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TERATOGENICITY STUDY OF RECOMBINANT HUMAN INTERFERON α A (LBD-007) IN RATS

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ABSTRACT: *LBD-007, a newly developed recombinant human interferon α A, was at dose levels of 0, 3×10^6, 6×10^6 and 12×10^6 IU/kg/day administered subcutaneously to pregnant Sprague-Dawley rats during the organogenetic period. Ethylenethiourea was used as a positive control. 2/3 of dams per group were subjected to caesarean section on day 20 of pregnancy and the remaining 10 dams per group were allowed to deliver. Effects of test substance on dams, embryonal development of F1 fetuses, as well as growth, behaviour and mating performance of F1 offspring were examined.*

1. *No treatment-related changes in clinical signs, food consumption, body weight and necropsy findings of dams were observed.*
2. *There were no growth retardation and teratogenic effects on F1 and F2 fetuses from the dams treated with LBD-007.*
3. *Parameters of growth, behaviour and reproductive performance of F1 offspring showed no changes related to treatment.*

The results show that the no-effect dose levels (NOELs) of LBD-007 are over 12×10^6 IU/kg/day for dams, for F1 and F2 fetuses and for F1 offspring.

Key Words: *LBD-007, Recombinant human interferon α A, Teratogenicity study, Rats, Subcutaneous application.*

INTRODUCTION

LBD-007, a recombinant human interferon α A, is an anti-virus and anti-cancer agent, which was newly developed by Lucky R & D center, Biotechnology (Yousung-Koo, Daejon, Korea).

As a part of toxicological screening of test agent LBD-007, teratogenicity of Sprague-Dawley rats was studied. This study was performed to assess the potential

toxic effects of test substance on dams, embryonal development of F1 fetuses, as well as growth, behavioural function and mating capability of F1 offspring.

MATERIALS AND METHODS

Animal Maintenance and Mating Procedure

Sprague-Dawley rats (KRICT Toxicology Center Breeding Facility) were kept under spf-conditions at a constant day/night cycle (light : 7 h to 19 h). Standard laboratory rodent diet (Jeil Feed Co., Daejon, Korea) and sterilized water were available ad libitum. For mating one female was placed into the cage of one male overnight and the first 24 h period following the mating procedure was designated as day 0 of pregnancy if copulation plug or sperm were detected.

Test Substance

LBD-007 (Lot. NO. AI004) was supplied by the Lucky R & D Center, Biotechnology (84 Jang-Dong, Yousung-Koo, Daejon, Korea) with a titer of 10^7 IU/m*l*, pH of 7.4 and on osmotic pressure of 281 mOsm. The vehicle, phosphate buffered saline (pH 7.4), was used as the control solution. Dilutions were made up weekly according to the body weight on day 7 and day 14 of gestation and all solutions were stored at 4℃. Ethylenethiourea (Hwa Kwang Co./Japan, Lot No. : ECH 7290) was used as a positive control.

Treatment and Observation of Dams

LBD-007 was administered subcutaneously to pregnant rats from days 7 to 17 of gestation. Per experimental group 35 females with successful copulation were used, aber there were only 23 females in the positive control group. There were three treatment groups (3×10^6, 6×10^6 and 12×10^6 IU/LBD-007/kg body wt.), one vehicle control group and one positive control group. Positive controls received 50 mg Ethylenethiourea/kg body wt. Pregnant females were observed for food consumption, weight development and sign of intoxication. At autopsy of the animals at the end of gestation, the following organs were weighed : liver, kidney, spleen, heart, adrenals, ovaries.

Caesarian Section on Day 20 of Gestation

The females of the caesarian section groups (2/3 of females of the vehicle control and treatment groups) and all positive controls were sacrificed by an overdose of CO_2 on day 20 of gestation. The implantation sites were numbered and recorded. The number of corpora lutea, living fetuses, dead fetuses and resorptions were registered. All living fetuses were immediately weighed, sexed and evaluated for externally visible abnormalities. Alternate fetuses were selected for either skeletal or visceral examination. The evaluation of skeletal abnormalities was performed after clearing the 95% ethanol-fixed fetuses with KOH, after staining the skeleton with alizarin red and after dyeing the cartilage with alcian blue (Inouye, 1976; Lorke, 1977). Alizarin red colors the calcified bone anlagen (Dawson, 1926). For visceral examination of Bouin's fluid fixed-fetuses we have adapted a Wilson's technic (Wilson *et al.*, 1972) for the head and abdomen and Nishimura method

(Nishimura, 1974) for the thorax.

Observation of F1 Animals

The remaining one third of pregnant females of all groups (except positive control group) were allowed to litter. The litters were examined for litter size and gross abnormality. On day 4 after delivery, each litter was reduced to eight pups (four males and four females when possible) to have comparabe litter size. The fetuses left over after litter size control were used for either skeletal or internal examination. Viability during lactation of F1 pups, growth, behaviour and mating performance of F1 offspring were evaluated. At the end of the 21-day lactation period, one male and one female weanlings per litter were killed and subjected to autopsy. Postnatal weight development was monitored weekly for one male and one female per litter. In addition, signs of physical development were recorded for each of the animals; these included separation of auricle, emergence of abdominal hair, eruption of incisor, separation of eyelids and descent of testis or opening of vagina. At autopsy of 10 weeks old F1 animals, the following organs were weighed : liver, kidney, spleen, heart, adrenals, ovaries or testes. One male and one female per litter were selected for behavioural function test. From day 9 of lactation, traction test was performed. At 8 weeks old, water-filled multiple T-maze test was carried out for the evaluation of learning ability of F1 offspring.

At 9 weeks old, one male and one female F1 animal per litter were mated within the groups, for the evaluation of reproductive capability avoiding sister-brother mating. Their male partners and the litter mates not copulated were also killed and autopsied. F1 pregnant females were observed for weight development. On day 20 of gestation, the female animals were sacrified. All parameters were evaluated as at caesarian section of F0 dams.

Statistical Analysis of the Data

Statistical significance was tested using Analysis of Variance (using Dunnett's or Scheffe's test). Kruskal-Wallis test and X^2-test. The positive and vehicle control groups were compared using the Mann-Whitney U-test. A difference was considered statistically significant at a $p<0.05$.

RESULTS

Effect of LBD-007 on Dams

No remarkable changes in behaviour or clinical signs were observed among controls and animals of treated groups. Also, no substance-related deaths occurred in all groups. There were no significant differences in the food consumption and body weight development of dams between the groups, except that in the positive control group, a significantly lower food consumption and retarded body weight was observed (Figure 1, 2). At necropsy of dams on day 20 of gestation or day 21 of lactation, no substance-related pathologic findings were discovered in all groups. Relative organ weights compared well between the groups, except that positive controls showed a decreased relative weight of spleen (Table 1).

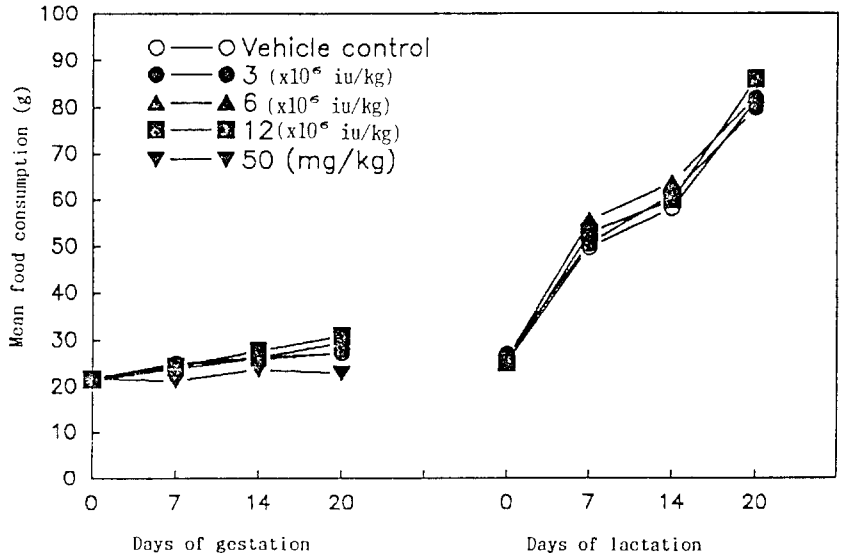

Figure. 1. Mean food consumption during gestation and lactation of FO dams treated with LBD-007.

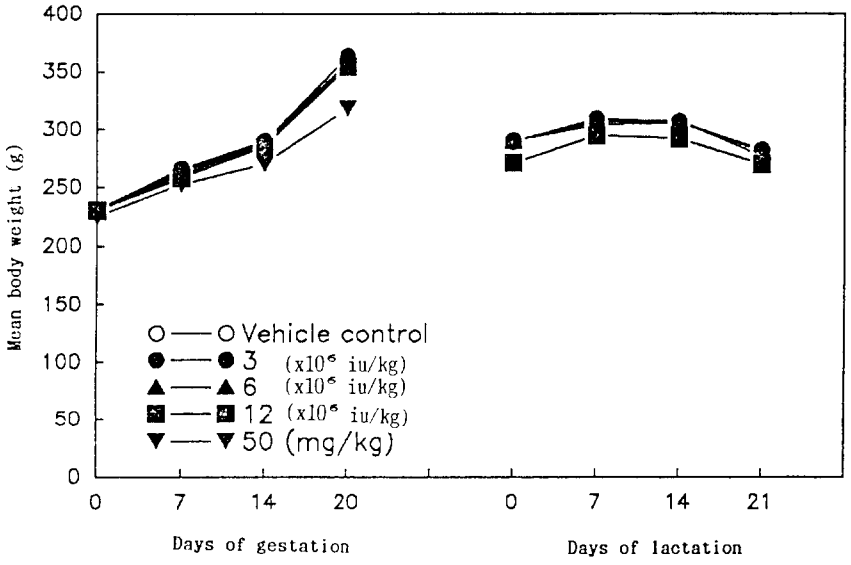

Figure. 2. Mean body weight changes during gestation and lactation of F0 dams treated with LBD-007.

Effect of LBD-007 on F1 Fetuses

Sectio-data

The litter parameters did not differ significantly between the groups, except a lower fetal weight of both sexes in the positive control group (Tabe 2). There were externally malformed fetuses; namely one acaudate, and curvature of thoracic vertebrae (Photo 1) among vehicle controls, one acaudate and curvature of thoracic

Table 1. Relative organ weights of F0 dams treated with LBD-007

Dose : ($\times 10^6$ IU/kg)	0	3	6	12	ETU[a] 50 (mg/kg)
Number of animals	21	24	19	20	20
Body weight (g)	371.8± 21.88	364.2± 30.47	367.2± 22.11	368.0± 28.04	330.9± 30.23**
% Body weight					
Liver (g)	4.365± 0.258	4.450± 0.367	4.503± 0,175	4.405± 0.336	4.226± 0.344
Kidney-Left (g)	0.291± 0.036	0.296± 0.050	0.290± 0.028	0.288± 0.036	0.286± 0.031
Kindney-Right (g)	0.297± 0.037	0.307± 0.053	0.295± 0.025	0.297± 0.036	0.294± 0.034
Spleen (g)	0.182± 0.022	0.178± 0.019	0.170± 0.022	0.175± 0.019	0.161± 0.019**
Heart (g)	0.249± 0.020	0.251± 0.030	0.246± 0.019	0.253± 0.024	0.247± 0.027
Adrenal Gland-Left (g)	0.009± 0.001	0.009± 0.002	0.009± 0.001	0.009± 0.002	0.008± 0.001
Adrenal Gland-Right (g)	0.008± 0.001	0.008± 0.001	0.008± 0.001	0.008± 0.002	0.008± 0.001
Overy-Left (g)	0.015± 0.004	0.015± 0.004	0.015± 0.003	0.015± 0.003	0.017± 0.005
Ovary-Right (g)	0.016± 0.004	0.015± 0.003	0.016± 0.004	0.015± 0.002	0.016± 0.003

Values are Mean± S.D.
*and **indicate significant difference at $p<0.05$ and $p<0.01$ levels when compared with control group.
[a] ETU = Ethylenethiourea

Table 2. Findings at caesarean section of FO dams treaed with LBD-007

Dose : ($\times 10^6$ IU/kg)	0	3	6	12	ETU 50 (mg/kg)
No. of pregnant animals	21	24	19[a]	20[a]	20
Corpora lutea(Mean± S.D.)	15.05± 3.71	15.04± 3.06	15.74± 2.66	15.25± 3.96	14.45± 3.32
Implantations(Mean± S.D.)	13.76± 3.33	13.96± 2.80	14.68± 2.67	14.05± 3.69	13.30± 3.11
% to corpora lutea(Mean± S.D.)	91.38± 6.15	92.64± 7.07	93.17± 5.04	91.67± 6.22	91.87± 6.13
Fetal deaths (resorptions + dead fetuses)	10	12	4	12	4
Resorptions	10	11	4	12	4
Early	10	11	4	11	4
Late	0	0	0	1	0
Dead fetuses	0	1	0	0	0
Live fetuses					
Male/Female	138/141	149/174	129/146	134/135	136/126
Litter size(Mean± S.D.)	13.29± 3.36	13.46± 2.84	14.47± 2.67	13.45± 4.05	13.10± 3.23
% to implantations(Mean± S.D.)	96.46± 7.39	98.46± 5.05	98.59± 3.83	92.14± 22.39	97.95± 4.95
Sex Ratio (male/female)	0.98	0.86	0.88	0.99	1.08
No. of fetuses with external anomalies(%)	2(0.7)	2(0.6)	0(0)	1(0.4)	262[b](100.0)
Acaudate	1	0	0	0	
Vestigial tail	0	0	0	1	
Acaudate, curvature of thoracic vertebrae	1	1	0	0	
Short tail, dwarf	0	1	0	0	

Table 2. Continued

Dose : ($\times 10^6$ IU/kg)	0	3	6	12	ETU 50 (mg/kg)
Body weight of live fetuses					
Male (Mean± S.D.)	3.35± 0.24	3.42± 0.29	3.38± 0.80	3.27± 0.23	2.66± 0.34**
Female (Mean± S.D.)	3.24± 0.24	3.24± 0.23	3.21± 0.17	3.13± 0.77	2.60± 0.31**

[a] In one animal data were excluded from statistical analysis because of premature delivery.

[b] Among positive controls following malformations occurred; Hydrocephalus (8), Kinky tail, club foot (1), Hydrocephalus, short tail (36), Hydrocephalus, kinky tail (46), Hydrocephalus, club foot (15), Hydrocephaius, acaudate (6), Hydrocephalus, short tail, club foot (2), Hydrocephalus, club foot, kinky tail (13), Hydrocephalus, short tail, kinky tail (54), Hydrocephalus, club foot, short tail (13), Hydrocephalus, short tail, acaudate (1), Hydrocephalus, club foot, acaudate (6), Hydrocephalus, short tail, kinky tail, club foot (60), Hydrocephalus, short tail, acaudate, club foot (1)

* and **indicate significant difference at $p<0.05$ and $p<0.01$ levels when compared with control group.

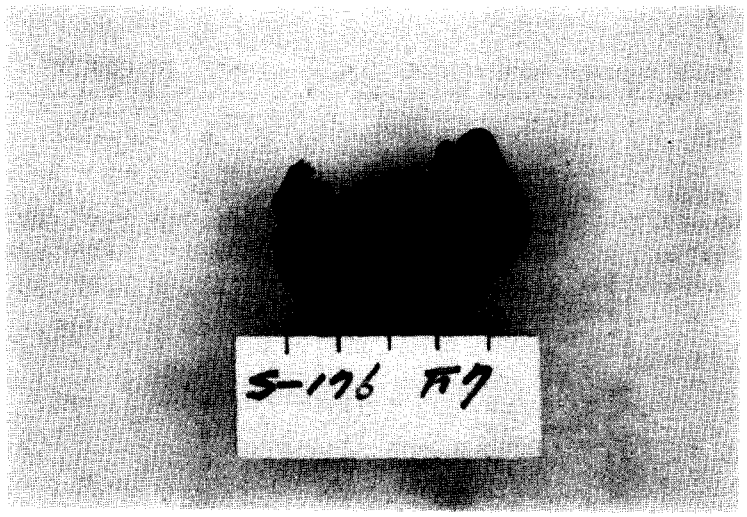

Photo. 1. Curvature of thoracie vertebrae in an F1 fetus in vehicle control group.

vertebrae, one short tail and dwarf in 3×10^6 IU/kg group and one vestigial tail in 12×10^6 IU/kg group. Malformations were rare and not dose-related. Among positive controls a high incidence of malformed fetuses were observed (Photo 2).

Visceral findings

No malformations occurred among examined fetuses, except that in the positive control group, numerous malformed fetuses were observed (Photo 3). Fifty two fetuses of the vehicle control and LBD-treated groups showed variations, namely dilatation of renal pelvis and/or dilatation of ureter (Table 3). They were not dose-related. The frequency of variations of the positive control group is increased, when compared with that of the vehicle control group. Among remaining fetuses after litter size control no malformations were observed in all groups.

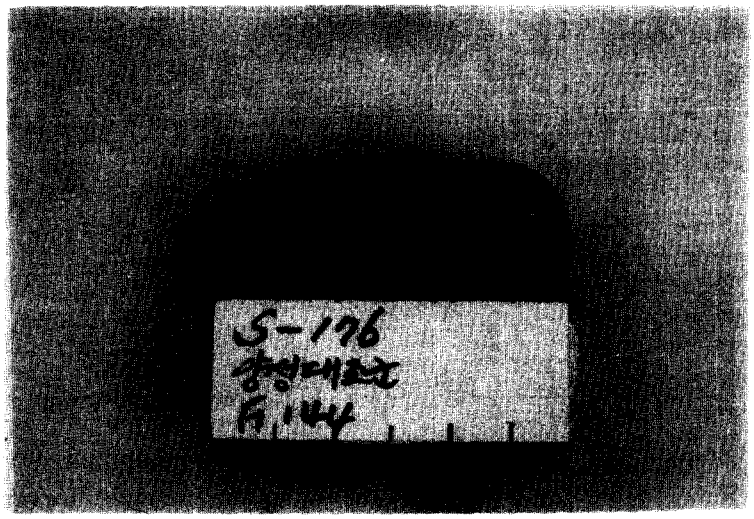

Photo. 2. Hydrocephalus in an F1 fetus in positive control group.

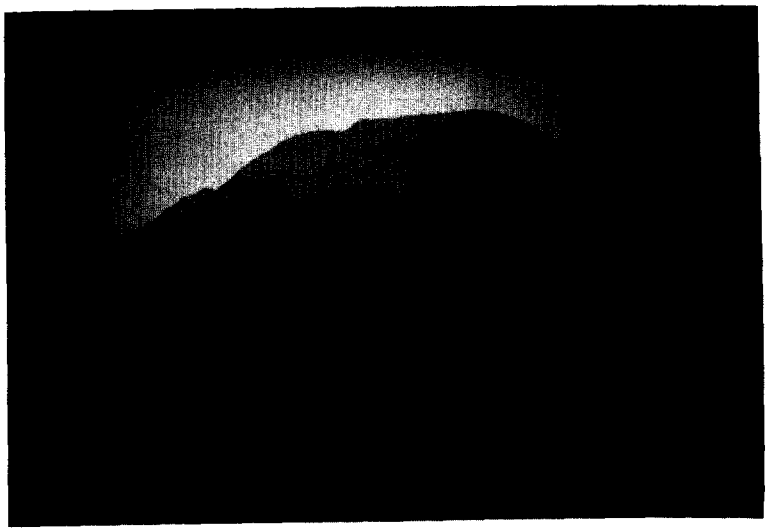

Photo. 3. Cleft palate in an F1 fetus in positive control group.

Skeletal findings

Eleven malformed fetuses were found; namely three shortend 13th rib among controls, five shortend 13th rib, one absent 13th rib, one shortend and absent 13th rib at 3×10^6 IU/kg, one shortend 13th rib at 6×10^6 IU/kg.

Malformations were rare and not dose-related. Ten variated fetuses were found, namely 14th rib and/or cleaved thoracic or lumbar vertebral body. They were as well not related to dose. Fetuses of the positive control group showed a high incidence of malformations and variations (Photo 4). The rate of ossification of eva-

Table 3. Findings in F1 fetuses from F0 dams treated with LBD-007

Dose : ($\times 10^6$ IU/kg)	0	3	6	12	ETU 50 (mg/kg)
No. of fetuses examined	133	156	133	130	128
No. of fetuses with anomalies(%)	0(0)	0(0)	0(0)	0(0)	126(98.4)
Hydroencephaly	0	0	0	0	111
Cleft palate, hydroencephaly	0	0	0	0	15
No. of fetuses with variations(%)	11(8.3)	23(14.7)	6(6.0)	10(7.7)	44(34.4)
Dilatation of renal peivis	7	6	2	2	2
Dilatation of ureter	0	12	1	2	10
Dilatation of renal pelvis and dilatation of ureter	4	5	5	2	32

Photo. 4. Multiple abnormality (Fused and wavy ribs, absent thoracic vertebral bodies and fused lumbar vertebral bodies) in an F1 fetus in positive control group.

luated skeletal districts compared well between the groups, except that in the positive control group, retarded ossification was observed (Table 4). No malformations occurred among examined fetuses left over after litter size control.

Effect of LBD-007 on F1-Offspring

In the treatment groups no substance-related effect could be seen in all parameters examined. There were no malformed newborns in all groups. The survival

Table 4. Skeletal findings in F1 fetuses from F0 dams treated with LBD-007.

Dose : ($\times 10^6$ IU/kg)	0	3	6	12	ETU 50 (mg/kg)
No. of fetuses examined	145	166	142	139	135
No. of fetuses with anomalies(%)	6(4.1)	14(8.4)	2(1.4)	0(0)	119(88.1)
Absent and/or fused vertabral body (thoracic or lumbar)	0	0	0	0	28[a]
Fused sternebrae	0	0	0	0	5
Rib	3	7	1	0	86[b]
Shortend 13th rib	3	5	1	0	
Absent 13th rib	0	1	0	0	
Shortend, absent 13th rib	0	1	0	0	
No. of fetuses with variations(%)	7(4.8)	0(0)	1(0.7)	2(1.4)	110(81.5)
14th rib	6	0	1	1	4
Wavy rib	0	0	0	0	2
Cleaved sternebrae	0	0	0	0	1
Asymmetric sternebrae	0	0	0	0	3
Cleaved vertebral body (thoracic or lumbar)	1	0	0	1	100[a]
Degree of ossifications					
No. of sternebrae	4.6± 0.5	4.6± 0.6	4.6± 0.4	4.9± 0.3	3.8± 1.6**
No. of metacarpals in both forelimbs	6.5± 0.6	6.5± 0.5	6.3± 0.3	6.4± 0.6	4.3± 1.5**
No. of 1st phalanges in both forelimbs	0.0	0.0	0.0	0.0	0.0
No. of metatarsals in both hindlimbs	8.0± 0.1	8.0± 0.1	7.9± 0.2	8.0± 0.0	6.3± 2.0**
No. of 1st phalanges in both hindlimbs	0.0	0.0	0.0	0.0	0.0
No. of sacral and caudal vertebrae	7.3± 0.5	7.5± 0.5	7.6± 0.4	7.6± 0.3	3.7± 1.1**

[a] Two types of abnormalities (absent and cleaved vertebral body) were found in 26 fetuses.

[b] Among positive controls following rib anbormalities were observed; Fused ribs (40), Flying rib (6), Fused flying ribs (29), Fused, flying ribs, shortend 13th rib (1), Fused, broken ribs, shortend 13th rib (1), Fused ribs, shortend 13th rib (5), Shortend 13th rib (4).

*and **indicate significant difference at $p<0.05$, and $p<0.01$ levels when compared with control groups

Table 5. Viability during lactation of F1 pups from F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
No. of live F1 pups (Mean± S.D per litter)				
At birth	128(12.8± 1.93)	144(14.4± 1.65)	135(13.5± 1.90)	136(13.6± 2.07)
Postpartum day 4	127(12.7± 1.89)	143(14.3± 1.64)	134(13.4± 1.84)	136(13.6± 2.07)
4[a]	80(8.0± 0.00)	80(8.0± 0.00)	80(8.0± 0.00)	80(8.0± 0.00)
21	80(8.0± 0.00)	79(7.9± 0.32)[d]	80(8.0± 0.00)	80(8.0± 0.00)
Viability index(%)				
Postpartum days 0~4[b]	99	99	99	100
4~21[c]	100	99	100	100

[a] After litter size control

[b] (No. of live pups at day 4/No. of live pups at birth)$\times 10$

[c] (No. of live pups at day 21/No. of live pups at day 4)$\times 100$

[d] One male pup died accidentally after traction test on day 16 postnatally

Table 6. Postnatal physical development results of F1 pups from F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
No. of pups examined	10	10	10	10
Separation of auricle				
Male	2.5± 0.71	2.6± 0.70	2.8± 0.63	2.4± 0.52
Female	2.4± 0.52	2.7± 0.67	2.8± 0.63	2.2± 0.42
Emergence of abdominal hair				
Male	9.0± 0.00	9.0± 0.00	8.9± 0.32	8.9± 0.32
Female	9.0± 0.00	9.1± 0.32	9.2± 0.42	9.1± 0.32
Eruption of incisor				
Male	10.4± 0.84	10.7± 1.06	10.8± 1.03	10.3± 0.82
Female	10.3± 0.95	10.5± 1.18	10.7± 0.95	10.3± 0.82
Separation of eyelids				
Male	13.7± 0.82	13.6± 0.70	13.8± 0.92	13.6± 0.52
Female	13.6± 1.17	13.7± 0.95	13.8± 0.63	13.4± 0.52
Descent of testis				
Male	21.1± 0.32	21.0± 0.00	21.2± 0.42	21.2± 0.42
Opening of vagina				
Female	31.1± 0.32	31.0± 0.00	31.2± 0.63	31.0± 0.00

Values are Mean± S.D. (days).

Table 7. Traction test results of F1 pups from F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
Male				
No. of pups examined	10	10	10	10
Days(Mean± S.D.)	16.0± 1.3	16.3± 1.1	16.0± 1.2	16.3± 0.9
Female				
No. of pups examined	10	10	10	10
Days(Mean± S.D.)	15.7± 1.5	16.0± 1.1	16.2± 1.1	15.8± 0.6

rate of F1 pups during the lactation period was not affected by the test substance (Table 5). At necropsy of F1 pups on day 21 of lactation, no substance-related pathologic findings were discovered. The physical development of the three treatment groups did not differ significantly from the control value (Table 6). All four groups had comparable results in the traction test and water T-maze test (Table 7, 8). No substance-related effect could be found. In F1 male and female animals, no notable clinical signs were observed from 0 to 70 days post partum. There were no significant differences in the body weight development of F1 animals between the groups (Figure 3, 4). At necropsy of 10 weeks old F1 offspring, no substance-related pathologic lesions could be seen. Relative organ weights compared well between the groups (Table 9, 10).

There was no indication of a decrease in fertility of F1 parent animals in the treatment groups (Table 11). No significant differences were observed in the body weight development of pregnant F1 females (Figure 5). At necropsy of F1 females on day 20 of gestation, no pathologic findings were discovered.

Table 8. Water filled multiple T-maze test results of F1 male and female rats from F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg)		0	3	6	12
No. of male rats examined (8 weeks old)		10	10	10	10
Straight channel:	Time(seconds)				
	1st	10.6± 3.9	26.6± 14.9*	24.5± 18.0	11.4± 2.4
	2nd	6.9± 2.2	8.4± 5.8	7.6± 5.5	6.1± 2.2
	3rd	6.5± 4.2	5.8± 1.5	5.9± 3.6	5.8± 1.2
Multiple T-maze					
Day 1	No. of errors				
	S.E	2.2± 0.6	2.4± 0.5	2.2± 0.6	2.2± 0.9
	B.E	0.7± 0.6	0.8± 0.4	0.6± 0.4	0.7± 0.6
	Z.E	2.1± 1.2	2.3± 0.8	2.1± 1.0	2.1± 1.0
	Time(seconds)	39.8± 13.7	44.9± 11.8	40.3± 11.1	42.5± 12.3
Day 2	No. of errors				
	S.E	1.3± 0.6	1.1± 0.7	1.3± 0.6	1.1± 0.5
	B.E	0.6± 0.6	0.3± 0.4	0.3± 0.3	0.3± 0.5
	Z.E	1.1± 0.9	0.6± 0.5	0.7± 0.4	0.7± 0.7
	Time(seconds)	28.5± 7.3	25.2± 7.9	24.5± 5.6	24.1± 8.5
Day 2	No. of errors				
	S.E	0.6± 0.4	0.4± 0.5	0.7± 0.6	0.4± 0.4
	B.E	0.3± 0.3	0.7± 0.9	0.6± 0.8	0.1± 0.2
	Z.E	0.2± 0.3	0.5± 0.7	0.3± 0.4	0.1± 0.2
	Time(seconds)	25.7± 8.9	28.2± 13.5	25.0± 7.4	19.8± 5.3
No. of female rats examined (8 weeks old)		10	10	10	10
Straight channel:	Time(seconds				
	1st	23.9± 15.6	18.4± 7.7	27.0± 15.1	25.1± 15.4
	2nd	7.6± 5.6	9.4± 8.0	6.6± 2.2	8.2± 4.1
	3rd	8.9± 7.8	7.1± 4.1	7.8± 9.0	9.2± 11.9
Multiple T-maze					
Day 1	No. of errors				
	S.E	2.5± 0.5	2.6± 0.4	2.4± 0.5	1.9± 0.6
	B.E	0.7± 0.5	0.6± 0.4	0.6± 0.4	0.4± 0.6
	Z.E	2.0± 0.7	2.2± 0.8	2.0± 0.9	2.1± 1.3
	Time(seconds)	38.8± 9.0	44.1± 8.0	38.6± 4.2	37.5± 12.5
Day 2	No. of errors				
	S.E	1.3± 0.6	1.2± 0.9	1.1± 0.8	1.3± 0.6
	B.E	0.3± 0.5	0.7± 0.5	0.4± 0.4	0.6± 0.5
	Z.E	0.7± 0.4	1.0± 0.8	0.6± 0.7	0.8± 0.6
	Time(seconds)	26.1± 8.6	28.0± 16.1	22.8± 8.5	27.4± 9.0
Day 2	No. of errors				
	S.E	1.0± 0.7	0.7± 0.7	0.7± 0.4	0.7± 0.4
	B.E	1.2± 1.3	0.5± 0.6	0.6± 0.4	1.1± 1.1
	Z.E	0.7± 1.0	0.4± 0.5	0.2± 0.3	0.3± 0.5
	Time(seconds)	33.1± 17.2	25.5± 10.7	24.7± 5.7	26.9± 12.1

Values are Mean± S.D.

S.E: Selecting error, B.E: Backing error, Z.E: Zone error

*indicate significant difference at $p<0.05$ level when compared with control group.

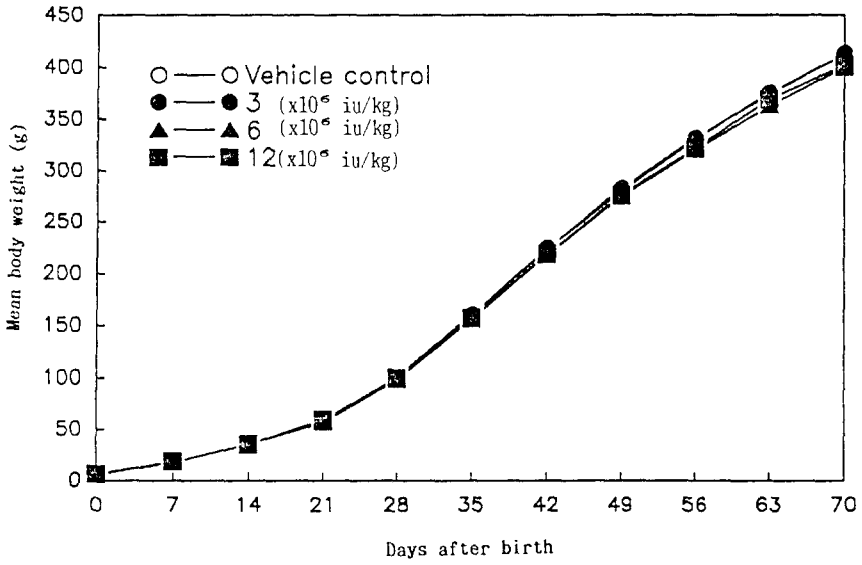

Figure. 3. Mean body weight changes of F1 male rats from F0 dams treated with LBD-007.

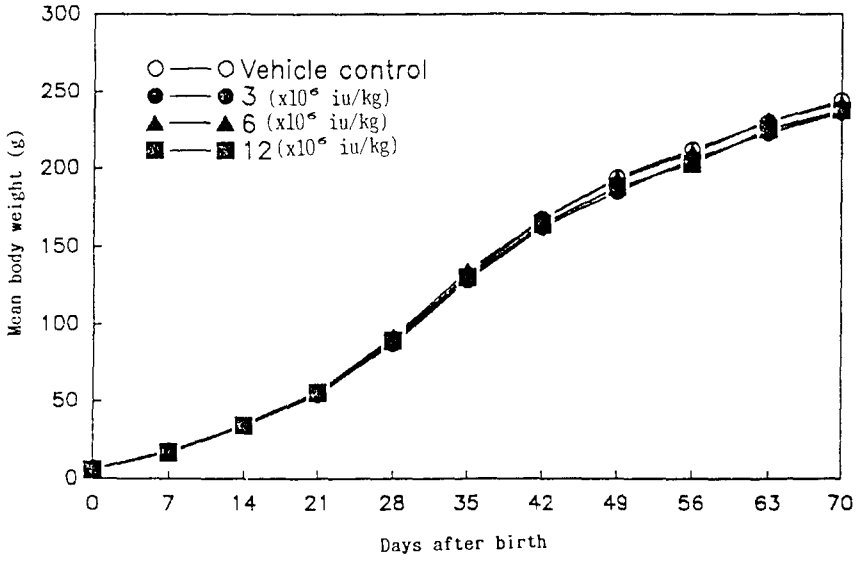

Figure. 4. Mean body weight changes of F1 female rats from F0 dams treated with LBD-007.

Effect of LBD-007 on F2 Fetuses

The litter parameters of the treatment groups did not differ significantly from the control values. There were no externally malformed fetuses (Table 12). In both skeletal and internal examination, no abnormalities were observed among examined fetuses. The variations found in the visceral examination of F2 fetuses were trival and not dose-related (Table 13).

Table 9. Relative organ weights of F1 male rats from F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
Number of animals	10	10	10	10
Body Weight(g)	413.1± 24.12	413.0± 25.34	400.0± 29.37	384.4± 59.45
% Body weight				
Liver(g)	3.893± 0.496	3,913± 0.264	4.194± 0.7841	4.577± 1.157
Kidney-Left(g)	0.393± 0.041	0.408± 0.032	0.425± 0.042	0.464± 0.114
Kidney-Right(g)	0.399± 0.038	0.410± 0.032	0.424± 0.035	0.467± 0.123
Spleen(g)	0.200± 0.030	0.201± 0.027	0.192± 0.031	0.209± 0.043
Heart(g)	0.333± 0.072	0.310± 0.023	0.333± 0.027	0.378± 0.098
Adrenal Gland-Left(g)	0.007± 0.001	0.008± 0.001	0.007± 0.001	0.007± 0.003
Adrenal Gland-Right(g)	0.007± 0.001	0.008± 0.002	0.007± 0.001	0.007± 0.002
Testis-Left(g)	0.392± 0.022	0.427± 0.051	0.392± 0.032	0.429± 0.130
Testis-Right(g)	0.398± 0.021	0.420± 0.039	0.390± 0.028	0.435± 0.131

Values are Mean± S.D.

Table 10. Relative organ weights of F1 female rats from F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
Number of animals	10	10	10	10
Body Weight(g)	248.1± 31.34	240.4± 20.70	242.1± 19.82	255.7± 57.73
% Body weight				
Liver(g)	4.087± 0.806	3.779± 0.294	3.978± 0.261	3.789± 0.685
Kidney-Left(g)	0.432± 0.080	0.428± 0.037	0.429± 0.030	0.418± 0.070
Kidney-Right(g)	0.447± 0.085	0.444± 0.043	0.450± 0.032	0.427± 0.067
Spleen(g)	0.201± 0.031	0.228± 0.034	0.195± 0.030	0.217± 0.051
Heart(g)	0.367± 0.045	0.370± 0.035	0.357± 0.019	0.343± 0.050
Adrenal Gland-Left(g)	0.013± 0.003	0.013± 0.002	0.013± 0.003	0.014± 0.004
Adrenal Gland-Right(g)	0.013± 0.004	0.012± 0.002	0.012± 0.002	0.013± 0.003
Ovary-Left(g)	0.016± 0.002	0.017± 0.004	0.015± 0.002	0.014± 0.003
Ovary-Right(g)	0.017± 0.002	0.016± 0.003	0.015± 0.004	0.016± 0.003

Values are Mean± S.D.

Table 11. Fertility data of F1 rats from F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
Male				
No. of mated animals	10	10	10	10
Copulation index[a] (%)	10/10(100)	10/10(100)	10/10(100)	8/10(80)
Fertility index[b] (%)	8/10 (80)	10/10(100)	8/10 (80)	5/8 (63)
Female				
No. of mated animals	10	10	10	10
Copulation index[a] (%)	10/10(100)	10/10(100)	10/10(100)	8/10(80)
Pregnancy index[c] (%)	8/10 (80)	10/10(100)	8/10 (80)[d]	5/8 (63)

[a] No. of animals with successful copulation/No. of mated animals

[b] No. of impregnating animals/No. of animals with successful copulation

[c] No. of pregnant animals/No. of animals with successful copulation

[d] In one animal neither vaginal plug nor sperm was detected, but she was found pregnant at autopsy.

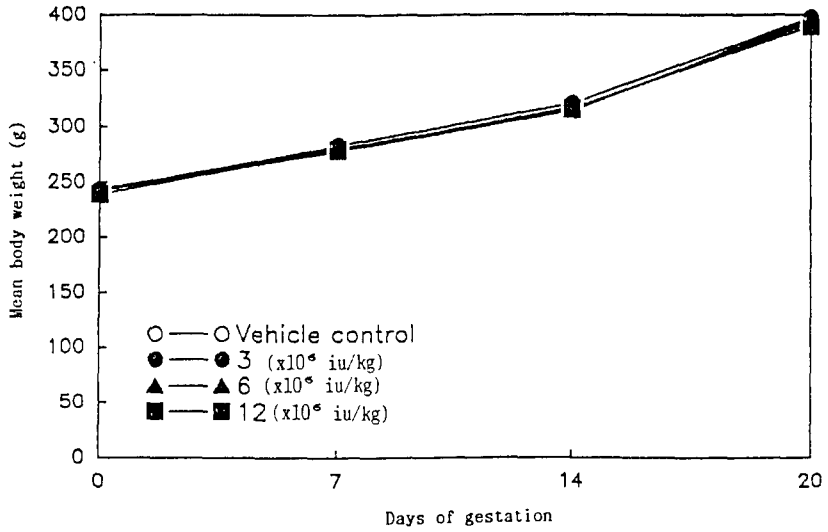

Figure. 5. Mean body weight changes during gestation of F1 rats from F0 dams treated with LBD-007

Table 12. Caesarean section data of F1 female rats from F0 dams treated with LBD-007

Dose : ($\times 10^6$ IU/kg)	0	3	6	12
No. of pregnant animals	8	10	8	5
Corpora lutea(Mean± S.D.)	15.88± 3.52	15.30± 1.16	16.25± 1.39	15.60± 2.07
Implantations(Mean± S.D.)	14.00± 3.63	13.60± 2.50	15.63± 0.92	14.20± 3.11
% to corpora lutea(Mean± S.D.)	87.76± 7.88	89.23± 16.38	96.41± 4.21	90.38± 12.78
Fetal deaths (resorptions+dead fetuses)	3	3	5	9
Resorptions	3	3	5	9
Early	3	3	5	9
Late	0	0	0	0
Dead fetuses	0	0	0	0
Live fetuses				
Male/Female	50/59	65/68	62/58	35/27
Litter size(Mean± S.D.)	13.63± 3.11	13.30± 2.45	15.00± 1.20	12.40± 3.21
% to implantations(Mean± S.D.)	97.99± 3.95	97.91± 3.38	95.99± 4.87	86.51± 5.64
Sex Ratio (male/female)	0.85	0.96	1.07	1.30
External anomalies of fetuses	0	0	0	0
Body weight of live fetuses				
Male(Mean± S.D.)	3.46± 0.21	3.43± 0.21	3.45± 0.25	3.60± 0.23
Female(Mean± S.D.)	3.38± 0.21	3.31± 0.30	3.30± 0.21	3.40± 0.21

Table 13. Visceral and skeletal findings in F2 fetuses from F0 dams treated with LBD-007

Dose : ($\times 10^6$ IU/kg)	0	3	6	12
Visceral examination				
No. of fetuses examined	52	63	57	30
No. of fetuses with anomalies	0	0	0	0
No. of fetuses with variations(%)	3(5.8)	6(9.5)	3(5.3)	1(3.3)

Table 13. Continued.

Dose : ($\times 10^6$ IU/kg)	0	3	6	12
Dilatation of renal pelvis	1	3	2	1
Dilatation of ureter	2	3	1	0
Skeletal examination				
No. of fetuses examined	56	71	63	31
No. of fetuses with anomalies	0	0	0	0
No. of fetuses with variations	0	0	0	0
Degree of ossifications				
No. of sternebrae	5.3± 0.3	5.1± 0.5	5.0± 0.6	5.1± 0.2
No. of metacarpals in both forelimbs	6.6± 0.6	6.5± 0.4	6.3± 0.5	6.5± 0.3
No. of 1st phalanges in both forelimbs	0.0	0.0	0.0	0.0
No. of metatarsals in both hindlimbs	8.0± 0.0	7.9± 0.2	8.0± 0.1	7.9± 0.1
No. of 1st phalanges in both hindlimbs	0.0	0.0	0.0	0.0
No. of sacral and caudal vertebrae	7.4± 0.2	7.5± 0.4	7.4± 0.3	7.4± 0.4

DISCUSSION

Interferon is a immunomodulating agent in the human being and animals. In toxicological testing of gentechnological recombinant interferon α A through animal experiment, careful attention must be paid not only to the antigenicity, but also to the reaction, which results from pharmacological profile of the test agent (Hohbach *et al.*, 1987). In addition, the identity and purity has to be defined with the natural active agent. The toxic potential of circulating metabolic fragments of this protein comes into question, because recombinant interferon α A cannot pass directly the placental barrier with too large molecule weight.

All LBD-007 (recombinant human interferon α A) doses tested did not induce any signs of intoxication in dams. No treatment-related changes in food consumption and body weight were seen in the groups treated with test substance. Dams of the treated groups showed no substance-related pathologic findings.

The litter parameters of dams, namely number of implantations, resorptions, dead fetuses, viable fetuses, external abnormality, litter size, sex ratio and fetal weight, showed no changes related to treatment of LBD-007. No substance-related visceral malformations occurred among examined fetuses of the treated groups. The variations found in the visceral examination of fetuses are common and known for the Sprague-Dawley rat (Morita *et al.*, 1987). They were not dose-related. The malformations and variations observed in the skeletal examination of fetuses are trival and not dose related. The fetuses left over after litter size control were not affected by the test agent. Even the highest dose induced neither teratogenicity nor growth retardation. Interferon Γ is reported to be non-teratogenic in rats (Harada, 1988). ETU (Ethylenethiourea)-induced toxic effects could be seen; namely a decrease in food consumption and body weight of dams, a lower fetal weight and a high incidence of visceral and skeletal malformations in F1 fetuses. Retarded ossification of skeletal districts was also seen in F1 fetuses.

No treatment-related effects were observed at all LBD-007 doses in following

parameters; external abnormality and litter size of newborns, viability during lactation of F1 pups, growth, behaviour and mating performance of F1 offspring. F2 fetuses were not adversely affected by the treatment.

From the results mentioned above, it may be concluded that LBD-007 does not appear to influence general signs of pregnant rats, embryonal develpment of F1/F2 fetuses, growth, behavioural function and mating capability of F1 offspring, even when injected subcutaneously at dose level of 12×10^6 IU LBD-007/kg body wt., which is about two hundred times the assumed human clinical dose.

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