**A 4-Week Repeated Oral Dose Toxicity Study of PROJECT B in Dogs Followed by a 4-Week Recovery Period**

**11 SUMMARY**

PROJECT B (Lot No. 001WKM) was suspended in 10 w/v% TC-5E solution and orally administered once daily for 4 weeks at dose levels of 0 (vehicle control), 3, 10, 30, and 300 mg/kg as PROJECT B to 4 male and 4 female beagle dogs per group in order to investigate its potential toxicity. Three males and two females were added to the 300 mg/kg group in order to assess the reversibility of toxicity during a subsequent 4-week recovery period.

The following observations and examinations were performed in this study: clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, gross pathology, organ weights, histopathology, and toxicokinetics.

One female at 300 mg/kg was sacrificed due to moribundity on Day 11 of dosing, and 1 female at 300 mg/kg was found dead in the morning (before dosing) on Day 13 of dosing. Tachypnea and lateral position, and loss of food consumption were observed before sacrifice in the moribund female. In the dead female, deteriorated conditions in clinical signs were not observed on the day before death. In these females, soft stool, diarrhea, vomiting, and salivation were occasionally observed. In hematology and blood chemistry, the following changes were noted at sacrifice in the moribund female: increased counts of leukocyte, neutrophil, and monocyte, increased fibrinogen, troponin I, troponin T, creatine kinase, total bilirubin, total protein, albumin, glucose, urea nitrogen, creatinine, calcium, and creatine kinase-MM type, and decreased eosinophil count, inorganic phosphorus, sodium, potassium, and chloride. In gross pathology, the following changes were observed in dead and/or sacrificed animals: red focus in the endocardium at the left ventricle and right atrium in the heart, red focus in the lung, and brown discoloration in the esophagus, pleural fluid, and red discoloration in the ileum. High lung weight and low thymus weight were noted. In histopathology, the following changes were observed: multifocal arteritis, multifocal inflammatory cell infiltration with hemorrhage, multifocal myocardial necrosis in the heart, atrophy of the thymus, spleen, submandibular and mesenteric lymph node, and mucosal necrosis with edema in the esophagus, inflammatory cell infiltration in the lamina propria and submucosa, subepithelial edema in the ileum, focal peribronchiolar inflammatory cell infiltration, focal inflammatory cell infiltration in the bronchiolar lumen and intraepithelium, hypertrophy of the bronchiole epithelium, and congestion and edema in the lung. Therefore, it was considered that, the cardiovascular system was one of the main target organs of toxicity of PROJECT B, and that effects on the cardiovascular system possibly caused deterioration in general condition or death in 2 females.

No test article-related changes were noted at 3 mg/kg.

At 10 mg/kg and more, soft stool and vomiting were observed in males and females.

At 30 mg/kg, high lung weight was noted in a male.

At 30 mg/kg and more, diarrhea was observed in males and females. Focal peribronchiolar inflammatory cell infiltration in the lung in histopathology was observed in males at 30 mg/kg, and in males and females at 300 mg/kg. Hypertrophy of the bronchiole epithelium, and foam cell accumulation in the lung in histopathology were observed in males at 30 and 300 mg/kg.

At 300 mg/kg in the survived animals, the following changes were noted in males and females: salivation, increased heart rate, shortened QT, PR, and QTc in electrocardiogaphy, decreased erythrocyte count, hematocrit value, hemoglobin concentration, eosinophil count, prolonged activated partial thromboplastin time, increased troponin I and urea nitrogen in hematology and blood chemistry, red focus in the endocardium at the right atrium in the heart in gross pathology, multifocal arteritis, multifocal inflammatory cell infiltration with hemorrhage in the heart, and atrophy of the thymus in histopathology. In males in addition to above changes, red focus in the lung in gross pathology, focal inflammatory cell infiltration in the bronchiolar lumen, and intraepithelium in the lung were observed in histopathology. And in females, increased fibrinogen, troponin T, sodium, and chloride, and decreased potassium in hematology and blood chemistry, red focus in the stomach body serosa in gross pathology, high heart weight and low thymus weight, multifocal myocardial necrosis, and thickening of the artery tunica intima in the heart, and perivascular hemorrhage in the muscle and serosa in the stomach body were noted in histopathology.

No test article-related changes were observed in body weight, ophthalmology, or urinalysis at any dose level.

After a 4-week recovery period, the only histopathological changes in the heart and lung were observed at 300 mg/kg as follows: thickening of the artery tunica intima, and lymph vessel dilatation in the heart accompanied by gross lesions, and fibrosis and brown pigment deposition in the lung. Thickening of the artery tunica intima that were observed in the heart were considered to be a state of repair process of the arteritis that were observed at the end of the dosing period, and lymph vessel dilatation in the heart might be caused the lymph vessel obstruction accompanied with arteritis or myocardial damage. Changes in the lung were considered to be a scarring related to the change of focal peribronchiolar inflammatory cell infiltration in the lung at the end of the dosing period. Accordingly, all test article-related changes noted at the end of the dosing period, except for histopathological change of repair process or scarring accompanied by gross lesions, showed full recovery during the 4-week recovery period.

tmax, Cmax, and AUC24 values for PROJECT B are shown in the table below.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dose Level (mg/kg/day) | | 3 | | 10 | | 30 | | 300 | |
| Males | Females | Males | Females | Males | Females | Males | Females |
| tmax (h) | Day 1 | 1.75 | 1.00 | 0.88 | 0.88 | 0.69 | 0.63 | 2.86 | 2.43 |
| Day 14 | 1.00 | 1.00 | 0.75 | 0.75 | 0.56 | 0.50 | 1.86 | 2.40 |
| Day 28 | 1.00 | 1.00 | 1.00 | 0.88 | 0.56 | 0.69 | 1.14 | 1.20 |
| Cmax (ng/mL) | Day 1 | 311 | 377 | 1660 | 1440 | 6460 | 10400 | 16000 | 18200 |
| Day 14 | 373 | 262 | 1950 | 1460 | 8130 | 9200 | 21900 | 24100 |
| Day 28 | 323 | 276 | 1710 | 1400 | 7330 | 9910 | 21800 | 25700 |
| AUC24  (ng·h/mL) | Day 1 | 1330 | 1070 | 5390 | 4810 | 18500 | 25500 | 136000 | 143000 |
| Day 14 | 1500 | 859 | 5750 | 4890 | 19500 | 25300 | 146000 | 184000 |
| Day 28 | 1250 | 849 | 5630 | 4200 | 16900 | 22900 | 127000 | 149000 |

In toxicokinetics, mean tmax in all groups was 0.50 to 2.86 h (Individual values of tmax was 0.25 to 8.00 h). Cmax and AUC24 of PROJECT B increased more than dose-proportional from 3 to 30 mg/kg/day, and increased less than dose-proportional from 30 to 300 mg/kg/day in both sexes on each day. There was no apparent sex difference and no apparent change by repeated dose.

It was concluded that, under the conditions of this study, the no-observed-adverse-effect level of PROJECT B was 3 mg/kg/day as PROJECT B for males and females because soft stool and vomiting were observed at 10 mg/kg/day and more. Moreover, moribund sacrifice and death were noted at 300 mg/kg/day. The test article-related changes noted during the dosing period, except for histopathological change of repair process or scarring accompanied by gross lesions, showed full recovery during the 4-week recovery period.