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Original Article

Toxicological effects of repeated subcutaneous administration of corn oil for 4 weeks in rats

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ABSTRACT — In toxicity studies, it is important to select a vehicle that does not affect the toxicity assessment of the test substance. To obtain toxicity information on corn oil as a Vehicle for subcutaneous toxicity studies of non-aqueous test substances, Crl:CD (SD) rats (6 animals/sex/group) were repeatedly dosed with corn oil at 0 (negative control; saline), 1, 2 and 5 mL/kg/day for 4 weeks. High body weight due to retention of the administered substance were found in corn oil groups in both sexes at 2 mL/kg/day or higher. Some relative organ weights at 2 mL/kg/day or higher were decreased due to high body weight or body weight gain. Subcutaneous retention of the administered substance found in all corn oil-treated groups at necropsy was observed on histopathological analysis as accumulation of the administered substance and granulation tissue. Accumulation of the administered substance in inguinal skin, the axillary lymph node, and alveolus of the lung and bronchus was observed. Multiple white nodules in the abdominal cavity and liver observed in a female at 5 mL/kg/day at necropsy were considered to be related to lipogranuloma at the peritoneum and hepatic capsule. In hematology, RBC was lower in females at the 5 mL/kg/day than in the control group.. In conclusion, since the effects of corn oil administration were found in groups at all volumes, it is necessary to take into account that corn oil induces these changes when used as a vehicle of a test substance in toxicity studies.

Key words: Corn oil, 4-week repeated subcutaneous toxicity, Rat, Retention of administered substance, Vehicle

INTRODUCTION

Subcutaneous administration is often the route of choice for toxicity studies of test substances. If the test substance is insoluble in water, a suspension is used, or an oil-based or organic solvent system is chosen (Strickley, 2004). However, in preclinical studies, it is important that the vehicle does not affect physiological and biological functions in order to correctly estimate the toxicity of a compound (Gad and Cassidy, 2006).

Vegetable oils can dissolve lipophilic compounds and are often used as a vehicle for subcutaneous administration (Strickley, 2004). Olive oil in particular has been used in subcutaneous administration studies in rodents (Sugiura *et al*., 2006). However, olive oil has strong antioxidative potential and is believed to exert favorable effects against free radicals and carcinogenesis (Borzi *et al*., 2019). In other words, it does not exert undesirable effects on the body, but it may mask the toxic effects of the compound. Corn oil is also a vegetable oil, and comparisons with olive oil have frequently been reported (Gao *et al*., 2019; Drehmer *et al.*, 2022). Corn oil does

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not exhibit antioxidant properties and has not been shown to differ from olive oil as a subcutaneously administered vehicle (Wu *et al*., 1994).

The maximum dose volume for repeated subcutaneous administration in rats is 5 mL/kg, according to the good practice guide (Diehl *et al*., 2001), but there are no reports on the maximum dose volume for corn oil. In the case of a non-aqueous vehicle, the time for absorption before redosing should be considered. It is perceivable that vegetable oils such as corn oil cannot be easily absorbed by subcutaneous administration, so the maximum dose volume would need to be evaluated.

In this study, corn oil was evaluated for local and systemic effects after repeated subcutaneous administration to rats for 4 weeks and to assess the dosage levels for repeated subcutaneous administration toxicity studies using corn oil as a vehicle.

MATERIALS AND METHODS

Reagents and animals

Corn oil was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Saline was purchased from Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan).

This study was conducted in compliance with the Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co., Ltd., and was approved by the Institutional Animal Care and Use Committee of the testing facility. Five-week-old male and female Sprague Dawley (SD) rats (n = 24/each sex) were purchased from Hino Breeding Center (Shiga, Japan) of Charles River Japan, Inc. The rats were maintained on a 12 hr light/dark cycle and housed for 5 weeks in a facility with a room temperature of 23 ± 2°C and humidity of 60 ± 10% before undergoing experimentation. Rats were allowed free access to a pelleted diet (CRF-1, sterilized by radiation, Oriental Yeast Co.), although powdered food was provided during the urinalysis, and in-house tap water via an automatic watering device except for about 16 hours of water consumption measurement during which tap water was provided via a glass bottle.

Animal experiments

The present study was conducted in compliance with the Guidelines for Animal Care and Use at Otsuka Pharmaceutical Co, Ltd., and was approved by the Institutional Animal Care and Use Committee of the testing facility.

Six- or 7-week-old SD rats received repeated subcutaneous administration of corn oil or saline as a negative control group in the morning for 4 weeks. The volume of corn oil was set at 1, 2, and 5 mL/kg for both sexes, with 6 animals in each group. Considering that the administered corn oil remained on the subcutaneous region, the dorsal skin was divided into seven areas: the back of the neck, the right and left shoulders, and the sides of the abdomen and lower back were administered in rotation. The administration volume was set at a maximum volume of 5 mL/kg, which is considered the maximum volume for subcutaneous administration in rats in the good practice guide (Diehl *et al*., 2001), with a ratio of approximately 2. The negative control group received 5 mL/kg of saline to be matched with the highest volume of corn oil.

Clinical sign, body weight, food consumption, clinical pathology, necropsy, organ weight and histopathology

All animals were observed for clinical signs twice daily before and approximately 2 hr after dosing, and their body weight and food consumption were measured once a week. All animals were placed in the metabolism cages and the early accumulated urine (fresh urine) sample was obtained under fasting conditions in Week 4. Ophthalmology and urinalysis were performed for standard laboratory procedures. Blood for hematology and blood biochemistry was also collected from the posterior vena cava under anesthesia on the day after the last dose. Ethylene-diamine-tetraacetic-acid was added to the blood sample for the hematology test and sodium citrate to the blood sample for the coagulation test. Tests included hemoglobin (Hb), hematocrit (Ht), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte, mean white blood cell count, platelets, pro-thrombin time, and activated partial thromboplastin time. Blood samples for the blood biochemistry test were heparinized blood. Tests included creatinine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, γ-glutamyl transpeptidase, total cholesterol, triglycerides, phospholipids, glucose, total protein, protein fractions, albumin/globulin ratio, urea nitrogen, creatinine, sodium, potassium, calcium, inorganic phosphate, and chloride. At the end of the dosing periods, each animal was fasted for 18 to 24 hr before necropsy. The animals were anesthetized with isoflurane and euthanized by exsanguination from the posterior vena cava and aorta on the day after the final dosing. Necropsy consisted of gross pathology of the external morphology and all major internal organs. Organ weights and histopathological analyses were performed on vari-

ous organs as described in the ICH-S4 guidelines. Bilateral organs were weighed together. The organ-to-body weight ratio (relative weights) was calculated based on the body weights recorded on the day of necropsy. Organs were collected and fixed in 10% neutral buffered formalin when dissected at the time of necropsy. The eyes were fixed in Davidson's solution, and the testes and epididymides were fixed in formalin- sucrose-acetic acid solution. The vertebrae including spinal cord, sternum, and femur were decalcified with formic acid and sodium citrate mixture after fixation. Fixation of skin at administration sites was performed at the oldest dosing site (7 days before necropsy) and at the most recent dosing site (the day before necropsy). The tissues for the microscopic evaluation were trimmed, dehydrated with a graded series of ethyl alcohol, dealcoholized with xylene, and embedded in paraffin. The paraffin block tissues were thinly sectioned, and stained with hematoxylin and eosin (HE).

Statistical analysis

The following data were analyzed by Dunnett's test: body weight, body weight gain, food consumption, organ weight, hematology, blood biochemistry, and urinalysis (except for the semiquantitative paper test). Dunnett's test was used for comparisons between the negative control and all corn oil-treated groups. The semiquantitative urinalysis data were analyzed by the cumulative chi-square test. The significance tests were performed two-tailed, and statistical significance was presented at either the 5% or 1% level.

RESULTS

Clinical sign, body weight, and food consumption

Subcutaneous swelling at the sites of dosing due to corn oil retention was observed in all animals, and swelling was widely distributed according to increased corn oil volume. The body weights of both sexes showed a tendency to increase generally with the volume, and a significant increase was found at 5 mL/kg/day in females (Fig. 1 and Table 1). There was no effect of corn oil dosing on food consumption.

Clinical pathology and organ weight

At 5 mL/kg/day, RBC was lower in females at the 5 mL/kg/day than in the control group. (95% vs. control; Fig. 2). There were no other changes in hematology, biochemistry, or urinalysis, including parameters related to red blood cells.

At 2 and 5 mL/kg/day, the relative weight of the liver was decreased in males (Table 1). At 5 mL/kg/day, the relative weight of the brain, heart, kidney, adrenal gland, and ovary was decreased in females.

Urinalysis and ophthalmologic and blood biochemical analysis showed no effects of corn oil administration.

Necropsy and histopathology

Retention of the administered substance at injection sites (one or more sites) was observed in all animals administered corn oil. The severity of the incidence increased with increasing dose (Fig. 3). Some of the retained administered substance flowed out during necropsy. The skin at dosing sites obtained from the animals showed that even with rotational dosing at 2 and 5 mL/kg/day, the doses were not clearly divided into sep-

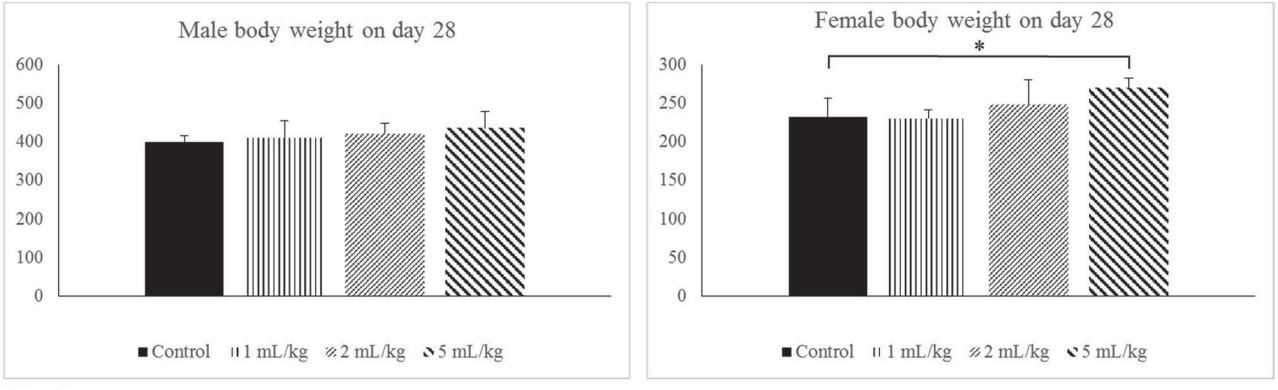

Fig. 1. Body weight of rats on Day 28. Significant weight gain was observed at 5 mL/kg in females. Significantly different from Corn Oil 0 mL/kg (The t-test with F-test for homogeneity of variance or Dunnett test): * $P < 0.05$

Table 1. Body weight and relative organ weights of rats.

Sex	Organ	Group			
		Control	1 mL/kg	2 mL/kg	5 mL/kg
Male	Body weight	374.7 ± 17.0	383.8 ± 41.3	396.8 ± 22.6	410.2 ± 37.5
	Liver #	3.05 ± 0.22	3.02 ± 0.15	2.82 ± 0.08*	2.78 ± 0.12*
Female	Body weight	216.3 ± 22.1	216.3 ± 9.6	234.2 ± 30.6	256.3 ± 11.3**
	Brain #	0.898 ± 0.062	0.855 ± 0.034	0.838 ± 0.126	0.730 ± 0.037**
	Heart #	0.402 ± 0.033	0.383 ± 0.038	0.360 ± 0.028	0.330 ± 0.023**
	Kidney #	0.822 ± 0.057	0.762 ± 0.015	0.782 ± 0.053	0.698 ± 0.037**
	Adrenal gland ##	35.60 ± 8.33	32.30 ± 5.74	29.77 ± 5.8	25.53 ± 5.98*
	Ovaries ##	50.7 ± 7.6	46.7 ± 8.5	41.8 ± 8.0	33.8 ± 4.8**

Significantly different from Corn Oil 0 mL/kg (The t-test with F-test for homogeneity of variance or Dunnett test) : * P < 0.05, ** P < 0.01
Unit: #; g/100 g, ##; mg/100 g

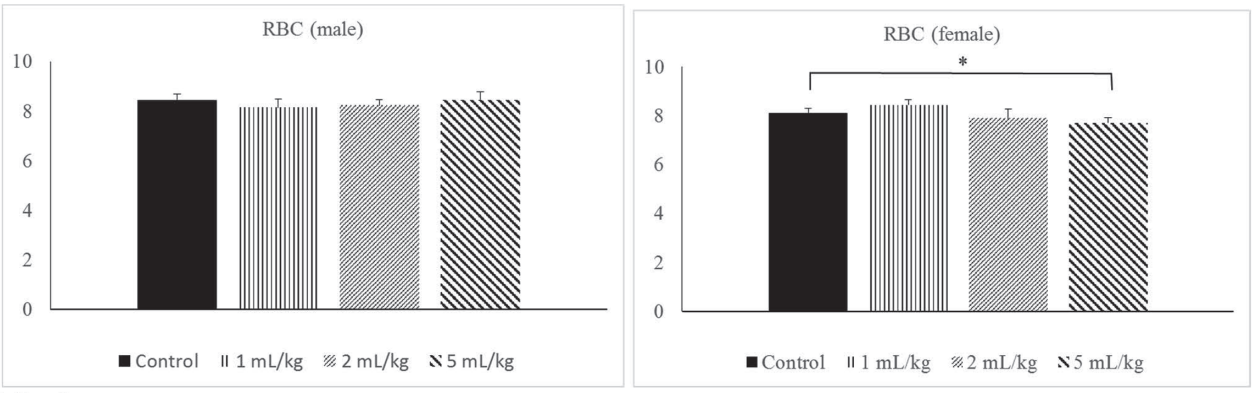

Fig. 2. Results of red blood cell count (×10⁶/mm³) in male and female rats. Significantly different from Corn Oil 0 mL/kg (The t-test with F-test for homogeneity of variance or Dunnett test): * P < 0.05

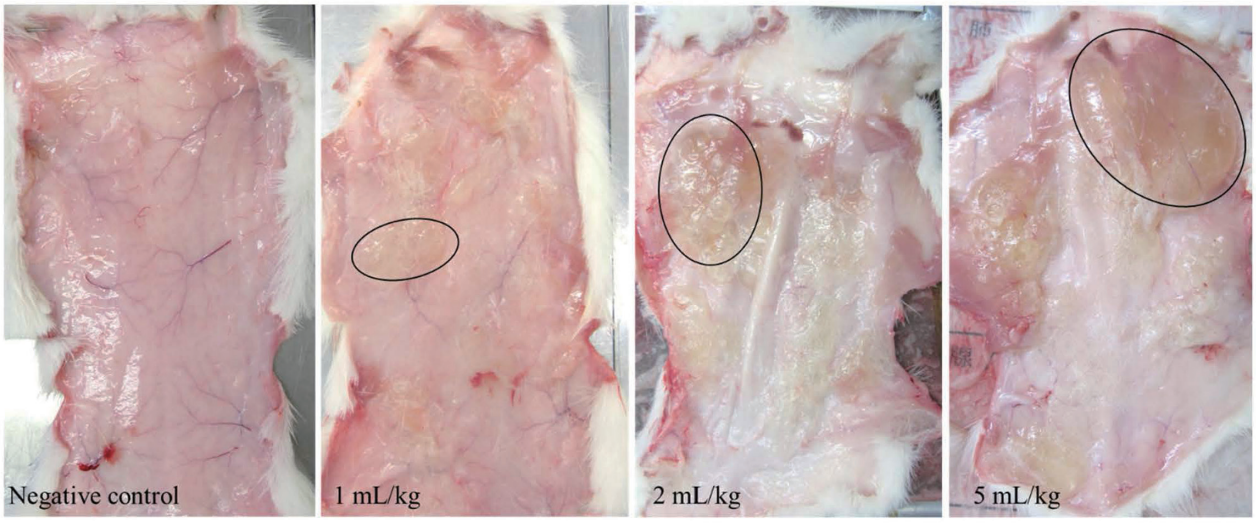

Fig. 3. Gross subcutaneous appearance at necropsy. The areas with the largest retention of the administered substance are indicated by circles. Retention of the administered substance was observed in all groups, and it was confirmed that the dosing sites were not clearly divided even with rotational dosing at 2 and 5 mL/kg. The area of corn oil retention increased with volume.

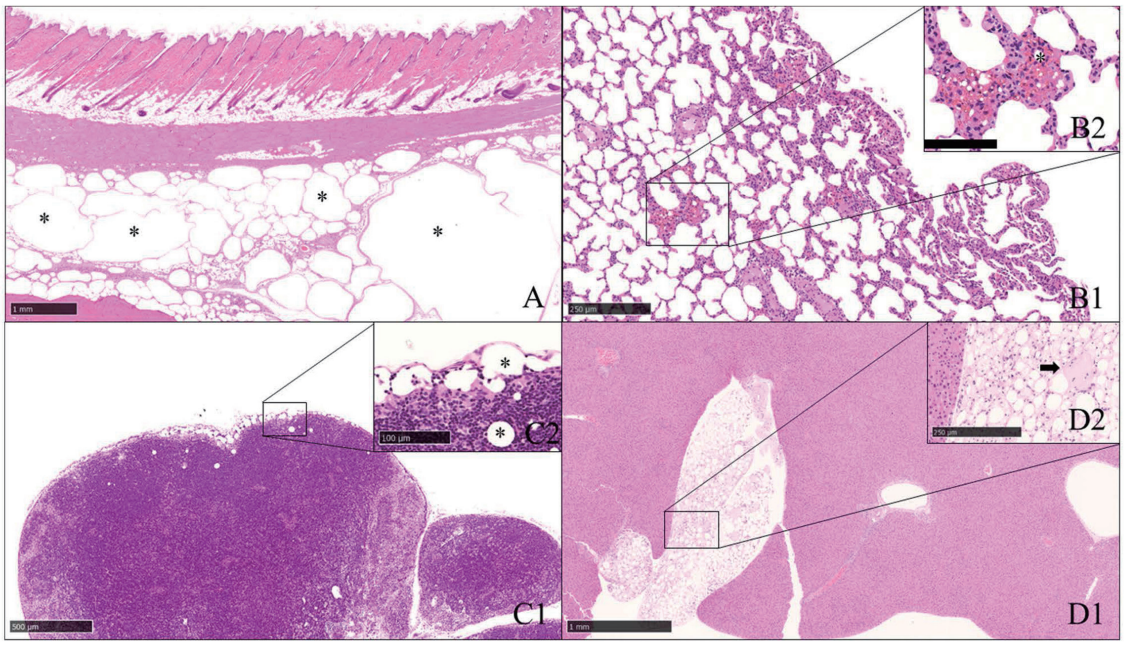

Fig. 4. Histopathological image of an organ with retention of the administered substance. A: Dorsal skin, the site of dosing. Retention of the administered substance was observed (asterisks). Bar = 1 mm. B1 and B2: Accumulation of macrophages in the lungs. Bar = 250 µm. Retention of administered substances was observed and deposition of hemosiderin was also observed (B2: high magnification, asterisks: retention of administered substances, bar = 100 µm). C1: Retention of the administered substance was observed in the axillary lymph node. Bar = 500 µm (C2: high magnification, asterisks: retention of administered substances, bar = 100 µm). D1: Lipogranuloma of the liver under the capsule. The border with the parenchyma of the liver was clear and multinucleated giant cells (arrow in D2: high magnification, bar = 250 µm) were observed. Bar = 1 mm.

arate areas. Brown or dark red foci were observed in a few cases in both sexes at 2 and 5 mL/kg, with a higher number of cases at 5 mL/kg. In 1 female at 5 mL/kg, multiple small white nodules in the abdominal cavity and liver were observed.

In histopathology, accumulation of the administered substance was observed in almost all animals treated with corn oil at the dosing sites, and granulation tissue was observed at many of these sites (Fig. 4A and Table 2). Inflammatory reactions were also observed in some of these areas. There was no difference between old and new dosing sites. In 1 male at 5 mL/kg, the administered substance also extended subcutaneously into the inguinal region. Retention of the administered substance was also observed in alveolus of the lung and bronchus and the axillary lymph node of both sexes in several animals at 1, 2, and/or 5 mL/kg (Fig. 4B and 4C). In some of these animals, macrophage accumulation was observed with retention of the administered substance. Hemosiderin deposition in macrophage, hemorrhage of focal alveolus, and/or infiltration of eosinophil was also observed in these animals at the lung and bronchus, and hyperplasia of lymphoid follicle was observed in 1 female in the axillary lymph node. The number of cases of these changes found in the lungs and bronchus and axillary lymph nodes was not responsive to volume.

Foreign body granuloma at the capsule in the liver and peritoneum in the abdominal cavity were observed in 1 female at 5 mL/kg/day (Fig. 4D).

DISCUSSION

Corn oil was subcutaneously administered once daily for 4 weeks to rats at a dose of 1, 2, and 5 mL/kg in order to assess the dosage for repeated subcutaneous administration toxicity studies using corn oil as a vehicle.

No deaths occurred in any of the corn oil-treated groups throughout the study period.

The body weights of both sexes showed a tendency to increase generally with the volume. Subcutaneous swelling at the site of dosing was observed earlier in the dosing period as the corn oil volume increased. Subcutane-

Table 2-1. Histopathological findings in four-week repeated subcutaneous dose toxicity study of corn oil in rats.

Organ	Sex	Male																			
	Volume (mL/kg)	0					1					2					5				
	Number of Animals	6					6					6					6				
Findings	Grade	-	1+	2+	3+	4+	-	1+	2+	3+	4+	-	1+	2+	3+	4+	-	1+	2+	3+	4+
Axillary lymph node																					
Accumulation, administered substance		6	0	0	0	0	3	3	0	0	0	4	2	0	0	0	5	1	0	0	0
Accumulation, macrophage		6	0	0	0	0	6	0	0	0	0	5	1	0	0	0	6	0	0	0	0
Injection site A																					
Accumulation, administered substance		6	0	0	0	0	0	1	5	0	0	0	3	3	0	0	0	2	4	0	0
Granulation tissue		6	0	0	0	0	3	3	0	0	0	4	2	0	0	0	1	4	1	0	0
Infiltrate, mononuclear cell		6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	5	1	0	0	0
Injection site B																					
Accumulation, administered substance		6	0	0	0	0	0	2	4	0	0	0	1	5	0	0	1	0	5	0	0
Granulation tissue		6	0	0	0	0	4	2	0	0	0	2	3	1	0	0	2	4	0	0	0
Infiltrate, mononuclear cell		6	0	0	0	0	3	3	0	0	0	5	1	0	0	0	6	0	0	0	0
Lung and Bronchus																					
Accumulation, administered substance, alveolus		6	0	0	0	0	6	0	0	0	0	4	2	0	0	0	4	2	0	0	0
Accumulation, macrophage, alveolus		6	0	0	0	0	6	0	0	0	0	3	3	0	0	0	3	3	0	0	0
Deposit, hemosiderin, macrophage		6	0	0	0	0	6	0	0	0	0	4	2	0	0	0	5	1	0	0	0
Hemorrhage, focal		6	0	0	0	0	5	1	0	0	0	5	1	0	0	0	4	2	0	0	0
Skin, inguinal*																					
Accumulation, administered substance		6	0	0	0	0	NE					NE					5	1	0	0	0

< > : Number of animals examined

Grade: -, not remarkable; 1+, minimal; 2+, mild; 3+, moderate; 4+, severe

*: One and 2 mL/kg groups were not examined because it was judged that no serious changes were detected in thses groups.

Injection site A: The oldest dosing site (7 days before necropsy)

Injection site B: The most recent dosing site (the day before necropsy)

NE: Not examined

Table 2-2. Histopathological findings in four-week repeated subcutaneous dose toxicity study of corn oil in rats- continued.

Organ	Sex	Female																			
	Volume (mL/kg)	0					1					2					5				
	Number of Animals	6					6					6					6				
Findings	Grade	-	1+	2+	3+	4+	-	1+	2+	3+	4+	-	1+	2+	3+	4+	-	1+	2+	3+	4+
Abdominal cavity																					
Foreign body granuloma, petitoneum		6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	5	1	0	0	0
Axillary lymph node																					
Accumulation, administered substance		6	0	0	0	0	4	2	0	0	0	5	1	0	0	0	5	1	0	0	0
Hyperplasia, lymphoid follicle		6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	5	1	0	0	0
Injection site A																					
Accumulation, administered substance		6	0	0	0	0	0	2	4	0	0	2	2	2	0	0	0	2	4	0	0
Granulation tissue		6	0	0	0	0	3	2	1	0	0	4	2	0	0	0	1	5	0	0	0
Infiltrate, mononuclear cell		6	0	0	0	0	5	1	0	0	0	6	0	0	0	0	6	0	0	0	0
Injection site B																					
Accumulation, administered substance		6	0	0	0	0	0	4	2	0	0	0	1	5	0	0	0	1	5	0	0
Granulation tissue		6	0	0	0	0	4	2	0	0	0	3	3	0	0	0	2	4	0	0	0
Infiltrate, mononuclear cell		6	0	0	0	0	5	1	0	0	0	6	0	0	0	0	5	1	0	0	0
Liver																					
Foreign body granuloma, capsule		6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	5	1	0	0	0
Necrosis, hepatocyte, focal		6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	5	1	0	0	0
Vacuolation, hepatocyte		6	0	0	0	0	5	1	0	0	0	6	0	0	0	0	5	1	0	0	0

< > : Number of animals examined
Grade: -, not remarkable; 1+, minimal; 2+, mild; 3+, moderate; 4+, severe
Injection site A: The oldest dosing site (7 days before necropsy)
Injection site B: The most recent dosing site (the day before necropsy)

Table 2-3. Histopathological findings in four-week repeated subcutaneous dose toxicity study of corn oil in rats- continued.

Organ	Sex	Female																			
	Volume (mL/kg)	0					1					2					5				
	Number of Animals	6					6					6					6				
Findings	Grade	-	1+	2+	3+	4+	-	1+	2+	3+	4+	-	1+	2+	3+	4+	-	1+	2+	3+	4+
Lung and Bronchus																					
Accumulation, administered substance, alveolus		6	0	0	0	0	3	3	0	0	0	5	1	0	0	0	3	3	0	0	0
Accumulation, macrophage, alveolus		6	0	0	0	0	3	3	0	0	0	5	1	0	0	0	2	4	0	0	0
Deposit, hemosiderin, macrophage		6	0	0	0	0	4	2	0	0	0	6	0	0	0	0	4	2	0	0	0
Hemorrhage, focal		6	0	0	0	0	6	0	0	0	0	4	2	0	0	0	6	0	0	0	0
Infiltrate, eosinophil, perivascular		6	0	0	0	0	4	2	0	0	0	5	1	0	0	0	5	1	0	0	0

< > : Number of animals examined

Grade: -, not remarkable; 1+, minimal; 2+, mild; 3+, moderate; 4+, severe

ous retention of the administered substance found in all corn oil-treated groups at necropsy was observed on histopathological analysis as accumulation of the administered substance and granulation tissue. These findings were accompanied by foreign body granuloma and mononuclear infiltration in some individual animals. These results and no effect in food consumption suggest that subcutaneously administered corn oil was accumulated locally and increased body weight as a result. The relative organ weight of the liver, brain, heart, kidney, adrenal gland, and ovary was decreased in males and/or females at 2 and/or 5 mL/kg/day. However, these organs were observed no histological findings and no effects in any other examinations. We suggested that the relative organ weight decrease was due to the effect of body weight gain caused by subcutaneous corn oil retention. In other words, these organ weight changes were considered secondary. Accumulation of the administered substance in inguinal skin, the axillary lymph node, and alveolus of the lung and bronchus was observed regardless of sex and dosing volume of corn oil. With these findings, accumulation of macrophage in the axillary lymph node and alveolus of the lung and bronchus, hyperplasia of follicle in the axillary lymph node, and perivascular infiltration of eosinophil in the lung and bronchus were observed. In addition, brown or dark red foci of the lungs and bronchus observed in males and/or females at 2 and/or 5 mL/kg/day was corresponded to hemorrhage and hemosiderin deposition. Hemosiderin deposition at macrophages might be associated with focal hemorrhage (Greaves, 2012). And macrophages accumulated around the administered substance sometimes contained hemosiderin. We suggested that focal hemorrhage might occur secondary to accumulation of the administered substance in the lung and bronchus.

Multiple white nodules in the abdominal cavity and liver were observed at necropsy in 5 mL/kg/day females as lipogranuloma in the peritoneum and hepatic capsule. Similar findings were reported from subcutaneous injection of olive oil to SD rats (Ramot *et al*., 2009). In the report, it was suggested that the oil may have reached the peritoneal cavity from the subcutaneous tissue passively via the lymphatic vessels or blood vessels. In this study, accumulation of the administered fluid was also observed in the axillary lymph nodes, suggesting that the lipogranuloma observed in the peritoneum and liver was also caused by subcutaneous dosing of corn oil. Furthermore, although morphologically it was in the form of lipogranuloma, it was determined to be associated with accumulation of administered corn oil, and is classified as foreign body granuloma in this report.

In hematology, RBC was lower in females at the 5 mL/kg/day than in the control group. There were no other changes in hematology, biochemistry, or urinalysis, including parameters related to red blood cells. Therefore, we suggest that the decrease in RBC was slight and had little toxicological significance, possibly due to the effect of corn oil dosing.

Vehicles used in repeated dose toxicity studies should not affect the toxicity assessment as much as possible. In addition, when a non-aqueous vehicle is used, the first dose of fluid should be absorbed before the next dose is administered (Diehl *et al*., 2001). Accumulation of the administered substance and associated changes in the lung and bronchus and the axillary lymph node were observed at all volumes. At 2 mL/kg or more, we considered that the administered substance was not divided subcutaneously at the site of administration, and that an additional formulation was administered into the formulation pool. In addition, low relative liver weight were found in males. Furthermore, in both males and females at 5 mL/kg/day showed body weight gain, accompanied by lower relative weight in the liver of males and in several organs of females, as well as lower RBC in females. These changes observed at a volume of 5 mL/kg were thought to have a significant effect on the toxicity assessment of the test substance, and the volume was considered unsuitable for toxicity assessment. The 2 mL/kg volume also showed a trend toward weight gain and also affected the assessment of liver weight in males. The events at the 2 mL/kg volume, although milder than those at the 5 mL/kg volume, were considered to affect the toxicity assessment of the test substance. Although accumulation of the administered substance and associated changes in the lung and bronchus and the axillary lymph node were observed at all volumes, the effect of the 1 mL/kg volume on the evaluation of the test substance was considered to be slight because the administration site was mostly divided and these changes associated with accumulation of the administered solution were limited. In other words, even if the corn oil dosed previously had not been fully absorbed, it was thought that it was generally possible to divide the corn oil into areas where it had not been dosed. Volumes higher than 2 mL/kg are also permissible for use in toxicity studies; however, usage would have to depend on the solubility of the test substance and the purpose of the study, and the possibility that corn oil-induced changes such as those described in this report may mask toxicity changes attributable to the test substance must be fully considered.

In conclusion, as a result of repeated subcutaneous administration of corn oil at 1, 2, and 5 mL/kg/day for

4 weeks, high body weight or a tendency toward high body weight due to subcutaneous retention of administered corn oil was found at 2 mL/kg/day or higher, and accumulation of corn oil was observed on the treated site, axillary lymph nodes, and the lung and bronchus in all corn oil-treated groups. In the groups at 2 mL/kg/day or more, hemorrhage associated with corn oil accumulation was found in the lung and bronchus, and low relative organ weight were found in the liver, brain, heart, kidney, adrenal gland, and ovary. In repeated subcutaneous administration studies using corn oil as a vehicle, the dosing volume should be set in consideration of these effects.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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