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FERTILITY STUDY OF RECOMBINANT HUMAN INTERFERON αA (LBD-007) IN RATS

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ABSTRACT: *LBD-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 Sprague-Dawley male rats from premating to mating period and to females from premating to early gestation period. Effects of test agent on reproductive performance of both sexes and embryonic development were examined.*

1. *No treatment-related changes in food consumption, body weight and necropsy findings were observed in parent animals.*
2. *Mating performance and fertility of parent animals were not adversely affected by test substance.*
3. *F_1 fetuses showed no changes related to treatment of LBD-007.*

The results show that the no-effect dose levels (NOELs) of LBD-007 are over 12×10^6 IU/kg/day for parent animals on general toxicity and reproductive function and for fetuses on embryonic development.

Key words: *LBD-007, Recombinant human interferon αA, Fertility 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, fertility study was carried out in Sprague-Dawley rats. This study was performed to assess the potential toxic effects of test substance on gonadal function and mating behaviour in both male and female animals, as well as conception rates and embryonal develo-

pment. It was carried out according to the Guidelines for Reproduction Study for Safety Evaluation of Drugs for Human Use (National Institute for Safety Research, 1988), Japanese Toxicity Guidlines (Yakugyo Jiho Col, 1984; Inveresk Research International, 1984) and the requirements for trial performance stated in "Embryotoxikologische Probleme in der Arzneimittel ForschungL (BGA, 1978). Also, the study was done according to GLP and including this paper, inspected by the QAU of TRC KRICT (Toxicology Research Center, Korea Research Institute of Chemical Technology).

MATERIALS AND METHODS

Animal Maintenance

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.

Test Substance

LBD-007 (Lot No. AI004) was supplied by the Lucky R & D Center, Biotechnology (84 Jang-Dong, Yousung-Koo, Daejeon, Korea) with a titer of 1.2×10^7 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 recent body weight and all solutions were stored at 4℃.

Treatment and Observation of Parent Animals

LBD-007 was administered subcutaneously to:

1) Male rats for 60 days prior to mating and during the mating period.

2) Female rats for 14 days prior to mating, during the mating period and in the early stage of gestation.

Per experimental group 22 males and 22 females 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 the vehicle only. Parent animals were observed for food consumption, weight development and sign of intoxication. All males were subjected to autopsy after copulation. Females were acrificed at the end of gestation. At autopsy of all animals, the following organs were weighed: liver, kidney, spleen, heart, adrenals, brain, ovaries or testes.

Mating Procedure

At the end of the premating period of treatment, males and females were housed in couples within the groups. The first 24 h period following the mating procedure was designated as day 0 of pregnancy if sperm or vaginal plug were detected.

Caesarian section on Day 20 of Gestation

The female animals were sacrificed by an over-dose 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.

Statistical analysis of the data

Statistical significance was tested using Analysis of Variance (using Dunnett's or Scheffe's test), Kruskal-Wallis test and χ^2-test. A difference was considered statistically significant at a $p<0.05$.

RESULTS

Effect of LBD-007 on Parent Animals

No substance-related changes in behaviour or clinical signs were seen among controls and animals of treated groups. Also, no mortality was found in all groups of both sexes. The food consumption and body weight development of parent animals did not differ significantly between the groups (Figur 1~4). At necropsy of parent animals, no treatment-related findings were discovered in all groups. Relative organ weights compared well between the groups (Table 1, 2).

Effect of LBD-007 on Reproductive Function in Parent Animals

The copulation rate did not differ significantly between the groups (Table 3). The pregnancy rates of 91, 95, 81 or 86% compared well between control, low,

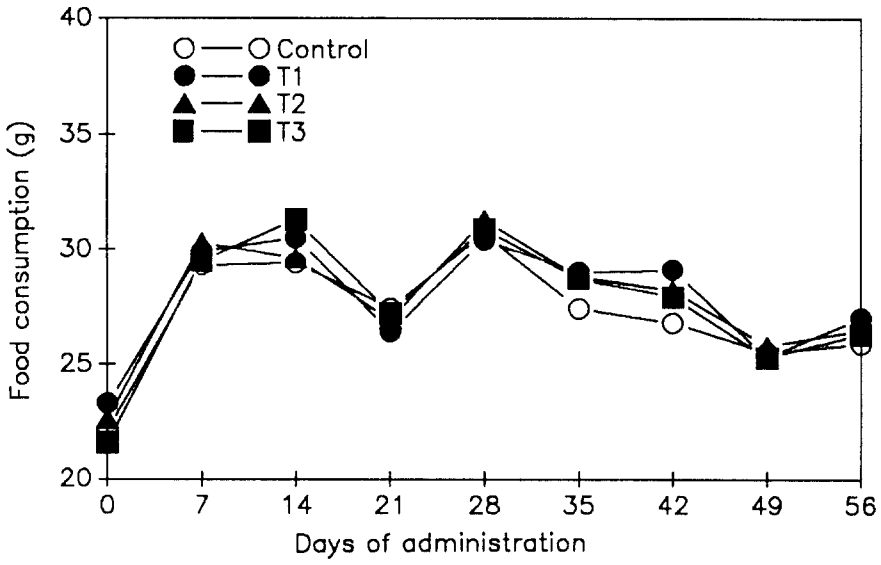

Figure 1. Changes in food consumption of male rats befor mating.

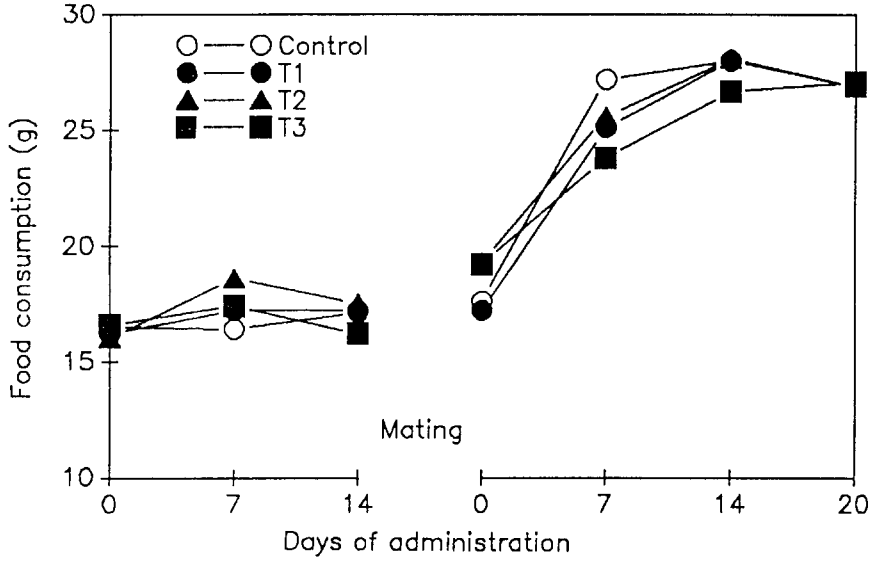

Figure 2. Changes in food consumptions of female rats before mating and in gestation period.

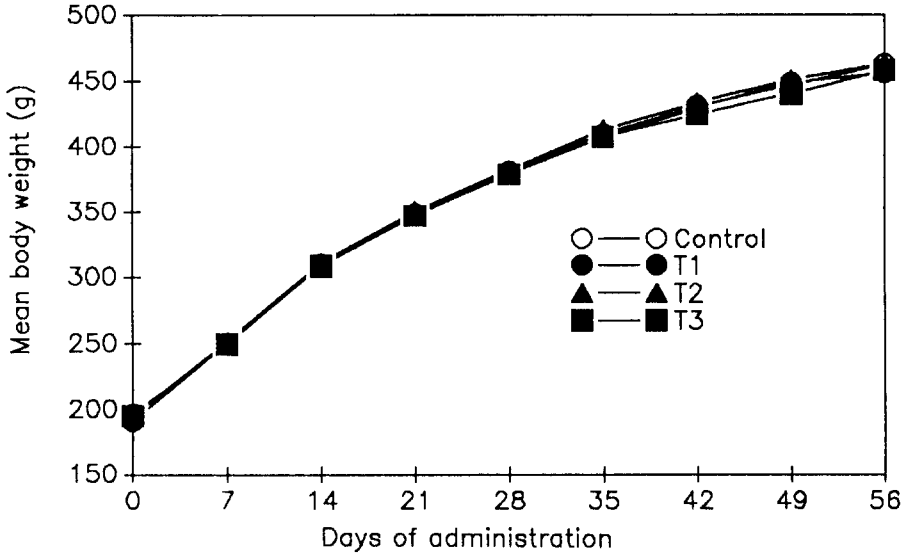

Figure 3. Changes in body weights of male rats before mating.

intermediated and high dose groups. Thus, there was no indication of a substance-related decrease in male or female fertility. We additionally showed mating sequence in a graphic form (Figure 5) and calculated the number of days needed for mating, after which the females became pregnant (Table 4). No sinificant differences between control and treated groups were also seen.

Effect of LBD-007 on F_1 fetuses

Sectio-data

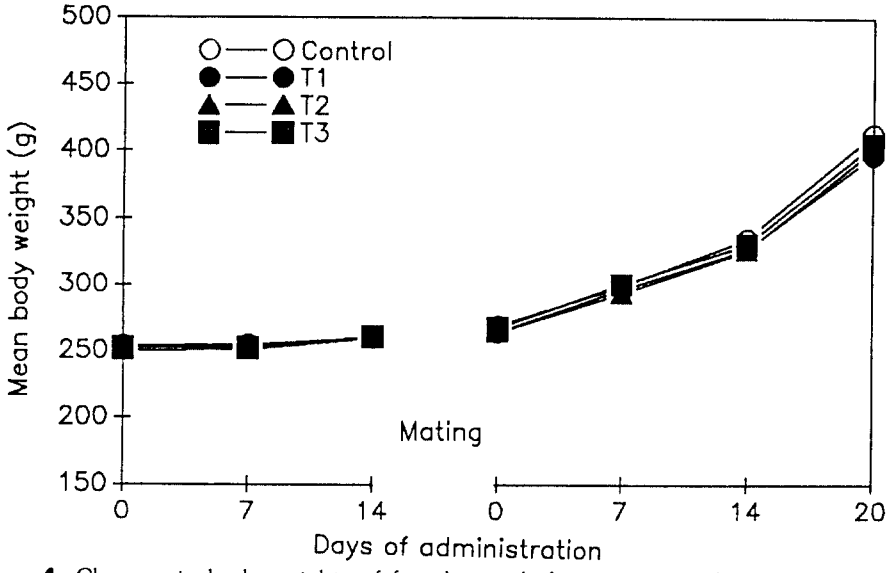

Figure 4. Changes in body weights of female rats before mating and in gestation period.

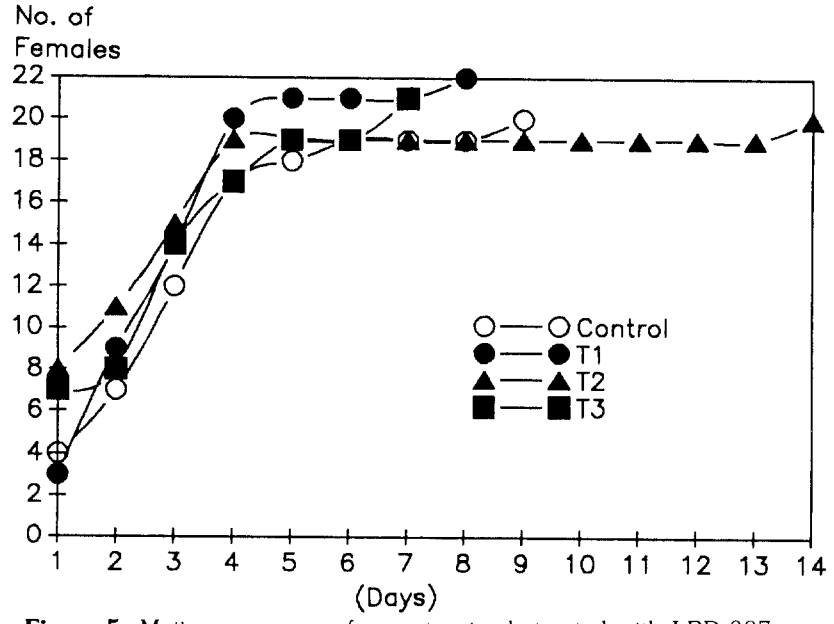

Figure 5. Mating sequence of parent animals treated with LBD-007.

The litter parameters, namely number of implantations, resorptions, dead fetuses, viable fetuses, litter size, sex ratio and fetal weight, compared well between the groups. There were three externally malformed fetuses, namely one vestigial tail among controls, one vestigical tail, one micrognathia and microcephaly among fetuses of 6×10^6 IU/kg group. Malformations were trivial and not dose-related (Table 5).

Visceral findings

Three malformed fetuses were observed, namely one double kidney at 3×10^6

Table 1. Relative organ weights of male rats treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
Number of Animals	22	22	22	22
Body Weight(g)	485.9± 56.16	485.6± 48.09	499.9± 43.23	487.0± 45.77
% Body weight				
Liver(g)	4.090± 0.3627	4.032± 0.4532	4.004± 0.3428	4.172± 0.4275
Kidney-Left(g)	0.423± 0.0406	0.389± 0.0446*	0.403± 0.0309	0.421± 0.0397
Kidney-Right(g)	0.429± 0.0411	0.393± 0.0456**	0.412± 0.0346	0.431± 0.0400
Spleen(g)	0.180± 0.0206	0.171± 0.0188	0.174± 0.0240	0.175± 0.0231
Heart(g)	0.309± 0.0254	0.299± 0.0293	0.291± 0.0203	0.298± 0.0195
Brain(g)	0.442± 0.0472	0.436± 0.0420	0.422± 0.0363	0.431± 0.0350
Adrenal Gland-Left(g)	0.006± 0.0009	0.007± 0.0010	0.007± 0.0008	0.007± 0.0011
Adrenal Gland-Right(g)	0.006± 0.0010	0.006± 0.0009	0.006± 0.0010	0.006± 0.0007
Testis-Left(g)	0.361± 0.0479	0.369± 0.0443	0.357± 0.0385	0.366± 0.0358
Testis-Right(g)	0.359± 0.0494	0.373± 0.0418	0.357± 0.0387	0.363± 0.0357

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

Table 2. Relative organ weights of female rats treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
Number of Animals	18	21	16	18
Body Weight(g)	413.1± 33.96	397± 30.58	401.8± 25.78	405.7± 26.58
% Body weight				
Liver(g)	4.256± 0.3398	4.284± 0.4535	4.281± 0.2596	4.204± 0.2365
Kidney-Left(g)	0.293± 0.0253	0.297± 0.0370	0.287± 0.0230	0.296± 0.0235
Kidney-Right(g)	0.320± 0.0242	0.310± 0.0446	0.292± 0.0274	0.298± 0.0217
Spleen(g)	0.162± 0.0197	0.165± 0.0192	0.174± 0.0160	0.172± 0.0147
Heart(g)	0.238± 0.0207	0.248± 0.0317	0.235± 0.0205	0.248± 0.0193
Brain(g)	0.473± 0.0312	0.493± 0.0439	0.481± 0.0326	0.476± 0.0282
Adrenal Gland-Left(g)	0.009± 0.0015	0.009± 0.0015	0.009± 0.0011	0.009± 0.0014
Adrenal Gland-Right(g)	0.008± 0.0014	0.009± 0.0013	0.008± 0.0014	0.009± 0.0012
Ovary-Left(g)	0.015± 0.0026	0.015± 0.0033	0.015± 0.0028	0.016± 0.0028
Ovary-Right(g)	0.014± 0.0026	0.015± 0.0041	0.016± 0.0042	0.015± 0.0040

Values are Mean± S.D.

Table 3. Fertility data of parent animals treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
No. of mated animals				
Male	22	22	22	22
Female	22	22	22	22
No. of animals with successful copulation				
Male	22	22	21	21
Female	22	22	21	21
No. of impregnating males	20	21	17	18

Table 3. Continued

DOSE($\times 10^6$ IU/kg):	0	3	6	12
No. of pregnant females	20[d]	21	17[e]	18
Male				
Copulation index(%)[a]	100	100	95	95
Fertility index(%)[b]	91	95	81	86
Female				
Copulation index(%)[a]	100	100	95	95
Pregnancy idex(%)[c]	91	95	81	86

[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 two animals neither vaginal plug nor sperm was detected, but they were found pregnant at delivery
[e] In one animal it is the same with the case of[d]

Table 4. Precoital time of parent animals treated with LBD-007

	Precoital time* (days)			
DOSE($\times 10^6$ IU/kg):	0	3	6	12
Mean	3.3	3.0	2.8	3.0

*Precoital time = No. of days needed for mating.

Table 5. Findings at caesarean section of dams treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
No. of pregnant animals	18	21	16	18
Corpora lutea(Mean± S.D.)	16.39± 3.96	15.24± 2.88	15.00± 3.67	15.83± 3.67
Implantations(Mean± S.D.)	15.11± 3.53	13.62± 2.54	13.88± 3.90	14.50± 3.55
Preimplantation loss(%)	7.19	10.50	8.62	9.01
Fetal deaths (resorptions + dead fetuses)	14	13	4	14
Resorptions				
Early	14	13	4	13
Late	0	0	0	0
Dead fetuses	0	0	0	1
Live fetuses				
Male/Female	130/128	130/143	116/102	135/112
Litter size(Mean± S.D.)	14.33± 3.91	13.00± 3.05	13.63± 3.79	13.72± 3.20
Fetal loss(%)	9.74	5.10	1.61	4.70
Sex Ratio(male/female)	1.02	0.91	1.14	1.21
No. of fetuses with external anomalies(%)	1(0.4)	0(0)	2(0.9)	0(0)
Vestigial tail	1	0	1	0
Micrognathia and Microcephaly	0	0	1	0
Body weight of live fetuses				
Male(Mean± S.D.)	3.04± 0.80	3.27± 0.27	3.35± 0.33	3.27± 0.33
Female(Mean± S.D.)	2.92± 0.76	3.15± 0.28	3.16± 0.30	3.16± 0.28

Table 6. Visceral and skeletal findings in fetuses from dams treated with LBD-007

DOSE($\times 10^6$ IU/kg):	0	3	6	12
Visceral examination				
No. of fetuses examined	124	131	104	118
No. of fetuses with anomalies(%)	0	1(0.8)	2(1.9)	0
Double kidney	0	1	1	0
Atelocephalia	0	0	1	0
No. of fetuses with variations(%)	5(4.0)	10(7.6)	7(6.7)	4(3.3)
Dilatation of renal pelvis	1	2	1	1
Dilatation of ureter	1	7	4	3
Dilatation of renal penal peivis and dilatation of ureter	3	1	2	0
Skeletal examination				
No. of fetuses examined	134	141	115	129
No. of fetuses with anomalies	0	0	0	0
No. of fetuses with variations(%)	0(0)	0(0)	2(1.7)	0(0)
Sacralization of lumbar vertebrae	0	0	1	0
Cervical rib	0	0	1	0
Degree of ossifications				
No. of sternebrae	4.4± 0.51	4.5± 0.73	4.9± 0.63	4.7± 0.60
No. of metacarpi in both forelimbs	6.1± 0.25	6.2± 0.41	6.5± 0.63	6.4± 0.57
No. of 1st phalanges in both forelimbs	0	0	0	0
No. of metatarsi in both hindlimbs	7.9± 0.17	7.9± 0.24	8.0± 0.00	8.0± 0.14
No. of 1st phalanges in both hindlimbs	0	0	0	0
No. of sacral and caudal vertebrae	7.0± 0.47	7.0± 0.75	7.3± 0.47	7.1± 0.55

and 6×10^6 IU/kg, respectively and one atelocephalia at 6×10^6 IU/kg. Malformations were rare and as well not dose-related. Among examined fetuses twenty six variated fetuses were found; namely dilatation of renal pelvis and/or dilatation of ureter. They were not related to dose (Table 6).

Skeletal findings

No malformations occurred in all groups. Two fetuses of 6×10^6 IU/kg group showed variatious, namely one cervical rib and one sacralization of lumbar vertebrae. They were rare and not dose-related. The ossification of evaluated skeletal districts did not differ significantly between the groups (Table 6).

DISCUSSION

Interferons are immunologically active, biological response modifiers in the human being and animals. In toxicological testing of gentechnological recombinant interferon αA through animal experiment, attention must be paid not only to the potential toxicity produced by impurities of the recombinant protein formulation, but also to the effects of supra-physiological levels of endogenous proteins (Lewa-

ndowski, 1988).

All LBD-007 (recombinant hmuan interferon αA) doses tested did not induce any signs of intoxication in parent animals. No substance-related changes in food consumption and body wight were seen in the treated groups. Males and females of the treated groups showed no treatment-related pathological findings. There was no indication of a substance-related impairment of fertility in parent animals. Harada (1988) reported that interferon Γ had no adverse effects on fertility of parent rats. In the Rhesus monkey, the reproductive function was adversely affected by interferon α (Trown *et al.*, 1986). The litter parameters of pregnant females, 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 treatment-related visceral malformations were observed among examined fetuses of treated groups. The variations found in the visceral examination of fetuses are common and known for the Sprague-Dawley rat (Palmer, 1977; Morita *et al.*, 1987; Manson *et al.*, 1989). They were not dose-related. The malformations and variations observed in the skeletal examination of fetuses are trivial and not dose-related.

From the results mentioned above, it may be concluded that LBD-007 does not appear to produce significant changes in general signs and fertility of parent rats and embryonic development of 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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