Tolerability and pharmacokinetics of inhaled bimosiamose disodium in healthy males

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Aims

The aim of these first-in-human studies was to investigate the tolerability and the pharmacokinetics of bimosiamose disodium (TBC1269Z) administered by inhalation.

Methods

Two randomized, double-blind, placebo-controlled Phase I trials were performed in healthy males. In a single-dose escalating study 48 subjects received doses of 2–140 mg bimosiamose disodium by inhalation and in a multiple-dose study 32 subjects received 8–70 mg bimosiamose disodium twice daily. In both studies 4 ml of the drug solution was administered via nebulizer over 15 min. Adverse events, vital signs, ECG, clinical laboratory parameters and forced expiratory volume in 1 s (FEV₁) data were recorded and nasopharyngeal examinations were performed to address the safety and tolerability. Blood was collected for the determination of plasma concentrations of bimosiamose.

Results

All subjects completed the study. No deaths or severe adverse events occurred. Eleven mild adverse events occurred in the dose-escalation study and 34 in the multiple-dose study after inhalation of bimosiamose disodium. Adverse events were more frequent at the highest dose (140 mg) of the dose-escalation study. For placebo treatment one moderate adverse event was observed in the dose-escalation study after placebo treatment, eight mild and three moderate adverse events occurred in the multiple-dose study. Bimosiamose was detected in plasma (maximum concentration 64 ng ml⁻¹) only at doses $\geq$ 50 mg given twice daily and 105 mg once daily. For the highest dose a median value of 5746 h ng ml⁻¹ was determined for the AUC over the entire period of treatment of the multiple-dose study.

Conclusion

The results suggest that single and multiple inhalation of bimosiamose disodium up to 70 mg is well tolerated in healthy males. Systemic bioavailability after inhalation is low.

Introduction

Lung inflammation comprises a heterogeneous group of acute and chronic diseases such as acute lung injury (ALI), adult respiratory distress syndrome (ARDS), asthma or chronic obstructive pulmonary disease (COPD). One characteristic they all have in common is

the increased presence of inflammatory cells including eosinophils, neutrophils, lymphocytes and mast cells in the airways of affected subjects [1]. Both onset and disease progression are, at least in part, associated with excessive recruitment of leucocytes from the circulation into the lung tissue which is mediated by cell adhesion

molecules [2]. Therefore, new agents such as cell adhesion antagonists are under development to prevent transmigration of leucocytes into the lung [3–9].

The selectin family of cell adhesion molecules is composed of three structurally related calcium-dependent carbohydrate binding proteins, E-, P- and L-selectin. The selectins mediate the initial rolling or 'tethering' of leucocytes on the vascular endothelium following inflammation or injury in most mammalian vascular systems [10]. As the process of leucocyte rolling is generally considered to be the primary event in the inflammatory response, selectins constitute a target for therapeutic intervention in the modulation of inflammation. However, based on the functional redundancy, especially between E- and P-selectin, in inflammatory disorders, pan-selectin antagonism targeted against all three selectins has been suggested as a therapeutic strategy [5, 11].

The pan-selectin antagonist bimosiamose (1,6-bis[3-(3-carboxymethylphenyl)-4-(2- α -D-mannopyranosyloxy)phenyl]hexane, Figure 1), a synthetic rationally designed drug [12], is the most advanced synthetic selectin antagonist in clinical development including asthma [9]. *In vitro* studies under static and dynamic flow conditions have demonstrated that this compound is able to block adhesion of leucocytes including neutrophils, eosinophils and lymphoid cells [13]. Consistent with these *in vitro* results, bimosiamose has demonstrated therapeutic efficacy in numerous *in vivo* disease models of acute and chronic inflammation, by prevention of influx of leucocytes into inflamed tissue [14–18]. In this context, an animal study using a sheep model of allergic inflammation suggested that inhalation of bimosiamose disodium is a viable route for therapeutic administration [15]. However, no observed effect levels (NOEL) of 1 mg kg⁻¹ day⁻¹ could be established in rats or of 40 mg kg⁻¹ day⁻¹ in beagle dogs after 28 days of inhalation (Inveresk report 19649, TBC1269Z: 4-week

inhalation toxicity study in rats, Tranent, Scotland, 2001; Inveresk report 19009, TBC1269Z: 4-week inhalation toxicity study in dogs, Tranent, Scotland, 2001; further details can be obtained from the authors).

In humans, the clinical effects and pharmacokinetics of bimosiamose disodium after intravenous administration have been described recently [19]. In addition, bimosiamose disodium is effective in attenuating the late asthmatic response in mild allergic asthmatic patients [20]. The aim of this first-in-human study was to describe the clinical effects and pharmacokinetics of inhaled bimosiamose disodium. To this end, we performed a dose-escalation study (Ia) followed by a multiple-dose study (Ib) with inhaled bimosiamose disodium in healthy male subjects. The clinical effects of the drug were monitored by ECG, vital signs, clinical laboratory data, forced expiratory volume in 1 s (FEV₁), nasopharyngeal inspection and physical examination.

Materials and methods

Study design

The trials were conducted in accordance with the current Declaration of Helsinki, current German drug law and the International Conference on Harmonization-Good Clinical Practice regulations. The studies were performed at 3C Clinical Research (now Parexel International GmbH, Hennigsdorf, Germany). The protocols were reviewed and approved by the Independent Ethics Committee of the Brandenburg Medical Council. All subjects participating in the studies gave written informed consent prior to screening.

Trial Ia was a randomized, double-blind, placebo-controlled dose-escalation study consisting of single inhalations of 2, 4, 8, 16, 35, 70, 105 and 140 mg bimosiamose disodium or placebo. Forty-eight healthy males between 22 and 54 years of age were enrolled and all subjects completed the study. The subjects were randomized into eight groups of six, of which five received the active substance and one received a placebo. Their mean ($\pm$ SD) age was 35 $\pm$ 9 years, their height was 180 $\pm$ 7 cm, their weight was 79 $\pm$ 11 kg and their body mass index (BMI) was 24.4 $\pm$ 2.6 kg m⁻².

Blood sampling was performed predose and at 0.25, 0.5, 1, 4, 8 and 24 h after dosing in trial Ia. Twelve-lead ECGs were recorded prestudy, predose and at 1, 4, 24 h after dosing and post study. Spirometry was performed prestudy, predose and at 20 and 45 min postdose, and the nasopharyngeal inspection was carried out predose and at 1 and 24 h postdose. Subjects were admitted to the unit 12 h before dosing and discharged after the 24-h postdose assessments.

Trial Ib had a randomized, double-blind, placebo-

Figure 1

The structure of bimosiamose

controlled design consisting of multiple-dose administration of 8, 35, 50 and 70 mg bimosiamose disodium or placebo twice daily over 7 days. Thirty-two healthy male volunteers between 21 and 54 years of age participated in this study. The participants were divided in four groups of eight individuals, of whom six received the active substance and two received placebo. Their mean ($\pm$ SD) age was 38 ± 10 years, their height was 181 ± 6 cm, their weight was 81 ± 11 kg and their BMI was 24.9 ± 2.6 kg m⁻².

In trial Ib blood samples were taken predose and at 0.25, 0.5, 1, 4, 8 and 12 h after the morning dose on days 1 and 7. On days 2–6 premorning dose samples were taken, together with a 24-h postdose sample which had been taken on day 8. Twelve-lead ECGs were recorded prestudy, predose, at 1 h post dose, at 24 h after dosing on day 7 and poststudy. Spirometry was performed prestudy, predose, at 20 and 45–60 min post dose and at 24 h postdosing on day 7. The nasopharyngeal inspection was carried out prestudy, predose, at 15 min post morning dose on days 2–7 and on day 8. The subjects were admitted to the study unit on the day prior to the first administration and discharged after their assessment on day 8.

Drug formulation

Bimosiamose disodium (Inveresk Research, Tranent, UK) was used as an aqueous solution for inhalation containing 100 mg ml⁻¹ in 5-ml ampoules. Four millilitres of an appropriately diluted solution with 0.9% sodium chloride was administered by nebulizer (Pari LC Star; Pari GmbH, Starnberg, Germany) over 15 min.

Analytical methods

A validated LC-ESI-MSMS method was applied to determine the concentrations of bimosiamose in human plasma [21]. Analyses were performed using a Finnigan MAT TSQ 7000 mass spectrometer interfaced with a JASCO high-performance liquid chromatographic system. Samples were loaded onto an YMC-Pack Phenyl, 150 $\times$ 2.1 mm, 5- μ m, 120-Å column, fitted with a YMC-Pack Phenyl, 10 mm $\times$ 4.6 mm guard column. Chromatography was performed at ambient temperature using an isocratic mobile phase composed of 0.1% formic acid/acetonitrile (60/40, v/v) delivered at 0.2 ml min⁻¹ with 100 μ l min⁻¹ into the ESI interface. The injection volume was 20 μ l. Negative ionization was performed with a capillary temperature of 250 °C and a sheath gas pressure of 345 kPa. The collision gas was argon with a pressure of 2 mTorr, used with an offset voltage of 45 V for the analyte bimosiamose and 35 V for the internal standard. The spray voltage was 4.5 kV. The molecular

ions were measured by selected reaction monitoring at m/z 861.2 $\rightarrow$ 449 for bimosiamose and m/z 404.8 $\rightarrow$ 185 for the internal standard. The lower limit of quantification (LOQ) was 30 ng ml⁻¹. The accuracy at the LOQ was 114% and the precision 11.8%.

Statistical analysis

Statistical analysis was performed using SAS software (SAS Institute, Inc., Cary, NC, USA) and program R for statistical computing integrated into the Revotar statistics and computational chemistry environment [22, 23]. For the demographic and safety data descriptive statistics were calculated. Pharmacokinetic data were summarized by descriptive statistics for selected subjects, as plasma concentrations of bimosiamose were detectable only at the highest concentrations in both studies. The AUC over the entire period of treatment was determined in the multiple-dose study.

Results

No deaths or serious adverse events were observed in either treatment group. All subjects recovered completely from the adverse events. In the single-dose escalation study, a total of 12 adverse events was reported in 11 out of 48 subjects (Table 1). All were of a mild (11 of 12 reports) or moderate (one report, placebo) intensity. Adverse events occurred most frequently at the highest dose (140 mg) (in four of 12 subjects). A decrease in FEV₁ to < 80% was recorded in three subjects, one after receiving 105 mg and two after receiving 140 mg bimosiamose disodium. The single administration of up to 70 mg bimosiamose disodium was indistinguishable from placebo with regard to FEV₁ or adverse events (Figure 2).

In the multiple dose study, a total of 45 adverse events was reported in 21 out of 32 subjects (Table 2). All were of a mild (42 reports) or moderate (three reports, placebo only) intensity and did not appear to be dose dependent.

There were no significant changes in ECG over time, or between dosing groups in both studies. Changes were observed in the QTc interval (Bazett); however, there were no values >500 ms in study Ia and 430 ms in study Ib, and no increase from baseline of >60 ms. Analysis of mean and individual QTc intervals revealed no abnormalities. Inhalation of up to 70 mg bimosiamose disodium was well tolerated after single- and multiple-dose administration in all the subjects.

Plasma concentrations of bimosiamose following single inhalation (Ia) were measurable only after the 105-mg (three subjects, five blood samples) and 140-mg doses (four subjects, 10 blood samples) (Table 3). All

Table 1

Summary of adverse events as a function of treatment and Medical Dictionary for Regulatory Activities (MedDRA) definition after single-dose administration of bimosiamose disodium by inhalation

Treatment	MedDRA definition	Severity of event		
		Mild	Moderate	Severe
Placebo (105-mg group)*	Pathological dislocation of ankle and foot joint	0	1	0
2 mg	Influenza-like symptoms	1	0	0
	Loose stools	1	0	0
4 mg	Vascular inflammation	1	0	0
8 mg	Headache	1	0	0
	Sore throat	1	0	0
16 mg	Headache	1	0	0
105 mg	Herpes zoster dermatitis	1	0	0
140 mg	Sore throat	1	0	0
	Dyspnoea	1	0	0
	Headache	1	0	0
	Increased salivation	1	0	0
Total		11	1	0

*The subject was in the 105-mg group and received placebo.

Figure 2

Changes of forced expiratory volume in 1 s (FEV_1) after inhalation in relation to dose. Error bars indicate SEM values. 20 min. post inhalation (●), 45 min. post inhalation (□)

plasma concentrations after single doses of up to 70 mg were below the LOQ of 30 ng ml⁻¹, and at higher doses no plasma concentrations higher than twice LOQ were found. The maximum concentrations (C_{max}) were in the range 37.4–58.0 ng ml⁻¹ and the times (t_{max}) corresponding to C_{max} ranged between 0.5 and 24 h.

In the multiple-dose study plasma concentrations above the LOQ were detected after the two highest doses. Measurable plasma concentrations were detected after inhalation of 50 mg in three of six subjects and after inhalation of 70 mg in four of six subjects (Table 3). For the highest dose a median value of 5746 h ng ml⁻¹ (range 3684–7378 h ng ml⁻¹) was obtained for the AUC over the entire period of treatment. C_{max} had a median value of 51.6 ng ml⁻¹ (range 45.1–64.4 ng ml⁻¹) at $t_{max} = 0.5$ h. The maximum plasma concentrations were achieved on day 7, except for one subject. No plasma bimosiamose concentration exceeding the LOQ was detected on day 1.

Discussion

Our findings indicate that single and multiple inhalations of bimosiamose of up to 70 mg via nebulizer were well tolerated. In the dose-escalating study, adverse events occurred more frequently only at the highest dose of 140 mg bimosiamose disodium compared with lower doses. Whereas a decrease in $FEV_1 < 80\%$ of the predicted value was recorded in three subjects after single inhalation of 105 and 140 mg bimosiamose disodium, the results of the multiple-dose study showed no trend for a decrease in FEV_1 with increasing dose levels (up to 70 mg twice daily). Consistent with this observation, no significant decrease in FEV_1 or peak flow rates was observed in a recent study using an aller-

Table 2

Summary of adverse events as function of treatment and the Medical Dictionary for Regulatory Activities (MedDRA) definition after multiple-dose administration of bimosiamose disodium by inhalation

Treatment	MedDRA definition	Severity of event		
		Mild	Moderate	Severe
Placebo (8-mg group)	Rhinitis	2	0	0
	Headache	1	0	0
	Coated tongue	1	0	0
Placebo (35-mg group)	Rhinitis	1	0	0
Placebo (70-mg group)	Erythematous rash	0	1	0
	Papular rash	1	0	0
	Sore throat	1	0	0
8 mg	Dizziness	0	1	0
	Palpitations	1	0	0
	Chest pain	0	1	0
	Headache	2	0	0
	Rhinitis	1	0	0
	Sore throat	2	0	0
	Coated tongue	1	0	0
	Redness of external ear, swelling of the ear	1	0	0
	Flatulence	2	0	0
	Rhinitis	3	0	0
35 mg	Headache	1	0	0
	Injection site pain	1	0	0
	Increased salivation	5	0	0
	Sore throat	1	0	0
	Headache	1	0	0
50 mg	Flatulence	2	0	0
	Heartburn	1	0	0
	Loose stools	1	0	0
70 mg	Subconjunctival haemorrhage	1	0	0
	Coated tongue	3	0	0
	Dry skin	1	0	0
	Headache	2	0	0
	Dry mouth	1	0	0
	Neck pain	1	0	0
	Total	42	3	0

gen challenge model in mild asthmatics after multiple inhalation of 70 mg of bimosiamose disodium twice daily [20]. An alternative explanation for the observed decline in FEV₁ after inhalation of 105 or 140 mg bimosiamose disodium is the presence of hidden airway hyperresponsiveness, which can occasionally be found in asymptomatic atopy as well as in healthy subjects [24, 25]. Nevertheless, inhalation of doses of >70 mg bimosiamose disodium should be monitored cautiously, particularly in patients with known airway hyperresponsiveness.

In the previously mentioned study in steroid-naïve asthmatic patients, a temporary shift occurred in

peripheral blood differentials, with increased numbers of lymphocytes and decreased neutrophils at 8 h after allergen challenge [20]. Furthermore, subcutaneous administration of an anti-CD11a monoclonal antibody (efalizumab) in the same model resulted in an increase in blood lymphocytes [6]. However, in the present studies no tendency for a dose-dependent effect of bimosiamose disodium was seen with respect to white blood cells or differential count in healthy males. This suggests that the observed effects could be restricted to the allergen challenge model, where the acute antigenic stimulus initiates a dynamic shift in peripheral blood leucocytes.

Table 3

Maximum plasma concentrations ($C_{\max}$), area under the plasma concentration curve (AUC) and time $t_{\max}$ after single and multiple bimosiamose disodium inhalation

Subject*	Nebulized dose (mg)	$N_{\text{LOQ}}^{\dagger}$	$C_{\max}$ (ng ml ⁻¹)	AUC (h ng ml ⁻¹)	$t_{\max}$
37	105	2	47.5	–	0.5 h
38	105	1	58.0	–	8 h
40	105	2	37.4	–	8 h
43	140	2	58.0	–	0.5 h
44	140	2	51.4	–	0.5 h; 1 h
47	140	2	52.8	–	0.5 h
48	140	4	49.8	952	24 h
122	50	4	35.6	3218	D 7, 1 h
123	50	1	32.5	–	D 7, 1 h
124	50	1	38.2	–	D 7, 1 h
125	70	12	64.4	7378	D 7, 0.5 h
128	70	11	50.5	6842	D 7, 0.5 h
131	70	11	52.6	4649	D 7, 8 h
132	70	9	45.1	3684	D4, predose

*Single dose: subjects 37–48; multiple dose twice daily over 1 week: subjects 122–132. $\dagger N_{\text{LOQ}}$: number of samples with plasma concentrations above the limit of quantification (LOQ) of 30 ng ml⁻¹. Single inhalation: three of five subjects at 105- and four of five subjects at 140-mg dose had plasma concentrations above LOQ. Multiple inhalation: three of six subjects at 50- and four of six subjects at 70-mg bimosiamose had plasma concentrations above LOQ.

Bimosiamose was detected in plasma after single and multiple inhalations of bimosiamose disodium. Plasma concentrations exceeding the LOQ of the assay were seen after single inhalation of at least 105 mg and after repeated inhalation of 50 mg given twice daily. Plasma bimosiamose concentration did not exceed the LOQ on day 1 of the multiple study. This result is in agreement with the observation in the dose-escalation study that single bimosiamose disodium doses exceeding 70 mg are required to obtain detectable plasma concentrations.

The low plasma concentrations of bimosiamose detected after its inhalation do not enable us to draw the conclusion that the drug reached the blood via the air-blood barrier of the lung. In the present studies, we used a breath-enhanced PARI LC Star jet nebulizer for inhalation. It generates a respiratory fraction of between 77 and 82%, which has been able to give an appropriate degree of pulmonary drug deposition [26–29]. However, deposition in the lung after inhalation via a nebulizer is less predictable than via a dry powder inhaler, and the breathing pattern of the patient influences nebulizer output and drug delivery [30, 31]. These findings, taken together with the observation that the oral bioavailability of bimosiamose is very low in the rat, do not allow us to distinguish between the fraction of bimosiamose that

reaches the systemic circulation via the lung and that via the gut.

The predose concentrations of approximately 40 ng ml⁻¹ measured after day 2 indicate that a relatively stable steady-state plasma concentration at the end of a dosing interval may be achieved by twice daily inhalation. Furthermore, the peak/trough concentration ratio is relatively small. In contrast to the low systemic exposure observed in the present inhalation study, a much higher $C_{\max}$ value (7.1 µg ml⁻¹) was achieved after a 15-min intravenous infusion of 1 mg kg⁻¹ bimosiamose disodium in healthy subjects [19]. The $\text{AUC}_{0-\infty} = 1360 \text{ h } \mu\text{g ml}^{-1}$ determined for the maximum single intravenous dose of 30 mg kg⁻¹ [19] exceeds the total AUC obtained for multiple inhalations over 1 week (present study) by several orders of magnitude. The low plasma concentrations found after inhalation of bimosiamose disodium do not permit a reliable estimation of AUC after a single inhalation. Thus, only a very crude estimate, of the order of a few percent at most, can be obtained for the upper limit of the apparent systemic bioavailability of the drug.

In conclusion, plasma concentration data for the pan-selectin antagonist bimosiamose disodium demonstrated that the drug has a very low apparent bioavail-

ability after inhalation. Concentrations were below the LOQ after inhalation (30 ng ml⁻¹) of up to 70 mg once or 35 mg twice daily, indicating limited systemic exposure. Bimosiamose was well tolerated at doses of up to 70 mg twice daily and <105 mg once daily.

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