Korean J. Toxicol.
Vol. 9, No. 1, pp. 99-105, 1993

TERATOGENICITY STUDY OF RECOMBINANT HUMAN INTERFERON αA (LBD-007) IN RABBITS

Moon-Koo Chung, Sung-Hoon Kim, Sang-Seop Han and Jung-Koo Roh

Toxicology Research Center, Korea Research Institute of Chemical Technology P.O. Box 9, Daedeog-Danji, Daejeon, 305-606, Korea

(Received February 16, 1993)
(Accepted May 29, 1993)

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 New Zealand White rabbits during the organogenetic period. Cyclophosphamide was used as a positive control.*

All pregnant females were subjected to the caesarean section on day 28 of pregnancy.

Effect of test substance on dams and embryonal development of fetuses were examined.

1. No treatment-related changes in clinical signs, food consuption, body weight and necropsy findings of dams were observed.

2. There were no growth retardation and teratogenic effects on fetuses from the 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 and for fetuses.

Key Words: *LBD-007, Recombinant human interferon αA, Teratogenicity study, Rabbits, 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, Daejeon, Korea). As a part of toxicological screening of test agent LBD-007, teratogenicity of New Zealand White rabbits was studed. This study was performe to assess the potential toxic effects of test substance on dams and embryonal development of fetuses.

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 o of pregnancy if copulatory acts were twice observed.

Test Substance

LBD-007 (Lot No. CI021) was supplied by the Lucky R & D Center, Biotechnology(84 Jang-Dong, Yousung-Koo, Daejeon, Korea) with a titer of 10^7 IU/m*l*, 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 6 and day 13 of gestation and all solutions were stored at 4℃

Cyclophosphamide (Sigma Co., Lot No.: 86F-0101) was used as a positive control.

Treatment and observation of dams

LBD-007 was administered subcutaneously to pregnant rabbits from days 6 to 18 of gestation. Per experimental group twenty females with copulatory acts were used. Ther were three treatment groups (3×10^6, 6×10^6 and 12×10^6 IU LBD-007/kg b.w.), one vehicle control group and one positive control group. Positive controls received 30 mg cyclophosphamide/kg body wt. once on day 11 of gestation intravenously. Pregnant females were observed for food consumption, weight development and sign of intoxication. All animals were subjected to autopsy at the end of gestation.

Caesarean 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 and 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. 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 clinical signs were observed among controls and animals of treated groups. No substance-related death occured, except that among positive controls, one animal died from paralysis of hindlimbs provoked by sudden movement of the anmimal by intravenous injection. Thee were no significant differences in the food consumption and body weight development of dams between the groups, except that positive controls consumed significantly less diet on day 13 of gestation and correspondingly had a significantly retarded body weight development (Figure 1, 2). At autopsy of the dams on daay 28 of gestation, no pathologic lesions were discovered.

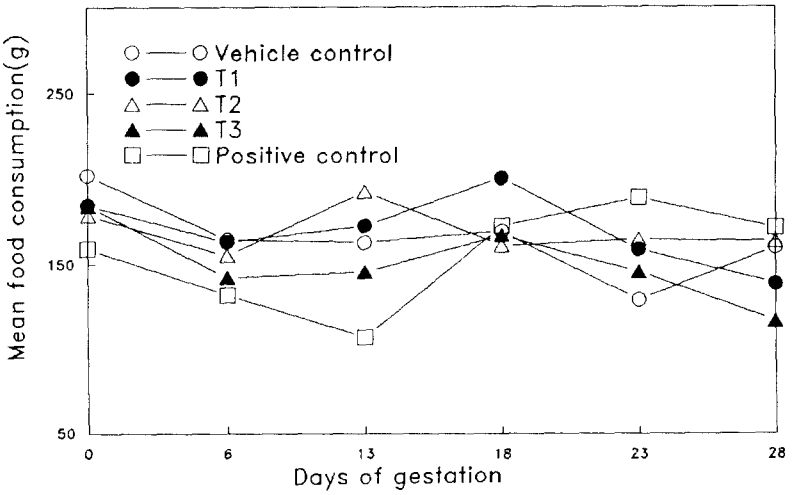

Figure 1. Mean food consumption during the gestation of rabbits treated with LBD-007.

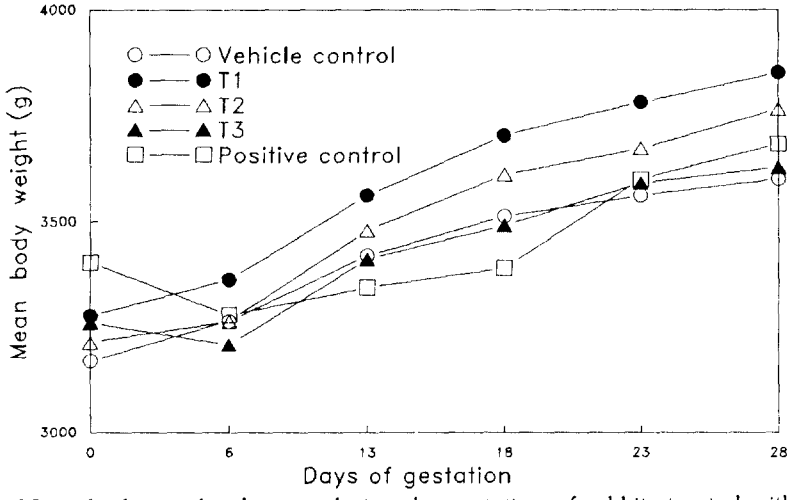

Figure 2. Mean body weight changes during the gestation of rabbits treated with LBD-007.

Effect of LBD-007 on fetuses

Sectio-data

The litter parameters compated well between the groups, except that in the positive control, a decreased fetal weight of both sexes was observed (Table 1). Exter-

Table 1. Findings at caesarean section of rabbits treated with LBD-007

DOSE($\times 10^6$ IU/kg)	0	3	6	12	CP[a] 30(mg/kg)
No. of pregnant animals	14	12	13	12	12
Corpora lutea(Mean± S.D.)	9.57± 2.24	9.08± 1.73	9.85± 2.48	8.50± 1.24	9.42± 1.16
Implantations(Mean± S.D.)	8.00± 1.36	8.33± 2.02	9.08± 2.69	7.42± 1.78	8.83± 2.32
% to corpora lutea (Mean± S.D.)	85.14± 12.79	91.77± 13.97	91.68± 10.97	87.12± 14.62	93.41± 14.34
Fetal deaths (resorptions + dead fetuses)	15	11	8	12	18
Resorptions	12	9	5	6	11
Early	12	8	4	6	10
Late	0	1	1	0	1
Dead fetuses	3	2	3	6	7
Live fetuses					
Male/Female	41/56	40/49	44/66	36/41	50/38
Litter size(Mean± S.D.)	6.93± 2.50	7.42± 2.97	8.46± 2.73	6.42± 2.07	7.33± 3.06
% to implantation (Mean± S.D.)	87.27± 26.44	88.18± 28.50	93.53± 9.99	86.92± 19.1	83.15± 28.52
Sex Ratio (Male/female)	0.73	0.82	0.67	0.88	1.32
No. of fetuses with external anomalies(%)	0(0)	0(0)	0(0)	0(0)	34(38.6)
Cleft palate	0	0	0	0	15
Cleft lip	0	0	0	0	16
Polydactyly	0	0	0	0	1
Oligodactyly	0	0	0	0	2
Body weight of live fetuses					
Male (Mean± S.D.)	35.48± 2.69	35.25± 4.36	35.05± 7.64	34.19± 4.46	30.72± 5.47*
Female (Mean± S.D.)	34.71± 5.53	35.05± 3.62	31.48± 5.35	31.94± 4.55	28.20± 4.80**

[a]; CP=Cyclophophamide

Values in parentheses represent occurrence rate.

* ; Significantly different from control value at $p<0.05$

**; Significantly different from control value at $p<0.01$

Table 2. Visceral findings in F1 fetuses from F0 dams treated with LBD-007

DOSE($\times 10^6$ IU/kg)	0	3	6	12	CP[a] 30(mg/kg)
No. of fetuses examined	97	89	110	77	87
No. of fetuses with anomalies(%)	0(0)	0(0)	0(0)	0(0)	4(4.6)
renal hypoplasia	0	0	0	0	2
renal agenesis	0	0	0	0	2
No. of fetuses with variations	0	0	0	0	0

[a] CP=Cyclophophamide

nally malformed fetuses were found only in the positive control group; nemely one cleft lip, one oligodactyly, fourteen cleft lip and cleft palate, one cleft palate, polydactyly and oligodactyly (combined).

Visceral findings

Neither malformations nor variations occurred among examined fetuses, except that in the positive control group, two renal hypoplasia and renal agenesis, respectively.

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

DOSE($\times 10^6$ IU/kg)	0	3	6	12	CP[a] 30(mg/kg)
No. of fetuses examined	98	89	110	77	87
No. of fetuses with anomalies(%)	0(0)	0(0)	1(0.9)	2(2.6)	15(17.2)[b]
Cleaved sternebrae	0	0	0	1	
Cleaved sternebrae, 8th lumbar vertetrae, 13th rib	0	0	1	0	
Fused ribs, 8th lumbar vertebrae, 13th rib	0	0	0	1	
No. of fetuses with variations(%)	34(34.7)	39(43.8)	44(40.0)	32(41.6)	21(24.1)
13th rib	22	27	33	23	15
Unilateral	10	10	8	8	6
Bilateral	12	17	25	15	9
8th lumbar vertebrae	0	0	1	0	1
Asymmetric sternebrae	2	0	0	0	0
Additional ossification centers of sternebrae	0	1	0	0	0
13th rib, 8th lumbar vertebrae	10	11	8	9	4
13th rib, asymmetric sternebrae	0	0	2	0	0
13th rib, 8th lumbar vertebrae, asymmetric sternebrae	0	0	0	0	1
Degree of ossifications					
No. of sternebrae	5.7± 0.4	5.9± 0.2	5.7± 0.4	5.8± 0.2	5.8± 0.2
No. of metacarpals in both forelimbs	9.6± 0.6	9.9± 0.1	9.7± 0.6	9.7± 0.5	9.6± 0.7
No. of 1st phalanges in both forelimbs	9.7± 0.7	10.0	9.9± 0.2	10.0	9.6± 0.8
No. of metatarsals in both hindlimbs	8.0	8.0	8.0	8.0	8.0± 0.1
No. of 1st phalanges in both hindlimbs	7.7± 1.0	8.0	8.0	8.0	8.0± 0.1
No. of sacral and caudal vertebrae	19.1± 1.1	19.3± 0.3	19.3± 0.4	19.1± 0.3	18.0± 0.9**

[a] CP=Cyclophosphamide

[b] Among positive controls following abnormalities were observed; Fused sternebrae(5), Fused caudal vertebral bodies(3), Fused caudal vertebral bodies, 13th rib(1), Fused sternebrae, asymmetric sternebrae, 8th lumbar vertebrae(1), Fused caudal vertebral bodies, 13th rib, 8th lumbar vertebrae(3), Fused caudal vertebral bodies, 13th rib, asymmetric sternebrae(1), Fused sternebrae, fused caudal vertebral bodies, 13th rib, 8th lumbar vertebrae(1).

**; Significantly different from control group ($p<0.01$).

Skeletal findings

Three malformed fetuses were found; namely one cleaved sternebrae, 8th lumbar vertebrae ad 13th rib (combined) in 6×10^6 IU/kg group, one cleaved sternebrae, one fused ribs, 8th lumbar vertebrae and 13th rib (combined) in 12×10^6 IU/kg group. There were variated fetuses in all groups; namely 13th rib (with a comparatively high frequency), 8th lumbar vertebrae, asymmetric sternebrae and additional ossification centers of sternebrae. They were not dose-related. The incidence of malformations and variations of positive control group is increased, when compared with that of vehicle control group. The ossification of evaluated skeletal districts compared well between the groups, except that in the positive control group, a retarded ossification of sacrocaudal vertebrae was observed (Table 3).

DISCUSSION

Interferon is a immunomodulating agent in the human being and animals. In toxicological testing of gentechnological recombinant interferon αA through animal experiment, careful attention must be paid to the reaction, which results from the exaggerated pharmacologic effect; supra-physiological concentrations of bioactive endogenous proteins affect receptors other than the target organ receptors (Lewandowski, 1988).

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. All pregnant animals of treated groups showed no substance-related pathologic findings.

No treatment-related changes were observed in litter parameters examined. No substance-related visceral malformations occurred among fetuses of the treatment groups. The skeletal malformations found in 6×10^6 and 12×10^6 IU/kg group were cleaved sternebrae and fused ribs. They have to be classified as "minor anomalies", which also accurrs in the controls at a very low frequency. They are rare and might not be substance-related. The variations found in all groups are trivial and known for the New Zealand White rabbit (Palmer, 1977; Morita *et al.*, 1987). Even the highest LBD-007-dose induced neither teratogenicity nor growth retardation. Interferon-γ is reported to be non-teratogenic in rabbits (Harada, 1988). CP(cylophosphamide)-induced toxic effects could be seen; namely a decreased food intake of dams, a lower fetal weight, a increased frequency of malformations and a retarded ossification of sacrocaudal vertebrae in fetuses.

From the results mentioned above, it may be concluded that LBD-007 does not appear to influence genral sign of pregnant rabbits and embryonal development of fetuses, even when injected 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.

These results are in good agreement with those of teratogenicity study of LBD-007 in rats. Thus, there was no species-diffeence between rat and rabbit with regard to the teratogenic potential of test agent.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. Jong-Choon Kim, Mr. Sang-Joon Lee for technical support and Miss Jeong Ran Kim for statistical analysis.

REFERENCES

Dawson, A.B. (1926): A note on the staining of the skeleton of cleared specimens with Alizarin Red S. Stain. Technol. **1**, 123-124.

Harada, Y. (1988): Experience in the safety testing for biotechnology products and problems generated by animal studies. In: Safety Assessment of Biotechnology Products. Y-N Cha *et al.*, eds, Proceedings of the International Symposium held at the Seoul Palace Hotel, Seoul, Korea, Dec. 2-3, 1988, pp. 35-52.

Lewandowski, M. (1988): The toxicology of industrial biotechnology products. In: Safety assessment of biotechnology products, Y.N. Cha *et al.*, eds, Proceedings of the international symposium held at the Seoul Palace Hotel, Seoul, Korea, Dec. 2~3, 1988. pp. 21-34.

Morita, H., Ariyuki, F., Inomata, N., Nishimura, K., Kasegawa, Y., Miyamoto, M. and Watanable, T. (1987): Spontaneous malformations in lavoratory animals: Frequency of external, internal and skeletal malformations in rats, rabbits and mice. Cong. Anom. **27**, 147-206.

Palmer, A.K. (1977): Incidence of sporadic malformations, anomalies and variations in radnom bred laboratory animals. In: Methods in Prenatal Toxicology. D. Neubert *et al.*, eds, Georg Thieme Verlag, Stuttgart. pp. 52-71.

Sterz, H. (1977): Routine examination of rat and rabbit fetuses for malformation of internal organ-Combination of Barrow's and Wilson's methods. In: Methods in Prenatal Toxicology. D. Neubert *et al.*, eds. Gerog Thieme Verlag, Stuttgart. pp. 113-122.