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Intravenous repeated-dose toxicity study of $ZnPcS_2P_2$-based-photodynamic therapy in beagle dogs

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Abstract

The purpose of current study was to investigate the potential repeated dosed toxicity of $ZnPcP_2S_2$-based photodynamic therapy (ZnPc-PDT) in Beagle dogs. Twenty-four Beagle dogs were randomly allocated to four groups, in which they were administered $ZnPcS_2P_2$ preparation intravenously at dosages of 0, 0.5, 1.5, and 4.5 mg/kg body weight and then were irradiated with 670 nm laser for 6 min at subsequent 48 and 72 h, once every four days for successively 10 times. There were no abnormal changes in clinical observations. After the administration most animals showed dysphoria and several had light edema on their faces. The results showed no mortality and no abnormity in ophthalmoscopy, electrocardiography, hematology, blood biochemistry and urinalysis, except that some statistical changes in specific indexes such as APTT in 1.5 and 4.5 mg/kg groups, and these changes disappeared at the end of recovery. Histopathological examination showed hepatic spotty and lytic necrosis in Beagle dogs of 4.5 mg/kg group, and at the end of recovery the damages alleviated. Additionally, some Kelly and Khaki granules presented in liver, spleen, lungs, kidneys, ovaries, lymph nodes, marrows and injection points. These drug pigmentations will provide valuable information about distribution of $ZnPcP_2S_2$. Based on the results; the NOAEL was considered to be 1.5 mg/kg and ZnPc-PDT appears to be a safety and promising approach in clinic.

Keywords: $ZnPcS_2P_2$; Photodynamic therapy (PDT); Repeated-dose toxicities; Dogs; Drug pigmentation; Intravenous

1. Introduction

Photodynamic therapy (PDT) is a well-investigated minimally invasive therapeutic modality for the treatment of neoplastic and vascular diseases. It has been applied clinically for the treatment of a variety of tumor types (Vrouenraets et al., 2003). In this approach, a photosensitizer is injected intravenously or intraperitoneally, and accumulated with more or less selectivity in tumors. Activation of a photosensitizer by laser light at specific wavelengths leads to the production of singlet oxygen and radical species, resulting in direct tumor cell killing, immune inflammatory responses, and damage to the microvasculature of the tumor (Pass, 1993). The main advantages of PDT include high degree selectivity of drug accumulation in tumor tissues, absence of systemic toxicity of the drug alone, and the possibility of treating multiple lesions simultaneously (Weishaupt et al., 1976).

During the past two decades, photosensitizer (a photosensitive dye) has attracted significant research efforts as a tool for photodynamic therapy (PDT) (Dougherty et al., 1998). Recently, the group of phthalocyanines (Pcs) has been used as photosensitizer for PDT and was found to be highly promising (Ali and Lier, 1999; Castano et al., 2005). Di-sulfo-di-phthalimidomethyl phthalolcyanine zinc ($ZnPcS_2P_2$) is a chelate formed by Pcs, and seems

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to have high phototoxicity (Ambroz et al., 1991; Berg et al., 1989). It shows superiority such as high quantum yields of singlet oxygen, minimal toxicity in the dark and a lack of pharmacological interactions with other drugs (Huang et al., 2000). ZnPc-PDT was developed as a novel anticancer method that could eliminate the residual leukemic cells in normal mononuclear cells (MNCs), which plays an important role in autologous high-dose chemotherapy supported by hematopoietic stem cells transplantation (HSCT). Autologous HSCT is a promising approach to leukemic therapy but it also has the risk of reinfusing residual leukemic cells that lead to relapse. It has been reported that human leukemic cell lines exhibit greater photosensitizer uptake and higher susceptibility to PDT than normal bone marrow MNCs (Dazino et al., 1998; Villeneuve, 1999; Hrkal et al., 2002). ZnPc-PDT can eliminate the residual leukemic cells in normal MNCs and appears to be a perspective means for purging (Huang et al., 2005). In this study, $ZnPcS_2P_2$ was investigated as a new amphipathic photosensitizer used in PDT purging technique. Although it accumulates with high degree selectivity in leukemic cells, it also concentrates in normal tissues (Jori, 1996). The literature pertaining to repeated-dose toxicity of ZnPc-PDT (even any other metal phthalocyanines) is absent. For clinical purpose, the safety evaluation of ZnPc-PDT has been conducted in repeated-dose toxicity study in Beagle dogs through intravenous administration. The study was performed following the Good Laboratory Practice Guidelines.

2. Materials and methods

2.1. Test article

$ZnPcS_2P_2$ used in this study was a gift from Department of Chemistry, Institute of Research on Functional Materials, Fuzhou University (China). It is an odorless cyan liquid, insoluble in water. The liquid was identified to have a chemical purity of more than 95.0% via gas chromatography and infrared spectroscopy. $ZnPcS_2P_2$ was dissolved in a solution containing Cremophor EL 2% (V/V), propylene glycol 20% (V/V), NaCl 0.9% (W/W), to make a stock solution and was stored in the dark at 4 °C. A 150 μg/ml solution of $ZnPcS_2P_2$ was prepared for dosing at 4.5 mg/kg. This 150 μg/ml dosing solution was further diluted to prepare a 50 μg/ml solution for dosing at 1.5 mg/kg and a 16.67 μg/ml solution for dosing at 0.5 mg/kg. These dosing solutions were prepared immediately before use.

2.2. Animals and husbandry

The study was conducted on 24 Beagle dogs, half male and half female, obtained from Institute of Laboratory Animal Science, Peking Union Medical College & Chinese Academy of Medical Sciences (Beijing, China). The dogs were about 6–8 months in age and weighed ranging from 6.1 to 7.8 kg. They were clinically examined for general health and parasite infestation for one week prior to treatment. Then the animals were allocated to dosage groups by computerized random selection, according to body weight means and homogeneous variances of group body weight. The animals were housed individually in cages and allowed free access to solid chow and tap water. The animal room was maintained at 23 ± 5 °C temperature and $50 \pm 10\%$ relative humidity with a 12-h light–dark cycle (lights on at 7:00 a.m.) and was ventilated automatically.

2.3. Study design and dose selection

Three dogs per sex were randomly allocated to the following groups: $ZnPcS_2P_2$ 0.5, 1.5 and 4.5 mg/kg groups and a vehicle (0 mg/kg, control) group. $ZnPcS_2P_2$ preparation or vehicle was injected via the forelimb vein at an infusion rate of 25–35 ml/min once every four days for successively 10 times under unanesthetized condition. The dosing volume was 30 ml/kg in all groups. The injection sites of all animals were photo-irradiated for 6 minutes using SDS-DL3 laser (China) emitting red light at 670 nm at 48 and 72 h after intravenous administration. The power of the laser was 200 mW, and the energy density at the illumination area was 7.0 J/cm^2. Two thirds of the animals, half males and females, were sacrificed under pentobarbital anesthesia at the end of treatment period on Day 42, and the rest one third were examined after two- week recovery period on Day 56.

The dosages selected in the present study were based on data about possible clinical therapeutic application and the results of an acute toxicity study of ZnPc-PDT on mice. In the acute toxicity study, the LD_{50} was considered to be 52 mg/kg, and 95% confident interval was from 35 to 65 mg/kg. In clinical therapeutic plan, $ZnPcS_2P_2$ is intended to be given through intravenous infusion at dose of 0.04 mg/kg/day. In the present study, 4.5 mg/kg was specified as the highest dose level, and lower doses of 1.5 and 0.5 mg kg, which were respectively 112.5, 37.5 and 12.5 times as clinical application dosage.

2.4. Clinical observation

Clinical signs were assessed and recorded twice daily, before and after administration or irradiation. Body weight was measured once a week and daily food consumption was calculated by measuring the amount of unconsumed food every day. Temperature was recorded weekly.

Ophthalmoscopic examinations were performed on each dog before the start of treatment (Day 1) and after the last dosing and irritation (Day 42), and after recovery (Day 56), using a binocular indirect ophthalmoscpe (Topcon, Japan). The observation areas included cornea, conjunctiva, sclera, iris, lens and fundus. Electrocardiograms were conducted on Day 1, Day 42, and Day 56 of the study duration, for collecting the data on heart rate, P–R interval, QRS interval and QT value.

2.5. Laboratory testing parameters

Blood and urine samples were collected from all animals on Day 1, Day 42 and Day 56 of the study duration. All dogs were fasted for more than 12 h prior to blood/urine sample obtained. Blood samples were obtained from forlimb vena into evacuated blood collection tubes. Under anaesthetic condition, urine was collected from bladders onto a specimen test paper through a catheter. EDTA and sodium citrate were used as anticoagulants for blood coagulation study. The hematological parameters were assessed using an MEK-6318K Automated Hematology Analyzer (Nihon-Kodhen Co., Tokyo, Japan); including erythrocyte counts (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cells distribution width-variation coefficient (RDW-CV), platelets count (PL), mean platelet volume (MPV), platelet distribution width (PDW), leukocyte counts (WBC) and differential cell count (DC). Blood smears were stained with Wrigh-Giemsa brilliant-cresyl-blue (Heath and Daland, 1931), and the reticulocyte count (Reti) was numbered under light microscopy. The coagulation parameters including thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (Fbg) were evaluated using a DIAGNOSTICA STAGO STA-4 Coagmaster (Junior Instruments Co., Gennevilliers, France). Blood chemistry parameters were determined using a Vitalab Autoanalyzer (Vital Scientific, Dieren, Netherlands) and an AVL-9181 Autoelectrolyte Analyzer (AVL Scientific Co., Roswell, Georgia, USA). The parameters measured were: aspartate aminotransferase (AST), alanine aminotransferase

(ALT), alkaline phosphatase (ALP), glucose (GLU), blood urea nitrogen (BUN), total protein (TP), albumin (ALB), albumin–globulin ratio (A/G), total bilirubin (T-BIL), total cholesterol (T-CHO), triglycerides (TG), creatine kinase (CK), uric acid (UA), lactate dehydrogenase (LDH), serum levels of sodium (Na), potassium (K), and chloride (Cl). Urinalysis were conducted using Multistrix® Strips (Bayer Corp., Bridgend, South Wales, UK), for parameters including glucose (GLU), bilirubin (BIL), ketone (KET), specific gravity (SG), occult blood (BLO), PH, protein (PRO), urobilinogen (URO), nitrite (NIT), and leukocyte (LEU).

2.6. Histopathological examination

Two thirds of the animals, half male and half female, were sacrificed under ether anesthesia at the end of treatment period, and the rest one-third were sacrificed at the end of recovery period. The following organs were removed and weighted: brain, thyroid gland (including parathyroid glands), lungs, heart, liver, spleen, kidneys, adrenal glands, prostate gland, testes, uterus and ovaries. The relative weight (weight per 100 g body weight) of each organ was calculated. The above organs and thymus, sternum and bone marrow, spinal cord, pituitary gland, esophagus, stomach, intestines (duodenum, jejunum, ileum, and colon), epididymides, lymph nodes (mesenteric), urinary bladder, trachea, skeletal muscle, and optic nerves were fixed in 10% buffered formalin solution. The samples of these organs were Paraffen-embedded, cut at a nominal thickness of 5 μm and then stained with hematoxylin and eosin for histopathological examination.

2.7. Statistical analysis

The experimental groups were compared against the control group in terms of body weight, food consumption, hematological parameters (except for differential leukocyte count and reticulocyte count), blood chemistry values, and organ weights. If the data were found to be homogenous by Bartlett's test (Bartlett, 1937), one-way analysis of variance (ANOVA) was employed. If the data were not homogeneous (and for the differential leukocyte count and reticulocyte count), Kruskal–Wallis's test (Kruskal and Wallis, 1952) was employed. The parameters found to be significant in the one-way analysis of variance (ANOVA) were assessed by the Dunnett test (Dunnett, 1955, 1964) Percentage and ratio data in urialysis were evaluated by χ^2-test. Levels of significance for pair-wise comparisons were reported at $P < 0.05$ or $P < 0.01$ in this study.

3. Results

3.1. Clinical observations

All Beagle dogs survived until the scheduled necropsy. Most animals showed signs of dysphoria after administration, with several dogs having light edema on their faces, however, these clinical signs resolved by the following morning. No other abnormal clinical signs were found. During the administration period, no significant suppression of body weight gain was observed in $ZnPcS_2P_2$ groups (Table 1). Food consumption of the $ZnPcS_2P_2$ groups had no statistical difference with that of control group. There was no significant change in body temperature, and the numerical values of their temperature were in normal range (Table 2). The ophthalmoscopic and electrocardiographic examinations did not indicate any treatment-related adverse effects (Table 3).

Table 1
Body weight in Beagle dogs throughout the study

Day	Number of dogs	Dose (mg/kg)			
		0	0.5	1.5	4.5
D_{-18}	6	6.8 ± 0.7	6.9 ± 0.9	6.9 ± 0.7	6.7 ± 0.6
D_{-11}	6	7.2 ± 0.8	7.3 ± 1.0	7.4 ± 0.8	7.1 ± 0.7
D_1	6	7.7 ± 0.8	7.7 ± 0.9	7.8 ± 0.8	7.6 ± 0.6
D_7	6	8.2 ± 0.8	8.2 ± 1.0	8.3 ± 0.8	8.0 ± 0.6
D_{14}	6	8.3 ± 1.0	8.4 ± 1.2	8.4 ± 0.9	7.7 ± 0.8
D_{21}	6	8.3 ± 1.0	9.0 ± 1.4	8.4 ± 0.8	7.3 ± 0.5
D_{28}	6	9.7 ± 1.2	9.8 ± 0.8	9.0 ± 1.1	10.0 ± 0.0
D_{35}	6	9.3 ± 1.5	9.3 ± 1.0	8.5 ± 1.2	8.8 ± 0.8
D_{39}	6	9.2 ± 1.3	9.3 ± 1.0	8.8 ± 1.2	8.7 ± 0.8
D_{42}	6	10.0 ± 1.4	10.5 ± 0.7	10.0 ± 0.0	9.5 ± 0.7
D_{49}	2	10.0 ± 1.4	11.5 ± 0.7	10.0 ± 1.4	11.0 ± 0.0
D_{53}	2	10.0 ± 1.4	11.5 ± 0.7	10.0 ± 1.4	11.0 ± 0.0

Note: The data represent $\bar{x} \pm s$.

Table 2
Temperature in Beagle dogs throughout the study

Day	Number of dogs	Dose (mg/kg)			
		0	0.5	1.5	4.5
D_{-18}	6	39.1 ± 0.4	39.0 ± 0.3	39.0 ± 0.3	39.3 ± 0.2
D_{-11}	6	39.1 ± 0.4	39.3 ± 0.4	38.9 ± 0.4	39.0 ± 0.3
D_1	6	39.0 ± 0.3	39.3 ± 0.2	39.1 ± 0.3	39.0 ± 0.3
D_7	6	39.2 ± 0.1	39.3 ± 0.2	39.0 ± 0.2	39.1 ± 0.2
D_{14}	6	39.1 ± 0.3	39.1 ± 0.4	38.9 ± 0.1	38.9 ± 0.2
D_{21}	6	39.2 ± 0.2	39.2 ± 0.3	38.9 ± 0.4	39.2 ± 0.3
D_{28}	6	39.2 ± 0.2	39.1 ± 0.3	39.0 ± 0.2	39.2 ± 0.2
D_{35}	6	39.0 ± 0.2	39.1 ± 0.4	39.2 ± 0.3	39.2 ± 0.3
D_{39}	6	39.0 ± 0.3	39.0 ± 0.3	38.5 ± 0.3	38.8 ± 0.4
D_{42}	6	39.1 ± 0.1	39.3 ± 0.3	38.7 ± 0.1	38.9 ± 0.2
D_{49}	2	38.9 ± 0.1	38.6 ± 0.1	38.8 ± 0.3	38.7 ± 0.2
D_{53}	2	39.1 ± 0.1	38.8 ± 0.3	38.8 ± 0.4	38.8 ± 0.3

Note: The data represent $\bar{x} \pm s$.

3.2. Hematology, blood chemistry, and urinalysis

Hematological examinations were performed four times totally, two times before the start of treatment, one after last dosing and irradiation (Day 42) and one after the end of recovery (Day 56). Before the start of treatment, there were some minor statistical changes between groups but the changes were within normal ranges. On Day 42 and Day 56, there were no statistical differences between $ZnPcS_2P_2$ groups and control group. (Tables 4 and 5). Blood chemistry tests revealed no abnormality in all examined groups and no differences between groups were found (Tables 6–8). The coagulation tests at the end of dosing and irradiation revealed that APTT had a tendency toward decrease, and the high-dose group showed statistical difference with control group. There was also a tendency toward decrease of the value of PT (Table 9). In urinalysis, protein index was positive in all groups. In urinalysis tests, the dogs were under anaesthetic condition, and some animals showed dysphoria and moved, which probably affected the results. Additionally, no statistical differences showed

Table 3
Electrocardiographic parameters in Beagle dogs throughout the study

Date	0 mg/kg	0.5 mg/kg	1.5 mg/kg	4.5 mg/kg
First pretest				
Rate	156.5 ± 25.3	159.3 ± 19.3	143.3 ± 24.1	133.7 ± 18.8
P–R interval (s)	0.080 ± 0.006	0.083 ± 0.004	0.088 ± 0.013	0.082 ± 0.004
QRS interval (s)	0.043 ± 0.001	0.049 ± 0.009	0.043 ± 0.001	0.044 ± 0.002
QT (s)	0.181 ± 0.012	0.188 ± 0.015	0.177 ± 0.014	0.196 ± 0.007
Second pretest				
Rate	163.0 ± 26.1	153.0 ± 22.0	139.8 ± 27.3	156.2 ± 19.8
P–R interval (s)	0.080 ± 0.003	0.084 ± 0.008	0.080 ± 0.003	0.084 ± 0.008
QRS interval (s)	0.045 ± 0.002	0.044 ± 0.002	0.043 ± 0.001	0.045 ± 0.003
QT (s)	0.192 ± 0.005	0.186 ± 0.015	0.193 ± 0.009	0.199 ± 0.003
Day42				
Rate	131.7 ± 18.6	151.7 ± 23.4	149.7 ± 26.4	170.0 ± 36.4
P–R interval (s)	0.091 ± 0.010	0.087 ± 0.018	0.089 ± 0.016	0.088 ± 0.013
QRS interval (s)	0.044 ± 0.002	0.045 ± 0.004	0.046 ± 0.003	0.047 ± 0.002
QT (s)	0.193 ± 0.008	0.196 ± 0.003	0.194 ± 0.004	0.195 ± 0.005
Day56				
Rate	103 ± 30	116 ± 14	125 ± 26	130 ± 26
P–R interval (s)	0.082 ± 0.003	0.078 ± 0.008	0.098 ± 0.020	0.078 ± 0.003
QRS interval (s)	0.068 ± 0.011	0.044 ± 0.006	0.070 ± 0.042	0.074 ± 0.003
QT (s)	0.240 ± 0.000	0.230 ± 0.014	0.204 ± 0.011	0.202 ± 0.025

Note: The data represent $\bar{x} \pm s$.

Table 4
Hematological parameters in Beagle dogs before the study ($\bar{x} \pm s$)

Date	0 mg/kg	0.5 mg/kg	1.5 mg/kg	4.5 mg/kg
First pretest				
WBC (10^9/L)	15.4 ± 3.0	14.5 ± 4.5	19.8 ± 8.3	16.7 ± 3.8
RBC (10^{12}/L)	6.41 ± 1.47*	4.99 ± 0.47	5.84 ± 0.75	6.41 ± 0.55
HGB (g/L)	140 ± 33	111 ± 12	123 ± 14	143 ± 12
HCT (%)	45.8 ± 10.9*	36.2 ± 3.8	39.8 ± 4.8	46.3 ± 3.5
MCV (Fl)	71.3 ± 1.5	72.5 ± 2.1	68.4 ± 3.7	72.3 ± 2.6
MCH (pg)	21.9 ± 0.4	22.3 ± 0.5	21.2 ± 1.4	22.2 ± 0.7
MCHC (g/L)	307 ± 7	308 ± 5	310 ± 5	308 ± 5
RDW-CV (%)	14.6 ± 0.8	15.5 ± 0.8	14.9 ± 0.7	14.6 ± 1.3
PLT (10^9/L)	233 ± 89	289 ± 97	284 ± 51	221 ± 87
MPV (FL)	7.9 ± 1.2	8.4 ± 0.9	7.7 ± 0.8	8.3 ± 0.4
PDW (10/GSD)	17.5 ± 0.9	17.1 ± 0.2	17.4 ± 0.6	17.0 ± 0.8
PCT (mL/L)	0.182 ± 0.082	0.233 ± 0.07	0.212 ± 0.043	0.173 ± 0.064
LYM (%)	28.6 ± 7.6	20.4 ± 6.3*	19.1 ± 3.4*	14.3 ± 3.1**
MO (%)	9.4 ± 7.2	11.7 ± 8.3	14.0 ± 4.7	8.2 ± 4.3
GRAN (%)	62.0 ± 5.7	67.9 ± 14.0	67.0 ± 5.6	77.5 ± 6.3*
Reti (‰)	3.0 ± 1.4	2.5 ± 1.0	3.0 ± 1.8	3.2 ± 1.0
Second pretest				
WBC (10^9/L)	19.3 ± 3.4	14.2 ± 2.1**	18.4 ± 2.4	14.8 ± 2.7*
RBC (10^{12}/L)	5.21 ± 0.42	5.15 ± 0.33	5.27 ± 0.52	5.08 ± 0.42
HGB (g/L)	112 ± 8.6	109 ± 7	112 ± 7	108 ± 8
HCT (%)	38.3 ± 3.1	36.9 ± 2.4	37.8 ± 3.0	36.4 ± 2.9
MCV (Fl)	73.6 ± 2.5	71.6 ± 1.4	71.9 ± 2.0	71.3 ± 1.7
MCH (pg)	21.5 ± 1.0	21.3 ± 0.6	21.3 ± 0.8	21.2 ± 0.7
MCHC (g/L)	292 ± 6	297 ± 9	296 ± 6	295 ± 5
RDW-CV (%)	14.2 ± 0.6	14.2 ± 0.8	14.1 ± 0.7	14.1 ± 0.7
PLT (10^9/L)	319 ± 75	388 ± 128	407 ± 106	322 ± 124
MPV (FL)	8.6 ± 1.3	7.1 ± 1.5	8.2 ± 1.5	8.4 ± 2.1
PDW (10/GSD)	16.8 ± 0.8	17.4 ± 0.8	17.0 ± 0.5	17.0 ± 1.1
PCT (mL/L)	0.263 ± 0.061	0.272 ± 0.117	0.327 ± 0.086	0.248 ± 0.064
LYM (%)	18.9 ± 9.8	24.4 ± 7.9	26.8 ± 10.5	18.8 ± 5.8
MO (%)	8.3 ± 4.2	11.0 ± 3.8	11.2 ± 3.5	8.6 ± 3.7
GRAN (%)	72.9 ± 13.8	64.6 ± 8.8	62.0 ± 12.1	72.5 ± 8.5
Reti (‰)	3.3 ± 1.0	2.8 ± 0.8	3.2 ± 1.2	3.2 ± 0.8

Note: $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 5
Hematological parameters in Beagle dogs throughout the study ($\bar{x} \pm s$)

Date	0 mg/kg	0.5 mg/kg	1.5 mg/kg	4.5 mg/kg
Day42				
WBC (10^9/L)	17.6 ± 7.1	18.6 ± 5.9	14.3 ± 2.1	19.0 ± 6.3
RBC (10^{12}/L)	6.00 ± 1.33	6.57 ± 2.74	6.43 ± 1.57	6.72 ± 1.38
HGB (g/L)	132 ± 27	145 ± 60	140 ± 33	143 ± 32
HCT (%)	44.4 ± 8.8	48.8 ± 20.5	45.9 ± 10.7	46.6 ± 9.3
MCV (Fl)	74.3 ± 2.2	74.3 ± 0.8	71.6 ± 2.9	69.5 ± 2.8*
MCH (pg)	22.1 ± 0.8	22.0 ± 0.3	21.8 ± 0.9	21.36 ± 1.2
MCHC (g/L)	298 ± 7	296 ± 4	305 ± 5	307 ± 9
RDW-CV (%)	15.1 ± 0.5	15.5 ± 0.6	14.8 ± 0.5	14.7 ± 1.0
PLT (10^9/L)	299 ± 70	332 ± 41	343 ± 74	323 ± 70
MPV (FL)	7.5 ± 1.1	8.3 ± 0.6	7.1 ± 0.7	6.8 ± 0.7
PDW (10/GSD)	17.4 ± 0.6	16.9 ± 0.5	17.3 ± 0.3	17.3 ± 0.2
PCT (mL/L)	0.218 ± 0.056	0.272 ± 0.044	0.235 ± 0.050	0.208 ± 0.043
LYM (%)	35.2 ± 3.2	29.5 ± 10.3	36.9 ± 7.0	39.0 ± 3.5
MO (%)	9.7 ± 3.8	9.1 ± 1.5	8.6 ± 3.0	8.9 ± 4.3
GRAN (%)	55.1 ± 2.1	61.5 ± 10.9	54.5 ± 4.6	52.1 ± 4.3
Reti (‰)	3.0 ± 0.9	3.2 ± 0.8	2.8 ± 0.8	3.2 ± 1.2
Day56				
WBC (10^9/L)	10.8 ± 1.0	10.7 ± 0.0	12.8 ± 5.5	9.2 ± 0.8
RBC (10^{12}/L)	6.48 ± 0.86	7.03 ± 2.28	6.38 ± 1.84	6.21 ± 0.08
HGB (g/L)	153 ± 21	171 ± 51	152 ± 30	150 ± 4
HCT (%)	38.9 ± 4.7	42.9 ± 12.4	37.7 ± 8.1	37.5 ± 0.4
MCV (Fl)	60.1 ± 0.7	61.4 ± 2.2	59.7 ± 4.5	60.3 ± 0.3
MCH (pg)	23.6 ± 0.1	24.5 ± 0.6	24.1 ± 2.2	24.2 ± 0.4
MCHC (g/l)	392 ± 6	398 ± 3	404 ± 6	401 ± 8
RDW-CV (%)	14.7 ± 0.5	14.4 ± 0.5	15.5 ± 0.1	13.9 ± 0.4
PLT (10^9/L)	215 ± 43	209 ± 39	209 ± 98	268 ± 66
MPV (FL)	6.8 ± 0.1	6.1 ± 1.6	6.2 ± 0.5	6.8 ± 0.4
PDW (10/GSD)	17.3 ± 0.5	17.6 ± 0.1	18.0 ± 0.14	17.6 ± 0.6
PCT (mL/L)	0.140 ± 0.028	0.120 ± 0.014	0.125 ± 0.050	0.180 ± 0.057
LYM (%)	20.7 ± 0.2	28.6 ± 3.7	23.3 ± 9.1	26.1 ± 9.8
MO (%)	9.8 ± 0.1	9.6 ± 3.0	9.1 ± 2.4	8.1 ± 5.0
GRAN (%)	69.6 ± 0.4	61.9 ± 0.8	67.7 ± 11.5	65.9 ± 4.7
Reti (‰)	3.5 ± 0.7	4.0 ± 1.4	3.0 ± 1.4	4.0 ± 1.1

Note: $^*P < 0.05$.

within $ZnPcS_2P_2$ groups and control group, so we concluded that the abnormal founding in proteinuria test were resulted from our laboratory manipulation, not directly related with $ZnPcS_2P_2$ treatment. At the end of dosing and irradiation, SG showed statistical differences between $ZnPcS_2P_2$ groups and control group, but the absolute values of SG varied mildly, and at the end of recovery the differences disappeared (Table 10).

3.3. Organ weight

With regard to absolute and relative organ weights, there were no statistical changes in females in the study (Table 11). Notable increases in heart, kidney, and adrenal gland were observed in males in $ZnPcS_2P_2$ groups, but the relative organ weights showed no statistical differences and there was no relationship noted between the dosing levels and the increase of these organs (Table 12). At the end of recovery, there was no notable difference in absolute and relative organ weights, both in males and females (Tables 13 and 14).

3.4. Gross and microscopic pathology

At the necropsy after last dosing and irradiation, no abnormality showed in organs examined except that in 1.5 mg/kg group, two dogs' livers turned grey, their edge turned blunt and swelled mildly. At the end of recovery, the dogs examined showed no abnormality. In $ZnPcS_2P_2$ groups, lymph nodes and injection sites showed green color, and in high dose group, the livers and kidneys also turned to green, and it was more remarkable in females.

In microscopic examination, no abnormality was found in 0.5 and 1.5 mg/kg $ZnPcS_2P_2$ groups after the last dosing and irradiation. In 4.5 mg/kg $ZnPcS_2P_2$ group, hepatic spotty and lytic necroses were observed. At the end of recovery, the hepatic spotty and lytic necroses in high dose group were also seen, but alleviated. Some Kelly and khaki granules were presented in Kupffer cells and endothelial cells of livers, epithelia of renal tubules, marginal sinus and medulla of spleens, alveolar walls of lungs, reticular cells and macrophages of mesenteric lymph nodes, and follicular cells of ovaries from all or most of

Table 6
Clinical chemistry parameters in Beagle dogs before the study ($\bar{x} \pm s$)

Date	0 mg/kg	0.5 mg/kg	1.5 mg/kg	4.5 mg/kg
First pretest				
GPT (U/L)	38.0 ± 4.9	28.2 ± 5.4*	29.2 ± 4.1*	34.8 ± 6.3
GOT (U/L)	40.8 ± 6.6	35.0 ± 3.8	37.0 ± 3.7	36.5 ± 8.4
TP (g/L)	67.5 ± 3.5	58.3 ± 2.2**	59.9 ± 3.7**	60.3 ± 1.7**
ALB (g/L)	33.4 ± 1.0	29.9 ± 0.8**	30.4 ± 0.9**	30.9 ± 1.0**
TBIL (μmol/L)	4.70 ± 1.87	3.34 ± 0.81	4.65 ± 1.06	4.56 ± 0.92
ALP (U/L)	60.7 ± 26.4	136.8 ± 47.5*	127.2 ± 22.9*	138.3 ± 58.5*
CK (U/L)	245.7 ± 11.5	370.0 ± 27.0*	395.2 ± 81.9**	323.3 ± 110.1
LDH (U/L)	140.3 ± 21.2	200.2 ± 46.0	240.5 ± 70.3*	212.2 ± 66.1
GLU (mmol/L)	3.76 ± 0.99	4.10 ± 0.54	4.01 ± 0.56	4.09 ± 0.54
BUN (mmol/L)	5.98 ± 0.91	4.06 ± 0.44	6.35 ± 2.71	5.73 ± 1.53
UA (μmol/L)	21.2 ± 6.7	14.8 ± 6.2	29.4 ± 22.1	19.8 ± 10.5
TG (mmol/L)	0.38 ± 0.07	0.46 ± 0.10	0.36 ± 0.05	0.36 ± 0.11
A/G	0.98 ± 0.09	1.05 ± 0.10	1.03 ± 0.10	1.04 ± 0.06
CHO (mmol/L)	3.43 ± 0.29	4.09 ± 0.43	3.71 ± 0.44	3.69 ± 0.83
CRE (μmol/L)	86.6 ± 14.0	74.5 ± 7.5	67.8 ± 10.8*	86.3 ± 13.8
Second pretest				
GPT (U/L)	35.7 ± 6.6	24.2 ± 3.9*	30.7 ± 9.9	35.7 ± 6.6
GOT (U/L)	39.5 ± 8.2	36.5 ± 3.9	41.7 ± 4.8	41.7 ± 4.7
TP (g/L)	64.1 ± 3.6	61.4 ± 6.3	63.3 ± 4.1	67.6 ± 3.9
ALB (g/L)	32.6 ± 1.6	29.8 ± 0.8**	30.9 ± 0.5	32.4 ± 1.6
TBIL (μmol/L)	6.08 ± 0.29	4.84 ± 0.67**	5.58 ± 0.40	6.13 ± 0.91
ALP (U/L)	74.8 ± 29.9	95.5 ± 27.3	102.5 ± 26.1	165.8 ± 167.7
CK (U/L)	251.8 ± 89.8	329.2 ± 122.1	428.7 ± 56.2**	275.2 ± 51.8
LDH (U/L)	172.8 ± 65.5	282.2 ± 110.9	338.7 ± 90.3*	283.0 ± 90.6
GLU (mmol/L)	3.70 ± 0.58	3.77 ± 0.78	3.54 ± 0.80	2.53 ± 0.75*
BUN (mmol/L)	6.03 ± 1.03	4.75 ± 1.16	5.92 ± 1.26	5.26 ± 1.25
UA (μmol/L)	25.2 ± 9.7	22.3 ± 13.0	22.0 ± 8.5	35.1 ± 12.1
TG (mmol/L)	0.41 ± 0.06	0.48 ± 0.10	0.39 ± 0.08	0.45 ± 0.11
A/G	1.03 ± 0.07	0.98 ± 0.22	0.96 ± 0.11	0.92 ± 0.10
CHO (mmol/L)	3.51 ± 0.41	4.44 ± 0.78	3.56 ± 0.67	3.80 ± 0.93
CRE (μmol/L)	86.6 ± 14.0	74.5 ± 7.5	67.8 ± 10.8*	86.3 ± 13.8

Note: $^{*}P < 0.05$, $^{**}P < 0.01$.

animals in ZnPc-PDT groups. And the degree and range of the $ZnPcS_2P_2$ pigmentation showed a direct relationship with dose levels. No abnormal change was found in control group.

4. Discussion

Over the past few decades, PDT has been applied to treat a number of oncological diseases. Currently, over 30 different photosensitizers are used in preclinical studies, almost all of which are porphyrin derivatives, phthalocyanines, and chlorins (Allison et al., 2004). $ZnPcS_2P_2$ belongs to Phthalocyanines family and it is a recently developing second-generation sensitizers. ZnPc-PDT can induce mitochondria-dependent apoptosis in leukemic HL60 cells, K562 cells and EL9611 cells, so it is promising to be employed to purge remission marrow of residue leukemic cells before autologous hematopoietic stem cell transplantation (Auto-HSCT) (Huang et al., 2006). The current study assessed the potential repeated-dose toxicity of ZnPc-PDT in Beagle dogs.

The intravenous administration of $ZnPcS_2P_2$ preparation at dose levels of 0, 0.5, 1.5, and 4.5 mg/kg/day and photo-irritation 48 and 72 h later to beagle dogs in this 10 times repeated-dose toxicity study was well tolerated and no mortality was observed. A few minor changes observed in hematological, blood chemistry and urinalyses parameters were considered not of toxicological significance. The values of APTT and PT in $ZnPcS_2P_2$ groups had a tendency toward reduction, which suggested that the coagulation system may be affected by the treatment. When in clinic, the state of coagulation system should be monitored, and attentions should be paid for the safety of the patients' who have hematological diseases.

At the end of treatment period, there were green, yellow-green, and khaki granules in the livers, spleens, lungs, kidneys, ovaries, lymph nodes, sternums and marrows in $ZnPcS_2P_2$ groups. The amount of granules had a dose-effect relationship with dose levels. In lung alveolar walls, there were many $ZnPcS_2P_2$ pigments, and foreign body derived granuloma were observed. In high-dose group, the animals showed hepatic spotty and lytic necrosis. At the end of recovery, hepatic spotty lytic necrosis was still

Table 7
Clinical chemistry parameters in Beagle dogs throughout the study ($\bar{x} \pm s$)

Date	0 mg/kg	0.5 mg/kg	1.5 mg/kg	4.5 mg/kg
Day42				
GPT (U/L)	41.2 ± 4.3	33.8 ± 4.8	44.0 ± 13.0	35.5 ± 5.3
GOT (U/L)	36.8 ± 6.5	38.3 ± 8.0	33.5 ± 5.9	33.2 ± 5.8
TP (g/L)	62.8 ± 4.6	62.6 ± 3.4	63.0 ± 5.2	61.7 ± 4.2
ALB (g/L)	31.8 ± 1.1	31.8 ± 1.5	31.4 ± 1.3	31.5 ± 0.5
TBIL (μmol/L)	3.59 ± 0.39	3.54 ± 0.33	4.03 ± 0.42	4.26 ± 0.90
ALP (U/L)	115.8 ± 56.7	130.8 ± 50.2	104.0 ± 55.1	136.0 ± 38.8
CK (U/L)	231.3 ± 117.6	261.0 ± 125.2	233.2 ± 113.5	192.5 ± 62.9
LDH (U/L)	118.3 ± 48.9	106.3 ± 37.6	93.0 ± 17.9	93.7 ± 56.5
GLU (mmol/L)	4.39 ± 1.13	4.89 ± 0.67	4.83 ± 0.31	5.06 ± 0.21
BUN (mmol/L)	5.01 ± 0.82	6.02 ± 1.86	5.69 ± 2.14	5.58 ± 1.33
UA (μmol/L)	10.5 ± 1.0	9.6 ± 3.7	11.0 ± 3.7	9.7 ± 4.6
TG (mmol/L)	0.34 ± 0.10	0.42 ± 0.05	0.29 ± 0.04	0.47 ± 0.22
A/G	1.03 ± 0.11	1.05 ± 0.15	1.01 ± 0.13	1.06 ± 0.14
CHO (mmol/L)	3.46 ± 0.24	3.68 ± 0.65	3.52 ± 0.47	3.79 ± 1.89
CRE (μmol/L)	92.4 ± 11.4	92.8 ± 12.4	94.1 ± 13.5	96.5 ± 8.4
Day56				
GPT (U/L)	48.0 ± 1.4	37.0 ± 12.7	45.5 ± 6.4	45.5 ± 6.4
GOT (U/L)	49.5 ± 3.5	48.0 ± 7.1	43.5 ± 7.8	45.0 ± 1.4
TP (g/L)	64.4 ± 2.1	71.9 ± 5.4	63.9 ± 5.1	70.3 ± 0.3
ALB (g/L)	33.4 ± 2.2	33.4 ± 0.8	33.0 ± 2.1	34.2 ± 0.2
TBIL (μmol/L)	4.14 ± 1.12	6.90 ± 3.11	4.58 ± 0.35	5.61 ± 1.00
ALP (U/L)	171.0 ± 66.5	99.5 ± 24.7	107.0 ± 26.9	64.0 ± 5.7
CK (U/L)	315.5 ± 17.7	274.5 ± 112.4	210.0 ± 17.0	215.5 ± 14.8
LDH (U/L)	273.5 ± 46.0	414.5 ± 152.0	261.5 ± 62.9	384.0 ± 36.8
GLU (mmol/L)	3.29 ± 0.39	3.56 ± 0.41	3.81 ± 0.03	3.37 ± 0.17
BUN (mmol/L)	5.80 ± 2.21	8.13 ± 0.69	6.49 ± 1.45	7.96 ± 0.10
UA (μmol/L)	18.2 ± 7.7	21.5 ± 10.7	17.2 ± 8.8	16.3 ± 5.7
TG (mmol/L)	0.37 ± 0.08	0.36 ± 0.18	0.34 ± 0.03	0.28 ± 0.06
A/G	1.07 ± 0.07	0.87 ± 0.08	1.06 ± 0.04	0.94 ± 0.01
CHO (mmol/L)	3.84 ± 0.55	5.10 ± 2.54	3.80 ± 0.33	3.99 ± 0.81
CRE (μmol/L)	99.7 ± 12.9	119.7 ± 5.8	110.0 ± 13.6	133.4 ± 3.0

Table 8
Electrolyte parameters in Beagle dogs throughout the study ($\bar{x} \pm s$)

Date	0 mg/kg	0.5 mg/kg	1.5 mg/kg	4.5 mg/kg
First pretest				
Ca (mmol/L)	2.14 ± 0.25	2.33 ± 0.14	2.24 ± 0.20	2.23 ± 0.13
Cl (mmol/L)	105.00 ± 1.26	104.67 ± 2.34	104.67 ± 2.88	104.83 ± 2.48
K (mmol/L)	5.20 ± 0.80	5.35 ± 0.38	5.35 ± 0.38	5.02 ± 0.56
Na (mmol/L)	140.17 ± 2.48	139.17 ± 2.23	139.17 ± 1.72	140.33 ± 2.42
Second pretest				
Ca (mmol/L)	3.51 ± 0.41	4.44 ± 0.78	3.56 ± 0.67	3.80 ± 0.93
Cl (mmol/L)	106.50 ± 2.81	106.33 ± 3.39	105.00 ± 2.10	105.33 ± 2.16
K (mmol/L)	5.42 ± 0.64	5.33 ± 0.41	5.23 ± 0.91	4.92 ± 0.50
Na (mmol/L)	139.0 ± 4.24	140.83 ± 2.79	139.17 ± 2.23	141.00 ± 1.79
Day42				
Ca (mmol/L)	2.56 ± 0.13	2.73 ± 0.13	2.50 ± 0.12	2.79 ± 0.17
Cl (mmol/L)	108.17 ± 1.47	106.00 ± 2.10	107.17 ± 1.94	106.83 ± 1.72
K (mmol/L)	4.68 ± 0.39	4.75 ± 0.29	4.45 ± 0.30	4.65 ± 0.21
Na (mmol/L)	141.67 ± 2.34	137.50 ± 9.27	141.00 ± 1.90	140.33 ± 1.37
Day56				
Ca (mmol/L)	2.38 ± 0.02	2.63 ± 0.37	2.56 ± 0.03	2.83 ± 0.06
Cl (mmol/L)	112.50 ± 2.81	110.00 ± 3.39	110.00 ± 2.10	110.50 ± 2.16
K (mmol/L)	4.60 ± 0.64	5.15 ± 0.41	5.25 ± 0.91	5.15 ± 0.50
Na (mmol/L)	148.0 ± 4.24	145.00 ± 2.79	140.50 ± 2.23	143.00 ± 1.79

Table 9
Coagulation parameters in Beagle dogs throughout the study ($\bar{x} \pm s$)

Date	0 mg/kg	0.5 mg/kg	1.5 mg/kg	4.5 mg/kg
First pretest				
PT (s)	8.5 ± 2.9	8.0 ± 0.8	7.7 ± 1.8	8.6 ± 2.8
APTT (s)	12.3 ± 2.0	10.8 ± 1.1	10.8 ± 1.3	11.7 ± 1.4
Plg (mg/dl)	392.2 ± 48.6	395.0 ± 72.0	476.4 ± 75.3	377.5 ± 59.3
TT (s)	19.9 ± 2.7	18.2 ± 1.0	16.9 ± 1.9*	16.4 ± 1.3**
Second pretest				
PT(s)	8.6 ± 1.5	8.5 ± 0.9	6.9 ± 0.8	8.9 ± 1.7
APTT (s)	11.0 ± 2.2	10.9 ± 1.6	11.2 ± 2.1	11.2 ± 2.4
Plg (mg/dl)	359.3 ± 63.4	367.3 ± 74.9	369.2 ± 79.3	364.3 ± 75.7
TT (s)	21.7 ± 1.5	21.9 ± 3.4	21.4 ± 1.1	20.9 ± 1.3
Day42				
PT (s)	9.1 ± 1.0	8.0 ± 1.1	7.7 ± 1.7	7.5 ± 0.4
APTT (s)	12.7 ± 0.6	10.9 ± 2.4	10.8 ± 1.5	9.7 ± 1.1**
Plg (mg/dl)	357.6 ± 72.4	395.6 ± 152.9	349.1 ± 91.1	400.4 ± 75.4
TT (s)	19.7 ± 3.2	20.3 ± 1.3	18.9 ± 2.1	19.2 ± 1.2
Day56				
PT (s)	7.2 ± 1.1	6.8 ± 0.4	6.7 ± 0.4	6.9 ± 0.3
APTT (s)	10.5 ± 1.6	9.5 ± 0.3	8.9 ± 0.8	9.2 ± 0.3
Plg (mg/dl)	398.6 ± 267.1	357.5 ± 48.2	398.0 ± 129.7	416.2 ± 87.8
TT (s)	35.5 ± 11.5	34.7 ± 1.3	35.1 ± 5.6	34.6 ± 10.0

Note: $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 10
Urinalysis parameters in Beagle dogs throughout the study ($\bar{x} \pm s$)

Date	0 mg/kg	0.5 mg/kg	1.5 mg/kg	4.5 mg/kg
Day42				
GLU	—	—	—	—
BIL	—	—	—	—
KET	—	—	—	—
SG	1.016 ± 0.003	1.008 ± 0.003**	1.009 ± 0.005*	1.010 ± 0.004*
BLO	—	—	—	—
PH	7.125 ± 0.25	7.875 ± 0.75	7.375 ± 0.8539	6.88 ± 0.4787
PRO	3/4(+)	1/4(+)	4/4(+)	4/4(+)
URO	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
NIH	—	—	—	—
LEU	—	—	—	—
Day56				
GLU	—	—	—	—
BIL	—	—	—	—
KET	—	—	—	—
SG	1.001 ± 0.000	1.008 ± 0.004	1.005 ± 0.000	1.010 ± 0.000
BLO	—	—		—
PH	7.5 ± 0.00	8.25 ± 0.3536	8.00 ± 0.00	6.75 ± 0.3536
PRO	—	—	—	—
URO	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
NIH	—	—	—	—
LEU	—	—	—	—

Note: $^{*}P < 0.05$, $^{**}P < 0.01$.

found in high-dose group, but the degree and range of the pathological lesions alleviated. The $ZnPcS_2P_2$ pigments did not abate obviously.

Under the condition of our study, the animals in 4.5 mg/kg $ZnPcS_2P_2$ group showed some toxic effects, and liver was target organ in the drug adverse action. The dogs in 1.5 and 0.5 mg.kg showed no treatment-related abnormal changes, which suggest $ZnPcS_2P_2$ will not have significant toxic effects on Beagle dogs in these dose levels. The NOAEL is 1.5 mg/kg. The results from our study revealed that ZnPc-PDT has a favorable pre-clinical profile.

Table 11
Organ weights at necropsy for Beagle dogs at the end of treatment (♀, $n = 2$)

	Dose (mg/kg)			
	0	0.5	1.5	4.5
Body wt (g)	8000 ± 0	8500 ± 707.1	8000 ± 0	8500 ± 707.1
Organ wt (g)				
Heart	72.54 ± 11.68	78.89 ± 13.46	74.51 ± 10.68	75.57±
Liver	135.58 ± 164.08	238.35 ± 8.90	273.23 ± 27.26	233.50 ± 22.27
Spleen	34.66 ± 13.84	28.84 ± 10.50	24.72 ± 0.06	32.55 ± 0.45
Lung	124.42 ± 83.18	91.35 ± 3.28	95.87 ± 36.51	90.21 ± 0.98
Kidney	34.87 ± 8.22	36.11 ± 5.18	39.68 ± 4.20	43.73 ± 7.65
Brain	67.13 ± 3.28	79.71 ± 3.09	82.01 ± 6.77	80.17 ± 3.97
Adrenals	1.56 ± 0.6	1.33 ± 0.05	1.47 ± 0.07	1.63 ± 0.23
Thymus	7.87 ± 3.54	16.71 ± 1.11	13.80 ± 7.25	15.58 ± 2.60
Thyroid	1.08 ± 0.04	1.23 ± 0.41	0.99 ± 0.06	1.40 ± 0.33
Ovaries	1.92 ± 1.73	0.84 ± 0.12	1.34 ± 0.67	0.95 ± 0.28
Uterus	8.04 ± 8.50	2.76 ± 0.47	11.06 ± 13.75	2.87 ± 0.75
Relative wt (%)				
Heart	0.91 ± 0.15	0.93 ± 0.08	0.94 ± 0.13	0.89 ± 0.10
Liver	2.80 ± 0.50	2.81 ± 0.13	3.42 ± 0.35	2.75 ± 0.04
Spleen	0.44 ± 0.18	0.34 ± 0.09	0.29 ± 0.03	0.39 ± 0.04
Lung	1.56 ± 1.04	1.08 ± 0.13	1.20 ± 0.45	1.07 ± 0.08
Kidney	0.44 ± 0.11	0.43 ± 0.10	0.50 ± 0.05	0.52 ± 0.04
Brain	0.84 ± 0.04	0.94 ± 0.04	1.03 ± 0.08	0.97 ± 0.04
Adrenals	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.0	0.02 ± 0.0
Thymus	0.10 ± 0.04	0.2 ± 0.03	0.18 ± 0.09	0.18 ± 0.01
Thyroid	0.01 ± 0.0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Overies	0.03 ± 0.02	0.01 ± 0.0	0.05 ± 0.01	0.01 ± 0.0
Uterus	0.11 ± 0.11	0.04 ± 0.01	0.14 ± 0.17	0.04 ± 0.01

Note: The data represent $\bar{x} \pm s$.

Table 12
Organ weights at necropsy for Beagle dogs at the end of treatment (♂, $n = 2$)

	Dose (mg/kg)			
	0	0.5	1.5	4.5
Body wt (g)	10500 ± 707.1	9500 ± 707.1	9500 ± 2121.3	8500 ± 707.1
Organ wt (g)				
Heart	68.74 ± 0.23	77.63 ± 2.05*	76.99 ± 23.62	69.66 ± 3.17
Liver	249.77 ± 9.91	302.79 ± 19.30	268.06 ± 10.55	253.52 ± 12.44
Spleen	23.88 ± 5.73	36.95 ± 4.47	27.86 ± 11.21	30.86 ± 3.20
Lung	95.42 ± 17.14	155.74 ± 65.07	66.41 ± 35.50	107.56 ± 9.97
Kidney	46.47 ± 1.19	50.96 ± 0.41*	48.51 ± 2.04	41.94 ± 4.61
Brain	72.22 ± 0.34	77.37 ± 0.66	83.07 ± 5.81	76.62 ± 2.74
Adrenals	1.04 ± 0.03	1.72 ± 0.48	1.38 ± 0.11*	1.17 ± 0.27
Thymus	20.08 ± 2.61	19.32 ± 9.93	20.96 ± 4.70	15.1 ± 6.10
Thyroid	1.09 ± 0.15	1.18 ± 0.09	0.99 ± 0.36	1.07 ± 0.02
Tests	11.39 ± 9.45	9.70 ± 0.24	11.18 ± 10.28	3.56 ± 1.04
Prostate	3.97 ± 2.03	4.68 ± 3.10	7.70 ± 8.99	1.57 ± 0.33
Relative wt (%)				
Heart	0.66 ± 0.04	0.82 ± 0.04	0.80 ± 0.07	0.83 ± 0.11
Liver	2.39 ± 0.25	3.21 ± 0.45	2.88 ± 0.54	3.0 ± 0.40
Spleen	0.23 ± 0.04	0.24 ± 0.20	0.29 ± 0.06	0.37 ± 0.06
Lung	0.92 ± 0.23	1.67 ± 0.81	1.30 ± 0.66	1.28 ± 0.22
Kidney	0.44 ± 0.04	0.54 ± 0.04	0.52 ± 0.10	0.50 ± 0.10
Brain	0.69 ± 0.05	0.82 ± 0.06	0.89 ± 0.14	0.90 ± 0.04
Adrenals	0.01 ± 0.0	0.02 ± 0.0	0.02 ± 0.01	0.02 ± 0.01
Thymus	0.19 ± 0.01	0.20 ± 0.08	0.23 ± 0.10	0.18 ± 0.08
Thyroid	0.01 ± 0.0	0.01 ± 0.0	0.02 ± 0.01	0.01 ± 0.0
Tests	0.11 ± 0.08	0.11 ± 0.01	0.11 ± 0.08	0.05 ± 0.01
Prostate	0.04 ± 0.01	0.05 ± 0.04	0.08 ± 0.08	0.02 ± 0.01

Note: The data represent $\bar{x} \pm s$.
*$P < 0.05$.

Table 13
Organ weights at necropsy for Beagle dogs at the end of recovery (♀, $n = 1$)

	Dose (mg/kg)			
	0	0.5	1.5	4.5
Body wt (g)	9000	11000	9000	11000
Organ wt (g)				
Heart	82.6	80.67	74.97	64.63
Liver	225.4	252.3	216.55	311.35
Spleen	29.48	35.52	27.92	22.72
Lung	79.72	87.69	82.57	64.21
Kidney	36.65	41.82	31.24	37.17
Brain	92.02	83.52	87.17	64.57
Adrenals	1.41	1.41	1.45	1.66
Thymus	13.35	32.20	7.19	0.89
Thyroid	1.0	1.21	1.11	1.40
Ovaries	0.87	0.96	0.98	1.56
Uterus	2.61	2.01	9.40	12.26
Relative wt (%)				
Heart	0.92	0.73	0.83	0.59
Liver	2.50	2.29	2.41	2.83
Spleen	0.33	0.32	0.31	0.21
Lung	0.89	0.80	0.92	0.58
Kidney	0.41	0.38	0.35	0.34
Brain	1.02	0.76	0.97	0.59
Adrenals	0.02	0.01	0.02	0.02
Thymus	0.15	0.29	0.08	0.01
Thyroid	0.01	0.01	0.01	0.01
Ovaries	0.01	0.01	0.01	0.01
Uterus	0.03	0.02	0.10	0.11

Table 14
Organ weights at necropsy for Beagle dogs at the end of recovery (♂, $n = 1$)

	Dose (mg/kg)			
	0	0.5	1.5	4.5
Body wt (g)	11000	12000	11000	11000
Organ wt (g)				
Heart	82.26	81.93	83.09	76.66
Liver	320.97	289.32	269.35	252.18
Spleen	43.75	21.65	28.78	23.32
Lung	137.65	124.69	111.66	95.09
Kidney	53.17	55.14	44.52	31.94
Brain	81.34	75.14	74.15	77.42
Adrenals	1.44	1.35	1.07	0.85
Thymus	41.15	24.67	29.95	9.28
Thyroid	1.80	2.31	1.13	1.23
Tests	4.08	4.16	2.28	12.08
Prostate	1.88	1.77	1.47	3.93
Relative wt (%)				
Heart	0.75	0.68	0.76	0.70
Liver	2.92	2.41	2.45	2.29
Spleen	0.40	0.18	0.26	0.21
Lung	1.25	1.04	1.02	0.86
Kidney	0.48	0.46	0.40	0.29
Brain	0.74	0.63	0.67	0.70
Adrenals	0.01	0.01	0.01	0.01
Thymus	0.37	0.21	0.27	0.08
Thyroid	0.02	0.02	0.01	0.01
Tests	0.04	0.03	0.02	0.11
Prostate	0.02	0.01	0.01	0.04

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