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A 4-week Repeated Oral Dose Toxicity Study of CJ-10882 in Dogs

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ABSTRACT : *The present study was conducted to investigate the potential subacute toxicity of CJ-10882 by a 4-week repeated oral dose in dogs. The test article was administered once daily by gavage to dogs at dose levels of 0, 2, 10, and 50 mg/kg/day for 4 weeks. During the test period, clinical signs, mortality, body weights, food consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, gross finding, organ weight, and histopathology were evaluated. Several clinical signs were observed in treated dogs at 50 mg/kg, including salivation and vomiting. Increase in the serum levels of ALT and albumin observed in the female 50 mg/kg group was considered as a toxic effect related to the test article since the histopathological change in liver was accompanied. There were no treatment-related effects on mortality, food and water consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, necropsy findings and organ weights in any treatment group. Based on these results, target organ was not observed and the no-observed-adverse-effect level (NOAEL) was 10 mg/kg/day and the absolute toxic dose was 50 mg/kg for both male and female dogs.*

Key Words : *CJ-10882, Subacute toxicity study, Dogs*

I. INTRODUCTION

Phosphodiesterases (PDEs) inactivate cyclic nucleotides by catalyzing hydrolysis of the 3-phosphodiester bond to form the corresponding inactive 5-mononucleotide products (Soderling and Beavo 2000). PDE IV is the predominant family of PDEs expressed in inflammatory cells, including eosinophils, T lymphocytes, macrophages, neutrophils, dendritic cells, mast cells, and structural cells such as sensory cells and epithelial cells (Barnette *et al.*, 1996). Inhibition of PDE IV blocks cell trafficking and cell proliferation, and attenuates the production of inflammatory mediators and cytokines, which suggests PDE IV inhibitor is attractive therapeutic target of asthma and chronic obstructive pulmonary disease (COPD) (Conti and Jin 1999; Torphy 1998). CJ-10882 is a selective inhibitor of PDE IV with the formula {(E)-[(3-Cyclopentyloxy-4-methoxyphenyl)methylene]-hydrazine-carboxamide}, an anti-inflammatory drugs recently developed by Research Laboratory of Cheil Jedang Co. (Ichon, Republic of Korea), employed for the treatment of asthma and COPD. CJ-10882 (mol. wt. of 277.3 Da and melting point of 150°C) is white to off-white crystalline powder, which has relatively good water solubility in the range of 60~120 mg/ml and is now being considered for evaluation in phase 1 clinical trial to treat COPD. The most common adverse effects associated with the PDE IV inhibitor are gastrointestinal (GI) disturbances such as nausea, vomiting and salivation, myocardial degeneration and necrosis, necrotizing vasculitis and inflammation in the mesentery and interstitial areas of the liver (Banner and Page 1996; Larson *et al.*, 1996).

In the present study, we report the results of 4-week repeated oral dose toxicity study in dogs performed as a part of the preclinical safety evaluation program for CJ-10882. The present study was conducted according to the test guidelines from the Korea Food

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List of abbreviations: AAALAC International, Association for Assessment and Accreditation of Laboratory Animal Care International; KFDA, Korea Food and Drug Administration; NOAEL, no-observed-adverse-effect level; OECD, Organisation for Economic Cooperation and Development.

and Drug Administration (KFDA) and Organisation for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals under modern Good Laboratory Practice Regulations.

II. MATERIALS AND METHODS

1. Chemicals

CJ-10882 {(E)-[(3-Cyclopentyloxy-4-methoxyphenyl) methylene]-hydrazinecarbox-amide, Lot No. Xiang 99059025, 99.95%}, was supplied by Research Laboratory of Cheil Jedang Co. Other chemicals were of reagent grade or high-performance liquid chromatographic (HPLC) grade, and therefore were used without further purification.

2. Animals

Twelve male (weighing 6.7~7.4 kg) and twelve female (weighing 6.1~9.3 kg) Beagle dogs (Canis familiaris) of 5.5 months old were purchased from Covance Research Product Inc. (Cumberland, VA). After quarantine and acclimation periods of 62 days, oral studies were performed (weighing 8.2~11.4 kg for male dogs and 7.5~10.4 kg for female dogs). They were housed in a light-controlled room (light : 07:00~19:00, dark : 19:00~07:00) kept at temperature of 23±3°C, humidity of 5515% and with the light intensity of 150~300 lux (Korea Institute of Toxicology, Korea Research Institute of Chemical Technology, Daejon, Republic of Korea). Food (Japan Oriental Yeast Company, Tokyo, Japan) was restricted to 300 g per day (the remaining food was weighed to measure net food intake per animal) with water *ad libitum*. This study was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International), and animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996).

3. Administration

CJ-10882 [the CJ-10882 powder (no vehicles or excipients were added) was filled in gelatin capsule, size 12 (Torpac, Farfield, NJ)], in doses of 0, 2, 10 and 50 mg/kg/day, was administered orally for a 4-week period (n = 3 for male and female dogs for each dose) based on the results of preliminary study (data not shown). The oral administration was selected in the present study, because the oral route is a clinically intended route. The negative control dogs were received empty gelatin capsule alone.

4. Clinical observation and mortality

Through the study, all animals were daily observed for clinical signs of toxicity, moribundity, and mortality. Detailed clinical observations were recorded and printed by Labcat Computer System (Innovative Programming Associates Inc., NJ, USA), respectively.

5. Body weights

Body weight of each dog was measured at the initiation of treatment, twice a week thereafter, and on the day of scheduled autopsy.

6. Food consumption

Food consumption was measured at the start of treatment and at weekly intervals thereafter. The amounts of food was calculated before it was supplied to each cage and their remnants were measured next day to calculate the difference which was regarded as a daily food consumption (g/dog/day).

7. Ophthalmoscopy

Ocular fundus examinations, slit lamp examinations and macroscopic observations were carried out at the pre-administration phase and the last week of treatment period. Conjunctiva, sclera, cornea, lens and iris of each eye were also examined.

8. Urinalysis

During the last week of treatment, urinalysis was conducted with fresh urine to determine specific gravity, pH, protein, glucose, ketone body, occult blood, bilirubin, urobilinogen, and nitrite by using a N-Multistix (Ames Division, Miles Laboratory, USA). Urine sediment test was also carried out within three hours

after taking samples during the last week of administration period. The urine collected for 17 hours was measured for the volume. During the collection, the dogs were housed in metabolism cages which allowed for separate collection of urine and feces.

9. Hematology

Hematological examination was performed at the pre-administration phase and the last week of treatment period. The blood samples were collected into CBC bottles containing EDTA-2K (Green Cross Medical Industry, Korea), and were analyzed within 20 minutes in our laboratory. Red blood cell count (RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and white blood cell count (WBC) were determined using a Coulter counter T-540 (Coulter Counter Electronics, USA). Differential WBC counts were made with a glass-slide method using the remaining blood after automatic analysis. Smears were air-dried immediately and stained subsequently with Wrights stain. Then, 200 cells were randomly counted in each smear. Following evaluation of the differential cell counts, the resulting percentage data were converted into absolute numbers using the total WBC count. Reticulocyte count was carried out with blood smear samples that were stained with New methylene blue stain. Any red or white blood cell morphological changes were also noted from these blood films.

10. Serum biochemistry

To get the sera for serum biochemistry, blood samples were centrifuged at 3,000 rpm for 10 minutes within 1 hour after collection. The sera were stored in the -80°C freezer before they were analyzed. Serum biochemistry parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CPK), glucose, total protein (TP), albumin, albumin/globulin ratio (A/G ratio), blood urea nitrogen (BUN), creatinine, triglyceride, phospholipid, total cholesterol, total bilirubin, calcium, and inorganic phosphorus were evaluated by an autoanalyzer (Shimadzu CL-7200, Shimadazu Co., Japan). Serum electrolytes such as chloride, sodium, and potassium were measured by an ion autoanalyzer (644 Na/K/Cl Analyzer, Ciba-Corning Co., USA).

11. Gross findings

At scheduled termination, all surviving animals were anesthetized by intravenous injection of pentothal sodium for blood sample collection, taken blood samples, and then sacrificed by exsanguination from the axillary artery. Complete gross postmortem examinations were performed on all terminated animals.

12. Organ weights

The absolute and relative (organ-to-body weight ratios) weights of following organs were measured in all survivors when they were sacrificed: brain, pituitary gland, adrenal glands, liver, spleen, kidneys, heart, thymus, lung, salivary glands, thyroid glands, testes, epdidymides, seminal vesicles, prostates, ovaries, and uterus.

13. Histopathology

The following tissues were obtained from all animals: abnormal lesions, skin, mammary gland, spleen, pancreas, jejunum, stomach, duodenum, ileum, cecum, colon, mesenteric lymph node, salivary gland, submandibular lymph node, ovaries, uterus, vagina, urinary bladder, epididymides, prostates, seminal vesicles, rectum, kidneys, adrenal glands, liver, sternum, thymus, heart, lung, trachea, esophagus, thyroids (including parathyroids), tongue, aorta, sciatic nerve, skeletal muscle, femur, thoracic spinal cord, Harderian glands, brain, pituitary gland, eyes, and testes. Eyes and testes were preserved in Davidson's fixative and Bouin's fixative, respectively. Other tissues were fixed with 10% neutral buffered formalin solution. The tissues were routinely processed, embedded in paraffin, and sectioned at 3~5 μm. The sections were stained with Hematoxylin-Eosin stain for microscopic examination. All organs and tissues taken from all animals in the negative control and highest dose groups were examined microscopically. All gross lesions as defined by

the study pathologist were also included in the examination.

14. Statistical analysis

Statistical analyses were performed by comparing the treatment groups with the negative control group using either Labcat Computer System or Statistical Analysis Systems (SAS/STAT User's Guide Version 6.12, NC, USA). Whenever, the data were presented as mean±SD. Variance of numerical data was checked by Bartlett's test. If the variance was homogeneous, the data was subjected to one-way analysis of variance (ANOVA) and, if not, they were analyzed by the Kruskal-Wallis nonparametric ANOVA. If either of these tests showed a difference between the groups, the data were analyzed by the multiple comparison procedure of the Dunnett's post-hoc test. The level of significance was taken as $p < 0.05$ or $p < 0.01$.

III. RESULTS

1. Clinical signs and mortality

Death was not observed at any doses studied including control dogs. Vomiting and salivation were found in high frequencies in both male and female dogs at 10 mg/kg and 50 mg/kg, but in low frequencies in the male dogs at 2 mg/kg. Anorexia was observed in both male and female dogs at 50 mg/kg with low frequencies.

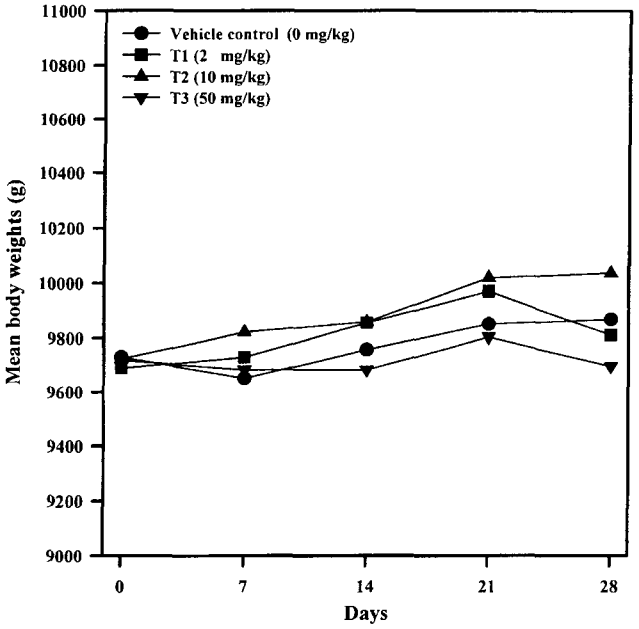

Fig. 1. Mean body weight changes of male dogs treated with CJ-10882.

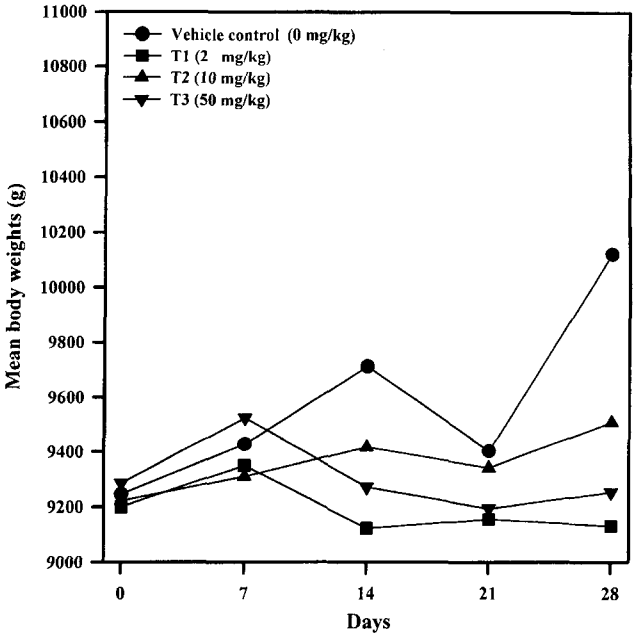

Fig. 2. Mean body weight changes of female dogs treated with CJ-10882.

2. Body weight changes

No significant differences on body weight were observed in male and female dogs among the groups. But, both male and female dogs at 50 mg/kg showed a tendency to decrease in body weight gain on the 28th days compared to the control dogs (Fig. 1 and Fig. 2).

Table 1. Mean food consumption in male and female dogs treated with CJ-10882 for 4 weeks

Dose (mg/kg/day)	0	2	10	50
Male				
Day 0	300±0.0	300±0.0	292±13.3	300±0.0
Day 8	300±0.0	300±0.0	300±0.0	254±79.7
Day 15	300±0.0	300±0.0	300±0.0	300±0.0
Day 22	300±0.0	300±0.0	300±0.0	300±0.0
Female				
Day 1	300±0.0	300±0.0	300±0.0	266±30.3
Day 8	300±0.0	300±0.0	300±0.0	285±26.0
Day 15	300±0.0	300±0.0	300±0.0	271±50.2
Day 22	233±59.3	300±0.0	300±0.0	300±0.0

Values are presented as means±SD (g).

3. Food consumption

In both male and female dogs at 50 mg/kg, they showed a tendency to decrease in food intake (Table 1).

4. Ophthalmoscopy

No treatment-related changes were observed in all dogs of both sexes (data not shown).

5. Urinalysis

In both male and female dogs at 50 mg/kg, they showed a significant increase in urine volume (Table 2). No significant difference between treatment groups and controls was seen for any other urinary parameters.

Table 2. Urinalysis findings in male and female dogs treated with CJ-10882 for 4 weeks

Dose (mg/kg/day)		Male				Female			
		0	2	10	50	0	2	10	50
Urine volume (ml)	Mean	175	160	166	339	175	268	256	606*
	SD	65.8	53.6	33.2	134	90.2	40.6	96.5	208
Glucose	-	3	3	3	3	3	3	3	3
Bilirubin	-	1	3	2	2	3	3	3	3
	1+	2	0	1	1	0	0	0	0
Ketone	-	2	1	2	3	2	3	3	3
	+/-	1	2	1	0	1	0	0	0
Specific gravity	≤1.005	1	2	1	1	0	1	1	1
	1.010	0	0	0	1	2	1	0	1
	1.015	0	0	0	1	0	1	0	0
	1.020	0	1	0	0	1	0	1	1
	1.025	2	0	2	0	0	0	1	0
pH	6.5	0	0	0	0	0	0	1	0
	7.0	1	1	2	0	0	1	1	0
	7.5	1	0	0	2	1	0	0	2
	8.0	0	1	0	0	1	1	0	1
	8.5	0	1	1	0	1	1	0	0
	9.0	1	0	0	1	0	0	1	0
Protein	-	0	1	1	1	1	2	1	2
	+/-	1	1	0	2	2	1	2	1
	1+	1	1	0	0	0	0	0	0
	2+	1	0	1	0	0	0	0	0
	3+	0	0	1	0	0	0	0	0
Urobilinogen	0.1	3	3	3	3	3	3	3	3
Nitrite	-	2	3	3	3	3	3	3	3
	+	1	0	0	0	0	0	0	0
Occult blood	-	2	2	1	0	3	3	2	1
	+/-	0	0	0	1	0	0	0	0
	1+	1	1	1	2	0	0	1	1
	2+	0	0	1	0	0	0	0	0
	3+	0	0	0	0	0	0	0	1
Sediment : Cast	-	3	3	3	3	3	3	3	3
EPI	-	0	0	1	0	0	0	0	1
	+/-	1	2	0	0	1	2	1	1
	1+	0	1	1	3	2	1	1	0
	2+	2	0	1	0	0	0	1	1
WBC	-	2	0	1	0	2	0	1	1
	+/-	0	1	0	1	1	2	2	1
	1+	0	0	0	1	0	0	0	1
	2+	1	0	1	1	0	1	0	0
	3+	0	2	0	0	0	0	0	0
	4+	0	0	1	0	0	0	0	0
RBC	-	3	0	1	0	1	2	0	3
	+/-	0	3	1	2	2	1	3	0
	+	0	0	1	1	0	0	0	0

EPI, epithelial cells; WBC, white blood cells; RBC, red blood cells.
*Indicates significant difference at $p < 0.05$ level when compared with the control group.

Table 3. Hematological findings in male and female dogs treated with CJ-10882 for 4 weeks

Dose (mg/kg/day)	0	2	10	50
Male				
Erythrocytes ($\times 10^{12}$/l)	6.74±0.40	6.93±0.89	6.52±0.14	6.42±0.41
Hemoglobin (g/dl)	15.2±0.61	15.6±1.95	15.3±0.23	14.9±0.82
Hematocrit (%)	47.3±2.14	46.5±5.10	44.8±0.15	43.9±2.91
MCV (fl)	70.1±1.06	67.2±1.45	68.8±1.48	68.3±0.25
MCH (pg)	22.6±0.46	22.5±0.17	23.4±0.91	23.2±0.35
MCHC (g/dl)	32.2±0.21	33.5±0.61*	34.0±0.57**	34.0±0.53**
Platelets ($\times 10^9$/l)	316±48.1	285±37.2	257±38.0	311±49.0
Reticulocytes (‰)	1.7±0.58	1.7±1.15	2.0±1.00	0.7±0.58
Leukocytes ($\times 10^9$/l)	8.96±1.48	9.73±1.88	10.4±0.696	11.1±1.43
Neutrophils ($\times 10^9$/l)	5.63±1.63	6.69±1.93	7.11±0.936	6.73±0.972
Eosinophils ($\times 10^9$/l)	0.15±0.11	0.09±0.15	0.20±0.19	0.25±0.12
Basophils ($\times 10^9$/l)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Lymphocytes ($\times 10^9$/l)	3.121±0.08	2.91±0.17	3.10±1.03	4.08±0.98
Monocytes ($\times 10^9$/l)	0.06±0.051	0.04±0.069	0.00±0.00	0.00±0.00
Female				
Erythrocytes ($\times 10^{12}$/l)	7.44±0.71	6.60±0.68	6.79±0.79	7.29±0.39
Hemoglobin (g/dl)	17.2±1.82	15.3±1.08	15.6±1.60	16.9±0.49
Hematocrit (%)	49.7±4.51	44.8±3.90	45.6±5.28	50.2±0.82
MCV (fl)	66.9±0.87	67.9±1.15	67.2±1.29	69.1±4.64
MCH (pg)	23.1±0.26	23.1±0.97	23.0±0.35	23.3±0.59
MCHC (g/dl)	34.5±0.81	34.1±1.22	34.2±0.80	33.7±1.54
Platelets ($\times 10^9$/l)	329±128	263±67.9	298±75.8	301±65.2
Reticulocytes (‰)	1.0±0.00	2.3±0.58	0.0±0.00	1.0±0.00
Leukocytes ($\times 10^9$/l)	11.6±0.83	20.3±1.34	10.9±2.42	10.1±0.83
Neutrophils ($\times 10^9$/l)	7.52±1.21	6.92±1.63	6.73±1.56	7.01±0.23
Eosinophils ($\times 10^9$/l)	0.19±0.12	0.13±0.04	0.28±0.29	0.06±0.06
Basophils ($\times 10^9$/l)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Lymphocytes ($\times 10^9$/l)	3.67±0.84	3.04±1.55	3.86±0.72	2.65±0.66
Monocytes ($\times 10^9$/l)	0.23±0.10	0.20±0.09	0.08±0.07	0.38±0.15

Values are presented as means±SD. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.
* and ** indicate significant difference at $p < 0.05$ and $p < 0.01$ levels, respectively, when compared with the control group.

6. Hematology

In male dogs, a significant increase in mean corpuscular hemoglobin concentrations was observed in all treatment groups compared with control dogs. In female dogs, no significant changes were observed (Table 3).

7. Serum biochemistry

In male dogs, no significant changes were observed. In female dogs, a significant increase in serum level of aspartate aminotransferase at 2 mg/kg, alanine aminotransferase at 50 mg/kg and a significant decrease in the serum level of triglyceride at 2, 10 and at 50 mg/kg was observed compared with control dogs (Table 4).

8. Gross findings

In male dogs at 50 mg/kg, white spot on the spleen was found in one male (data not shown). In all female dogs, no notable changes were observed.

9. Organ weights

In male dogs, no significant changes were observed. In female dogs, the absolute weight of right ovary was significantly lower at 10 mg/kg and at 50 mg/kg than that in control dogs (Table 5).

10. Histopathological findings

In males, inflammation of the lung, mineralization of the kidney and atrophy of the thymus were ob-

Table 4. Serum biochemical findings in female dogs treated with CJ-10882 for 4 weeks

Dose (mg/kg/day)	0	2	10	50
Aspartate aminotransferase (IU/l)	19.8±2.11	24.3±0.75*	19.5±1.65	19.4±1.70
Alanine aminotransferase (IU/l)	24.9±5.04	28.9±8.25	29.9±4.16	87.2±39.6*
Alkaline phosphatase (IU/l)	177±43.7	211±51.7	174±12.4	224±96.3
Blood urea nitrogen (mg/dl)	5.8±2.02	14.4±2.17	13.2±1.80	19.2±2.81
Creatinine (mg/dl)	0.83±0.06	0.77±0.15	0.86±0.13	0.95±0.19
Glucose (mg/dl)	99.1±2.51	97.3±0.23	98.0±2.35	104±11.7
Total cholesterol (mg/dl)	184±47.3	140±18.1	146±11.7	154±28.9
Total bilirubin (mg/dl)	0.01±0.01	0.02±0.01	0.04±0.02	0.06±0.04
Total protein (g/dl)	5.73±0.24	5.81±0.21	5.60±0.10	6.25±0.47
Albumin (g/dl)	3.50±0.09	3.42±0.17	3.59±0.24	4.00±0.20*
Creatine phosphokinase (IU/l)	205±51.6	200±24.0	213±74.3	140±19.7
Triglyceride (mg/dl)	60.1±11.9	39.8±3.11**	28.4±5.79**	38.3±6.85*
Calcium (mg/dl)	10.2±0.24	10.1±0.13	9.76±0.43	10.3±0.05
Inorganic phosphate (mg/dl)	5.79±0.46	5.61±0.38	5.31±0.70	5.58±0.35
Phospholipid (mg/dl)	395±68.7	309±36.0	317±21.4	356±56.5
Albumin/Globulin (ratio)	1.58±0.22	1.46±0.26	1.80±0.27	1.80±0.29
Sodium (nmol/l)	147±1.15	145±1.73	145±0.58	148±4.04
Potassium (nmol/l)	4.74±0.10	4.53±0.23	4.57±0.15	4.75±0.17
Chloride (nmol/l)	110±1.53	111±1.15	111±1.53	112±4.00

Values are presented as means±SD.
* and ** indicate significant difference at $p < 0.05$ and $p < 0.01$ levels, respectively, when compared with the control group.

Table 5. Absolute organ weights in female dogs treated with CJ-10882 for 4 weeks

Dose (mg/kg/day)	0	2	10	50
Body weight	10100±1930	9160±1590	9530±1100	9260±787
Spleen (g)	23.0±5.05	27.1±7.48	28.2±4.72	28.7±2.70
Liver (g)	283±10.8	262±46.7	269±11.2	249±15.6
Adrenal gland-left (g)	0.39±0.05	0.42±0.06	0.47±0.05	0.46±0.02
Adrenal gland-right (g)	0.40±0.11	0.45±0.07	0.50±0.08	0.46±0.02
Kidney-left (g)	20.6±0.82	22.3±1.37	21.1±1.26	23.5±0.91
Kidney-right (g)	20.2±0.87	21.4±1.01	21.7±2.07	22.7±1.19
Heart (g)	79.9±9.58	75.0±3.95	80.5±4.30	76.3±2.52
Lung (g)	75.8±10.7	81.2±12.1	83.1±3.48	78.7±0.62
Ovary-left (g)	0.88±0.63	0.36±0.10	0.40±0.02	0.34±0.04
Ovary-right (g)	0.55±0.18	0.36±0.07	0.32±0.04*	0.27±0.02*
Brain (g)	70.3±1.29	69.0±7.36	71.5±1.87	68.0±5.41
Pituitary gland (g)	0.06±0.01	0.05±0.01	0.05±0.02	0.05±0.01

Values are presented as means±SD.
*Indicates significant difference at $p < 0.05$ level when compared with the control group.

served at 2 mg/kg. Inflammation of the lung and liver, mineralization of kidney and atrophy of the thymus were observed at 10 mg/kg. Inflammation of the liver, pericholangitis, mineralization of the kidney, atrophy of the thymus were observed at 50 mg/kg. In females, atrophy of the thyroid gland, atrophy of the thymus were observed at 2 mg/kg. Inflammation of the lungs, liver and salivary gland, atrophy of the thyroid gland and the thymus, hyperplasias of the urinary bladder were observed at 10 mg/kg. Inflammation of the liver, atrophy of the thymus, mineralization of the kidney were observed at 50 mg/kg (Table 6).

IV. DISCUSSION

The present study was conducted to investigate the potential subacute toxicity of CJ-10882. It was administered orally to dogs at dose levels of 0, 2, 10 and 50 mg/kg/day for 4 weeks. Salivation and vomiting were observed in a dose dependent manner in the degree and frequency in the 10 and 50 mg/kg groups. PDE IV inhibitors are known to influence on the central nervous system and to cause vomiting. Because CJ-10882 is a PDE IV inhibitor, it is considered that it caused vomiting by influencing on the CNS. Consid-

Table 6. Histopathological findings in male and female dogs treated with CJ-10882 for 4 weeks

Dose (mg/kg/day)	Male				Female			
	0	2	10	50	0	2	10	50
Lung								
Inflammation	1	1	1	0	1	0	1	0
Liver								
Inflammation	0	0	1	1	0	0	1	1
Pericholangitis	0	0	0	1	0	0	0	0
Kidney								
Mineralization	1	1	1	1	1	0	0	2
Inflammation	0	0	2	0	0	0	0	0
Thymus								
Atrophy	2	1	3	3	1	3	2	3
Spleen								
Capsular hyperplasia	1	0	0	1	0	0	0	0
Thyroid gland								
Atrophy	0	0	0	0	0	1	1	0
Salivary gland								
Inflammation	0	0	0	0	1	0	1	0
Urinary bladder								
Hyperplasia	0	0	0	0	0	0	1	0

ering the report that salivation was observed in all the treatment groups when rolipram, a kind of PDE IV inhibitors, was repeatedly administered to rats for 2 weeks at dose levels of 10, 30 and 100 mg/kg, CJ-10882 is considered to induce salivation as other PDE IV inhibitors do. Body weight decreasing tendency observed in 50 mg/kg group of both sexes is caused by decrease of some food intake and stress resulted from vomiting and salivation. An increase in the urine volume in 50 mg/kg group of both sexes considered as a treatment -related toxic effect, but we could not conclude the exact cause. Increase in the MCHC value observed in the male 10 and 50 mg/kg groups was not recognized in the female groups and was within the normal physiological range of beagle dogs. Therefore, it was not considered as a treatment-related toxic effect (Wolford *et al.*, 1986). Also, a significant change of triglyceride observed in the female 10 and 50 mg/kg groups was within the normal physiological value of beagle dogs. White spot on the spleen observed in the male 50 mg/kg group and decrease of the absolute organ weights of the ovary in the female 10 and 50 mg/kg groups were considered as an accidental change because the histopathological changes of those organs were not observed. An Increase in the serum levels of ALT and albumin observed in the female 50 mg/kg group was considered as a toxic effect related to the test article since the histopathological change in related organs (liver) was accompanied although it was not observed in the male group with the same dose. Atrophy of the thymus in 50 mg/kg group accompanied by the depletion of the thymocytes was considered as a toxic effect related to the test article. It was reported that PDE IV inhibitors restrain various cytokine secretion and inhibit proliferation of the immune cells (Larson *et al.*, 1996). The inflammation of the lung, kidney, and salivary gland, pericholangitis, mineralization of kidney and capsular hyperplasia of the spleen had no dose-dependent relationship and could be observed often spontaneously (Turton and Hooson 1998). Therefore, these lesions are not considered as treatment-related toxic effects.

Based on these results, we concluded that CJ-10882 caused salivation and vomiting in 50 mg/kg group of both sexes. The absolute toxic dose of CJ-10882 was 50 mg/kg, and no observed adverse effect level was 10 mg/kg for both male and female dogs.

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