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# Bimosiamose, an inhaled small-molecule pan-selectin antagonist, attenuates late asthmatic reactions following allergen challenge in mild asthmatics: A randomized, double-blind, placebo-controlled clinical cross-over-trial

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**Abstract**

*Background*: Asthma is characterized by increased recruitment of inflammatory cells from the circulation into the airways. As selectins mediate tethering and rolling of leukocytes on the vascular endothelium, they constitute a promising target for the therapeutic modulation of inflammation. We evaluated the effect of inhaled bimosiamose (TBC1269), a synthetic pan-selectin antagonist, on allergen-induced late asthmatic reactions (LAR) in mild asthmatics.

*Methods*: Twelve male subjects with mild allergic asthma (only beta-agonists prn) with demonstrable LAR (fall of $FEV_1$ 3–8 h after allergen inhalation $>15\%$ of baseline) at screening completed a randomized, double-blind, placebo-controlled clinical cross-over-trial. Subjects were treated with inhaled bimosiamose 70 mg bid or matching placebo on days 1–3 and 70 mg once on the morning of day 4. On day 4 following the last inhalation of study drug, an allergen challenge was performed. The primary endpoint was the maximum fall in $FEV_1$ between 3 and 8 h after allergen inhalation on active treatment vs. placebo. Secondary endpoints included early asthmatic response, exhaled nitric oxide, and airway hyperresponsiveness to methacholine 24 h post allergen.

*Results*: Bimosiamose significantly attenuated the maximum LAR compared to placebo by 50.2% (placebo mean $\pm$ SEM fall $-13.10\pm 2.30\%$, bimosiamose $-6.52\pm 3.86\%$, treatment effect $p=0.045$; linear mixed-effects model). There was no effect of active treatment on early asthmatic response, post allergen airway hyperresponsiveness or exhaled nitric oxide, and peripheral blood cells.

*Conclusions*: Administration of the pan-selectin antagonist bimosiamose is effective in a human allergen challenge model of asthma. The result of this proof-of-concept exploratory trial is the first study that demonstrates clinical efficacy of selectin-antagonists as novel therapeutic strategy in asthma.

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*Keywords:* Late asthmatic reactions; Exhaled nitric oxide; Early asthmatic response; Selectins; Adhesion molecules

## 1. Introduction

Asthma is characterized by increased presence of inflammatory cells including eosinophils, neutrophils, lymphocytes and mast cells in the airways of affected subjects. Allergen-induced late asthmatic reactions (LAR) mimick chronic asthma, in as much as they are characterized by an delayed and persistent influx of eosinophils and neutrophils between 4 and 24 h after allergen inhalation, increased airway hyperresponsiveness, and exhaled nitric oxide [1]. Therefore, allergen-induced LAR is considered a valid model to study anti-inflammatory actions of established drugs, e.g. corticosteroids [2], but also investigational compounds [3,4].

Induction and maintenance of inflammation in asthma is mediated by recruiting inflammatory cells from the

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circulation via lung tissue to the airways. In this concerted cascade of molecular events, e.g. adhesion, transmigration, maturation and activation, the initial tethering and rolling of blood cells mediated by selectins are recognized to be the primary step [5,6]. Therefore, selectins constitute a promising target for therapeutic intervention in the modulation of inflammation [7,8].

The selectin family of vascular cell adhesion molecules is comprised of three structurally related calcium-dependent carbohydrate binding proteins, E-, P- and L-selectin. P-selectin is a rapidly inducible form found primarily on activated platelets and endothelium. E-selectin is found primarily on activated endothelial cells and maximal expression occurs 3–6 h after the inflammatory process begins. L-selectin is constitutively expressed on the surface of several leukocyte subtypes, including neutrophils, eosinophils, monocytes, the majority of circulating B and T cells, and on a subset of natural killer cells [9].

Bimosiamose (TBC1269 = 1,6-Bis[3-(3-carboxymethyl-phenyl)-4-(2-α-D-mannopyrano-syloxy)-phenyl]hexane) is a synthetic computer designed pan-selectin antagonist targeted against all three identified selectins [10]. Different in vitro studies under static and dynamic flow conditions demonstrated that this compound is able to block leukocytes including neutrophils, eosinophils and lymphoid cells. In this context, a preferential blockage of neutrophils as compared to eosinophils was observed [11,12]. Consistent with these in vitro results, bimosiamose demonstrated in numerous in vivo disease models of acute and chronic inflammation therapeutic efficacy by prevention of influx of polymorphonuclear neutrophils into inflamed tissue [11,13–15].

Recently, an initial observation evaluating the effect of a single intravenous dose of bimosiamose in asthmatic subjects was reported. In this study, bimosiamose had only a minor effect on sputum eosinophils following allergen challenge, and failed to attenuate the allergen-induced LAR [16]. Animal data using a sheep model of allergic inflammation, however, suggested that inhalation of bimosiamose may offer a more efficacious approach as compared to intravenous injection [17]. Therefore, this randomized, double-blind, placebo-controlled clinical cross-over trial was performed to evaluate the effect of 4 days treatment with inhaled bimosiamose on allergen-induced LAR in mild asthmatics.

## 2. Methods

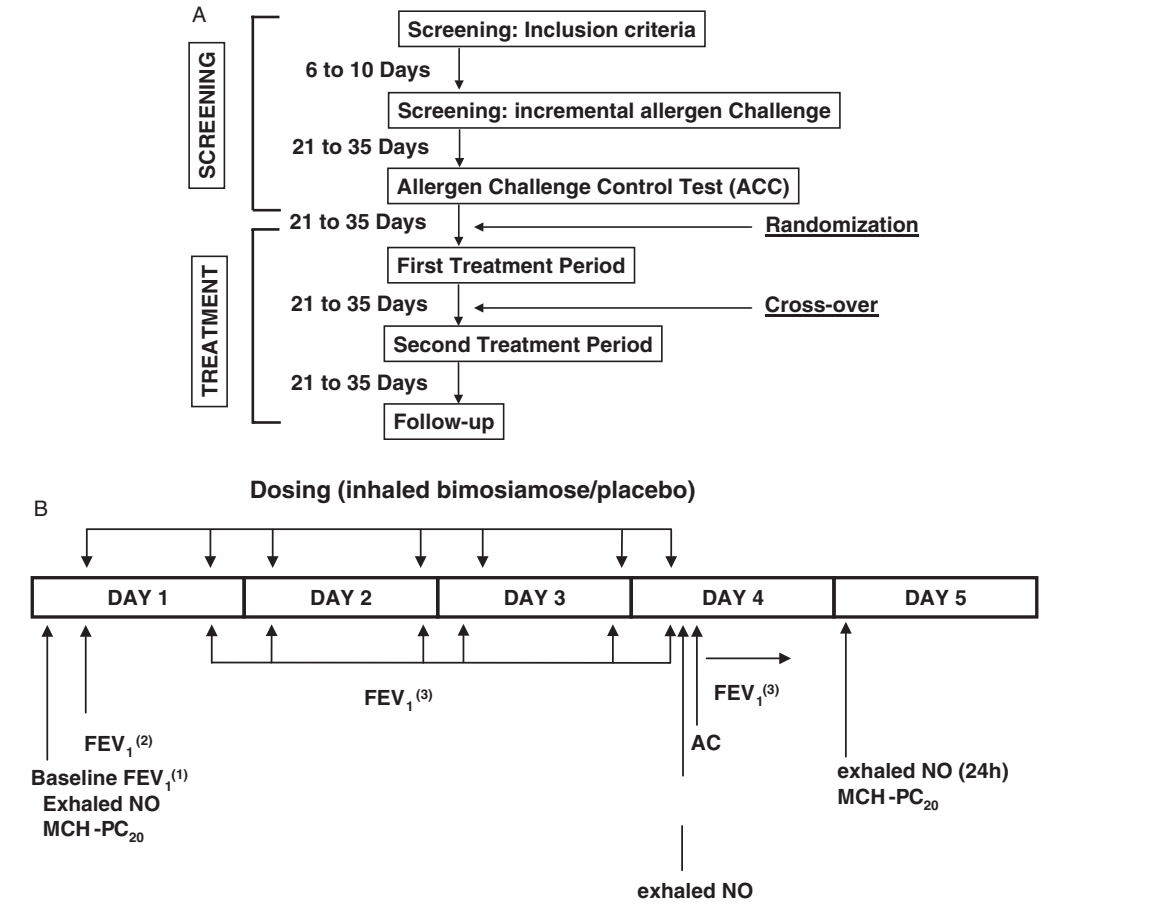
### 2.1. Subjects

We enrolled 12 male subjects with a history of mild asthma [18]. All subjects used only short-acting beta-agonists as needed (prn), had a baseline forced expiratory volume in 1 s ($FEV_1$) of greater than 80% of predicted, a provocative concentration of methacholine causing a 20% fall in $FEV_1$ ($PC_{20}$) $<8$ mg/ml [19], positive skin prick tests to at least one inhalant allergen, and dual-phase responses to inhaled allergen-challenge. Exclusion criteria were use of any anti-inflammatory medications including inhaled, nasal or systemic corticosteroids, leukotriene antagonists, anti-histamines, cromones, theophylline or non-steroidal anti-inflammatory drugs within 4 weeks before study entry, actual smoking, smoking within 6 months prior to study entry or any smoking history of $>10$ packyears, any significant co-morbidity other than asthma, upper respiratory tract infection within 4 weeks before screening, and ongoing specific immunotherapy. The protocol was approved by the local ethics committee. All subjects signed informed consent prior to any study related procedure. The study was performed between August 2002 and April 2003. All procedures were performed out of seasonal allergen exposure in subjects with seasonal allergies (grass pollen).

### 2.2. Study design

The overall study design is summarized in (Fig. 1A). At screening (Visit 1), spirometry, exhaled nitric oxide measurements (eNO), methacholine challenge, skin prick test, ECG, vital signs measurements were performed and blood specimen drawn for clinical chemistry, hematology, specific IgE (RAST), and hepatitis antibody screening. Then, an allergen challenge was performed with an antigen prior identified by skin test or RAST using an incremental allergen challenge protocol. Bolus allergen challenge was then performed after a minimum washout of 3 weeks (allergen challenge control test) [20]. Following further 3 weeks washout, patients were randomly assigned to receive either 4 days of inhaled bimosiamose (70 mg bid on days 1, 2, and 3, and 70 mg on the morning of day 4) or matching placebo (0.08% citrate buffered 0.9% NaCl), delivered through a PARI LC STAR nebulizer (Pari, Sternberg, Germany) (Fig. 1B). On day 1 (prior to first dose of study drug), spirometry, eNO, methacholine challenge, and repeated safety ECG recordings were performed. On day 4, another eNO was measured and bolus allergen challenge using the same allergen dose as during the allergen challenge control test, was performed 30 min after end of drug inhalation, and spirometry was recorded for full 8 h. Subjects returned to the clinic 24 h after allergen challenge (day 5) for spirometry, eNO and methacholine challenge. After a washout period of at least 3 weeks, patients entered the second cross-over treatment period according to the randomization order. The chosen length of washout period is substantially longer than the terminal elimination half-life of bimosiamose (approximately 3 h) after intravenous administration [21].

Blood hematology, pharmacokinetics and clinical chemistry samples were obtained on days 1, 4, and 5,




| | |
|---|---|
| Baseline $FEV_1$ (1): | Highest value of 3 x $FEV_1$ |
| $FEV_1$ (2): | 0, 15, 30, 60, 120, 240 min |
| $FEV_1$ (3): | 0, 3, 6, 10, 15,30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, 480 |
| AC | allergen challenge |
| MCH | methacholine challenge |

Fig. 1. (A and B) Study design.

respectively. A follow-up visit was performed at least 7 days after the end of the second treatment period.

### 2.3. Methacholine challenge

Methacholine challenge was performed according to recommendations of the American Thoracic Society [19] using a five-breath-dosimeter protocol (DeVilbiss Dosimeter 406, Hounslow, UK, connected to a ZAN nebulizer, Oberthulba, Germany). Quadrupling concentrations of methacholine were inhaled until a $>10\%$ fall in forced expiratory volume in 1 s ($FEV_1$) was noted. In that case, doubling doses were further used and the procedure was stopped, when a $>20\%$ fall in $FEV_1$ was observed, or the final concentration was reached (16 mg/ml methacholine). To ensure comparable baseline characteristic between cross-over-periods, the $PC_{20}$ methacholine on each day 1 prior to treatment had to be within one doubling concentration.

### 2.4. Allergen challenge

Screening allergen challenge was performed with incrementing doses of specific allergen (ALK, Hamburg, Germany, 100.000 Standard Quality Units (SQ) of allergen per ml stock solution), using a five-breath-dosimeter protocol (DeVilbiss Dosimeter 406, Hounslow, UK) with a Mefar nebulizer (Mefar, Brescia, Italy), until an early asthmatic response (EAR, $>20\%$ fall in $FEV_1$ compared to post-saline baseline values) was achieved [20,22]. After determination of baseline $FEV_1$ (best of three post allergen diluent inhalation recordings), allergen administration was started with an initial concentration of 100 SQ/ml allergen, and doubling concentrations were inhaled until occurrence of an EAR or the maximum dose of 20.000 SQ/ml was reached. Patients inhaled five slow breaths of nebulized allergen solution from functional residual capacity to total lung capacity followed by breath holding for another 2 s.

Triplicate measurement of $FEV_1$ were made 90 s after each allergen inhalation according to ATS guidelines [23] and the highest value was recorded as baseline. Spirometry was measured after 3, 6, 10, 15, 30, 45, and 60 min, followed by repeated measurements every 30 min for a total of 8 h. If $FEV_1$ fell by $>10\%$ from baseline, additional spirometry was performed in 15 min intervals. LAR was defined as a fall in $FEV_1 > 15\%$ from post-saline baseline between 3 and 8 h after allergen inhalation. The provocative dose of allergen causing a 20% reduction in $FEV_1$ ($PD_{20}$) was calculated as described elsewhere [24]. This screening $PD_{20}$ was then used for subsequent bolus allergen challenges throughout the trial, as described by Taylor et al. [20].

To ensure an adequate reproducibility of the LAR, we incorporated an allergen control test scheduled at least 3 weeks after screening allergen challenge and at least 3 weeks prior to treatment, in which the ability of the calculated $PD_{20}$ allergen to effectuate comparable early and late asthmatic response when inhaled as bolus was tested. Only patients with reproducible LAR at screening and during the control test were rendered eligible to enter the treatment period [2].

### 2.5. Measurement of exhaled nitric oxide

eNO was quantified according to American Thoracic Society guidelines at a constant flow rate of 50 ml/s using a EcoMedics CLD88sp chemoluminescence analyzer. All measurements were performed in triplicate [25].

### 2.6. Peripheral blood counts

Peripheral blood cell counts and differentials were analyzed by the local Mainz University Hospital central lab using a flow cytometer.

### 2.7. Investigational drug dosage and administration

Seventy milligram (0.7 ml of 100 mg/ml bimosiamose 0.08% citrate buffered 0.9% NaCl solution) dissolved in 3.3 ml sterile saline (final volume 4 ml) was administered by aerosol inhalation through a PARI LC STAR nebulizer (output 440 mg/min) whereas solvent containing 0.7 ml of 0.08% citrate buffered 0.9% NaCl in 3.3 ml sterile saline was used as placebo (final volume 4 ml). The dose of 70 mg bid corresponds to the maximum tolerated dose determined in phase I studies [26,27].

Each subject inhaled the study medication by tidal breathing trough a mouthpiece with a nose clip in place twice daily on day 1, 2, 3 and once in the morning on day 4 in house. Vital signs and ECG were recorded before and 30, 60, 120 and 240 min and $FEV_1$ was measured before and 15, 30, 60, 120 and 240 min after the first dose on day 1 of each treatment period in the morning. For dose administration on day 1 evening and days 2 and 3 peak expiratory flow values were recorded before 5 and 15 min after the inhalation of the study drug. On day 4 $FEV_1$ was measured according to the protocol of the allergen challenge and vital signs and ECG were measured before 30, 60, 120 and 240 min after final inhalation.

### 2.8. Statistical analysis

The statistical analysis was performed with the program R for statistical computing [28,29]. Particularly, methods provided by the base-package, the ctest-package for classical statistical tests and the lme4-package for mixed-effect models were used. Descriptive statistics were applied to summarize clinical characteristics of the subjects. Unless otherwise stated, data are given as arithmetic mean values with standard errors of mean (SEM). To compare screening (incremental) allergen challenge and bolus allergen challenge control test, non-parametric Wilcoxon signed rank test was used. To analyze the cross-over treatment period, a linear mixed-effects model [30] based on the restricted maximum likelihood (REML) was applied to the LAR data in order to analyze treatment effect, period effect and a potential carry-over effect similar to the methods described by Senn for the analysis of cross-over studies [31].

Primary endpoint of the analysis was the comparison of the mean maximum fall in $FEV_1$ during LAR (3 8 h post allergen inhalation) expressed as percentage drop from baseline (post-saline $FEV_1$) on day 4 of active (bimosiamose) vs. placebo treatment. Secondary endpoints for analysis were area under the curve (AUC) for LAR, airway hyperresponsiveness ($PC_{20}$ methacholine) 24 h after allergen challenge on day 5, eNO 24 h after allergen on day 5, and comparison of peripheral blood cells prior to and following allergen challenge on day 4. The AUC of $FEV_1$ during LAR was calculated using the trapezoidal rule for a time range between $t = 180$ and 480 min post allergen inhalation.

A $p$-value of less than 0.05 was considered as statistically significant. Based on prior studies in similar patients [2,32,33], the study was designed to give at least 80% power to detect a $>50\%$ attenuation of the late asthmatic response (active over placebo) with a one-sided

Table 1
Characteristics of subjects

| | $N = 12$ |
|---|---|
| Age (years, median, range) | 27.5 (24–44) |
| $FEV_1$ ($l$, mean ± SEM) | 4.68 ± 0.63171 |
| $FEV_1$ (% of predicted, mean ± SEM) | 104 ± 2.6 |
| $PC_{20}$ methacholine (mg/ml, geom. mean ± SD) | 1.6 ± 2.44 |
| RAST class (median, range) | 3 (2–6) |
| Allergen used ($n$) | |
| Timothy grass | 8 |
| House dust mite | 3 |
| Horse dander | 1 |

alpha level of 0.05 using complete data of $n=12$ subjects (primary efficacy endpoint).

## 3. Results

### 3.1. Patients

Baseline data of all subjects are given in Table 1. Of 19 screened patients, 12 patients were eligible to be enrolled, randomized and completed both treatment periods. Reasons for premature discontinuation during screening were screening failure (absent airway hyperresponsiveness, asthma severity > step 1, failure to demonstrate early or late phase asthmatic response during allergen challenge, $n=6$) or absence of late asthmatic reaction during the allergen challenge control test ($n=1$).

### 3.2. Primary outcome

#### 3.2.1. Allergen-induced late asthmatic responses (LAR) and effect of treatment

During the screening period, the mean maximum late fall in $FEV_1$ (3–8 h post allergen) was comparable and not significantly different between the initial incremental allergen challenge and bolus allergen challenge control test (maximum LAR $-27.9\pm2.1$ and $-23.7\pm4.5\%$ fall from baseline, $p=0.380$).

During treatment period, inhalation of bimosiamose or corresponding placebo was safe and well tolerated. After allergen challenge following placebo treatment, there was a $-13.10\pm2.30\%$ fall from baseline in $FEV_1$. In contrast, after allergen challenge following bimosiamose treatment the fall from baseline reduced to $-6.52\pm3.86\%$. Compared to placebo, this 50.2% attenuation after bimosiamose treatment was statistically significant (treatment effect $p=0.045$) (Fig. 2B). No evidence for period or carry-over effects has been found according to the linear mixed-effects model applied to the analysis of all cross-over LAR data ($p>0.7$).

### 3.3. Secondary outcomes

#### 3.3.1. Area under the curve (AUC) for LAR

AUC for LAR was also reduced by bimosiamose vs. placebo ($-414\pm593$ vs. $-896\pm375$, although this reduction failed to reach statistical significance ($p>0.05$). However, this failure was largely due to one patient behaving opposite to what was expected, i.e. complete attenuation of LAR during placebo and marked LAR during bimosiamose. If this patient was excluded from analysis, the AUC for the fall of $FEV_1$ relative to the baseline value vs. time (LAR) was significantly reduced by bimosiamose ($-68\pm528$ vs. $-955\pm506$, $p=0.02$).

#### 3.3.2. Early asthmatic responses (EAR)

There was no significant difference between inhalation of bimosiamose and placebo on the early asthmatic response ($30.1\pm6.0$ vs. $28.7\pm3.3\%$, $p=0.52$) (Fig. 2A).

### 3.4. Exhaled nitric oxide, airway hyperresponsiveness

The baseline values of eNO on each day 4 were comparable between treatment periods (placebo: $76.5\pm22.8$ ppb; bimosiamose: $56.0\pm13.7$ ppb, $p=$ns), and were not different from values obtained on day 1 prior to first dose of treatment (placebo: $71.3\pm21.5$ ppb; bimosiamose: $56.4\pm13.7$ ppb), i.e. there was no effect of multiple doses of bimosiamose on baseline eNO ($p=$ns). eNO increased significantly 24 h after allergen challenge during both treatment periods (placebo: $96.0\pm24.7$ ppb, $p<0.01$; bimosiamose: $86.6\pm17.0$ ppb, $p<0.01$). There was no effect of bimosiamose on allergen-induced eNO 24 h post allergen challenge as compared to placebo ($p=0.76$) (Fig. 3).

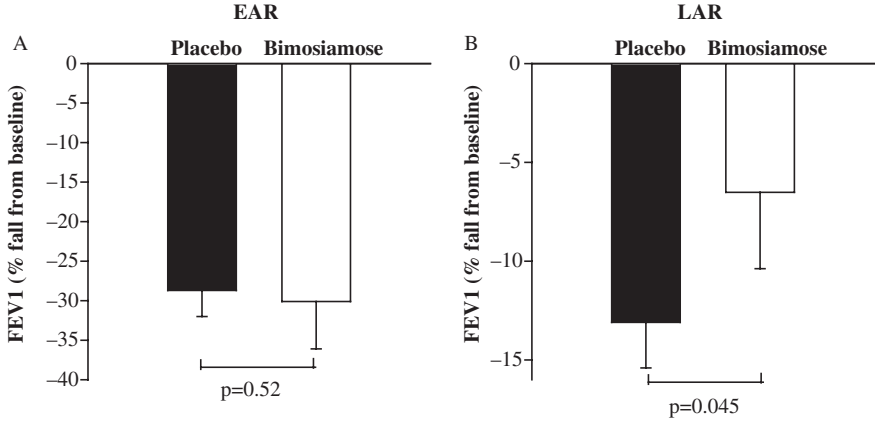



Fig. 2. (A and B) Mean (SEM) maximum fall in $FEV_1$ during (A) early asthmatic reactions, and (B) late asthmatic reactions following treatment with inhaled bimosiamose or matching placebo.

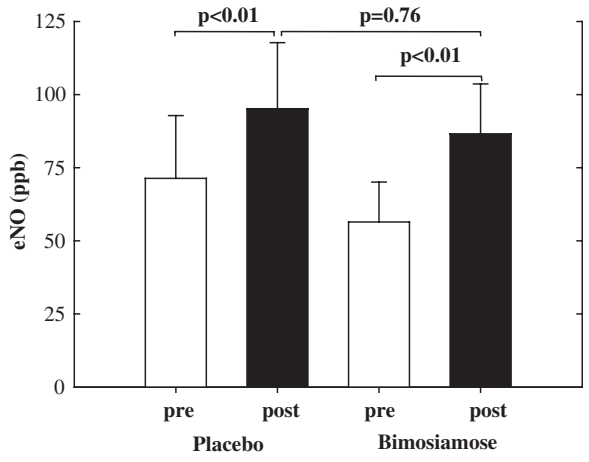



Fig. 3. Levels of exhaled nitric oxide prior to (white bars, day 4) and 24 h after (black bars, day 5) allergen challenge following bimosiamose or matching placebo. Data are mean values with SEM.

There was an increase of methacholine responsiveness following allergen challenge during both periods (placebo: 1.2±4.1 mg/ml, post 0.7±5.5 mg/ml, $p=0.151$; bimosiamose: pre 1.2±3.4 mg/ml, post: 0.9±4.2 mg/ml, $p=0.034$; geometric mean±geometric standard deviation). This increase was only significant during bimosiamose treatment. However, there was no statistically significant effect of bimosiamose on post-allergen $PC_{20}$ methacholine compared to placebo ($p=0.91$).

### 3.5. Peripheral blood cells

There was no difference in baseline white blood cell (WBC) counts between placebo and active treatment. Differential cell analysis of WBC revealed that on active treatment, there was a small temporarily increase of peripheral blood lymphocytes only at 8 h after allergen compared to placebo ($p=0.04$), which was accompanied by a relative decrease of blood neutrophils ($p=0.003$). However, when taking into account absolute numbers of changes in blood cells after allergen, this difference was no longer significant. Further, monocytes, eosinophils and basophils showed no differences in cell numbers between placebo and bimosiamose treatment (Table 2).

### 3.6. Safety

Treatment with inhaled bimosiamose was generally well tolerated. There was one serious adverse event not related to trial medication. In total, there were 35 adverse events during bimosiamose, and 19 during placebo, and these events were nearly exclusively related to the inhalation procedure (sore throat, pharyngeal discomfort, temporal vertigo). Bimosiamose did not affect pulmonary function assessed by $FEV_1$ (measured prior to and 15, 30, 60, 120 and 240 min after morning inhalation on day 1) or peak expiratory flow rates (measured prior to and 5, 10, and 15 min after inhalation on days 1 (evening dose), 2 and 3).

There were no significant changes in lab clinical chemistry, vital signs and ECG during the trial.

## 4. Discussion

This study is the first human trial reporting clinical efficacy of a pan-selectin antagonist, bimosiamose, in an allergen challenge model of asthma by showing attenuation of the maximum fall of $FEV_1$ during the late phase allergic response (LAR) by approximately 50% over placebo.

The demonstrated clinical effectiveness of bimosiamose on the LAR after inhalation is in line with earlier preclinical and clinical reports. Using the allergen sheep model, both pre- and postallergen challenge inhalative administration of bimosiamose resulted in a dose and time dependent inhibition of the LAR [17]. In addition, the results of this preclinical model suggested that inhalation route of administration seems to be more effective than the intravenous route of administration. A recent human study evaluated the effect of a single intravenous dose of bimosiamose on allergen challenge in asthmatic subjects. In this trial, bimosiamose had only a minor effect on sputum eosinophils following allergen challenge, and failed to attenuate the allergen-induced LAR [16]. As compared to intravenous route of administration, these findings support the strategy of inhalative administration of bimosiamose.

The results of our trial concur with recent data where an anti-CD11a monoclonal antibody (efalizumab) was used as adhesion blocker in a human allergen challenge model of

Table 2
White blood cell count (WBC) and peripheral blood cell differentials prior to and 8 h after allergen challenge

| Parameter | Unit | Baseline (pre allergen) | | Eight hours post allergen | | *p*-value | | |
|---|---|---|---|---|---|---|---|---|
| | | Bimosiamose | Pbo. | Bimosiamose | Pbo. | Bimo. vs. Pbo. | Bimo. vs. baseline | Pbo. vs. baseline |
| WBC | (Cells/ml) | 6.1 | 6.1 | 7.3 | 7.8 | ns | ns | ns |
| Neutrophils | (%) | 57.8 | 62.4 | 55.3 | 66.6 | 0.003 | ns | ns |
| Lymphocytes | (%) | 29.6 | 26.3 | 31.6 | 22.2 | 0.04 | ns | ns |
| Monocytes | (%) | 7.9 | 7.3 | 7.8 | 6.6 | ns | ns | ns |
| Eosinophils | (%) | 4.3 | 3.3 | 4.5 | 3.9 | ns | ns | ns |
| Basophils | (%) | 0.7 | 0.7 | 0.8 | 0.7 | ns | ns | ns |

asthma [34]. In this study, compared to baseline eight subcutaneous administration of efalizumab inhibited the maximum LAR by 48% whereas the matching placebo inhibited the maximum LAR only by 18%. Unfortunately, this observation did not reach significance over placebo [34].

Except from the study cited above, there is—at least to our best knowledge—little information about the magnitude of placebo effects during allergen challenge studies, in particular when prolonged treatment periods are involved. Our prospective study was designed to give sufficient power to detect more than 50% attenuation of the late asthmatic response, the primary efficacy endpoint (bimosiamose over placebo). The initial power calculation was based on the landmark study by Inman et al. [33], who rendered as few as 8–10 subjects sufficient to demonstrate at least 50% attenuation of LAR with 90% power, based on a mean maximum fall during LAR of $-23.3 \pm 2$ and $-24.5 \pm 2.2\%$ from baseline. In this regard, it has to be considered, that the study by Inman et al. was a mere repeatability study not taking into account potential placebo effects. Nevertheless, their degree of LAR is fully consistent with the degree of LAR in our group of patients during screening ($-27.9 \pm 2.1\%$) and the bolus allergen control test ($-23.7 \pm 4.5\%$ fall from baseline), and also with own data from a previous study [32]. Moreover, our study design included a 4 day treatment period with placebo which for blinding purposes consisted of 0.08% citric acid dissolved in saline, hence one also has to take into account the sensation of bad taste reported by many subjects during either treatment period. It is therefore possible, that the mode of delivery, duration of treatment, and particularly taste of placebo treatment may have influenced the magnitude of the placebo effect during our trial. Since no anti-inflammatory or bronchodilator properties of citric acid have been reported this far, we consider the placebo effect observed in our patients likely to be linked with the taste of our placebo solution.

Despite this placebo effect, the results of our study clearly indicate, that inhalation of bimosiamose reduced the maximum late asthmatic response, although the degree of protection in absolute numbers appeared less than the one seen with inhaled corticosteroids [35,36]. In contrast, treatment with a single intravenous dose of bimosiamose resulted in a minor decrease in sputum eosinophils after allergen challenge, but had no effect on the late asthmatic response [16]. This difference in efficacy between inhalative and intraveneous administration is consistent with previous published results. As described in an animal model of allergic asthma, as compared to intraveneous administration, inhaled bimosiamose attenuated much stronger the late asthmatic reaction in sheep [17].

In our study, we did not observe an effect of bimosiamose on exhaled nitric oxide. Since exhaled nitric oxide is generally accepted as a marker of eosinophlic inflammation, this finding would suggest that blocking selectins by bid inhalation of 70 mg of bimosiamose had no major effect on eosinophilic airway inflammation post allergen challenge. Thus, the protective effect of bimosiamose on LAR in our study cannot be attributed to a reduction in exhaled NO and/or eosoinophilic inflammation.

The data on peripheral blood cells in our study, however, do not shed further light on the possible mechanism by which bimosiamose protected against LAR, since we did not observe significant changes in peripheral blood cells apart from a minor, temporarily increase in the relative proportion of lymphocytes and reduced neutrophils at 8 h after allergen, the latter losing significance when calculated as absolute changes in cells from pre-allergen values. The fact that inhalative administration of bimosiamose did not alter peripheral blood differentials prior to and after challenge is consistent with the low concentration of bimosiamose in the circulation after inhalation. In both a ascending dose tolerance study and multiple dose tolerance study of bimosiamose, systemic blood levels of bimosiamose were only in singular cases quantitatively detectable after inhalation of doses of $\geq 105$ mg bid [26,27].

As an alternative mechanism, blockage of selectins also influence signal transduction during inflammatory pathways. Besides mediation of leukocyte recruitment, selectins and their ligands effect cellular activation of the mitogen-activated protein kinase pathway [37], shifts in intracellular $Ca^{2+}$ [38] and expression of proinflammatory cytokines like TNF-alpha or interleukin-8 [39]. In the allergic sheep study, a marked reduction of bronchoalveolar lavage histamine and kallikreine content was observed, and the authors attributed this finding to an involvement of selectins in mast cell signaling and degranulation [17].

In our study, there was no effect of bimosiamose treatment on airway hyperresponsiveness 24 h post allergen. From a mechanistic point of view, it is reasonable to speculate that the observed effect on LAR was somehow associated with a modulation of cellular inflammation, although we have no direct evidence from our trial to support this hypothesis. Nevertheless, discordance of airway inflammation and hyperresponsiveness has been demonstrated by several authors [4,40].

We used a breath-enhanced PARI LC Star jet nebulizer for pulmonary delivery of bimosiamose. This nebulizer usually generates a respiratory fraction of drug between 78 and 82% [41]. The fraction of particles sized $<6.1$ μm mass diameter was shown to be 77.2 for budesonide [42], and the fraction of particles $<5$ μm was 68% for salbutamol, respectively [43]. A radiolabeling study with a similar therapeutic approach (inhaled VLA-4 antagonist) revealed a lung deposition of 18% of the administered dose after inhalation of drug through the Pari LC Star [44]. Thus, the nebulizer device chosen is able to deliver an appropriate degree of pulmonary drug deposition [45]. Nevertheless, drug delivery to the lungs by nebulizer is much more variable and lung deposition far less predictable than e.g. with dry powder inhalers, thus necessitating the use of higher drug doses (e.g. for bronchodilators) and eventually leading to higher systemic exposure and toxicity [46]. As a

consequence it may be useful to study lung deposition of radiolabelled bimosiamose by scintigraphy in subsequent trials. As bimosiamose is not orally bioavailable, observed plasma levels in phase I trials probably exclusively reflect lung deposition of bimosiamose and absorption of drug via the lungs [47].

In summary, this study represents the first proof-of-concept trial demonstrating clinical effectiveness of an inhaled pan-selectin-antagonist (bimosiamose) in an allergen challenge model of asthma. Targeting selectins appears an attractive future approach in chronic-inflammatory disorders.

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