5$)	Neutrophils ($\times10^5$)	Macrophages ($\times10^5$)
Negative control		1·7 ± 0·2	0·1 ± 0·0	1·6 ± 0·2
Positive control		6·6 ± 0·7††	4·8 ± 0·7††	1·8 ± 0·3
Erdosteine	10	4·3 ± 0·6	3·0 ± 0·6	1·3 ± 0·2
	30	3·1 ± 0·8*	1·9 ± 0·6	1·2 ± 0·2
	100	3·0 ± 0·7*	1·4 ± 0·6*	1·5 ± 0·2

Each mouse was administered orally with distilled water (negative control and positive control) or erdosteine 1 h before the intratracheal instillation of saline or 1 ng LPS. Each value represents the mean ± s.e.m., n = 5–6. ††$P < 0.01$ compared with the corresponding value in the negative control group. *$P < 0.05$ compared with the corresponding value in the positive control group.

liver. The liberated sulphhydryl groups of this metabolite are suggested to display free radical scavenging activity (Dechant & Noble 1996). *N*-Acetylcysteine, which has an oxidizable thiol group, is a scavenger of HOCl (Gillissen et al 1997). Similarly, erdosteine seems to have a scavenging activity against HOCl, which could contribute to the inhibition of HOCl-induced lung injury. S-CMC did not suppress the increase in lung wet wt/BW and lung dry wt/BW at any of the doses tested. Although the effect of S-CMC on HOCl is unknown, the lack of free sulphhydryl groups may be the reason for its weak activity. Ambroxol tended to suppress the increase in lung wet wt/BW at a concentration of $10\,mg\,kg^{-1}$ and significantly inhibited it at $30\,mg\,kg^{-1}$, although the inhibition was just 29 and 23%, respectively. Chemically, ambroxol and *N*-acetylcysteine are completely different molecules. Ambroxol has an aromatic ring with an amine group and a hydroxycyclohexyl ring. Nevertheless, an HOCl scavenging action by ambroxol has been reported (Lapenna et al 1994; Gillissen et al 1997). In this study, however, ambroxol did not significantly inhibit lung injury. Considering that erdosteine but not ambroxol is a prodrug-type compound, the possibility remains that ambroxol is almost oxidized before arriving at airway tissues.

We previously reported that congestion, infiltration of inflammatory cells, oedema and epithelium disintegration were observed in the lung of mice 3 days after paraquat treatment, and that erdosteine reduced these changes, delayed the onset of the lethal event, and finally prevented the mortality (Kaise et al 1993). The toxicity in acute paraquat poisoning is believed to result from free radical injury (Bus et al 1974). These findings support the notion that erdosteine suppresses ROS in-vivo, and thus that the prevention of HOCl-induced lung injury by erdosteine in this study was mediated via its ROS scavenging action.

The effect of erdosteine on LPS-induced airway inflammation was tested. We observed increases in neutrophil counts and TNF-α level in BAL fluid 3 h after intratracheal instillation of LPS. de Moraes et al (1996) have previously reported increases in TNF-α level and neutrophil counts in BAL fluid 3 h after LPS inhalation in mice. In addition, they demonstrated that TNF-α was produced before neutrophil recruitment and that anti-TNF-α antibodies blocked neutrophil recruitment. In this study, erdosteine did not significantly inhibit the neutrophil influx at 3 h after the LPS challenge, presumably because of the inability of the drug to prevent the production of TNF-α. In contrast, at 24 h after the LPS instillation, erdosteine significantly reduced the increased neutrophil counts at a dose of $100\,mg\,kg^{-1}$. These results indicate that erdosteine exerts an inhibitory effect on LPS-induced inflammation in a later phase rather than in the TNF-α-mediated early phase.

This study showed that erdosteine inhibited neutrophil infiltration without affecting TNF-α production in mice intratracheally treated with LPS. Although erdosteine did not significantly inhibit the TNF-α generation, it may have influenced the expression of adhesion molecules and/or the related chemotactic factors in the process between TNF-α and neutrophil recruitment. The recruited neutrophils release a variety of products, such as elastase and ROS (McCusker & Hoidal 1988), the latter of which can damage various cells to reinforce the activity of elastase by inactivating α_1-antitrypsin (Carp & Janoff 1980). Moreover, neutrophil elastase is shown to cause detachment and deformation of human bronchial epithelial cells and to induce gene expression and protein production of interleukin-8 (Shibata et al 1996). Thus, erdosteine may have inhibited the production of neutrophil chemotactic factors via prevention of airway epithelium injury because of its scavenging action against ROS from recruited neutrophils. *N*-Acetylcysteine is reported to suppress nuclear factor-κB activation, cytokine-induced neutrophil chemo-attractant messenger RNA expression, and neutrophilic lung inflammation in rats (Blackwell et al 1996). As regards the exact mechanism for the anti-inflammatory effect of erdosteine without affecting TNF-α production, further investigation is needed.

In conclusion, this study demonstrated that erdosteine inhibited HOCl-induced acute lung injury in mice, suggesting that this drug exerts a scavenging effect on ROS in-vivo. The antioxidative effect may be involved in the prevention by erdosteine of LPS-induced neutrophil recruitment into the airway. Our results suggest that the attenuation of ROS and neutrophil influx may contribute to the clinical efficacy of erdosteine in the therapy of chronic bronchitis.

References

Blackwell, T. S., Blackwell, T. R., Holden, E. P., Christman, B. W., Christman, J. W. (1996) In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. J. Immunol. 157: 1630–1637

Bus, J. S., Aust, S. D., Gibson, J. E. (1974) Superoxide- and singlet oxygen-catalyzed lipid peroxidation as a possible mechanism for paraquat (methyl viologen) toxicity. Biochem. Biophys. Res. Commun. 58: 749–755

Carp, H., Janoff, A. (1980) Potential mediators of inflammation: phagocyte-derived oxidants suppress the elastase-inhibitory capacity of alpha$_1$-proteinase inhibitor in vitro. J. Clin. Invest. 66: 987–995

Dechant, K. L., Noble, S. (1996) Erdosteine. Drugs 52: 875–881

de Moraes, V. L. G., Vargaftig, B. B., Lefort, J., Meager, A., Chignard, M. (1996) Effect of cyclo-oxygenase inhibitors and modulators of cyclic AMP formation on lipopolysaccharide-induced neutrophil infiltration in mouse lung. Br. J. Pharmacol. 117: 1792–1796

Ghio, A. J., Marshall, B. C., Diaz, J. L., Hasegawa, T., Samuelson, W., Povia, D., Kennedy, T. P., Piantadosi, A. (1996) Tyloxapol inhibits NF-kappa B and cytokine release, scavenges HOCl, and reduces viscosity of cystic fibrosis sputum. Am. J. Respir. Crit. Care. Med. 154: 783–788

Gillissen, A., Schärling, B., Jaworska, M., Bartling, A., Rasche, K., Schultze-Werninghaus, G. (1997) Oxidant scavenger function of ambroxol in vitro: a comparison with N-acetylcysteine. Res. Exp. Med. 196: 386–398

Guo, Y., Schneider, L. A., Wangensteen, O. D. (1995) HOCl effects on tracheal epithelium: conductance and permeability measurements. J. Appl. Physiol. 78: 1330–1338

Inglesi, M., Nicola, M., Fregnan, G. B., Bradamante, S., Pagani, G. (1994) Synthesis and free radical scavenging properties of the enantiomers of erdosteine. II Farmaco 49: 703–708

Kaise, T., Hosoe, H., Sano, J., Ohmori, K. (1993) Effects of KW-9144 on paraquat toxicity in mice and bleomycin-induced pulmonary injury in rats. Jpn. J. Pharmacol. 61 (Suppl. 1): 81P

Lapenna, D., De Gioia, S., Ciofani, G., Cuccurullo, F. (1994) Ambroxol is a scavenger of hypochlorous acid and monochloramine. Pharmacology 49: 132–135

McCusker, K. T., Hoidal, J. (1988) Leukocyte function and chronic bronchitis. Semin. Respir. Infect. 3: 5–13

Miyata, T., Kai, H., Isohama, Y., Takahama, K. (1998) Current opinion of muco-active drug research: strategies and problems. Eur. Respir. J. 11: 480–491

Ochoa, L., Waypa, G., Mahoney, J. R., Rodriguez, L., Minnear, F. L. (1997) Contrasting effects of hypochlorous acid and hydrogen peroxide on endothelial permeability: prevention with cAMP drugs. Am. J. Respir. Crit. Care. Med. 156: 1247–1255

Repine, J. E., Bast, A., Lankhorst, I. (1997) Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. Am. J. Respir. Crit. Care. Med. 156: 341–357

Schraufstätter, I. U., Browne, K., Harris, A., Hyslop, P. A., Jackson, J. H., Quehenberger, O., Cochrane, G. (1990) Mechanisms of hypochlorite injury of target cells. J. Clin. Invest. 85: 554–562

Shibata, Y., Nakamura, H., Kato, S., Tomoike, H. (1996) Cellular detachment and deformation induce IL-8 gene expression in human bronchial epithelial cells. J. Immunol. 156: 772–777

Weiss, S. J., Klein, R., Slivka, A., Wei, M. (1982) Chlorination of taurine by human neutrophils: evidence for hypochlorous acid generation. J. Clin. Invest. 70: 598–607