Effect of a mucoactive compound (CO 1408) on airway hyperreactivity and inflammation induced by passive cigarette smoke exposure in guinea-pigs

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ABSTRACT: Environmental exposure to tobacco smoke contributes to the onset of several lung diseases, e.g. chronic bronchitis and asthma, including an increase in airway reactivity. We have investigated the effect of a new mucoactive compound, CO 1408, on airway hyperreactivity and lung inflammation induced in guinea-pigs by passive cigarette smoke exposure.

Animals were exposed to cigarette smoke in a Plexi-glass box, three times a day for four days. Airway reactivity to histamine was assessed ex-vivo in lung parenchymal strips. As a measure of lung inflammation, the number of leucocytes was evaluated in bronchoalveolar lavage (BAL) fluids and histological sections.

Passive smoke exposure potentiated histamine-induced contraction in lung parenchymal strips, a phenomenon associated with an increase in proinflammatory cells in the BAL fluids and enhanced eosinophil infiltration into parenchymal tissues. Pretreatment with oral CO 1408 at 400 mg·kg\(^{-1}\) but not 100 mg·kg\(^{-1}\), completely prevented the cigarette smoke-induced airway hyperreactivity. 400 mg·kg\(^{-1}\) CO 1408 also inhibited the increase in cell numbers in the BAL fluids, but not eosinophil recruitment in parenchymal tissues.

The present data indicate the ability of CO 1408 to modulate smoke-induced airway hyperreactivity and, to some extent, lung inflammation, an effect which might be of value in the therapy of obstructive pulmonary diseases.
was administered at the doses of 100 and 400 mg·kg\(^{-1}\) was dissolved each day in distilled water. The compound in the Plexi-glass box but not exposed to cigarette smoke. (Auergesellschaft GMBH, Berlin). (n=3), as measured by "carbon monoxide detector tube" in the box during each smoke exposure was 0.26±0.01% days. As reported previously [7], the average CO% volume separated by a 30 min interval, each day, for 4 consecutive filled chamber for three exposures of 10 min duration, a respiration pump. The animals were kept in the smoke-box (30 \(\times\) 30 cm) and the smoke of one cigarette (Stop, Monopoli di Stato, Italy) was delivered into the box using a respiration pump. The animals were placed in a Plexi-glass box but not exposed to cigarette smoke.

Passive smoke exposure

Passive smoke exposure was performed as described previously [7]. The animals were placed in a Plexi-glass box (30 \(\times\) 15 \(\times\) 15 cm) and the smoke of one cigarette (Stop, Monopoli di Stato, Italy) was delivered into the box using a respiration pump. The animals were kept in the smoke-filled chamber for three exposures of 10 min duration, separated by a 30 min interval, each day, for 4 consecutive days. As reported previously [7], the average CO% volume in the box during each smoke exposure was 0.26±0.01% (n=3), as measured by "carbon monoxide detector tube" (Auergesellschaft GMBH, Berlin). The animals of the sham-exposed groups were placed in the Plexi-glass box but not exposed to cigarette smoke. CO 1408 (supplied by Camillo Corvi manufacturer) was dissolved each day in distilled water. The compound was administered at the doses of 100 and 400 mg·kg\(^{-1}\) per os, 30 min before the first smoke or sham exposure on each day (four treatments).

In vitro functional studies

Guinea-pigs were sacrificed, 30 min after the last smoke or sham exposure, by excess urethane (Fluka) anaesthesia and exsanguination. The lungs were removed and parenchymal strips (3 cm in length) prepared according to Hernández et al. [9]. Tissues were mounted in conventional organ baths (20 ml) containing Krebs-bicarbonate solution (37°C) aerated with O\(_2\)/CO\(_2\) (95/5%). A load of 1 g was applied to the tissues, and changes in their length were recorded by isotonic transducers (Basile 7006; Comerio, Italy) connected to a Gemini polygraph (Basile 7070; Comerio, Italy). The signals from the transducers were conveniently amplified before drawing the traces on the paper graph. The amplification was maintained constant for all the tissues examined. After 30 min stabilization, cumulative concentration-response curves for histamine (10\(^{-10}\)–10\(^{-4}\) M; histamine dihydrochloride, Carlo Erba) were performed in lung parenchymal strips obtained from sham- and smoke-exposed guinea-pigs.

Data were expressed in millimetres of change in amplitude of the recorded signal, as measured on the amplified paper graph.

Bronchoalveolar lavage

Guinea-pigs were anaesthetized with urethane 30 min after the last sham or smoke exposure, and a BAL was performed as described previously [7]. Briefly, the lungs were gently washed through the trachea with 3\(\times\)10 ml of 0.9% NaCl (37°C). The recovered fluid (more than 97%) was centrifuged for 10 min at 800 \(\times\) g and the pellet resuspended in phosphate-buffered saline. Total cell count was determined after dilution with Unopette microcollection system (Becton-Dickinson), using a Burker counting chamber and phase contrast microscopy. Differential cell count was made on the same samples stained with May-Grünwald-Lösung (Merck) and Giemsa-Lösung (Merck) under light microscopy, counting 200 cells in each sample. Data were expressed as cell\(\times\)10\(^6\) recovered in 30 ml BAL fluid.

Histological analysis

Animals were sacrificed 30 min after the last sham or smoke exposure, by excess urethane anaesthesia. The chest was opened and the pulmonary artery rapidly cannulated through a ventricular incision. The lungs were perfused in situ for 10 min with 0.9% NaCl (25 ml\(\times\)min\(^{-1}\)), and subsequently fixed by perfusing for 20 min with 4% formalin phosphate-buffered saline. After removal, the lungs were immersed in fixative and stored at 4°C. Left caudal lobes were later processed for wax histology and 4 mm sections cut (12 from each lobe) and stained with haematoxylin and eosin. The number of infiltrated eosinophils was determined for each section by light microscopy. Data were expressed as mean cells counted in 12 consecutive sections of each lung.

Statistical analysis

Data were expressed as mean±SEM from (n) replications. Comparisons between the linear portion of the concentration-response curves in the different experimental groups were performed by means of a computer-aided program, following the variance analysis for parallel regression lines [10]. This procedure allows initial calculation of the presence or absence of parallelism between the lines under comparison. Once established that, as in our case, no significant difference from parallelism could be demonstrated, the dose-ratio and its 95% confidence limits (95% CL) were calculated from the analysis of variance according to Finney [11], as the antilogarithm to the horizontal distance between the curves.

Other comparisons were performed by a multiple variance analysis (Duncan’s test). A probability level of p<0.05 was considered statistically significant.

Results

We have investigated the activity of CO 1408 on the effects of passive cigarette smoke exposure of guinea-pigs. CO 1408 (400 mg·kg\(^{-1}\) per os) itself did not modify the histamine induced contractions (fig. 1). The dose-ratio calculated between concentration-response curves to histamine in isolated lung parenchymal strips taken from sham exposed control guinea-pigs (n=4) and those taken from sham exposed CO 1408 treated guinea-pigs (n=4) was 1.79 (95% CL 0.70–4.52).
Airway hyperreactivity testing

Passive cigarette smoke exposure in conscious guinea-pigs resulted in airway hyperreactivity, shown by an increase in histamine induced contractions in isolated parenchymal strips. As reported in figure 2, histamine concentration-response curves performed in lung parenchymal strips taken from cigarette smoke-exposed animals (n=6) were significantly (p<0.01) shifted to the left, when compared to those obtained in sham-exposed preparations (n=4). The dose-ratio calculated in these experiments was 7.38 (95% CL 1.32–41.33). Maximal contraction to histamine in sham- and smoke-exposed tissues were not significantly different, being 72.6±8.3 and 102.0±15.7 mm respectively (p>0.05). CO 1408, administered per os, at a dose of 100 mg·kg⁻¹ before smoke exposure did not significantly reduce the degree of smoke-induced hyperreactivity (fig. 2). However, histamine-induced contraction was significantly (p<0.01) increased in lung parenchymal strips taken from these animals (n=4), when compared to sham-exposed controls (dose-ratio=3.17; 95% CL 1.31–7.69).

At a dose of 400 mg·kg⁻¹ per os, CO 1408 completely inhibited the cigarette smoke induced increased reactivity to histamine (fig. 3). Histamine-induced contractions in these treated lung parenchymal strips were similar to those from sham-exposed control guinea-pigs but were significantly different (p<0.01) from those from untreated animals exposed to cigarette smoke. The dose-ratio calculated for this comparison was 7.01 (95% CL 1.59–30.85).

Bronchoalveolar lavage

There was a significant (p<0.01) increase in total cell count, eosinophils and macrophages, in the BAL fluids obtained from smoke-exposed animals, when compared to sham-exposed controls (table 1). CO 1408 (400 mg·kg⁻¹ per os) inhibited the changes in number of inflammatory cells in animals exposed to smoke (table 1). Total cell, eosinophil and macrophage numbers were similar in BAL fluids obtained from sham-exposed control guinea-pigs and animals exposed to cigarette smoke after CO 1408 treatment. However, the number of total cells and eosinophils in BALs of CO 1408 treated animals were significantly lower than in untreated guinea-pigs exposed to smoke. Preliminary experiments (data not reported) did not show a significant difference in the number and type of cells in BAL between sham-exposed animals receiving CO 1408 and sham-exposed controls.

Fig. 1. – Effect of CO 1408, 400 mg·kg⁻¹ per os, on histamine-induced contraction. Data are expressed as millimetres of change in amplitude of the recorded signal induced by histamine in lung parenchymal strips, and represent the mean±SEM of n replications. –: sham-exposed control guinea-pigs (n=4); –: sham-exposed guinea-pigs after CO 1408 treatment (n=4).

Fig. 2. – Effect of CO 1408 (100 mg·kg⁻¹ per os) on airway hyperreactivity induced by passive cigarette smoke exposure. Data are expressed as millimetres of change in amplitude of the recorded signal induced by histamine in lung parenchymal strips, and represent the mean±SEM of n replications. –: sham-exposed control guinea-pigs (n=4); –: smoke-exposed control guinea-pigs (n=6); –: guinea-pigs exposed to smoke after CO 1408 treatment (n=4).

Fig. 3. – Effect of CO 1408 (400 mg·kg⁻¹ per os) on airway hyperreactivity induced by passive cigarette smoke exposure. Data are expressed as mm of change in amplitude of the recorded signal induced by histamine in lung parenchymal strips, and represent the mean±SEM of n replications. –: sham-exposed control guinea-pigs (n=4); –: smoke-exposed control guinea-pigs (n=6); –: guinea-pigs exposed to smoke after CO 1408 treatment (n=6).
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extend these findings. In particular, the histological exam-  
sults of 12 subsequent lung parenchymal sections taken from smoke-exposed guinea-pigs, as com-  
pared to sham-exposed controls. CO 1408 (400 mg·kg-1 per os) did not reduce eosinophil infiltration within the lung parenchymal tissue. The mean number of eosinophils counted in 12 subsequent lung parenchymal sections was 1.94±0.18 (n=3), 4.20±0.39 (n=3) and 4.08±0.71 (n=5) in sham-exposed animals, smoke-exposed animals and guinea-pigs exposed to smoke after CO 1408 treatment, respectively.

**Discussion**

The capacity of cigarette smoke to enhance airway responsiv-  

ease to different stimuli has been confirmed by several experimental studies [12–14]. In this regard, we have previously demonstrated [7] that passive cigarette smoke exposure induces airway hyperreactivity, as shown by increased bronchial contractions to histamine, both in vivo and in isolated lung parenchymal strips in vitro. This was associated with an increase in proinflammatory cells, particularly eosinophils, within the airway lumen, as shown by BAL studies.

The results reported in this study confirm and further extend these findings. In particular, the histological examination showed that passive cigarette smoke exposure also led to increased numbers of eosinophils in lung parenchymal sections. These data seem to be in contrast with those recently reported by NISHIKAWA et al. [14]. These authors claimed that airway hyperreactivity induced by cigarette smoke was not accompanied by inflammatory changes, since they could not detect an increase in neutrophil numbers in tracheal tissue. The different experimental procedures used in the two studies might account for this discrepancy. In our previous experiments [7, 12], we were also unable to detect an increase in neutrophil numbers in the BAL fluids of smoke exposed animals, suggesting that smoke-exposure mainly affects eosinophil recruitment. Moreover, the trachea might not represent the appropriate tissue in which to evaluate the inflammatory reaction, since airway hyperreactivity induced by passive smoke exposure specifically involves changes in the contractile responsiveness of peripheral airways (parenchymal strips) [7]. Therefore, our data support a parallelism between the induction of hyperreactivity and the inflammatory reaction, and particularly show that similar changes in inflammatory cell recruitment can be detected both by BAL and histological analysis. However, the relative importance of leucocytes in BAL fluid or in tissues, in the development of airway hyperreactivity is still debated. In this regard, data obtained with CO 1408 may provide further insights.

CO 1408 is a new mucoactive drug with potential effective-  

ness in some obstructive lung diseases [8]; it possesses a bronchosecretogogue activity in rats and increases the mucociliary transport rate. Our results demonstrate that this compound also possesses an inhibitory effect against the smoke-induced airway hyperreactivity and inflammation, which was dose-dependent only at the highest dose tested (400 mg·kg⁻¹ per os) did CO 1408 completely prevent smoke-induced hyperreactivity and the increase in eosinophils in the lavage fluid. However, CO 1408 pre-treatment did not prevent the increase in eosinophil numbers in parenchymal tissue induced by smoke.

These data suggest that recruitment of inflammatory cells into the airway lumen could be more relevant to the induction of the airway hyperreactivity to histamine than recruitment in lung parenchyma. It is thus possible that the efficacy of CO 1408 in this model of airway hyperreactivity might depend on its ability to differentially modulate leucocyte activation, subsequent to their recruitment either into the airway lumen or parenchymal tissue. Thus, it would be interesting to test the capacity of CO 1408 to modulate the release, from activated leucocytes, of those mediators, such as eosinophil-derived proteins, eicosanoids and free radicals, which have been involved in the genesis of airway hyperreactivity in other experimental models [15, 16].

In conclusion, our data demonstrate that passive cigarette smoke exposure in guinea-pigs causes airway hyperreactivity to histamine, associated with signs of lung inflammation. Moreover, these results indicate that CO 1408 may prevent the smoke-induced airway hyperreactivity and, to some extent, the associated recruitment of inflammatory cells. Allied to its mucoactive properties, such effects of this compound might increase its potential usefulness in the therapy of obstructive pulmonary diseases.

### Table 1. – Effect of CO 1408, 400 mg·kg⁻¹ per os, on the increase in number of inflammatory cells in the airway lumen induced by passive cigarette smoke exposure

<table>
<thead>
<tr>
<th></th>
<th>Sham-exposed control</th>
<th>Smoke-exposed control</th>
<th>Smoke-exposed CO 1408</th>
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<tr>
<td></td>
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<td>n=4</td>
<td>n=5</td>
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<tr>
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<td>23.2±0.8##</td>
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<td>0.9±0.5*</td>
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<td>Macrophages</td>
<td>13.7±1.9</td>
<td>22.3±2.9*</td>
<td>19.0±1.3</td>
</tr>
</tbody>
</table>

Data are expressed as cells×10⁶ recovered in 30 ml BAL fluid, and represent the mean±SEM of n replications. *: p<0.05; **: p<0.01 as compared to control; #: p<0.05; ##: p<0.01 as compared to smoke exposed controls (Duncan’s test). BAL: bronchoalveolar lavage.
References


