

Effect of a single dose of the selectin inhibitor TBC1269 on early and late asthmatic responses

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Summary

Background Selectins participate in the initial phase of leucocyte migration from circulation to inflamed tissues and may play a role in inflammatory cellular influx into airways in asthma. In the sheep asthma model, TBC1269, a pan-selectin antagonist, reduced late allergen response by 74%.

Objective To determine whether a single dose of TBC1269 inhibits early (EAR) and late (LAR) asthmatic responses, and whether it inhibits sputum leucocyte influx after inhalation allergen challenge in atopic asthmatic subjects treated with bronchodilators only.

Methods Twenty-one asthmatic subjects (mean $\pm$ SD, age = 32.5 ± 6.7 years, 8 males, FEV₁ percent predicted = $84 \pm 15\%$) with known late asthmatic response based on a screening inhalation allergen challenge were randomly assigned to receive intravenous treatment with either placebo ($n = 11$) or TBC1269 ($n = 10$, 30 mg/kg) infused over 15 min immediately prior to a second (post-treatment) allergen challenge at least 4 weeks after the screening challenge. After each challenge, EAR and LAR were monitored for 7 h. In addition, sputum was induced 1 day before and 1 day after each allergen challenge.

Results TBC1269 did not attenuate the EAR compared with placebo (largest fall in FEV₁ within 1 h of $34.1 \pm 13.9\%$ vs. $31.8 \pm 12.2\%$ for TBC1269 and placebo groups respectively, $P = 0.61$) or the LAR (largest fall in FEV₁ between 3 and 7 h of $39.3 \pm 15.3\%$ vs. $32.6 \pm 13.8\%$, $P = 0.24$). TBC1269 had only minor effects on allergen-induced sputum eosinophilia.

Conclusion We conclude that TBC1269 administered before allergen challenge as a single intravenous dose does not attenuate early or late asthmatic responses to allergen in asthmatic subjects.

Keywords adhesion molecules, allergen challenge, asthma, selectin, selectin antagonist, TBC1269

Submitted 17 December 2002; revised 25 July 2003; accepted 16 September 2003

Introduction

Selectins mediate the tethering and rolling of circulating leucocytes over endothelial cells, the first steps for leucocyte transvascular migration [1]. There are three types of selectins: L, E and P. L selectin is expressed on the surface of leucocytes, E selectin on activated endothelial cells and P selectin on activated platelets and endothelial cells. Selectins mediate cell adhesion by binding to glycoproteins and glycolipids on the surface of leucocytes and endothelial cells. After rolling, leucocytes adhere firmly to endothelial cells and traverse the vascular wall, a process mediated by integrins. In asthmatic subjects, serum concentrations of E selectin increase during asthma attacks [2], probably as a result of increased endothelial expression. Thus, blocking the interaction between selectins and their ligands is a possible strategy for preventing cellular infiltration during inflammatory responses.

Animal and clinical studies suggest that selectins are involved in the recruitment of inflammatory cells into the airway after allergen challenge. Using a mouse model of airway allergic inflammation, De Sanctis et al. [3] showed that, compared with wild-type mice, knockout mice deficient in P selectin have decreased airway hyper-reactivity, and attenuated influx of eosinophils and lymphocytes in broncho-alveolar lavage (BAL) after sensitization and intra-tracheal challenge with ovalbumin. Similarly, L-selectin knockout mice show a marked decrease of airway hyper-reactivity and mild attenuation of T cell recruitment in BAL in the same model of allergic inflammation [4, 5]. Broide et al. [6], using a mouse model of ragweed induced eosinophilic peritonitis, showed that P-selectin-deficient mice have a 75% decrease in eosinophil recruitment into peritoneal fluid after intra-peritoneal challenge. In asthmatic subjects, E-selectin expression is increased on endothelial cells of bronchial mucosal biopsies [7] compared with healthy subjects, and it is further increased 5–6 h after allergen challenge, coinciding with the recruitment of eosinophils, neutrophils and lymphocytes into the airway mucosa [8]. In addition, sialyl Lewis x glycans, the ligand for selectins, are increased in lung endothelial cells in

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asthmatic subjects [9]. Taken together, these animal and clinical studies suggest that selectins may play an important role in the recruitment of leucocytes into the airway during allergic inflammatory responses, and provide the rationale for considering selectins as novel therapeutic targets in asthma [10].

Recently, small non-oligosaccharide molecules have been designed to inhibit the interaction between selectins and sialyl-Lewis(x) tetrasaccharide (sLe(x)). Because sLe(x) is a natural ligand for all 3 selectins, these compounds are potential antagonists for all selectins [11]. Indeed, one such compound, TBC1269 (Texas Biotechnology Corporation, Houston, TX, USA), effectively inhibits the binding of sLe(x) to all 3 selectins [12]. *In vitro*, TBC1269 decreases the binding of human eosinophils and neutrophils to P selectin under static and dynamic conditions [13]. In the sheep model of airway allergic inflammation caused by sensitization and aerosol challenge with *Ascaris suum* antigen, TBC1269 reduces early and late airway responses, prevents allergen-induced airway hyper-reactivity and reduces allergen-induced airway neutrophilia during the late airway response [14].

In this study, we tested the hypothesis that TBC1269 could inhibit airway responses to inhaled allergen in asthmatic subjects. To test our hypothesis, we conducted a clinical trial in which we tested the efficacy of TBC1269 in attenuating early- and late-phase responses in asthmatic subjects with documented dual-phase responses to allergen. Another outcome that we measured was eosinophil percentage in induced sputum before and after allergen challenge.

Methods

Subjects and study design

Asthmatic subjects ($n = 21$) were recruited through advertisement to the public. Inclusion criteria were $FEV_1 \geq 65\%$ of predicted [15], asthma treated only with short-acting bronchodilators, methacholine airway hyperactivity ($PC_{20} = 8 \text{ mg/mL}$), cutaneous reactivity to at least one allergen and dual-phase responses to inhalation allergen challenge. Methacholine challenge was performed as part of another study [16] immediately before participation in this study. The exclusion criteria were use of anti-inflammatory medications or upper respiratory infection within 6 weeks; use of leukotriene

antagonists, antihistamines, cromolyn and nedocromil within 4 weeks; tobacco use within 1 year or lifetime smoking history greater than 5 pack-years; and any significant co-morbidity besides asthma. All subjects signed informed consent and the study was approved by the Committee on Human Research of the University of California at San Francisco. The study protocol is described in Fig. 1.

An additional 11th subject was enrolled in the placebo group because a previous subject had all 4 induced sputum samples of inadequate quality for having more than 80% of cells of the squamous type indicating excessive saliva contamination. Although this subject's sputum samples were excluded from data analysis, her EAR and LAR data were included.

Allergen skin test

Allergen prick skin test was performed as previously described [17] using 4 allergen extracts (*Dermatophagoides pteronyssinus*, *D. farinae*, cat, ryegrass from Greer Laboratories, Lenoir, NC, USA) and controls (diluent and histamine base 1.8 mg/mL from Allarmed Laboratories, San Diego, CA, USA). Skin reactivity was assessed 15 min after the prick and considered to be positive if it provoked a weal at least 2 mm greater than the weal for the negative control (diluent) and surrounding erythema. The allergen extract that produced the largest weal was selected for the inhalation allergen challenge, and skin prick test titration was carried out with 2-fold dilutions to find the lowest concentration that caused a 2-mm weal with erythema.

Sputum induction

Sputum was induced and processed as described previously [18]. Briefly, subjects inhaled four puffs ($360 \mu\text{g}$) of albuterol and 10 min later, while wearing a nose clip, they breathed nebulized 3% saline solution through a mouth piece for 12 min (100 mL of saline solution in the reservoir of a Ultra-Neb 99 nebulizer, DeVilbiss, Somerset, PA, USA). At 2-min intervals, subjects spat saliva into a cup (discarded saliva) and coughed sputum into another cup (sputum sample). Airway calibre was monitored with peak flow measurements and, if peak flow declined $\geq 20\%$ from the post-albuterol value, spirometry was performed. Sputum was processed [18] to

Visit:	V1	V2	V3	V4	V5	V6	V7	V8	
Time:	3-14 days	1 st day	2 nd day	3 rd day	4 weeks	1 st day	2 nd day	3 rd day	4 weeks
Procedures:	ST S BD ECG	STit S IS	S IAC BD	S IS BD		STit S IS BD	S Drug IAC BD ECG	S IS BD	S BD

Fig. 1. Study Design. After an initial visit (V1) to confirm asthma and atopy, subjects returned 3–14 days later on 3 consecutive days (V2–V4). On the first day (V2), subjects underwent allergy skin prick test titration (STit) and sputum induction (IS). On the second day (V3), they underwent inhalation allergen challenge (IAC) after which FEV_1 was monitored for 7 h (at 10, 20, 30, 45 and 60 min to assess EAR, and then at 2, 3, 4, 5, 6 and 7 h to assess LAR) and on the third day (V4), sputum induction was repeated. LAR was defined as a drop of $\geq 15\%$ from post-diluent FEV_1 between 3 and 7 h after the challenge. Subjects who developed LAR returned 4 weeks later to repeat these 3 consecutive days (V5–V7) – sputum induction, allergen challenge and sputum induction – but this time placebo or TBC1269 (Drug) was administered intravenously just prior to the challenge. Blood was drawn (BD) before and after drug infusion, before allergen challenge and after challenge at 1, 3, 7 and 24 h for complete blood count, tryptase concentrations and biochemistry. Lastly, a final visit took place 4 weeks after the second allergen challenge for a discharge interview and examination. ST, allergy skin prick test; S, spirometry; ECG, electrocardiogram; EAR, early asthmatic response; LAR, late asthmatic response.

obtain a differential cell count based on non-squamous cells and the supernatant was frozen at minus 70 °C until used to measure eosinophil cationic protein and tryptase concentrations. Induced sputum samples that had 80% squamous cells were excluded from analysis (6 out of 44 samples were excluded).

Quantification of ECP and tryptase

ECP and tryptase were measured in serum and sputum samples using the RIA kit and UniCap System kit, respectively (Pharmacia & Upjohn, Kalamazoo, MI, USA) following the manufacturer's instructions. The sensitivities of the assays were 2 and 1 µg/L, respectively.

Allergen challenge

Our whole-lung inhalation allergen challenge protocol has been described in detail elsewhere [16]. The initial dose of allergen for inhalation was four serial dilutions below that predicted to cause a 20% decrease in FEV₁ based on skin test titration and methacholine reactivity [19]. The baseline FEV₁ was taken as the highest of three values measured 10 min after inhalation of allergen diluent. Subjects then took 5 breaths (from functional residual capacity to total lung capacity) of each allergen concentration, which increased in serial doubling doses at 12-min intervals. FEV₁ was measured twice, 10 min after each dose step, and the higher value was recorded. If the post-allergen FEV₁ was >85% of baseline, the next dose step was delivered. If it was 80–85% of baseline, then FEV₁ was measured 10 min later. If the FEV₁ remained 80–85% of baseline, the same allergen dose was repeated; however, if it was >85% of baseline then the next dose step was delivered. The challenge was stopped when dosing with allergen caused the FEV₁ to fall to <80% baseline.

In the post-treatment inhalation allergen challenge, which was preceded by either placebo or TBC1269 infusion, the initial allergen dilution was three 2-fold dilutions below the last allergen dilution given in the first challenge. Progressive dilutions were administered as in the first challenge until the FEV₁ fell to <80% baseline value or until the subject received one doubling dilution more concentrated than the last allergen dilution given in the first challenge.

Drug infusion

Either placebo (normal saline solution) or TBC1269 diluted in normal saline solution were prepared by a pharmacist unaware of the study measurements and delivered in bags of identical appearance. The TBC1269 dose was 30 mg/kg because this was the maximum dose tested and well tolerated in earlier phase I studies (Texas Biotechnology Corporation, data in file). The adverse effects observed in phase-I studies were mild and consisted of fatigue, dizziness, headache and nausea. In these studies, TBC1269 half-life was 4 h when administered intravenously at a single dose of 30 mg/kg. In this study, either placebo or TBC1269 was administered intravenously in a single dose through a peripheral vein over 15 min, starting 15 min before the beginning of the allergen challenge.

Safety measures

Subjects were monitored for adverse effects such as unexpected symptoms and changes in vital signs, electrocardiogram, urinalysis, complete blood count and serum chemistry for liver and renal functions. Complete blood counts with a differential count of leucocytes were obtained prior to drug infusion and at 1, 3, 7 and 24 h after the post-treatment challenge.

Statistical analysis

Data were analysed using SAS (v6.07. SAS Institute Inc., Cary, NC, USA) and Stata 5 (Stata Corporation, College Station, TX, USA) programs using analysis of covariance (ANCOVA). In the ANOVA model, treatment was a factor and the corresponding variable at the screening allergen challenge was a covariate. Induced sputum data were ranked and ranks were analysed in the same manner using ANCOVA. Within-group variables were compared using a paired *t* test and proportions were analysed with Fisher's exact test. A *P*-value <0.05, using two-tailed tests, was considered to be statistically significant. Values are expressed as mean ± SE. Because in the sheep model intravenous TBC1269 reduced late-phase response (LAR) by 74% [14], we aimed at enrolling 10 subjects per group to provide 80% power to detect a 50% or higher attenuation in LAR with an alpha error of 0.05.

Results

At baseline, there were no significant differences between the clinical characteristics of the 10 subjects assigned to the TBC1269 group and the 11 subjects assigned to the placebo group (Table 1).

Early and late asthmatic responses

TBC1269 (30 mg/kg) administered intravenously 15 min before inhalation allergen challenge did not attenuate the late asthmatic response when compared with placebo infusion (largest fall in FEV₁ between 3 and 7 h after challenge of 39.3 ± 15.3% vs. 32.6 ± 13.8% for TBC1269 and placebo groups respectively, *P* = 0.24, see Fig. 2). Similarly, TBC1269 did not attenuate early airway response to allergen challenge (largest fall in FEV₁ between 10 and 60 min after challenge of 34.1 ± 13.9% vs. 31.8 ± 12.2%, *P* = 0.61). Furthermore, there were no differences within each group comparing EAR and LAR from screening allergen challenge with EAR and LAR from the post-treatment allergen challenge (Fig. 2). The same results were found when EAR and LAR were analysed as areas under the curve.

Episodes of excessive bronchoconstriction occurred more frequently in both groups during the second allergen challenge (Table 3). Two subjects in the TBC1269 group required treatment with inhaled albuterol and inhaled fluticasone in the 12-h period following the second allergen challenge. Thus, these two subjects received inhaled fluticasone before the post-challenge sputum induction the following day. The results of EAR, LAR and sputum do not change significantly when these two subjects are excluded from data

Table 1. Baseline characteristics of study subjects

Characteristic	TBC1269 (n = 10)	Placebo (n = 11)
Age (years)	32.1 ± 7.3	32.8 ± 8.3
Gender (M:F)	4:6	4:7
Caucasian (n)	8	5
PC ₂₀ methacholine (mg/mL)	0.25 ± 0.43	0.30 ± 0.56
FEV ₁ , % predicted		
Pre-screening challenge	83 ± 17	84 ± 13
Pre-treatment challenge	81 ± 15	78 ± 11
Blood eosinophils (%)		
Pre-screening challenge	4.7 ± 2.5	5.1 ± 2.3
Pre-treatment challenge	5.6 ± 3.0	5.6 ± 2.7
Sputum eosinophils (%)*	6.0 ± 8.8	7.9 ± 7.8
Sputum neutrophils (%)	41.2 ± 24.3	34.2 ± 16.3
Sputum ECP (µg/L)	502 ± 407	337 ± 326
Sputum tryptase (µg/L)	16.5 ± 43	21.4 ± 42
Screening EAR (%)	34.7 ± 8	38.8 ± 13
Screening LAR (%)	32.4 ± 11	39.3 ± 10

FEV₁, forced expired volume in one second; FVC, forced vital capacity; PC₂₀methacholine, concentration of methacholine causing a 20% fall in FEV₁; ECP, eosinophils cationic protein. Early asthmatic response (EAR) and late asthmatic response (LAR) during screening allergen challenge. Values are expressed as mean ± SD, except for PC₂₀ methacholine, which is expressed as geometric mean ± SD. There were no significant differences between groups for pre-screening challenge values by the Mann-Whitney Rank Test, and for pre-treatment challenge values by ANCOVA using ranks and adjusting for pre-screening values. For sputum pre-treatment challenge values, see Table 2.

analysis. A third subject in the TBC group was also treated with inhaled fluticasone, but only after the post-challenge sputum induction the following day. One of the subjects treated with inhaled fluticasone before the post-treatment sputum induction had reported cough for 2–3 days just prior to the post-treatment challenge. Allergen challenge was carried out because the FEV₁ was within 10% of baseline, although blood drawn on the morning of the challenge was subsequently noted to have had an increased percentage of neutrophils, raising the possibility that he may have had a respiratory tract infection.

Nine subjects (82%) in the placebo group and 7 subjects (70%) in the TBC1269 group ($P = 0.64$ by Fisher's exact test) received the same allergen dilution as the last challenge dose in both screening and post-treatment allergen challenges. The remaining 5 subjects received a last allergen dose in the post-treatment challenge that was 1 ($n = 4$) or 2 ($n = 1$ in TBC1269 group) dilutions less concentrated than the last allergen dose received in the screening challenge.

Sputum induction

As expected, the percentage of eosinophils in induced sputum increased significantly after screening allergen challenge in both groups. In the TBC1269 group, the percentage of sputum eosinophils increased significantly from $6.5 \pm 8.8\%$ at baseline to $18.8 \pm 18.3\%$ ($P = 0.006$) after screening allergen challenge. Similarly, in the placebo group, the percentage of sputum eosinophils increased significantly from $7.9 \pm 7.8\%$ at baseline to $28.0 \pm 12.9\%$ ($P = 0.0003$) after screening allergen challenge.

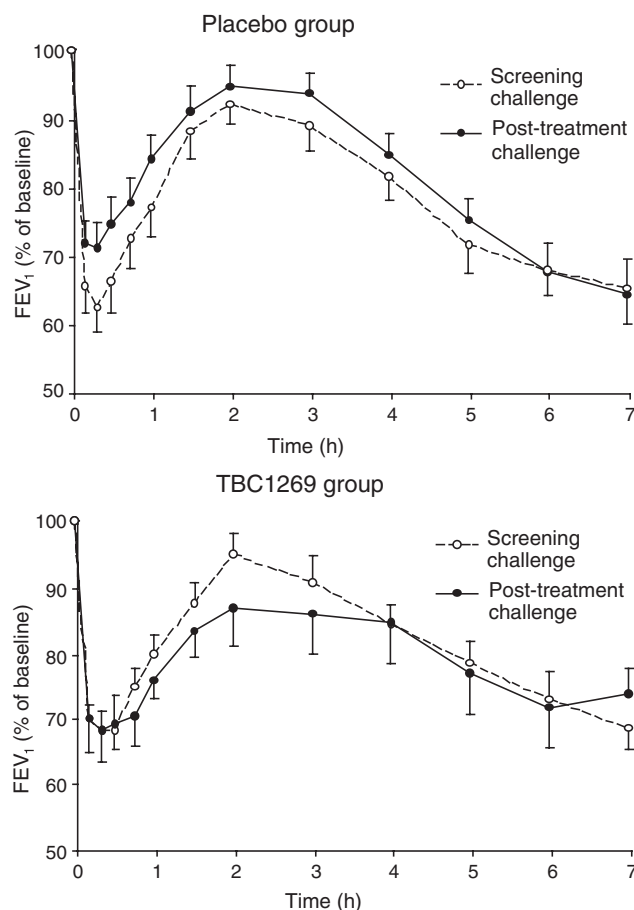


Fig. 2. Changes in FEV₁ as percent of baseline during early (EAR) and late (LAR) asthmatic responses after whole-lung inhalation allergen challenges in 21 atopic asthmatic subjects. Initially subjects underwent a screening inhalation allergen challenge to select LAR responders (Screening Challenge – dashed line). Four weeks later, subjects received either TBC1269 or placebo as a single intravenous dose prior to the allergen challenge (post-treatment challenge – continuous line). Each graph shows data from both challenges for a single group. There were no significant differences between groups in the degree of early (EAR) or late (LAR) asthmatic responses after either challenge. Similarly, there were no significant differences within groups in the degree of EAR and LAR between screening and post-treatment challenges. Values are expressed as mean and bars represent standard error.

The effect of the study drug on allergen-induced sputum eosinophil percentages is shown in Table 2 and Fig. 3. The interpretation of these data is complicated by the fact that three subjects in the active treatment group had declines in FEV₁ > 60% following the post-treatment allergen challenge. These three subjects were treated with repeated doses of bronchodilators, and two of them also received inhaled corticosteroids before sputum induction the next day. Excluding all three of these subjects, the increase in sputum % eosinophils was smaller in the TBC group than in the placebo group ($4.4 \pm 10.3\%$ vs. $15.1 \pm 9.2\%$, $P = 0.027$ by ANCOVA taking corresponding values at screening challenge as a covariate). However, excluding only those 2 subjects in the TBC group who had received inhaled corticosteroids renders the between group sputum eosinophil difference no longer statistically significant ($P = 0.06$). Further exploration of the difference between groups in sputum eosinophils following treatment also did not support a large effect of TBC1269 on

Table 2. Results of induced sputum 24 h before and 24 h after post-treatment allergen challenge in subjects with atopic asthmatic subjects treated with TBC1269 or placebo before challenge

Variable	TBC1269 group (<i>n</i> = 10)		Placebo group (<i>n</i> = 11)	
	Before	After	Before	After
Total cells ($\times 10^4/\text{mL}$)	225 $\pm$ 259	280 $\pm$ 323	80 $\pm$ 77	124 $\pm$ 141
Epithelial cells ($\times 10^4/\text{mL}$)	22.8 $\pm$ 31.0	16.3 $\pm$ 25.1	13.6 $\pm$ 9.8	18.0 $\pm$ 31.6
Epithelial cells (%)	12.2 $\pm$ 10.6	6.9 $\pm$ 6.0	23.4 $\pm$ 12.0	12.8 $\pm$ 6.7
Macrophages ($\times 10^4/\text{mL}$)	59.2 $\pm$ 53.2	36.4 $\pm$ 29.5	36.3 $\pm$ 48.0	35.8 $\pm$ 33.4
Macrophages (%)	34.4 $\pm$ 16.3	22.9 $\pm$ 14.2	42.2 $\pm$ 14.3	32.4 $\pm$ 10.6
Eosinophils ($\times 10^4/\text{mL}$)	16.7 $\pm$ 22.5	27.7 $\pm$ 39.8	5.4 $\pm$ 7.4	32.5 $\pm$ 54.7
Eosinophils (%)	8.7 $\pm$ 12.2	14.0 $\pm$ 12.6	6.8 $\pm$ 6.3	22.5 $\pm$ 10.3
Neutrophils ($\times 10^4/\text{mL}$)	122.9 $\pm$ 194.7	196.7 $\pm$ 297.0	23.5 $\pm$ 24.5	35.0 $\pm$ 32.8
Neutrophils (%)	43.7 $\pm$ 21.2	55.3 $\pm$ 25.0	26.6 $\pm$ 13.2	30.5 $\pm$ 11.0
Lymphocytes ($\times 10^4/\text{mL}$)	3.4 $\pm$ 7.6	2.7 $\pm$ 5.4	1.2 $\pm$ 1.8	2.6 $\pm$ 3.5
Lymphocytes (%)	1.0 $\pm$ 1.0	0.8 $\pm$ 0.7	1.1 $\pm$ 0.7	1.8 $\pm$ 0.9
ECP ($\mu\text{g/L}$)	562 $\pm$ 666	872 $\pm$ 788	196 $\pm$ 161	1169 $\pm$ 1070
Tryptase ($\mu\text{g/L}$)	15.3 $\pm$ 34.8	15.4 $\pm$ 34.9	25.4 $\pm$ 67.3	13.9 $\pm$ 25.3

Values are expressed as mean $\pm$ SD. There were no significant differences between groups by ANCOVA adjusted for the baseline values at the screening challenge as a covariate.

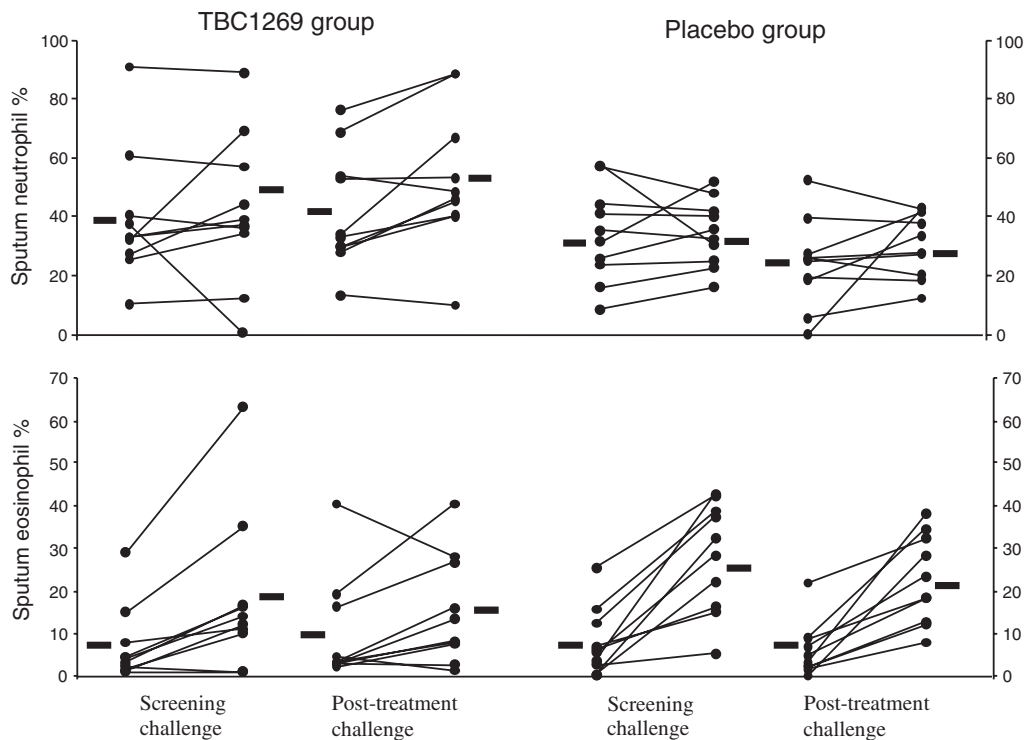


Fig. 3. Changes in percentage of neutrophils and eosinophils in induced sputum before and after whole-lung inhalation allergen challenges for TBC1269 and placebo groups. Bars represent means. Eosinophil percentage increased significantly after challenge compared with values before challenge in both groups. However, for either group, changes in percentage eosinophils or in percentage neutrophils at the post-treatment challenge were of similar magnitude as the changes at the screening challenge. In addition, there were no significant differences between groups in terms of changes of either cell type at the screening or at the post-treatment challenges.

allergen-induced changes in sputum eosinophils. For example, if no subject is excluded, after treatment there was no statistically significant difference in increases in sputum eosinophil percentage between the two groups by ANCOVA ($P = 0.157$) using values at the screening allergen challenge as a covariate and treatment group as a factor (Fig. 3). Similarly,

there were no statistically significant differences between the two groups if only post-challenge values were compared (Table 2).

After screening challenge, the percentage of sputum neutrophils in induced sputum did not change significantly in all subjects analysed together ($37.7 \pm 20.5\%$ pre-challenge

vs. $41.4\% \pm 19.6\%$ post-challenge, $P = 0.23$) or in each group separately (Fig. 3). TBC1269 treatment did not affect the percentage of neutrophils in induced sputum after allergen challenge when compared with placebo treatment (Fig. 3) regardless of inclusion or exclusion of subjects who experienced excessive bronchoconstriction at the post-treatment challenge. Similarly, TBC1269 treatment did not affect other cell types in sputum after allergen challenge when compared with placebo treatment (Table 2).

ECP concentrations in sputum increased significantly after the screening challenge in the TBC1269 group (from $502 \pm 406 \mu\text{g/L}$ before challenge to $1091 \pm 671 \mu\text{g/L}$ after challenge, $P = 0.02$) and in the placebo group (from 337 ± 326 to $973 \pm 820 \mu\text{g/L}$, $P = 0.02$). TBC1269 did not attenuate the increase in sputum ECP after the post-treatment allergen challenge when compared with placebo treatment (Table 2).

Sputum tryptase did not change significantly from 24 h before to 24 h after the screening allergen challenge (data not shown) or after the post-treatment allergen challenge (Table 2) in either group.

Complete blood count and tryptase serum concentrations

Complete blood counts with a differential count of leucocytes were obtained prior to drug infusion and at 1, 3, 7 and 24 h after the post-treatment challenge. There were no statistically significant differences in blood eosinophils and neutrophils at each of these time-points between the TBC1269 group and placebo group. Within either the TBC1269 group or the placebo group, there were no statistically significant differences between baseline values and values after drug infusion and challenge (data not shown).

Serum tryptase was measured at baseline before allergen challenge and at 1 and 7 h after challenge. There were no statistically significant differences between groups and within groups comparing screening with post-treatment values (data not shown).

TBC1269 serum concentrations were obtained prior to drug infusion and at 1, 3, 7 and 24 h after challenge. TBC1269 maximum concentration occurred at 1 h after the challenge ($376.5 \pm 100.1 \mu\text{g/mL}$) and its half-life was 3.1 ± 0.7 h. Volume distribution was $189.2 \pm 120.2 \text{ mL/kg}$ and clearance $39.9 \pm 18.5 \text{ mL/h/kg}$.

Safety data

Apart from instances of excessive bronchoconstriction during the post-treatment allergen challenge, there were no suggestions of adverse effects of TBC1269 treatment. Minor adverse events occurred in both groups as shown in Table 3. There were no significant adverse effects of intravenous TBC1269 administration in serum biochemistry (electrolytes, glucose, calcium, and renal and hepatic functions), electrocardiograms and urinalyses.

Discussion

In this double-blinded, placebo-controlled, parallel-group study, treatment with TBC1269, a pan-selectin inhibitor, did not attenuate early or late asthmatic response to whole-lung

Table 3. Adverse events in each group after treatment

Adverse event	TBC1269 (n = 10)	Placebo (n = 11)
Fatigue	0	2
Dizziness	1	2
Headache	1	3
Nausea	2	2
Rash	0	1
Bronchitis	1	0
FEV ₁ decline >50% after challenge*	4	3
FEV ₁ decline >60% after challenge*	3	0

*After the post-treatment allergen challenge.

inhalation allergen challenge, nor it did inhibit influx of inflammatory cells into the airway to a great extent. This is a disappointing result for the first clinical trial of an adhesion molecule inhibitor in human asthma, particularly because the drug was so efficacious in an animal model of lung allergic inflammation. In the sheep model of ascaris antigen-induced lung allergic inflammation, TBC1269 given at a dose 10 times smaller than that used in this clinical study inhibited early allergen response by 42% and late response by 74%. It also attenuated the increase in airway hyper-reactivity and the neutrophil lung influx after allergen challenge [14].

Several reasons could explain the discrepancy between the successful effects of TBC1269 in animal and *in vitro* studies with human leucocytes and our results showing a lack of effect of TBC1269 in asthmatic subjects. First, selectins may play a more important role in acute allergic inflammation in animal models that have been newly sensitized than in human asthma where airway allergic inflammation is long standing. Second, the dose of TBC1269 could have been insufficient to achieve the desired effect. The main pre-clinical data supporting the efficacy of TBC1269 have been its efficacy in the sheep model of asthma [14]. This model was used to guide dosing in the human studies and, following safety testing in Phase I studies, the dose chosen for the allergen challenge study reported here was 10-fold higher than the dose efficacious in sheep. The sheep model of asthma relies on sensitization and challenge to ascaris and is characterized by prominent allergen-induced airway neutrophilia. Allergen-induced eosinophilia, not neutrophilia, is prominent in human allergen challenge models. TBC1269 inhibits neutrophil adhesion more than eosinophil adhesion based on an *ex vivo* model [13]. In this model, the inhibitory effect of TBC1269 on adhesion of human eosinophils and neutrophils to P-selectin-coated plates under static and dynamic (tethering under flow) were examined. Dynamic adhesion of neutrophils to the P-selectin-coated plates was inhibited by 60% and 80% at concentrations of 100 and 1000 $\mu\text{g/mL}$ of TBC1269, respectively. In contrast, dynamic adhesion of eosinophils was only inhibited by 35% using TBC1269 1000 $\mu\text{g/mL}$, the maximum concentration tested. Although the data from this *ex-vivo* model cannot be directly extrapolated to the *in vivo* setting where all 3 selectins are present, its findings suggest that the achieved serum TBC1269 concentrations in our study (maximal of 376.5 $\mu\text{g/mL}$ at 1 h, and $\sim 94.3 \mu\text{g/mL}$ at 7 h

post-challenge) may not have been sufficient to inhibit eosinophil influx. These TBC1269 concentrations or lower may be effective in inhibiting allergen-induced neutrophilia in the sheep model of asthma.

The third reason that could explain the failure of TBC1269 to inhibit allergen airway response in asthmatic subjects was the administration of an excessive dose of TBC1269. It is conceivable that TBC1269 may not work at very high doses, although this is unlikely. Fourth, in the sheep model, the inhibitory effect of TBC1269 was more marked when it was administered by aerosol than by intravenous infusion [14]. It is thus possible that TBC1269 could have inhibited the airway response to allergen challenge if we had given it by inhalation rather than by intravenous route. Fifth, selectins may not play an essential role in the airway influx of inflammatory cells in human asthma. Indeed, integrins can mediate the selectin functions of supporting tethering and rolling of leucocytes [20, 21], and thus, compensate for the inhibition of selectin function caused by the administration of TBC1269. A recent report [22] showed that in a murine model of ovalbumin-induced lung allergic inflammation, L-selectin knockout mice had cellular lung infiltration similar to wild mice after challenge, but did not develop increased airway hyper-responsiveness. On the other hand, ICAM-1 knockout mice had attenuation of both cellular infiltration and airway hyper-responsiveness [22]. The sixth possible explanation for our findings is that eosinophils are already present in the bronchial mucosa, and may be responsible for the initial cellular influx into airway lumen after allergen challenge. Therefore, inhibiting endothelial adhesion to circulating eosinophils may not affect the initial influx of eosinophils into the airway lumen. Indeed, haematopoietic stem cells committed to eosinophil differentiation (CD34-positive cells bearing IL-5 receptor α) have been found in the bronchial mucosa of asthmatic subjects [23], and may differentiate *in situ* into eosinophils and contribute to the initial eosinophil influx into the airway lumen after challenge. Again, this mechanism may not be blocked by inhibition of selectins in the intravascular compartment. It is possible that airway eosinophilia could be inhibited if selectin inhibitors were administered repeatedly for days or weeks prior to the allergen challenge to reduce the population of eosinophils and eosinophil progenitors in the mucosa. Therefore, additional studies evaluating different doses, routes of administration and repeated dosing are necessary before TBC1269 can be definitively considered to be ineffective in attenuating allergic airway responses in asthma.

It is noteworthy that several subjects in both groups had reductions in FEV₁ >50% in the post-treatment allergen challenge (Table 3), even though most had received a dose of allergen similar to that given in the screening allergen challenge. These events demonstrate that repeated allergen challenges in asthmatic subjects can be associated with excessive bronchoconstriction. Therefore, whole-lung inhalation allergen challenges in asthmatic subjects require careful monitoring of pulmonary function and prompt availability of treatment for excessive bronchoconstriction.

In conclusion, the pan-selectin inhibitor TBC1269 administered as a single intravenous dose immediately before inhalation allergen challenge did not attenuate early- or late-phase responses in asthmatic subjects.

Acknowledgements

Texas Biotechnology Corporation, Houston, TX, supported this study.

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