

EFFECTIVENESS OF CARBOCYSTEINE LYSINE SALT MONOHYDRATE ON MODELS OF AIRWAY INFLAMMATION AND HYPERRESPONSIVENESS

C. ASTI, G. MELILLO, G. F. CASELLI, L. DAFFONCHIO, A. HERNANDEZ*, G. CLAVENNA and C. OMINI

*Dompe' S.p.A., Department of Pharmacology, L'Aquila, and *Institute of Pharmacological Sciences, University of Milan, Italy*

Accepted 19 May 1995

We investigated the possible effects of the mucoactive drug Carbocysteine lysine salt monohydrate (CLS.H₂O) on experimentally-induced airway inflammation and hyperresponsiveness. CLS.H₂O given by the oral route (300 mg kg⁻¹) significantly reduced neutrophil infiltration into the airway lumen induced by intratracheal injection of IL-1 β in rats. In addition, CLS.H₂O inhibited dose-dependently (100–300 mg kg⁻¹ p.o.) the formation of pleural exudate and leukocyte recruitment induced by intrapleural injection of carrageenan in rats. Because of the close interaction between the inflammatory process and the development of airway hyperresponsiveness we also tested CLS.H₂O on cigarette-smoke-induced inflammation and hyperreactivity in anaesthetized guinea-pigs. The drug, given either by oral (300 mg kg⁻¹) or aerosol route (30–100 mg ml⁻¹), was able to reduce the increase in airway responsiveness induced by smoke and the associated cell recruitment detected in the bronchoalveolar lavage (BAL) fluids. These results suggest that CLS.H₂O can exert an anti-inflammatory action in addition to its mucoregulatory activity. The anti-inflammatory and anti-hyperreactivity effect of the drug within the airways may be of advantage in the treatment of inflammatory lung diseases where mucus secretion together with airway inflammation and hyperreactivity contribute to airway obstruction.

KEY WORDS: lung inflammation, airway hyperreactivity, carbocysteine lysine salt monohydrate.

INTRODUCTION

Mucus hypersecretion, airway inflammation and bronchial hyperreactivity are common pathological features of several respiratory diseases, such as chronic bronchitis and asthma [1–3]. In particular, the inflammatory process is recognized as one of the major mechanisms amplifying and sustaining the airway disease. After acute mucosal injury or chronic inflammation, infiltrating cells and activated resident cells release a wide spectrum of secretagogues which promote mucus secretion by direct stimulation of secretory cells or by mucus gland hyperplasia. Mucus hypersecretion which contributes in asthma to airflow obstruction, seems to be due to release of eicosanoids, PAF, histamine, eosinophil cationic protein. In chronic and acute bronchitis neutrophil products (e.g. elastase, cathepsin G, tryptase and chymase) and macrophage products are the main secretagogue agents [4]. These evidences strongly support the contribution of airway inflammation to abnormal mucus generation and plugging.

Carbocysteine lysine salt monohydrate (CLS.H₂O) is a well-known mucoactive drug, in current clinical use for the therapy of patients with mucus secretion disorders. Several evidences demonstrated that CLS.H₂O does not have a direct mucolytic activity. Its efficacy on normalization of mucus secretion is believed to be related to its ability to restore the correct balance between sialo- and fuco-mucins, thereby increasing mucus fluidity and removal [5–8]. In addition, CLS.H₂O is able to increase the chloride transport in the airway epithelium, an effect that might contribute to its mucoregulatory action [9]. More recently, it has been demonstrated that CLS.H₂O can ameliorate impairment of mucociliary clearance induced by human neutrophil elastase, possibly through a direct inhibition of the mucus discharge mediated by elastase [10].

These observations suggest that CLS.H₂O possesses properties additional to direct mucus regulation which might contribute to its clinical effectiveness. Since airway inflammation is the underlying alteration contributing to airway obstruction in different respiratory pathologies, also characterized by abnormal mucus production, we investigated the potential anti-inflammatory

Correspondence to: Dr G. Melillo, Dompe' S.p.A. Research Center, via Campo di Pile, 67100 L'Aquila, Italy.

properties of CLS.H₂O in different experimental models of lung inflammation and airway hyperreactivity.

MATERIALS AND METHODS

IL-1 β -induced airway inflammation

Female rats (200–230 g, Iffa Credo, L'Arbresle, France), under ketamine–xylazine anaesthesia, were injected intratracheally with IL-1 β solution in saline (5 ng per rat, 250 μ l) after blunt dissection of the soft tissues of the neck to expose the trachea [11]. Four hours after IL-1 β injection, the animals were killed by excess ether anaesthesia and the inflammation evaluated as the number of total cells and neutrophils in bronchoalveolar lavage (BAL) fluids.

BAL was performed by gently washing the lung cavities with 5 ml saline by repeated lavage with a total volume of 45 ml and a recovery of 42 to 43 ml [12].

BAL fluid was centrifuged at 250 g for 10 min. The pellet was dissolved in 2 ml saline. Total and differential cell counts were performed as described below.

CLS.H₂O (300 mg kg⁻¹), bethametasone (1 mg kg⁻¹), ketoprofen lysine salt (30 mg kg⁻¹) or vehicle (CMC 0.5% w/v) were administered per os 1 h before IL-1 β .

Carrageenan-induced pleurisy

Ether-anaesthetized female rats (190–210 g, Iffa Credo, L'Arbresle, France) were injected intrapleurally with 2% carrageenan type I solution in saline (200 μ l for each pleural cavity) [13].

The animals were killed 6 h after carrageenan injection by ether hyperanaesthesia and the pleural cavity opened for collection of the inflammatory exudate. The volume of the pleural fluid was measured by a graduate tube. Pleural exudate was diluted 1:10 with saline. Total and differential cell count determination is described in detail below.

CLS.H₂O (100 and 300 mg kg⁻¹), or ketoprofen lysine salt 100 mg kg⁻¹ or vehicle (CMC 0.5% w/v) were administered per os 1 h before carrageenan injection.

Smoke-induced airway inflammation and hyperreactivity

Male guinea-pigs (450–550 g, Iffa Credo, L'Arbresle, France) were anaesthetized with urethane (1.2 g kg⁻¹ i.p.). The trachea was cannulated and the lung mechanically ventilated (Rodent Ventilator, Basile, Comerio, Italy) at 60 strokes min⁻¹ with 1 ml of room air per 100 g body weight. Spontaneous breathing was suppressed with pancuronium bromide 2 mg kg⁻¹ i.m. Pulmonary inflation pressure (PIP mmHg), an index of intrathoracic airway calibre, was measured through a pressure transducer (Bentley

Trantec 800, Basile, Comerio, Italy) connected to the respiration circuit and signals registered on a pen recorder (model 7070, Basile, Comerio, Italy). Animals were exposed for 15 s min⁻¹ for 10 min to the smoke generated by a commercially available cigarette directly to the ventilation circuit [14].

The development of airway hyperreactivity was assessed as the increase in the bronchoconstriction induced by acetylcholine (Ach, 10–300 μ g kg⁻¹ i.v.) 5 min after smoke exposure (or 15 min after the initial stabilization in control animals). Bronchoconstriction, calculated at the peak effect, was expressed as the increase in PIP (mmHg) over the basal values.

Airway inflammation was studied in a separate set of animals exposed to smoke as above. The percentage of eosinophils in BAL was evaluated as an inflammatory index.

BAL was performed 5 min after smoke inhalation by gently washing the lung through the trachea three times with 10 ml of saline (37°C). The recovered fluid (more than 97%) was centrifuged at 800 g for 5 min and the pellet resuspended in 0.5 ml of phosphate-buffered saline. Total and differential cell counts were performed as described below.

CLS.H₂O or vehicle (CMC 0.5% w/v or saline) were administered either per os (300 mg kg⁻¹) 1.5 h before smoke exposure, or by aerosol (30–100 mg ml⁻¹ aerosol \times 60 s) 3 min before smoke. The aerosol was generated by an ultrasonic nebulizer (deVilbiss, Pulmosonic, PA, USA) connected to the respiration apparatus.

Total and differential cell count determination

Pleural and BAL fluids were diluted 1:10 with Türk's solution and total cell count determined using a 'Burker' counting chamber and phase contrast microscopy (Wilovert \times 200, Leitz Italiana, Milan, Italy).

For differential cell count analysis, the pulmonary fluids were diluted with saline to obtain 5 \times 10⁴ cells (100 μ l saline)⁻¹. Cell populations were identified by morphological examination of smears prepared by cytocentrifugation (Cytospin 3 centrifuge Shandon, UK) and stained with Diff-Quik staining set (Fixative solution, Stain solution I and II). Two hundred cells were examined in each slide by immersion microscopy (Zeiss, Oberkochen, Germany).

Drugs

Acetylcholine, ketamine, xylazine, urethane, carrageenan type I, carboxymethylcellulose (CMC) and phosphate-buffered saline (PBS) were obtained from Sigma (St. Louis, MO, USA); pancuronium bromide (Pavulon) was from Organon; Türk's solution from Merck; Diff-Quik staining set was purchased from Baxter (Switzerland), Bethametasone (Bentelan) from Glaxo. Carbocysteine lysine salt monohydrate, Ketoprofen lysine salt, human recombinant IL-1 β (mature fragment 117–269,

expressed in *E. coli* or *B. Subtilis*, specific activity $1-3 \times 10^8$ IU mg^{-1} ; endotoxin contamination <0.1 EU/mg as measured by LAL chromogenic assay) were from Dompé S.p.A. [15].

Statistical analysis

Data are reported as means \pm SE. Statistical analysis was performed by ANOVA followed by the multiple *t*-test LSM (least-square estimates of marginal means for unbalanced design) or by Mann-Whitney *U*-test, when the criteria of homoscedasticity was not satisfied (Shapiro-Wilk test) [16]. Non-linear regression analysis of Ach log-concentration-response curves were evaluated using ALLFIT version 2.0 program. This procedure allows to calculate also the dose-ratio (DR) \pm SE.

RESULTS

IL-1 β -induced airway inflammation

Intratracheal injection of IL-1 β (5 ng per rat) induced a marked pulmonary inflammation within 4 h, characterized by a recruitment of inflammatory cells recovered in the BAL fluids (Table I). Among the

different classes of cells, IL-1 β significantly ($P < 0.01$) increased the number of neutrophils when compared to controls. The changes in other cells did not reach a statistical significance.

Oral pretreatment with CLS.H₂O (300 mg kg^{-1}) significantly ($P < 0.05$) reduced the number of total inflammatory cells and neutrophils by about 50%. Bethametasone, administered orally (1 mg kg^{-1}) in the same experimental conditions, prevented (86% inhibition) the cell recruitment. No inhibition was observed with a classic NSAID, ketoprofen lysine salt (KLS), administered at 30 mg kg^{-1} p.o..

Carrageenan-induced pleurisy

As shown in Table II, carrageenan injected into the rat pleural cavity evoked an inflammatory reaction characterized by pleural exudation and intense recruitment of inflammatory cells. Neutrophils were the most abundant cells recovered in pleural fluids 6 h after carrageenan. Oral treatment with CLS.H₂O (300 mg kg^{-1} , but not 100 mg kg^{-1}) 1 h prior to carrageenan significantly ($P < 0.01$) reduced exudate volume by about 25% (Table II and Fig. 1). Furthermore, CLS.H₂O dose-dependently inhibited the recruitment of inflammatory cells within the

Table 1
Effect of Carbocysteine lysine salt monohydrate (CLS.H₂O), bethametasone and ketoprofen lysine salt on airway inflammatory cell recruitment induced by intratracheal instillation of IL-1 β in rats

| Treatment | Total cells | | Neutrophils | |
|--|-------------------|----------------|-------------------|----------------|
| | $\times 10^6$ | Inhibition (%) | $\times 10^6$ | Inhibition (%) |
| Saline | 2.32 \pm 0.40 | | 0.08 \pm 0.037 | |
| IL-1 β (5 ng per rat) | 6.71 \pm 0.68† | | 3.91 \pm 0.59† | |
| IL-1 β (5 ng per rat)+CLS.H ₂ O (300 mg kg^{-1}) | 4.34 \pm 0.47* | 53.99 | 1.83 \pm 0.30* | 54.3 |
| IL-1 β (5 ng per rat)+Bethametasone (1 mg kg^{-1}) | 2.93 \pm 0.22** | 86.1 | 0.60 \pm 0.01** | 86.4 |
| IL-1 β (5 ng per rat)+Ketoprofen lysine salt (30 mg kg^{-1}) | 7.55 \pm 0.68 | +19.1 | 3.21 \pm 0.66 | 18.3 |

Data are expressed as mean \pm SE ($n=4-15$). Drugs were administered per os 1 h before IL-1 β injection. Inflammatory cells were evaluated in BAL fluids 4 h after IL-1 β . (LSM's test * $P < 0.05$, ** $P < 0.01$ vs IL-1 β ; † $P < 0.01$ vs control).

Table 2
Effect of Carbocysteine lysine salt monohydrate (CLS.H₂O) on carrageenan-induced pleurisy in rats

| Treatment | Volume (ml) | Total cells $\times 10^7$ | Neutrophils $\times 10^7$ | Eosinophils $\times 10^7$ | Macrophages $\times 10^7$ |
|--|----------------------------|---------------------------|---------------------------|----------------------------|---------------------------|
| Vehicle | 2.04 \pm 0.09 | 13.0 \pm 1.2 | 10.5 \pm 1.0 | 0.21 \pm 0.04 | 2.3 \pm 0.3 |
| CLS.H ₂ O 100 mg kg^{-1} | 1.88 \pm 0.10 (-7.8%) | 9.3 \pm 0.9* (-28.5%) | 7.4 \pm 0.7* (-29.5%) | 0.16 \pm 0.02 (-23.8%) | 1.7 \pm 0.2 (-23.3%) |
| CLS.H ₂ O 300 mg kg^{-1} | 1.52 \pm 0.06** (-25.5%) | 7.0 \pm 0.7** (-46.1%) | 5.3 \pm 0.5** (-49.5%) | 0.08 \pm 0.02** (-62.9%) | 1.6 \pm 0.2* (-30.8%) |

Data are reported as mean \pm SE from nine replications. In brackets is indicated the percentage inhibition vs vehicle. CLS.H₂O was administered per os 1 h before carrageenan injection. Inflammatory events were evaluated 6 h after carrageenan.

* $P < 0.05$, ** $P < 0.01$ as compared to vehicle (Mann-Whitney *U*-test).

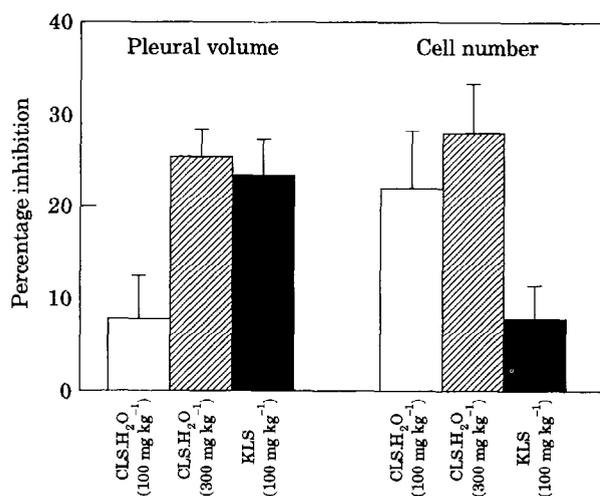


Fig. 1. Effect of Carbocysteine lysine salt monohydrate (CLS.H₂O) and ketoprofen lysine salt (KLS) on carrageenan-induced pleurisy in rats. CLS.H₂O and KLS were administered per os 1 h before carrageenan injection. Inflammatory events were evaluated 6 h after carrageenan. Data are reported as percentage inhibition vs control ($n=5-9$).

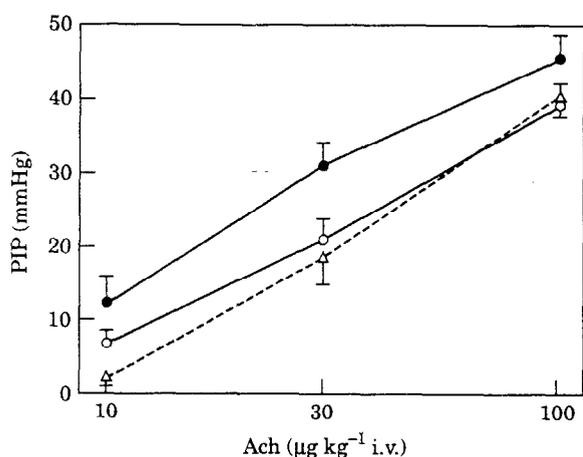


Fig. 2. Effect of Carbocysteine lysine salt monohydrate (CLS.H₂O) on airway hyperreactivity induced by cigarette smoke exposure in anaesthetized guinea pigs. CLS.H₂O was administered per os (300 mg.kg⁻¹) 1.5 h before smoke exposure. Ach-induced bronchoconstriction was evaluated 5 min after smoke and expressed as increase in pulmonary inflation pressure (PIP). Data are reported as mean \pm SE ($n=6-8$). [Dose ratio (DR) control/smoke: 1.77 \pm 0.02]. ○, control; ●, smoke; △, CLS. H₂O 300 mg kg⁻¹ p.o.+smoke.

pleural space. In particular, after oral treatment with both the doses (100 and 300 mg kg⁻¹), a significant reduction in the number of total cells and neutrophils was observed. Moreover, in animals treated with the highest dose, a significant decrease in eosinophils and macrophages was also evident (Table II). No changes in lymphocyte count was observed at any tested dose. Ketoprofen lysine salt inhibited the formation of pleural exudate by an extent similar to that of CLS.H₂O, but unlike the mucoactive drug it did not affect cell influx (Fig. 1).

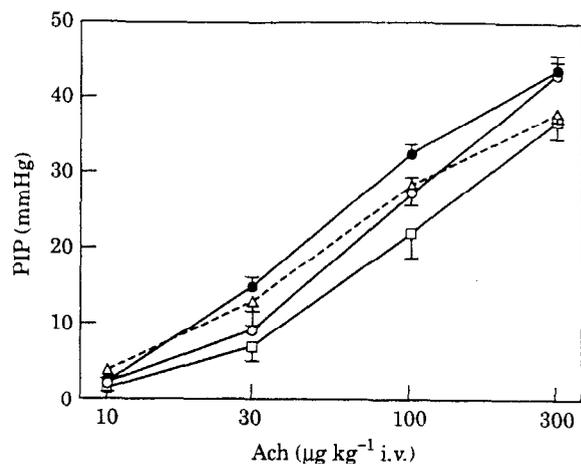


Fig. 3. Effect of Carbocysteine lysine salt monohydrate (CLS.H₂O) on airway hyperreactivity induced by cigarette smoke exposure in anaesthetized guinea pigs. CLS.H₂O was administered by aerosol (30 and 100 mg ml⁻¹×60 s) 3 min before smoke exposure. Ach-induced bronchoconstriction was evaluated 5 min after smoke and expressed as increase in pulmonary inflation pressure (PIP). Data are reported as mean \pm SE ($n=5$). (DR control/smoke: 1.41 \pm 0.11). ○, control; ●, smoke; △, CLS. H₂O 30 mg ml⁻¹+smoke; □, CLS. H₂O 100 mg ml⁻¹+smoke.

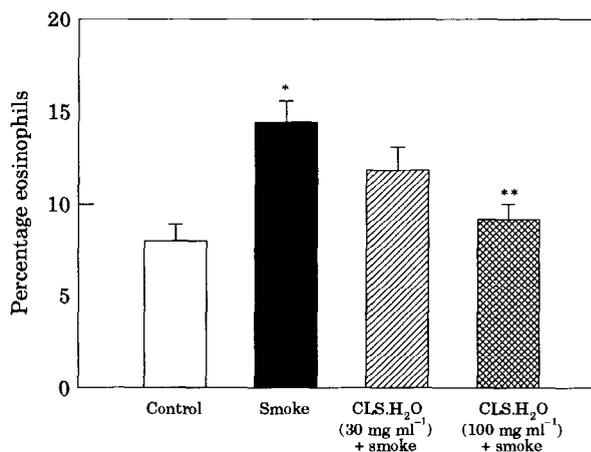


Fig. 4. Effect of Carbocysteine lysine salt monohydrate (CLS.H₂O) on airway inflammation induced by cigarette smoke exposure in anaesthetized guinea-pigs. CLS.H₂O was administered by aerosol (30 and 100 mg ml⁻¹×60 s) 3 min before smoke exposure. The percentage of eosinophils in BAL fluids was evaluated 5 min after smoke. Data are reported as mean \pm SE ($n=5$). * $P<0.05$, as compared to control animals. ** $P<0.05$, as compared to smoke exposed animals (LSM's test).

Smoke-induced airway inflammation and hyperreactivity

Active cigarette smoke exposure (15 s min⁻¹ for 10 min) in anaesthetized guinea-pigs triggered airway hyperreactivity, measured by an increase in the bronchoconstrictor effect of Ach. As shown in Figs 2 and 3, Ach dose-response curves obtained in smoke-exposed animals were significantly ($P<0.01$) shifted to the left when compared to control curves.

CLS.H₂O, administered by oral route (300

mg kg⁻¹), 1.5 h before smoke exposure, completely prevented the development of smoke induced airway hyperreactivity (Fig. 2). In fact, dose-response curves performed with Ach in animals treated with CLS.H₂O were significantly ($P < 0.05$) shifted to the right of those obtained in smoke-exposed group (DR treated/smoke: 1.96 ± 0.15).

After aerosol administration (30 and 100 mg ml⁻¹ × 60 s), CLS.H₂O also counteracted the ability of cigarette smoke to increase airway reactivity (Fig. 3), in a dose-dependent manner (DR treated/smoke: 1.35 ± 0.11 and 2.07 ± 0.14 respectively; $P < 0.05$ and $P < 0.01$). In a parallel set of experiments we evaluated the development of the inflammatory reaction associated with airway hyperreactivity. A significant ($P < 0.05$) recruitment of eosinophils was observed into the BAL fluids collected 5 min after smoke exposure ($14.5 \pm 1.10\%$) when compared to control animals ($8.0 \pm 0.90\%$) (Fig. 4). Other cells recovered in the BAL were not modified by smoke exposure (data not shown). In parallel with functional results, CLS.H₂O inhibited eosinophil infiltration in a dose-related manner when administered by the aerosol route. The significance level was reached at the concentration of 100 mg ml⁻¹ ($9.2 \pm 0.86\%$; $P < 0.05$) (Fig. 4).

DISCUSSION

Airway inflammation is the underlying feature of several obstructive lung diseases such as chronic bronchitis and asthma. Indeed, inflammatory cells and the many mediators they release are involved in airway hyperreactivity, mucus hypersecretion and mucosal oedema [4, 12], all together these pathological features contributing to airway obstruction.

Carbocysteine lysine salt monohydrate (CLS.H₂O) is a mucoregulatory drug characterized by a spectrum of activities other than a direct effect on mucus secretion. Indeed, normalization of mucus secretion is also associated to its ability to increase the chloride transport in the airway epithelium [9] and to counteract mucus discharge mediated by elastase [10].

On the basis of these experimental evidences we hypothesized that CLS.H₂O could possess other properties additive to its mucoregulatory action. Therefore, in order to verify this hypothesis we tested CLS.H₂O in different experimental models of airway inflammation and hyperreactivity.

The experimental results obtained in the first set of experiments indicate that CLS.H₂O is able to reduce neutrophil infiltration induced within the airways by intratracheal injection of IL-1 β . In this model, neutrophil recruitment is completely prevented by oral pretreatment with glucocorticoids, but is not affected by classical NSAID, as ketoprofen lysine salt (KLS). The ability of CLS.H₂O to counteract an inflammatory reaction was also confirmed in an other experimental model where pleural inflammation was induced by

injection of carrageenan. Indeed, in this experimental condition oral treatment with CLS.H₂O reduced significantly both the exudate volume and the leukocyte recruitment, in particular neutrophil and eosinophil influx. The protective effect on proinflammatory cells was not observed after oral administration of KLS, whereas this drug was able to reduce the increased pleural fluids by an extent similar to that of CLS.H₂O. These studies suggest that CLS.H₂O exerts its anti-inflammatory activity by a mechanism of action different than that of classical NSAIDs. Indeed, while CLS.H₂O can modulate also inflammatory cell recruitment, it is well known that NSAIDs such as KLS can prevent inflammatory exudate formation without affecting cell movement [17].

To better investigate and characterize the anti-inflammatory profile of CLS.H₂O, we evaluated its effectiveness after oral and aerosol treatment on airway inflammation and hyperreactivity induced by cigarette smoke. In these experiments, CLS.H₂O counteracted in a dose-related manner both the increased bronchial reactivity and eosinophil recruitment within the airway lumen. From the Ach-curves analysis it was also observed that aerosol pretreatment with CLS.H₂O, 100 mg ml⁻¹, induced a rightward-shift of the curve in comparison to the control curve (without smoke). This finding would suggest that CLS.H₂O could have a possible direct bronchodilator effect on airway smooth muscle. Up to now, there is no evidence for a muscle relaxing effect of CLS.H₂O, even if further experiments are required to clarify this point.

In conclusion, this study clearly demonstrated the capacity of CLS.H₂O, either per os or by aerosol, to counteract lung inflammatory events in different experimental models, supporting an anti-inflammatory action of CLS.H₂O at the respiratory level.

The mechanism by which CLS.H₂O exerts this effect it is not known. Clearly, as discussed above, blockade of the cyclooxygenase in a manner similar to NSAIDs can be ruled out. Since different patterns of mediators are involved in the three models of lung inflammation studied, CLS.H₂O activity observed in all of them does not seem to depend on a specific drug mediator interaction. However, the capacity of CLS.H₂O to prevent cell recruitment in the carrageenan-induced pleurisy at both the doses (100 and 300 mg kg⁻¹), whereas only the higher dose tested was able to inhibit pleural exudation, seems to suggest a preferentially effect of CLS.H₂O on cell recruitment. It is possible that the reported effect of CLS.H₂O on epithelial cells and/or on mucin secretion might contribute to reduce cell infiltration, together with antioxidant properties of the molecule. The anti-inflammatory activity noticed for CLS.H₂O, whatever mechanism is involved, represents a property relevant to its therapeutic effect. Indeed, inhibition of the inflammatory reaction and the associated release of

secretagogues might contribute to the control of the quality and quantity of mucus secretion. On the other hand, reduction in exudate formation and inflammatory cell recruitment would account for inhibition of other related pathological events, such as oedema and bronchial hyperreactivity, which contribute to airway obstruction. Therefore, the overall effect shown by CLS.H₂O might be of advantage in the therapy of lung diseases, such as asthma and COPD, where mucus hypersecretion is associated to airway inflammation and hyperreactivity.

REFERENCES

- O'Byrne PM. Airway inflammation and airway hyperresponsiveness. *Chest* 1986; **90**: 575-7.
- Lundgren JD, Shelhamer JH. Pathogenesis of airway mucus hypersecretion. *J Allergy Clin Immunol* 1990; **85**: 399-417.
- Hargreave FE, Gibson PG, Ramsdale EH. Airway hyperresponsiveness, airway inflammation and asthma. *Immunol Allergy Clin* 1990; **10**: 439-48.
- Lundgren JD, Baraniuk JN. Mucus secretion and inflammation. *Pulmonary Pharmacol* 1992; **5**: 81-96.
- Braga PC, Allegra L, Rampoldi C, Ornaghi A, Beghi G. Long-lasting effect on rheology and clearance of bronchial mucus after short-term administration of high doses of Carbocysteine-Lysine to patients with chronic bronchitis. *Respiration* 1990; **57**: 353-8.
- Puchelle E, Aug F, Polu JM. Effect of the mucoregulator S-Carboxy-methyl-Cysteine in patients with chronic bronchitis. *Eur J Clin Pharmacol* 1978; **14**: 177-84.
- Nobilini A. Meeting of the Italian Society of Respiratory Medicine, 1994, abstract book 86A.
- Havez R, Degand P, Roussel P, Randoux A. Mode d'action biochimique des dérivés de la cystéine sur le mucus bronchique. *Poumon Coeur* 1970; **26**: 81-90.
- Colombo B, Turconi P, Daffonchio L, Fedele G, Omini C, Cremaschi D. Stimulation of Cl⁻ secretion by the mucoactive drug S-carboxymethylcysteine lysine-salt in the isolated rabbit trachea. *Eur Respir J* 1994; **7**: 1622-8.
- Daffonchio L, De Santi MM, Gardi C, Lungarella G, Omini C. Effect of S-carboxymethylcysteine lysine salt on mucociliary clearance in rabbits with secretory cell metaplasia. *Res Comm Mol Path Pharmacol* 1994; **86**: 59-74.
- Ulich TR, Watson LR, Yin S, Guo K, Wang P, Thang H, del Castillo J. The intratracheal administration of endotoxin and cytokines. *Am J Pathol* 1991; **138**: 1485-96.
- Döring G. The role of neutrophil elastase in chronic inflammation. *Am J Respir Crit Care Med* 1994; **150**: S114-17.
- Tesson F, Ruffié C, Hidi R, M.R. e Silva P, Vazeux G, Vargaftig BB, Bon C. Cell-associated and soluble phospholipases A₂ increase during carrageenan and zymosan-induced pleurisy in rat. *Eur J Pharmacol* 1993; **248**: 27-32.
- Daffonchio L, Hernandez A, Gallico L, Omini C. Airway hyperreactivity induced by active cigarette smoke exposure in guinea-pigs: possible role of sensory neuropeptides. *Pulmon Pharmacol* 1990; **3**: 161.
- Boraschi D, Bossù P, Ruggiero P, Tagliabue A, Bertini R, Macchia G, Pellegrini L, Melillo G, Ulisse E, Visconti U, Bizzarri C, Del Grosso E, Mackay AR, Frascotti G, Frigerio F, Grifantini R, Grandi G. Substitution of an active domain of IL-1 selectively abolishes binding capacity for IL-1R_I. *J Immunol* in press.
- SAS/STAT User's Guide. Version 6, IV Ed. Cary, NC, USA 1990.
- Goodman Gilman A, Rall TW, Nies AS, Taylor P. (Eds) *The pharmacological basis of therapeutics*. Chapter 26, 638-670. VIII edition. Pergamon Press, 1990.