ACUTE TOXICITY OF PIERISIN-1, A CYTOTOXIC PROTEIN FROM *PIERIS RAPAE*, IN MOUSE AND RAT

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ABSTRACT — To investigate the toxicity of pierisin-1, a cytotoxic protein present in the cabbage butterfly, *Pieris rapae*, pierisin-1 was administered via intraperitoneally in mice and rats and the effects examined. Common findings in these experiments were hypoactivity with a gradual decrease in body weight due to decreased food intake, relative polycythemia with low serum albumin concentration and atrophy of the thymus, spleen, seminal vesicles and adipose tissue. Characteristic findings were diarrhea, fusion and atrophy of the villi and dilatation of the crypts in the small intestine at 6-100 µg/kg in BALB/c mice as well as elevation of LDH activity and creatinine value, hemolysis and renal and hepatic injuries at 1,000 and 10,000 µg/kg in BALB/c mice. In the case of ICR mice, severer renal injury was observed. On the other hand, in Fischer 344/Du rats, sudden stop of food intake, elevation of both AST and ALT activities, interlobar adhesion of the right hepatic lobe, capsular thickening, septal fibrosis and single cell necroses of subcapsular hepatocytes in the liver and basophilic tubules in the kidneys were observed. Oral administration of pierisin-1 at a dose of 10,000 µg/kg in BALB/c mice did not exert any obvious effects. Thus, existence of species and strain differences in toxicity of pierisin-1 to animals was demonstrated.

KEY WORDS: Pierisin-1, Cytotoxic protein, Acute toxicity, Rat, Mouse

INTRODUCTION

Pierisin-1 is a 98-kDa protein isolated from the cabbage butterfly, *Pieris rapae*, exhibits strong cytotoxicity to mammalian cells (Koyama *et al.*, 1996; Watanabe *et al.*, 1998). The IC₅₀ values to a variety of cell lines range from 0.043 ng/ml to 270 ng/ml (Kono *et al.*, 1999; Kanazawa *et al.*, 2001). Pierisin-1 induces apoptosis to most cells, including HeLa cells, within 48 hr (Watanabe *et al.*, 1998; Kono *et al.*, 1999). Activation of caspases and release of cytochrome c have been documented (Kanazawa *et al.*, 2002a) and overexpression of Bcl-2 protein in HeLa cells results in acquisition of resistance, so that, pierisin-1-induced apoptosis may be mediated primarily via a mitochondrial pathway involving Bcl-2 and caspases (Kanazawa *et al.*, 2002a).

Amino acid sequence similarities suggested that pierisin-1 is an ADP-ribosylating toxin (Kanazawa *et al.*, 2001). Such bacteria-derived ADP-ribosylating

toxins feature an enzyme domain exhibiting mono (ADP-ribosyl) transferase activity and a receptor-binding domain allowing entry into target cells (Kanazawa *et al.*, 2001; Watanabe *et al.*, 1999). Although all of the acceptor molecules for ADP-ribosyl moieties catalyzed by bacteria-derived ADP-ribosylating toxins so far reported are proteins, pierisin-1 was found to transfer the moiety to the N-2 position of guanine bases in DNA (Takamura-Enya *et al.*, 2001). Thus, pierisin-1 is classified as a guanine-specific ADP-ribosylating toxin. Moreover, it is known to induce mutations in the *HPRT* gene in CHL cells (Totsuka *et al.*, 2003). However, damage signaling and mechanisms of apoptosis induction caused by the modified DNA remain to be clarified.

Immunological and structural analyses of fractionated HeLa cellular components have demonstrated receptors for pierisin-1 to be neutral glycosphingolipids, globotriaosylceramide (Gb3) and globotetraacylceramide (Gb4) (Matsushima-Hibiya *et al.*, 2003).

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Globopentaosylceramide (Gb5) also exhibits receptor activity for pierisin-1, and such glycolipids capable of binding to pierisin-1, including Gb3 and Gb4, are abundant in pierisin-1-sensitive cell lines, HeLa, TMK-1 and MCF-7, whereas they are lacking in the insensitive cell line, MEB4 (Matsushima-Hibiya *et al.*, 2003). These observations indicate that the amount of receptor in cells is one of the main factors affecting sensitivity.

Despite the variation in strength, the cytotoxicity suggests that pierisin-1 could be a potent mammalian toxin with significant antitumor activity against certain cancers. Indeed, a single intraperitoneal injection of pierisin-1 suppressed HeLa tumor growth in BALB/c nude mice, and in a preliminary experiment the dose of 10 µg/kg i.p. was sufficient to kill the animals (Kanazawa *et al.*, 2002b).

The present study, carried out to determine acute toxicity in detail, included pathological and biochemical analyses. As expected, pierisin-1 exhibited strong toxicity to BALB/c mice, ICR mice and Fischer 344 rats on intraperitoneal injection. Several strain/species-specific lesions such as dilatation of small intestine at low dose (6-100 µg/kg) and hemolysis at high dose (1,000 and 10,000 µg/kg) in BALB/c mice, renal injury in ICR mice at 30 µg/kg and emaciation due to no intake of food in Fischer 344 rats were observed. Possible target organs and cause of death are discussed.

MATERIALS AND METHODS

Test substance

Pierisin-1 was supplied from the National Cancer Center Research Institute (Dr. Kanazawa, Dr. Watanabe and Dr. Wakabayashi).

Study outline and animals

The present study encompassed five experiments. Protocols for each experiment, including the number of animals per group, animal age, and the doses and administration route used are presented in Table 1. The study objects in experiments 1-4 were as follows. Experiment 1: single intraperitoneal administration with BALB/c mice (Japan Slc Inc., Shizuoka, Japan) at doses of 3, 6, 10, 30, 100, 1,000 and 10,000 µg/kg. Experiment 2: single oral administration with BALB/c mice at a dose of 10,000 µg/kg. Experiment 3: single intraperitoneal administration with ICR mice (Japan Slc Inc.) at a dose of 30 µg/kg. Experiment 4: single-dose intraperitoneal administration with F344 rats (Japan Charles River Inc., Kanagawa, Japan) at doses

of 10 and 30 µg/kg. Pierisin-1, purified from the larvae of *Pieris rapae* as described previously (Watanabe *et al.*, 1998) were dissolved in physiological saline with a volume of 0.1 mL per 20 g per body weight.

In Experiments 1, 3 and 4, the main general effects in mice and rats administered pierisin-1 was a decrease in body weight resulting from a decrease of food intake. Whether lesions observed in the pierisin-1-treated animals were due to a decrease of food intake and what the cause of the disturbance of food intake needs to be investigated. Therefore, to clarify the direct effects of pierisin-1 on the animal body overall, fasting experiments with BALB/c mice and F344 rats were carried out and designated as experiment 5.

All these experiments were carried out according to the Guidances for Animal Testing at An-Pyo Center.

Housing conditions

Animals were housed individually in aluminum cages with stainless steel wire mesh fronts and floors placed over an automatic water-flushing breeding rack in an air-conditioned animal room (temperature, 23±3°C; relative humidity, 55±20%; lighting cycle, 12 hr light-dark, illumination at intensity of 150-300 lx) with a barrier system. The animals were given commercial diet MF (Oriental Yeast Co., Ltd. Tokyo, Japan) and tap water *ad libitum*. Animal husbandry and handling were conducted in accordance with the An-Pyo Center's animal use and care guidelines.

Examinations

Clinical observation was performed and body weight and food consumption were measured once a day, at the same time each day during the experimental period. Blood sampling for clinical laboratory tests was performed just before necropsy on all moribund and schedule-sacrificed animals, and thereafter pathological examinations were conducted. All major organs were removed and fixed in 10% phosphate buffered formalin, processed routinely, and embedded in paraffin. Paraffin sections 3 µm in thick were cut and stained with hematoxylin and eosin. Parameters of clinical laboratory tests for each experiment are presented in Table 1.

RESULTS

The time-courses for death and mortality and the representative data in each experiment are summarized in Table 2. Changes in mean body weights and mean food consumption in Experiment 1 are presented in

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Table 1. Experimental design.

Experiment No.	Species	Strain	Age (Week)	Sex ¹⁾	Dose (µg/kg)	Route of administration ²⁾	No. of animals	Parameters	
								Hematology ³⁾	Blood chemistry ⁴⁾
1	Mouse	BALB/c	6	M	0	i.p.	3	WBC, RBC, Hb, Ht, MCV, MCH, MCHC, Differential leukocyte count	TP, Alb, A/G
					3		3		
					6		3		
					10		3		
					30		3	ND	ND
2	Mouse	BALB/c	6	M	100	i.p.	3		
					1000		3		
					10000		3	WBC, RBC, Hb, Ht, MCV, MCH, MCHC	LDH, Crea
					10000		3	ND	
					0		2	WBC, RBC, Hb, Ht, MCV, MCH, MCHC, Differential leukocyte count	TP, Alb, A/G, Glu, TG, T-cho, Crea
3	Mouse	ICR	6	M	10000	p.o.	2		BUN, AST, ALT, ALP, Ca, P, LDH
					0		2		
					30		3	WBC, RBC, Hb, Ht, MCV, MCH, MCHC	TP, Alb, A/G, AST, ALT
					30		3	WBC, RBC, Hb, Ht, MCV, MCH, MCHC, Differential leukocyte count	
					0		3		
4	Rat	F344	6	M	10	i.p.	3		TP, Alb, A/G, Glu, TG, T-cho, Crea
					30		3		
					0		3	WBC, RBC, Hb, Ht, MCV, MCH, MCHC	BUN, AST, ALT, ALP, Ca, P, CPK
					10		2		LDH
					30		2		
5 *	Mouse	BALB/c	6	M	–	–	3	WBC, RBC, Hb, Ht, MCV, MCH, MCHC	TP, Alb, A/G, Glu, TG, T-cho, Crea
	Rat	F344	6	M	–	–	3		BUN, AST, ALT, ALP, Ca, P

¹⁾ M: Male, F: Female. ²⁾ i.p.: intraperitoneal, p.o.: per os.

³⁾ WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, Ht: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

⁴⁾ TP: total protein, Alb: albumin, A/G: albumin/globulin ratio, Glu: glucose, TG: triglyceride, T-cho: total cholesterol, Crea: creatinin, BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, CPK: creatine phosphokinase, LDH: lactate dehydrogenase, Ca: calcium, P: phosphate.

*: fasted, –: not applicable.

Figs. 1 and 2. Changes in mean body weights in Experiment 3 and 4 are presented in Figs. 3 and 4.

Experiment 1

Time-courses of change in mean body weight and food consumption are presented in Figs. 1 and 2, respectively. The interval from dosing until death was 7 days in the 6 µg/kg group and 1 day in the 10,000 µg/kg group. LD₁₀₀ in BALB/c mice was 30 µg/kg. The dose and the time-course of death were well related (Table 2). Suppression of body weight gain due to decrease in food consumption occurred and was followed by the appearance of clinical signs. The interval from onset of clinical signs till death was approximately 1 day, irrespective of the dose. At doses of 6–100 µg/kg, the first clinical sign was hyperactivity, followed by hypoactivity, and mice in the 10 µg/kg group showed diarrhea and abdominal distension. In addition, adoption of a hunchback position was also observed in mice in the 30 µg/kg group, suggesting the presence of abdominal pain. In clinical laboratory tests, high hematocrit value and red blood cell (RBC) counts were noted, whereas serum albumin concentrations were low. Therefore, these hematological findings were considered indicative of relative polycythemia with low albumin. This was a characteristic finding in pierisin-1-treated mice and rats as described here and after. At necropsy, atrophy of the spleen, thymus and both white and brown adipose tissues and dilatation of the lumen due to an increase in the watery contents of the small intestine were observed as common findings in dead and moribund sacrificed animals. On histopathological examination, atrophy and fusion of the villi and dilatation of the crypts in the small intestine (Photo 1) were characteristic findings. Single cell necrosis in the spleen, thymus, lymph node, small and large intestine, liver, testes, prostate, preputial glands and Harderian glands and atrophy of hepatocytes and both white and brown adipocytes were also observed.

With doses of 1,000 and 10,000 µg/kg, mice showed hypoactivity and a prone position. Relative polycythemia with low serum albumin concentrations, hemolysis and elevation of LDH activity and creatinine value were demonstrated. Hydrothorax and ascites in the 1,000 µg/kg group and red coloration and relaxation in the small intestine and lobulation in the liver in the 10,000 µg/kg group were macroscopically characteristic findings. Moreover, systemic enlargement and/or red coloration of lymph nodes were observed in 1 mouse each of the two groups. Histopathological

examination demonstrated hemolysis, decreased erythropoiesis (Photo 2), single cell necrosis in the thymus, spleen, lymph node, bone marrow, cardiac endothelium, liver, kidneys, prostate and seminal vesicles, and edema in the pancreas and white adipose tissue. In addition, hepatitis (Photo 3) and dilatation of glands in the glandular stomach in 10,000 µg/kg group and pulmonary edema in 1,000 µg/kg group and tubular dilatation in the kidneys in both groups were noted. The degree of injuries in the lymphoid tissues was mild in the 10,000 µg/kg group but strong in the 1,000 µg/kg group.

Experiment 2

Mice orally administered at 10,000 µg/kg of pierisin-1 did not show any abnormalities. Clinical laboratory tests and pathological examination also exhibited no significant effect.

Experiment 3

Time-courses of mean body weight and food consumption changes were similar to those in Experiment 1. The first clinical sign was hypoactivity, then mice adopted a hunchback position with abdominal distension, and gradually became emaciated, as seen in the 10 µg/kg group in Experiment 1. Relative polycythemia with a low serum albumin concentration was a characteristic hematological finding. At necropsy, atrophy of the thymus, spleen and both white and brown adipose tissue was observed. Histopathologically, basophilic change, vacuolar degeneration and single cell necrosis in the tubular epithelium, cell debris within the tubules and tubular dilatation in the kidneys (Photos 4 a and b) were characteristic findings in this experiment. The degree of these renal lesions was stronger than that of mice that received the same dose in Experiment 1. The presence of single cell necrosis in the tubular epithelium and cell debris in the uriniferous tubules indicated strong cell injury. In the small intestine, atrophy of the villi and dilatation of the crypts were observed. However, the degree of these intestinal lesions was milder than in Experiment 1. Single cell necrosis in the thymus, spleen, liver, large intestine and prostate, and atrophy of hepatocytes and both white and brown adipocytes were also observed. Single cell necroses of hepatocytes were particularly prominent in mice showing higher activity of both AST and ALT.

Experiment 4

Almost all abnormalities are limited to rats of the

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Table 2. Summary of representative data from gross and histopathological assessment.

Experiment No.	Dose level (µg/kg)	Route of administration	General condition	Hematology ¹⁾	Blood chemistry ²⁾	Gross findings	Histopathology	Mortality (%)	Days of death after treatment
1	0		-	WBC (2.61), RBC (9.49), Ht (50.2), MCV (52.8), PLT (900)	Alb (3.29), LDH (409), Crea (0.08)	-	-	0	
	3			ND	ND			0	
	6		hyperactivity, hypoactivity, diarrhea, abdominal distention, hunchback position	RBC ↑ (12.43), Ht ↑ (60.22), Hb ↑ (20.38), MCV ↓ (48.4)	Alb ↓ (3.07)	dilatation of lumen in the small intestine	atrophy and fusion of villi and dilatation of crypts in the small intestine	33	7
	10			ND	ND			67	7 and 8
	30		hyperactivity, hypoactivity	ND	ND			100	6
1000	100	i.p.	hyperactivity, hypoactivity	ND	ND	dilatation of lumen in the small intestine	atrophy and fusion of villi and dilatation of crypts in the small intestine	100	5
			hyperactivity, prone position	WBC ↑ (12.74), RBC ↑ (11.78), Ht ↑ (61.3), Hb ↑ (17.9), MCV ↑ (52.1), PLT ↓ (260)	LDH ↑ (1904), Crea ↑ (0.26)	hydrothorax, ascites	hemolysis, decreased erythropoiesis, edema in the lung and pancreas	100	2 and 3
	10000		hyperactivity, prone position	ND	LDH ↑ (5204), Crea ↑ (0.31)	red coloration and relaxation in the small intestine, evident lobule of the liver	hemolysis, decreased erythropoiesis, single cell necrosis of hepatocytes, hepatitis-type liver injury	100	1

-: no abnormal changes detected, ND : Not determined, Numbers within parentheses indicate measurement values.

¹⁾ unit : WBC (white blood cell ; ×10³/mL), RBC (red blood cell ; ×10⁶/mL), Ht (hematocrit ; %), Hb (hemoglobin ; g/dL), MCV (mean corpuscular volume ; fL), PLT; platelet (×10³/mL).²⁾ unit : Alb (albumin ; g/dL), LDH (lactate dehydrogenase ; IU/L), Crea (creatinine ; mg/dL).

Table 2. -continued Summary of representative data from gross and histopathological assessment.

Experiment No.	Dose level (µg/kg)	Route of administration	General condition	Hematology ¹⁾	Blood chemistry ²⁾	Gross findings	Histopathology	Mortality (%)	Days of death after treatment
2	0	p.o.	-	Ht (51.5)	Alb (3.54), AST (55), ALT (45)	-	-	0	
	10000		-	Ht (51.6)	Alb (3.41), AST (52), ALT (48)	-	-	0	
	0		-	Ht (45.1)	ND	-	-	0	
3	30	i.p.	hypoactivity, hunchback position, abdominal distention	Ht ↑ (65.4)	Alb (3.34) ↓, AST ↑ (2518), ALT ↑ (696)	dilatation of lumen in the small intestine	atrophy and fusion of villi and dilatation of crypt in the small intestine, basophilic tubule in the kidneys	100	6
	0		-	Ht (41.6)	Alb (3.27), AST (66), ALT (34)	-	-	0	
	10		-	Ht (43.7)	Alb (3.26), AST (69), ALT (44)	-	capsular thickening in the liver	0	
4	30	i.p.	no intake of food, hypoactivity	Ht ↑ (51.2)	Alb ↓ (2.62), AST ↑ (216), ALT ↑ (77)	interlobar adhesion of right hepatic lobe	capsular thickening, septal fibrosis, atrophy and single cell necrosis of hepatocytes in the liver, basophilic tubule in the kidneys	100	7
5*			hypoactivity	Ht ↑ (51.2)	Alb ↑ (3.55)	atrophy of the spleen, thymus and seminal vesicle	single cell necrosis in the spleen and thymus, hyposecretory change in the seminal vesicle	100	3

* : fasted (rats), - : No abnormal changes detected, Numbers within parentheses indicate measurement values.

¹⁾ unit ; Ht (hematocrit ; %).²⁾ unit ; Alb (albumin ; g/dL), AST (aspartate aminotransferase ; IU/L), ALT (alanine aminotransferase ; IU/L), ND : not determined.

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30 $\mu\text{g}/\text{kg}$ group. The body weights gradually decreased and the rate of suppression of body weight gain was about 28%, and there was no food intake seen as the first clinical sign for 4 days after dosing. Thereafter,

rats showed hypoactivity, lacrimation and piloerection. Relative polycythemia with low serum albumin concentration and elevation of AST and ALT activities were found by clinical laboratory tests. At necropsy,

Fig. 1. Time-course of change in mean body weights (Experiment 1).
X axis : Days after administration, Y axis : Mean body weight (g),
Dose Level ($\mu\text{g}/\text{kg}$) : $\blacklozenge$ 0, $\square$ 6, $\blacktriangle$ 30, $\triangle$ 100, $\blacksquare$ 1,000, $\diamond$ 10,000.

Fig. 2. Time-course of change in mean food consumption (Experiment 1).
X axis : Days after administration, Y axis : Mean food consumption (g),
Dose Level ($\mu\text{g}/\text{kg}$) : $\blacklozenge$ 0, $\square$ 6, $\blacktriangle$ 30, $\triangle$ 100, $\blacksquare$ 1,000, $\diamond$ 10,000.

interlobar adhesion in the right lobe of the liver was characteristic (Photo 5). The other macroscopic finding was atrophy of the thymus, spleen, seminal vesicle and both white and brown adipose tissue. Histopatho-

logical examination demonstrated septal fibrosis, capsular thickening and single cell necrosis of subcapsular hepatocytes in the liver (Photo 6) and basophilic tubules in the kidney. The sudden stop in food intake

Fig. 3. Time-course of change in mean body weights (Experiment 3)
X axis : Days after administration, Y axis : Mean body weight (g), Dose Level ($\mu\text{g/kg}$) : $\blacklozenge$ 0, $\square$ 10, $\blacktriangle$ 30.

Fig. 4. Time-course of change in mean food consumption (Experiment 3)
X axis : Days after administration, Y axis : Mean food consumption (g), Dose Level ($\mu\text{g/kg}$) : $\blacklozenge$ 0, $\square$ 10, $\blacktriangle$ 30.

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Photo 1. BALB/c mouse, 10 µg/kg group (Experiment 1), Ileum ; Note the atrophy and fusion of villi and dilatation of the crypts, HE stain, ×200.

Photo 2. BALB/c mouse, 1000 µg/kg group (Experiment 1), Bone marrow (sternum); Note hemolysis and decreased erythropoiesis, HE stain, ×350.

suggested an effect on the center regulating food intake, but there were no histological abnormalities detected in the ventromedial nucleus of hypothalamus; a regulatory center of food intake. As other findings, hemorrhage and decreased hematopoiesis in the bone marrow, atrophy of the thymus and spleen and hyposecretory change in the seminal vesicle were observed. Capsular thickening in the liver was also observed in both males and females in the 10 µg/kg group.

Experiment 5

Fasted mice showed atrophy of the spleen, thymus and both white and brown adipose tissue, and dilatation of the lumen in the gallbladder on gross examination. Histopathological examination demonstrated single cell necroses in the spleen, lymph node, thymus, liver, prostate and preputial glands, atrophy of hepatocytes, ulceration in the glandular stomach, and atrophy of hepatocytes and both white and brown adipocytes.

In fasted rats, relative polycythemia with high serum albumin concentration, hemorrhage and decreased hematopoiesis in the bone marrow, single cell necrosis in the thymus, atrophy of the spleen and both white and brown adipocytes and hyposecretory

changes in the seminal vesicles were observed.

DISCUSSION

Pierisin-1 toxicity in mice in the present study increased in degree and incidence of lesions in a dose-related manner. A variety of toxic effects including a decrease in food intake, malabsorption due to injury of the intestinal mucosa at doses of 6-100 µg/kg, and hepatic and renal injury, hemolysis and circulatory disturbance at doses of 1,000 and 10,000 µg/kg were considered to be due to direct toxicity of pierisin-1 in BALB/c mice and the cause of death varied depending on the dose level. Elevated LDH was considered to be due to hemolysis, this finding being noted in mice without abnormalities in the heart, liver or brain. Expression of stronger renal toxicity to ICR mice than BALB/c mice suggests that there are strain differences in the toxicity of pierisin-1 in mice. The cause of death in F344 rats could be considered to be emaciation due to no intake of food, although toxic effects were observed in the liver and kidney.

Recently, globotriosylceramide (Gb3) and globotetraosylceramide (Gb4), which are receptors for verotoxin from *Shigera* bacteria, were proven to serve

Photo 3. BALB/c mouse, 10000 µg/kg group (Experiment 1), Liver ; Hepatitis-type hepatic injury, HE stain, ×400.

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as pierisin-1 receptors (Matsushima-Hibiya *et al.*, 2003). Gb3 is distributed in the vascular endothelium, spleen, kidney, mucosa of the small intestine, while Gb4 is found in the kidney, spleen, erythrocytes. In fact, characteristic findings in the present experiments were observed in the small intestine, kidney and erythrocytes and *in vitro* studies have demonstrated that the amount of receptors in cells is one of the main factors affecting sensitivity of cells (Matsushima-Hibiya *et al.*, 2003).

Gradual decrease in body weight gain is also induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the sign is referred to the wasting syndrome, which is related to altered retinoid homeostasis (Fletcher *et al.*, 2001). The heterodimeric partner of peroxisomal proliferator activated receptor γ (PPAR γ) receptor is the retinoid X receptor (RXR)

(Mangelsdorf and Evans, 1995). PPAR γ agonist induces atrophy of adipose tissue and its side effect is systemic fluid retention (Guan *et al.*, 2005), which are seen in the present study. However, the roles of retinoid and/or PPAR γ in pierisin-treated animals were unknown.

Lesions which cannot be explained by the presence of receptors induce relative polycythemia with a low albumin concentration in mice and rats, hydrothorax, ascites, hepatitis-like injuries in mice at doses of 1,000 or 10,000 $\mu\text{g/kg}$ and the sudden stop of food intake in rats. Pierisin-1 is a toxin derived from insects and that is anticipated as a new candidate for cancer therapy. Likewise, immunotoxins which are chimeric molecules consisting of cell binding ligands and toxins or their subunits are used for chemotherapy. However, they are known to induce vascular leak syndrome

Photo 4. ICR mouse, 30 $\mu\text{g/kg}$ group (Experiment 2), Kidney ; (a) Basophilic change of the tubular epithelium and cell debris in the tubules(arrows), HE stain, $\times 192$.
(b) Dilatation of uriniferous tubules, HE stain, $\times 288$.

Photo 5. F344 rat, 30 μ g/kg group (Experiment 3),
Liver ; Fibrous adhesion (arrows) between the cranial (CR) and caudal part (CA) of the right lobe , HE stain, $\times 300$.

Photo 6. F344 rat, 30 μ g/kg group (Experiment 3),
Liver ; Capsular thickening (*) and single cell necrosis of subcapsular hepatocytes (arrows), HE stain, $\times 200$.

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(VLS), characterized with hypoalbuminemia, edema and body weight gain, although their mechanism is yet unclear (Vieta *et al.*, 1993). In the present study, these human VLS-like symptoms were also observed in mice treated with 1,000 µg/kg of pierisin-1. Moreover, relative polycythemia was characteristic of hematological findings, although serum albumin concentration was low. This phenomenon may be suggested to be an increase in vascular permeability as seen in VLS, rather than a mere dehydration.

Immunotoxins, BR96 sFv-PE40 can induce VLS in rats as established as an animal model of VLS (Siegall *et al.*, 1994). BR96 sFv-PE40-induced VLS are known to be suppressed by pretreatment of dexamethazone (Siegall *et al.*, 1994) and nonsteroidal anti-inflammatory drugs (NASIDs) (Siegall *et al.*, 1997) and but not by histamine or serotonin (Siegall *et al.*, 1994, 1997) or not reproduced in athymic rats (Siegall *et al.*, 1994), suggesting a lack of T-cell response. Interleukin-2 (IL-2) induces VLS in mice and tumor necrosis factor- α (TNF- α) plays a central role in their mechanism (Rosenstain *et al.*, 1986; Dubinett *et al.*, 1994). Immunosuppression of mice by pretreatment with irradiation (500 rad) or by injection of cyclophosphamide or by concurrent use of cortisone acetate reduced or eliminated markedly the development of VLS (Onda *et al.*, 2000). Similarly, VLS was not observed in nude mice receiving IL-2 (Onda *et al.*, 2000). Based on these findings, immune response was considered to have an important role in the pathogenesis of VLS. In fact, lymphoid organs injuries were evident in mice showing VLS-like symptoms in the present study.

Hepatic injuries are also one of the side effects due to immunotoxins (Vieta *et al.*, 1993). As for immunotoxins-induced hepatic injuries, TNF- α produced by Kupffer cells has an important role in pathogenesis (Schilling *et al.*, 1992). TNF- α and agonistic Fas are known to have also anticancer activity and administration of high dose TNF- α in humans (Schilling *et al.*, 1992) and anti-Fas antibody in mouse (Ogasawara *et al.*, 1993) is reported to cause hepatotoxicity by an unknown mechanism. In mice, an administration of BR96 sFv-PE40 induced no VLS but hepatic injuries (Siegall *et al.*, 1994). In the present study, large administration of pierisin caused only hepatitis-type hepatic injury in mice without showing lymphoid organs injuries. This change was considered a dose-limited toxicity and host immune response may be participated in their pathogenesis.

Mice administered orally 10,000 µg/kg of pieri-

sin-1 did not exhibit any abnormal changes in any of the parameters examined, even though the dose is 2,000 times the LD₅₀ by the i.p. route. Thus the cytotoxic activity of pierisin-1 may be lost with proteolytic enzyme digestion. Similar toxicity appeared with administration via i.v. route. Approximately 50-fold dose were requires for exertion of pierisin-1 toxicity by s.c. routes. Therefore, direct entrance of pierisin-1 into the blood may be needed to exert toxicity.

In pierisin-1-treated rats, the sudden stop in food intake, relative polycythemia with low serum albumin concentration and interlobar adhesion of the right lobe in the liver were characteristic findings lacking in fasted rats. However, the cause for the loss of appetite could not be identified morphologically, although immune cytokines such as interleukin-1 or interferon- α are suspected to play a role in anorexia (Hori *et al.*, 1991). The pathogenesis of the interlobar adhesion of hepatic right lobe was intriguing given the lack of hepatic necrosis. It pointed to a remodeling of the hepatic architecture.

In conclusion, pierisin-1 shows species and strain differences in toxicity to animals. Investigation of possible causative factors such as receptor for pierisin-1 would be very helpful to understand the toxic mechanism of pierisin-1 in animals.

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