2 Synopsis

Title of the study:

Effects of 500 mcg roflumilast on inflammatory cells and mediators in BAL fluid after segmental pulmonary LPS challenge in healthy volunteers – ERIC

Study center(s):

Fraunhofer Institut Toxikologie und Experimentelle Medizin, Germany

Publication (reference):

Not applicable

Studied period (years):

23-Jul-2004 (first subject in) to 31-Mar-2005 (last subject out)

Clinical phase: I

Objectives:

This segmental pulmonary LPS-challenge study sought to investigate the anti-inflammatory properties of roflumilast in human beings and thus to provide insights into the mode of action of roflumilast and a link to the clinical efficacy seen in COPD patients.

Methodology:

This study was designed as a 4-week, randomized, double blind, monocenter study including two parallel treatment arms (placebo and roflumilast 500 µg od [once daily], respectively).

The study consisted of a 4-week treatment period (V1 to V5) with a prior, variable 3 to 7 days post-screening period (V0 to V1). If required, due to the occurrence of adverse events, one or several follow-up visits were performed.

Healthy, non-smoking female and male subjects who met all inclusion criteria without fulfilling any exclusion criterion on Screening Visit V0, were enrolled into the study.

On Randomization Visit V1 subjects were re-evaluated and those who met the randomization criterion were randomized in a 1:1 ratio to receive once-daily either roflumilast 500 µg, or placebo.

The subjects were asked to return to the study site once weekly during the treatment period for Visits V2 to V5. During these visits, compliance was checked by drug accountability and

AEs were monitored. Furthermore, blood samples were taken for retrospective assessment of roflumilast N-oxide plasma levels after unblinding of the study.

After 28 days of treatment, on Visit V5, a first bronchoscopy was performed: following a baseline BAL (broncho-alveolar lavage) in one bronchial segment, LPS and saline were instilled, each in different segments of contra-lateral lungs.

Twenty-four hours later during a second bronchoscopy on visit V6, a BAL was performed both in the LPS-challenged and saline-control segments. Total and differential cell counts and inflammatory markers were determined in the recovered BAL fluid. Additionally, inflammatory markers were assessed in the blood immediately before challenge, and 4, 8 and 24 hours post challenge.

No. of subjects (total and for each treatment):

A total of 48 subjects were enrolled during screening period of the study. Of these, five subjects were not eligible for inclusion in the treatment period. As there were no major protocol violations, the SAF (safety set), FAS (full analysis set) and VCS (valid cases set) were identical with 22 subjects in the roflumilast 500 µg od group and with 21 subjects in the placebo group.

Diagnosis and criteria for inclusion:

Inclusion criteria

- written informed consent
- age between 18 to 45 years
- body weight acc. BMI (Body Mass Index) 18 to 28
- assessed as healthy individual by the investigator, based on a screening examination including medical history, physical examination, BP (blood pressure), PR (pulse rate), ECG (electrocardiogram), and clinical laboratory results
- FEV_1 ≥80% predicted
- non-smoker for ≥2 years, with a smoking history of ≤1 pack year
- not suffering from any condition that might interfere with study procedures or evaluation
- negative skin prick test (performed within ≤1 year before study entry)

Exclusion criteria:

The presence of any of the following excluded a subject from study participation:

- any active disease, acute or chronic (also psychiatric diseases);
- any signs of cardiac diseases (eg QTc acc. to Bazett: males ≥430 ms, females ≥450 ms; PR ≥220 ms);

- respiratory tract infection not resolved 4 weeks prior to the Screening Visit V0;
- clinically relevant abnormal laboratory values suggesting an unknown disease and requiring further clinical evaluation (as assessed by the investigator);
- elevated serum Immunoglobulin E (IgE) levels ≥100 IU (international units)/mL (performed within ≤1 year before study entry);
- HIV (human immnunodeficiency virus) I and II or hepatitis screening positive or not performed (in case of a positive HIV test, the subject had to be informed by a physician in personal communication);
- diagnosis, treatment, or remission of any cancer (other than basal cell carcinoma) within 5 years prior to study start;
- any medication two weeks before start of this study or within less than 10 times the elimination half-life of the respective drug, if not expressively allowed by the investigator;
- pregnancy, breast feeding, oocyte donation or oocyte implantation planned during the trial;
- female subjects of childbearing potential not using and not willing to use a medically reliable method of contraception for the entire study duration, such as oral, injectable, or implantable contraceptives, or intrauterine contraceptive devices, unless she was surgically sterilized or had undergone a hysterectomy or any other criteria considered sufficiently reliable by the investigator in individual cases;
- participation in another study (use of investigational product) within 30 days preceding the Screening Visit V0 or re-entry of subjects previously enrolled in this study;
- suspected inability or unwillingness to comply with study procedures;
- alcohol or drug abuse;
- inability to follow study procedures due to eg language problems, psychological disorders;
- suspected hypersensitivity to any ingredients of the study medication or medication in line with bronchoscopy;
- vulnerable subjects (eg persons kept in detention).

Randomization criterion

Subjects had to be judged to be clinically healthy, as evidenced by the compliance with all inclusion criteria without showing any exclusion criterion to be eligible for randomization into the double-blind treatment period at Randomization Visit V1.

Test product: Roflumilast

Dose: 500 µg/tablet

Mode of administration: one tablet od, oral intake each morning

Batch No.: 420210

Duration of treatment: 4 weeks

Reference product: Matching placebo

Dose: not applicable

Mode of administration: one tablet od, oral intake each morning

Batch No.: 130290

Criteria for evaluation:

Efficacy

Primary variable:

- the change of monocyte cell count [cells/mL] in BAL (broncho-alveolar lavage) fluid from baseline to 24 hours after LPS challenge (V6-V5), counted by flow cytometry

Key-secondary variable:

- change of neutrophil cell count [cells/mL] in BAL fluid from baseline to 24 hours after LPS challenge (V6-V5)

Efficacy evaluation (secondary variables):

- mean differences to baseline in total and differential (neutrophil, eosinophil, macrophage and monocyte, lymphocyte) cell counts in BAL fluid 24 hours after LPS challenge;
- mean differences to baseline in inflammatory markers in BAL fluid 24 hours after LPS challenge (TNFα, IL-6, IL-8, MMP-9, MCP-1, nitrite, nitrate);
- mean differences to pre-challenge in inflammatory markers in blood at 4, 8 and 24 hours after LPS challenge (TNFα, IL-6, IL-8, CRP [C-reactive protein], E-Selectine).

Safety

- adverse events;
- changes in laboratory values;
- changes in physical examination findings including ECG [PR, QRS, QT intervals]);
- changes in vital signs BP, PR and body temperature;

- changes in spirometry parameters (FEV_1 [Forced expiratory volume in one second] and FVC [Forced vital capacity]).

Statistical methods:

The primary variable was the change of monocyte cell count [cells/mL] in BAL fluid (V6-V5), counted by flow cytometry, and was analyzed parametrically by an ANCOVA (analysis of covariance) model. The test was one-sided with $\alpha = 0.025$.

The main analysis was based on the change from baseline at V5 induced by LPS challenge (ie V6-V5), where baseline, sex, age and treatment were used as factors and covariables in the ANCOVA model. The following additional models were applied:

1. the saline control measurement at V6 was added to the model above as additional covariate;
2. the change of the saline control measurement at V6 from baseline was analyzed in complete analogy as in the model used for the main analysis. This analysis was not intended as further efficacy analysis, but to assess potential effects of treatment to saline measurements. Significance of the statistical test was not expected;
3. the LPS measurement at V6 was compared between placebo and roflumilast without using baseline as covariate and only by including age and sex in addition to treatment in the ANCOVA model;
4. this model was similar to the previous model (model 3), but included saline control at V6 as an additional covariate.

An additive model and normal distribution of the means by treatment group were assumed for the efficacy parameters, which were also analyzed non-parametrically by the Wilcoxon test for reasons of robustness. Point estimates and 95% confidence intervals were calculated for the difference of medians.

All safety assessments were analyzed by appropriate descriptive measures.

Summary - Conclusions

Demography:

The total set consisted of all subjects enrolled, including subjects withdrawn prior to randomization (non-eligible subjects) and comprised 48 subjects, of whom 43 (89.6%) were randomized.

All 43 randomized subjects took at least one dose of study medication and were therefore included in the SAF. Furthermore, as all subjects participated without any major protocol violation, the FAS was identical with the VCS. In addition, no invalid values were determined, therefore the PP analysis is identical to the ITT analysis. In 37 FAS subjects, bronchoscopic measurements were available.

Slightly more males than females were included in the study (53.5% and 46.5%) and 93.0% of the subjects in the ITT had never smoked. Demographic data and baseline characteristics were similar in the ITT and in ITT subjects with available bronchoscopy data.

Analysis of Efficacy:

Efficacy results

All results are described for the ITT analysis.

Primary variable: change of monocyte cell count from V5 to V6

The change of monocyte cell count in BAL fluid was less pronounced under treatment with roflumilast 500 µg od, but the ANCOVA showed no statistically significant difference between roflumilast 500 µg od and placebo for the change of monocyte cell count (LSMean = -23.90*10^3 cells/mL; one-sided p = 0.2053).

Change of monocyte cell count [10^3 cells/mL] in BAL fluid 24 h after LPS challenge: within- and between-treatment differences, endpoint analysis (ITT)

WITHIN	V5		V6 LPS		V6 LPS - V5			
	N	Mean	n	Mean	n	LSMean ± SE	95% CI	p-value[a]
Rof500	18	1.33	17	125.53	17	123.08 ± 19.81	82.69, 163.48	<0.0001
Pbo	19	2.06	19	148.00	19	146.98 ± 18.62	109.01, 184.96	<0.0001

BETWEEN					Difference Test - Ref for V6 LPS - V5		
	Test	Ref	n Test	n Ref	LSMean ± SE	95% CI	p-value sup.[b]
	Rof500	Pbo	17	19	-23.90 ± 28.66	-82.35, 34.55	0.2053

[a] Two-sided p-value for within-treatment differences, significance level 5%.
[b] One-sided p-value for superiority, significance level 2.5%.
BAL = Broncho-alveolar lavage, CI = confidence interval, ITT = intention-to-treat analysis, LPS = lipopolysaccharide, LS = least squares, n = number of patients with data available at V5 and V6, Pbo = placebo, Rof500 = roflumilast 500 µg od, SE = standard error, V5 = Visit 5, V6 = Visit 6.
Data source: Table 15.2.1.1 and 15.2.1.2

Key-secondary variable: change of neutrophil cell count from V5 to V6

The change in neutrophil cell count in BAL fluid after LPS challenge in subjects treated with roflumilast 500 µg od was lower compared to placebo. The difference between roflumilast 500 µg od and placebo for the change of neutrophil cell count was statistically significant (LSMean = -499.84*10^3 cells/mL; one-sided p = 0.0232).

Change of neutrophil cell count [10^3 cells/mL] in BAL fluid 24 h after LPS challenge: within- and between-treatment differences, endpoint analysis (ITT)

WITHIN	V5		V6 LPS		V6 LPS – V5			
	n	Mean	n	Mean	n	LSMean ± SE	95% CI	p-value[a]
Rof500	18	0.64	17	836.31	17	792.93 ± 169.11	448.04, 1137.83	<0.0001
Pbo	19	1.42	19	1255.98	19	1292.77 ± 159.28	967.92, 1617.63	<0.0001

BETWEEN					Difference Test - Ref for V6 LPS - V5		
	Test	Ref	n Test	n Ref	LSMean ± SE	95% CI	p-value sup.[b]
	Rof500	Pbo	17	19	-499.84 ± 241.00	-991.36, -8.32	0.0232

[a] Two-sided p-value for within-treatment differences, significance level 5%.
[b] One-sided p-value for superiority, significance level 2.5%.
BAL = Broncho-alveolar lavage, CI = confidence interval, ITT = intention-to-treat analysis, LPS = lipopolysaccharide, LS = least squares, n = number of subjects with data available at V5 and V6, Pbo = placebo, Rof500 = roflumilast 500 µg od, SE = standard error, V5 = Visit 5, V6 = Visit 6.
Data source: Table 15.2.2.1 and 15.2.2.2

Secondary variables

Cell count in BAL fluid

Cell count in BAL fluid was evaluated at Treatment Visit V5 before LPS challenge and at Treatment Visit V6 after LPS challenge.

The change of total cell count in BAL fluid was lower under treatment with roflumilast 500 µg od compared with placebo, the difference between roflumilast 500 µg od and placebo for the change of total cell count was statistically significant (LSMean = $-0.61*10^6$ cells/mL; one-sided p = 0.0209). This corresponded to the reduction of cell influx that was found for the monocyte cell count and the neutrophil cell count. The change of eosinophil cell count in BAL fluid was lower under treatment with roflumilast 500 µg od compared with placebo, the difference between roflumilast 500 µg od and placebo for the change of eosinophil cell count was statistically significant (LSMean = $-7.08*10^3$ cells/mL; one-sided p = 0.0108).

The change in cell counts of lymphocytes and of the sum of monocytes and macrophages did not show any statistically significant differences between the treatment groups.

The absolute cell counts of all cell types (eosinophils, neutrophils, lymphocytes, macrophages and monocytes) were increased after LPS challenge, but changed only slightly after saline instillation in both treatment groups.

At Treatment Visit V5, the sum of monocytes and macrophages was close to 95% in both treatment groups while lymphocytes, eosinophils and neutrophils were only small fractions in BAL fluid. After LPS challenge, the proportion of neutrophils increased from 0.7% to 65.7% in the roflumilast 500 µg od group and from 1.1% to 68.1% in the placebo group while the sum of monocytes and macrophages (as percentage) decreased correspondingly.

Inflammatory markers in BAL fluid

Inflammatory markers in BAL fluid were evaluated at Treatment Visit V5 before LPS challenge and at Treatment Visit V6 (24 h after LPS challenge). With the exception of nitrite and nitrate, all inflammatory markers in BAL fluid were increased after LPS challenge, but changed only slightly after saline instillation in both treatment groups. There were no statistically significant differences between the treatments for any of the inflammatory markers in BAL fluid.

Inflammatory markers in blood

Of the blood inflammatory markers CRP, E-selectine, TNFα, IL-6 and IL-8, only CRP and IL-6 showed an increase after LPS challenge in both treatment groups. For CRP, the change estimated by LSMean was 4.66 mg/L; two-sided p = 0.0001 in the roflumilast 500 µg od group and 4.62 mg/L; two-sided p = 0.0003 in the placebo group 24 hours after LPS challenge. For IL-6, the change estimated by LSMean was 13.76 pg/mL; two-sided p = 0.0008 in the roflumilast 500 µg od group and 13.24 pg/mL; two-sided p = 0.0200 in the placebo group 4 hours after LPS challenge. There were no statistically significant differences between the treatments for any of the blood inflammatory markers (ITT, Table 15.2.3.2, Table 15.2.3.3).

Summary

So far the anti-inflammatory properties of roflumilast have only been clearly demonstrated in *in vitro* experiments and in animal models. The findings of significant reductions of pulmonary influx of neutrophils, eosinophils and cells in total after LPS challenge under treatment with roflumilast 500 µg od compared with placebo indicate anti-inflammatory properties of roflumilast in humans as well.

There were no statistically significant differences between the treatments for inflammatory markers in BAL fluid and blood. The time point chosen for the BAL post LPS challenge was optimized for cell influx. Peak concentrations of inflammatory markers can usually be found 6-8 hours post challenge, therefore the measured levels were probably too low to allow the detection of any differences between treatments.

In summary, roflumilast 500 µg od had a positive influence on the cell count in BAL fluid after LPS challenge.

Analysis of Safety:

Overall, 102 treatment-emergent AEs were reported by 39 (90.7%) subjects, thereof 65 AEs in 22 (100.0%) subjects in the roflumilast 500 µg od group and 37 AEs in 17 (81.0%) subjects in the placebo group. Most AEs were mentioned only in 1 or 2 subjects, a relevant proportion of the AEs could be seen as typical for the underlying bronchoscopy with LPS challenge procedure (eg 20 AEs of throat irritation in 18 subjects and 10 AEs of decreased lung function test in 7 subjects). Frequent and typical AEs in the roflumilast 500 µg od group were headache in 11 (50.0%) subjects, diarrhea in 8 (36.4%) subjects and nausea in 7 (31.8%) subjects.

In 14 (63.6%) subjects in the roflumilast 500 µg od group, treatment-emergent AEs were assessed as likely related to the study drug by the investigator. With respect to intensity, all AEs were mild with the exception of three moderate AEs, no AE was of severe intensity. All AEs in this study were completely recovered.

There were no deaths or serious AEs. Five AEs led to premature discontinuation, thereof four cases of mild respiratory tract infections and one case of a moderate headache that were completely recovered. One further case of elevated liver enzymes that led to premature discontinuation was a baseline abnormality and therefore not reported as AE. Based on the AEs reported in this study, there was no safety concern associated with the use of roflumilast 500 µg od.

Physical examination, blood pressure, and heart rate measurements did not reveal any influence of the study medication. There was a decrease of median body weight in the roflumilast 500 µg od group and no change of median body weight in the placebo group. The change in mean body weight was -1.4 kg in the roflumilast 500 µg od group and -0.1 kg in the placebo group.

Taken together, roflumilast 500 µg od was well tolerated. The pattern of AEs observed did not suggest any adverse effect of the study medication. Safety results did not reveal any unknown risk of treatment in terms of frequency or intensity of AEs.

Conclusions:

The change of monocyte cell count in BAL fluid after LPS challenge was less pronounced in subjects treated with roflumilast 500 µg od compared with placebo, but the ANCOVA showed no statistically significant difference between roflumilast 500 µg od and placebo for the change of monocyte cell count (LSMean = $-23.90*10^3$ cells/mL; one-sided p = 0.2053). The change in neutrophil cell count in BAL fluid after LPS challenge in subjects treated with roflumilast 500 µg od was lower compared to placebo. The difference between roflumilast 500 µg od and placebo for the change of neutrophil cell count was statistically significant (LSMean = $-499.84*10^3$ cells/mL; one-sided p = 0.0232).

The findings of significant inhibition of LPS-induced increase in neutrophils, eosinophils and total cell count by roflumilast in this study indicate anti-inflammatory properties of roflumilast 500 µg od in humans.

In this study, there were no safety concerns associated with the use of roflumilast 500 µg od, the medication was well tolerated.

In conclusion, 4 weeks treatment with roflumilast 500 µg od resulted in a reduction of the LPS induced influx of inflammatory cells into the airways.