Korean J. Toxicol.
Vol.3, No.1, pp. 33-44, 1987

STUDIES OF RECOMBINANT HUMAN INTERFERON-αA (rHuIFN-αA) ON FERTILITY IN RATS

Yong-Soon Lee, Jae-Hak Park, Tae-Gyu Kang, Hyun-Su Kim,*
Nam-Sin Cho* and Moo-Young Yoo*

*College of Veterinary Medicine, Seoul National University and *Genetic Engineering Division, Cheil Sugar Co., Ltd.*

ABSTRACT: *A fertility study was carried out in Sprague Dawley rats which have been given the intravenous or intraperitoneal injections of rHuIFN-αA, a commecially available therapeutic agent, at dose levels of 1×10^5, 4×10^5 and 1.2×10^6 I.U/kg/day. Male rats were treated with rHuIFN-αA from 60 days before pairing and until the completion of mating. Female rats received rHuIFN-αA for 22days prior to mating and up to day of gestation. All pregnant females were sacrificed on day 20 of gestation and all fetuses were examined for abnormalities. Both the male and female animals treated with rHuIFN-αA did not show any abnormal responses. No abnormal signs were seen in reproducibility for the rats treated with rHuIFN-αA. No External, internal and skeletal anomalies attributable to rHuIFN-αA were observed in the fetuses. It was concluded that rHuIFN-αA had no harmful effect on mating, fertilization, implantation, or embryonic development.*

INTRODUCTION

Recombinant human interferon-αA (rHuIFN-αA) which has been produced by gene-manipulated E. coli is a commercially available therapeutic agent used for, such cliseasee as viral disease and anti-tumors. This present paper deals with the effect of rHuIFN-αA on the fertility upon administration via tail vein or intraperitonium to rats from pre-mating to early gestation. This study was carried out in accordance with the "Guidelines for Reproduction Experiments to Evaluate the Safety of Drugs" issued by the Ministry of Health and Welfare, Korea National Institute of Health.

MATERIALS AND METHODS

Materials

Injectable rHuIFN-αA which has been produced by Cheil Sugar Cooperation, was used and those levels were devided into two groups, 1×10^6 I.U/vial and 3×10^6 I.U/vial.

Methods

100 rats were used per sex. Male rats, 3 weeks of age, and female rats, 5 weeks of age, of the Sprague-Dawley strain were used under the approval of specific pathogen free-rats by serological tests.

The animals were housed in polycarbonate cages ($26 \times 42 \times 18$ cm) bedded with autoclaved wood-shavings at an ambient temperature of $23 \pm 3°C$, and a relative humidity of $55 \pm 10\%$. The ventilation of the breeding room was maintained by optimal air condition, and the room was lighted with 12-hour photoperiod. Animals were allowed free access to Sam-Yang Laboratory Animal Diet (Sam-Yang Feedstuff Co. Korea) and to water bottle. Water was changed daily. Bottles and cages were autoclaved every three days.

During the attenuation period, 5 rats were housed per cage until the mating. During the mating period, the female rats were paired at 1 : 1 basis with the male rats. Thereafter, 3 rats, which were confirmed pregnant by the presence of vaginal copulation plugs and sperms in their vaginal smears, were housed in one cage.

Dosage range was determined, based on 3 kinds of treated groups-one vehicle, non-treated group, and three treated groups were devided by high dose levels (1.2×10^6 I.U/kg/day), medium dose levels (4×10^5 I.U/kg/day), and low dose levels (1×10^5 I.U/kg 1 day).

Test materials (1×10^6 I.U/vial and 3×10^6 I.U/vial) which were diluted in 1 ml distilled water treated via tail vein or peritonium at 19 : 00 hour daily. All dosages were matched by the weights of the rats. Male rats, 3 weeks of age, were treated with the drug for 60 days from pre-mating until the completion of mating, and female rats, 5 weeks of age were treated 14 days before mating. Pregnated rats were also treated up to day 7.

All visible responses of treated animals were observed 3 times per day. Body weights of the male rats were recorded one time per 10 days from the day of first treatment to the day of mating, and those of the female rats were recorded at 1st, 3rd, 6th, 12th, and 14th day of treatment before the day of mating, and at 0, 9th, 12th, 15th, 18th, and 20th day after gestation.

In the reproducibility test, estrus cycles were calculated, between the pre-treatment and post-treatment, by the vaginal smear using methylene blue (0.5%) during the 14 days of pre-treatment periods to the 0 day of gestation periods. In the treated groups, male rats which were treated for 60 days, 14 weeks of age, and female rats which were treated 14 days, 10 weeks of age, of the same strain were paired at a 1 : 1 basis.

It was confined 14 days to the maximum mating periods. In that period, non-mated rats were re-mated with non-treated rats. Mating was also confirmed by the presence of viginal copulation plugs and sperms in their viginal smear. The day on which the mating was confirmed at 9 : 00 AM or the day after mating was designated day 0 of gestation.

In the pathological test carried out on the 20th day of gestation, all pregnant females in each group were autopsied by the Cesarean section, and all fetuses were carefully examined. And, observations of the tissue were processed particularly for the abnormal fetuses. In the late period of gestation, on the twentieth day of gestation, all pregnant females in each group were anesthetized with pentobarbital sodium. After bleeding and autopsied by the Cesarean section, ovaries and uteruses were collected. And then, numbers of corpora luteum, implant, viable and dead fetuses were also collected. External abnormalities were also examined in both sexes. The weight of underdeveloped fetus was 60% lighter than that of control fetus. The weights of the maternal spleen were also measured. The half viable fetuses per litter were used in the screening of organ anomalies, and the rest was used in skeletal examination.

Organ examination

Collected fetuses were fixed in Bouin's solution for 7 days, and modified examinations were processed under the method of Wilson's (1965), and Nishimura's method which was mainly used in the screening of the thoracic organs.

Skeletal examination

All anomalies of the skeletal system were observed under a vertical microscope, and all fetuses were double-stained by Inouye's method. In the case of delayed ossification, we mainly observed cervical vertebra, coccygeal vertebra, thoracic vertebra, metacarpus, and metatarsus. The proportion of rats with abnormal skeletal development was calculated (Hoshi *et al* 1985) In the statistical method,student-T-test and Mann-Whitney Wilcoxon rank sum tests were used.

RESULTS

1. Male rats

The treated animals did not show any abnormal responses. As indicated in Fig. 1, the body weight changes of the treated groups and control groups did not have any reduction trend, and

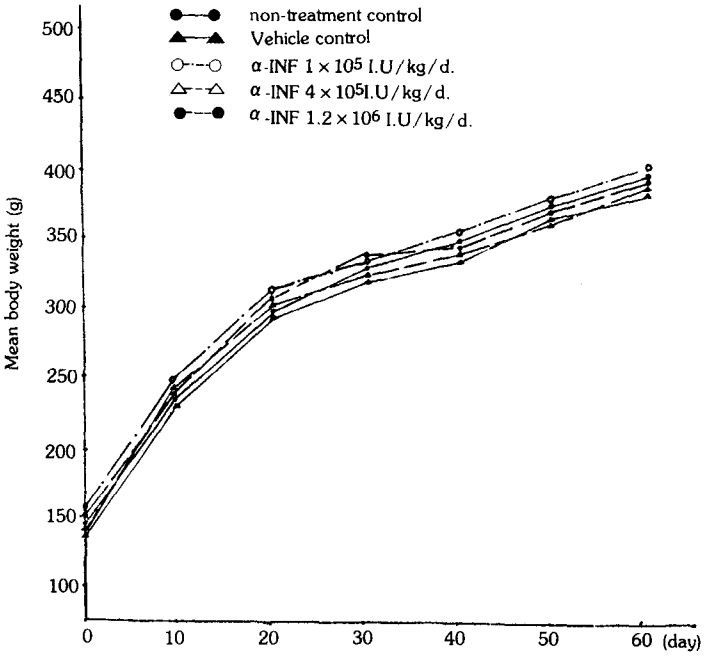

Fig. 1. Mean Body weight changes of male rats before mating.

there were no abnormal effects on the reproductive system and the other whole organs which were examined in copulated males. Testicular weights of the treated groups and the control groups also did not show any significant differences. (Table 1).

2. Female rats

During the test, as indicated in Fig. 2 and 3, the body weight changes of the treated groups and the control groups in pre-gestation and post-gestation period did not show any significant differences as well.

There were no anomalies in non-pregnanted rats after the twentieth day of mating, grossly.

Results of the spleen weight changes in each group, as indicated in Table 3, showed remarkable significant differences between non-treated group and vehicle-treated group.

Table 1. Testicular weights of males (F_0) after mating period.

Dose IU/kg/day	Control	vehicle control	1×10^5	4×10^5	1.2×10^6
No. of animal	19[a)]	19[b)]	20	20	19[c)]
Total	3.4472 ±0.065	3.4466 ±0.058	3.4679 ±0.072	3.4642 ±0.042	3.3727 ±0.070
Testis Right	1.7179 ±0.035	1.7168 ±0.030	1.7543 ±0.028	1.7259 ±0.024	1.6862 ±0.037
Left	1.7293 ±0.031	1.7298 ±0.032	1.7135 ±0.051	1.7383 ±0.023	1.6865 ±0.033

. Mean + S.E
. a)c): Not possible to mate even with cross mating.
. b) : Sacrificed due to mycoplasma symptoms.
. Not significantly different.

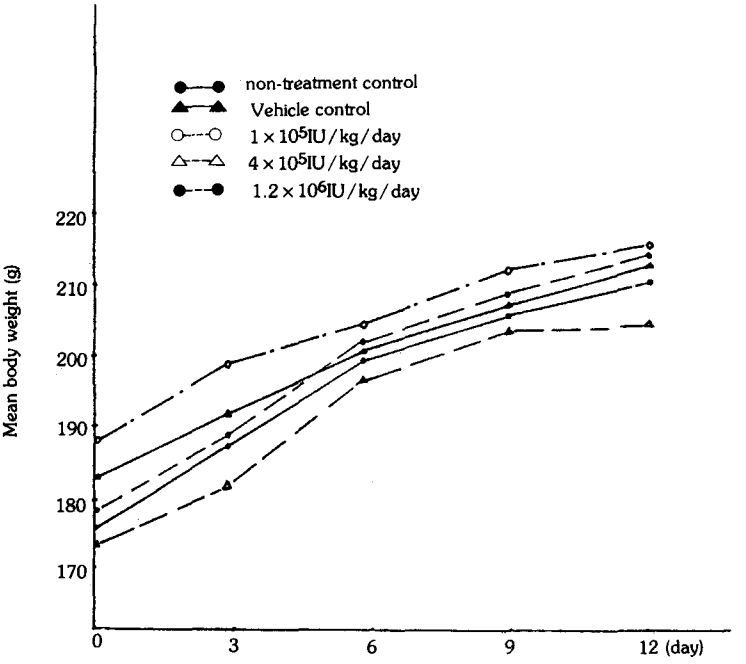

Fig. 2. Mean Body weight changes of female rats before mating.

3. Reproductability and affection to the fetus

Data of reproductability, which was presented in Table 2, showed decrease of gestation, but statistical probability was not recognized.

As a general rule, 89% of the rate of pregnancy were approved in normal conditions. (Neubert et al 1977)

It was not confirmed that estrus cycle changes were not altered from pre-gestation to post-gestation periods (Table 2).

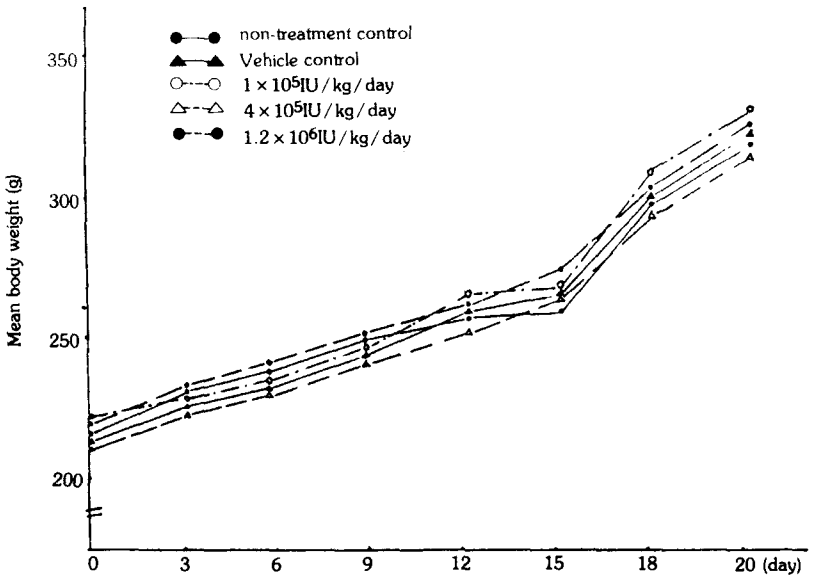

Fig. 3. Mean Body weight changes of dams during gestation period.

Table 2. Influence of α-interferon on reproductive ability in fertility study

Dose IU/kg/day	Control	Vehicle c.	1×10^5	4×10^5	1.2×10^5
(male) No. of male rats examined	20	19	20	20	20
No. of copulated rats	19	19	20	20	19
copulation rate (%)	95	100	100	100	95
No. of pregant rats	19	18	18	19	16
Pregnancy rate (%)	95	94.7	90	95	89
(female) No. of female rats examined	20	20	20	20	20
Estrus cycle Pretreatment	4.0±0.1	4.0±0.0	4.0±0.1	4.0±0.1	4.0±0.1
treatment	4.0±0.1	4.0±0.1	4.0±0.0	4.0±0.1	4.0±0.1
No. of copulated rats	20	20	20	20	20
Copulation rate(%)	100	100	100	100	100
No. of pregnant rats	19	18	18	19	16
Pregnancy rate(%)	95	94.7	90	95	89

The percentage was calculated in the abscence of dead animals.

Table 3. Mean spleen weights of dams.

Dose IU/kg/day	Control	vehicle control	1×10^5	4×10^5	1.2×10^6
No. of dams	19	18	18	19	16
Mean ± S.E	0.7874 ±0.028	0.8667 ±0.063	* 0.9139 ±0.043	* 0.7042 ±0.026	* 0.7481 ±0.32

*$p<0.05$ VS control

Table 4. Influence of α-Interferon on Embryonic Development.

Dose (IU/kg/day)	control	Vehicle C.	1×10^5	4×10^5	1.2×10^6
No.of dams	19	18	18	19	16
Maternal mortality	0	1	0	0	2
No.of corpora luteum	284	243	252	265	241
Mean+S.E	14.94±0.408	*13.50±0.556	14.00±0.45	*13.90±0.27	15.06±0.56
Total Implantation	239	229	229	236	189
Mean+S.E	12.58±0.520	12.72±0.630	12.72±0.311	12.42±0.579	11.81±0.825
No.of undeveloped embryo	5	9	10	5	11
Mean±S.E	0.26±0.129	0.50±0.232	0.55±0.166	0.26±0.129	0.68±0.678
early stage a)	4	8	10	5	11
late stage b)	1	1	0	0	0
Undevelopment rate (%) c)	2.09	3.93	4.36	2.11	*5.80
No.of dead fetuses (%) d)	2(0.83)	2(0.87)	2(0.87)	0(0.00)	2(0.83)
No.of live fetuses (%) e)	232(97)	216(95)	216(95)	227(96)	185(98)
Mean±S.E	12.21+0.585	12.00+0.583	12.00+0.388	11.94+0.492	11.56+0.428
Live fetuses rate (%) f)	96.79±0.015	*95.17±0.027	94.22±0.014	96.84±0.014	97.88±0.015
No.of female fetuses(%) g)	122(55.0)	98(44.5)	96(46.4)	102(45.7)	104(57.2)
No.of male fetuses (%) h)	116(49.26)	115(54.33)	120(55.44)	125(54.21)	81(42.75)
Sex ratio (M:F)	1.26	1.41	1.39	1.42	0.93
Mean fetal weight (g): M	4.36±0.59	4.04±0.36	3.94±0.37	4.04±0.28	4.05±0.32
: F	3.98±0.54	3.70±0.34	8.58±0.56	4.01±0.34	3.75±0.40
Placental weight (g) I)	8.66±0.401	7.98±0.501	8.58±0.56	8.30±0.33	8.04±0.58
Implantation rate (%) Mean+S.D	84±0.14	92±0.11	92±0.07	90±0.08	84±0.13

a) early stage: Implantation site, placental remnant, early resorption
b) late stage: late Resorption, macerated fetuses
c) No.of undevelopment/No of implantation
d) No.of dead fetuses/No of implantation
e),f) No.of live fetuses/No of implantation
g),h) No.of ♀♂ fetuses/No of ♀♂ fetuses
I) Placental wt./One litter
* $p<0.05$ vs control
Not significantly different from the saline control.

Photo. 1. Control
Lateral ventricle dilatation

Photo. 2. Vehicle control (Saline)
Pelvic dilatation

Photo. 3. rHuIFN-αA, 1×10^5IU/kg
Littleness rib (Right)

Photo.4. Vehicle control (Saline)
Lumbar rib

Photo. 5. Vehicle control (Saline)
Fission of sternebrae

In the observation of the later phases of gestation, as totally arranged Table 4, there were any significant differences in the rate of implantation, viable and dead fetuses, sexes, and placental weights.

But, the number of corpora luteum showed significant difference between vehicle-treated groups and medium dose treated groups.

Table 5. Malformation of fetuses born of dams administered α-interferon.

Dose (IU/kg/day)	control	vehicle. c.	1×10^5	4×10^5	1.2×10^6
External (%)	1/232(0.43)	0/216(0.00)	0/216(0.00)	1/227(0.44)	1/185(0.54)
Edema	1	0	0	0	1
lack of tail	0	0	0	1	0
Internal (%)	2/121(1.65)	2/112(1.78)	0/119(0)	7/115(6.08)	3/94(3/19)
lateral ventricle dilatation	1	0	0	3	!
Third ventricle dilatation	1	0	0	0	0
pelvic dilatation	0	2	0	4	2
Skeletal	4/111(3.6)	8/104(7.6)	7/ 97(7.2)	2/112(1.7)	1/91(1/09)
fission of thoracic v. center	1	0	0	0	1
lack of thoracic v. sacral v. caudal v. rib	0	0	0	1	0
little rib of 13th rib	0	2	2	0	0
fission of sternebrae	3	6	5	1	0

Table 6. Effects on skeletal development in fetuses

Dose (IU/kg/day)	Control	Vehicle C.	1×10^5	4×10^5	1.2×10^6
No. of fetuses examined	111	104	97	112	91
No.of fetuses with skeletal vertebrae (%)	9(8.1)	*17(16.3)	*22(22.6)	**20(17.8)	*15(16.4)
Dumbell type of thoracic vertebrae	1	3	10	14	5
Asymetry of sternebrae	1	1	1	0	10
Lumber rib	7	13	10	6	10
Dumbell type of lumbar vertebrae	0	0	1	0	0
State of ossification (Mean + S.E)					
Cervical vertebra Arch	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0
Body	0.19±0.03	0.16±0.04	0.29±0.06	0.18±0.04	0.18±0.05
Thoracic Vertebra Arch	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0
Body	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0
Lumbar vertebra Arch	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0
Body	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0
Caudal vertebra	3.03±0.14	**3.48±0.11	3.35±0.15	**3.70±0.08	2.98±0.14
Rib	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0
Metacarpus	5.98±0.25	6.66±0.14	6.26±0.28	7.25±0.11	6.19±0.15
Metatarsus	5.00±0.34	7.49±0.14	6.29±0.29	7.66±0.07	5.87±0.32
Sternebra	4.15±0.10	**4.55±0.07	4.10±0.14	4.38±0.07	3.06±0.10

**$p < 0.01$ vs control, *$p < 0.05$ vs control, .$p < 0.01$ vs vehicle, ..$p < 0.05$ vs vehicle.

The rate of resorbed embryos showed significant difference in high dose treated group in comparison to non-treated groups, but it was not confirmed to vehicle treated groups. Baker et al (1980) confirmed as common data such a degree of variation.

In the external anomalies of fetuses, only one fetus among 227 fetuses had no tail in the medium dose treated group, one of the 185 fetuses in high dose treated group was also observed as congestion, the rest were normal.

The rate of external anomalies generally approved as normal level. In observed results of fetal internal organs, lateral ventricle dilatation (Photo 1), 3rd ventricle dilatation, renal pelvic dilation (Photo 2), the non-treated group, significant differences were not confirmed (Table 5). Synostosis of sternebral (Photo 3), shortening of the rib (Photo 4) and fission of sternebrae (Photo 5 was observed in each group overall. Absence of thoracic, lumbar, sacral, coccygeal vertebrae and sternum, rib were also observed without showing the significant difference (Table 5).

The ratios of skeletal variation were observed with a great significant difference in vehicle-treated group and all of the test groups. As compared with the vehicle-treated group, all of the test groups did not show the probabilities (Table 6).

In the rate of ossification, the vehicle and medium dose treated group showed more ossified pattern than the non-treated group, and there was no significant difference between vehicle treated group and medium treated group. In the case of thoracic vertebrae, all of the treated group did not represent the significant differences in comparison with the non-treated group except for the vehicle-treated group.

DISCUSSION

During the test period, one rat of the vehicle-treated group was diagnosed as mycoplasmosis, therefore it was excluded. Non-copulating two rats, which were each in high dose treated and non-treated group were autopsied, and were diagnosed each as submeningial hemorrhages and scrotal atrophy, which were considered that kinds of symptoms did not derived from the effect of the test material in two males. One veicle treated rat and two high dose treated rats were dead during the test periods. The former was diagnosed as purulent pneumonia and the latter were diagnosed as corneal inflammation by mycoplasmosis and fibrosarcoma in the dermal area. Even though there are different thoughts, as showed in Table 3, probability between non-treated group and veicle treated group is not much of consideration. The reason is that pathological abnormalities did not exist.

In the number of corpora luteum, significant differences between vehicle treated group and medium dose treated group which was statistically approved did not indicate the problem of test materials, because any significant differences could not be found between high dose treated and non-treated groups.

REFERENCE

Baker, H.J., Ldndsey, J. rR. and Weisbroth, S.H. (1980): The Laboratory rat (Vol. 2), Research Applications, Academic Press, 77 page.

Hoshi, N., Ueno, K., Lgarashi, T., Kitagawa, H., Fujita, T., Ichigawa, N., Kondo, Y. and Isoda, M. (1985): Teratological study of Hydroxypropyl methyl-cellulose acetate succinate in rabbits. J. Toxicol. Sci. vol. 10, Supplement II; 187-201.

Inouye, M.(1976): differential staining of cartilage and bone in fetal mouse skeleton by alcian blue and alizarin red S, Cong. Anam., 16: 171-173.

Neubert, D., Merker, H.J. and Kwasigroch, T.E. (1977): Methods in Prenatal Toxicology, Evaluation of Embriotoxic Effects in Experimental Animals, Teratology Workshop, April 1977, Berlin, Georg Thieme Publishers Stuttgart, 81P.

Wilson, J.G. (1965): *Teratology-Principles and Techniques (eds. Wilson, J.G., Warkany, J.,)*, Univ. Chicago Press.: 262-277.

Aliverti, V. and Bonanomi, L. (1979): The Extent of Fetal Ossitication as an Index of Delayed Development in Teratogenic Studies on the Rat. Teratology, 20: 237-242.