AstraZeneca

Clinical Study Report Synopsis

Drug Substance	AZD7624
Study Code	D2550C00004
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A Double-blind, Randomised, Placebo-controlled, 2-period, Cross-over Study in Healthy Volunteers to Investigate the Effects of a Single Dose of Inhaled AZD7624 on White Blood Cells and Inflammatory Markers in Induced Sputum and Blood after Oral Inhalation of 45,000 Endotoxin Units Lipopolysaccharide (LPS)

Study dates: First subject enrolled: 18 October 2013
Last subject last visit: 29 April 2014

Phase of development: Clinical pharmacology (I)

This study was performed in compliance with Good Clinical Practice, including the archiving of essential documents.

Publications

None at the time of writing this report.

Objectives and criteria for evaluation

Table S1 Objectives and outcome variables

Objective			Outcome Variable
Priority	**Type**	**Description**	**Description**
Primary	Proof of pharmacology biomarker	To investigate the effect of a single dose of inhaled AZD7624 on neutrophils in induced sputum after oral inhalation of LPS, compared to placebo	Change in sputum neutrophil % differential from pre-LPS challenge to 6 hours after LPS challenge Change in total neutrophils per gram of sputum from pre-LPS challenge to 6 hours after LPS challenge was evaluated as a supportive endpoint
Secondary	Proof of pharmacology biomarker	To investigate the effect of a single dose of inhaled AZD7624 on TNF-α concentration in induced sputum after oral inhalation of LPS compared to placebo	The effect of a single dose of inhaled AZD7624 on levels of TNF-α in induced sputum after oral inhalation of LPS was evaluated compared to placebo
Secondary	Safety	To assess the safety and tolerability after single dose administration of inhaled AZD7624	AEs, safety laboratory parameters (AZD7624-specific monitoring of CK and myoglobin), vital signs, 12-lead ECG, body temperature and forced expiratory volume in 1 second (FEV_1)
Exploratory	PK	To measure exposure after single dose administration of inhaled AZD7624	Plasma observed maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), and area under the plasma concentration-time curve from time zero to t hours postdose ($AUC_{(0-t)}$)

Objective			Outcome Variable
Priority	**Type**	**Description**	**Description**
Exploratory	Biomarker	To evaluate the effect of a single dose of AZD7624 on additional inflammatory biomarkers in induced sputum and blood	The effect of a single delivered dose of 2053 µg of AZD7624 (corresponding to 1191 µg lung deposited dose) on additional inflammatory biomarkers in blood (IL-6, MIP-1β, CRP and neutrophils) and induced sputum (leucocytes, macrophages/monocytes, lymphocytes, eosinophils, pHSP-27 [is not a part of this CSR], IL-6, IL-8, and MIP-1β)

AE Adverse event; $AUC_{(0-t)}$ Area under the plasma concentration-time curve from time zero to t hours postdose; CK Creatine kinase; C_{max} Observed maximum plasma concentration; CRP C-reactive protein; CSR Clinical Study Report; ECG Electrocardiogram; FEV_1 Forced expiratory volume in the first second; IL Interleukin; LPS Lipopolysaccharide; MIP Macrophage inflammatory protein; pHSP Phosphorylated heat shock protein; t_{max} Time to reach maximum plasma concentration; TNF-α Tumour necrosis factor-alpha

Study design

This was a double-blind, randomised, placebo-controlled, 2-way cross-over study to investigate the effects of a single dose of inhaled AZD7624 on white blood cells and inflammatory markers in induced sputum and blood after oral inhalation of lipopolysaccharide (LPS) as well as the safety, tolerability and PK of AZD7624 following a single inhaled dose.

Healthy male and female volunteers of non-childbearing potential aged 18 to 55 years were to be screened (Visit 1) within 28 days before the first administration of the investigational product, followed by a second pre-entry visit (Visit 2) for sputum induction and methacholine challenge 7 to 14 days before Visit 3.

If sputum quality acceptance criteria for baseline sputum sampling were not met at Visit 2, the sputum induction was to be repeated and a maximum of 2 additional sputum inductions was to be conducted prior to Visit 3. A total of 30 volunteers were randomised at Visit 2 to one of the 2 below treatment groups (ie, sequences) in a 1:1 ratio.

- Group 1: AZD7624 (Visit 3) followed by placebo (Visit 5) after a washout period of ≥28 days
- Group 2: Placebo (Visit 3) followed by AZD7624 (Visit 5) after a washout period of ≥28 days

At Visit 3 volunteers were admitted to the study centre the day before the administration of the investigational product (Day -1) and remained at the study centre for at least 24 hours after the administration of the investigational product. A single dose of AZD7624 or placebo using

SPIRA was administered at Visit 3. Lipopolysaccharide challenge took place 0.5 hours after administration of the investigational product, followed by sputum induction 6.5 hours after administration of the investigational product. For the LPS challenge 45,000 endotoxin units (EU) of LPS was delivered by a breath activated Mefar dosimeter. Volunteers returned to the study centre on Day 4 and Day 7 after administration of the investigational product at Visit 3 to provide a blood sample for monitoring of selected safety variables.

There was at least a 28-day wash-out period between Visit 3 and Visit 5.

At Visit 4 (4 to 7 days before Visit 5), baseline sputum for the Treatment Period 2 was collected. If induced sputum at Visit 4 did not fulfil the quality criteria (ie, if sputum total, neutrophil and macrophage counts in the sputum sample at this visit was not within the range of –80% to +100% of those measured at first baseline at Visit 2), a maximum of a further 2 sputum inductions was to be made prior to Visit 5 (after which the volunteer was discontinued if quality criteria was not achieved). The second baseline sputum quality criteria were additional to the acceptance criteria for first baseline sputum sampling.

At Visit 5 volunteers were admitted to the study centre the day before the administration of the investigational product (Day -1) and remained at the study centre for at least 24 hours after the administration of the investigational product. A single dose of AZD7624 or placebo using SPIRA was administered at Visit 5. Volunteers who received AZD7624 at Visit 3 received placebo at Visit 5 and vice versa. Lipopolysaccharide challenge took place 0.5 hours after administration of the investigational product, followed by sputum induction 6.5 hours after administration of the investigational product. For the LPS challenge 45,000 EU of LPS was delivered by a breath activated Mefar dosimeter.

Volunteers returned to the study centre on Day 4 of Visit 5 after administration of the investigational product to provide a blood sample for monitoring of selected safety variables.

Visit 6 (Follow-up Visit) took place 7 days after Visit 5.

Target subject population and sample size

Up to 30 healthy male and female volunteers (of non-childbearing potential) aged 18 to 55 years (inclusive) who had a body mass index between 18 and 30 kg/m^2 (inclusive) and weighed at least 50 kg and no more than 100 kg.

A total of 30 healthy male volunteers were randomised, of which 21 male volunteers completed the study and treatment.

Investigational product and comparator(s): dosage, mode of administration and batch numbers

Table S2 Details of investigational product

Investigational product	Dosage form and strength	Manufacturer	Batch number
AZD7624	Nebuliser solution; 20 mg/mL	AstraZeneca	13-000896AZ
AZD7624 placebo	Nebuliser solution	AstraZeneca	13-000895AZ
LPS from *Escherichia coli*	Lyophilised powder; 5.10 mg/mL	Sigma-Aldrich	043M4033V

All the volunteers were randomised to receive either AZD7624 or matching placebo in Treatment Period 1. Volunteers who received AZD7624 in Treatment Period 1 received matching placebo in Treatment Period 2 and vice versa.

Duration of treatment

Each volunteer received one dose of AZD7624 nebuliser solution 20 mg/mL and one dose of placebo nebuliser solution separated by a washout period of ≥28 days.

Statistical methods

Descriptive statistics were provided by treatment for observed and change-from-baseline values of neutrophil % differential at baseline and each post-baseline assessment time.

Analyses of neutrophil % differential was performed by fitting an analysis of covariance model for change in neutrophil % differential with fixed effects for treatment, period, sequence, volunteer as random, and neutrophil % differential period specific baseline as a continuous covariate. The estimated least-squares (LS) treatment means from the fitted model, together with corresponding 95% confidence interval (CI) (2-sided) were presented. Also, the estimated LS mean treatment difference (AZD7624 treatment versus placebo) was presented together with corresponding 90% CI (2-sided) and p-value.

Similar analyses were performed for the supportive endpoint of change in total neutrophil count.

For the exploratory objective (tumour necrosis factor-alpha [TNF-α] induced LPS response), similar analyses were performed as for the primary endpoint, by fitting an analysis of covariance model for change from baseline in TNF-α at 6 hours post LPS challenge with fixed effects for treatment, period, sequence, and TNF-α period specific baseline as a continuous covariate.

For the secondary exploratory objective (LPS response in exploratory biomarkers), descriptive statistics were provided by treatment for observed and change-from-baseline values at baseline and each post LPS challenge assessment time point.

No formal statistical hypothesis testing were performed for PK and safety and tolerability. All safety, tolerability, and pharmacokinetic data recorded during the study were listed and summarised using descriptive statistics as appropriate.

Subject population

Of the 30 male volunteers randomised in this study, 21 volunteers completed the study and the treatment, while 9 volunteers discontinued from the study. None of the volunteers were excluded from the safety analysis set. The reasons for discontinuation is presented below,

- Volunteer E0001180 discontinued due to an AE of pyrexia
- Volunteer E0001003 withdrew consent
- Volunteer E0001148 was discontinued as per the physician's decision as the volunteer had an elective knee surgery
- Volunteers E0001066, E0001071, E0001088, E0001138 and E0001168 were discontinued as they failed Treatment Period 2 baseline sputum assessment
- Volunteer E0001197 was discontinued as the postdose sputum sample produced in Treatment Period 1 was insufficient

All the volunteers in this study were males with a mean age of 32 years. Most of the volunteers were non-Hispanic whites. All the volunteers had a BMI within the protocol-specified range of 19 kg/m^2 to 30 kg/m^2.

Summary of pharmacokinetic results

The mean plasma concentration profile was characterised by rapid absorption (about 5 minutes after start of inhalation), and a decline with rapid distribution phase followed by a slower elimination phase.

Only key exposure parameters were evaluated in this study, as PK sampling interval was limited (0 to 24 hours postdose) and the pharmacokinetic profile of AZD7624 has been well characterised in previous studies.

The maximum AZD7624 plasma concentration occurred at a median time of 6 minutes (range 4.8 to 15 minutes) coinciding with completion of inhalation dosing. The estimated exposure parameters (Area under the plasma concentration-time curve [AUC] and observed maximum plasma concentration [C_{max}]) after a single inhaled dose of AZD7624 were in the range of previously observed results reported in the multiple ascending dose study (D2550C00002) with similar dose administration.

Summary of primary and secondary pharmacodynamic results

As expected, oral inhalation of 45000 endotoxin units (LPS) caused a neutrophilic inflammatory response in the lungs in healthy volunteers. The mean results exhibited an increase in primary (neutrophils) and secondary (TNF-α) PD biomarkers in induced sputum at 6 hours post LPS challenge in both active (AZD7624) and placebo-treated volunteers. However, pretreatment with AZD7624 one-half hour prior to LPS challenge attenuated this inflammatory response significantly ($p<0.0001$ for neutrophil %differential, $p=0.0006$ for total neutrophil, and $p=0.0009$ for TNF-α in induced sputum) compared to placebo pretreatment. Graphical presentation of the primary PD biomarkers indicated these primary variables were not impacted by treatment and period effect in this crossover study.

Summary of exploratory pharmacodynamic results in sputum

Exploratory PD biomarkers including the cytokines (interleukin [IL]-6 and IL-8), macrophage inflammatory protein (MIP)-1β, and total cell count exhibited an increase in induced sputum at 6 hours post LPS challenge in both AZD7624 and placebo cohorts. However, the observed inflammatory response was substantially attenuated following 0.5 hour pretreatment with a single inhaled dose of 2053 µg of AZD7624. Changes in differential counts (ie % differential for eosinophils, and lymphocytes) and total macrophages count (which remained unchanged) were minimal between the active and placebo treatments.

Change-from-baseline results were consistent with the observed data outcome for both active and placebo treatments.

Summary of exploratory pharmacodynamic results in blood

Both observed and change-from-baseline results showed systemic increases in exploratory biomarkers (C-reactive protein [CRP], IL-6, and MIP-1β) following the LPS challenge. These systemic inflammatory biomarkers were blocked by a single inhalation dose of AZD7624 administered at 0.5 hour prior to endotoxin administration. Neutrophil % differential also exhibited elevation in blood circulation at 6 hours and 12 hours postdose with a return to baseline within 24 hours. This inflammatory response was consistently attenuated by a single dose of AZD7624.

Summary of safety results

- There were no deaths, SAEs or OAEs reported in this study
- Volunteer E0001180 had an AE of severe pyrexia which led to the discontinuation of the investigational product as well as from the study
- Overall 13 (43.3%) of the 30 volunteers reported at least one AE. A total of 6 (22.2%) of the 27 volunteers who received AZD7624 reported at least 1 AE, whilst, 9 (37.5%) of the 24 volunteers who received placebo reported at least 1 AE
- The most commonly reported AE belonged to the SOC respiratory, thoracic and mediastinal disorders (5/30; 16.7%) and the PT upper respiratory tract infection

(3/30; 10.0%). However none of the events of upper respiratory tract infection was considered to be related to the investigational product. A total of 6 volunteers reported AEs considered causally related to the investigational product. Most of the AEs reported were considered to be of mild intensity by the Investigator

- Variation, with no trends over time was observed in mean and median laboratory results, except for neutrophil count and neutrophils percentage. In volunteers receiving both AZD7624 and placebo, the mean neutrophil count and neutrophils percentage showed an increase from baseline to12 hours postdose on Day 1 and then decreased to almost baseline value on Day 7, 144 hours postdose. With the exception of the neutrophil count, and neutrophil percentage, none of the laboratory results were considered to be clinically significant changes. None of the volunteers had abnormal myoglobin or creatine kinase values. Although abnormal urinalysis results were reported, none of these values were considered to be clinically significant by the Investigator

- Variation, with no trends over time was observed in mean and median vital signs measurements. Abnormal 12-lead electrocardiogram readings were reported for healthy volunteers, but none were considered clinically significant by the Investigator

- Volunteer E0001051 (placebo) had an erythematous patchy macular rash on torso-level of the 5^{th} intercostal space down to the umbilicus reported on Day 2, and was reported as an AE (see CSR Appendix 12.2.10.3). No other abnormal physical examination findings were reported in the study. No abnormal spirometry readings were reported in the study