P-74

アセトアミノフェン投与により発生したマイクロミニピッグのNSAIDs不耐症を疑う症例

Cases of suspected NSAIDs intolerance of *microminipigs* caused by acetaminophen administration

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Introduction

Microminipig (MMP, Fuji Micra Inc.) is raising attention as a new test system due to their ultra-small size and easy handling.

The purpose of this study was to evaluate the potential toxicity of acetaminophen (APAP) when administered by oral gavage to MMPs at 0, 80, 200, and 600 mg/kg/day for 28 consecutive days.

Symptoms very similar to human “NSAIDs intolerance (urticaria/angioedema)” were observed specifically in all MMPs at 600 mg/kg and died.

Through this study, the usefulness of MMPs is confirmed and the clinical, gross, and histopathological features caused by APAP administration are presented.

Methods

Materials

<Test article>
A generic term ： Acetaminophen(APAP)
Lot No. ： 5IXZKEF
Supplier : Tokyo Chemical Industry Co., Ltd.

<Animals>
Sus scrofa domestica,
Microminipig (MMP, Fuji Micra Inc.)
Male, 16-25 months old

<Caging>
Individual housing
< Housing and Environmental Conditions >
Temperature : 17.5～28.4℃
Humidity : 29.5～80.4%
Lighting : 12-hour light/dark cycle

<Diet>
Pellet chow NS（Nosan Corporation.）
<Water>
Tap water, provided ad libitum,
Through an automatic waterer

<Adinistration>
Route of administration: Oral
Dosage : 0, 80, 200, and 600 mg/kg body weight/day
Dosing volume : 2 mL/kg
(Individual dosing volume was determined from body weight on the day of administration)
Administration : Gavage administration by a disposable syringe and stomach tube.
Dose frequency : Once a day

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Measurement

Clinical sign	: Once a day
Body weight	: Day 1*, 8, 15, 22, 29 and the day of autopsy
Food consumption	: Day 1, 8, 15, 22, 28 and the day of autopsy
Electrocardiogram	: pre, Day 27
Blood pressure	: pre, Day 27
Ophthalmology	: pre, Day 27
Urinalysis	: pre, Day 27
Hematology	: Day 28
Blood biochemistry	: Day 28
Organ weights	: the day of autopsy
Necropsy	: At the day after the final administration
Histopathology	: At the day after the final administration

＊: The first dosing date was defined as Day 1

Results

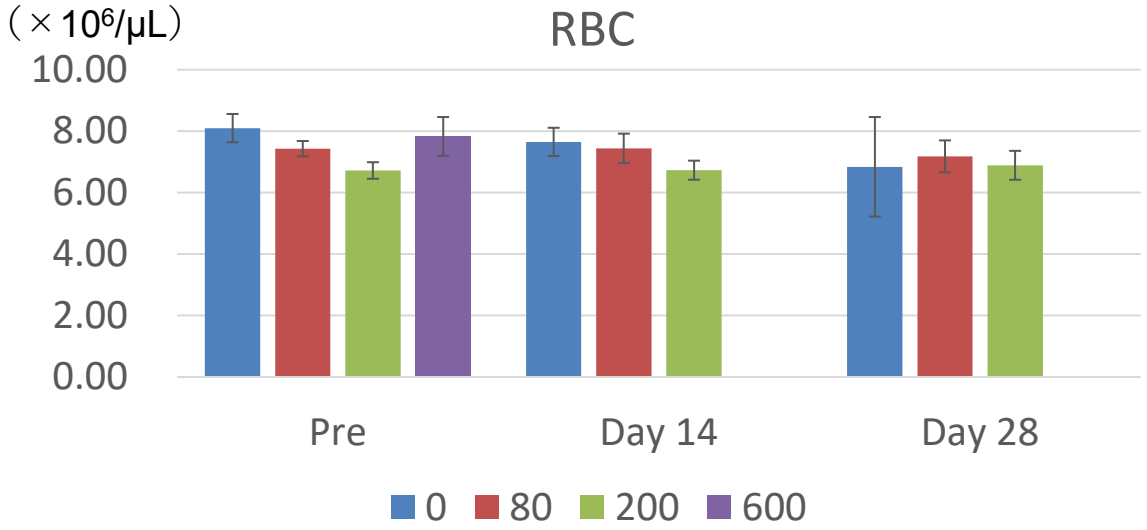
Clinical sign

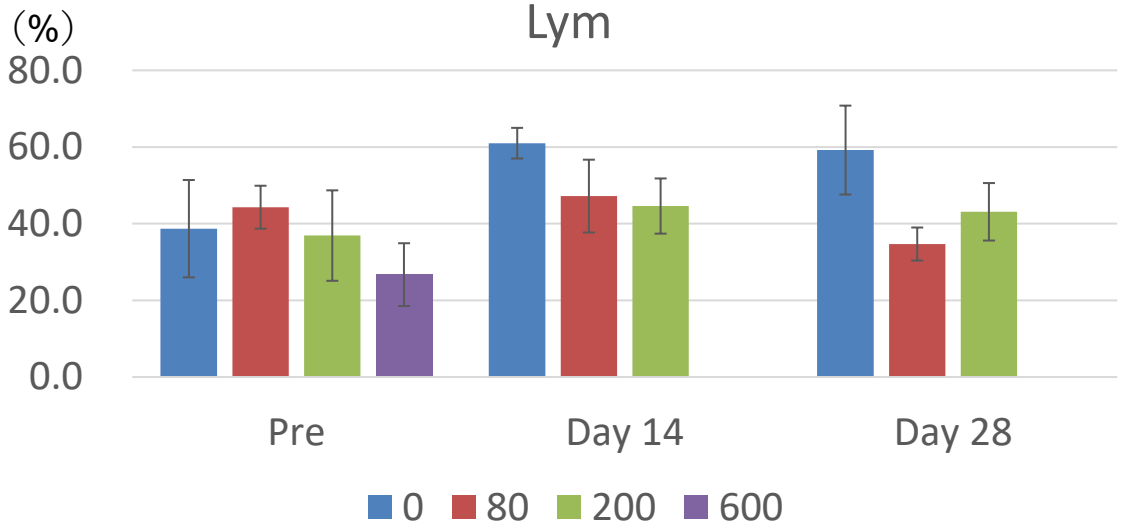
Dose (mg/kg/day)	Animal No.	Findings	Day1 B	Day1 A	Day2 B	Day2 A	Day3 B	Day3 A	Day4 B	Day4 A	Day5 B	Day5 A	Day6 B	Day6 A	Day7-28 B	Day7-28 A
200	1301	Decreased locomotor activity	-	-	-	-	+	-	-	-	+	+	-	-	-	-
		Lying on belly	-	-	-	-	+	-	-	-	+	+	-	-	-	-
	1302	Decreased locomotor activity	-	-	+	-	-	-	+	+	-	-	-	-	-	-
		Lying on belly	-	-	+	-	-	-	+	+	-	-	-	-	-	-
	1303	Decreased locomotor activity	-	-	+	-	-	-	+	+	+	-	+	-	-	-
		Lying on belly	-	-	+	-	-	-	+	+	+	-	+	-	-	-
		Average of food consumption (g)	500.2		500.0		500.0		500.0		500.0		500.0		500.0	
600	1401	Death	-	-	-	-	+	·	·	·	·	·	·	·	·	·
		Depression	-	-	+	+	·	·	·	·	·	·	·	·	·	·
		Head swelling	-	-	+	+	·	·	·	·	·	·	·	·	·	·
	1402	Death	-	-	-	-	-	+	·	·	·	·	·	·	·	·
		Decreased locomotor activity	-	-	-	-	+	·	·	·	·	·	·	·	·	·
		Lying on belly	-	-	-	-	+	·	·	·	·	·	·	·	·	·
		Head swelling	-	-	+	+	+	·	·	·	·	·	·	·	·	·
		Itching	-	-	+	+	+	·	·	·	·	·	·	·	·	·
		Cyanosis	-	-	-	-	+	·	·	·	·	·	·	·	·	·
	1405	Death	-	-	-	-	+	·	·	·	·	·	·	·	·	·
		Decreased locomotor activity	-	-	+	+	·	·	·	·	·	·	·	·	·	·
		Lying on belly	-	-	+	+	·	·	·	·	·	·	·	·	·	·
		Head swelling	-	-	+	+	·	·	·	·	·	·	·	·	·	·
		Redness	-	-	+	+	·	·	·	·	·	·	·	·	·	·
		Cyanosis	-	-	+	+	·	·	·	·	·	·	·	·	·	·
		Average of food consumption (g)	197.4		71.3		0.6		·	·	·	·	·	·	·	·

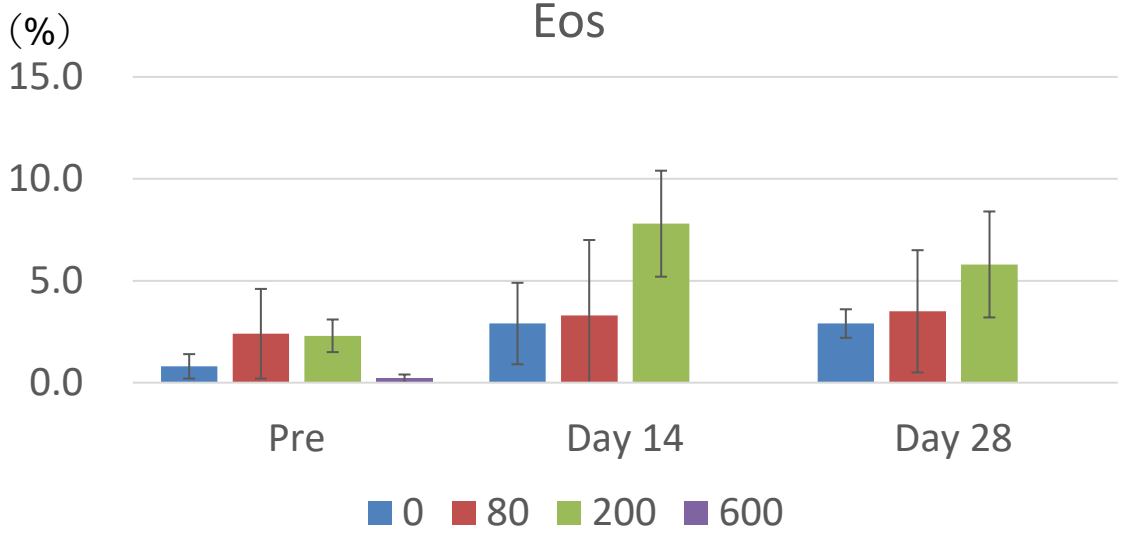
B : Before to immediately after administration, A : 1 to 4 hours after administration　– : No noteworthy findings, +: The finding is observed　·: No data

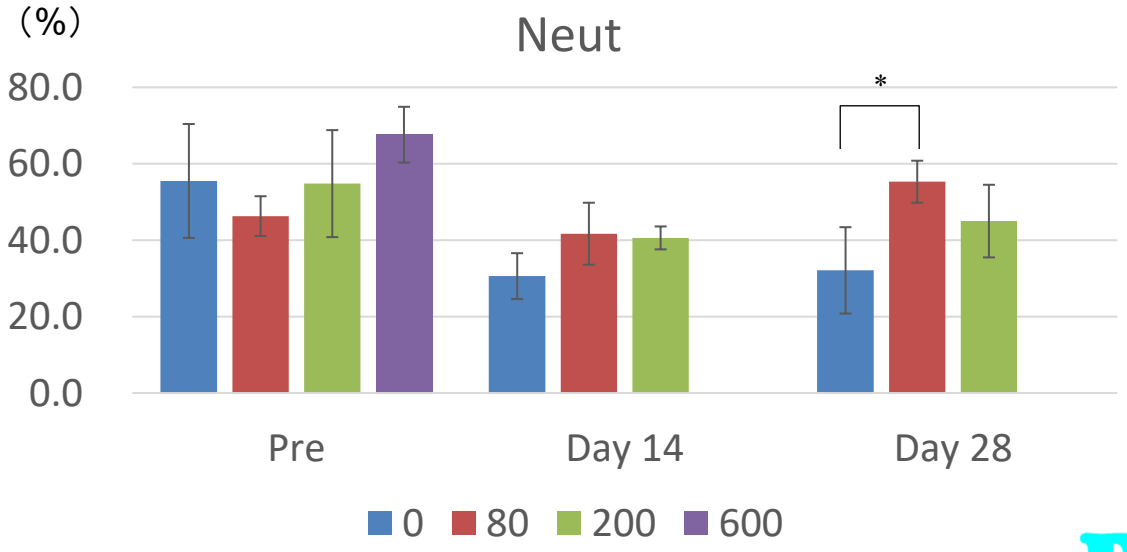
No note worthy findings were observed at 0 and 80 mg/kg.

Hematology



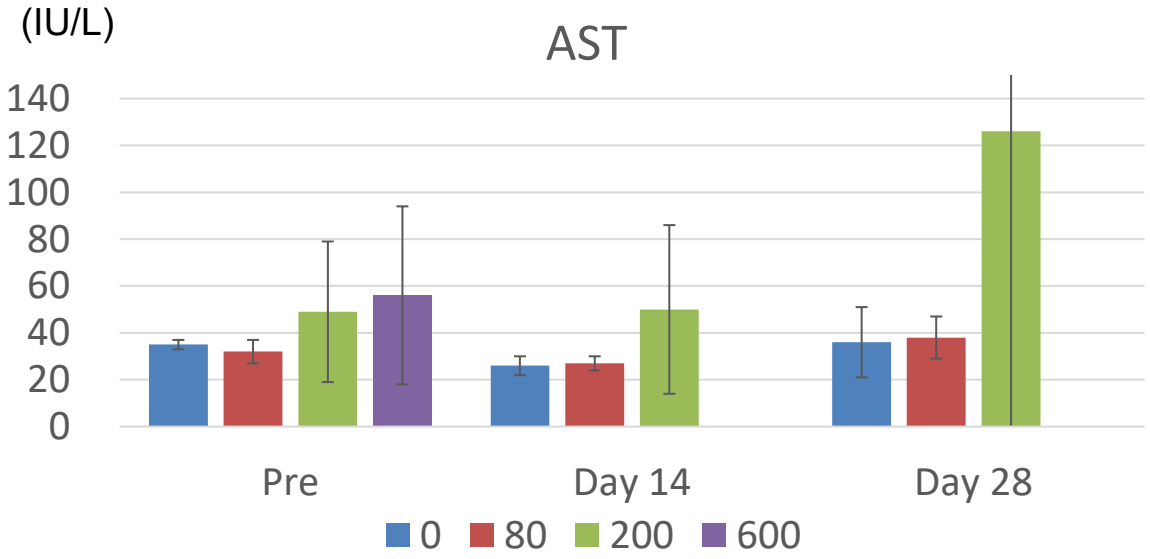


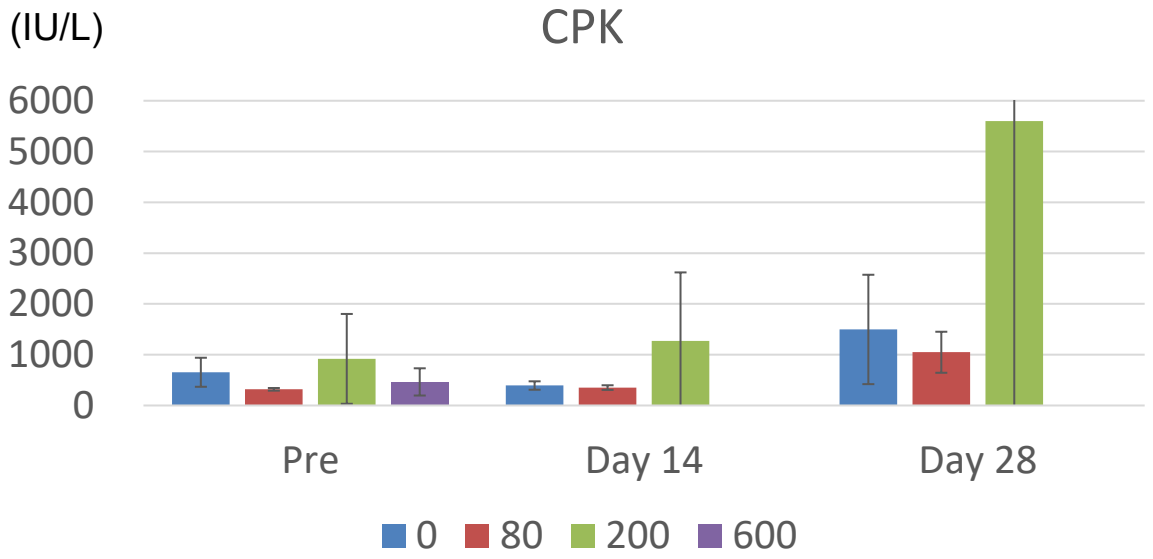


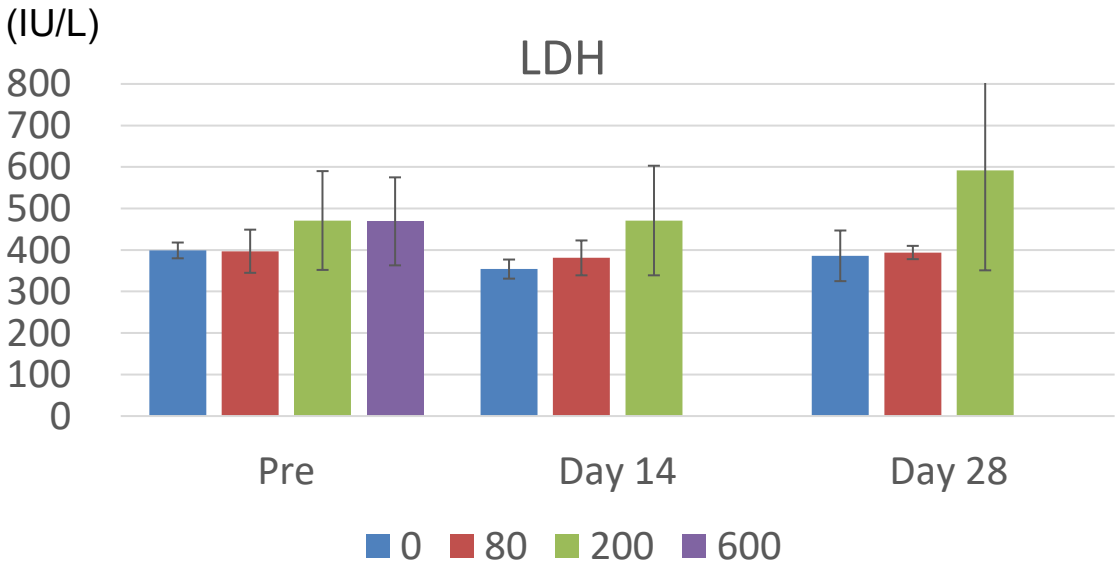


* : $p<0.05$ vs. control（Dunnett test）

Blood biochemistry







Necropsy

Organ/tissue		Findings	Dose of acetaminophen(mg/kg/day)			
			0[a)]	80	200	600
		No. of animal examined	3	3	3	3
External examination	Skin ※	Redness to cyanosis-like dark purple discoloration	0 #	0	0	3
	Skin of head and neck	Swelling = subcutaneous edema (geratinous), severe	0	0	0	3
Head and neck	Submandibular lymph nodes	Edema and/or dark reddish discoloration	0	0	0	3
	larynx	Edematous thickening, mucosal	0	0	0	3
	Thymus (thoracic)	Small sized	0	0	1	3
	Skull	Morphologic anomaly, foramen magnum stenosis	0	0	1	0
Thoracic cavity	Lung	Pale to dark reddish discoloration and edema, lobar to whole lung	3	1	3	3
		Adhesion with pleura	0	2	1	0
	Heart	Petechiae to ecchymosis ＊	0	0	0	3
Abdominal cavity	Stomach (fundus-pylorus)	Dark red discoloration	0	0	0	3
	Small intestine	Dark red discoloration, intestinal wall	1	0	0	3
	Liver	Dark red discoloration, all lobes	0	0	0	3
	Kidney	Dark red discoloration, medulla	0	0	0	3
	Mesenteric lymph nodes	Dark red discoloration	0	0	0	3
Pelvic cavity	Urinary bladder	Red discoloration, mucosal	0	0	0	2
	Testis and Epididymis	failure of testicular descent, Left, small sized	1	0	0	0
		Adhesion with capsule	0	0	1	0

a) : 0.5% Methylcellulose administered at a rate of 2 mL/ kg　　#: The number of animals with findings

: The findings highlighted in yellow were specifically observed at 600 mg/kg.

※ : Eyelids, auricle, Inside of the limbs, abdomen, around the dewlap, nipple, foreskin, perianal, perineum, and scrotum.

＊: Adipose tissue around coronary arteries and veins, right atrial auricle

No noteworthy change was observed in the following organ/tissue : Eyeball, submandibular gland, tongue, thymus (cervical), aorta, spleen, pancreas, large intestine, bile duct, adrenals, prostates, seminal vesicles, brain, spinal cord, pituitary gland, skeletal muscles, sciatic nerve, femur, sternum.

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Histopathology

Organ/tissue	Findings		Dose of acetaminophen（mg/kg/day） 0[a)]	80	200	600
Integumentary system						
Abdominal skin with nipple		No. of animals examined	3	3	3	3
	Hemorrhage		0 #	0	0	1
Skin of head		No. of animals examined	2	3	3	3
Upper lip	Edema, dermis and subcutis（with granulocyte infiltration and fibrin deposition, perivascular and around sweat glands）		0	0	0	3
		mild	0	0	0	1
		moderate	0	0	0	2 ＊
	Mononuclear cell infiltration, dermis and subcutis（around sweat glands ）		1	3	3	0
		mild	1	2	2	0
		moderate	0	1	1	0
Lower lip	Edema, dermis and subcutis		0	0	0	1
	Ulcer, focal		0	0	1	0
Eyelid	Edema, dermis（with degeneration / necrosis and fibrin deposition in muscular layer）		0	0	N/A	3
		moderate	0	0	N/A	3
	Mononuclear cell infiltration, dermis and subcutis（perivascular and around hair follicles）		0	2	N/A	0
Auricle		No. of animals examined	2	2	3	3
	Edema, dermis（with granulocyte infiltration and fibrin deposition）		0	0	0	3
		moderate	0	0	0	1
		severe	0	0	0	2
	Mononuclear cell infiltration, dermis and subcutis（perivascular and around hair follicles）		0	1	0	0

a) : 0.5% Methylcellulose administered at a rate of 2 mL / kg.　# : The number of animals with findings and the grade is mild unless otherwise noted.
＊ : With dermal degeneration / necrosis (characterized by vacuolation) in one animal. : The findings highlighted in yellow were specifically observed at 600 mg/kg/day.

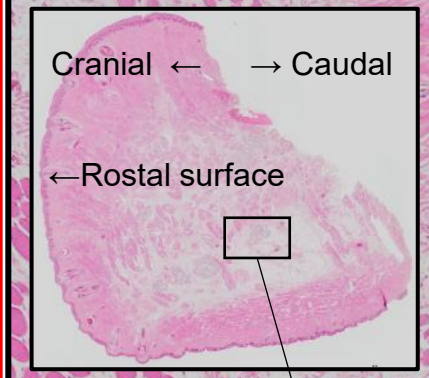
Histopathology

	Organ/tissue	Findings	Dose of acetaminophen (mg/kg/day) 0[a)]	80	200	600
		No. of animals examined	3	3	3	3
Cardiovascular system	Heart	Hemorrhage, subepicardial adipose tissue	0 #	0	0	3
Hematopoietic system	Spleen	Congestion	0	0	1	2
	Thymus	Atrophy / degeneration	2	3	3	3
	Mesenteric lymph node	Congestion	0	0	0	2
	Submandibular lymph node	Hemorrhage	0	0	0/1	2
Respiratory system	Trachea	Edema, lamina propria	0	0	0	1
	Lung	Alveolar septal thickening and / or Inflammatory cell infiltration	3	3	3	2
		Congestion	1	2	2	3
Digestive system	Tongue	Edema	0	0	0	3
	Esophagus	Hemorrhage, muscle layer	0	0	0	1
		Edema, submucosa	0	0	0	1
	Pancreas	Fatty infiltration	3	3	2	3
		Cellular infiltration	0	0	1	0
	Liver	Congestion	0	1	1	3
		Cellular infiltration	0	0	1	3
		Degeneration / necrosis, hepatocellular	0	0	0	3
		Hemorrhage	0	0	0	1
	Gallbladder	Erosion	0	0	0/1	1/2
Urinary system	Kidney	Mononuclear cell infiltration	3	1	1	2
		Dilatation, distal tubular	0	0	0	2
		Congestion	0	0	0	3
		Granuloma	0	1	0	0
Reproductive system	Testis	Degeneration / atrophy, seminiferous tubule	1	1	1	3
		Spermatocele	0	1	1	1
	Prostate	Mineralization	2	3	1	2
Endocrine system	Thyroid	Dilatation, follicle	0	1	0	1
		Fatty infiltration	0	0	1	0
	Adrenal	Focal hyperplasia, cortical cells	1	0	0	0
		Degeneration / necrosis, with hemorrhage	0	0	0	3
Musculoskeletal system	Femur	Disarrangement of epiphyseal cartilage	2	1	3	2

a) : 0.5% Methylcellulose administered at a rate of 2 mL / kg. # : The number of animals with findings. The findings highlighted in yellow were specifically observed at 600 mg/kg/day

No noteworthy change was observed in the following organ/tissue : Eyeball, submandibular gland, aorta, stomach, small intestine, large intestine, urinary bladder, epididymis, seminal vesicles, brain, spinal cord, pituitary gland, skeletel muscles, siatic nerve, bone(sternum), bone marrow(femur and sternum).

Histopathology



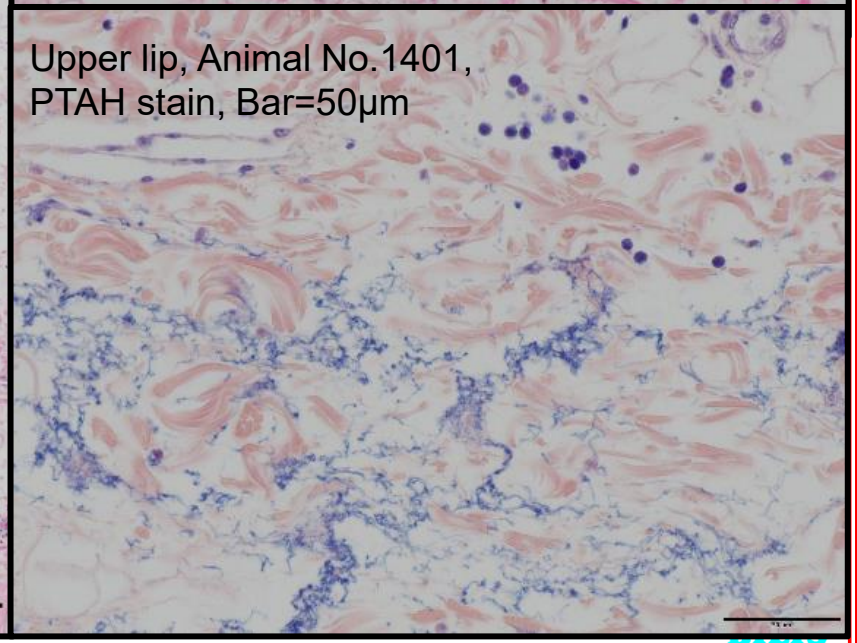
Sweat gland

Subcutaneous edema,with granulocyte infiltration and fibrin deposition, perivascular and around sweat glands

Sagittal section of upper lip, Animal No.1401,HE stain, Bar=500μm

granulocyte infiltration and fibrin deposition

Upper lip, Animal No.1401,
PTAH stain, Bar=50μm



Summary of results

Clinical sign : Swelling of head, decreased locomotor activity, loss of appetite, skin redness, pruritus and cyanosis were observed in all of animals at 600 mg/kg from Day 2 and they died on Day 3. At 200 mg/kg, decreased locomotor activity was observed intermittently from Day 2 to 6.

Hematology : Decreases of red blood cell counts on Day 14. Decreases of lymphocyte ratio of leukocyte on Day 14 and 28. Increase of eosinophil and neutrophil ratio of leukocyte on Day 14 and 28.

Blood biochemistry : Increases of AST, CPK and LDH on Days 14 and 28.

Necropsy : Subcutaneous edema in the head, petechiae on the epicardial fat, and dark reddish discoloration of the lung, liver, and kidney were observed in the dead animals.

Histopathology : Edema with granulocyte infiltration and fibrin deposition in dermis and / or subcutis of the lips, eyelids, and auricles, hemorrhage in heart, and congestion of the lungs, liver, and kidneys were observed in the dead animals.

Discussion

1. The well known toxicity of APAP was confirmed using MMP, suggesting MMP is useful for the toxicity study.

2. In human, NSAIDs intolerance is defined as the symptoms of hypersensitivity to all NSAIDs that have prostaglandin synthase inhibitory action. Urticaria / angioedema is a type of NSAIDs intolerance and characterized by swelling of lips, eyelids or face a few minutes to half a day after using an antipyretic analgesic. It is said it can be caused by antipyretic analgesics including APAP.

In this study, dead animals showed edema of the head within a day after administration and histopathological changes suggesting increased vascular permeability were observed, so that these cases were considered very similar to human NSAIDs intolerance (urticaria / angioedema) .
Although the differential diagnosis of NSAIDs intolerance includes allergies, the involvement of NSAIDs intolerance was suspected because these animals had no history of APAP administration before the study.

Further toxicological and pathological investigations are needed to clarify the disease.

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Acknowledgements

We would like to thank Professor Kazuichi Nakamura of Kitasato University for giving opportunity of this research and his support.