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TERATOGENICITY STUDY OF RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (LBD-005) IN RABBITS

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ABSTRACT: *LBD-005, a newly developed recombinant granulocyte-macrophage colony-stimulating factor, was at dose levels of 0, 20, 80 and 320 μg/kg/day administered subcutaneously to pregnant New Zealand White rabbits during the organogenetic period. The dams were subjected to caesarean section on day 28 of pregnancy. Effects of test substance on dams and embryonal development of fetuses were examined. No treatment-related changes in clinical signs and necropsy findings of dams were observed in all groups. At 80 and 320 μg/kg, a significant decrease in food consumption followed by a loss in body weight was found. At 320 μg/kg* a increased resorption rate and a lower fetal weight of both sexes were also seen. There were no growth retardation and teratogenic effects on fetuses from dams treated with LBD-005. The results show that the no-effect dose levels (NOELs) of LBD-005 are *20μg/kg/day for dams and 80 μg/kg/day for fetuses.*

Key Words: *LBD-005, recombinant granulocyte-macrophage colony stimulating factor, teratogenicity study, rabbits, subcutaneous application.*

INTRODUCTION

LBD-005, a recombinant Granulocyte-Macrophage Colony Stimulating Factor, is a hematogenic 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-005, teratogenicity of New Zealand White rabbits was studied. This study was performed to assess the potential toxic effects of test substance on dams and embryonal development of fetuses.

It was carried out according to the Guidelines for Reproduction Studies for Safty Evaluation of Drugs for Human Use (National Institute for Safety Research, 1988), Japanese Toxicity Guidelines (Yakugyo Jiho Co., 1984; Inveresk Research International, 1984) and the requirements for trial performance stated in "Embryotoxikologische Problem in der Arzneimittel Forschung" (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 and Mating Procedure

Pregnant New Zealand White rabbits were supplied by Samyook Laboratory Animal Center (Hwasung, Korea). They were kept under conventional conditions. Standard laboratory rabbit diet (Purina Korea Co., Kunsan, Korea) and sterilized water were available ad libitum. For mating one female was placed into the cage of one male in the evening and the day of copulation was designated as day 0 of pregnancy if copulatory acts were twice observed.

Test Substance

LBD-005 (Lot No. CI021) was supplied by the Lucky R & D Center, Biotechnology (84, Jang-Dong, Yousung-Koo, Daejon, Korea) with a protein content of 1 mg/1 ml w/v, pH of 7.4 and an osmotic pressure of 283 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 6 and 12 of gestation and all solutions were stored at 4℃.

Treatment and Observation of Dams

LBD-005 was administered subcutaneously to pregnant rabbits from days 6 to 18 of gestation. Per experimental group twenty three females with copulatory acts were used. Based on the results of pilot study, 320 μg/kg was selected for the high dose. There were three treatment groups (20, 80 and 320 μg LBD-005/kg b.w) and one control group which received the vehicle only. Pregnant females were observed for food consumption, weight development and sign of intoxication. All animals were subjected to autopsy at the end of gestation.

Caesarian Section on Day 28 of Gestation

On day 28 of gestation the pregnant females of all groups were killed by a blow at the base of the skull. 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. Each living fetus was evaluated for externally visible abnormalities. After sexing, all fetuses were examined for internal malformations. For visceral examination of fetuses we have adapted Barrow's and Wilson's methods (Sterz, 1977). The evaluation of skeletal abnormalities was performed after clearing the 95% ethanol-fixed fetuses with KOH and after staining the skeleton with alizarin red (Dawson, 1926).

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-005 on Dams

No notable changes in behaviour or clinical signs were observed. There were, however, a few exceptions: one dam given 20 μg/kg showed lacrimation on several days. Also at 20 μg/kg, two animals showed an early delivery on day 23 and day 26 of gestation, respectively. No substance-related deaths occurred. There were no significant differences in the food consumption of pregnant animals, except that dams of 80 μg/kg and 320 μg/kg groups consumed significantly less diet on day 6 and day 12 of gestation. The body weight development of the 20 μg/kg group compared well with the control. At 80 μg/kg, the body weight of day 18 of gestation was significantly lower. Dams givein 320 μg/kg had a loss in body weight from day 12 to the end of gestation. At autopsy of dams on day 28 of gestation, no pathologic lesions were observed.

Effect of LBD-005 on Fetuses

Sectio-data

No significant differences were observed in litter parameters, except at 320 μg/kg, a lower fetal weight of both sexes and a increased number of resorptions. At 80 μg/kg, there was a slight, aber not significant increase of resorption rate

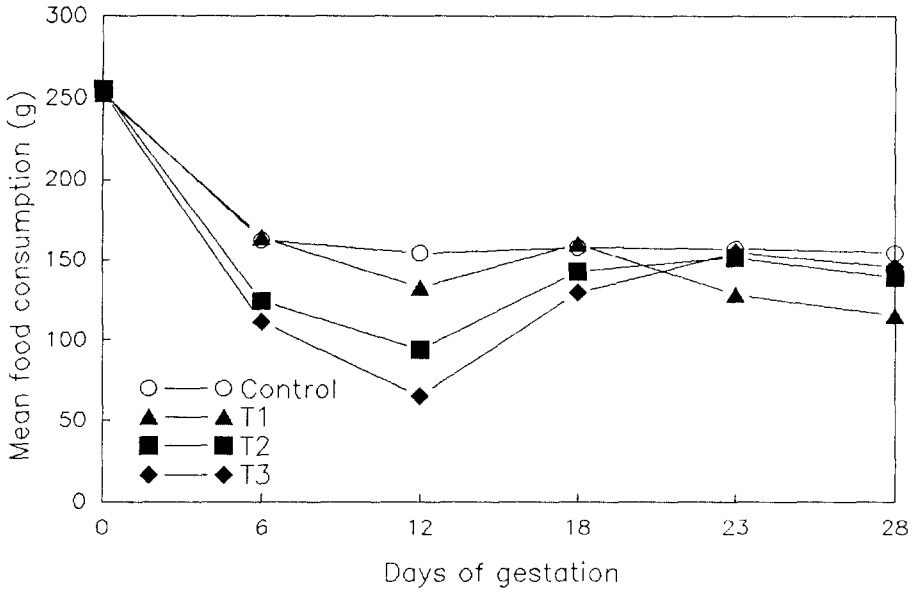

Figure 1. Mean food consumption during the gestation of rabbits treated with LBD-005.

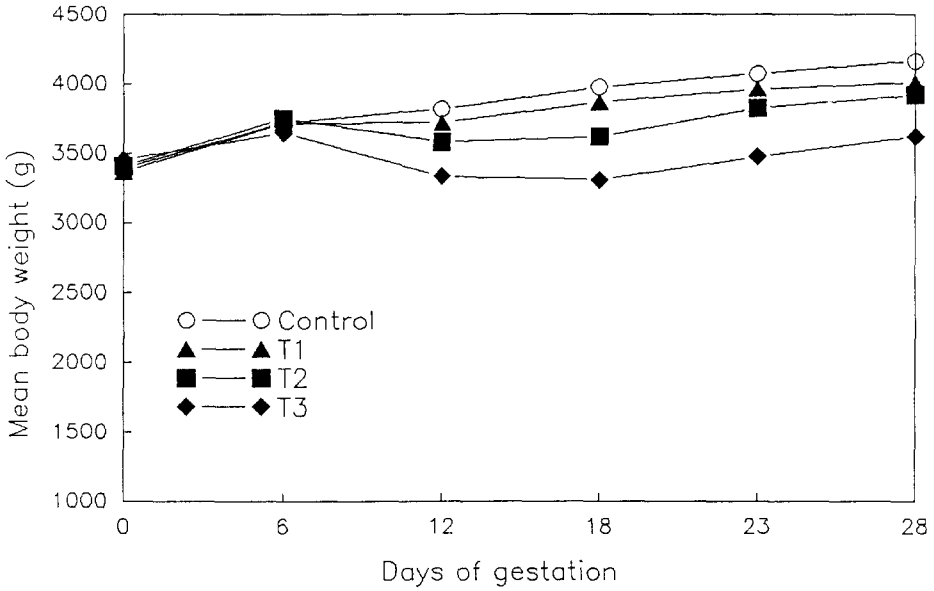

Figure 2. Mean body weight changes during the gestation of rabbits treated with LBD-005.

Table 1. Findings at caesarean section of rabbits treated with LBD-005

Dose (μg/kg)	0	20	80	320
No. of pregnant animals	16	15	17	14[a]
Corpora lutea (Mean± S.D.)	8.69± 1.62	9.00± 1.36	8.65± 1.46	8.71± 2.20
Implantations (Mean± S.D.)	7.94± 1.73	7.67± 1.59	7.41± 1.37	7.29± 2.95
% to corpora lutea (Mean± S.D.)	91.21± 9.51	85.23± 13.37	86.14± 11.91	80.97± 22.63
Fetal deaths (resorptions + dead fetuses)	8	7	18	46
Resorptions	1	4	13	39**
Early	1	4	13	39
Late	0	0	0	0
Dead fetuses	7	3	5	7
Live fetuses				
Male/Female	60/59	64/44	56/52	28/28
Litter size (Mean± S.D.)	7.44± 1.63	7.20± 1.37	6.35± 1.46	6.22± 3.03
% to implantations (Mean± S.D.)	94.00± 6.31	94.87± 9.99	86.89± 16.45	68.01± 28.08**
Sex ratio (male/female)	1.02	1.45	1.08	1.00
External anomalies of fetuses	0	0	0	0
Body weight of live fetuses				
Male (Mean± S.D.)	35.73± 4.38	36.20± 3.66	33.93± 4.35	29.24± 6.19*
Female (Mean± S.D.)	35.62± 4.84	34.91± 5.76	34.68± 4.14	25.87± 7.02**

[a] Five of fourteen does had resorptions only.
* and ** indicate significant difference at $p<0.05$ and $p<0.01$ levels when compared with control group.

followed by a lower number of viable fetuses. No external malformations occurred among examined fetuses of all groups (Table 1).

Table 2. Visceral findings in F1 fetuses from F0 dams treated with LBD-005

Dose (μg/kg)	0	20	80	320
No. of fetuses examined	119	108	108	56
No. of fetuses with anomalies(%)	0(0)	0(0)	1(0.9%)	0(0)
Renal hypoplasia	0	0	1	0
No. of fetuses with variations	0	0	0	0

Table 3. Skeletal findings in F1 fetuses from F0 dams treated with LBD-005

Dose (μg/kg)	0	20	80	320
No. of fetuses examined	119	108	108	56
No. of fetuses with anomalies(%)	0(0)	1(0.9%)	2(1.9%)	1(1,8%)
Cleaved sternebrae, 13th rib	0	1	0	0
Fused ribs, 13th rib, 8th lumbar vertebrae	0	0	1	0
Fused ribs, abscence of 1st sacral vertebral arch, 13th rib	0	0	1	0
Fused sternebrae, abscence of sacral vertebrae, asymmetric sternebrae, 6th lumbar vertebrae	0	0	0	1
No. of fetuses with variations(%)	40(33.6%)	51(47.2%)	47(43.5%)	29(51.8%)
13th rib	39	50	38	25
Unilateral	16	11	4	4
Bilateral	23	39	34	21
8th lumbar vertebrae	0	0	1	0
13th rib, 8th lumbar vertebrae	1	0	8	4
13th rib, additional ossification centers of sternebrae	0	1	0	0
Degree of ossifications				
No. of sternebrae	5.9± 0.2	5.9± 0.1	5.9± 0.2	5.9± 0.2
No. of metacarpals in both forelimbs	9.9± 0.4	9.9± 0.2	9.7± 0.3	9.2± 0.6
No. of 1st phalanges in both forelimbs	10.0± 0.1	10.0	10.0	9.9± 0.2
No. of metatarsals in both hindlimbs	8.0	8.0	8.0	8.0
No. of 1st phalanges in both hindlimbs	8.0	8.0	8.0	8.0
No. of sacral and caudal vertebrae	19.5± 0.4	19.5± 0.5	19.6± 0.4	18.7± 1.6

*Multiple abnormalities (fused sternebrae, asymmetric sternebrae and abscence of sacral vertebrae) were found in one fetus from dam (No. 83) of 320 μg/kg group.

Visceral findings

No visceral malformations occurred among examined fetuses except one renal hypoplasia in the 80 μg/kg group (Table 2).

Skeletal findings

There were four malformed fetuses; namely one cleaved sternebrae and 13th rib (combined) in 20 μg/kg group, one fused ribs, 13th rib and 8th lumber verteb-

rae (combined) and one fused ribs, abscence of 1st sacral vertebral arch and 13th rib (combined) in 80 μg/kg group and one fused sternebrae, abscence of sacral vertebrae, asymmetric sternebrae, 6th lumbar vertebrae (combined) in 320 μg/kg group.

Variated fetuses were found in all groups: namely 13th rib, 8th lumbar vertebrae and additional ossification centers of sternebrae. They were not dose-related. The ossification of evaluated skeletal districts compared well between the groups (Table 3).

DISCUSSION

GM-CSF (granulocyte-macrophage colony stimulating factor) stimulates the proliferation of granulocyte and macrophage colonys and regulates some of the functional activities of stem cells in the human being and animals (Metcalf *et al.*, 1986). In toxicological testing of gentechnological recombinant GM-CSF 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 (Hochbach *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 glycoprotein comes into question, because recombinant GM-CSF cannot pass directly the placental barrier with two large molecule weight.

All LBD-005 (recombinant GM-CSF) doses tested did not induce any signs of intoxication in dams. One lacrimation and two early deliverys of 20 μg/kg group are not considered to be treatment-related. A lower food intake of 80 μg/kg and 320 μg/kg groups followed by a significant retardation must be the result of treatment-related anorexia. No substance-related pathologic findings were seen in the groups treated with test substance. The litter parameters of dams, namely number of implantations, dead fetuses, external abnormality, litter size, sex ratio, showed no changes related to treatment of LBD-005. A increased resorption rate of 320 μg/kg group concomitant with the lower number of viable fetuses must be substance-related and has to be called embryocidal effect. A lower fetal weight of 320 μg/kg group is as well treatment-related and has to be classified as a sign of retardation. No substance-related visceral malformations occurred. One renal hypoplasia of 80 μg/kg group is regared as a spontaneous finding. The variations found in the skeletal examination of fetuses are common and known for the New Zealand White rabbit (Palmer, 1977; Morita *et al.*, 1987). They were not dose-related.

The skeletal malformations of the three treated groups were cleaved sternebrae, fused sternebrae, fused ribs, abscence of sacral vertebrae and abscence of 1st sacral vertebral arch. They were rare and might be the result of maternal toxicity.

From the results mentioned above, the NOELs (no observed effect level) of LBD-005 on the dams and fetuses were considered to be 20 μg/kg and 80 μg/kg, respectively.

We reported recently that LBD-005 was non-teratogenic in rats. But the general signs of pregnant rats and embryonal development of F1 fetuses were not influenced even when injected subcutaneouly at dose level of 1000 μg/kg LBD-005/kg body wt; the NOELs were 1000 μg/kg. Thus, there was a clear-cut species-differe-

nce between rat and rabbit concerning the toxicological response of test agent.

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