Final Report

Study Title	Ciclesonide Nasal Spray: 28 Day Nasal Administration Toxicity Study in the Dog with a 28 Day Treatment-free Period
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There were no decedents and no signs of reaction to treatment during the study. There were no effects on food intake, heart rates and haematology parameters that were attributed to treatment and there were no ophthalmic abnormalities.

Low weight gains and/or slight weight loss were seen in one or two animals fromn all treated groups during the treatment period. Following cessation of treatment, weight gains in most animals previously treated at 480 mcg/kg/day were higher than control values.

Increased total plasma cholesterol concentrations were seen in two animals receiving 240 mcg/kg/day and five animals receiving 480 mcg/kg/day. Increased plasma triglyceride concentrations were seen in one or two males or females in all treated groups. After 4 weeks of recovery, a slightly high triglyceride concentration was seen in one male that had been affected in Week 4 of treatment.

Low thymus weights were seen in some animals in all treated groups, although there was no clear dose-relationship. Slightly low adrenal weights were seen in three animals receiving 480 mcg/kg/day. In animals killed after 4 weeks of the treatment-free period, thymus and adrenal weights were broadly comparable to control weights.

At the terminal kill, there were no macroscopic findings due to effects of the test article. Microscopically, there was no evidence of local irritation in the nasal cavity, but the lymphoid aggregates normally associated with the respiratory mucosa tended to be smaller in the intermediate and high dose group compared with controls. There was also atrophy in the thymus of some intermediate and high dose animals and atrophy of the adrenal cortex of one high dose male due to systemic effects of the test article.

At the treatment-free kill, there were no treatment-related macroscopic findings. Microscopic assessment of reversibility was limited by the small number of animals available, but the data were suggestive of incomplete reversal of the local lymphoid atrophy in the nasal cavity. The adrenal and thymus of high dose treatment-free animals were comparable with controls indicating reversal of systemic effects.

Intranasal administration of Ciclesonide to dogs at dosages of 120, 240 or 480 mcg/kg/day was well-tolerated with no clear evidence of systemic or local toxicity. A number of reversible changes in the thymus, adrenal and nasal mucosa and effects on weight gain were attributed to the glucocorticosteriod pharmacology of the test article and are of no toxicological significance. As pharmacological effects

INTRODUCTION

The objective of the study was to determine the local tolerance, together with an evaluation of the systemic toxicity and the toxicokinetic profile of the test article, Ciclesonide Nasal Spray (Glucose-free), following nasal administration for 28 days to the dog. The reversibility of any effects observed were then assessed over a 28-day treatment-free period.

The nasal route of administration was chosen because it is the human therapeutic route.

The dog was selected because it is a non-rodent species recommended by various regulatory authorities. Background data are available.

Study dates

Protocol signed by Study Director:	29 May 2001
Animals on site & experimental start date:	19 April 2001
First treatment:	14 June 2001
Study termination:	9 August 2001
Pathology completed & experimental completion date:	28 January 2002

The study completion date is the date the final report is signed by the Study Director.

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.

Formulations analysis

The unused dosing devices were returned to the Sponsor on completion of the study. The dosing devices were analysed after the end of the treatment period of the study by The Principal Investigator (Dr Yasuhide Uejima, Teijin Limited, 4-3-2 Asahigaoka, Hino, Tokyo 191-8512, Japan). The results of these analyses were communicated to Covance Laboratories for inclusion in the final report.

TEST SYSTEM

Species, strain and supplier

Thirty-eight purpose-bred beagle dogs were obtained from B & K Universal, Hull, in order to provide 19 healthy animals of each sex.

Specification

The animals were between 5 and 9 months old and in the weight range of 8.40 to 13.15 kg on arrival. Contrary to the protocol, not all animals were less than 9 months old at the start of dosing. They were approximately 7 to 10½ months old and weighed 8.94 to 14.03 kg at the start of dosing.

Environment

The animals were housed in four exclusive rooms, air-conditioned to provide a minimum of 10 air changes/hour. Routinely, the temperature and relative humidity ranges were 16 to 22°C and 40 to 80% respectively. The temperature and humidity were occasionally outside the protocol ranges; the actual ranges recorded were 15 to 23°C and 29 to 82% respectively. Fluorescent lighting was controlled automatically to give a cycle of 12 hours light (0700 h to 1900 h) and 12 hours dark.

Each animal was allocated to a pen with a floor area of 2.25 m^2. Where possible, animals of the same sex and group were routinely housed in groups of three by removal of the barrier dividing the pens. Animals remained separated each day from before dosing and during the feeding period.

Environmental enrichment

A number of methods are used at Covance to enrich the environment of the animals as detailed in standard operating procedures.

Diet and water

Each animal was offered 400 g of Harlan Teklad Dog Maintenance Diet (Harlan Teklad, Bicester) each morning, after completion of the fourth dosing session. Any residual food was removed and weighed in the afternoon. Each batch of diet was analysed for specific constituents and contaminants. All animals received a Winalot Shapes biscuit (Friskies Petcare, Suffolk) immediately after the fourth dosing session. Certificates of diet analysis are presented in Appendix 13.

Mains water was provided *ad libitum* via an automatic watering system. The water was periodically analysed for specific contaminants.

No contaminants were present in diet or water at levels which might have interfered with achieving the objective of the study.

PRE-EXPERIMENTAL PROCEDURES

Acclimatisation and health procedures

Prior to arrival, the animals received a course of treatment for endo-parasites and a course of vaccinations, details of which are contained in the supplier's data.

On arrival, all animals were given a clinical inspection for ill-health. They were acclimatised for a period of about eight weeks. Details of any prophylactic treatments and immunisations were maintained in the study records.

A veterinary inspection was performed before the start of dosing to ensure their suitability for the study.

Allocation to treatment group

The animals were assigned to treatment groups during the acclimatisation period using a randomisation procedure based on stratified body weight, which used the most recent body weight data.

Ophthalmoscopy

Investigations were performed on all animals pre-treatment and towards the end of Week 4. A mydriatic agent was instilled into the eyes before examinations.

Electrocardiography

Investigations were performed on all animals pre-treatment and towards the end of Week 4. Readings were taken approximately one hour after the completion of the fourth dosing session. Recordings were taken using the fixed limb leads I, II and III and the augmented leads aVR, aVL and aVF. Heart rate was derived from lead II.

Toxicokinetics

Blood sampling

Blood samples for toxicokinetics (5 mL nominal to provide at least 2.0 mL serum) were taken from all animals on Days 1 and 27 of treatment, not Day 28 as stated in the protocol. Samples were collected pre-dose (before the first dosing session), immediately on completion of the second and fourth dosing sessions for each animal, and 0.5, 1, 2, 4, 6, 8 and 24 hours after completion of the fourth dosing session.

The times of the bleeds as specified in the protocol were not always strictly adhered to, but generally only deviated a few minutes from the scheduled time. The greatest deviation was 10 minutes late at the 0.5 hour bleed on Day 27. The deviations are not considered to have compromise the evaluation of the toxicokinetic data.

Samples were taken from the jugular vein into plain tubes. The plasma was separated as soon as practicable, the serum transferred to uniquely labelled polypropylene tubes and the samples stored frozen (-20°C nominal) prior to analysis. On Day 27 at the 6 hour timepoint, the prepared serum from Group 3 (Nos 29, 30 and 31), Group 4 (Nos. 32, 33 and 34) and Group 5 (Nos. 35 and 36) was placed into 0.5 mL tubes due to a tube labelling error. For these animals 0.5mL of serum was collected and the rest discarded.

Samples from treated animals were analysed by the Bioanalytical Department at Covance Laboratories; samples from negative control and placebo control animals (Groups 1 and 2) were not analysed.