Effect of cilomilast (Ariflo) on TNF-α, IL-8, and GM-CSF release by airway cells of patients with COPD

M Profita, G Chiappara, F Mirabella, R Di Giorgi, L Chimenti, G Costanzo, L Riccobono, V Bellia, J Bousquet, A M Vignola

Background: Inflammation in chronic obstructive pulmonary disease (COPD) is characterised by increased neutrophilic infiltration of the airways. Cilomilast, a novel selective phosphodiesterase 4 inhibitor in clinical development for COPD treatment, exerts anti-inflammatory effects. The ability of cilomilast to inhibit the release of neutrophil chemoattractants such as tumour necrosis factor (TNF-α), interleukin (IL)-8, and granulocyte-macrophage colony stimulating factor (GM-CSF) by bronchial epithelial cells and sputum cells isolated from 10 patients with COPD, 14 normal controls, and 10 smokers was investigated.

Methods: Bronchial epithelial cells obtained by bronchial brushing and sputum cells isolated from induced sputum samples were cultured for 24 hours in the presence or absence of cilomilast (1 μM). After incubation the supernatants were harvested and the levels of mediators measured by ELISA. Chemotactic activity in supernatants was also measured using a Boyden chamber.

Results: TNF-α and IL-8 release by bronchial epithelial cells and sputum cells was higher in patients with COPD than in controls (p<0.0001) and smokers (p<0.0001). GM-CSF was only detectable in sputum cell supernatants and its level was higher in patients with COPD than in controls and smokers (p<0.0001, respectively). Cilomilast significantly reduced TNF-α release by bronchial epithelial cells and sputum cells (p=0.005) and GM-CSF release by sputum cells (p=0.003), whereas IL-8 release was not statistically inhibited. Supernatants of sputum cells and bronchial epithelial cells treated with cilomilast significantly decreased neutrophil chemotaxis (p<0.006 and p<0.008, respectively).

Conclusions: Cilomilast inhibits the production of some neutrophil chemoattractants by airway cells. This drug may play a role in the resolution of neutrophilic inflammation associated with COPD and cigarette smoke.
COPD patients with radiographic or CT evidence of emphysema were excluded. Healthy smokers (median 40 pack years, range 32–45) had pulmonary function within the normal range. Normal subjects (controls) had never suffered from asthma or chronic bronchitis or from any bronchial or respiratory tract infection during the month preceding the study. All were lifelong non-smokers and their pulmonary function was within the normal range.

Subjects were excluded if they had had a bronchial infection during the month preceding the study. No subject had received corticosteroids or theophylline in any form during the 2 months before the study. The study was approved by the local ethics committee and subjects gave their informed consent.

### Table 1: Assessment of inhibitory effects of different concentrations of cilomilast on mediators released by bronchial epithelial cells and sputum cells isolated from patients with COPD

<table>
<thead>
<tr>
<th>Bronchial epithelial cells</th>
<th>Sputum cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Cilomilast 0.1 µM</strong></td>
</tr>
<tr>
<td>TNF-α</td>
<td>166 (165.2–201.2)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>NA</td>
</tr>
</tbody>
</table>

Results are expressed as median and 25–75th percentiles of three consecutive experiments. NA=not available because of undetectable levels of GM-CSF.

**Isolation of bronchial epithelial cells**

Bronchial epithelial cells were recovered by bronchial brushing as previously described.\(^5\) Most of the patients were volunteers; two underwent bronchoscopy for diagnostic purposes. Briefly, the cells were pelleted by centrifugation (400g, 4°C, 10 minutes), washed three times, and resuspended in 1 ml RPMI at a final density of 1 × 10⁶ cells/ml. Slides prepared by cytospin centrifugation (800g, 10 minutes, Cytospin, Shandon Southern Products, Runcorn, Cheshire, UK) of 200 µl of the cell suspension were stained with May-Grunwald-Giemsa for differential cell counts and by immunocytochemistry for cytokeratin using an alkaline phosphatase, anti-alkaline phosphatase (APAAP) system (Dako, Versailles, France) to assess their epithelial origin. Negative controls were prepared using a mouse IgG1 MOPC cell line antibody (Organon Laboratories, West Chester, PA, USA) as the primary antibody.

**Treatment of bronchial epithelial cells with cilomilast**

Bronchial epithelial cells were cultured in the absence or presence of cilomilast (SB-207499 BULK, SmithKline Beecham, UK) 1 µM for 24 hours at 37°C in a humidified 5% CO₂. This concentration of cilomilast was determined from three consecutive dose-response curves in which the effects on the release of TNF-α and GM-CSF of three concentrations of cilomilast (0.1, 1 and 10 µM) were assessed (table 1). At the end of the incubation period the cell viability was >90% as assessed by trypan blue exclusion and the cell supernatants were harvested and stored at −80°C for further analysis (TNF-α, IL-8, and GM-CSF) and chemotaxis assay.

**Sputum induction and processing**

Sputum induction and processing were performed according to the methods of Hargreave et al without modification.\(^6\) Differential cell counts were performed on cytospin centrifugation preparations stained with May-Grunwald-Giemsa. In all cases 400 cells were counted by two blind observers and the results are expressed as a percentage of the total cells.

**Treatment of sputum cells with cilomilast**

Sputum cells were resuspended at a concentration of 1 × 10⁶/ml in RPMI 1640 + 10% heat inactivated (56°C, 30 minutes) FCS + 1% penicillin-streptomycin solution + 1 mM/l-Glutamine (RPMI buffer). The sputum cells were cultured in the absence or presence of cilomilast 1 µM for 24 hours at 37°C in a humidified 5% CO₂. This concentration of cilomilast was determined on the basis of three consecutive dose-response curves in which the effects of three concentrations of the drug (0.1, 1 and 10 µM) on the release of TNF-α and GM-CSF were assessed. Dose-response curve experiments with a higher number of concentration points were precluded by the limited number of cells. At the end of the incubation time the cell viability was >85%, as assessed by trypan blue exclusion, and supernatants were harvested and stored at −80°C for further analysis (TNF-α, IL-8, and GM-CSF) and chemotaxis assay.

**Measurement of TNF-α, IL-8 and GM-CSF**

The absolute values of TNF-α, IL-8, and GM-CSF in supernatants of bronchial epithelial cells and sputum cells were assessed using commercially available specific enzyme immunoassay kits (EIA). TNF-α, IL-8, and GM-CSF kits were purchased from R&D Systems (Minneapolis, USA) and their limits of detection were <0.18 pg/ml, 3 pg/ml, and 10 pg/ml, respectively.

**Neutrophil chemotaxis assay**

To lend further support to the hypothesis that cilomilast may have a role in decreased neutrophil migration within the airways of subjects with COPD, we evaluated the ability of supernatants of bronchial epithelial cells and sputum cells incubated in the presence or absence of cilomilast 1 µM for 24 hours at 37°C in humidified 5% CO₂ to induce neutrophil chemotaxis. Neutrophils were obtained from the peripheral blood of healthy donors as previously described,\(^2\) resuspended at a concentration of 1 × 10⁷/ml in PBS buffer, and incubated with supernatants of bronchial epithelial and sputum cells for chemotaxis assay.

To determine how much of the supernatant chemotactic activity was due to IL-8 (whose activity was not affected by cilomilast), the sputum cell supernatants were cultured in four blocking experiments for 24 hours with or without cilomilast 1 µM, then incubated for 30 minutes with an anti-IL-8 monoclonal antibody (Genzyme, Cambridge, MA, USA) in a concentration of 1 mg/ml at 37°C before loading the chemotaxis chamber.\(^3\) Chemotaxis was performed using a 48-well microchemotaxis chamber (Costar; Neuro Probe, Cabin Jonh, MD, USA) as previously described. Briefly, neutrophils were loaded into the upper well and supernatants of induced bronchial epithelial and sputum cells were in the bottom chamber. The two wells were separated by a polycarbonate filter paper with a pore size of 3 µm. The chamber was incubated at 37 °C for 1 hour, after which the filter was fixed, stained, and mounted on a glass microscope slide (observed at 400x). The numbers of cells migrating beyond a certain depth into the filter were counted. Each experimental condition was performed in duplicate and 3–4 fields were assessed. The
chemotactic activity of the supernatants was also evaluated and the results expressed as medians with 25–75th percentiles. The number of cells migrating spontaneously in the presence of RPMI 10% FCS under the same conditions as the supernatants (negative control) was subtracted from all measurements before data analysis.

Statistical analysis
The results are expressed as median values with 25–75th percentiles. Statistical analysis was performed with a non-parametric Kruskal-Wallis test with Bonferroni Dunn’s post hoc test to assess differences between groups. Statistical analysis of the effects of bronchial epithelial and sputum cell supernatants cultured in the presence of cilomilast was performed using the Wilcoxon test.

RESULTS

Demographic characteristics of patients
The differences in the median ages of the controls, healthy smokers, and subjects with COPD were not statistically significant. Forced expiratory volume in 1 second (FEV1) was lower in patients with COPD than in smokers (p<0.0001; table 2). All patients with COPD had moderate disease (FEV1 43–75% predicted). Eight were classified as IIA moderate COPD; eight as IIB moderate COPD (FEV1 43–45% predicted). The percentage of squamous cells in sputum samples was significantly higher in patients with COPD than in controls and smokers (p<0.0001 and p=0.0005, respectively); and the percentage of eosinophils was higher in patients with COPD than in controls (p=0.005). No statistical differences were observed in lymphocytes and bronchial epithelial cells (table 3).

Effects of cilomilast on TNF-α, IL-8, and GM-CSF release
Cultured bronchial epithelial cells isolated from patients with COPD released higher levels of TNF-α (165 (89–180) pg/ml) than cells isolated from smokers (65 (50–85) pg/ml) and control subjects (17 (12–25) pg/ml; p<0.0001). In addition, bronchial epithelial cells isolated from smokers with COPD released higher levels of TNF-α than those isolated from controls (p<0.0001; fig 1A). When bronchial epithelial cells isolated from COPD patients were cultured in the presence of cilomilast there was a significant inhibition of TNF-α release (116 (40–134) pg/ml; p<0.0001, fig 1B). Cultured sputum cells isolated from patients with COPD released higher levels of TNF-α (1650 (1421–1720) pg/ml) than sputum cells isolated from smokers (40 (320–500) pg/ml) and control subjects (97.5 (90–115) pg/ml; p<0.0001). In addition, sputum cells isolated from smokers released higher levels of TNF-α than cells isolated from controls (p=0.005; fig 1C). Furthermore, when the sputum cells isolated from COPD patients were cultured in the presence of cilomilast there was a significant inhibition of TNF-α release (1230 (1020–1320) pg/ml; p=0.005, fig 1D).

Cultured bronchial epithelial and sputum cells isolated from patients with COPD released higher levels of IL-8 than cells isolated from smokers and controls (bronchial epithelial cells: 4423.5 (4325–4589) pg/ml; sputum cells: 4458.5 (4255–4577) pg/ml; p<0.0001). In addition, sputum cells isolated from COPD patients released higher levels of IL-8 than sputum cells isolated from smokers (404 (320–500) pg/ml) and control subjects (97.5 (90–115) pg/ml; p<0.0001). In addition, sputum cells isolated from smokers released higher levels of IL-8 than cells isolated from controls (p=0.005; fig 1C). Furthermore, when the sputum cells isolated from COPD patients were cultured in the presence of cilomilast there was a significant inhibition of IL-8 release (1230 (1020–1320) pg/ml; p=0.005, fig 1D).

Cilomilast did not significantly reduce IL-8 release by bronchial epithelial cells (baseline: 4423.6 (4325–4589) pg/ml; cilomilast: 4336.5 (4247–4526) pg/ml; % inhibition compared

Table 2  Demographic characteristic of patients

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>Smoker (S)</th>
<th>COPD</th>
<th>p value†</th>
<th>C v S*</th>
<th>C v COPD*</th>
<th>S v COPD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>8</td>
<td>10</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Sex [M/F]</td>
<td>9/5</td>
<td>6/2</td>
<td>6/4</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>50 (40–70)</td>
<td>41 (33–59)</td>
<td>63 (56–71)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Smoke (% of current smokers)</td>
<td>0%</td>
<td>100%</td>
<td>70%</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Packs/year*</td>
<td>0</td>
<td>40 (32–45)</td>
<td>37.5 (32.5–52)</td>
<td>0.0001</td>
<td>p=0.009</td>
<td>p=0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>FEV1 (%) *</td>
<td>105 (100–110)</td>
<td>93 (90–103)</td>
<td>54 (50–62.5)</td>
<td>0.0001</td>
<td>p=0.009</td>
<td>p=0.0001</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Results are expressed as median with 25–75th percentiles in parentheses.
* p value for non-parametric Bonferroni Dunn’s post hoc test.
† p value for non-parametric Kruskal-Wallis test comparing the differences between the groups.

Table 3  Cell analysis of sputum samples

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>Smoker (S)</th>
<th>COPD</th>
<th>p value†</th>
<th>C v S*</th>
<th>C v COPD*</th>
<th>S v COPD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability (%)</td>
<td>86.8 (67–89.3)</td>
<td>75.7 (73.4–83.3)</td>
<td>80.5 (74.6–87.8)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Squamous cells (%)</td>
<td>4.3 (2.9–8.0)</td>
<td>2.8 (1.1–4.9)</td>
<td>4.1 (2.5–4.4)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total cell counts (10⁶/g sputum)</td>
<td>2.4 (1.9–3.0)</td>
<td>5 (4.2–6.4)</td>
<td>9.7 (2.5–17.2)</td>
<td>NS</td>
<td>NS</td>
<td>p&lt;0.0023</td>
<td>NS</td>
</tr>
<tr>
<td>Differential cell counts (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>83.1 (72.4–87.8)</td>
<td>57.2 (49.4–72.4)</td>
<td>25.2 (15.1–39.3)</td>
<td>p&lt;0.0001</td>
<td>NS</td>
<td>p&lt;0.0001</td>
<td>p=0.0002</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>15.1 (12.7–26.0)</td>
<td>40.6 (25.8–47.0)</td>
<td>68.2 (54.4–81.0)</td>
<td>p&lt;0.0001</td>
<td>NS</td>
<td>p&lt;0.0001</td>
<td>p=0.0005</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0 (0–0.3)</td>
<td>0.9 (0.1–1.7)</td>
<td>1.9 (0.8–4.8)</td>
<td>p&lt;0.008</td>
<td>NS</td>
<td>p=0.008</td>
<td>NS</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>0.4 (0.1–0.8)</td>
<td>0 (0–0.2)</td>
<td>0.2 (0–0.9)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as median with 25–75th percentiles in parentheses.
* p value for non-parametric Bonferroni Dunn’s post hoc test.
† p value for non-parametric Kruskal-Wallis test comparing the differences between the groups.
with baseline 1.4% (0–3.3), fig 2B) and sputum cells (baseline: 4458.5 (4255–4677) vs 4345 (4125–4581) pg/ml; % inhibition compared with baseline 3.1% (0–6.6, fig 2D) isolated from patients with COPD.

Bronchial epithelial cells from control subjects, smokers, and patients with COPD released very low or undetectable levels of GM-CSF. Cultured sputum cells isolated from patients with COPD (599.5 (454–657) pg/ml) released higher levels of GM-CSF than sputum cells isolated from smokers (294 (253–325) pg/ml) and control subjects (125 (100–150) pg/ml; p=0.0002 and p<0.0001, respectively; fig 3A). Furthermore, cilomilast significantly inhibited GM-CSF release by sputum cells isolated from patients with COPD (322 (250–350) pg/ml; p=0.005, fig 3B).

**Effect of cilomilast on neutrophil chemotaxis assay**

Supernatant fluids harvested from bronchial epithelial and sputum cells from patients with COPD recovered and cultured in the presence or absence of cilomilast were tested for their capacity to induce neutrophil chemotaxis. Supernatants recovered from bronchial epithelial cells incubated with cilomilast had significantly less chemotactic activity for neutrophils than supernatants from cells untreated with cilomilast (200.5 (150–221) vs 130 (120–169) cells high power field; p=0.008, fig 4A). Furthermore, supernatants recovered from sputum cells incubated with cilomilast had significantly lower chemotactic activity for neutrophils than those from cells untreated with cilomilast (198.5 (167–267) vs 124 (119–172.5) cells high power field; p=0.006, fig 4B).

In addition, blocking the experiments showed that the combination of an anti-IL-8 antibody with cilomilast exerted a greater inhibitory effect on neutrophil chemotaxis than either the anti-IL-8 antibody or cilomilast alone (from 80 (75–115) to 149 (127.5–200) or 124 (119–172.5) cells high power field).

**DISCUSSION**

This study shows that cilomilast inhibits the release of TNF-α and GM-CSF by bronchial epithelial cells and sputum cells isolated from patients with COPD. In addition, cilomilast significantly reduced the chemotactic activity of supernatant fluids harvested from cultured bronchial epithelial and sputum cells of COPD patients.

The levels of cAMP play an important role in the modulation of the functional activation of several airway cells. Increased levels of cAMP have been shown to decrease the release of neutrophil chemotactic activity as well as neutrophil adhesion to bronchial epithelial cells via MAC-1. In airway epithelial cells the rise in cAMP was found to be protective against oxidants and to enhance the release of prostaglandin E2. It is therefore conceivable that increases in the cellular concentration of cAMP not only mediate the relaxation of airway smooth muscle but also inhibit the activation of inflammatory cells.

Inflammatory and immunomodulatory cells contain PDE4 and many functions of these cells are inhibited by selective PDE4 inhibitors. In addition, PDE4 seems to be the most important isoenzyme in both the microsomal and cytoplasmic compartments of airway cells.

Cilomilast is a second generation PDE4 inhibitor effective in the treatment of COPD. It has high selectivity for the cyclic AMP specific (or PDE4) isoenzyme. In vitro, cilomilast suppresses the activity of many proinflammatory and immune cells implicated in the pathogenesis of COPD and is highly active in animal models. In vitro studies have also suggested that cilomilast can inhibit antigen driven IL-5 production, cytokine induced adhesion to endothelial cells, and chemotaxis. However, most of these effects have been evaluated using cell lines or animal models and, to date, no studies have assessed the effects of cilomilast on airway cells isolated from patients with COPD.
In this study sputum cells were cultured to assess their ability to release inflammatory mediators and to respond to a drug such as cilomilast. It should be noted that, in reality, drugs or stimuli usually target the various cell types present in the airways and rarely just a single cell population, so we used airway cells from COPD patients to confirm the ability of cilomilast in a concentration of 1 \( \mu \text{M} \) to influence the functional activation of airway cells. This concentration of cilomilast was chosen based on the results of the dose-response curve experiments as well as on previous evidence.

Cilomilast significantly inhibited the release of TNF-\( \alpha \) and GM-CSF by both epithelial and sputum cells, while there was no inhibitory effect on IL-8 release.

It is unlikely that the heterogeneity of the cell population is the main cause of the different effects of cilomilast on mediator release and on the low or absent inhibitory effect on IL-8 as similar results were obtained with cultured bronchial epithelial cells which were virtually pure. There are several possible explanations for the lack of inhibition by cilomilast on IL-8 release. It is possible that IL-8 release might have been inhibited by a higher concentration or a different incubation time. In addition, as IL-8 release by airway cells is regulated by complex intracellular pathways, it is possible that some of them may not be targeted by cilomilast. This hypothesis is supported by a previous study which showed that enhanced cAMP levels in epithelial cells did not inhibit IL-8 release, suggesting that IL-8 release is not solely modulated by cAMP. Moreover, the extent of the inhibitory effect of cilomilast may differ according to the clinical severity of COPD—being greater in patients with severe COPD than in those with mild COPD in whom treatment with theophylline reduced sputum
levels of IL-8 by 24% compared with baseline. However, Cul-pitt et al[2] assessed the effects of theophylline on IL-8 levels in sputum supernatants obtained immediately after sputum processing while we evaluated the effects of cilomilast on IL-8 release from sputum cells cultured for 24 hours. Whether this effect reflects different modes of action of theophylline and cilomilast or whether it depends on the different profile of airway inflammation in moderate and severe COPD needs to be studied further.

The findings of this study enable us to define better the scientific rationale for the use of cilomilast in the treatment of COPD, and provide important information about the mechanisms by which it may affect the inflammatory process in the airways of these subjects. Interestingly, the inhibitory effect of cilomilast on TNF-α and GM-CSF release by airway cells was associated with a significant reduction in neutrophil chemotactic activity exerted by supernatants harvested from cultured bronchial epithelial cells and sputum cells incubated for 24 hours with the drug. It is also likely that cilomilast may exert a direct inhibitory effect on neutrophil chemotaxis, as has already been demonstrated on fibroblast migration.[24] It is of note that, although significant, the inhibitory effect on neutrophil chemotaxis was not complete. This might be attributed to the low inhibitory effect of cilomilast on IL-8 release by both bronchial epithelial and sputum cells, as well as to the presence of other chemotactic mediators for neutrophils such as LTβ4.

Taken together, the results of the present study suggest that cilomilast has the potential to inhibit the development of neutrophilic inflammation in the airways of patients with COPD. Indeed, it is believed that neutrophil recruitment and activation is an important step in the pathogenesis of airway inflammation in this disease, as shown by the increased numbers of neutrophils in both central and peripheral airways, as well as by their distribution in the epithelial layer and within mucous glands. It is well known that neutrophil activation leads to the release of a wide variety of inflammatory mediators such as proteases and oxygen free radicals that not only amplify the recruitment of inflammatory cells, but also cause damage to lung tissues.

It is therefore conceivable that drugs capable of inhibiting neutrophil recruitment and function may be a promising strategy in the treatment of COPD for several reasons. Firstly, so far very few drugs have been shown to inhibit neutrophil function as well as the release of mediators by airway cells isolated from patients with COPD. [5] Secondly, the effects of cilomilast on the release of inflammatory mediators further support the concept that this drug not only provides bronchodilatory effects but is also characterised by anti-inflammatory properties which appear to be effective on neutrophils. Thirdly, the inhibitory effects of cilomilast on the release of neutrophil chemotactants by bronchial and sputum cells isolated from patients with COPD suggest that these cells have a certain degree of responsiveness to this drug, a finding which has not always been observed with other drugs including corticosteroids.[8]

This study has indicated several anti-inflammatory effects of cilomilast on airway cells isolated from patients with COPD and supports its potential usefulness in the treatment of this disease.

Figure 4 Neutrophil chemotactic activity of supernatants of (A) bronchial epithelial cells and (B) induced sputum cells stimulated in the presence or absence of cilomilast (1 µM) for 24 hours at 37°C. Statistical analysis was performed by the Wilcoxon U test. The results are expressed as medians (horizontal bars) with 25th and 75th percentiles (dotted bars).

REFERENCES

Authors' affiliations

V Bellia, A M Vignola, Istituto di Medicina Generale e Pneumologia, Università di Palermo, Italy
M Profita, G Chiappara, F Mirabella, R Di Giorgi, L Chimenti, G Costanzo, L Riccobono, A M Vignola, Istituto di Fisiopatologia Respiratoria, CNR, Via Trabucco, Palermo, Italy
J Bousquet, INSERM, U-454 Montpellier, France

This study was supported by GlaxoSmithKline USA and by INSERM-CNR.
LUNG ALERT

Contamination of rhinovirus stocks with poliovirus type 1

One of the major successes of public health vaccination programmes has been the eradication of circulating poliovirus from the majority of countries in the world. Reinroduction of wild type poliovirus may occur following spread from sources of virus stored in virological and other laboratories for research purposes or from countries where polio remains endemic. It is therefore of obvious concern that the authors at the National Collection of Pathogenic Viruses (NCPV), whilst characterising stocks of rhinovirus from the MRC Common Cold Research Unit, found that five of 22 cell cultures had abnormal cytopathic effects when infected with putative rhinovirus. PCR and sequencing confirmed that four of these putative rhinovirus stocks (1A, 1B, 4 and 6) had similarities to poliovirus type 1 sequences and were confirmed to contain poliovirus type 1 with likely contamination of two further serotypes. Genomic analysis of the poliovirus showed similarity to wild type virus, although the possibility that this was a vaccine strain that has undergone genetic drift remains. These findings should alert the medical and scientific communities to the risk of possible contamination of rhinovirus and, indeed, other virus stocks with poliovirus which has the ability to replicate in most cell lines and to overgrow more slowly replicating viruses. The authors advise that working stocks of virus should be checked regularly and eventually replaced with fresh virus from appropriate reference strains.

T Wilkinson
St Bartholomew’s Hospital, London, UK
tomw1970@hotmail.com

www.thoraxjnl.com
Effect of cilomilast (Ariflo) on TNF-α, IL-8, and GM-CSF release by airway cells of patients with COPD

M Profita, G Chiappara, F Mirabella, R Di Giorgi, L Chimenti, G Costanzo, L Riccobono, V Bellia, J Bousquet and A M Vignola

Thorax 2003 58: 573-579
doi: 10.1136/thorax.58.7.573

Updated information and services can be found at:
http://thorax.bmj.com/content/58/7/573

These include:

References

This article cites 30 articles, 8 of which you can access for free at:
http://thorax.bmj.com/content/58/7/573#BIBL

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections

Articles on similar topics can be found in the following collections

- Health education (1223)
- Smoking (1037)
- Tobacco use (1039)
- Inflammation (1020)
- Health effects of tobacco use (211)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/