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PERI-AND POSTNATAL STUDY OF RECOMBINANT HUMAN INTERFERON αA (LBD-007) IN RATS

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ABSTRACT: *LDB-007, a newly developed recombinant human interferon αA, was at dose levels of 0, 3×10^6, 6×10^6 and 12×10^6 IU/kg/day administered subcutaneously to pregnant and subsequent delivered SpragueDawley rats from day 17 of gestation throguh day 21 of lactation.*

Effects of test substance on dams and growth, behaviour and mating performance of F1 offspring were examined.

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

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 offspring and for F1/F2 fetuses.

Key words: *LBD-007, Recombinant human interferon αA, Peri-and postnatal study, Rats, Subcutaneous application.*

INTRODUCTION

LBD-007, a recombinant human interferon αA, is an anti-virus and anti-cancer agent, which was newly developed by Lucky R & D Center, Biotechnology (Yousung-Koo, Daejeon, Korea). As a part of toxicological screening of test agent LBD-007, peri-and postnatal study was performed in Sprague-Dawley rats. This study was performed to assess the potential toxic effects of test substance on dams, 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., Daejeon, 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. AI014) was supplied by the Lucky R & D Center, Biotechnology (84 Jang-Dong, Yousung-Koo, Daejeon, Korea) with a titer of 10^7 IU/ml, pH of 7.4 and an 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 17 of gestation and on day 0 of lactation. All solutions were stored at 4℃.

Treatment and Observation of Dams

LBD-007 was administered subcutaneously to pregnant and subsequent delivered dams from day 17 of gestation through day 21 of lactation. Per experimental group 25 females with successful copulation were used. There were three treatment groups (3×10^6, 6×10^6 and 12×10^6 IU/LBD-007/kg body wt.) and one control group which received vehicle only. Pregnant females were observed for food consumption, weight development, pregnant period and sign of intoxication. Effect on delivery and nursing of dams were also examined. At autopsy of the animals at the end of lactation, the following organs were weighed: liver, kidney, spleen, heart, adrenals, ovaries.

Observation of F1 Animals

All dams of the groups were allowed to litter. The litters were examined for litter size, sex ratio and gross abnormality. On day 4 after delively, each litter was reduced to eight pups (four males and four females when possible) to have comparable 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 autopsid. F1 pregnant females were observed for weight development. On day 20 of gestation, the female animals were sacrificed. All parameters were evaluated, as at caesarian section of F0 dams.

Caesarean Section on Day 20 of Gestation

On day 20 of gestation the pregnant F1 females of all groups were sacrificed by an overdose of CO_2. 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.

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. A difference was considered statistically significant at a $p<0.05$.

RESULTS

Effect of LBD-007 on Dams

No notable changes in behaviour or clinical signs were observed among controls and animals of treated groups. Also, no mortality was seen in all groups. The food consumption and body weight development of dams did not differ significantly between the groups (Figure 1, 2). The length of gestation of the treated groups was 22 days and did not differ from the control value. At necropsy of dams on day 21 of lactation, no substance-related pathologic findings were discovered in the treated groups. Relative organ weights compared well between the groups, except that at 12×10^6 IU/kg, a increased weight of left ovary was seen (Table 1).

Effect of LBD-007 on F1-Offspring

In all treatment groups no substance-related effect could be seen in all parameters examined. There were no malformed newborns in all groups. The sex ratio of newborns did not differ significantly between the groups. Neonatal deaths during the lactation period was not affected by the test substance (Table 2). A few deaths found in the control, 6×10^6 and 12×10^6 IU/kg group are considered to

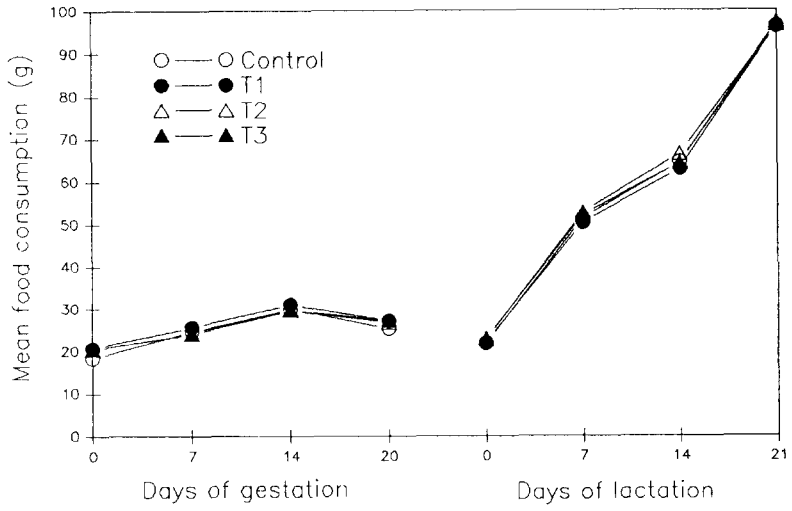

Figure 1. Changes in food consumptions of F0 dams treated with LBD-007.

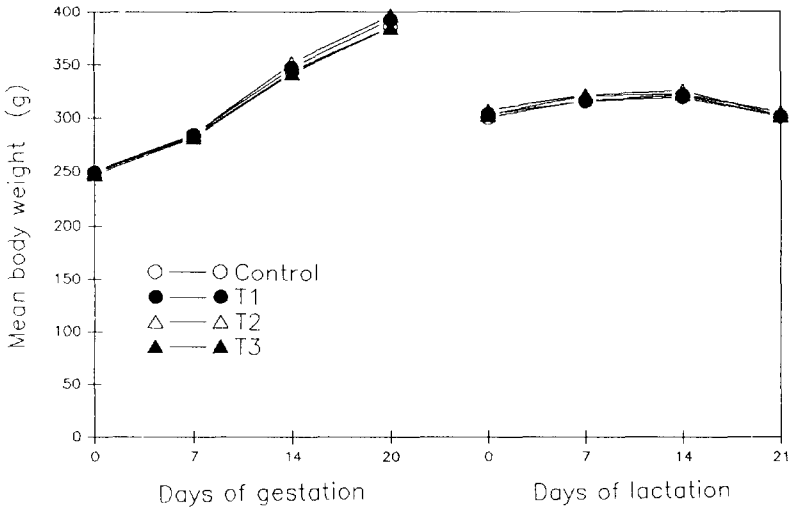

Figure 2. Changes in body weights of F0 dams treated with LBD-007.

be due to poor suckling or cannibalism. 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 values (Table 3). The traction test results compared well between the groups (Table 4). The results of water-filled multiple T-maze test are as follows: in males, a decreased number of zone errors (day 1) was observed in 12×10^6 IU/kg group. In females, there was a decreased number of zone errors (day1) in 3×10^6 and 6×10^6 IU/kg group. Both the time required for the straight channel (1st and 2nd) and the number of zone errors (day 2) showed a significant increase in 12×10^6 IU/kg group. No substance-related effect could be found. In F1 male and female animals, no notable clinical signs were observed from 0 to 70 days

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

DOSE($\times 10^6$ IU/kg)	0	3	6	12
Number of animals	24	24	22	23
Body weight (g)	300.7± 23.28	301.0± 26.47	301.6± 31.16	304.8± 30.01
% Body weight				
Liver (g)	4.794± 0.440	4.609± 0.585	4.942± 0.547	5.079± 0.505
Kidney-Left (g)	0.396± 0.050	0.386± 0.038	0.393± 0.057	0.409± 0.049
Kindney-Right (g)	0.497± 0.049	0.398± 0.035	0.403± 0.051	0.414± 0.043
Spleen (g)	0.180± 0.018	0.178± 0.028	0.181± 0.019	0.189± 0.019
Heart (g)	0.333± 0.022	0.340± 0.030	0.341± 0.034	0.348± 0.036
Adrenal Gland-Left (g)	0.012± 0.002	0.012± 0.002	0.013± 0.002	0.013± 0.002
Adrenal Gland-Right (g)	0.011± 0.002	0.011± 0.002	0.011± 0.002	0.011± 0.001
Overy-Left (g)	0.015± 0.003	0.015± 0.002	0.014± 0.002	0.017± 0.003*
Ovary-Right (g)	0.016± 0.002	0.015± 0.002	0.015± 0.002	0.015± 0.002

Values are Mean± S.D.
*; Significantly different from control value at $p<0.05$

Table 2. Reproductive and littering findings of F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg)	0	3	6	12
Number of animals	24	24	22	23
No. of live neonates				
Male (Mean± S.D.)	6.3± 2.05	6.5± 1.82	7.2± 1.93	7.2± 2.41
Female (Mean± S.D.)	6.8± 1.85	6.8± 2.73	7.0± 2.06	6.2± 1.87
Total (Mean± S.D.)	13.0± 2.11	13.3± 3.43	14.2± 2.42	13.3± 2.66
Sex ratio (Male/Female)	0.93	0.95	1.04	1.16
No of neonates with external anomalies	0	0	0	0
Pregnant period(day) (Mean± S.D.)	22.1± 0.45	22.1± 0.45	22.2± 0.50	22.3± 0.47
Viability index[a]	99.0	99.4	99.0	98.7
Lactation index[b]	99.5	100.0	100.0	100.0
Delivery index[c]	24/24	24/24	22/22	23/23

[a] (No. of live offspring at day 4/No. of live offspring at birth)$\times$100
[b] (No. of live offspring at day 21/No. of live offspring after litter size control)$\times$100
[c] No. of dams with live newborns/No. of pregnant dams

post partum. Ther 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 6, 7).

There was no indication of a decrease in fertility of F1 parent animals in the treatment groups (Table 8). 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.

Effect of LBD-007 on F1 fetuses

Among fetuses left over after litter size control, neither visceral nor skeletal mal-

Table 3. 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	24	24	22	23
Separation of auricle				
Male	2.2± 0.41	2.4± 0.50	2.5± 0.51	2.3± 0.45
Female	2.4± 0.49	2.3± 0.48	2.4± 0.50	2.3± 0.47
Emergence of abdominal hair				
Male	8.0± 0.55	8.1± 0.50	8.0± 0.49	7.9± 0.46
Female	8.1± 0.54	8.2± 0.59	8.1± 0.61	8.1± 0.60
Eruption of incisor				
Male	10.1± 0.95	9.7± 1.00	9.6± 0.85	9.8± 0.72
Female	9.7± 0.87	9,6± 0.82	9.6± 0.85	9.7± 0.98
Separation of eyelids				
Male	13.6± 0.65	13.5± 0.78	13.5± 0.67	13.2± 0.49
Female	13.5± 0.66	13.4± 0.88	13.3± 0.55	13.2± 0.74
Descent of testis				
Male	21.6± 0.77	21.6± 1.02	21.5± 0.74	21.6± 0.72
Opening of vagina				
Female	31.8± 0.74	31.5± 0.59	31.6± 0.60	31.6± 0.58

Values are Mean± S.D. (days).

Table 4. 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	24	24	22	23
Days (Mean± S.D.)	11.9± 3.4	11.3± 2.9	11.2± 2.8	11.7± 3.2
Female				
No. of pups examined	24	24	22	23
Days (Mean± S.D.)	11.0± 2.7	11.3± 2.8	12.9± 3.8	11.3± 2.2

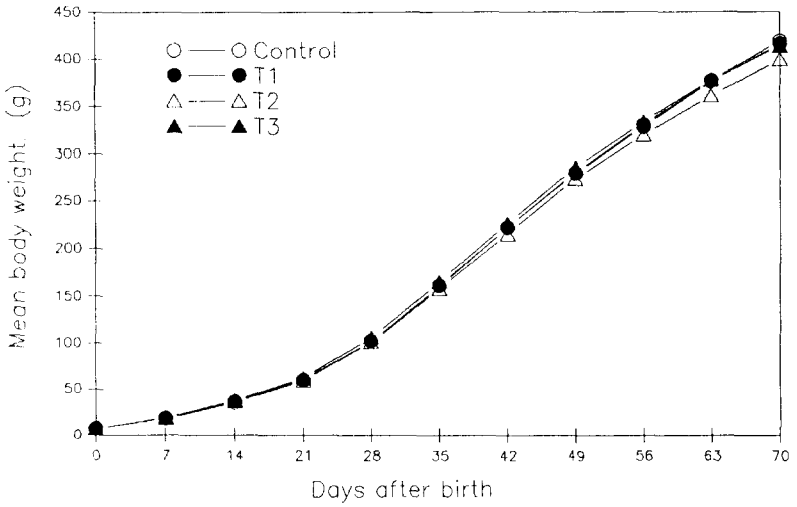

Figure 3. Changes in body weights of F1 male rats from F0 dams treated with LBD-007.

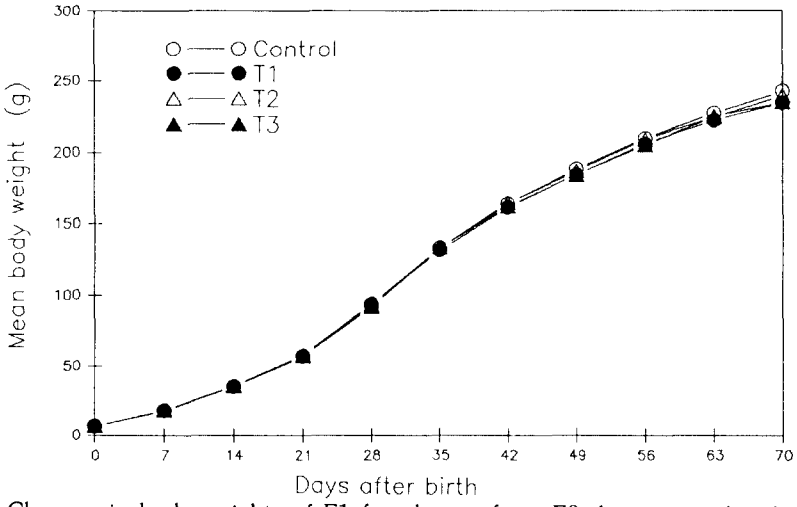

Figure 4. Changes in body weights of F1 female rats from F0 dams treated with LBD-007.

Table 5. 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)		24	24	22	23
Straight channel:	Time(seconds)				
	1st	17.5± 12.5[a]	17.0± 6.5	17.2± 9.8	15.4± 5.3
	2nd	7.0± 3.3	5.8± 2.0	6.8± 3.4	6.1± 1.7
	3rd	6.2± 2.4	6.1± 3.7	5.9± 2.6	8.2± 6.6
Multiple T-maze					
Day 1	No. of errors				
	S.E	2.7± 0.8	2.8± 0.8	2.5± 0.6	2.5± 1.0
	B.E	0.9± 0.7	1.1± 0.9	0.8± 0.7	1.0± 1,1
	Z.E	2.9± 1.1	3.1± 1.4	2.7± 1.3	1.5± 0.9**
	Time(seconds)	53.3± 19.0	59.1± 24.8	52.8± 16.9	52.6± 26.2
Day 2	No. of errors				
	S.E	1.2± 0.6	1.3± 0.6	1.6± 0.9	1.5± 0.9
	B.E	0.5± 0.8	0.6± 0.6	0.7± 0.8	0.8± 1.0
	Z.E	0.6± 0.6	0.7± 0.7	0.5± 0.5	0.7± 0.8
	Time(seconds)	29.1± 12.3	30.6± 9.7	35.3± 16.9	33.2± 20.3
Day 3	No. of errors				
	S.E	0.9± 0.6	0.9± 0.7	0.6± 0.5	0.9± 0.6
	B.E	0.7± 0.8	0.5± 0.5	0.3± 0.4	0.6± 0.5
	Z.E	0.2± 0.3	0.1± 0.2	0.1± 0.2	0.3± 0.4
	Time(seconds)	30.1± 13.6	30.0± 10.2	27.9± 12.0	29.0± 9.7
No. of female rats examined (8 weeks old)		24	24	22	23
Straight channel:	Time(seconds)				
	1st	13.4± 8.6	14.4± 7.2	17.4± 10.1	22.5± 17.9*
	2nd	5.8± 3.5	5.9± 2.3	6.5± 3.2	8.2± 4.6*
	3rd	5.7± 3.3	4.7± 1.5	5.0± 2.2	7.8± 6.5

Table 5. Continued.

DOSE($\times 10^6$ IU/kg)		0	3	6	12
Multiple T-maze					
Day 1	No. of errors				
	S.E	2.2± 0.5	2.6± 0.9	2.7± 1.0	2.7± 0.9
	B.E	1.3± 0.7	1.4± 1.1	1.2± 0.8	1.2± 1.2
	Z.E	1.9± 0.9	1.1± 0.5**	1.1± 0.6**	2.3± 1.4
	Time(seconds)	50.3± 11.6	56.6± 21.1	55.8± 15.9	55.8± 21.4
Day 2	No. of errors				
	S.E	1.9± 0.9	2.2± 0.7	2.1± 1.2	2.5± 1.0
	B.E	1.1± 1.0	0.8± 1.0	0.7± 0.7	1.3± 0.9
	Z.E	0.6± 0.7	0.8± 0.7	0.8± 0.8	1.6± 1.4*
	Time(seconds)	40.4± 17.1	40.5± 13.9	38.7± 16.1	51.3± 22.7
Day 3	No. of errors				
	S.E	1.2± 0.7	1.1± 0.9	1.4± 0.8	1.3± 0.8
	B.E	1.0± 0.7	0.9± 0.8	1.1± 1.0	0.9± 0.8
	Z.E	0.4± 0.6	0.3± 0.5	0.5± 0.6	0.5± 0.5
	Time(seconds)	34.3± 14.2	36.2± 19.1	37.9± 18.4	36.9± 13.3

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.
**indicate significant difference at $p<0.01$ level when compared with control group.

Table 6. 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	24	24	22	23
Body weight (g)	418.7± 32.23	416.0± 24.77	399.5± 30.62	414.0± 33.45
% Body weight				
Liver (g)	4.441± 0.337	4.594± 0.611	4.290± 0.361	4.937± 0.480
Kidney-Left (g)	0.430± 0.034	0.418± 0.037	0.411± 0.032	0.425± 0.422
Kindney-Right (g)	0.437± 0.031	0.422± 0.038	0.412± 0.030	0.435± 0.045
Spleen (g)	0.193± 0.021	0.197± 0.031	0.199± 0.023	0.197± 0.024
Heart (g)	0.338± 0.029	0.339± 0.044	0.328± 0.014	0.331± 0.028
Adrenal Gland-Left (g)	0.008± 0.001	0.007± 0.001	0.008± 0.001	0.008± 0.000
Adrenal Gland-Right (g)	0.007± 0.001	0.007± 0.001	0.007± 0.001	0.007± 0.001
Testls-Left (g)	0.410± 0.035	0.404± 0.041	0.424± 0.041	0.410± 0.039
Testls-Right (g)	0.409± 0.031	0.405± 0.032	0.425± 0.038	0.414± 0.035

Values are Mean± S.D.

Table 7. 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	24	24	22	23
Body weight (g)	242.3± 19.53	234.1± 22.92	239.2± 20.15	234.5± 22.23
% Body weight				
Liver (g)	4.181± 0.323	4.336± 0.446	4.000± 0.321	4.100± 0.345
Kidney-Left (g)	0.434± 0.043	0.446± 0.046	0.413± 0.025	0.431± 0.035
Kindney-Right (g)	0.448± 0.042	0.458± 0.042	0.428± 0.026	0.449± 0.037

Table 7. Continued.

DOSE($\times 10^6$ IU/kg)	0	3	6	12
Spleen (g)	0.215± 0.032	0.220± 0.029	0.224± 0.020	0.225± 0.025
Heart (g)	0.363± 0.033	0.371± 0.039	0.358± 0.041	0.380± 0.070
Adrenal Gland-Left (g)	0.015± 0.002	0.014± 0.002	0.016± 0.002	0.015± 0.003
Adrenal Gland-Right (g)	0.014± 0.002	0.014± 0.002	0.015± 0.002	0.014± 0.001
Overy-Left (g)	0.018± 0.004	0.018± 0.003	0.019± 0.003	0.019± 0.002
Ovary-Right (g)	0.019± 0.004	0.018± 0.004	0.019± 0.003	0.020± 0.003

Values are Mean± S.D.

Table 8. 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	24	24	23	23
Copulation index[a] (%)	21/24(88)	19/24(79)	18/23(78)	22/23(96)
Fertility index[b] (%)	15/21(71)	18/19(95)	17/18(94)	16/22(73)
Female				
No. of mated animals	24	24	23	23
Copulation index[a] (%)	21/24(88)	19/24(79)	18/23(78)	22/23(96)
Pregnancy index[c] (%)	15/21(71)	18/19(95)	17/18(94)	16/22(73)

[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

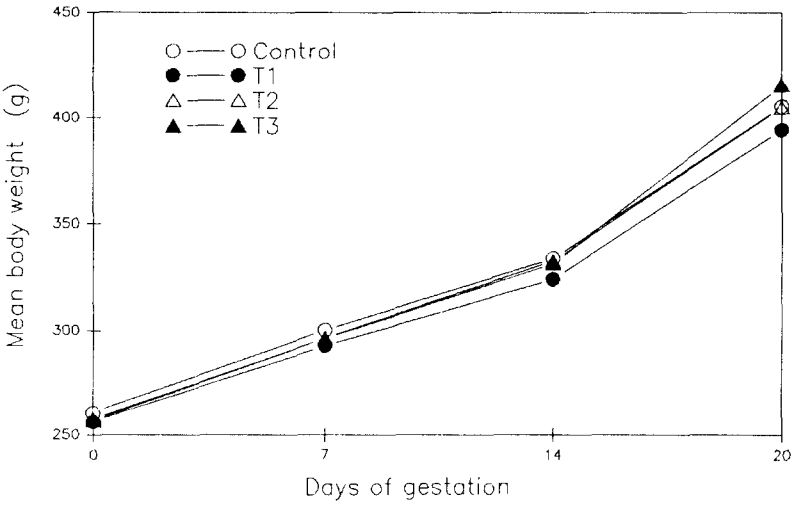

Figure 5. Mean body weight changes during gestation of F1 rats from F0 dams treated with LBD-007.

formations occurred in all groups.

Effect of LBD-007 on F2 fetuses

The litter parameters of the treated groups did not differ significantly from the

Table 9. 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	15	17	16	16
Corpora lutea(Mean± S.D.)	16.8± 2.43	15.3± 2.89	16.1± 1.98	17.7± 2.52
Implantations(Mean± S.D.)	15.1± 2.23	13.9± 3.67	15.2± 2.17	16.1± 2.66
% to corpora lutea(Mean± S.D.)	90.2± 6.01	89.1± 12.2	94.5± 6.76	91.0± 5.56
Fetal deaths (resorptions + dead fetuses)	7	2	4	0
Resorptions	7	2	3	0
Early	7	2	3	0
Late	0	0	0	0
Dead fetuses	0	0	1	0
Live fetuses				
Male/Female	114/106	123/111	129/110	131± 127
Litter size(Mean± S.D.)	14.7± 2.53	13.8± 3.99	14.9± 2.08	16.1± 2.66
% to implantations(Mean± S.D.)	96.7± 5.34	97.6± 9.70	97.6± 4.67	100.0± 0.00
Sex Ratio (male/female)	1.08	1.11	1.17	1.03
No. of fetuses with external anomalies(%)	0(0)	0(0)	0(0)	1(0.4)
Acaudate	0	0	0	1
Body weight of live fetuses				
Male (Mean± S.D.)	3.5± 0.31	3.4± 0.30	3.5± 0.21	3.5± 0.28
Female (Mean± S.D.)	3.4± 0.32	3.2± 0.32	3.4± 0.21	3.3± 0.32

Table 10. 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	107	114	117	125
No. of fetuses with anomalies	0	0	0	0
No. of fetuses with variations(%)	1(0.9)	1(0.9)	1(0.9)	3(2.4)
Dilatation of renal pelvis	0	1	0	1
Dilatation of ureter	1	0	0	0
Dilatation of renal peivis and dilatation of ureter	0	0	1	2
Skeletal examination				
No. of fetuses examined	113	120	122	133
No. of fetuses with anomalies(%)	2(1.8)	1(0.8)	0(0)	0(0)
Shortend 13th rib	2	1	0	0
No. of fetuses with variations(%)	4(3.5)	10(8.3)	7(5.7)	12(9.0)
14th rib	0	6	1	1
Wavy ribs	0	1	0	0
Asymmetric sternebrae	0	1	0	0
Dumbbell-shaped thoracic vertebral body	2	2	4	6
Cleaved thoracic vertebral body	2	0	2	5
No. of ossification centers				
No. of sternebrae	4.8± 0.7	4.7± 0.8	4.8± 0.4	5.0± 0.5
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

Table 10. Countinued.

Dose : ($\times 10^6$ IU/kg)	0	3	6	12
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

control values. No externally malformed fetuses were found, except one acaudate in 12×10^6 IU/kg group (Table 9). No visceral malformations occurred among examined fetuses. Variations found in the visceral examination of F2 fetuses were trivial and not dose-related. There were three skeletally malformed fetuses among examined F2 fetuses; namely two shortend 13th rib in the control group and one shortend 13th rib at 3×10^6 IU/kg. The variations found in the skeletal examination of F2 fetuses were dumbbell-shaped thoracic vertebral body, cleaved thoracic vertebral body, 14th rib, wavy rib and asymmetric sternebrae. They were rare and not dose-related (Table 10).

DISCUSSION

Interferons can inhibit cell proliferation and modulate immune response in the human being and animals. In toxicological testing of gentechnological recombinant interferon αA through animal experiment, attention must be paid to the antigenicity, namely the potential of toxicity due to the production of antibodies. In addition, adverse effects on fetal development, which can be induced indirectly by effect of the test agent on physiology or biochemistry, are to take into account (Hohbach *et al.*, 1987).

All LBD-007 (recombinant human interferon αA) 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. The pregnant period was not adversely affected by the test agent. Dams of the treated groups showed no substance-related pathologic findings. A increased weight of left ovary found in 12×10^6 IU/kg group is considered to be a spontaneous finding.

No treatment-related effects were observed at all LBD-007 doses in following parameters; external abnormality, sex ratio and litter size of newborns, viability during lactation of F1 pups, growth, behaviour and mating performance of F1 offspring. A reduced number of zone errors (day 1) found in the treated male or female groups must be a accidental finding. A significant increase in the time required for the straight channel (1st and 2nd) and the number of zone rrors (day 2) is not considered to be treatment-related. Even the highest dose did not influence F1 offspring adversely. Harada (1988) reported that interferon Γ had no adverse effect on growth and reproductive capability of F1 rats. The abortifacient activity of interferon α was observed in cynomolgus monkeys, not in rats; interferon α has minimal cross-species activity. F1 fetuses left over after litter size control were not adversely affected by the treatment. The litter parameters of F1 dams showed no changes related to treatment of LBD-007. One external malformation observed in 12×10^6 IU/kg group is considered to be a spontaneous finding. No substance-

related visceral malformations occurred among examined fetuses of the treated groups. The variations found in the visceral examination of F2 fetuses are common and known for the Sprague-Dawley rat (Morita *et al.*, 1987; Manson *et al.*, 1989). They were not dose-related. The malformations and variations observed in the skeletal examination of F2 fetuses are trivial and not dose-related.

From the results mentioned above, it may be concluded that LBD-007 does not appear to influence general signs of pregnant rats, growth, behavioural function and mating capability of F1 offspring and F1/F2 fetuses, 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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