Definitive Protocol

Study Title	AZD5501 and AR-C154669XX: Effects on General Activity and Behaviour in the Mouse Following Oral Administration
Study Director	Sharon Rowton, BSc (Hons)
Sponsor	AstraZeneca UK Ltd Safety Assessment UK Mereside, Alderley Park Macclesfield, Cheshire, SK10 4TG
Sponsor's Study Monitor	Alan Robinson BSc (Hons)
Test Facility	Covance Laboratories Ltd Otley Road, Harrogate North Yorkshire, HG3 1PY
Test Sites	AstraZeneca UK Ltd Safety Assessment UK Mereside, Alderley Park Macclesfield, Cheshire, SK10 4TG AstraZeneca UK Ltd R&D Charnwood Development Drug Metabolism and Bioanalysis Bakewell Road, Loughborough Leicestershire, LE11 5RH
Covance Study Number	0088/385
Sponsor's Study Reference Number	TSM1268
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The content of this protocol represents our interpretation of the study objectives and the requirements of the regulatory guidelines.

INTRODUCTION

Objective

The objective of this study is to determine the effects of AZD5501 and AR-C154669XX on general activity and behaviour in the mouse. This test will be conducted by administering the test articles to mice and then observing them for general behavioural, autonomic and motor effects based on Irwin's method (Irwin, 1968). Comparisons will be made with a vehicle control group. A chlorpromazine hydrochloride-treated group will also be included as a positive control.

Justification

The Irwin screen will provide useful information on potential adverse effects of the drug under test on general physiological and behavioural functions. The test will be conducted in the mouse, which is a frequently used species in such studies and for which background data are available. The test articles will be administered orally as this is the intended clinical route. The dose levels employed in this study have been agreed by the Study Director and Sponsor. The dose levels selected are 3, 30 and 300 mg/kg and encompass up to 100 times the anticipated therapeutic dose. The test articles are intended as a therapy for asthma and rhinitis. The pharmacological class/activity is defined as dual cytokine CCR3 receptor and histamine H1 receptor antagonist.

TEST ARTICLES, REFERENCE COMPOUND AND VEHICLE

Description, identification and storage

The test articles are identified as AZD5501 and AR-C154669XX.

The Sponsor will provide as much information as possible on the physical appearance, known hazardous properties, purity, stability, a date of expiry and Certificates of Analysis.

When not in use the test articles will be stored in sealed containers, at room temperature (nominally 10 to 30°C) and protected from light.

The second sample will be retained at Covance as back-up samples and kept frozen (nominally -20°C) until notification from the Sponsor that they are not required or until issue of the final report.

The results of the formulation analysis will be issued in a Principal Investigator's contributory report, including a Principal Investigator's GLP statement and QA statement, and will be included in the final report as an Appendix.

TEST SYSTEM

Species, strain and supplier

An adequate number of male Crl:CD-1 (ICR) BR mice will be obtained from Charles River (UK) Ltd., Margate, Kent.

Specification

The mice will be approximately 4 weeks of age and weigh between 18 and 22 g on arrival. The age and weight of the animals on their respective day of dosing will be documented in the raw data and final report. The mice will be approximately 5 weeks of age on the day of dosing.

Environment

The animals will be housed in groups of up to five in polypropylene cages (33 x 15 x 13 cm) with solid floors and Grade 10 woodflakes (Datesand Ltd., Cheshire, UK) as bedding. The cages will be cleaned and dried before use. Aspen chew blocks will be placed within the cages as a form of environmental enrichment.

Routinely, holding rooms will be maintained within acceptable limits for temperature and relative humidity (nominally 19 to 25°C and 40 to 70%, respectively). These rooms are illuminated by fluorescent light for 12 hours (06:00 to 18:00 hours) out of each 24 hour cycle and designed to receive at least 15 fresh air changes per hour.

Concentrations of AZD5501 or AR-C154669XX in plasma will be determined using high performance liquid chromatography (HPLC-MS). Details of the methodology will be contained in the Principal Investigator's plan.

The results of the pharmacokinetic analysis will be issued in a Principal Investigator's contributory report, including a Principal Investigator's GLP statement and QA statement, and will be included in the final report as an Appendix.

TERMINAL PROCEDURES

At the end of the study (Day 8 for the Irwin animals, or following the 2 hour post-dose bleed for the PK animals), the animals will be humanely killed by a Schedule 1 method (e.g. cervical dislocation) and discarded without necropsy.

If an animal shows any sign of serious discomfort during the study it may be killed immediately and humanely at the discretion of the Study Director.

Any animal found dead or killed prematurely during the study may be subjected to a necropsy, at the discretion of the Study Director. A macroscopic examination will be performed, after opening the thoracic and abdominal cavities, by observing the appearance of the tissues *in situ*. Any abnormalities will be recorded.

GOOD LABORATORY PRACTICE COMPLIANCE

The component of the study conducted at Covance and AstraZeneca will be performed in compliance with:

United Kingdom Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999.

OECD Principles on Good Laboratory Practice (revised 1997, Issued January 1998) ENV/MC/CHEM(98)17

FDA Good Laboratory Practice for Non-clinical Laboratory Studies 21 CFR Part 58, December 1978 and subsequent amendments.

EXPERIMENTAL DESIGN

Regulatory test guidelines

This report is designed to meet the known requirements of EC Directive 75/318/EEC and all subsequent amendments together with any relevant International Conference on Harmonisation (ICH) guidelines. These requirements and guidelines were current at the time the report was issued.

Test article administration

The test article was administered daily, for at least 28 days excluding the day of necropsy, by nasal administration. Each dog received three instillations per nostril per session and were treated for four sessions, each separated by at least 30 minutes.

The start and finish times of each dosing session were recorded. Although the start of each dosing session was separated by at least 30 minutes, on several occasions dosing was completed within 30 minutes of the previous session. The shortest time separating the completion of dosing sessions was 18 minutes.

Administration was on at least alternate breaths to alternate nostrils, just as the animal was about to inhale, starting with the left nostril. The head was held slightly upwards to prevent forward drainage of the test article. To avoid losing the prime from the spray device, after each actuation the spray device was returned to the vertical prior to the release of the actuator, thus allowing the pump to recharge without the introduction of air.

The dosing device was shaken gently before use and primed prior to the start of each day's dosing. To prime each dosing device, it was actuated eight times or until a fine spray was emitted. The device was not primed between sessions, unless there was a mishap in dosing resulting in loss of the existing prime.

The dosing devices for Groups 2 and 5 were not primed prior to the start of the day's dosing on Day 13, in error.

The dosing device nozzle was cleaned before the start of each session and between dogs using suitable antiseptic or alcoholic wipes.

The five nominated mice from each group were examined for general behaviour, according to Irwin's method (Irwin, 1968). The parameters in Table 1 were systematically evaluated in accordance with the relevant Covance Standard Operating Procedure (current version of SOP PHARM 8.10). Irwin observations were performed at 0.5, 2, 4, and 24 hours post-dose.

Animals were kept for a further 7 days, during which time they were observed daily for gross signs of toxicity and mortality.

Bodyweights were recorded on Day 1, 3, 5 and 8.

PHARMACOKINETICS

At two hours post-dose (±15 minutes), all three PK animals within Groups 2 to 7 were terminally anaesthetised under gaseous halothane and approximately 250 µl of blood was taken *via* the orbital sinus and transferred into tubes containing EDTA.

After thorough mixing, the blood samples were cooled on ice and plasma was prepared within 30 minutes of sampling by centrifugation (3500rpm for 10 minutes at approximately 4°C). The plasma was transferred to neutral polypropylene tubes, immediately frozen upright on dry ice. Samples were then transferred to the freezer, where they were stored frozen (nominally -20°C) prior to dispatch.

The animals were killed by cervical dislocation whilst under anaesthesia.

Plasma samples were shipped frozen on dry ice by courier to the Sponsor.

The Principal Investigator for the Pharmacokinetics was Julie Doherty.

The pharmacokinetic results are shown in the Results Section with the full method and results in Appendix 4.

TERMINAL PROCEDURES

At the end of the study (Day 8 for the Irwin animals, or following the 2 hour post-dose bleed for the PK animals), the animals were humanely killed by a Schedule 1 method (cervical dislocation) and discarded without necropsy.

RESULTS AND DISCUSSION

Irwin Screen

The Irwin observations are presented in Table 1. Group mean bodyweights are summarised in Table 2; individual animal data are presented in Table 3.

No behavioural or physiological changes were observed in vehicle-treated animals (Groups 1 and 8) in this study.

Effects of AZD5501

Oral administration of AZD5501 at dose levels of 3, 30 or 300 mg/kg, produced no noteworthy behavioural or physiological changes in mice during this study.

Effects of AR-C154669XX

AR-C154669XX administered orally at dose levels of 3, 30 or 300 mg/kg, produced no noteworthy behavioural or physiological changes in mice in this study.

One animal (animal number 32) given AR-C154669XX (300mg/kg) displayed slight signs that, in the context of this study, were considered to be of little or no biological significance. This animal had no pupil response to light due to the pupils already constricted at 0.5 hours post-dose, slight piloerection and slightly 'scruffy' appearance suggestive of slightly decreased grooming at 24 hours post-dose and slight piloerection on Day 5.

Note: Animal number 31 dosed orally with AR-C154669XX at a dose level of 300 mg/kg displayed slight piloerection, a slightly hunched posture, slight apathy, slight decreases in grooming and transfer arousal and decreased alertness (no response) at 24 hours post-dose. By Day 5 this animal was generally subdued with substantial piloerection and a hunched posture, in addition a large swelling was present towards the left side of its abdomen and marked weight loss was apparent. The animal was killed by intraperitoneal overdose of Euthatal and a macroscopic examination was performed by a veterinary surgeon. The macroscopic examination revealed the swelling to be a subcutaneous accumulation of undigested food as a result of a dosing injury. It was concluded that the signs exhibited by this animal were a consequence of mis-dosing, and therefore its results were not considered in this report.