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PERI-NATAL AND POST-NATAL STUDY OF THE RECOMBINANT HUMAN INTERFERON αA (rHuIFN-αA) IN RATS

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ABSTRACT: *A Peri-and Postnatal Study was Carried out to examine the effects of rHuIFN-αA, produced by gene-manipulated E. coli, on offsprings of Wistar rats. The substance was administered intraperitoneally to dams at dose levels of 1×10^5, 4×10^5 and 1.2×10^6 I.U/kg/day during the period from day 17 of gestation to day 21 after delivery. All the pregnant dams were allowed to deliver naturally, and the postnatal development of the offsprings was observed. No noticeable toxic effects and pathological changes on dams were observed, and no detectable variations in postnatal development of offsprings occured.*

Key words: *rHuIFN-αA, peri-and postnatal study.*

INTRODUCTION

The present paper deals with the effect of rHuIFN-αA on offsprings by oral administration of rHuIFN-αA to the dams during peri-and postnatal periods. This study was carried out in accordance with the "Guidelines for the Test Method of Specific Toxicity Test on Drugs (KNIH, 1985)" issued by the Ministry of Health and Welfare, Korea National Institute of Health.

MATERIALS AND METHODS

Materials

The injectable rHuIFN-αA which was produced by Genetic Engineering Division, Cheil Sugar Co., Ltd., was used, and dose level was 2×10^7 I.U./vial.

Methods

Male rats, 9 weeks of age (283-313g), and female rats, 9 weeks of age (200-232g), of Wistar strain were used under the approval of specific pathogen free-rats by serological tests.

The animals were housed in polycarbonate cages (26 × 42 × 18cm) bedded with autoclaved wood-shavings at an ambient temperature of 23 ± 3°C, and a relative humidity of 55 ± 10%.

The ventilation of the breeding room was maintained in order to get optimal air condition and the room was lighted by 12 hours photoperiod. They were allowed free access to Sam-Yang Laboratory Animal Diet (Sam-Yang Feedstuff Co., Korea) and water bottles. Water was changed daily. Bottles and cages were autoclaved every three days.

After the acclimatization and quarantine for one week, each female was paired with male at 1:1 basis. The mating was confirmed by the presence of vaginal plugs and sperm in vaginal smear on the next day morning (9:00 A.M.); the day was determined to be day 0 of gestation.

The pregnant rats were divided into five groups and kept individually in a cage.

The agents (2×10^7 I.U/vial) were diluted subsequently with saline into 3 dose levels; 1.2×10^6, 4×10^5 and 1×10^5 I.U/0.1 ml. Each rat in 3 treated groups was administered intraperitoneally once a day per kg. body weight during the period from day 17 of gestation to day 21 after delivery. The rats of vehicle control were administered with the same volume of saline, and the rats of non-treatment control were not administered.

All dams in each group were allowed to deliver naturally and to nurse to day 21 afterward.

Observations:

Maternal

1) Throughout the experiment, all visible responses of treated animals were observed once a day; abnormalities on delivery, gestation periods, lactation dams, nursing instinct, and effects of drug or its metabolites on neonates through dams, etc.
2) Body weights were recorded once a week during the acclimatization period, once at mating, nine times on days 0,7,9,11,13,15,17,19 and 20 during gestation and six times on days 1,3,6,11,16 and 21 after delivery.
3) After the weaning period, all dams were autopsied on day 22 for gross and histological examinations.

Offsprings

1) At birth, mortality rate of newborns was calculated. Dead fetuses were observed for external abnormalities and were stained with alcian blue-alizarin red S double staining method (Simons, E.V., *et al.*,971; Inouye, M., 1976; Aliverti, V., *et al.*,1979) for skeletal examination and viable fetuses were observed for sex and external abnormalities.
   The weaning period was determined as 21 days.
2) As for the survival rate of fetuses, the number of survived fetuses was recorded on days 1,3,6,11,16 and 21 after delivery. Nursing rates were calculated on day 7 versus the numbers of viable fetuses at birth, and on day 21 versus that of on day 7.
3) During the test period, all visible responses in behavior and signs were observed once a day, and body weights were measured on days 1,3,11,16 and 21 after delivery.
4) Eruption of incisor teeth at about the day 11 and Separation of eyelids at about the day 15 were observed (Imoto, S. *et al.*, 1985; Buelke-Sam, J., *et al.*, 1970; Butcher, R.E., *et al.*, 1979; Vorhees, C.V., *et al.*, 1979; Vorhess, C.V., *et al.*, 1981).

Data were analyzed statistically by one-way ANOVA and Student's t-test. The differences between treated groups and control groups were estimated at the levels of 95% ($p<0.05$) and 99% ($p<0.01$).

RESULTS

1) General condition and mortality of dams.

No remarkable toxic effects were detected in treated groups. One dam of low dosage group died from dystocia. It was excepted from the experiment. No abnormal behavior and signs were also observed in both treated groups and control groups.

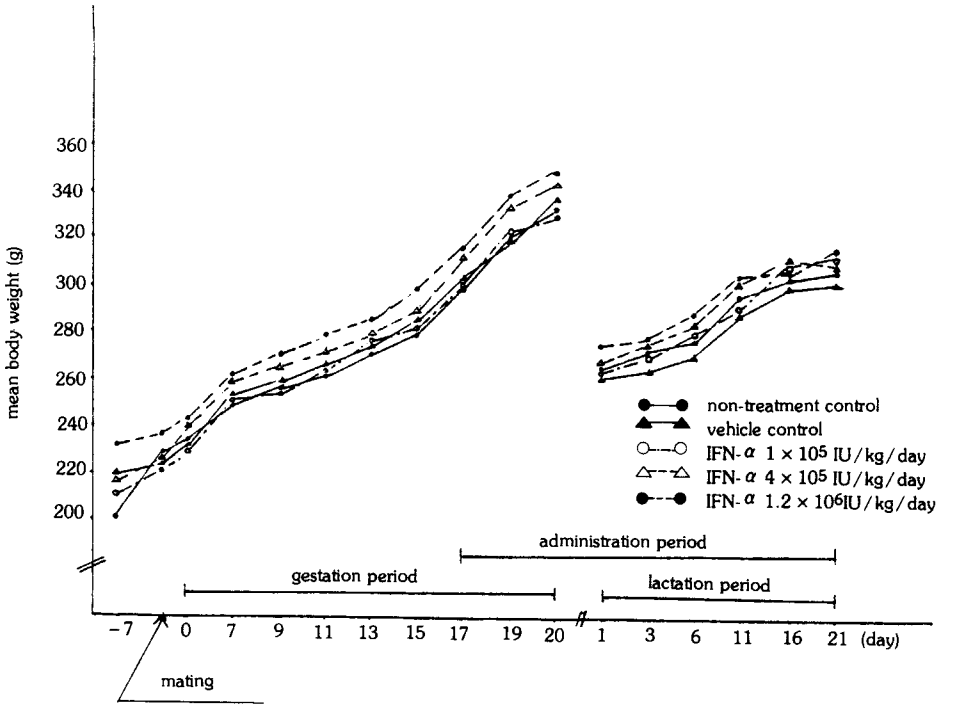

Fig. 1. Body weight changes of dams treated intraperitoneally with α-Interferon in peri and postnatal test.

No difference was shown in the gestation period.

2) Maternal body weights.

The body weight changes in treated groups and control groups were shown in Fig. 1 during gestation and lactation periods. No difference was found between treated groups and control groups.

3) Pathological findings.

No pathological changes of organs were observed.

4) Skeletal abnormalities of dead newborns.

No external abnormalities were observed.

Six of ten dead newborns in non-treatment control, five of nine in vehicle control, seven of twelve in low dosage group, five of ten in middle dosage group, and sixteen of seventeen in high dosage group were examined for skeletal variations (Table 1). The rest which were lost in each group may be canibalized by dams.

One case of lumbar rib was seen in vehicle control (photo 1). In high dosage group, one case of lumbar rib only (photo 2), one case of costal malformations only (photo 3), and two cases of lumbar rib with costal malformations (photo 4) were seen. These four malformed fetuses in high dosage group were a litter.

There was no significant difference between treated and control groups.

Table 1. Delivery and nursing aspects of youngs of rats after intraperitoneal administration of IFN-a from day 17 of gestation to day 21 after delivery.

Dose (IU1kg1day)		non-treatment	vehicle control	1 ± 10^5	4 ± 10^5	1.2 ± 10^6
No. of dams (Fo)		19	20	19	19	20
Gestation period (day). mena ± S.D.		21.8 ± 0.42	21.4 ± 0.50	21.7 ± 0.45	21.7 ± 0.48	21.7 ± 0.49
Litter size,[a]	mean ± S.D.	10.8 ± 3.32	11.0 ± 2.90	10.0 ± 3.25	10.7 ± 3.71	11.1 ± 3.30
Live born	Total	203	218	196	200	219
	male	106	109	107	93	117
	female	97	109	89	107	102
Dead born		3	2	5	3	2
Sex ratio (male1female)[b]		1.09	1.00	1.20	0.87	1.15
Viability index at birth(%)[c]		98.54	99.09	97.75	98.52	99.10
1 day	male	104(2)[d]	109	107	93	116(1)
	female	97	109	89	107	99(3)
3 day	male	103(1)	107(2)	102(5)	91(2)	113(3)
	female	95(2)	105(4)	88(1)	104(3)	92(7)
6 day	male	101(2)	107	102	91	113
	female	95	104(1)	88	103(1)	92
11 day	male	101	107	102	91	113
	female	95	104(1)	88	103(1)	92
16 day	male	101	107	102	91	112
	female	95	104	87(1)	102(1)	92
21 day	male	101	107	102	91	112
	female	95	104	87	102	92
Nursing rate(%)	at 6 day,[e] mean ± S.D.	97.0 ± 7.52	97.2 ± 4.66	97.4 ± 5.91	97.3 ± 4.11	93.7 ± 11.73
	at 21 day.[F] mean ± S.D.	100.0 ± 0.00	100.0 ± 0.00	99.2 ± 0.00	99.6 ± 1.64	99.4 ± 2.48
External anomaly		0/206	0/220	0/201	0/203	0/221
Skeletal Variations		0/6	1/5	0/7	0/5	4/16

a) : Total borns (live + dead born)/litter.
b) : No. of male young/No. of female youngs.
c) : (No. of live borns/total borns) × 100.
d) : Each value in parenthesis represents the No. of dead newborn.
e) : Percentage of live youngs vs birth.
f) : percentage of live youngs vs 6 day.

5) Litter size and body weights of offsprings.

Although there is no significant difference between treated and control groups, litter size was slightly increased in high dosage group, but slightly decreased in low dosage group. Otherwise, body weights were significantly decreased in females of high dosage group vs. non-treatment control on day 1 and 15 ($P < 0.05$), and significantly increased in low ($P < 0.01$) and middle dosage ($P < 0.05$) groups (Table 2, Fig. 2)

6) Survival rates.

Viable rates at birth, and nusing rates on day 6 and 21 did not show any probabilities (Table 1)

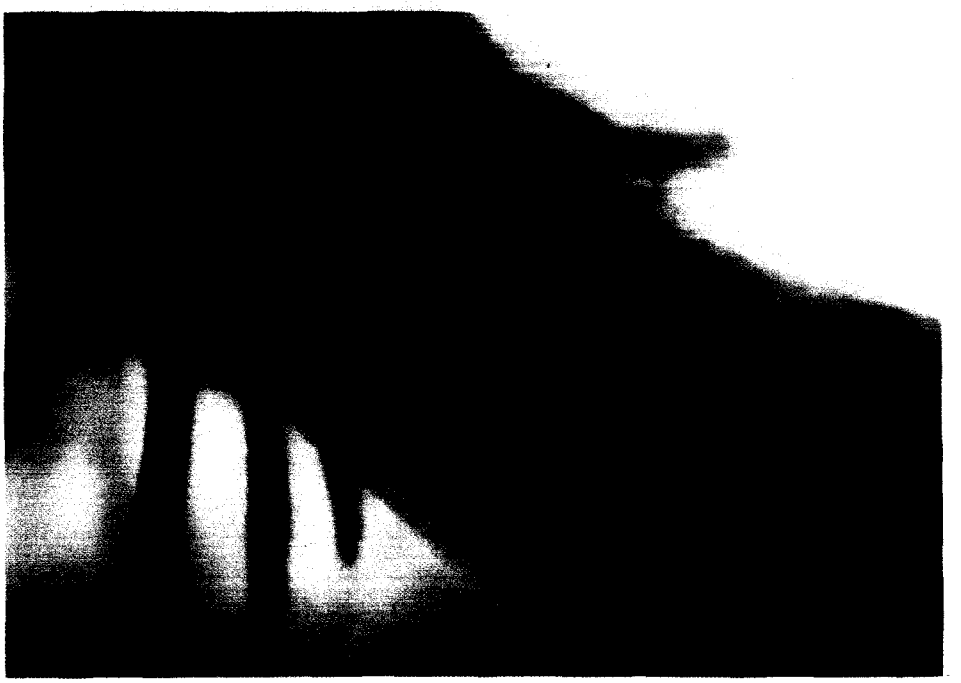

Photo. 1. Vehicle control Lumbar rib

Photo. 2. rHuIFN-αA, 1.2×10^6 IU/kg/day treated group. Lumbar ribs

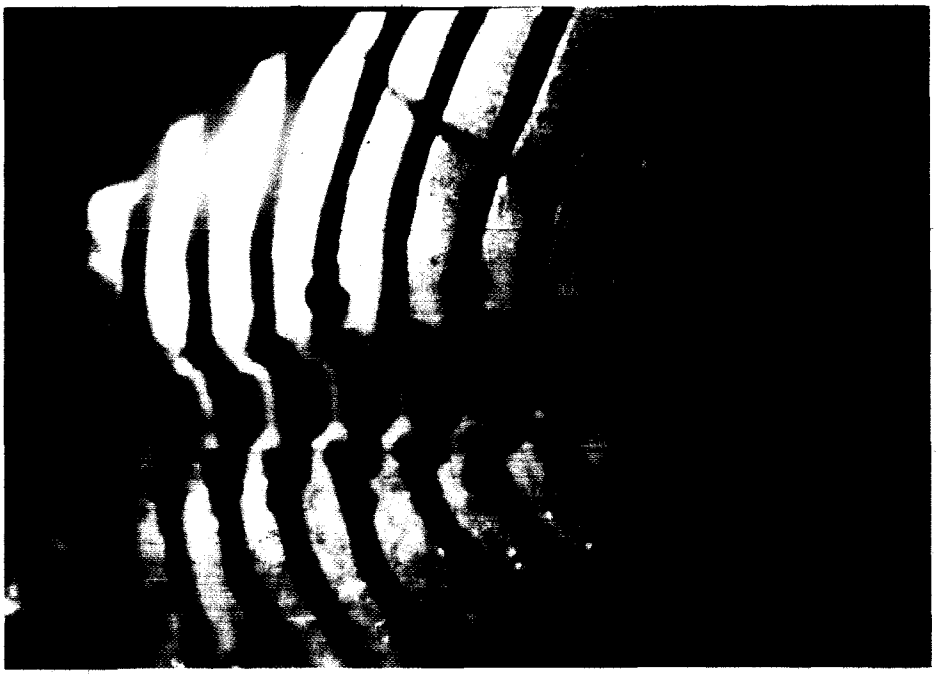

Photo. 3. rHuIFN-αA, 1.2×10^6 IU/kg/day treated group. Costal malformations

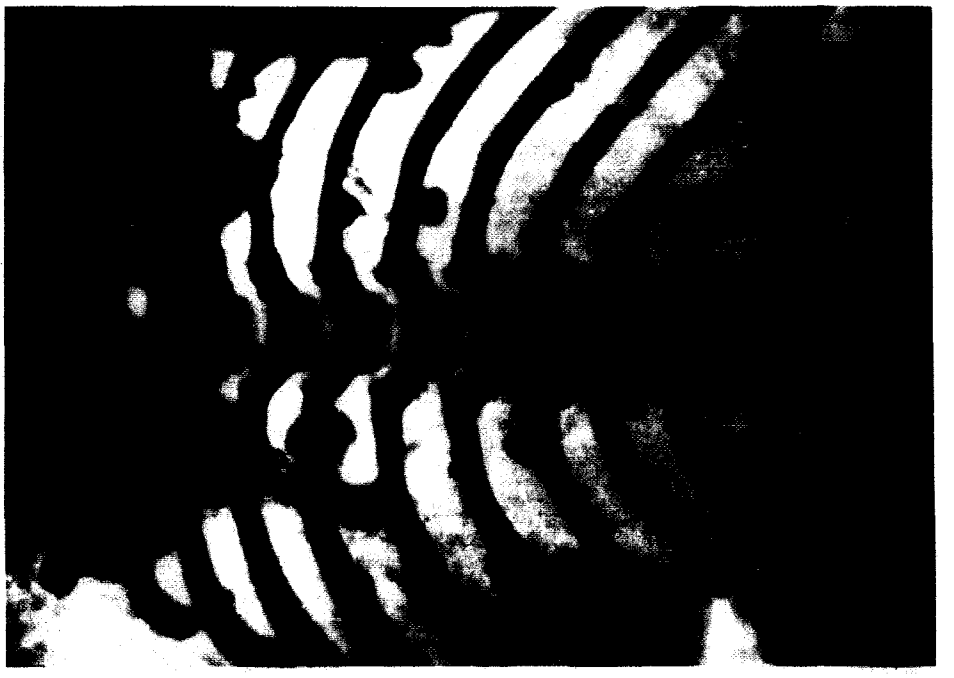

Photo. 4. rHuIFN-αA, 1.2×10^6 IU/kg/day treated group. Lumbar and costal malformations

Table 2. Effects of IFN-a on postnatal development of young (F1) from dams administered with IFN-a intraperitoneally from day 17 of gestation to day 21 after delivery.

Dose (IU/kg/day)	non-treatment control		vehicle control		1×10^5		4×10^5		1.2×10^6	
Sex	male	female	male	female	male	female	male	female	male	female
1 day	6.50 ±0.518	6.29 ±0.561	6.43 ±0.743	6.08 ±0.636	6.95 ±0.561	6.63^{+} ±0.655	7.31^{*+} ±1.511	6.52 ±0.878	6.52 ±0.881	5.79* ±0.759
3 day	8.63 ±1.013	8.18 ±0.927	8.23 ±1.149	7.82 ±1.003	9.32^{+} ±0.844	8.87^{*+} ±0.836	9.01^{+} ±1.622	8.52^{+} ±1.165	8.32 ±1.089	8.06 ±1.012
6 day	12.12 ±1.464	11.89 ±1.691	11.80 ±1.759	11.41 ±1.692	13.99** ±1.399	13.29** ±1.421	13.15^{+} ±2.233	12.46^{+} ±1.867	12.07 ±1.495	11.85 ±1.197
11 day	20.47 ±3.112	20.11 ±3.262	19.60 ±3.231	18.59 ±2.222	23.56^{**++} ±2.851	22.50^{**++} ±2.684	21.97^{+} ±3.411	20.94^{+} ±3.248	20.14 ±2.497	19.85^{+} ±1.950
16 day	28.57 ±5.498	28.52 ±5.831	27.08 ±4.787	26.58 ±5.283	32.78^{**++} ±3.731	31.37^{+} ±3.728	31.79^{*+} ±5.314	29.19 ±4.258	28.44 ±3.698	27.32 ±3.991
21 day	37.58 ±8.672	38.12 ±7.506	36.37 ±7.207	35.92 ±7.558	43.13^{*++} ±4.853	42.71^{*+} ±4.633	39.64 ±8.654	36.71 ±6.932	38.69 ±5.545	36.71 ±5.747
Eruption of incisor teeth (day), mean ± S.D.	10.9 ± 0.45		11.1 ± 0.52		10.7 ± 0.42		10.7 ± 0.43^{+}		10.8 ± 0.52^{+}	
Separation of eyelids (day), mean ± S.D.	15.2 ± 0.31		15.6 ± 0.47		15.1 ± 0.29		15.2 ± 0.37		15.5 ± 0.94	

a) : mean ± S.E calculated from mean values per litter.

* : Significantly different from non-treatment control ($p < 0.05$), **: ($p<0.01$).

$^{+}$: Significantly different from vehicle control ($p<0.05$), $^{++}$: ($p<0.01$).

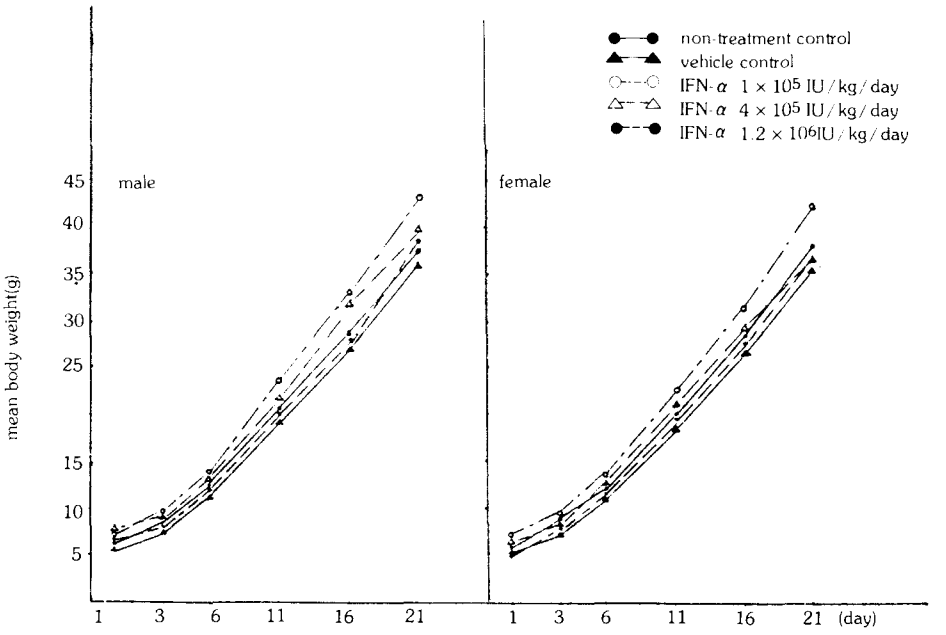

Fig. 2. Growth curves of male and female offsprings in pre and postnatal test.

7) Physical development.

As to the postnatal development, the dates of eruption of incisor teeth in all treated groups were significantly early ($P < 0.05$) compared to vehicle control, but not nontreatment control. No difference in eye openings was observed among group (Table 2).

8) Discrimination.

In view of the whole data, this test meterial is not toxic substance in adult rats and their offsprings.

DISCUSSION

The present study was carried out to examine the effect of rHuIFN-αA, produced by gene-manipulated E. coli, on offsprings of rats.

The substance was administered intraperitoneally to dams at dose levels of 1×10^5, 4×10^5 and 1.2×10^6 I.U/kg/day during the period from day 17 of gestation to day 21 after delivery.

No noticeable toxic effects on the nursing ability of dams were detected. No pathological changes of organs were also observed.

No remarkable variations in survival rates of offsprings were observed. Litter size was slightly increased in high dosage group, but slightley decreased in low dosage group. Otherwise, body weights were significantly increased in low dosage group, but slightly decreased in females of high dosage group.

As to postnatal development, the dates of eruption of incisor teeth in all treated groups were significantly early compared to vehicle control, but not non-treatment control.

From the results mentioned above, it might be considered that α-interferon has none of toxic effects in rats and their offsprings.

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