

## Perinatal and Postnatal Study of LBD-001, a Recombinant Human Interferon $\gamma$ , in Rats

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**ABSTRACT** : LBD-001, a recombinant human interferon  $\gamma$  produced by genetically engineered yeast as a host system, was intravenously administered to pregnant female rats (Sprague-Dawley) from day 17 of gestation to day 21 of lactation at dose levels of  $0.35 \times 10^6$ ,  $0.69 \times 10^6$ , and  $1.38 \times 10^6$  I.U./kg/day. In vasopressin-treated group, vasopressin (5 I.U./kg/day) was intravenously injected only for 5 days of perinatal period. (1) No significant changes by the treatment of LBD-001 were observed in the body weights, food and water consumption, feeding and nursing behaviors, and the weights of main organs of mother rats. In vasopressin-treated group, no significant changes were observed except the decrease in the food consumption on day 18 of gestation and one case of abnormal offspring with bleeding spots on the skin. (2) No significant changes in the body weights, survival rates, locomotor activity, emotional development, and the motor coordination of offsprings (F1) by the treatment of LBD-001 were observed except the fact that increase of ambulation in the female offsprings of LBD-001 ( $0.69 \times 10^6$  or  $1.38 \times 10^6$  I.U./kg/day)-treated groups and the increase of rearing in the males of LBD-001 ( $1.38 \times 10^6$  I.U./kg/day)-treated group, and the increase of the weight of liver and ovaries in the female offsprings in the LBD-001 ( $1.38 \times 10^6$  I.U./kg/day)-treated group were observed. Altogether, the results show that LBD-001 at the dose of  $1.38 \times 10^6$  I.U./kg/day or less does not significantly affect the mother rats and their offsprings (F1) except the minor influences when treated during the perinatal and postnatal period.

**Key Words** : LBD-001, Recombinant human interferon  $\gamma$ , Vasopressin, Perinatal and postnatal study, Rats, Intravenous injection

### I. INTRODUCTION

LBD-001 is a recombinant human interferon  $\gamma$  produced by fermentation of genetically engineered yeast containing the DNA which encodes for the human protein. The main action mechanism of naturally occurring interferon  $\gamma$ , a biological response modifier which is secreted from antigen-stimulated T-lymphocyte is related with enhancement of phagocytic function. LBD-001 was developed by Biotech Research Institute, Lucky Chemical Ltd. (84 Jang-Dong, Yousung-Ku, Daejeon, Korea). Previously, we have reported the fertility study of LBD-001 in rats (Lee and Cho, 1996), the teratological study of LBD-001 in rats (Cho and Lee, 1996), and the teratological study of LBD-001 in rabbits (Lee and Cho, 1997). As a part of toxicological tests of

LBD-001, effects of LBD-001 on mother rats and their offsprings (F1) during the perinatal and postnatal period were examined (National Institute of Safety Research of Korea, 1986).

### II. MATERIALS AND METHODS

#### 1. Treatment of Test Substance

LBD-001 was supplied by Biotech Research Institute, Lucky Chemical Ltd. and serially diluted in 5% dextrose in phosphate buffered saline (PBS; pH 7.4) to make three doses of preparations. The dose of LBD-001 was  $0.35 \times 10^6$ ,  $0.69 \times 10^6$ , or  $1.38 \times 10^6$  I.U./kg/day. In vehicle-treated group, 5% dextrose in PBS (1 ml/kg/day) was treated. In nontreated group, none was treated. The day with sperm positivity in the vaginal smears was regarded as day 0 of gestation. And the birthday of offsprings (F1)

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was regarded as day 1 of lactation. The test substance was administered intravenously to tail vein of rats from day 17 of gestation to day 21 of lactation period. However, vasopressin (Hanil Co.; 5 I. U./kg/day) used as a reference drug was administered only from day 17 to 21 of gestation. The number of animals per group ranged from 10 to 20.

## 2. Animal Maintenances

Sprague-Dawley rats (over 40-day-old male rats and over 42-day-old female rats) bred in our Institute were kept at the temperature of 22~25°C and under the constant bright (6 a.m. to 7 p.m.) and dark (7 p.m. to 6 a.m.) cycle. Standard laboratory rodent diet (Samyang Feed Co.) and sterilized water were fed *ad libitum*.

## 3. Observation of Mother Rats

Body weight, food consumption, and water consumption of mother rats were measured on day 0, 7, 14, 16, 18, and 20 of gestation and on day 1, 3, 7, 10, 14, and 21 of lactation period. All the rats were naturally delivered. General behaviors and lactating or nursing behaviors of the mother rats were observed every day. On day 22 of lactation, the mother rats were sacrificed by ether and autopsied. The weights of main organs such as heart, liver, lungs, kidneys, adrenals, spleen, thymus, and ovaries were measured. From the uterine horns, traces of implantation sites were counted and then the delivery rates (=the number of delivered offsprings/the number of implantation sites × 100) were calculated.

## 4. Observation of Delivered Offsprings (F1)

After the delivery, the number of live or dead offsprings, their external malformations, sex, and dead or stillborn offsprings were observed. The dead or stillborn offsprings were stained for skeletal observation according to the method of Dawson (1926) using alizarin red S (Sigma Chem Co.). The body weights of live offsprings were measured on day 1, 3, 7, 10, 14, 17, and 21 of lactation period.

During the lactation period, the detachment of ears, the appearance of abdominal hairs, the eruption of lower incisors, and the opening of eyelids were observed to check the physical development of the offsprings. In addition, in order to check the sexual differentiation, the descent of testes and opening of vagina were observed. For checking the development of sensory function of the offsprings, the visual placing reflex, the preyer reflex, the righting reflex, the response to the pain which was added to the tail by pinching with an aortic clamp, and the free fall reflex were tested on day 20 of lactation period (Irwin, 1968; Buelke-Sam, 1979). Locomotor activity and emotionality were measured by the open field test (Hall, 1934; Cory-Slechta, 1989) on day 28 of lactation. In the test, the number of ambulation for 3 min, rearing, face-washing, fur-licking, defecation, and urination was counted. Motor coordination was measured by the method of Lynch *et al.* (1975) in which the number of falls for 3 min from the rotating rotarod (12 r.p.m.; 5 cm of diameter) was counted. On day 28 of lactation period, some of the offsprings received the test of locomotor activity and emotionality were sacrificed by ether and the weights of main organs such as heart, liver, lungs, kidneys, adrenals, spleen, thymus, testes, and ovaries were measured.

## 5. Statistical Analyses

To analyze the statistical significance, Student's *t*-test or  $\chi^2$ -test was used. Experimental data in a group were compared with those of vehicle-treated group. When the difference shows  $p < 0.05$ , the data were considered significantly different.

# III. RESULTS AND DISCUSSION

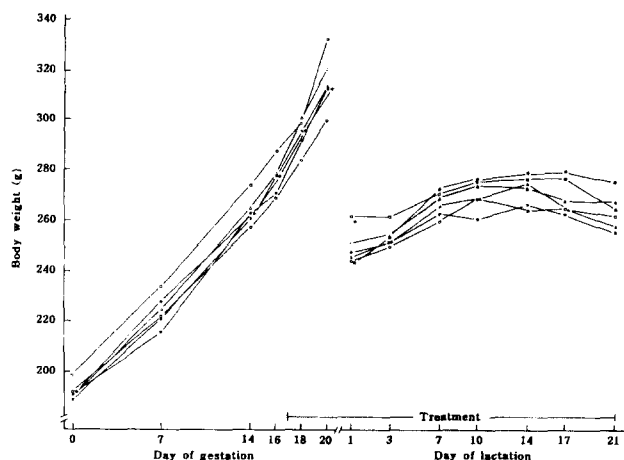
## 1. Influences on Mother Rats

### 1) General Symptoms

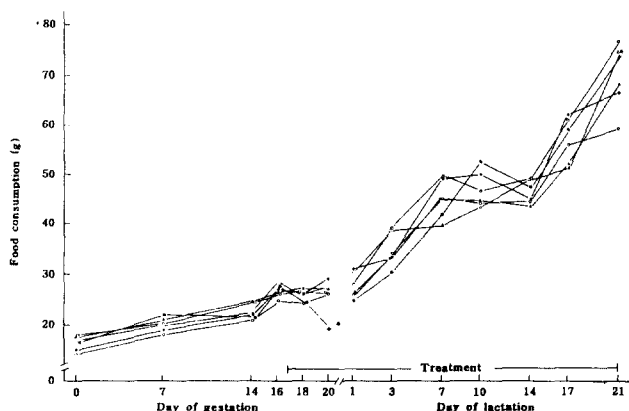
Any abnormal symptoms and behaviors by the treatment of LBD-001 was not detected.

### 2) Changes on Body Weight and Food or Water Consumption

Changes of the body weight during the test



**Fig. 1.** Body weight changes of rats treated with LBD-001 during perinatal and postnatal period. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 ( $0.35 \times 10^6$  I.U./kg/day), □: LBD-001 ( $0.69 \times 10^6$  I.U./kg/day), △: LBD-001 ( $1.38 \times 10^6$  I.U./kg/day), ★: Vasopressin (5 I.U./kg/day; only treated from day 17 to 21 of gestation).

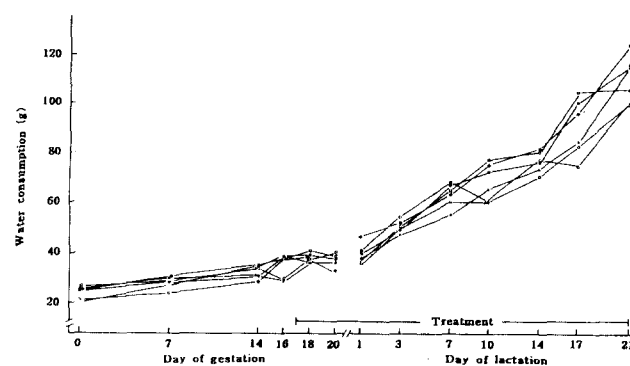


**Fig. 2.** Food consumption of rats treated with LBD-001 during perinatal and postnatal period. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 ( $0.35 \times 10^6$  I.U./kg/day), □: LBD-001 ( $0.69 \times 10^6$  I.U./kg/day), △: LBD-001 ( $1.38 \times 10^6$  I.U./kg/day), ★: Vasopressin (5 I.U./kg/day; only treated from day 17 to 21 of gestation). \* $p < 0.01$ ; Significantly different from the vehicle-treated control group.

period were shown in Fig. 1. The consumption of food and water was shown in Fig. 2 and Fig. 3. No significant differences were observed among the groups in the body weight and the food or water consumption, except the significant decrease ( $p < 0.01$ ) of the food consumption was observed compared with the vehicle-treated group on lactation day 20 of gestation.

### 3) Observation of Main Organs of Mother Rats

The weights of the main organs autopsied on



**Fig. 3.** Water consumption of rats treated with LBD-001 during perinatal and postnatal period. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 ( $0.35 \times 10^6$  I.U./kg/day), □: LBD-001 ( $0.69 \times 10^6$  I.U./kg/day), △: LBD-001 ( $1.38 \times 10^6$  I.U./kg/day), ★: Vasopressin (5 I.U./kg/day; only treated from day 17 to 21 of gestation).

day 22 of lactation period were shown in Table 1. In LBD-001 ( $0.35 \times 10^6$  I.U./kg)-treated group, the weights of lungs and kidneys were significantly higher compared with those of the vehicle-treated group. However, the increases were not dose-dependent and accidental, since higher or lower dose of LBD-001 ( $0.69$  or  $1.38 \times 10^6$  I.U./kg/day)-treated group did not show significant differences. In the weight of other organs, no significant differences were observed among the groups.

## 2. Influences on Fetuses

### 1) Delivery Records

The results were shown in Table 2. Delivery rates were a little decreased in the LBD-001 ( $1.38 \times 10^6$  I.U./kg)-treated group and vasopressin-treated group. As an external malformation, bleeding spots around the snout and eyes were found in one case of vasopressin (5 I.U./kg)-treated group. Hirayama (1980) reported that the offsprings born from the mother rats subcutaneously injected with vasopressin (2 I.U./kg/day) from day 17 to 21 of gestation showed outstanding bleeding spots on the skin. The difference of severity of the bleeding spots in our study might be partly from the difference of the administration routes. As a reference, one mother rat which was intravenously injected with vasopressin (10 I.U./kg/day) also delivered 3 stillborn offsprings with bleeding spots on the skin among 10 offsprings (data not shown).

**Table 1.** Organ weights of rats treated with LBD-001 during perinatal and posnatal period

Parameters	Nontreated group	Vehicle group	LBD-001 (I.U./kg/day)			Vasopressin (I.U./kg/day)
			0.35×10 <sup>6</sup>	0.69×10 <sup>6</sup>	1.38×10 <sup>6</sup>	5
No. of dams	14	20	18	16	16	10
Body weight (g, Mean±S.D.)	256.6±13.4	259.4±18.8	260.2±23.4	262.8±20.2	263.8±21.3	252.0±16.1*
Heart (mg)	929.6±64.0	944.3±42.3	1014.0±157.7	991.5±94.6	975.3±122.1	952.2±53.7
Liver (g)	16.650±0.765	15.876±1.590	15.806±2.439	16.851±2.546	16.810±2.500	16.121±1.650
Lungs (g)	1.650±0.287	1.593±0.147	1.748±0.149*	1.668±0.271	1.649±0.200	1.630±0.382
Kindneys (g)	2.324±0.099	2.253±0.167	2.482±0.158**	2.364±0.212	2.491±0.300	2.217±0.154
Adrenals (mg)	75.4±9.9	79.0±14.3	80.6±11.0	84.1±7.8	76.5±14.5	79.9±8.4
Spleen (mg)	695.6±91.5	673.5±111.0	801.1±204.7	776.4±118.3	585.5±236.8	705.3±202.0
Thymus (mg)	233.8±61.4	198.6±57.1	234.4±84.8	234.7±57.9	260.4±87.3	240.0±71.0
Ovaries (mg)	66.7±9.9	65.6±11.7	70.9±19.0	70.7±8.2	72.4±12.0	67.0±10.7

\*p<0.05, \*\*p<0.01; Significantly different from the vehicle-treated group.

**Table 2.** Delivery records of rats treated with LBD-001 during perinatal and postnatal poeriod

Parameters	Nontreated group	Vehicle group	LBD-001 (I.U./kg/day)			Vasopressin (I.U./kg/day)
			0.35×10 <sup>6</sup>	0.69×10 <sup>6</sup>	1.38×10 <sup>6</sup>	5
No. of dams	14	20	18	16	16	10
Duration of gestation (days) (Mean±S.D.)	21.7±0.45	21.8±0.40	21.7±0.47	21.9±0.33	21.7±0.45	22.0±0.00
Total no. of implantation traces	128	214	188	182	164	114
No. of implantation traces per litter (Mean±S.D.)	9.1±2.29	10.7±2.10	10.4±1.57	11.4±1.22	10.3±2.1	11.4±1.96
Total no. of newborns	128	202	174	172	142	100
No. of newborns per litter (Mean±S.D.)	9.1±2.29	10.1±2.02	9.7±1.49	10.8±1.20	8.9±1.90	10.0±2.28
Delivery rate (%) <sup>b</sup>	100	94.4	92.6	94.5	86.6	87.7
Total no. of stillborns	2	10	12	2	4	6
Stillborn rate (%) <sup>c</sup>	1.6	5.0	6.9	1.2	2.9	6.0
Total no. of live offsprings	126	192	162	170	138	94
No. of live offsprings per litter (Mean±S.D.)	9.0±2.20	9.4±1.74	9.0±2.58	10.6±1.11	8.6±1.80	9.2±3.06
Sex ratio (no. of males/no. of females)	0.77 (55/71)	1.04 (98/94)	0.80 (72/90)	1.18 (92/78)	1.23 (76/62)	1.24 (52/42)
Total no. of newborns with external malformations	0	0	0	0	0	1 <sup>d</sup>

<sup>a</sup>Total no. of newborns=Total no. of live newborns+Total no. of stillborns. <sup>b</sup>Total no. of newborns/Total no. of implantation traces. <sup>c</sup>Total no. of stillborns/Total no. of newborns. <sup>d</sup>Bleeding spots around the snout and eyes.

## 2) Effects on Viability of Offsprings (F1)

The results were shown in Table 3. On day 14 and 21 of lactation period, the survival rates showed a little lower tendency in the female offsprings of LBD-001 (0.69×10<sup>6</sup> I.U./kg)-treated group. However, it might be negligible since the rates were neither dose-dependent nor significantly different from those of the vehicle-treated group. Except this, the survival rates of the male and female offsprings were not significantly different among the groups.

## 3) Body Weight of Offsprings

The body weight changes of the male and female offsprings were shown in Table 4. The body weight gains of the male or female offsprings grown from day 1 to 21 of lactation period were not significantly different among the groups. The body weight gain in the males or female offsprings was 34.1 or 34.2 g in nontreated group, 31.1 or 30.0 g in vehicle-treated group, 32.3 or 32.6 g in LBD-001 (0.35×10<sup>6</sup> I.U./kg)-treated group, 33.0 or 31.1 g in LBD-001 (0.69×10<sup>6</sup> I.U./kg)-treated group and 30.



**Table 5.** Physical development, sexual differentiation, and sensory function of offsprings (F1) born from rats treated with LBD-001 during perinatal and postnatal period

Parameters	Nontreated group		Vehicle group		LBD-001 (I.U./kg/day)		Vasopressin (I.U./kg/day)
	male	female	male	female	male	female	
Physical development (days)							
Detachment of ears	2.6±0.53 (124)	2.7±0.7 (192)	2.4±0.61 (162)	2.5±0.50 (138)	2.4±0.64 (164)	2.5±0.50 (138)	2.8±0.37 (92)
Appearance of abdominal hairs	7.6±0.49 (124)	7.4±0.49 (192)	7.4±0.49 (158)	7.4±0.44 (150)	7.3±0.44 (150)	7.4±0.48 (136)	7.8±0.80 (90)
Eruption of lower incisors	9.9±0.73 (124)	10.0±0.76 (192)	10.7±0.87 (156)	10.1±0.76 (136)	9.9±0.76 (136)	10.1±0.76 (136)	10.3±0.51 (88)
Opening of eyelids	14.3±0.51 (124)	14.3±0.67 (192)	14.4±0.91 (154)	14.3±0.71 (152)	14.3±0.71 (152)	14.9±0.68 (134)	14.7±0.48 (88)
Sexual differentiation (days)							
Descent of testes	16.5±1.04 (48)	17.2±0.68 (94)	16.8±0.74 (66)	16.7±0.79 (90)	16.7±0.79 (90)	17.1±0.73 (72)	17.3±0.79 (48)
Opening of vagina	34.8±2.04 (30)	36.0±2.95 (30)	36.2±2.72 (30)	36.6±2.64 (30)	36.6±2.64 (30)	35.8±3.55 (30)	35.6±2.67 (30)
Sensory function on day 20 of lactation period							
No. of offsprings	124	186	150	150	150	134	90
Visual placing reflex <sup>a</sup>	0	0	0	0	0	0	0
Preyer reflex <sup>a</sup>	0	0	0	0	0	0	0
Righting reflex <sup>a</sup>	0	0	0	0	0	0	0
Response to pain <sup>a</sup>	0	0	0	0	0	0	0
Free fall reflex <sup>a</sup>	0	0	0	0	0	0	0

Values represent mean ± S.D. or the number of offsprings in case of the figures in parentheses. <sup>a</sup>No. of offsprings with abnormal sensory function.

**Table 6.** Locomotor activity and emotionality measured by the open field test in offsprings (F1) born from rats treated with LBD-001 during perinatal and postnatal period

Parameters	Nontreated group		Vehicle group		LBD-001 (I.U./kg/day)		Vasopressin (I.U./kg/day)		
	male	female	male	female	male	female			
Sex	20	20	20	20	20	20	20		
No. of offsprings	38.2±16.1	39.4±24.0	39.4±15.8	29.2±15.7	51.4±20.5**	38.2±18.7	36.5±24.1	44.6±19.5* 49.5±23.7* 47.3±18.7**	30.8±14.6 35.1±17.1 30.8±14.6
Ambulation <sup>a</sup>	13.5±7.4	11.9±6.1	12.0±6.5	15.6±9.7	14.2±6.6	12.3±5.6	11.8±9.2	14.3±7.3	23.3±18.7* 15.7±7.0 9.6±6.6
Rearing	2.6±1.7	2.8±1.6	2.4±1.6	3.2±2.4	2.5±2.0	4.1±2.1	3.2±1.6	4.6±3.6	3.6±2.4 4.1±2.5 3.2±1.4
Face-washing	0±0	0±0	0.1±0.4	0.1±0.2	0±0	0.1±0.4	0.1±0.2	0.1±0.2	0±0 0±0 0±0
Fur-licking	9	9	7	10	6	5	5	9	5 11 5
Defecation	3	5	4	4	2	4	4	3	4 2 2
Urination									

Values represent mean ± S.D. or the total number. <sup>a</sup>No. of squares crossed for 3 minutes. \*p<0.05. \*\*p<0.01; Significantly different from the vehicle control group t-test or  $\chi^2$ -test.

0 or 29.0 g in LBD-001 ( $1.38 \times 10^6$  I.U./kg)-treated group and 33.3 or 32.7 g in vasopressin-treated group. All the groups which received the treatment of injection showed a little lower body weight gains compared with that of nontreated group. In part, a stress by the injection might make the mother rats lactate less to the offsprings.

#### 4) Effects on Physical Development, Sexual Differentiation, and Sensory Function of Offsprings

The results were shown in Table 5. Physical development was not significantly different among the groups; the mean day of the detachment of ears, the appearance of abdominal hairs, the eruption of the incisors, and the opening of eyelids, the descent of testes, and the opening of vagina

was about day 2.5, 7.5, 10, 14.5, 17, and 35, respectively. All the 20-day-old offsprings received the sensory reflex tests such as the visual placing reflex, the preyer reflex, the righting reflex, the response to the pain, and the free fall reflex did not show any abnormalities except that one offspring in LBD-001 ( $0.69 \times 10^6$  I.U./kg)-treated group showed negative response against the pain reflex, but died of severe diarrhea after 3 days.

#### 5) Effects on Development of Sensory Locomotor Activity, and Emotionality of Offsprings

The results of locomotor activity and emotionality were measured by the open field test on day 28 of lactation period were shown in Table 6 and Table 7. In the test, the number of ambulation for 3 min was

**Table 7.** Motor coordination measured by the rotarod test in offsprings (F1) born from rats treated with LBD-001 during perinatal and postnatal period

Parameters	Nontreated group	Vehicle group	LBD-001 (I.U./kg/day)			Vasopressin (I.U./kg/day)
			$0.35 \times 10^6$	$0.69 \times 10^6$	$1.38 \times 10^6$	5
No. of falls for 3 min (mean $\pm$ S.D.)	male $3.6 \pm 3.04$ (20) female $3.2 \pm 1.94$ (20)	male $3.6 \pm 2.26$ (20) female $2.6 \pm 1.80$ (20)	male $3.2 \pm 2.44$ (20) female $2.9 \pm 2.40$ (20)	male $2.8 \pm 2.49$ (20) female $2.9 \pm 2.70$ (20)	male $3.5 \pm 2.04$ (20) female $2.6 \pm 2.08$ (20)	male $3.9 \pm 3.11$ (10) female $3.3 \pm 2.73$ (10)

Values in parentheses represent the number of examined offsprings.

**Table 8.** Organ weights of offsprings (F1) born from rats treated with LBD-001 during perinatal and postnatal period

Parameters	Nontreated group	Vehicle group	LBD0-001 (I.U./kg/day)			Vasopressin (I.U./kg/day)
			$0.35 \times 10^6$	$0.69 \times 10^6$	$1.38 \times 10^6$	5
No. of offsprings	12	12	15	12	12	12
Body weight (g) <sup>a</sup>	male $67.67 \pm 10.96$ female $65.59 \pm 11.51$	male $70.44 \pm 5.24$ female $67.95 \pm 2.89$	male $70.00 \pm 7.25$ female $69.17 \pm 7.85$	male $73.6 \pm 9.9$ female $66.84 \pm 8.88$	male $70.30 \pm 5.60$ female $67.83 \pm 3.49$	male $71.39 \pm 8.25$ female $69.83 \pm 5.94$
Organ weight <sup>a</sup>						
Heart (mg)	male $349.3 \pm 55.4$ female $346.1 \pm 61.3$	male $376.8 \pm 40.4$ female $342.0 \pm 30.4$	male $351.6 \pm 37.6$ female $346.3 \pm 33.0$	male $362.1 \pm 38.0$ female $335.2 \pm 33.7$	male $354.1 \pm 33.9$ female $352.1 \pm 40.3$	male $360.8 \pm 51.0$ female $341.2 \pm 42.1$
Liver (g)	male $4.081 \pm 0.795$ female $3.703 \pm 0.774$	male $4.235 \pm 0.740$ female $4.084 \pm 0.600$	male $3.951 \pm 0.570$ female $3.957 \pm 0.512$	male $4.343 \pm 0.497$ female $3.799 \pm 0.488$	male $4.296 \pm 0.63$ female $4.954 \pm 0.267^{**}$	male $4.321 \pm 0.693$ female $4.311 \pm 0.527$
Lungs (mg)	male $561.5 \pm 75.8$ female $537.2 \pm 88.2$	male $622.0 \pm 73.7$ female $586.8 \pm 90.2$	male $580.4 \pm 99.6$ female $565.4 \pm 92.9$	male $650.6 \pm 68.7$ female $610.3 \pm 105.6$	male $612.2 \pm 119.7$ female $567.8 \pm 44.9$	male $600.1 \pm 82.3$ female $570.1 \pm 89.1$
Kidneys (mg)	male $941.2 \pm 155.6$ female $881.8 \pm 158.7$	male $959.3 \pm 105.0$ female $944.9 \pm 88.3$	male $963.4 \pm 116.7$ female $911.8 \pm 96.2$	male $1040.5 \pm 158.5$ female $955.9 \pm 156.4$	male $953.9 \pm 116.2$ female $874.0 \pm 43.1^*$	male $963.0 \pm 154.1$ female $929.9 \pm 78.0$
Adrenals (mg)	male $19.7 \pm 3.5$ female $18.1 \pm 2.8$	male $19.0 \pm 2.7$ female $18.6 \pm 2.4$	male $18.0 \pm 2.6$ female $18.5 \pm 2.1$	male $20.8 \pm 2.7$ female $20.2 \pm 2.5$	male $19.3 \pm 2.1$ female $18.9 \pm 1.9$	male $18.9 \pm 3.1$ female $18.7 \pm 2.5$
Spleen (mg)	male $342.1 \pm 12.5$ female $348.3 \pm 57.2$	male $365.0 \pm 64.2$ female $337.0 \pm 48.7$	male $346.5 \pm 92.7$ female $302.9 \pm 66.6$	male $384.9 \pm 90.1$ female $326.7 \pm 65.1$	male $394.2 \pm 65.6$ female $366.0 \pm 69.7$	male $353.1 \pm 72.7$ female $329.0 \pm 59.1$
Thymus (mg)	male $269.1 \pm 78.7$ female $274.7 \pm 15.8$	male $241.0 \pm 45.8$ female $262.2 \pm 27.4$	male $254.8 \pm 52.0$ female $267.8 \pm 45.2$	male $289.0 \pm 65.5$ female $264.0 \pm 63.4$	male $261.1 \pm 46.2$ female $266.3 \pm 48.6$	male $273.3 \pm 54.4$ female $279.3 \pm 52.1$
Testes (mg)	male $507.7 \pm 135.8$	male $540.9 \pm 54.5$	male $535.4 \pm 47.1$	male $569.1 \pm 96.2$	male $570.3 \pm 80.0$	male $541.7 \pm 63.7$
Ovaries (mg)	female $15.8 \pm 2.9$	female $16.6 \pm 2.3$	female $17.6 \pm 1.9$	female $15.7 \pm 2.2$	female $18.5 \pm 1.8^*$	female $16.5 \pm 2.5$

<sup>a</sup>Weights were represented as mean  $\pm$  S.D. \* $p < 0.05$ , \*\* $p < 0.01$ ; Significantly different from the vehicle-treated group.

significantly higher in the females of LBD001 ( $0.69 \times 10^6$  or  $1.38 \times 10^6$  I.U./kg)-treated group and in the males of LBD001 ( $0.35 \times 10^6$  or  $1.38 \times 10^6$  I.U./kg)-treated group compared with that of vehicle-treated group. The number of rearing was significantly higher in the male offsprings of LBD001- ( $1.38 \times 10^6$  I.U./kg)-treated group compared with that of vehicle-treated group. Except these, no significant dose-dependent effects were observed in the number of rearing, face-washing, fur-licking, defecation, and urination among the groups. Motor coordination measured by counting the number of falls for 3 min from the rotating rotarod was not significantly different among the groups.

#### 6) Weights of Main Organs of Offsprings

The results were shown in Table 8. No significant differences in the weights of heart, liver, lungs, kidneys, adrenals, spleen, thymus, testes, and ovaries which were measured on day 28 of lactation period were observed among the groups except that significant increases of the weights of liver and ovaries and significant decrease of the weight of kidneys of the female offsprings in LBD-001 ( $1.35 \times 10^6$  I.U./kg)-treated group as compared with the vehicle-treated group ( $p < 0.01$  in the weight of liver;  $p < 0.05$  in the weights of ovaries and kidneys). The decrease of the weight of kidneys in the females might be accidental because the kidney weight was not significantly different as compared with that of non-treated group and failed to show clear dose-dependent decrease. Why the weight of only the female livers, not male livers, and ovaries showed significant increases is a little hard to explain even though the increases also was not clearly dose-dependent. However, there were no abnormalities in macroscopic observation of the autopsied organs.

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