Efficacy of the novel phosphodiesterase-4 inhibitor BAY 19-8004 on lung function and airway inflammation in asthma and chronic obstructive pulmonary disease (COPD)

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Abstract

Selective inhibitors of phosphodiesterase-4 (PDE4) inhibit the hydrolysis of intracellular cAMP, which may result in bronchodilation and suppression of inflammation. We examined the effect of 1 week treatment with BAY 19-8004 (5 mg once daily), a novel orally administered PDE4 inhibitor, on trough FEV1 and markers of inflammation in induced sputum in patients with asthma or chronic obstructive pulmonary disease (COPD). Seven patients with asthma (mean [SD] FEV1 69.5 [9.3]% predicted; reversibility in FEV1 26.2 [10.1]%; all non-smokers) and 11 patients with COPD (FEV1 58.6 [8.3]% predicted; reversibility in FEV1 6.5 [4.7]%; median [range] 44 [21–90] pack years of smoking) were included in this randomized, double-blind, placebo-controlled trial. FEV1 was measured before and after 1 week of treatment; sputum was induced by 4.5% saline inhalation on the last day of treatment. FEV1 did not improve during either treatment in both patient groups (p > 0.2). Sputum cell counts were not different following placebo and BAY 19-8004 treatment in asthma and COPD patients (p > 0.2). However, only in patients with COPD, small but significant reductions in sputum levels of albumin and eosinophil cationic protein were observed (p < 0.05). In conclusion, 1 week of treatment with the selective PDE4 inhibitor BAY 19-8004 does not affect FEV1 and sputum cell numbers in patients with asthma or COPD. However, such treatment does seem to reduce levels of albumin and eosinophil cationic protein in sputum samples obtained from patients with COPD.

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Keywords: Induced sputum; Phosphodiesterase-4 inhibitor; BAY 19-8004; Microvascular leakage; Inflammation

1. Introduction

Phosphodiesterases (PDEs) are intracellular enzymes involved in the inactivation of the second messengers cAMP and cGMP [1]. The subtype PDE4 is expressed in smooth muscle cells and inflammatory cells and as such represents a potential target in asthma and chronic obstructive pulmonary disease (COPD) [2,3]. Inhibition of PDE4 results in increases in intracellular cAMP which in turn results in smooth muscle relaxation and suppression of activation of inflammatory cells [1,2]. In vivo administration of ‘second generation’ PDE4 inhibitors such as cilomilast has been shown to be clinically effective. In patients with severe asthma using inhaled steroids, PDE4 inhibition by a single dose of cilomilast provides rapid bronchodilation which is sustained during prolonged treatment [4]. Furthermore, in COPD patients with poorly reversible lung function, PDE4 inhibition by cilomilast during 6 weeks of treatment results in additional bronchodilation, on top of β2-agonist use [5].

BAY 19-8004 is a novel, selective second generation PDE4 inhibitor which is under development for the treatment of patients with asthma or COPD [6]. Phase I studies in healthy volunteers have indicated that once daily dosing with 5 mg of BAY 19-8004 is optimal [6]. To date, there have been no studies reporting on the effects of BAY 19-8004 in patient populations. Therefore, the aim of this study was to investigate the acute effect of a single dose of BAY 19-8004 on lung function in patients with asthma and in patients with COPD. Furthermore, we aimed to examine whether such acute bronchodilation was sustained during 1 week of treatment with BAY 19-8004 and whether this
effect was associated with changes in markers of inflammation. To that end, both patient groups were treated for 1 week with PDE4 inhibitor BAY 19-8004 (5 mg once daily) or placebo using a cross-over design. Lung function was recorded during 7 h following the first (acute effect) and last dose (medium term effect) of study medication. Markers of inflammation were studied in induced sputum samples, collected following 1 week of treatment. Finally, to evaluate systemic effects of BAY 19-8004, whole blood was stimulated ex vivo with bacterial lipopolysaccharide (LPS) and subsequent cytokine production was measured.

2. Methods

2.1. Patients

Eleven patients with COPD (3 female) and seven patients with asthma (3 female), aged between 18 and 80 years, were recruited for this study. Patients had to fulfill the following inclusion criteria. COPD: a clinical diagnosis of the disease [7], post-bronchodilator FEV1 40–70% of predicted value, reversibility in FEV1 following inhalation of 400 μg of salbutamol <12% from pre-bronchodilator value and smoking history ≥20 pack years. Asthma: a clinical diagnosis of the disease [8], pre-bronchodilator FEV1 40–80% of predicted value, reversibility in FEV1 following inhalation of 400 μg of salbutamol ≥15% from pre-bronchodilator value and smoking history ≥10 pack years. All patients had to have stable disease, with no exacerbation in the 8 weeks prior to inclusion and no upper respiratory tract infection within 1 week prior to inclusion. During the study, only treatment with short-acting bronchodilators was allowed. Treatment with inhaled corticosteroids (ICS) and long-acting β2-agonists was excluded during the study and discontinued 1 week prior to inclusion in the study. Patients were excluded if they used theophylline, aminophylline, or leukotriene receptor antagonists or synthesis inhibitors. Following these in- and exclusion criteria, patients with stage II moderate COPD [9] and patients with moderate persistent asthma [8] were included. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center. Written informed consent was obtained from all patients.

2.2. Design

The study had a double-blind, randomized, placebo-controlled, cross-over design, including a screening visit and two 7-day treatment periods with a wash-out period in between. At the first visit, medical history and eligibility were assessed, spirometry with reversibility in FEV1 to 400 μg salbutamol was determined, and induced sputum, blood for clinical chemistry and hematology, and a 12-lead ECG recording were obtained. After 5–10 days, patients returned to the lung function laboratory and received the first tablet of study medication. Prior to and at 1, 2, 4, 6, and 7 h after intake of study medication, spirometry was performed. Patients were then instructed to continue the use of one tablet of study medication once daily (during breakfast, with food) for the next 5–9 days. The last dose of study medication was administered at the department with assessment of spirometry prior to and at 1, 2, 4, 6, and 7 h after intake of the study medication. Immediately after spirometry assessment at 7 h, sputum induction was performed. Furthermore, a 12-lead ECG recording and blood sample were obtained during this visit for safety monitoring. In addition, blood samples were collected for determination of plasma levels of BAY 19-8004 and LPS stimulation test. After a wash-out period of 5–9 days, patients returned for the cross-over treatment of 5–9 days, with identical assessments at the first and last day of treatment. Adverse events were documented on all study visits.

Treatment with ICS and long-acting β2-agonists was not allowed during the study. Therefore, these medications were discontinued 1 week prior to inclusion and during the study. In case of deterioration of the disease and worsening of symptoms, patients restarted with their original treatment and dropped out of the study. Patients withheld from short-acting β2-agonists and anticholinergics ≥4 h before lung function measurements at each of the study visits.

2.3. Spirometry

FEV1 was recorded from maximal expiratory flow-volume curves on a calibrated pneumotachograph (Koko Spirometer, PDS Instrumentation, Louisville, CO, USA) according to standards [10]. Measurements were compared to the reference values of Crapo et al. [11]. Reversibility in FEV1 was determined by measuring FEV1 before and 30 min after inhalation of 400 μg salbutamol, administered by pressurized metered dose inhaler connected to an Aerosol chamber. Reversibility was calculated as difference between post- and pre-bronchodilator value and expressed in ml or % change from pre-bronchodilator value.

2.4. Safety assessments

Twelve-lead ECGs were recorded using a Cardiovit CS6/12 (CardioKinetics Ltd, Schiller AG, Baar, Switzerland) with the patient in supine position. Blood for routine evaluation of clinical chemistry and hematology was collected in tubes containing serum–gel clotting activator and EDTA, respectively.

2.5. Induction and processing of sputum

Sputum was induced by inhalation of aerosolized 4.5% (w/v) hypertonic saline (DeVilbiss Ultraneb 2000, Somerset, PA, USA) according to the method of In’t Veen et al. [12]. Saline was inhaled for three periods of 5 min.
Within 2 h after collection, whole sputum samples were processed following a standardized protocol [13]. Cytospins were stained with May–Grünewald–Giemsa. Differential cell counts of 500 non-squamous cells were performed by a qualified cytopathologist on coded slides and expressed as percentage of non-squamous cells. Sputum samples containing >80% of squamous cells were excluded from analysis because of poor cytopsin quality. Levels of albumin in sputum supernatants were measured by nephelometric assay and expressed as μg/ml [12]. Fibrinogen (Kordia, Leiden, The Netherlands) and IL-8 (Central Laboratory for Blood Transfusion, Amsterdam, The Netherlands) were determined by ELISA (lower limit of detection 120 ng/ml and 7.8 pg/ml, respectively) [12]. ECP was measured by FEIA (Pharmacia Cap System ECP FEIA, Kabi Pharmacia Diagnostics AB, Sweden; lower limit of detection 20 ng/ml) [12].

2.6. Levels of BAY 19-8004 in plasma

At the last day of each of the treatment periods, blood was collected in tubes containing heparin for determination of BAY 19-8004 levels in plasma. Within 1 h after collection, plasma was separated by centrifugation at 1500g for 10 min. BAY 19-8004 levels were determined by high pressure liquid chromatography, with post-column derivatisation and fluorescence detection. This method has a working range of 0.5–100 μg/l, with a lower limit of quantification of 0.5 μg/l.

2.7. LPS stimulation test of whole blood cultures

TNF-α release was assessed in whole blood cultures that were stimulated with LPS essentially as described [14] with minor modifications. First, 200 μl of blood was pre-incubated for 5 min at 37 °C and then stimulated by the addition of 20 μl LPS (LPS 1 mg/ml (w/v)) 1:90 in 0.1% (v/v) hydroxylamine in PBS. After mixing, the sample was further incubated for 4 h at 37 °C. The assay was stopped by the addition of 1.8 ml ice-cold PBS/BSA (3% (w/v)) and centrifugation for 5 min at 1000g. Two hundred μl aliquots of plasma were stored at −80 °C. TNF-α was determined by ELISA (Central Laboratory for Blood Transfusion, Amsterdam, The Netherlands) with lower limit of detection of 10 pg/ml.

2.8. Statistical analysis

Values of sputum eosinophils, albumin, fibrinogen, IL-8 and ECP were not normally distributed and (natural) log-transformed prior to statistical analysis. Primary efficacy variables were trough FEV1 value (just before patients took the last dose of study medication) and differential cell counts in sputum at the last day of treatment in each period. Secondary efficacy variables were maximum increase in FEV1 between 1 and 7 h following administration of treatment on the first and last day of treatment; albumin, fibrinogen, IL-8 and ECP in sputum supernatants, and TNF-α in plasma from LPS-stimulated whole blood cultures, as measured on the last day of treatment. Data were analyzed using an analysis of variance according to the cross-over model containing factors for treatment sequence, patient within treatment sequence, period and treatment. Analyses of lung function parameters at the end of treatment were corrected for baseline values. p < 0.05 was considered as the level of statistical significance.

Sample size calculations showed that with 10 evaluable patients, a mean change in the primary efficacy variable of one standard deviation could be detected with a power of 85% and two-sided alpha of 5%.

3. Results

All patients completed the study. Patient characteristics at baseline are shown in Tables 1 and 2.

3.1. Trough FEV1

In patients with COPD, mean (SD) FEV1 during 1 week of placebo treatment changed from 1.78 (0.37) l prior to the first dose, to 1.77 (0.37) l prior to the last dose of treatment (p > 0.8), and from 1.83 (0.41) l to 1.87 (0.41) l during BAY 19-8004 treatment (p > 0.2). In patients with asthma, FEV1 changed from 2.35 (0.8) l to 2.38 (0.85) l during placebo treatment (p > 0.6) and from 2.38 (0.78) l to 2.42 (0.91) l during BAY 19-8004 treatment (p > 0.7). The changes in FEV1 during active and placebo treatment were not statistically significant in either the COPD (p > 0.3) or the asthma group (p > 0.9).

3.2. FEV1 in response to the first and last dose of BAY 19-8004

Fig. 1 shows the changes in FEV1 during 7 h following the first and last dose of each treatment. In both patient

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COPD</td>
</tr>
<tr>
<td>Gender, male:female (n)</td>
<td>8:3</td>
</tr>
<tr>
<td>Age (year)</td>
<td>64.2 (6.7)</td>
</tr>
<tr>
<td>Smoking history (pack years)*</td>
<td>44 (21–90)</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV1 (% pred)</td>
<td>58.6 (8.3)</td>
</tr>
<tr>
<td>Post-bronchodilator FEV1 (% pred)</td>
<td>62.3 (8.2)</td>
</tr>
<tr>
<td>Reversibility in FEV1 (ml)</td>
<td>114.5 (88.5)</td>
</tr>
<tr>
<td>Reversibility in FEV1 (% baseline)</td>
<td>6.5 (4.7)</td>
</tr>
<tr>
<td>Prior inhaled steroid usage (n)</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation), age and medication usage are in actual numbers.

* Median (minimum–maximum).
groups, the bronchodilator response to either the first or the last dose of study medication, as assessed by area under the FEV1-time curves, was not different between BAY 19-8004 and placebo ($p = 0.3$).

### 3.3. Inflammatory cell counts and mediators in induced sputum

Differential cell counts in induced sputum samples, obtained at the end of the last treatment day, were not different between placebo and BAY 19-8004 treatment in patients with asthma or with COPD (Fig. 2, $p > 0.2$). Soluble markers in sputum were not different between placebo and active treatment in patients with asthma ($p > 0.6$). In contrast, in patients with COPD, small but significant reductions in sputum levels of albumin ($p < 0.05$) and ECP ($p < 0.01$) were observed following 1 week treatment with BAY 19-8004 as compared to placebo, whereas the decrease in IL-8 did not reach statistical significance ($p = 0.09$).

### 3.4. LPS-induced TNF-α release in whole blood

The mean (SD) TNF-α production in whole blood upon LPS stimulation following placebo and BAY 19-8004 treatment was 19.3 (7.8) and 14.4 (8.4) ng/ml, respectively ($p > 0.3$), in patients with asthma, and 15.8 (6.6) and 16.8 (11.3) ng/ml, respectively ($p > 0.7$), in patients with COPD.

### 3.5. Plasma levels of BAY 19-8004

Following 1 week of treatment, mean (SEM) plasma levels of BAY 19-8004 were 29.0 (4.9) ng/ml in patients with asthma and 39.5 (7.7) ng/ml in COPD patients.

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**Table 2**

Sputum characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>COPD</th>
<th>Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous epithelial cells (%)</td>
<td>12.6 (8.4)</td>
<td>39.6 (22.2)</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>33.3 (11.1)</td>
<td>32.0 (14.8)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>62.6 (11.1)</td>
<td>50.8 (20.6)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.8 (0–10.2)</td>
<td>1.7 (0–43.4)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>1.2 (0.6)</td>
<td>1.2 (0.7)</td>
</tr>
<tr>
<td>Bronchial epithelial cells (%)</td>
<td>1.9 (1.0)</td>
<td>8.1 (6.8)</td>
</tr>
<tr>
<td>Albumin (µg/ml)</td>
<td>105.7 (20–202.8)</td>
<td>265.8 (77.2–989.2)</td>
</tr>
<tr>
<td>Fibrinogen (µg/ml)</td>
<td>3.4 (0.2–22.5)</td>
<td>2.5 (0.8–4.3)</td>
</tr>
<tr>
<td>IL-8 (ng/ml)</td>
<td>3.2 (1.6–5.6)</td>
<td>1.2 (0.7–2.4)</td>
</tr>
<tr>
<td>ECP (ng/ml)</td>
<td>108.1 (21.0–458.0)</td>
<td>87.2 (27.6–1526.0)</td>
</tr>
</tbody>
</table>

Data are mean (SD) or geometric mean (range).
3.6. Safety

During the study, no changes were observed in ECG recordings, blood pressure, heart rate, and laboratory values in blood and urine in either patient group. A total of 41 adverse events were recorded, of which 71% occurred during BAY 19-8004 treatment. Most common reported adverse events were gastrointestinal complaints including nausea, diarrhea, vomiting and dyspepsia (10 events during BAY 19-8004 and 2 events during placebo) and headache (4 events during BAY 19-8004 and 1 event during placebo).

4. Discussion

The results of this study show that 1 week of treatment with the novel PDE4 inhibitor BAY 19-8004, 5 mg once daily, reduces albumin and ECP levels in induced sputum of patients with COPD. These reductions in inflammatory mediators were not accompanied by acute or medium term changes in lung function, nor in cell numbers in induced sputum. Furthermore, BAY 19-8004 treatment did not change any of the endpoints in patients with asthma. These results may suggest that in patients with COPD treated with BAY 19-8004, markers of the activity of inflammatory cells in sputum can be reduced by 1 week of treatment, whereas a longer treatment period may be needed to affect inflammatory cell numbers and possibly FEV₁.

This is the first clinical study to examine the effects of BAY 19-8004 treatment in patients with asthma or COPD. Several in vitro and in vivo animal studies have demonstrated that the pre-clinical profile of BAY 19-8004 includes potent inhibition of leukotriene D₄-induced bronchoconstriction, of LPS-induced mucus hypersecretion and of tobacco smoke-induced inflammatory cell influx in guinea pigs [6]. The anti-inflammatory properties of BAY 19-8004 in animal models have, at least to some extent, been confirmed in our study since we have demonstrated for the first time that the PDE4 inhibitor BAY 19-8004 attenuates albumin and ECP levels in sputum of patients with COPD. However, despite a reduction in markers of inflammation, cell numbers in sputum did not change in these COPD patients. Recently, it was shown in patients with COPD that following 12 weeks of PDE4 inhibition by cilomilast, inflammatory cell numbers including CD4 and CD8⁺ T lymphocytes, macrophages and neutrophils in the lamina propria of bronchial biopsies were reduced by approximately 50%, although cell numbers in sputum were not affected [15]. This may suggest that the intervention period with PDE4 inhibitor BAY 19-8004 was too short to have a reflection on inflammatory cell numbers.

Fig. 2. Differential cell counts in induced sputum (left panel) from patients with asthma (upper panel) or COPD (lower panel) were not significantly different between placebo (open symbols) and BAY 19-8004 (closed symbols) treatment. Soluble markers in sputum (right panel) were not significantly different between treatment in patients with asthma. In patients with COPD, there were significant differences between placebo and BAY 19-8004 treatments for albumin and ECP. Lines represent the group median.
In the present study, neither a single dose nor 1 week treatment with BAY 19-8004 changed FEV\textsubscript{1} in either COPD or asthma. Previous studies with the PDE4 inhibitor cilomilast have shown that single dose treatment does not result in acute bronchodilation in patients with COPD [16], whereas it might have such an effect in patients with asthma [4]. Furthermore, extended treatment with cilomilast for 6 weeks has been shown to induce bronchodilation in patients with COPD [5] and in patients with asthma [4]. Again, this may suggest that for BAY 19-8004, the intervention period of 1 week in the current design was not long enough to observe changes in lung function.

In order to minimize bias, this study was designed carefully. Using strict criteria, two groups of patients were included who had comparable level of airway obstruction, but responded differently to bronchodilators: patients with COPD were poorly reversible to \beta\textsubscript{2}-agonists (stage II COPD [9]), while patients with asthma had (partially) reversible lung function upon \beta\textsubscript{2}-agonist inhalation (moderate persistent asthma [8]). These distinctly different patient groups were studied in the same design. ICS use was not allowed during and 1 week before start of the study. At the time of start of the first week of randomized treatment, ICS had been stopped for 2 weeks. Although it cannot be ruled out that the withdrawal period of ICS was too short, it is unlikely that this explained the negative results in the asthma group since patients who received active treatment during the second treatment period did not respond better to BAY 19-8004 than those who received placebo up to 1 week after stopping ICS [4]. Furthermore, PDE4 inhibition by cilomilast treatment improves lung function in asthmatics, on top of regular treatment with ICS [4], which may suggest that BAY 19-8004 was less effective in our patients with asthma. In the present study, a relatively short intervention in a cross-over design with a small group of patients was used rather than a long(er) intervention in larger parallel patient groups, which might have been more successful in demonstrating efficacy of BAY 19-8004 treatment on FEV\textsubscript{1} and cell counts in induced sputum. Nevertheless, in our group of COPD patients we would have been able to detect a difference in FEV\textsubscript{1} between BAY 19-8004 and placebo treatment of 5% predicted, given the observed standard deviation in FEV\textsubscript{1} during placebo treatment (6% predicted), a two-sided \alpha of 5% and one-sided \beta of 10%. Conversely, based on the observed effects of treatment on FEV\textsubscript{1} and sputum neutrophils, it appeared that a sample size of 80 and 1743 COPD patients, respectively, would be necessary to demonstrate a significant difference between placebo and BAY 19-8004 treatment (power 85%, two-sided \alpha of 5%). Finally, the lack of efficacy of BAY 19-8004 on FEV\textsubscript{1} may suggest that the dosage used (5 mg once daily) was not sufficient. However, Phase I data showed that 5 mg of BAY 19-8004 once daily was the most suitable dose in terms of safety and efficacy. A higher dose would probably have resulted in more class-related side effects.

How can the present results be explained? Microvascular leakage is regulated through formation and closure of intercellular gaps between endothelial cells, which facilitate the extravasation of fluid, macromolecules and cells [17]. Elevation of cAMP levels decreases intercellular gap formation and permeability, thereby restoring the pulmonary endothelial barrier integrity [17]. Markers of microvascular leak, including albumin and fibrinogen, can be measured in induced sputum samples [18]. Previously, it has been shown in healthy subjects in vivo that microvascular leakage, induced by inhalation of histamine, can be attenuated by pre-treatment with formoterol [19], which causes intracellular cAMP elevation by stimulation of the \beta\textsubscript{2}-receptor [20]. Similarly, induced hyperpermeability in cultured pulmonary endothelial monolayers can be reduced by PDE3 and PDE4 inhibitors [21]. In the present study, these in vitro results were confirmed, as judged by the decrease in sputum albumin concentrations following 1 week of treatment with the PDE4 inhibitor BAY 19-8004.

TNF-\alpha production by activated peripheral blood mononuclear cells is regulated by cAMP. Such TNF-\alpha production following LPS in vivo in rats can be inhibited by BAY 19-8004 [6]. Similarly, in human whole blood stimulated ex vivo with LPS, TNF-\alpha production can be attenuated during treatment with the PDE4 inhibitor rolflumilast [22]. However, in the present study in humans in vivo no effect of BAY 19-8004 on TNF-\alpha production by LPS stimulated whole blood was observed. These results may suggest that either the plasma levels of BAY 19-8004 in our patients were too low to affect the TNF-\alpha production as observed in rats [6], or BAY 19-8004 is less potent than rolflumilast in attenuating TNF-\alpha production in human whole blood ex vivo.

What are the clinical implications? The current treatment options for patients with asthma and COPD mainly consist of inhaled steroids in combination with bronchodilators [8, 9]. While in patients with asthma this approach is successful [23, 24], such treatment does not seem to affect the rate of decline in lung function in patients with COPD [25–28]. Therefore, in particular for patients with COPD, PDE4 inhibition might be a novel treatment. However, follow-up studies are needed to study the effect of long-term treatment with PDE4 inhibitors in general and BAY 19-8004 in particular, on airway inflammation and progression of the disease.

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