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Letter

Physiological conditions in iPRECIO® -implanted rats

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ABSTRACT — Any devices used in toxicity studies should be validated. In the present study, the physiological conditions of rats implanted with a new micro-infusion pump, iPRECIO®, were examined to evaluate its availability for toxicity studies. Five or six animals/group of 6-week-old male CD(SD) rats received either sham surgery or the implantation surgery for either the iPRECIO® (iP) pump or a standard osmotic infusion pump (OSM) under the back skin. This was followed by 4 weeks (experiment I) or 13 weeks (experiment II) of observation. During the observation period, the iP- and OSM-animals received a continuous infusion of 2.0 or 2.5 μL/hr of saline via the external jugular vein. In experiment I, standard hematologic and blood chemical parameters used in toxicity studies were measured at weeks 1, 2, and 4. The iP-animals showed no abnormal changes in any parameters at any point when compared with the OSM-/SHAM-animals. In experiment II (only iP- and SHAM-animals), necropsy and histopathological examination were performed at weeks 1, 2, 4, and 13. The histopathological examination revealed foreign material-induced inflammatory changes in the dorsal subcutaneous tissue (implantation site) of the iP-animals, including the infiltration of polymorphonuclear or mononuclear cells, edema, granulation and fibrous capsule formation. However, the abnormalities were limited to the implantation site. These results suggest that the implantation of iPRECIO® exerted no significant impact on the physiological condition of the rats. Therefore, we concluded that iPRECIO® is applicable for toxicity studies.

Key words: Infusion pump, Implantation, Rat, iPRECIO®, Physiological condition

INTRODUCTION

Any toxic responses elicited by xenobiotics start with exposure to chemicals. Therefore, administration of test chemical to animals is one of the most important elements of *in vivo* toxicity studies. At present, there are few methods applicable for continuous dosing (for example, for several weeks) of test chemicals to unrestrained animals. A new type of implantable micro-infusion pump, iPRECIO®, may become a useful tool in such settings (Abe *et al.*, 2009; Tan *et al.*, 2011; Turner *et al.*, 2011) because of its features, which including high-quality compact machinery and a battery promising long and high-precision runs.

Occasionally, foreign materials implanted in the living body can trigger non-specific reactions locally and/or systemically to a slight or severe degree (Anderson, 2001; Anderson *et al.*, 2008; Wagner *et al.*, 2012). It is important to understand the behavior of such background noises in non-treated animals for proper evaluations of toxicity because general toxicity studies are essentially based on the comparisons of a wide-range of endpoints between chemical-treated and non-treated animals (Baird *et al.*, 2013). However, no comprehensive information about such background noise is available in the case of iPRECIO®-implanted rats.

In the present study, we examined the physiological conditions of iPRECIO®-implanted rats using various parameters commonly used in toxicity studies (hematologic and blood chemical parameters) and microscopic changes at the implantation site to evaluate the validity of iPRECIO® for *in vivo* toxicity studies.

MATERIALS AND METHODS

Overview

The present study consisted of experiment I (4-week) and experiment II (13-week). Group composition of each

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experiment is presented in Table 1.

In both experiments, male Crl:CD(SD) rats were purchased at 5 weeks old from Charles River Laboratories Japan, Inc. (Atsugi, Japan), and thereafter kept in a barrier facility under the following environmental conditions: 1) Temperature, 20 to 26°C; 2) Relative humidity, 35 to 70%; 3) Ventilation, 12 times/hr or more; 4) Lighting, 12 hr/day. The animals were allowed free access to the pellet diet CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. The animals were subjected to the implantation surgery at 6 weeks old. They were individually housed in a plastic cage (Width, 34.5 × Depth, 40.3 × Height, 17.7 cm) with a Sunflake floor-bed (Charles River Laboratories Japan, Inc.) until 1 week after the surgery, and thereafter, kept in a steel wire mesh cage (Width, 29.1 × Depth, 26.3 × Height, 18.0 cm).

In the experiment I, a total of 54 animals were assigned to iP-, OSM-, or SHAM-group (18 animals/group), and received either the iPRECIO® (iP-group) or an osmotic infusion pump (OSM-group; existing available device), or sham operation (SHAM-group). The OSM- and SHAM-groups' conditions were designed to be compared with the iPRECIO®-implanted conditions (iP-animals). For the iP- and OSM-animals, 2.5 μL/hr of saline was continuously infused up to a maximum of 4 weeks. At weeks 1, 2, and 4 after the implantation surgery, six animals/group were euthanized and subjected to hematological and blood chemical examinations.

In experiment II, a total of 40 animals were assigned to two groups (iP- or SHAM-group; 20 animals/group) and received the iPRECIO® (iP-group) or sham operation (SHAM-group). No OSM-group was designated in the experiment II because of the pump's functional limitation for 13-week dosing. For the iP animals, 2.0 μL/hr of saline was continuously infused up to a maximum of 13 weeks. At weeks 1, 2, 4, and 13 after the surgery, five animals/group were euthanized and subjected to macroscopic (necropsy) and histopathological examinations of the systemic organs (macroscopic) and implantation site (histopathological). In addition, the hematological examination (for erythrocyte- and leukocyte-related parameters) was conducted only at week 13.

Infusion pumps and implantation surgery

Two types of implantable infusion pumps, iPRECIO® SMP200 and Alzet™ 2ML4 (as representative osmotic infusion pump), were used for the iP-group and OSM-group, respectively (Abe *et al.*, 2009; Joles *et al.*, 1992; Sherratt *et al.*, 1988; Ronis *et al.*, 2001; Shobo *et al.*, 2011; Tan *et al.*, 2011; Turner *et al.*, 2011). The iPRECIO® pump (38.7 × 19.2 × 9.7 mm/7.9 g; reservoir volume, 0.9 mL; Fig. 1) was provided by PRIME-TECH Corporation (Tokyo, Japan). The Alzet™ 2ML4 pump (diameter, 1.4 × length, 5.1 cm/5.1 g; reservoir volume, 2.0 mL) was purchased from ALZA Corporation (Mountain View, CA, USA). The product profiles of these pumps are referred to the web pages (http://www.iprecio.com/products/tabid/128/Default.aspx, and http://www.alzet.com/products/guide_to_use/pump_selection.html#, ascertained on December 17, 2015).

The pumps were handled in accordance with the manufacturers' instructions. The saline loaded in the iPRECIO® pump was exchanged once a week under its refillable feature (Fig. 1). In contrast, the osmotic pump-loaded saline is not exchangeable. Instead, 2.0 mL of saline was loaded once into the osmotic pump at the implantation surgery.

Just before the implanting, the fur on the surgical sites was shaved clean while animals were under pentobarbital anesthesia (37.8 mg/kg, intraperitoneal). For both devices, the pump unit was placed subcutaneously in the dorsal region, and the catheter (diameter, 0.55 mm) connected to the pump unit was inserted into the external jugular vein. After the implantation, the incised part was clipped on and gummed with Aron-alpha® A (Dai-Ichi Sankyo, Tokyo, Japan). The SHAM-animals received the surgical operation at the same site, but had no implanted device.

Post-surgical care was performed with reference to previous reports (Flecknell, 2009; Shoieb *et al.*, 2012) under the guidance of an in-house attending veterinarian. All the animals received 5 mg/kg of carprofen (subcutaneously) at the surgery and at 24 hr after the implantation, and also, 10 mg/kg of enrofloxacin (subcutaneously)

Table 1. Group composition.

Experiment	Group	Pump	Total No. of animals	Sacrificed at
I	iP	iPRECIO®	18	Weeks 1, 2 and 4 (6 animals/week)
	OSM	Alzet® 2ML4	18	Weeks 1, 2 and 4 (6 animals/week)
	SHAM	-	18	Weeks 1, 2 and 4 (6 animals/week)
II	iP	iPRECIO®	20	Weeks 1, 2, 4 and 13 (5 animals/week)
	SHAM	-	20	Weeks 1, 2, 4 and 13 (5 animals/week)

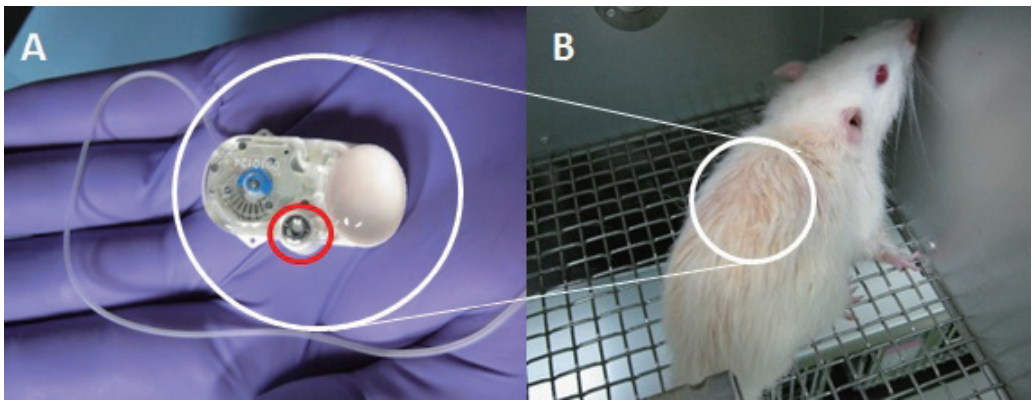

Fig. 1. The micro-infusion pump iPRECIO® (panel A) was implanted subcutaneously in the dorsal area of the rats (panel B, about 2.5 months after the implantation). Septum (red circle in the panel A), an injection port equipped on the pump unit, was palpable on the skin, allowing repeated refilling of the sample. At each time of the exchange, the saline remaining in the reservoir was removed via the septum using a needle and syringe, and 900 μL of fresh sterilized saline was then injected into the reservoir tank.

Table 2. Hematologic parameters (erythrocyte-related parameters) in the pump-implanted rats.

Exp.	Sacrificed	Group name	HCT (%)	HGB (g/dL)	RBC ($\times 10^6$/mm^3)	MCV (μm^3)	MCH (pg)	MCHC (%)	RET (%)	PLT ($\times 10^3$/mm^3)
I	Week 1	iP (n = 6)	37.8 ± 2.9	13.0 ± 0.5	6.0 ± 0.6	63.5 ± 1.6	21.9 ± 2.2	34.5 ± 2.7	7.6 ± 1.6	1331 ± 115
		OSM (n = 6)	38.7 ± 1.8	12.9 ± 0.5	6.0 ± 0.3	64.7 ± 0.9	21.5 ± 0.6	33.3 ± 0.4	8.2 ± 1.2	1501 ± 164
		SHAM (n = 6)	38.8 ± 1.4	13.1 ± 0.5	6.1 ± 0.2	63.4 ± 2.0	21.3 ± 0.5	33.6 ± 0.5	6.5 ± 2.1	1288 ± 90
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Week 2	iP (n = 6)	40.0 ± 1.9	13.2 ± 0.6	6.4 ± 0.2	62.0 ± 2.1	20.7 ± 0.8	33.4 ± 0.5	5.8 ± 1.1	1278 ± 177
		OSM (n = 6)	40.8 ± 1.3	13.7 ± 0.4	6.7 ± 0.2	60.9 ± 1.7	20.5 ± 0.4	33.7 ± 0.7	5.1 ± 0.6	1150 ± 264
		SHAM (n = 6)	39.6 ± 2.0	13.4 ± 0.6	6.5 ± 0.3	60.8 ± 1.3	20.5 ± 0.6	33.8 ± 0.4	5.6 ± 1.0	1062 ± 141
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Week 4	iP (n = 6)	40.5 ± 2.2	13.8 ± 0.7	7.1 ± 0.4	57.0 ± 1.5	19.3 ± 0.5	33.9 ± 0.2	3.6 ± 0.5	995 ± 270
		OSM (n = 6)	40.5 ± 0.9	13.9 ± 0.2	7.1 ± 0.2	57.0 ± 2.0	19.6 ± 0.7	34.3 ± 0.3	3.3 ± 0.3	1010 ± 80
		SHAM (n = 6)	42.4 ± 1.6	14.5 ± 0.5	7.5 ± 0.3	57.0 ± 1.8	19.5 ± 0.5	34.3 ± 0.5	3.0 ± 0.2	1067 ± 74
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
II	Week 13	iP (n = 5)	38.0 ± 1.7	12.2 ± 0.5	6.2 ± 0.3	61.6 ± 2.9	19.8 ± 0.7	32.2 ± 0.5	2.4 ± 0.2	1076 ± 75
		SHAM (n = 5)	39.2 ± 0.9	12.9 ± 0.4	6.3 ± 0.3	62.0 ± 1.8	20.3 ± 0.6	32.8 ± 0.5	2.1 ± 0.3	1049 ± 48
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	iP-Sham	n.s.	n.s.

Hematologic parameters at weeks 1, 2 and 4 were determined in experiment I, and those at week 13 were in experiment II. Data are presented as mean ± standard deviation. Significant comparison-pairs are shown in the line "SIG." [Welch's test with the Holm adjustment, $P \leq 0.05$ (n.s: no significant pairs)]. HCT, hematocrit; HGB, hemoglobin; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET, reticulocyte; PLT, platelet.

at the surgery, and thereafter, twice a day for 5 days.

Blood examinations

The examined hematologic and blood chemical parameters, which are commonly employed in general toxicity studies, were shown in Tables 2, 3 and 4.

At each examination point, six animals/group in experiment I (at week 1, 2, or 4) or five animals/group in experiment II (only at week 13) were euthanized by exsanguination under isoflurane anesthesia. Whole blood from the animals was collected from the abdominal aorta. Potassium ethylenediaminetetraacetate-containing blood samples

Table 3. Hematologic parameters (leukocyte-related parameters) in the pump-implanted rats.

Exp.	Sacrificed	Group name	WBC ($\times 10^3$/mm^3)	NEUT ($\times 10^3$/mm^3)	LYMPH ($\times 10^3$/mm^3)	MONO ($\times 10^3$/mm^3)	EOSN ($\times 10^3$/mm^3)	BASO ($\times 10^3$/mm^3)
I	Week 1	iP (n = 6)	11.5 ± 2.2	3.7 ± 1.6	7.0 ± 2.2	0.37 ± 0.10	0.09 ± 0.02	0.02 ± 0.01
		OSM (n = 6)	10.1 ± 2.4	2.2 ± 1.0	7.1 ± 1.3	0.32 ± 0.16	0.08 ± 0.05	0.02 ± 0.01
		SHAM (n = 6)	11.2 ± 2.0	3.2 ± 0.8	7.5 ± 1.3	0.26 ± 0.08	0.08 ± 0.03	0.02 ± 0.01
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Week 2	iP (n = 6)	10.8 ± 3.2	2.7 ± 1.7	7.5 ± 1.9	0.28 ± 0.08	0.07 ± 0.03	0.02 ± 0.01
		OSM (n = 6)	11.2 ± 2.2	2.2 ± 0.4	7.8 ± 1.3¶	0.24 ± 0.08	0.08 ± 0.03	0.01 ± 0.00
		SHAM (n = 6)	10.1 ± 3.3	1.6 ± 0.7	8.0 ± 2.7	0.23 ± 0.09	0.06 ± 0.03	0.01 ± 0.01
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Week 4	iP (n = 6)	10.6 ± 2.0	1.7 ± 0.6	8.4 ± 1.7	0.24 ± 0.07	0.09 ± 0.03	0.02 ± 0.01
		OSM (n = 6)	10.7 ± 3.6	1.9 ± 0.8	8.3 ± 2.8	0.22 ± 0.08	0.09 ± 0.04	0.02 ± 0.01
		SHAM (n = 6)	12.3 ± 2.2	2.0 ± 0.9	9.8 ± 2.2	0.29 ± 0.03	0.09 ± 0.04	0.03 ± 0.01
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
II	Week13	iP (n = 5)	10.7 ± 2.7	1.8 ± 0.5	8.3 ± 2.4	0.29 ± 0.07	0.18 ± 0.04	0.01 ± 0.01
		SHAM (n = 5)	10.8 ± 1.5	2.2 ± 1.4	8.2 ± 1.3	0.23 ± 0.11	0.15 ± 0.06	0.01 ± 0.01
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Hematologic parameters at weeks 1, 2 and 4 were determined in experiment I, and those at week 13 were in experiment II. Data are presented as mean ± standard deviation. Significant comparison-pairs are shown in the line "SIG." [Welch's test with the Holm adjustment, $P \leq 0.05$ (n.s: no significant pairs)]. ¶: calculated from 5 animals' values [1 animal exhibited extraordinary value (719×10^3/mm^3)]. WBC, white blood cell; NEUT, neutrophil; LYMPH, lymphocyte; MONO, monocyte; EOSN, eosinophil; BASO, basophil.

were used for the hematologic parameters. Sodium citrate (3.13%)-containing plasma was used for prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB). Heparin lithium-containing serum was used for creatine kinase (CK). Serum collected from the coagulator-coated tubes was used for other blood parameters.

The hematologic parameters (erythrocyte- and leukocyte-related parameters) were determined using ADVIA™ 120 (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Sodium (Na), potassium (K), and chloride (Cl) were determined using EA06R (A&T Corp., Yokohama, Japan). C-reactive protein (CRP) was determined using the Rat C-Reactive Protein ELISA kit (Helica BioSystems, Inc., Santa Ana, CA, USA). PT, APTT, and FIB were determined using STA Compact™ (Roche Diagnostics K.K., Tokyo, Japan). The other blood chemical parameters were determined using Hitachi 7170 (Hitachi High-Technologies Corp., Tokyo, Japan).

Necropsy and histopathological examination

In experiment II, all of the animals were subjected to necropsy. In the necropsy, systemic organs (the external surface, orifices, brain, and organs in the abdominal, thoracic, and pelvic cavities) were observed macroscopically in addition to the soft tissues around the implanted part of the pump unit (subcutaneous tissue in the dorsal area). The tissue around the implantation site was immersed and fixed in 10% neutral-buffered formalin, and then embedded in paraffin. The paraffin-embedded tissue was sliced at approximately 3 μm thickness, followed by hematoxylin-eosin staining. The section was examined microscopically.

Statistical analyses

Quantitative data were analyzed with Welch's test. The family-wise error was adjusted by the Holm method. Significance level was designed to be $P \leq 0.05$.

Animal ethics

This study was conducted in compliance with the Japanese law and standard according to "Act on Welfare and Management of Animals (Act No. 105 in 1973, latest revision; Act No. 38 in 2013)" and "Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Ministry of the Environment, Notification No. 88 in 2006, latest revision: Notification No. 84, 2013)". In addition, this study was approved by the in-house committee on animal ethics and conducted in accordance with the in-house guidance, "Guidance for Animal Testing of the Public Interest Incorporated Foundation BioSafety Research Center (BSRC)".

RESULTS AND DISCUSSION

In both experiment I and II, no deaths or abnormal appearances were observed in any of the groups throughout the continuous infusions. There were no differences in

Table 4. Blood chemical parameters in the pump-implanted rats.

Exp.	Sacrificed	Group name	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	Ca (mg/dL)	P (mg/dL)	GLU (mg/dL)	TG (mg/dL)	T-CHO (mg/dL)
I	Week 1	iP (n = 6)	142.3 ± 0.8	5.0 ± 0.2	105.3 ± 1.5	10.3 ± 0.3	8.6 ± 1.8	238 ± 41	102 ± 39	74 ± 6
		OSM (n = 6)	142.3 ± 1.2	5.1 ± 0.3	104.4 ± 1.0	10.5 ± 0.3	9.5 ± 1.6	249 ± 25	87 ± 22	74 ± 3
		SHAM (n = 6)	142.2 ± 1.3	5.0 ± 0.2	104.5 ± 2.0	10.6 ± 0.4	7.6 ± 1.0	227 ± 37	132 ± 55	77 ± 12
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Week 2	iP (n = 6)	143.2 ± 1.3	5.0 ± 0.2	106.2 ± 1.7	10.1 ± 0.2	6.8 ± 1.3	256 ± 56	118 ± 58	73 ± 11
		OSM (n = 6)	143.0 ± 0.7	4.9 ± -.2	105.4 ± 2.1	10.4 ± 0.2	7.3 ± 1.1	248 ± 54	135 ± 42	72 ± 10
		SHAM (n = 6)	143.3 ± 0.4	5.0 ± 0.3	105.3 ± 0.7	10.0 ± 0.2	7.0 ± 0.7	243 ± 55	120 ± 41	70 ± 7
		SIG.	n.s.	n.s.	n.s.	OSM-SHAM	n.s.	n.s.	n.s.	n.s.
	Week 4	iP (n = 6)	142.6 ± 0.8	4.9 ± 0.3	106.1 ± 1.4	10.0 ± 0.1	6.5 ± 0.4	225 ± 13	128 ± 39	68 ± 10
		OSM (n = 6)	142.4 ± 0.8	4.8 ± 0.3	104.5 ± 0.9	10.0 ± 0.4	6.8 ± 0.8	263 ± 36	133 ± 67	72 ± 10
		SHAM (n = 6)	143.2 ± 0.6	5.0 ± 0.2	105.1 ± 1.2	9.9 ± 0.2	6.3 ± 0.8	227 ± 14	174 ± 66	63 ± 6
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Blood chemical parameters were determined only in the Experiment I. Data are presented as mean ± standard deviation. Significant comparison-pairs are shown in the line "SIG." [Welch's test with the Holm adjustment, $P \leq 0.05$ (n.s: no significant pairs)]. Na, sodium; K, potassium; Cl, chloride; Ca, calcium; P, Inorganic phosphorus; GLU, glucose; TG, triglyceride; T-CHO, total cholesterol.

Table 4. (continued).

Exp.	Sacrificed	Group name	T-PRO (g/dL)	ALB (g/dL)	A/G	AST (Unit/L)	ALT (Unit/L)	ALP (Unit/L)	γ-GTP (Unit/L)	T-BIL (mg/dL)
I	Week 1	iP (n = 6)	4.9 ± 0.3	2.4 ± 0.3	0.99 ± 0.12	82 ± 18	32 ± 5	1155 ± 211	0.3 ± 0.1	0.03 ± 0.01
		OSM (n = 6)	4.8 ± 0.1	2.3 ± 0.2	0.95 ± 0.13	74 ± 16	30 ± 3	1235 ± 342	0.4 ± 0.1	0.03 ± 0.01
		SHAM (n = 6)	4.9 ± 0.2	2.5 ± 0.1	1.01 ± 0.04	70 ± 8	36 ± 4	1194 ± 224	0.4 ± 0.2	0.01 ± 0.01
		SIG.	n.s.	n.s.	n.s.	n.s.	OSM-SHAM	n.s.	n.s.	n.s.
	Week 2	iP (n = 6)	5.1 ± 0.1	2.6 ± 0.2	1.03 ± 0.17	68 ± 8	31 ± 4	1364 ± 327	0.4 ± 0.3	0.02 ± 0.01
		OSM (n = 6)	5.1 ± 0.2	2.5 ± 0.1	1.00 ± 0.05	78 ± 11	32 ± 3	1247 ± 122	0.3 ± 0.3	0.01 ± 0.01
		SHAM (n = 6)	5.1 ± 0.1	2.6 ± 0.1	1.04 ± 0.05	67 ± 7	32 ± 4	1324 ± 168	0.3 ± 0.3	0.02 ± 0.01
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Week 4	iP (n = 6)	5.2 ± 0.1	2.5 ± 0.1	0.96 ± 0.09	73 ± 12	29 ± 2	965 ± 188	0.5 ± 0.2	0.02 ± 0.01
		OSM (n = 6)	5.3 ± 0.2	2.6 ± 0.1	1.00 ± 0.07	69 ± 9	35 ± 7	985 ± 225	0.5 ± 0.2	0.03 ± 0.01
		SHAM (n = 6)	5.3 ± 0.3	2.7 ± 0.1	1.04 ± 0.06	86 ± 24	31 ± 4	1230 ± 171	0.5 ± 0.4	0.02 ± 0.01
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Blood chemical parameters were determined only in the 4-week experiment. Data are presented as mean ± standard deviation. Significant comparison-pairs are shown in the line "SIG." [Welch's test with the Holm adjustment, $P \leq 0.05$ (n.s: no significant pairs)]. T-PRO: total protein. ALB: albumin. A/G: albumin/globulin ratio. AST: aspartate aminotransferase. ALT: alanine aminotransferase. ALP: alkaline phosphatase. γ-GTP: γ-glutamyl transpeptidase. T-BIL: total bilirubin.

Table 4. (continued).

Exp.	Sacrificed	Group name	CRE (mg/dL)	BUN (mg/dL)	CK (Unit/L)	CRP (ng/mL)	PT (second)	APTT (second)	FIB (mg/dL)
I	Week 1	iP (n = 6)	0.16 ± 0.02	16 ± 1	213 ± 46	266 ± 48	9.2 ± 0.3	18.5 ± 1.6	393 ± 109
		OSM (n = 6)	0.16 ± 0.02	16 ± 2	173 ± 30	249 ± 77	9.2 ± 0.3	18.4 ± 1.2	369 ± 119
		SHAM (n = 6)	0.18 ± 0.03	16 ± 3	236 ± 68	255 ± 66	9.1 ± 0.3	18.8 ± 1.6	361 ± 23
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Week 2	iP (n = 6)	0.22 ± 0.02	19 ± 3	189 ± 55	226 ± 27	9.2 ± 0.2	18.0 ± 1.6	358 ± 54
		OSM (n = 6)	0.20 ± 0.02	18 ± 2	156 ± 30	246 ± 53	9.3 ± 0.2	19.3 ± 1.7	347 ± 43
		SHAM (n = 6)	0.21 ± 0.03	19 ± 2	198 ± 30	234 ± 38	9.4 ± 0.2	20.2 ± 1.1	308 ± 39
		SIG.	n.s.	n.s.	n.s.	n.s.	n.s.	iP-SHAM	n.s.
	Week 4	iP (n = 6)	0.22 ± 0.03	18 ± 3	164 ± 18	192 ± 22	9.3 ± 0.2	19.9 ± 1.3	345 ± 36
		OSM (n = 6)	0.24 ± 0.03	19 ± 1	138 ± 12	222 ± 43	9.5 ± 0.3	21.1 ± 1.2	321 ± 34
		SHAM (n = 6)	0.24 ± 0.04	17 ± 1	147 ± 25	192 ± 28	9.6 ± 0.2	20.9 ± 2.7	309 ± 29
		SIG.	n.s.	n.s.	iP-OSM	n.s.	n.s.	n.s.	n.s.

Blood chemical parameters were determined only in the 4-week experiment. Data are presented as mean ± standard deviation. Significant comparison-pairs are shown in the line "SIG." [Welch's test with the Holm adjustment, $P \leq 0.05$ (n.s: no significant pairs)]. CRE: creatinine. BUN: blood urea nitrogen. CK: creatine kinase. CRP: C-reactive protein. PT: prothrombin time. APTT: activated partial thromboplastin time. FIB: fibrinogen.

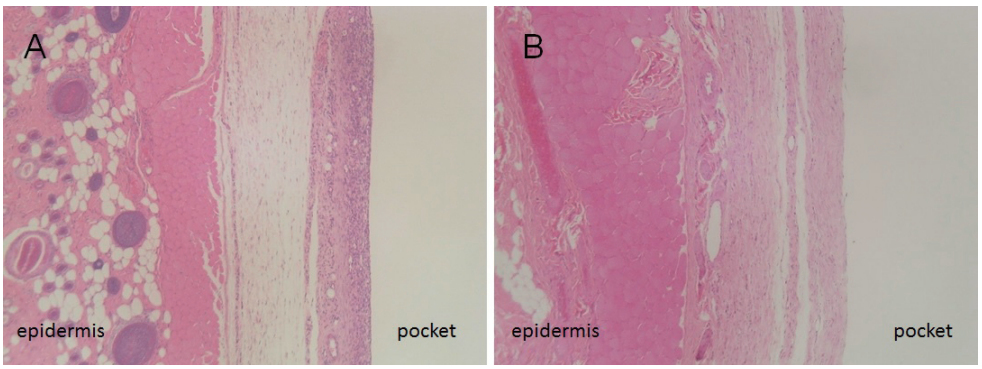

Fig. 2. Typical images around the implantation site (dorsal subcutaneous area) of the iPRECIO®-implanted rats at week 1 (panel A) and week 13 (panel B) after the implantation surgery. The images are photographed at × 40 magnification. In each image, the right side is the subcutaneous pocket in which the pump was placed, and the left side is the dermal tissue of the back skin. At week 1, the infiltration of various leukocytes (blue-stained) was observed in the subcutaneous tissue adjacent to the pocket. At week 13 (panel B), the corresponding part was changed into fibrous tissue instead of cellular infiltrations of leukocytes.

the body weight and food consumption between the iP-/OSM- and SHAM-groups (data not shown). These results indicate that the implantation of the devices did not lead to any critical internal damage to rats because the body weight and food consumption in life are early, sensitive, and convenient indices of serious homeostatic failure.

Hematologic parameters were determined at weeks 1, 2, and 4 in experiment I, and at week 13 in experiment II (Table 2, erythrocyte-related parameters; Table 3, leukocyte-related parameters). There were no critical differences in any of the parameters among the groups. The mean corpuscular hemoglobin concentration (MCHC) at week 13 in the iP-group was significantly lower than that in the SHAM-group ($P = 0.0394$), but the change was considered to be meaningless because of the small difference [32.2 ± 0.5% (iP) *vs.* 32.8 ± 0.5% (SHAM)]. In the OSM-group in experiment I, the lymphocyte (LYMPH) at week 2 was calculated from the data of 5 animals, because 1 rat showed an extremely high value ($719 \times 10^3/mm^3$). This extraordinary value in this 1 animal would be unrelated to the physiological conditions judging from the absence of any other alterations in other leukocyte-related parameters.

Blood chemical parameters were determined at weeks 1, 2, and 4 in experiment I (Table 4). There were no critical differences in any of the blood chemical parameters among the groups. The CK at week 4 in the iP-group was significantly higher than that in the OSM-group ($P = 0.0466$). However, no significant difference was observed in this parameter between the iP- and SHAM-groups at any examination points. Therefore, the difference of the CK between the iP- and OSM-groups was concluded not to be a biological concern. The APTT at week 2 in the iP-group was significantly lower than that in the SHAM-group ($P = 0.0455$), but the change was also considered not to be a concern judging from the changing direction and difference [18.0 ± 1.6 seconds (iP) *vs.* 20.2 ± 1.1 seconds (SHAM)]. Between the OSM- and SHAM-groups, the calcium (Ca) at week 2 and alanine aminotransferase (ALT) at week 1 showed significant changes ($P = 0.0304$ and 0.0416, respectively), but the small differences indicated that these changes were meaningless [Ca at week 2: 10.4 ± 0.2 mg/dL (OSM) *vs.* 10.0 ± 0.2 mg/dL (SHAM), ALT at week 1: 30 ± 3 Unit/L (OSM) *vs.* 36 ± 4 Unit/L (SHAM)].

It is well known that hematologic and blood chemical parameters are regulated by various physiological systems, such as endocrine, immune, and the nervous systems, and are useful indices of systemic condition. Thus, the data from our extensive hematologic and blood chemical examinations, in which no toxicologically meaningful changes were observed, would imply the absence of any significant influence on such homeostatic systems in both iP- and OSM-animals.

In experiment II, the 5 animals/group/week were euthanized at weeks 1, 2, 4, and 13, respectively, and subjected to necropsy. The necropsy revealed no noteworthy changes in any organs and tissues of any of the animals. Therefore, the histopathological examination focused on the implantation site (the dorsal subcutaneous tissue; Table 5). In this site, the infiltration of polymorphonuclear cells, edema, infiltration of mononuclear cells, and granulation, were observed in the iP-animals (Fig. 2). These changes, which are known as initial inflammatory changes, were

Table 5. Inflammatory changes in the implantation site.

Week	FBR changes	iP (n = 5 / week)				SHAM (n = 5 / week)			
		-	+/-	+	++	-	+/-	+	++
1	Polymorphonuclear	4	0	1	0	5	0	0	0
	Edema	0	1	4	0	5	0	0	0
	Mononuclear	4	1	0	0	4	1	0	0
	Granulation	0	0	4	1	4	1	0	0
	Fibrous capsule formation	5	0	0	0	3	2	0	0
2	Polymorphonuclear	5	0	0	0	5	0	0	0
	Edema	3	2	0	0	5	0	0	0
	Mononuclear	2	1	2	0	4	1	0	0
	Granulation	0	2	1	2	5	0	0	0
	Fibrous capsule formation	2	2	1	0	5	0	0	0
4	Polymorphonuclear	5	0	0	0	5	0	0	0
	Edema	5	0	0	0	5	0	0	0
	Mononuclear	2	3	0	0	4	1	0	0
	Granulation	1	2	2	0	5	0	0	0
	Fibrous capsule formation	1	0	4	0	5	0	0	0
13	Polymorphonuclear	5	0	0	0	5	0	0	0
	Edema	5	0	0	0	5	0	0	0
	Mononuclear	5	0	0	0	5	0	0	0
	Granulation	0	4	1	0	5	0	0	0
	Fibrous capsule formation	0	0	5	0	5	0	0	0

Histopathological examination was performed at weeks 1, 2, 4 and 13 in experiment II. Data are presented as the number of animals exhibiting the microscopic inflammatory change in the implantation site (dorsal subcutaneous tissue). -: Not observed. +/-: Minimum. +: Slight. ++: Moderate. "Polymorphonuclear" means the cellular infiltration of polymorphonuclear cells (mainly, granulocytes). "Mononuclear" means the cellular infiltration of mononuclear cells (mainly, macrophages, lymphocytes).

observed frequently at weeks 1 and 2 (Table 5). However, most of these changes tended to lessen or weaken by week 4 or later. At week 13, the inflammatory changes shifted to the fibrous capsule, an end-form of inflammatory reaction (Table 5). This type of the serial change is known as typical reactions induced by non-biological materials (Anderson, 2001; Anderson *et al.*, 2008). In contrast to the iP-animals, very few of the SHAM-animals showed these changes in the corresponding site. The foreign material-induced changes in the iP animals were concluded to be limited locally to the implantation site of the iPRECIO®. In fact, the necropsy revealed no significant changes in the near regional lymph nodes, and furthermore, none of the hematological parameters were suggestive of any such inflammation.

Foreign materials implanted in the living body sometimes induce tumors. However, the histopathological examinations in this study revealed no proliferative changes in the iPRECIO®-implanted areas.

As described above, the iP-animals (including OSM-animals) revealed no abnormalities in appearance, body weight, food consumption, hematological parameters, and blood chemical parameters. The necropsy also revealed no visible changes in any organs and tissues of the iP-animals. These results suggest that the iPRECIO®-implantation into rats exerted no significant impact on systemic conditions. On the other hand, the iP-animals revealed some local microscopic inflammation at the implantation site. These types of local changes are predictable reactions because foreign devices placed in the living body generally induce some degree of local inflammation at implantation sites (Anderson, 2001; Anderson *et al.*, 2008). Thus, as long as the changes remain within the area of the implantation sites, they would not disrupt the evaluations of chemicals in most general toxicity studies.

In conclusion, the implantation of iPRECIO® exerted no significant impact on the physiological condition of the rats. The pump is, therefore, applicable for a continuous exposure model in *in vivo* toxicity studies.

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

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