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Fertility Study of LBD-001, a Recombinant Human Interferon γ, in Rats

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Abstract – LBD-001, a recombinant human interferon γ produced by genetically engineered yeast as a host system, was administered intraperitoneally to Sprague-Dawley male rats from premating to mating period at least for 60 days and to female rats from at least for 2 weeks before mating to early gestation period (from day 0 to 7 of gestation) at dose levels of 0.35×10^6, 0.69×10^6, and 1.38×10^6 I.U./kg/day. In the positive control group, ethynylestradiol (EE_2; 40 μg/kg/day) was subcutaneously administered only to female rats during the early gestation period. Effects of the test agents on reproductive performances of the male or female rats and embryonic development were as followings; (1) No significant changes by the treatment of LBD-001 were observed in general behaviors, body weight, food and water consumption, and necropsy of parent animals. However, significant decreases of body weight, food consumption, and water consumption were observed in EE_2-treated female rats. (2) Mating performances and fertility of parent animals were not significantly affected by the treatment of LBD-001. In EE_2-treated females, however, the fertility was completely inhibited. (3) No changes in resorption rate and external abnormality of F1 fetuses were observed by the treatment of LBD-001. The results show that LBD-001 at the dose of 1.38×10^6 I.U./kg/day or less does not affect general toxicity and reproductive function of parent animals and embryonic development of F1 fetuses.

Keywords □ LBD-001, recombinant human interferon γ, ethynylestradiol, fertility study, rats, intraperitoneal injection.

LBD-001 is a recombinant human interferon γ produced by fermentation of genetically engineered yeast containing the DNA which encodes for the human protein. The main action mechanism of naturally occuring interferon γ, 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). As a part of toxicological tests of LBD-001, fertility study was carried out in Sprague-Dawley rats (National Institute of Safety Research of Korea, 1986; Lee, E. B., 1985; Manson, J. M. *et al.*, 1994). This study was performed to assess the potential toxic effects of the test substance on gonadal function and mating behavior in both male and female animals, as well as gestation rate of the females and early embryonic development of their fetuses.

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MATERIALS AND METHODS

Treatment of the 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). Since maximal tolerance dose could not be determined in preliminary subacute toxicity test, we selected the lowest dose level (1.38×10^6 I.U./kg/day) used in the subacute toxicity test as the highest dose level in our fertility test. The dose of LBD-001 was 0.35×10^6, 0.69×10^6, or 1.38×10^6 I.U./kg/day. In the positive control group, ethynylestradiol (EE_2; Sigma Chem. Co.) suspended in 0.5% sodium carboxymethylcellulose was used. In vehicle-treated group and the male counterpart group against EE_2-treated female group, 5% dextrose in PBS (1 ml/kg/day) was treated. In nontreated group, none was treated. Even though intravenous injection could be expected in clinical application of LBD-001, intraperitoneal application was used in this test to minimize possible tissue damage

to rat tails by long-term injection. The test substances or the vehicle was intraperitoneally administered to male rats from premating to mating period at least for 60 days and to female rats from at least 2 weeks before mating to early gestation period (from day 0 to day 7 of gestation). But, EE_2 (40 μg/kg/day) was subcutaneously administered only to female rats during the early gestation period. The number of animals per group ranged from 20 to 23.

Animal maintenance

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 ℃ 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 Food Co.) and sterilized water were fed *ad libitum.*

Observation method

Mating was started on 60 days after the treatment of the test substance to male rats and 14 days after the treatment to female rats. A virgin female rat in proestrous stage was kept overnight (from 5 *p.m.* to 10 *a.m.* of the next day) in a cage with a male. In the next morning, the copulated female rats with vaginal plugs and sperms in their vaginal smears were isolated individually to different cages. The day with a positive sperm smear was considered day 0 of gestation. If a female rat was not copulated in the first mating, the female rat was allowed to be mated more with other male partners belonging to the same group. But the additional mating period did not exceed 2 weeks. General body condition, general behavior of live rats, and the presence of dead rats were checked every day during all the test period. Body weight was basically measured once a week. However, the body weight of the female rats was measured every day during the gestation period. Consumption of food and water was measured twice a week. On day 19 of gestation, the female rats were anesthetized by ether and their abdominal cavity and uterine horns were opened to observe the number of implantation sites, number of corpora lutea, number of live or dead fetuses, their external malformations, and number of fetuses resorbed at early or late period of gestation. Female rats noncopulated even after several matings were also autopsied.

Statistical analyses

To analyze the statistical significance, Student's *t*-test or χ^2-test was applied. 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.

RESULTS AND DISCUSSION

General signs

LBD-001-treated groups did not show differences of general behaviors and symptoms both in males and females, compared with the vehicle-treated group. EE_2 treatment also did not evoke abnormal behaviors.

Effects on body weight

Effects on body weight changes of male rats were shown in Fig. 1. In nontreated group, the average body weight increased from 154.4 to 331.9 g after 9 weeks (63 days). In vehicle-treated group, the average body weight increased from 160.9 to 342.8 g. In LBD-001-treated

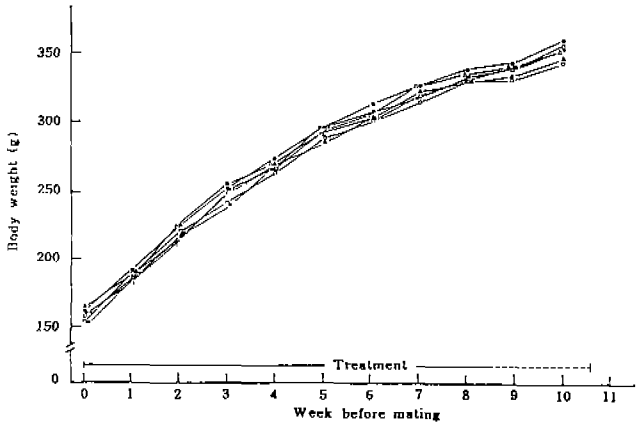

Fig. 1. Changes of body weight in male rats. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 (0.35×10^6 I.U./kg/day), □: LBD-001 (0.69×10^6 I.U./kg/day), △: LBD-001 (1.38×10^6 I.U./kg/day), ■: EE_2-counterpart group.

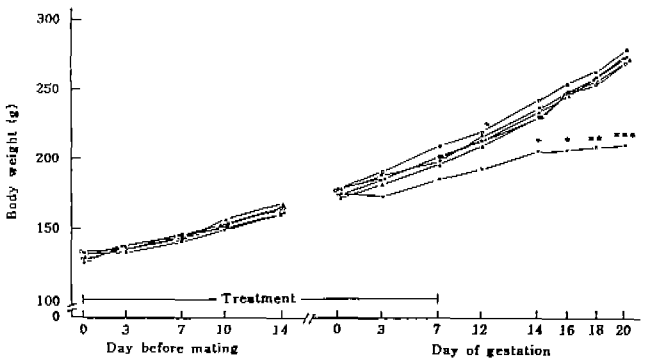

Fig. 2. Changes of body weight in female rats. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 (0.35×10^6 I.U./kg/day), □: LBD-001 (0.69×10^6 I.U./kg/day), △: LBD-001 (1.38×10^6 I.U./kg/day), ■: EE_2 (40 μg/kg/day; treated only from day 0 to 7 of gestation). *p<0.05, **p<0.01, and ***p<0.001; Significantly different from the vehicle-treated group.

group, the average body weight increased from 161.5 to 340.5 g at the dose of 0.35×10^6 I.U./kg, from 157.1 to 337.1 g at the dose of 0.69×10^6 I.U./kg, and from 164 to 335.1 g at the dose of 1.38×10^6 I.U./kg. In EE_2-counterpart group, body weight of male rats changed from 152.2 to 335 g. The body weight changes showed no significant differences among the groups.

Effects on body weight changes of female rats were shown in Fig. 2. The average body weight of female rats from the beginning day of the treatment to 2 weeks after the treatment increased from 128.9 to 166.5 g in nontreated group, from 133.7 to 160.6 g in vehicle-treated group, from 129.0 to 163.1 g in LBD-001 (0.35×10^6 I.U./kg)-treated group, from 133.4 to 166 g in LBD-001 (0.69×10^6 I.U./kg)-treated group, from 131.9 to 168.6 g in LBD-001 (1.38×10^6 I.U./kg)-treated group, and from 126.4 to 162.7 in EE_2-treated group in which EE_2 was not treated yet during this period. The body weight changes showed no significant differences among the groups.

The average body weight of female rats from day 0 to day 19 of gestation changed from 174.7 to 270.8 g in nontreated group, from 179 to 274 g in vehicle-treated group, from 174 to 273.8 g in LBD-001 (0.35×10^6 I.U./kg)-treated group, 178.1 to 280.1 g in LBD-001 (0.69×10^6 I.U./kg)-treated group, from 180.3 to 274.3 g in LBD-001 (1.38×10^6 I.U./kg)-treated group, and 176.4 to 210.0 g in EE_2-treated group. The body weight changes showed no significant differences among the groups except EE_2-treated group in which the body weights of the female rats were significantly lower, compared with vehicle-treated group ($p<0.05$ on day 14 and 16 of gestation, $p<0.01$ on day 18 of gestation, $p<0.001$ on day 20 of gestation). The inhibition was considered to result from antifertility activity of EE_2.

Effects on food consumption and water consumption

In male rats, changes of food and water consumption among the groups were not significantly different from vehicle-treated group during 60 days of the treatment (Fig. 3). In female rats, the changes among the groups were not significantly different from vehicle-treated group, except significant difference in EE_2-treated group (Fig. 4; $p<0.01$ on day 14 of gestation and $p<0.001$ on day 19 of gestation in food consumption; $p<0.01$ on day 14 and 19 of gestation in water consumption). The lower consumption of food and water might be related with the fact that EE_2-treated rats did not gestate because EE_2 inhibited the implantation stage of the fertilized ovua.

Effects on copulation rate and fertility capability

The results were shown in Table I. Copulation rates (=No. of rats copulated/No. of rats examined × 100) ranged from 87 to 96% in male rats and 83.3 to 91.7% in female rats, but were not much different among the groups. Pregnancy rates (= No. of pregnant rats/No. of copulated female rats × 100) were over 95.7% and not much different among the groups, except 0% in EE_2-treated group in which the fertility was completely inhibited from the implantation stage ($p<0.001$ as compared with vehicle-treated group).

Effects on fetuses

Effects on fetuses observed on day 20 of gestation were shown in Table II. Implantation rate (=No. of im-

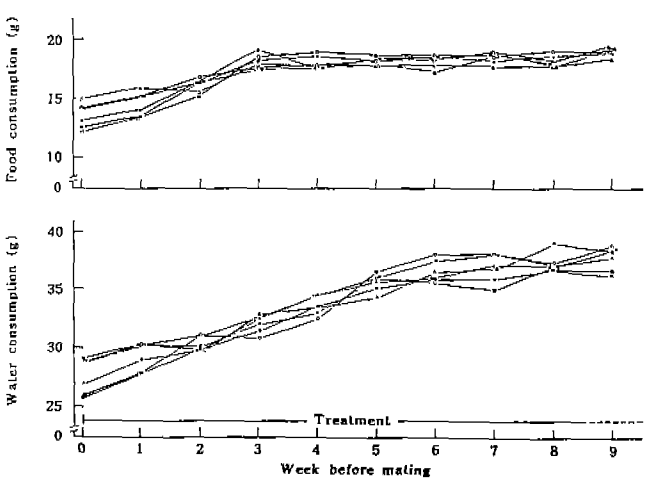

Fig. 3. Changes of food and water consumption in male rats. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 (0.35×10^6 I.U./kg/day), □: LBD-001 (0.69×10^6 I.U./kg/day), △: LBD-001 (1.38×10^6 I.U./kg/day), ■: EE_2-counterpart group.

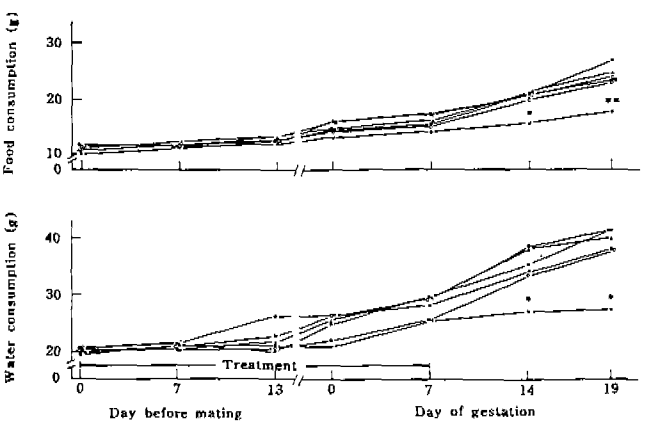

Fig. 4. Changes of food and water consumption in female rats. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 (0.35×10^6 I.U./kg/day), □: LBD-001 (0.69×10^6 I.U./kg/day), △: LBD-001 (1.38×10^6 I.U./kg/day), ■: EE_2 (40 μg/kg/day; treated only from day 0 to 7 of gestation.). *p<0.01, **p<0.001; Significantly different from the vehicle-treated group.

Table I. Influences of LBD-001 on fertility of male and female rats

Sex	Parameters	Nontreated group	Vehicle group	LBD-001 (I.U./kg/day, *i.p.*)			EE_2 (μg/kg/day)[a]
				0.35×10^6	0.69×10^6	1.38×10^6	40
Male	No. of examined rats	23	23	23	23	23	23
	No. of copulated rats	20	21	22	21	20	20
	Copulation rate (%)[b]	87.0	91.3	95.7	91.3	87.0	87.0
Female	No. of examined rats	24	24	24	24	24	24
	No. of copulated rats	20	21	22	21	20	20
	Copulation rate (%)[b]	83.3	87.5	91.7	87.5	83.3	83.3
	No. of pregnant rats	20	20	22	21	19	0*
	Pregnancy rate (%)[c]	100	95.2	100	100	95.0	0*

[a]EE_2 (Ethynylestradiol) subcutaneously administered only to female rats. [b]No. of copulated rats/No. of examined rats $\times$ 100. [c]No. of pregnant rats/No. of copulated rats $\times$ 100. *$p<0.001$; Significantly different from the vehicle-treated group.

Table II. Effect of LBD-001 on pregnant female rats

Parameters	Nontreated group	Vehicle group	LBD-001 (I.U./kg/day, *i.p.*)			EE_2 (μg/kg/day)[a]
			0.35×10^6	0.69×10^6	1.38×10^6	40
No. of examined rats	23	20	20	20	20	20
No. of pregnant rats	20	20	20	20	19	0*
No. of corpora lutea[b]	10.3±1.29	10.0±1.32	10.7±1.73	10.8±2.31	11.1±2.52	12.8±3.65
No. of implantation sites[b]	9.9±0.91	9.3±1.59	10.2±1.46	10.3±1.52	9.6±2.62	0.0±0.00*
Implantation rate (%)[c]	96.1	93.0	95.3	95.4	86.5	0*
No. of normal fetuses[b]	9.2±1.46	8.9±1.55	9.4±1.50	9.6±1.70	9.2±2.81	–
Total no. of resorbed fetuses	15	8	15	13	9	–
at early stage	15	7	14	13	8	–
at late stage	0	1	1	0	1	–
% of resorbed fetuses[d]	7.6	4.3	7.4	6.3	5.4	–

[a]EE_2 (Ethynylestradiol) was subcutaneously administered. [b]Mean±S.D. [c]No. of implantation sites/No. of corpora lutea $\times$ 100. [d]No. of resorbed fetuses/(No. of normal fetuses+No. of resorbed fetuses) $\times$ 100. *$p<0.001$; Significantly different from the vehicle-treated group.

plantation sites/No. of corpora lutea $\times$ 100) was 96.1% in nontreated group, and 93.0% in vehicle-treated group. In LBD-001-treated groups, the rate was 95.3% at the dose of 0.35×10^6 I.U./kg, 95.4% at the dose of 0.69×10^6 I.U./kg, and 86.5% at the dose of 1.38×10^6 I.U./kg respectively. High dose (1.38×10^6 I.U./kg) of LBD-001 showed increased tendency of implantation rate, but not significant as compared with the vehicle-treated group. In EE_2-treated group, no implantation sites were observed ($p<0.001$). The numbers of resorbed fetuses ranged from 4.3 to 7.6% among the groups. LBD-001, however, did not show dose-dependent and significant effects on the resorption rates.

Autopsy of experimental animals

One female rat in the vehicle-treated group and two female rats in the EE_2-treated group, showed edema of unilateral side of ovary. Except these, no miscellaneous and abnormal outer appearances of the organs were observed in autopsied male and female rats.

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