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TERATOGENIC STUDY OF THE RECOMBINANT HUMAN INTERFERON-αA(rHuIFN-αA) IN RABBITS

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ABSTRACT: *A teratogenic study was carried out on New zealand White rabbits in order to examine the teratogenic potentiality of the recombinant human interferon-αA(rHuIFN-αA), an available therapeutic agent. The rHuIFN-αA was intravenously administered at dose sevels of 1×10^5, 4×10^5 and 1.2×10^6 IU/kg/day for a period of 13 days from day 6 to day 18 of gestation. Two-thirds of the pregnant females in each group were sacrificed on day 29 of gestation and their fetuses were examined. The remaining dams were allowed to litter naturally, and the postnatal develpment of the offsprings was observed. The administration of rHuIFN-αA during a period of organogenesis produced no embryotoxic and teratogenic effects.*

INTRODUCTION

The present paper deals with investigation on the teratogenic potentiality of recombinant human interferon-αA(rHuIFN-αA), as an available therapeutic agent.

The 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.

MATERIALS AND METHODS

Materials

Injectable rHuIFN-αA, which was produced by Genetic Engineering Division, Cheil Sugar Co. Ltd., was used and dose levels were divided into two groups, 1×10^6 I.U/vial and 3×10^6 I.U/vial.

Methods

Female rabbits of New Zealand White strain, 2.60 to 3.25kg of body weight, were used.

Pregnant rabbits which were confirmed mating with male rabbits of the same strain, were identified as healthy animals by the clinical examinations, such as parasitosis-mainly coccidiosis and respiratory infection-mainly snuffle (Fox, *et al*, 1984)

The pregnant rabbits were housed in stainless wire cages at all ambient temperature of $23 \pm 3°C$, and a relative humidity of $55 \pm 10\%$. The ventilation of the breeding room was maintained in order to get optimal air condition, and the room was lighted by 12-hour photoperiod. They were allowed free access to Sam-Yang Rabbit Diet (Sam-Yang Feedstuff Co., Korea) and water. Water was changed two times per day.

Dosage range was determined based on the data of preliminary study in pregnant rats. Sixty rabbits were separated into 5 groups of 12; Group I: non-treated control, Group II: control treated with the vehicle (physiological saline), Group III: 1×10^5 IU/kg/day of rHuIFN-αA was given via ear vein, Group IV: 4×10^5 IU/kg/day of rHuIFN-αA was given via ear vein, Group V: 1.2×10^6 IU/kg/day of rHuIFN-αA was given via ear vein. Each suspension was administered via ear vein to pregnant females for 13 days from day 6 to day 18 of gestation.

Observation Maternal

Pregnancy was identified by body weight gains and palpation technique. Actually, ten to eleven rabblits were pregnated per each group.

Two-thirds of the dams in each group were autopsied by Cesarean section on day 29 of gestation. One-third of the dams in each group were allowed to deliver spontaneously, and all fetuses were examined until the end of weaning period.

During the whole test periods, all symptoms were observed two times per day. Body weights were recorded on 0, 3rd, 6th, 9th, 12th, 18th, 21th, 24th, 27th, and 29th day of gestation.

On day 29 of gestation, Euthanasia was carried out via ear vein with 30 m*l* of air embolus. After the dissection of abdominal area, examined the corpora lutea, placental weights, resorbed fetuses, dead fetuses, the number of implantation, and viable fetuses. Microscopic observation of the tissues were carried out against the abnormal organs.

The distribution in uterine horns, sex, and weights of viable and dead fetuses were recorded individually, and the fetuses were examined for abnormal behaviors and external abnormalities.

On day 30 after birth, maternal rabbits were autopsied, and pathological examinations were carried out.

Fetuses

On day 29 of gestation, whole uteruses were collected by Casarean section. After the dissection of uterus, the colours and volumes of amnion fluids were observed, and the fetuses were carefully wrapped with gauze, arranged in order with marking (Hoshi, *et al*, 1985). The changes of fetal weights were measured. The thoracic and abdominal organs of all fetuses were examined on their position, color and form.

One-third of the fetuses per litter were used for internal organ examinations and the rest was used for skeletal examinations.

One-third of the fetuses was examined for visceral abnormalities: mainly in head, kidneys, liver, and heart and vasculature. Fetuses were fixed in formalin solution, and examined by New Toxicity Test Method (Shirasu and Hayama, 1985) under the vertical microscope.

The remaining two-thirds per litter were stained with alizarin red S solution, and examined for skeletal abnormalities (Dawson, 1926). Samples in glycerin solution were examined under the vertical microscope.

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

Maternal

There were no abnormal findings in animals during the experiment in regard to their behaviors. No abortion and body weight variations was observed in all groups (Table 1, Fig. 1).

Table 1. Influence of α-INF on embryonic development (F_1) in teratogenicity study.

Dose (IU/kg/day)	control	Vehicle control	1×10^5	4×10^5	1.2×10^6
No.of mothers	12	12	12	12	12
Dead slaughter	1	3	1	1	2
Infertility	2	0	2	2	1
Abortion	0	0	0	0	0
Total mother with of all resorption (Mean ± S.D.)	0	0	0	0	0
Total number of Corpora luteum (Mean ± S.D.)	49 (8.2 ± 1.6)	52 (8.7 ± 1.7)	49 (8.2 ± 0.7)	48 (8 ± 1.6)	51 (8.5 ± 0.8)
Total implantation (Mean ± S.D.)	44 (7.3 ± 1.1)	46 (7.7 ± 1.1)	46 (7.7 ± 0.5)	43 (7.2 ± 1.1)	48 (8 ± 1)
Total resorption	6	9	5	7	6
Early	4	7	4	6	5
Late	2	2	1	1	1
Total dead	1	2	0	0	1
Total live (Mean ± S.D.)	37 (6.2 ± 1.1)	35 (5.8 ± 1.3)	41 (6.8 ± 0.9)	36 (6 ± 2)	41 (6.8 ± 0.9)
Sex male	21	16	19	19	21
female	16	19	22	17	20
male/female	1.3	0.8	0.9	1.1	1.1
Mean fetal weight Male	41.20 ± 3.24	39.66 ± 4.43	41.61 ± 4.34	35.41 ± 4.69	45.67 ± 4.30
Female	41.12 ± 4.11	39.68 ± 4.35	41.17 ± 3.43	36.39 ± 3.56	41.30 ± 5.30
Mean placental weight (Mean ± S.D.)	5.67 ± 0.57	6.09 ± 0.34	6.11 ± 0.71	5.73 ± 0.67	5.88 ± 1.07

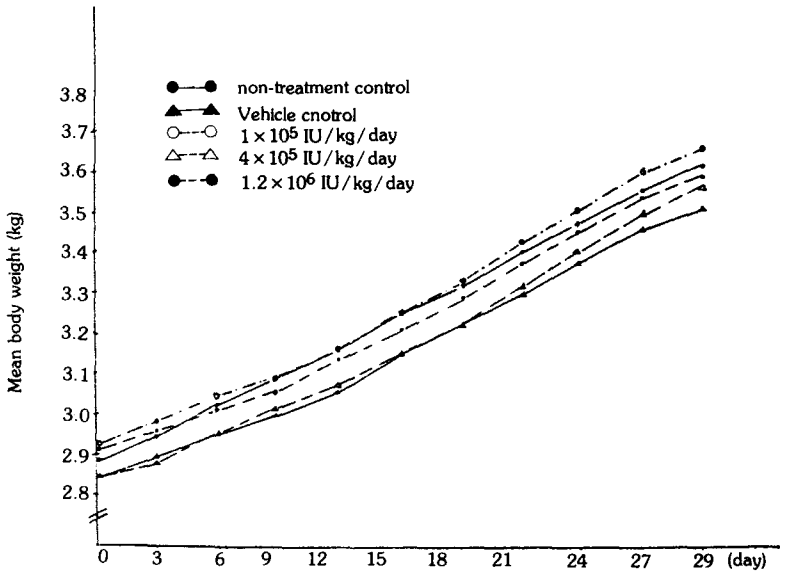

Fig. 1. Body weight changes of dams treated intravenously with α-interferon in teratogenicity study.

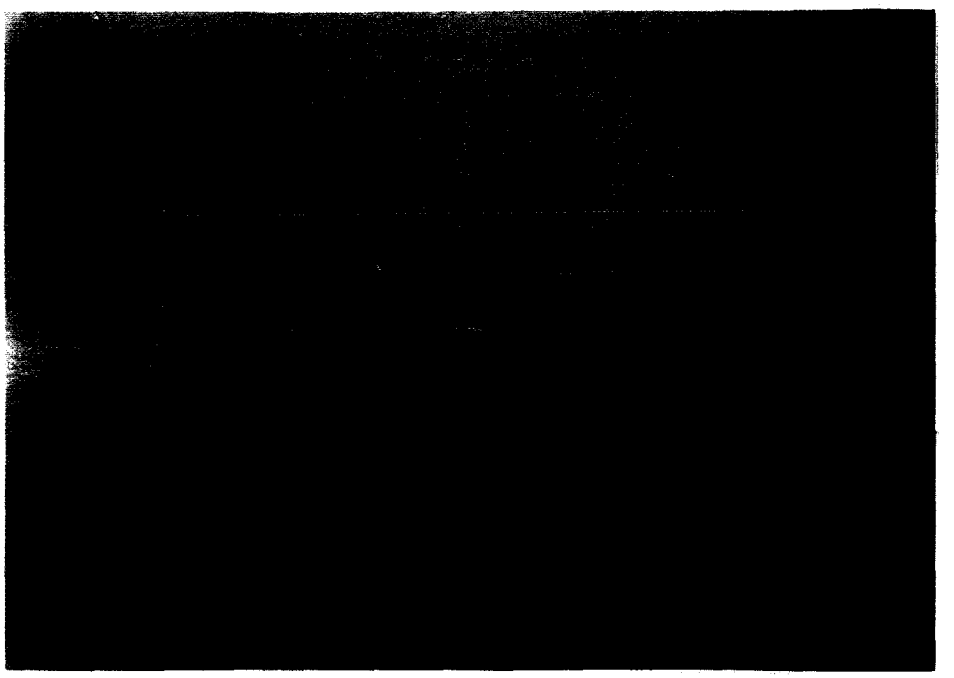

Photo. 1. Vehicle control
Early absorption

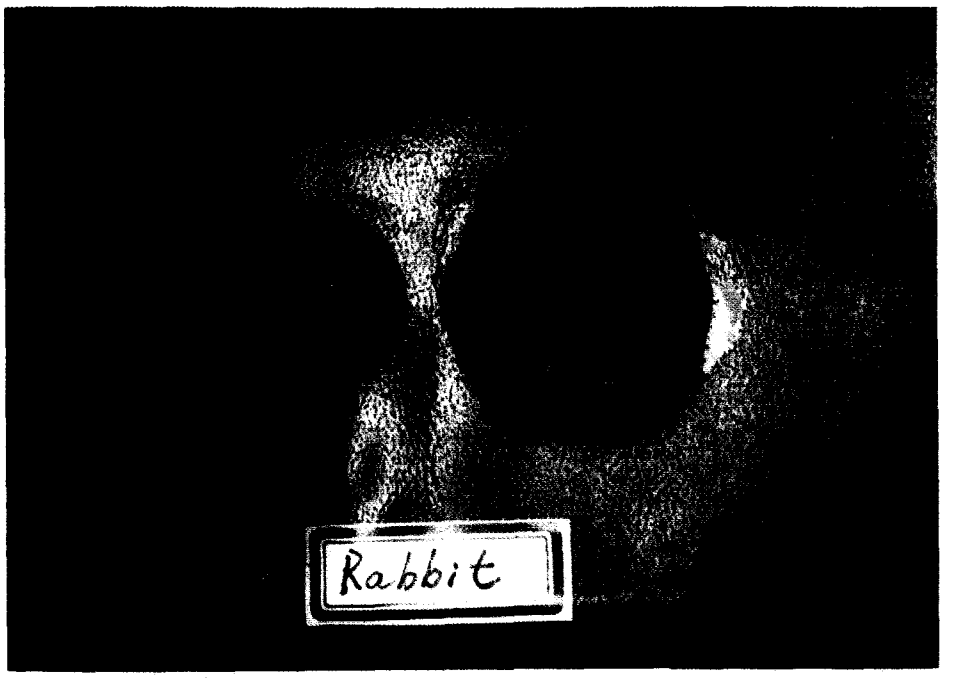

Photo. 2. Vehicle control
Late absorption(left) and normal placenta(right)

Photo. 3. rHuIFN-αA 4×10^5 IU/kg treated group.
Late absorption

Photo. 4. Control
Undeveloped embryo

Table 2. Malformations of fetuses (F_1) born of the dams intravenously or intraperitoneally administered α-INF.

Dose (IU/kg/day)	Control	Vehicle Control	1×10^5	4×10^5	1.2×10^6
External(%)	0/37(0.00)	0/35(0.00)	0/41(0.00)	0/36(0.00)	0/41(0.00)
Internal(%)	0/13(0.00)	0/12(0.00)	0/14(0.00)	0/12(0.00)	0/14(0.00)
Skeletal(%)	0/24(0.00)	0/23(0.00)	0/27(0.00)	0/24(0.00)	0/27(0.00)

Table 3. Influence of α-INF on skeletal development in rabbit fetuses (F_1)

Dose (IU/kg/day)		Control	Vchicle control	1×10^5	4×10^5	1.2×10^6
No.of fetuses examined		24	23	27	24	27
No.of fetuses with skeletal variation		0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Degree of ossification	cervical vertebrae	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0
	thoracic vertebrae	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0	13.0 ±0.0
	lumber vertebrae	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0	7.0 ±0.0
	Lack of sternebrae	3	2	1	1	2
	Caudal vertebrae	16.27±0.23	16.06±0.16	16.23±0.27	16.14±0.17	16.25±0.21
	12th rib(%)	12(50.00)	9(39.13)	11(40.74)	10(41.66)	10(37.04)
	unilateral 13th rib(%)	2(8.33)	4(17.39)	5(18.52)	2(8.33)	4(14.81)
	bilateral 13th rib(%)	10(41.67)	10(43.48)	11(40.74)	12(50.00)	13(48.15)
	ossifying rate of talus(%) (Mean±S.D.)	98.72±3.16	99.12±1.83	100.00±0.00	99.7±1.41	100.00±0.00

Several sudden deaths were observed in each group, and diagnosd as hemorragic colitis or coccidiosis (Photo 1). Except the vehicle control, non-pregnant rabbits were observed in each group (Table 1).

Number of corpora lutea and implantations.

The number of corpora lutea did not show any differences in each group, but high dose treated group showed some increasing trends (Table 1).

Resorption of fetuses.

Early resorption (Photo 1) and late resorption (Photo 2 and 3) were observed in each group. But all the treated groups showed decreasing trends rather than the vehicle control in the resorption rate.

Immatured and dead fetuses (Photo 4).

There was no significant difference between the vehicle and the treated groupe (Table 1).

Viable fetuses and body weights.

In the decrease of body weights of viable fetuses, medium dose treated group showed significant difference ($p<0.01$) in comparison with the vehicle control. But, high dose treated group showed increases of body weights with significant difference ($p<0.01$), as indicated in Table 2.

Table 4. Influence of α-interferon on postnatal development of youngs(F_1) from dams (rabbits)

Dose(IU/kg/day)		Control		Vehicle control		1×10^5		4×10^5		1.2×10^6	
No. of dams(F_1)		3		3		3		3		3	
Total born Total		15		18		20		24		20	
Male		9		10		10		12		11	
Female		6		8		10		12		9	
Sex ratio(Male/Female)		1.5		1.25		1.0		1.0		1.2	
Mean litter number (Mean ± S.D.)		5 ± 0.8		6 ± 0.8		6.7 ± 1.9		8 ± 0.8		6.7 ± 1.7	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Body weight of youngs(g)	at birth	65.6 ± 7.4	66.7 ± 7.6	65.4 ± 6.2	65.2 ± 7.1	59.5 ± 3.5	58.5 ± 4.1	52.7 ± 4.2	51.5 ± 2.4	59.9 ± 6.0	54.3 ± 6.6
	1 week	111.5 ± 17.8	114.0 ± 17.9	111.6 ± 14.2	111.3 ± 16.7	97.8 ± 6.5	95.3 ± 11.4	83.6 ± 5.1	79.8 ± 5.0	102.2 ± 16.5	95.7 ± 14.3
	2 week	243.3 ± 28.6	247.4 ± 27.8	232.5 ± 16.7	231.0 ± 19.5	214.1 ± 7.9	215.3 ± 7.4	184.9 ± 7.7	181 ± 6.4	211.2 ± 23.1	206.1 19.9
	3 week	375.5 ± 41.5	378.4 ± 39.6	352.2 ± 18.1	351.5 ± 21.5	328.1 ± 9.0	329.2 ± 8.3	288.7 ± 10.5	283.1 ± 8.3	319.6 ± 29.4	315.1 ± 24.4
	4 week	513.4 ± 52.3	509.6 ± 50.6	477.2 ± 17.4	470.7 ± 24.7	440.5 ± 9.7	440.1 ± 9.4	391.5 ± 14.1	385.0 ± 11.7	428.7 ± 35.4	424.8 ± 29.0

External anomalies in fetuses.

As indicated in Table 2, there were not any anomalies except for the immatured and dead fetuses.

Anomalies in internal organs and skeletal system.

There was no difference between the control and treated groups, as represented in Table 3.

External observations of offsprings.

Three dams in each group parturated, as shown in Table 4, there were not any anomalies.

Body weight changes of offsprings (F^1).

Control groups showed rather high increase than treated groups in their weight gains.

Autopsies of offsprings (F1).

Any anomalies of internal organs were not shown on day 30 postpartum.

DISCUSSION

Pregnant New Zealand White rabbits of 2.60 to 3.25 kg of body weights were treated with rHuIFN-αA, an available therapeutic agent. The substance was administered via ear vein at dose levels of 1.2×10^6, 4×10^5, and 1×10^5 I.U/kg/day from day 6 to day 18 of gestation.

The maternal rabbits showed no special alterations or toxic lesions in all groups.

Litter data were similar in all groups, except poor growth of furs. And they were recovered within a few day as normal states. Rather high weight gains of offsprings in control groups is not much of a consideration. The reason is that the weight gains show relative decreases in the case of the large litter size, especially in rabbits.

Therefore, it is concluded that rHuIFN-αA has none of embryonic toxicity and teratogenicity during organogenesis period.

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