Electronic Supplementary Material (ESI) for Toxicology Research.
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Appendix (Online publication only)

Toxicological Investigations

Clinical observations

Mortality observations were recorded twice daily, and clinical observations of physical appearance and behaviour were recorded daily during the predose, dosing, and "off-drug" phases of the studies. Body weight and food consumption measurements were generally recorded at least weekly and body temperature biweekly.

Physical and neurological examinations

Conscious animals (rats and monkeys) were assessed as part of the repeat dose toxicology studies. Physical examinations included abdominal palpation, eye, ear and nose examination, heart and lung auscultation, heart rate and respiratory rate. Neurological examinations were conducted in monkeys only and included an assessment of general sensorimotor expressions and cerebral/spinal nerve tests. Functional neurobehavioral assessments were performed prior to the initiation of dosing and on Days 16 and 81 in males only in the 12 week toxicity study, and in Weeks 11, 25 and 49 in the 52 week toxicity study, respectively. The potential for any delayed neurobehavioral effects was investigated at the end of an 8 or 16 week 'off-dose' period in the high dose groups (i.e. 300 or 500 mg/kg in the 12 and 52 week studies, respectively).

Electrocardiography

Electrocardiogram (ECG) readings were recorded in temporarily restrained conscious monkeys in general toxicology studies by a three-channel electrocardiograph. Tracings from all limb leads were recorded simultaneously on the same sheet of paper, for each animal and ECG tracings were recorded at various time points before dosing, and within 24 hours of dosing on Day 15 (4 week study), Days 67 and 72 (12 week study), or Weeks 13, 27 and 51 (52 week study) and also at the end of the treatment free period. Heart rate (beats per minute), RR and PR interval, QRS complex, and QT interval lengths and QTc in seconds were determined from DII tracings; calculated according to Fridericia's formula.

Ophthalmology examinations.

Assessments were performed in rats and mildly sedated monkeys at the following intervals: predose and around the final week of treatment for both rats and monkeys (4 and 12 week studies) or in Weeks 13, 24 and 52 in monkeys (52 week study only) as well as towards the end of the drug-free phase. Animals were examined in randomized order using slit lamp examination of the conjunctiva,

cornea, anterior chamber, iris, lens, vitreous body and by means of indirect ophthalmoscopy, following instillation of a mydriatic to both eyes.

Clinical pathology investigations.

Blood and urine samples were collected periodically for clinical pathology analyses during the predose and postdose periods (following overnight fasting). For example, monkey blood samples (approximately 4.5 mL) were drawn from all animals at various intervals twice during the predose phase and on Day 29 (4 week study), Days 36 & 85 (12 week study) or Weeks 11, 25 & 49 (52 week study) of the dosing phase, and also during the last week of the treatment-free phase. Rat blood samples were taken at scheduled necropsy i.e. Day 29 (4 week study) and Day 85 (12 week study). Comprehensive hematology analyses were conducted on ethylenediamine tetra-acetate (EDTA)-anticoagulated whole blood using an automated haematology analyzer (e.g. Sysmex XT 2000iV or similar apparatus). The following parameters were determined: reticulocyte and erythrocyte count, hemoglobin concentration, hematocrit, total and differential leukocyte counts, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count, hematocrit and mean corpuscular volume. Serum chemistry was analyzed using a Konelab 60i (Kone Instruments Co. Finland). The following parameters were determined: glucose, total cholesterol, triglycerides, urea, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gammaglutamyl transferase, glutamate dehydrogenase, total bilirubin, total protein, albumin, globulin, sodium, potassium, chloride, calcium, and inorganic phosphorus. Coagulation parameters were evaluated on sodium citrate-treated plasma samples collected from cynomolgus monkeys using an Amax 190 coagulometer (Trinity Biotech) to determine the activated partial thromboplastin time and prothrombin time. Urinalysis was performed on samples collected 16-h post-treatment period. Excreted urine volume was recorded, and urine samples (approximately 2 hours) were collected with the same interval as blood and analyzed using a Konelab 60i (Kone Instruments Co. Finland) for the following parameters: specific gravity, color, turbidity, pH, glucose, protein, and creatinine.

Assessment of Anti-GSK1223249 antibodies

The presence of anti-GSK1223249 binding antibodies in sera was evaluated by electrochemiluminescent assay (ECLIA) method at various time points. Sera were collected for analysis of anti-GSK1223249 antibodies pre-dose (week -1), during dosing on Day 43 (rat) or Day 36 (monkey) in the 12 week study, or Weeks 13, 27 and 51 (monkey only) in the 52 week study, and also during the off-dose periods. Samples were diluted 1:10 in assay buffer and added to a 96-well

AVIDIN-coated plate containing biotinylated GSK1223249. Ruthenium-labelled GSK1223249 was added to the plate, incubated for 30-60 minutes, and read out by a Meso Scale Discovery (MSD) Sector Imager 2400 (MSD Discovery Workbench software, v3.0). Affinity purified rabbit anti-GSK1223249 antibody prepared in 10% normal monkey serum was used as the reference standard. The assay lower limit of quantification (LLQ) for anti-GSK1223249 antibodies was 0.1 to 12.8 μg/mL.

Anatomic pathology.

The animals were euthanized by exsanguination while under deep anaesthesia induced with sodium pentobarbitone. A complete gross necropsy was performed and included collection of macroscopic observations, organ weight measurements, and a comprehensive collection of tissue samples for microscopic examination. Tissues were preserved in neutral buffered 10% formalin, routinely processed and embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy by board-certified veterinary pathologists. The eyes, testes and epididymides were fixed in modified Davidson's solution. Testes were stained with Periodic Acid-Schiff (PAS) and hematoxylin and spermatogenesis staging was performed. Bone tissue was decalcified using Kristenson's fluid.

A detailed microscopic survey of the central and peripheral nervous system was performed in the monkey, in addition to transverse and longitudinal sections of the vagus, median, sciatic, tibeal and peroneal peripheral nerves. The spinal cord was evaluated in transverse and/or longitudinal section at C1 (including spinal trigeminal nucleus and tract) and the cervical and lumbar intumescences. Dorsal root ganglia from the cervical and lumbar regions were evaluated. Hemicoronal sections of brain were evaluated at the following levels, with the approximate bregma level (mm) as referenced in The Rhesus Monkey Brain in Stereotaxic Coordinates [Paxinos, 2000] listed in parentheses; Level 1 (12.60), Level 2 (0.00), Level 3 (-4.05), Level 4 (-8.10), Level 5 (-12.15), Level 6 (-16.20), Level 7 (-19.75), Level 8 (-30.60) and Level 9: (-36.45). The following structures were generally present in one or more levels; cerebral cortex (frontal, temporal, parietal, occipital) and subcortical white matter, lateral olfactory tract, olfactory tubercle, basal ganglia, corpus callosum, internal capsule, external capsule, medial septal area, optic nerve, septal area, fornix, anterior commisure, hypothalamus, amygdala, optic tract, lateral ventricle, 3rd ventricle, hippocampus, oculomotor nucleus and nerve, lateral geniculate nucleus, medial geniculate nucleus, substantia nigra, midbrain, superior colliculus, inferior colliculus, pons, cerebral aquaduct, pineal gland, middle cerebellar peduncle, trochlear nerve, trigeminal nerve, 4th ventricle, cerebellar cortex, cerebellar nuclei, medulla oblongata,

vestibular nuclei, olivary nuclei, reticular area, gracile and cuneate nuclei, pyramindal tracts, hypoglossal nerve, spinal accessory nerve and central canal.

A peer review of selected microscopic tissue sections, and pathology data interpretation was completed. The peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.

Developmental assessments

The female animals were euthanized by deep anaesthesia induced with sodium pentobarbitone , necropsied and pregnancy status recorded. The ovaries were removed and the corpora lutea counted. The total number of implantation sites, resorptions, and live and dead fetuses were counted, and their status and relative positions recorded. Each live and dead fetus was examined externally. Abdominal and thoracic viscera of all fetuses were examined by a modified Staples' technique and sex was identified by internal examination of the gonads [Solomon, 1997; Staples, 1974]. Dead fetuses were discarded upon completion of the visceral examination, and approximately one-half of the live (euthanized) fetuses were decapitated and the heads preserved in Bouin's solution; these were subsequently sectioned and examined by the Wilson's technique [Wilson, 1965]. The remaining fetuses, and all intact live (euthanized) foetuses were processed for skeletal examination, fixed in Bouin's solution, and stained with Alizarin Red S. Malformations i.e. fetal observations judged to potentially affect survival, growth, development, functional competence or external appearance were determined, along with those representing retardations in development, transitory alterations or permanent alterations not believed to adversely affect survival, growth, development, function, longevity, or external appearance.