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Letter

Investigation of solvents that can be used as vehicles to evaluate poorly soluble compounds in short-term oral toxicity studies in rats

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ABSTRACT — In non-clinical toxicity studies of orally dosed small molecule drugs, methyl cellulose (MC) is commonly used as the vehicle. It is well tolerated and easy to prepare. However, it is not suitable as the vehicle for all poorly soluble compounds. The objective of this study was to evaluate the no-observed-effect levels (NOELs) of solvents that are possible alternative vehicles for 2-week oral administration of poorly soluble drugs. Five animals/group were dosed once daily with 25 mg/kg/day MC 400 (0.5% MC, control group), up to 5,000 mg/kg/day of polyethylene glycol 400 (PEG 400), 5,500 mg/kg/day of dimethyl sulfoxide (DMSO), 1,000 mg/kg/day of hydroxypropyl-β-cyclodextrin (HP-β-CD), 250 mg/kg/day of polysorbate 80 (Tween 80), 600 mg/kg/day of sodium dodecyl sulfate (SDS), 9,000 mg/kg/day of olive oil and sesame oil, and 600 mg/kg/day of lactic acid. Parameters evaluated included clinical signs, clinical pathology, organ weight, gross pathology, and histopathology. The NOELs were considered to be 1,250 mg/kg/day for PEG 400, 1,000 mg/kg/day for HP-β-CD, 250 mg/kg/day for Tween 80, 4,500 mg/kg/day for olive oil, 4,500 mg/kg/day for sesame oil, and 600 mg/kg/day for lactic acid. The NOELs of DMSO and SDS could not be determined, because rats dosed with DMSO or SDS showed DMSO-specific offensive odor or SDS-related significant irritant effects even at the lowest dose levels (DMSO, 1,100 mg/kg/day; SDS, 150 mg/kg/day). This study provides the NOELs of several solvents that could be used as vehicles in 2-week oral toxicity studies in rats.

Key words: Vehicle, Poorly soluble compound, Rat, Oral toxicity study, NOEL

INTRODUCTION

The ICH M3 guideline (ICH, 2009) requires that the target toxicity potentially elicited by candidate compounds be identified in toxicity studies. Therefore, it is necessary to find a solvent that can maximize exposure to the compound; generally these include pH buffers, co-solvents, cyclodextrins, surfactants, suspensions, and oils (Li and Zhao, 2007). However, solvent-specific effects on experimental animals have been reported. For example, surfactants, co-solvents, and lipid solvents are known to cause gastrointestinal effects such as vomiting and loose stools when administered orally to dogs and monkeys (Neervannan, 2006). Therefore, it is important to understand these solvent-specific effects in order to properly assess the toxicity of candidate compounds (Li and Zhao, 2007; Neervannan, 2006; Gad *et al*., 2006). The ICH S4 (ICH, 1998) recommends a clinically applicable route of administration for toxicity studies, and many small molecule drugs are oral formulations. Aqueous solutions of cellulose derivatives, which are among the least toxic solvents, are commonly used as vehicles for oral formula-

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tions of small molecule drugs because they are well tolerated by animals and easy to prepare and handle in toxicity studies (Thackaberry, 2013). The dosing formulation for such oral toxicity studies is generally a homogeneous suspension; however, when poorly soluble molecules are prepared with methyl cellulose (MC), the suspension may not be confirmed to be homogeneous. Therefore, a vehicle that can handle poorly soluble molecules is desired. It is necessary to select a less toxic solvent suitable for oral administration, but there are no guidelines for selecting a suitable vehicle for toxicity studies, and toxicological information on solvents is limited. There are some reviews on toxicological studies of solvents, but they contain only limited information on test conditions such as species, route of administration, duration of administration, dosage, and tolerability (Li and Zhao, 2007; Neervannan, 2006; Gad *et al*., 2006; Gad *et al*., 2016). Recently, detailed toxicity information (clinical observations, body weight, urinalysis, hematology, blood biochemistry, necropsy, organ weights, and histopathology) following oral administration of several solvents has been reported, but this is based on 5 days of oral administration (Gopinathan *et al*., 2013).

In the present study, we investigated the oral repeated toxicity (for 2 weeks) of eight solvents in female rats and determined the no-observed-effect level (NOEL) of each solvent. The eight solvents were the organic solvents, polyethylene glycol 400 (PEG 400) and dimethyl sulfoxide (DMSO); the cyclodextrin, hydroxypropyl-β-cyclodextrin (HP-β-CD); the surfactants, polysorbate 80 (Tween 80) and dodecyl sulfate sodium (SDS); the oils, olive oil and sesame oil; and a pH buffer, lactic acid.

MATERIALS AND METHODS

Chemicals

All excipients used were reagent or pharmacopoeial grade. Methyl cellulose 400 (MC) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Polyethylene glycol 400 (PEG 400), dimethyl sulfoxide (DMSO), hydroxypropyl-β-cyclodextrin (HP-β-CD), polysorbate 80 (Tween 80), sodium dodecyl sulfate (SDS), olive oil, sesame oil, and lactic acid were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

In this study, PEG 400 was selected as an organic solvent and HP-β-CD as cyclodextrin because they are widely used as vehicles in oral toxicity studies (Gad *et al*., 2006; Gad *et al*., 2016). In addition, DMSO was selected as an organic solvent due to their high solubility. Furthermore, Tween 80 was selected as a nonionic surfactant and SDS as an anionic surfactant. Moreover, olive oil was selected as an oil containing mostly monounsaturated fatty acids and sesame oil as oil containing a mixture of monounsaturated fatty acids and polyunsaturated fatty acids. Lactic acid was also selected as the pH buffer.

Animals

Animal usage was approved by the Institutional Animal Care and Use Committee of the Drug Research Division of Sumitomo Pharma Co., Ltd. Female Sprague-Dawley (Crl: CD) rats aged 5 weeks were purchased from The Jackson Laboratory Japan, Inc. (Kanagawa, Japan) and were subjected to quarantine and acclimation period of 1 week. Females were chosen because less toxicity information is available in females than in males. The animals were caged individually in a barrier-sustained room with a controlled temperature of 20-26°C, relative humidity of 40-70%, and 12-hr light/dark cycle. The rats were fed a commercial pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum, except during urine collection when the animals were fasted and fed bottled water ad libitum.

Animal experiments

In this study, each solvent was evaluated separately in 5 experiments, and the 0.5% MC-treated group was the control group in each experiment. At 6 weeks of age, the rats were randomly assigned to each group based on body weight. The group composition is shown in Table 1. The rats were dosed with 0.5% MC or the solvents by oral gavage once daily in the morning for 14 days. The dosing volume was 5 mL/kg for all solvents, except olive oil and sesame oil, which were administered at 2.5, 5, and 10 mL/kg, respectively. The volume was calculated based on the latest body weight. Clinical signs were observed daily before and within 4 hr after dosing. Body weights were measured on Days 1, 3, 8, 11, and 14 of dosing and body weight gains were calculated. Food consumptions were measured on Days 1-3, 6-8, and 12-14 of dosing, and food consumption in one day was calculated from food consumption in 2 days. Ophthalmological examinations using a slit lamp (SL-15; Kowa Co. Ltd., Nagoya, Japan), an ophthalmoscope (OMEGA 200 head-worn type; Heine Optotechnik GmbH & Co. KG, Herrsching, Germany), and a binocular indirect ophthalmoscope were performed during the quarantine or acclimation period and on week 2 of dosing.

Clinical pathology included urinalysis, hematology, and blood biochemistry. Urine samples were collected for 15-20 hr after administration on Day 14 of dosing. The volume was visually measured using a graduated cylin-

Table 1. Group composition.

Group	Concentration (% w/v)	Dose level (mg/kg/day)	Number of animals
Methyl cellulose 400	0.5	25	5
PEG 400	25	1250	5
	50	2500	5
	100	5000	5
DMSO	20	1100	5
	40	2200	5
	100	5500	5
HP-β-CD	5	250	5
	10	500	5
	20	1000	5
Tween 80	0.05	2.5	5
	0.5	25	5
	5	250	5
SDS	3	150	5
	6	300	5
	12	600	5
Olive oil	100	2250	5
	100	4500	5
	100	9000	5
Sesame oil	100	2250	5
	100	4500	5
	100	9000	5
Lactic acid	1	60	5
	5	300	5
	10	600	5

der or a measuring pipette, and sodium (Na), potassium (K), chloride (Cl), albumin (Alb), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine (Cre), N-acetyl-beta-D-glucosaminidase (NAG) and Na/K ratio (Na/K) were measured using an automated analyzer JCA-ZS050 (JEOL Ltd., Tokyo, Japan). After the final dosing, the animals were fasted for 15-20 hr, and whole blood was collected from the abdominal aorta at necropsy. In hematology, using blood treated with EDTA-2K, red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count (Ret), platelet count (PLT), white blood cells (WBC), and differential WBC count (basophil: Baso, lymphocyte: Lymp, eosinophil: Eos, neutrophil: Neut and monocyte: Mono) were measured by an automated blood cell analyzer XT-2000i (Sysmex Corporation, Hyogo, Japan). Also, using blood treated with 3.2% sodium citrate solution, prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (Fbg) were measured by an automated blood coagulation analyzer CS-2400 (Sysmex Corporation). In blood biochemistry, using blood treated with heparin lithium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP, LDH, total bilirubin (T-Bil), direct bilirubin (D-Bil), indirect bilirubin (I-Bil), γ-glutamyl transpeptidase (γ-GTP), creatine kinase (CK), total cholesterol (T-Cho), phospholipids (PL), triglycerides (TG), glucose (Glu), blood urea nitrogen (BUN), Cre, inorganic phosphorus (P), calcium (Ca), Na, K, Cl, total protein (TP), Alb, and albumin/globulin ratio (A/G) were measured by a JCA-ZS050 analyzer (JEOL Ltd., Tokyo, Japan).

At necropsy after whole blood collection, the external appearance and all the internal organs/tissues were examined. The lung, salivary glands, liver, heart, kidneys, ovaries, uterus, brain, spleen, thymus, pituitary gland, thyroid, and adrenal glands were weighed. The organ weights relative to the body weight (relative weight) were calculated using the body weight recorded on the day of necropsy. After the organ weight measurement, the lung, forestomach, glandular stomach, duodenum, jejunum, colon, pancreas, liver, heart, kidneys, urinary bladder, ovaries, uterus, vagina, cerebrum, cerebellum, pons, spinal cord, sciatic nerve, mesenteric lymph nodes, spleen, pituitary gland, adrenal glands, skeletal muscle, mammary gland, and gross lesions were fixed in 10% neutral-buffered formalin (NBF). The eyeballs were initially fixed in formaldehyde-glutaraldehyde in phosphate buffer and post-fixed in 10% NBF. Then, all fixed organs were embedded in paraffin, sectioned, stained with hematoxylin and eosin (HE), and examined by light microscopy.

Statistical analysis

Group means and standard deviations in the values of body weights, body weight gains, food consumptions, urinalysis, hematology, coagulation, blood biochemistry, and organ weights were calculated, and each value was statistically analyzed as follows using SAS versions 9.4 software (SAS Institute Inc., Cary, NC, USA). The differences in group means between the control group and each solvent-treated group were tested using the Dunnett test. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

PEG 400

No deaths were noted in any dose groups. Soft stool was noted at > 2,500 mg/kg/day. No changes were noted in any parameters evaluated at 1,250 mg/kg/day. Statistically significant lower Na and Cl in urinalysis and lower K in blood biochemistry (Supplemental Fig. 1) were considered not to be PEG 400-related because these values were not dose dependent, or were within the background data range at the facility, or both. Therefore, the NOEL of PEG 400 given orally to rats for 2 weeks was considered to be 1,250 mg/kg/day.

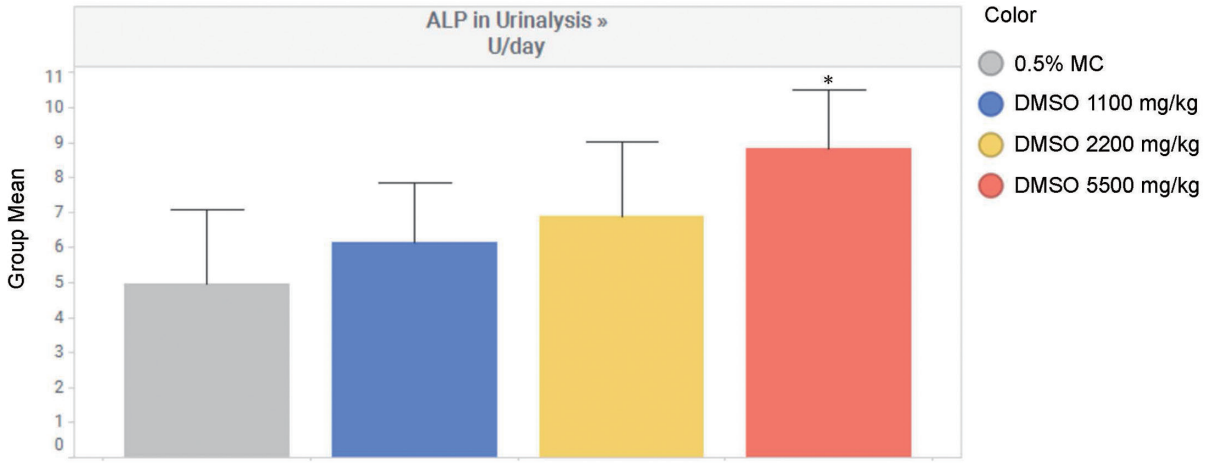

Fig. 1. Increased ALP in urinalysis in the DMSO 5,500 mg/kg/day group. Values represent group mean ± SD. n = 5. The asterisk (*) indicates a statistically significant difference from the 0.5% MC group (*: $p < 0.05$).

In previous reports, PEG 400 has a laxative effect and causes soft stool (Neervannan, 2006), qualitatively similar to the results of the present study. In addition, PEG 400 given orally to rats has been reported to cause soft stools at 2,000 mg/kg/day for 5 days and statistically significant increase in urine Cre at 500 mg/kg/day for 5 days with no dose dependency (Gopinathan *et al*., 2013); soft stools and slight decrease in body weights and food consumptions at > 2,800 mg/kg/day for 13 weeks, and slight increase in kidney weights and changes in some urinary parameters at > 1,100 mg/kg/day for 13 weeks (Hermansky *et al*., 1995). In our 2-week study, soft stool was noted as a PEG 400-related change, but other changes were not.

DMSO

No deaths were noted in any dose groups. Statistically significant higher ALP in urinalysis at 5,500 mg/kg/day (Fig. 1) and a DMSO-specific offensive odor as clinical signs at > 1,100 mg/kg/day were noted. Other statistically significant changes such as lower LDH in blood biochemistry and higher absolute and relative uterus weights (Supplemental Fig. 2) were considered not to be DMSO-related because these values were within the background data range in the facility.

The higher ALP level could affect toxicity assessment. The DMSO-specific offensive odor has been reported previously (Gopinathan *et al*., 2013). The odor was considered to come from the dimethyl sulfide (DMS), a degradation product of DMSO (Distefano and Borgstedt, 1964). However, it did not affect other parameters. Therefore, it was considered that the odor might be toxicologically insignificant. Based on these results, the NOEL of DMSO given orally to rats for 2 weeks was considered to be less than 1,100 mg/kg/day, though it might be 2,200 mg/kg/day if the offensive odor is not considered.

In previous reports in rats, oral DMSO has been reported to produce a DMSO-specific offensive odor at > 250 mg/kg/day for 5 days, statistically significant increase in serum cholesterol and gas retention in the large intestine at necropsy at 1,000 mg/kg/day for 5 days (Gopinathan *et al*., 2013); liver morphologic change at > 1,000 mg/kg/day for 6 weeks, slight decrease in body weights and some organ weights at > 1,000 mg/kg/day for 59 days; death at 14,100 mg/kg/day for 13 weeks, and atrophy of the spleen at > 400 mg/kg/day for 13 weeks (Smith *et al*., 1967). Gopinathan *et al*. reported an increase in serum cholesterol and gas retention in the large intestine at necropsy, which were not noted in our two-week study. Gopinathan *et al*. performed necropsy under non-fasting conditions, which may have resulted in the increase in serum cholesterol and gas retention. In addition, Smith *et al*. reported animal death, liver morphologic change, decrease in body weight, decrease in some organ weights, and atrophy of the spleen, which were not noted in our 2-week study.

HP-β-CD

No deaths or HP-β-CD-related changes were noted in any dose groups. Statistically significant higher body weights and body weight gains, lower PLT and

Eos in hematology, lower T-Bil and I-Bil in blood biochemistry, and higher absolute and relative lung weights (Supplemental Figs. 3 and 4) were considered not to be HP-β-CD-related because these values were not dose dependent, or were within the background data range in the facility, or both. Therefore, the NOEL of HP-β-CD given orally to rats for 2 weeks was considered to be 1,000 mg/kg/day.

In previous reports, oral administration of HP-β-CD to rats caused higher AST and ALT in blood biochemistry, indicating HP-β-CD affected the liver (Thackaberry, 2013; Gould and Scott, 2005; Gad *et al*., 2016). In our study, a tendency to higher AST (+ 28%) and ALT (+ 48%) at 1,000 mg/kg/day was noted; however, these changes were not statistically significant and were within the background data range in the facility and no liver abnormalities were noted on histopathology. Therefore, the slight changes in AST and ALT would not affect toxicity assessment in short-term toxicity studies in rats. In addition, oral dose of HP-β-CD to rats has been reported to cause soft stools and increases in AST and ALT at > 2,250 mg/kg/day for 7 days and at > 450 mg/kg/day for 28 days, increase water consumption and changes in some hematologic and blood biochemical parameters at 4,500 mg/kg/day for 28 days (Gould and Scott, 2005). These changes were not noted in our 2-week study.

Tween 80

No deaths or Tween 80-related changes were noted in any dose groups. Statistically significant higher Na and Na/K in urinalysis and lower relative pituitary weights (Supplemental Fig. 5) were considered not to be Tween 80-related because these values were not dose dependent, or were within the background data range in the facility, or both. Therefore, the NOEL of Tween 80 given orally to rats for 2 weeks was considered to be 250 mg/kg/day.

In a previous report, oral dose of Tween 80 to rats for 5 days has been reported to induce statistically significant lower Glu and higher Na in blood biochemistry at > 5 mg/kg/day and lower uric acid in blood biochemistry at > 50 mg/kg/day and lower Ca in blood biochemistry at 150 mg/kg/day (Gopinathan *et al*., 2013). These changes were not noted in our 2-week study. The differences in the results of blood biochemistry were considered to be dependent on whether rats were fasted before necropsy.

SDS

One animal given 600 mg/kg/day died on Day 5 of dosing. At 600 mg/kg/day, abnormal respiratory sounds, bradypnea, salivation, soft stool, urinary incontinence, and a scab in the dorsal neck as clinical signs, and statistically significant lower food consumption were noted (Fig. 2). Therefore, the dose level was considered intolerable.

At 150 and 300 mg/kg/day, salivation and abnormal respiratory sounds as clinical signs, rough surface in the forestomach and raised focus in the forestomach at necropsy, and/or erosion/ulcer, multifocal squamous cell hyperplasia and mixed inflammation in the forestomach on histopathology (Fig. 3) were noted. These changes were considered to be due to the irritant effects of SDS

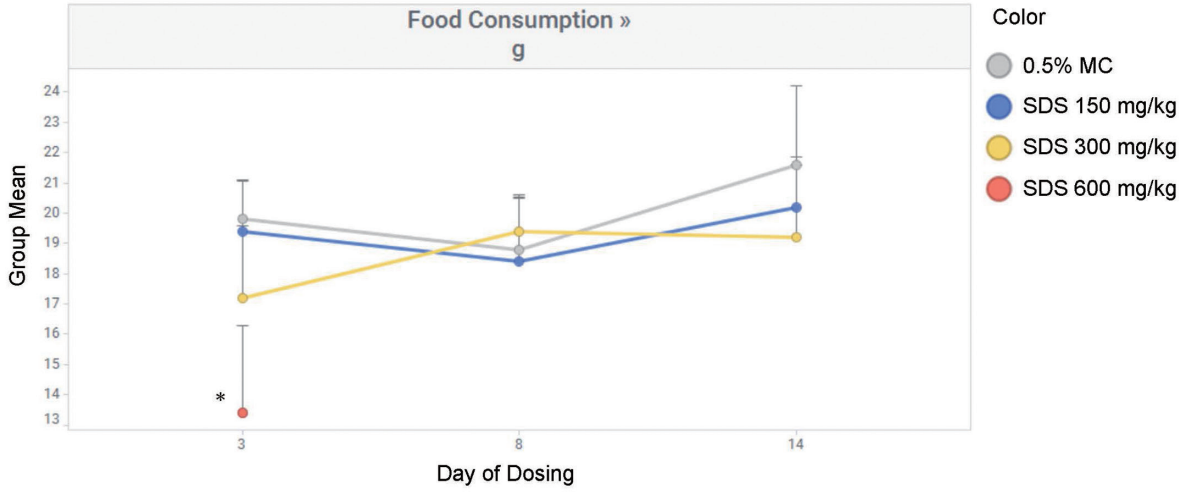

Fig. 2. Decreased food consumption in the SDS 600 mg/kg/day group. Values represent group mean ± SD. n = 5. The asterisk (*) indicates a statistically significant difference from the 0.5% MC group (*: $p < 0.05$).

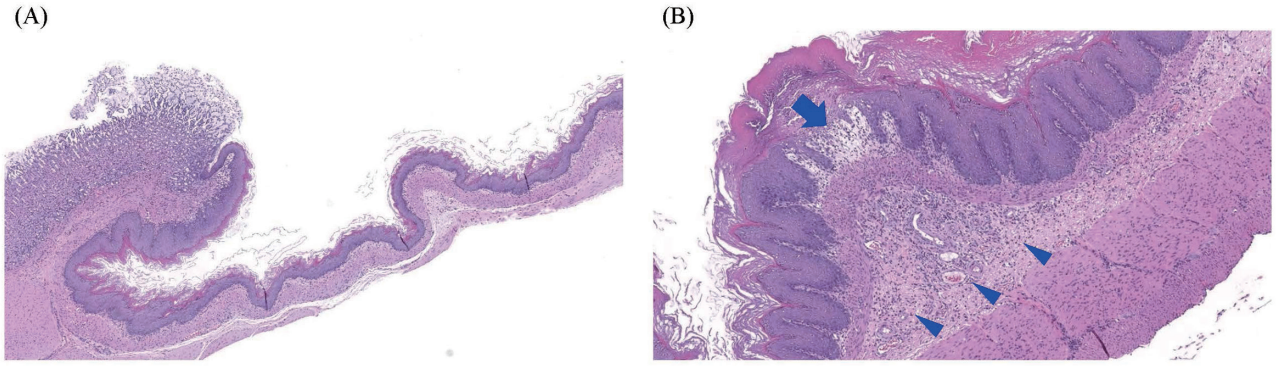

Fig. 3. Histopathological images of the forestomach of rats in the 0.5% MC group (A) and SDS 300 mg/kg/day group (B) (hematoxylin and eosin). Erosion/ulcer (arrows), hyperplasia of squamous cell and inflammatory cell infiltration (arrow heads) are observed.

(TOXNET). Other statistically significant changes such as Na in urinalysis (Supplemental Fig. 6) were not considered SDS-related because they were not dose dependent. Therefore, the NOEL of SDS given orally to rats for 2 weeks was considered less than 150 mg/kg/day.

In a previous report, oral SDS given at 100 mg/kg/day to rats for 1 month was the no-observed-adverse-effect-level (NOAEL) (Thackaberry, 2013), so a dose level of 150 mg/kg/day, which is close to 100 mg/kg/day, was set as the lowest dose level in the present study. However, irritant changes such as rough surface in the forestomach were observed at > 150 mg/kg/day.

Olive oil

No deaths were noted in any dose groups. Statistically significant lower body weights and body weight gains with lower food consumptions were noted throughout the period of dosing at 9,000 mg/kg/day (Figs. 4 to 6). The mean body weights were 6% lower than in the control group on Day 14 of dosing. No olive oil-related changes were noted in any parameters evaluated at 2,250 and 4,500 mg/kg/day. Other statistically significant changes such as lower food consumptions at 2,250 mg/kg/day, higher Na/K in urinalysis, higher AST in blood biochemistry, and lower T-Cho in blood biochemistry (Fig. 6 and Supplemental Fig. 7) were considered not to be olive oil-related because these values were not dose dependent, or were within the background data range in the facility, or both. Therefore, the NOEL of olive oil given orally to rats for 2 weeks was considered to be 4,500 mg/kg/day.

Administration of monounsaturated fatty acids such as olive oil has been reported to increase glucagon-like peptide 1 (GLP-1) (Mansour *et al*., 2013). Increase in GLP-1 suppresses gastric motility and slows the excretion of gastric contents from the stomach, which may result in decreased food consumption and body weight loss (Li *et al*., 2016; Baggio and Drucker, 2014; Habegger *et al*., 2013). The body weight loss with decreased food consumptions noted in our study was considered to be caused by the above mechanism.

In a previous report, olive oil orally given to rats for 5 days has been reported to cause oil in the stomach and discoloration of the intestinal wall at necropsy at > 10,000 mg/kg/day; discoloration of liver and spleen, and fecal mass in the large intestine at necropsy at 20,000 mg/kg/day; and changes in some clinical pathological parameters at > 5,000 mg/kg/day (Gopinathan *et al*., 2013). These changes were not noted in our 2-week study. The differences in the results were probably due to the difference in the timing of necropsy. Blood sampling and necropsy were conducted approximately 1 to 2 hr after the final dosing in the study reported by Gopinathan *et al*., and approximately 15 to 20 hr after the final dosing in our study.

Sesame oil

No deaths were noted in any dose groups. Statistically significant lower food consumption was noted throughout the period of dosing at 9,000 mg/kg/day, though there was no body weight loss (Fig. 7). No sesame oil-related changes were noted in any parameters evaluated at 2,250 mg/kg/day and 4,500 mg/kg/day. Other statistically sig-

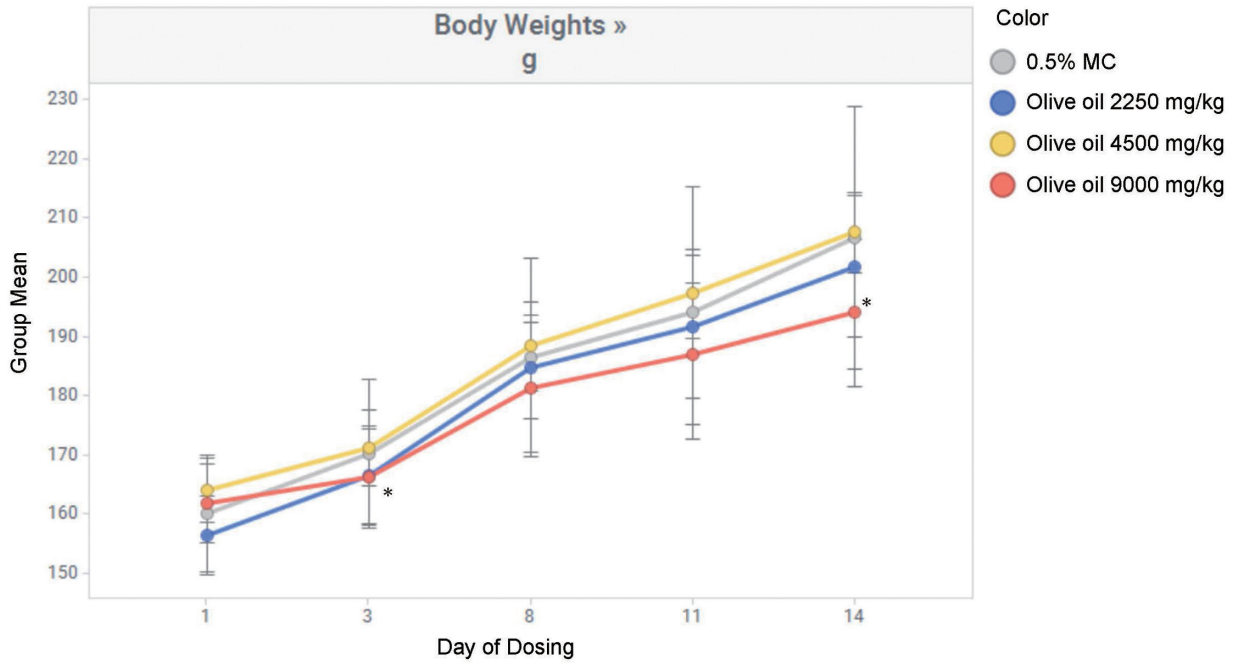

Fig. 4. Decreased body weights in the olive oil 9,000 mg/kg/day group. Values represent group mean ± SD. n = 5. The asterisk (*) indicates a statistically significant difference from the 0.5% MC group (*: $p < 0.05$).

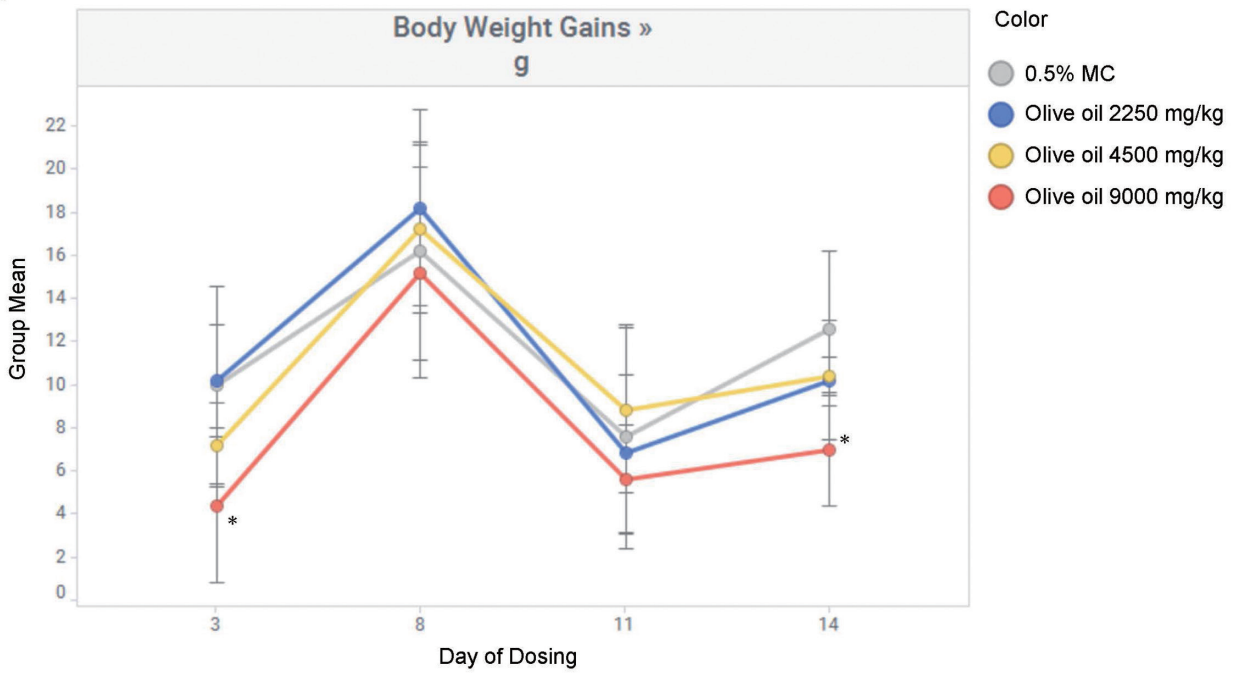

Fig. 5. Decreased body weight gains in the olive oil 9,000 mg/kg/day group. Values represent group mean ± SD. n = 5. The asterisk (*) indicates a statistically significant difference from the 0.5% MC group (*: $p < 0.05$).

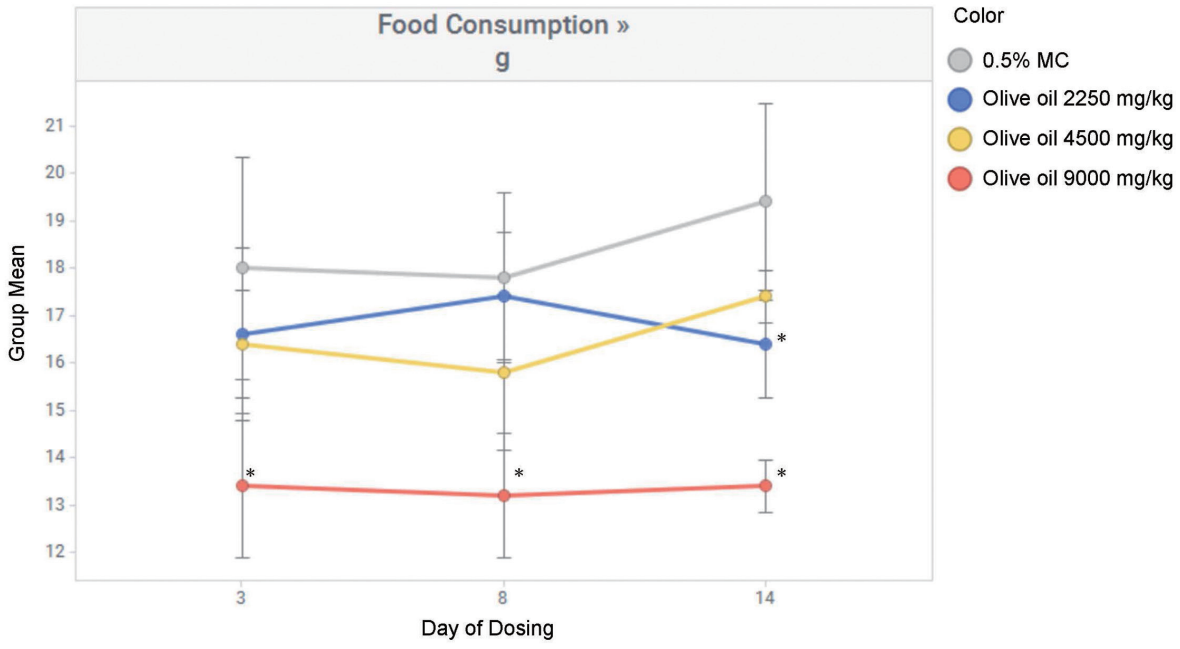

Fig. 6. Decreased food consumption in the olive oil 9,000 mg/kg/day group. Values represent group mean ± SD. n = 5. The asterisk (*) indicates a statistically significant difference from the 0.5% MC group (*: $p < 0.05$).

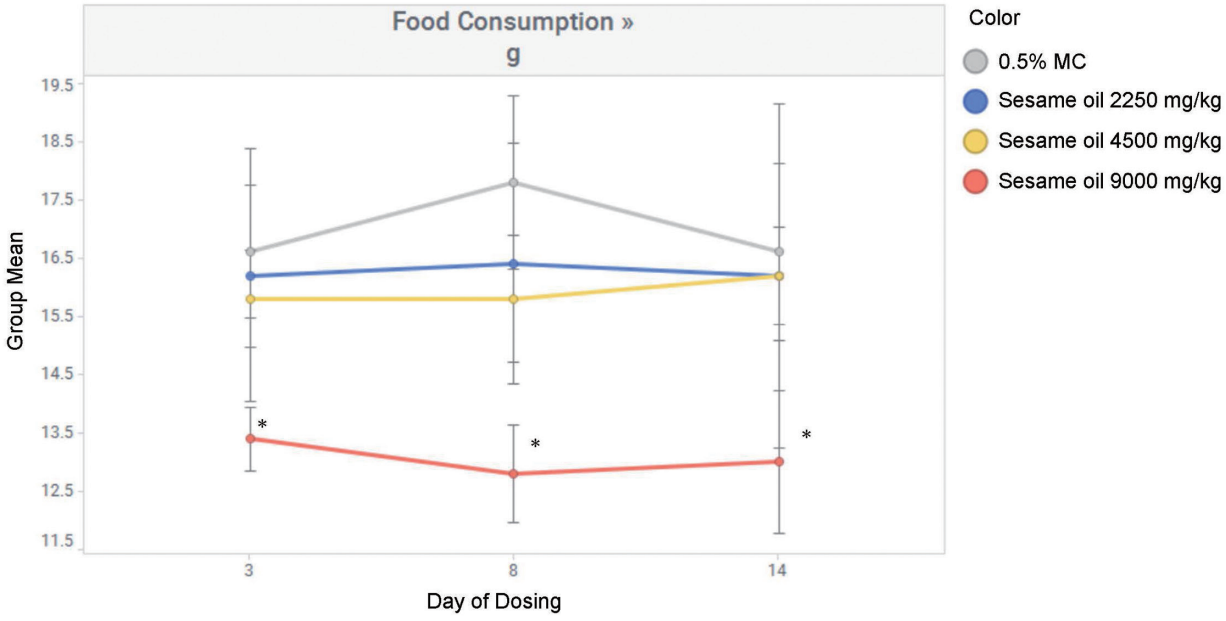

Fig. 7. Decreased food consumption in the sesame oil 9,000 mg/kg/day group. Values represent group mean ± SD. n = 5. The asterisk (*) indicates a statistically significant difference from the 0.5% MC group (*: $p < 0.05$).

nificant changes such as higher LDH in urinalysis, lower MCHC in hematology, higher Cl in blood biochemistry, and lower relative kidney weights (Supplemental Fig. 8) were considered not to be sesame oil-related because these values were not dose dependent, or were within the background data range in the facility, or both. Therefore, the NOEL of sesame oil given orally to rats for 2 weeks was considered to be 4,500 mg/kg/day.

The main components of sesame oil are oleic acid (monounsaturated fatty acid) and linoleic acid (polyunsaturated fatty acid). Therefore, the lower food consumption at 9,000 mg/kg/day sesame oil was considered to be caused by the increase in GLP-1 after the administration of monounsaturated fatty acids as in the case of olive oil. The reason for the lack of body weight loss in our study is unknown. However, the reduction in food consumption at 9,000 mg/kg/day sesame oil was similar to that at 9,000 mg/kg/day olive oil, suggesting that 9,000 mg/kg/day sesame oil may also cause body weight loss.

In a previous report, sesame oil given orally to rats for 1 month has been reported to be well tolerated at a dose volume of 1 mL/kg (but the dose level is unknown) (Gad *et al*., 2006; Gad *et al*., 2016). No sesame oil-related changes were noted at 4,500 mg/kg/day in our 2-week study.

Lactic acid

No deaths or lactic acid-related changes were noted in any parameters evaluated in any dose group. Therefore, the NOEL of lactic acid given orally to rats for 2 weeks was considered to be 600 mg/kg/day.

In a previous report, orally administered lactic acid had an LD_{50} of 3,730 mg/kg in rats and was well tolerated up to 2,000 mg/kg/day for 16 days (The joint FAO/WHO Expert Committee on Food Additives, 1973). Similar to our two-week study, this previous report found no abnormalities in any parameters evaluated up to the highest dose level of 600 mg/kg/day.

In conclusion, in the present study, we investigated the oral repeated toxicity (for 2 weeks) of eight solvents (PEG 400, DMSO, HP-β-CD, Tween 80, SDS, olive oil, sesame oil, and lactic acid) in female rats.

NOELs were confirmed to be 1,250 mg/kg/day for PEG 400, 1,000 mg/kg/day for HP-β-CD, 250 mg/kg/day for Tween 80, 4,500 mg/kg/day for olive oil, 4,500 mg/kg/day for sesame oil, and 600 mg/kg/day for lactic acid. However, the NOELs of DMSO and SDS could not be identified, because rats dosed with DMSO or SDS showed DMSO-specific offensive odor or SDS-related irritant effects even at the lowest dose levels. However, 2,200 mg/kg/day of DMSO may be acceptable if the DMSO-specific offensive odor is unlikely to affect toxicity assessment. It was concluded that the vehicles can be used in 2-week oral dose toxicity studies in rats without affecting toxicity parameters at dose levels below the NOELs identified in our research.

This report provides useful information for those performing detailed toxicity assessments of poorly soluble compounds in drug development.

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

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