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Acute Oral, Intramuscular and Intravenous Toxicity Studies of Recombinant Interferon-α2a in Sprague-Dawley Rats

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ABSTRACT *: Acute oral, intramuscular, and intravenous toxicity studies of recombinant human interferon α2a (rhIFN α2a) were performed in Sprague-Dawley (SD) rats. SD rats were administered with doses of 31.25, 62.5, 125, 250 and 500 MIU/kg. respectively, and clinical signs, mortality and body weight changes were observed for 2 weeks. In all animals administered with rhIFN α2a, there was neither dead animals nor significant changes of body weights. In addition, no differences were found between control and treated groups in clinical signs and autopsy findings. Therefore, LD_{50} of rhIFN α2a was considered to be higher than 500 MIU/kg in SD rats.*

Key Words *: Acute toxicity, Recombinant human interferon α2a, Sprague-Dawley rats*

I. INTRODUCTION

Interferon (IFN) was first reported as an antiviral agent (Issacs *et al.*, 1957). IFNs are classified as α, β, and γ: IFN α consists of at least 13 immunomodulatory 189-amino acid residue glycoproteins synthesized by macrophages and B cells (Cruse *et al.*, 1995). IFN β is released from fibroblast. IFN α and β have antiviral property and share a common receptor. Activated lymphocytes secrete IFN γ, which has its own receptor (Kuby *et al.*, 1992). IFNs also have immunomodulatory functions and antiproliferative effects by activating macrophages (Herberman *et al.*, 1980), natural killer cells (Silva *et al.*, 1980) and cytotoxic T-cells (Lindahl *et al.*, 1972). Besides antiviral effect of IFN α, antitumor effect was reported in non-Hodgkin's lymphoma (Foon *et al.*, 1984), metastatic breast cancer, multiple myeloma (Gutterman *et al.*, 1980), metastatic renal cell carcinoma (Quesada *et al.*, 1989), hairy cell leukemia (Quesada *et al.*, 1986), and chronic myelogenous leukemia (Talpaz *et al.*, 1986).

Although IFN α was useful in the treatment for many cancers and viral diseases, the amount of IFN α from human cells was not enough to treat many patients. Large production of IFN α2 could be realized using recombinant DNA techniques after Lawn *et al.* (1981) reported cDNA sequence of IFN α2. Gutterman *et al.* (1982) reported that when a recombinant leukocyte A interferon was administered to cancer patients, some patient showed tumor regression while caused mild clinical signs.

Recently, Korea Green Cross Corporation succeeded in the production of recombinant human interferon α2a (rhIFN α2a). In this study, we determined the acute toxicity of rhIFN α2a and compared the toxicities based on the administration routes in Sprague Dawley rats.

II. MATERIALS AND METHODS

1. Test substance

The test substance, rhIFN α2a was produced and supplied by Korea Green Cross Corporation based in Korea.

2. Animals

4 weeks old, 90 male and 90 female SD rats were

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Table 1. Mortality of male and female SD rats after single administration of recombinant human interferon α2a in various routes

Administration Route	Sex	Dose (MIU/kg)				
		31.25	62.5	125	250	500
IM[1]	Male	0/5[a] (0%)[b]	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
	Female	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
PO[2]	Male	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
	Female	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
IV[3]	Male	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
	Female	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)

1; Intramuscular injection, 2; *Per Os* administration, 3; Intravenous injection.
a; Number of dead animals/treated animals.
b; Percentage of dead animals.

obtained from Samyuk laboratory animal research center (Osan, Korea). All animals were acclimatized for 1 week prior to the administration of the test substance under the environmentally controlled room (temperature : 22±3°C, relative humidity : 55±5%, air circulation frequency : 10~12 times/hr, artificial light : 150-300 Lux from 7 am to 7 pm) in Experimental animal laboratory of Veterinary Medicine in Seoul National University. Rats were housed in polycarbonate cage (26×42×18 cm). All animals fed with the mouse-rat pellets (Samyang Foods. Co., Korea) and consumed tap water *ad libitum*.

3. Experimental design

Experiments were conducted according to the "Guidelines for Toxicity Testing of Pharmaceuticals" (KFDA No. 98-116, 1998. 12. 8.). 25 male and 25 female rats per each adminstration route were randomly divided into 5 groups respectively, according to the dosage levels. 500 MIU/kg which was equivalent to 10,000 fold of a clinical dose given to a man weighing 60 kg was the high dose of the test substance and the medium high dose, the medium dose, the medium low dose and the low dose were prepared to 250 MIU/kg, 125 MIU/kg, 62.5 MIU/kg, and 31.25 MIU/kg, respectively. The rhIFN α2a was administered into rats intramuscularly at quadrate muscle of thigh, orally and intravenously into the coccygeal vein, respectively.

All animals were observed for 2 weeks focusing on clinical signs and body weight changes. Clinical signs were observed for 6 hours following treatment of the test substance on the day of administration and once everyday thereafter for 2 weeks. Body weight was measured immediately prior to dosing of the test substance and on the day 4, 7 and 14 after treatment. Following the observation period, all animals were sacrificed by exsanguination. Autopsy was conducted on every animal and all major organs and tissues including brain, thymus, heart, lung, liver, stomach, intestine, kidney, adrenal gland, spleen, and ovary or testis were examined for gross lesions. Samples with any abnormal finding were fixed in 10% buffered formalin for further examination.

For estimation of LD_{50}, Litchfield-Wilcoxon was used. Body weight changes were compared by using one-way ANOVA and two-tailed Dunnett's t-test. χ^2 (Chi-square) test was used to analyze the incidence of abnormal findings at autopsy.

III. RESULTS AND DISCUSSION

No death and clinical abnormalities among all the animal intramuscularly, orally and intravenously

Table 2. Abnormal clinical findings of male and female SD rats after single administration of recombinant human interferon α2a in various routes

Administration Route	Sex	Dose (MIU/kg)				
		31.25	62.5	125	250	500
IM[1]	Male	0/5[a]	0/5	0/5	0/5	0/5
	Female	0/5	0/5	0/5	0/5	0/5
PO[2]	Male	0/5	0/5	0/5	0/5	0/5
	Female	0/5	0/5	0/5	0/5	0/5
IV[3]	Male	0/5	0/5	0/5	0/5	0/5
	Female	0/5	0/5	0/5	0/5	0/5

1; Intramuscular injection, 2, *Per Os* administration, 3; Intravenous injection.
a; Number of dead animals/treated animals.

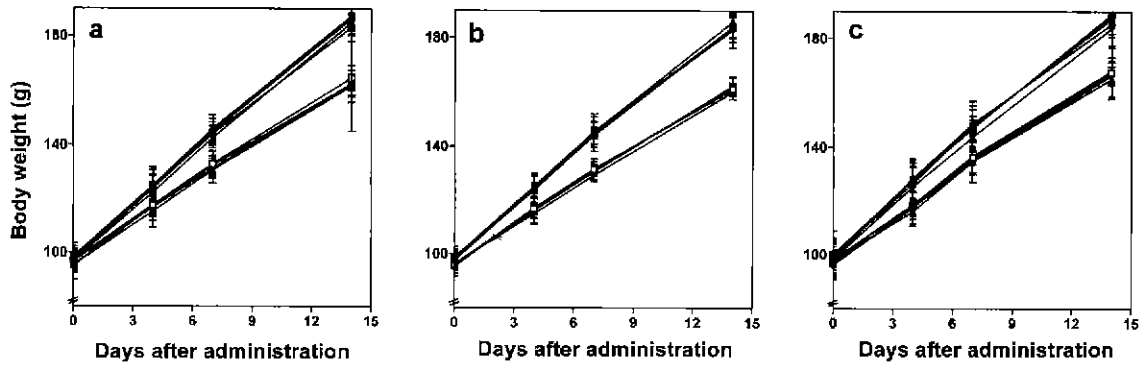

Fig. 1. Body weight changes of male (closed symbols) and female (open symbols) Sprague Dawley rats given recombinant human interferon α2a intramuscularly (a), orally (b) and intravenously (c). ■, □; 31.25 MIU/kg, ▲, △; 62.5 MIU/kg, ▼, ▽; 125 MIU/kg, ◆, ◇; 250 MIU/kg, ●, ○; 500 MIU/kg. Values are expressed as means±SD.

Table 3. Autopsy findings of male and female rats after single administration of recombinant human interferon α2a in various routes

Administration Route	Sex	Dose (MIU/kg)									
		31.25		62.5		125		250		500	
		tk	fd	tk	fd	tk	fd	tk	fd	tk	fd
IM[1]	Male	5[a](0)[b]	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)
	Female	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)
PO[2]	Male	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)
	Female	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)
IV[3]	Male	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)
	Female	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)	5(0)	0(0)

1: Intramuscular injection, 2; *Per Os* administration, 3; Intravenous injection
tk : Terminal kill; fd : Found dead.
a; Number of animals.
b: No. of abnormality detected animals.

treated with the test substance were observed during the observation period of 2 weeks and all animals appeared to be healthy and normal during this period (Tables 1 and 2). The Body weights of SD rats were measured on the day 0, 4, 7 and 14 after the administration of the rhIFN α2a. In body weight changes, there were no statistically significant differences observed among the treated groups (Fig. 1). In all animals, there were no abnormal lesions regardless of the treated dose and route (Table 3). In conclusion, rhIFN α2a showed no signs of the acute toxicity in SD rats when given intramuscularly, orally and intravenously at a dose as large as 500 MIU/kg and LD_{50} of rhIFN α2a was considered to be higher than 500 MIU/kg in SD rats

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REFERENCES

Cruse, J.M. and Lewis. R.E. (1995): Illustrated dictionary of immunology, CRC press. Boca Raton, pp. 168-169.

Foon, K.A , Sherwin. S.A., Abrams, P.L., Longo, D.L., Fer, M.F., Stevenson, H.C., Ochs, J.J., Bottino, G.C., Schone-Berger, C.S., Zeffren, J., Jaffe, E.S. and Oldham, PK. (1984): Treatment of advanced non-Hodgkin's lymphoma with recombinant leukocyte A interferon. *N. Engl. J. Med.*, **311**, 1148-1152

Gutterman, J.U., Blumenschein, G.R., Alexanian, R., Yap. H.Y., Buzdar. A.U., Cabanillas. F., Hortobagyi, G.N., Hersh, E.M , Rasmussen, S.L.. Harmon, M., Kramer. M. and Pestka. S. (1980)· Leukocyte interferon-induced tumor regression in human metastatic breast cancer, multiple myeloma. and malignant lymphoma, *Ann. Inter. Med.*. **93**, 399-406.

Gutterman, J.U.. Fire, S.. Quesada, J.. Horring, S.J., Leuine, J.F., Aleanian, R., Bernhardt, L., Kramer, M.,

Spiegal, H., Colburn, W., Trown, P., Merigan, T. and Dziewanowski, Z. (1982): Recombinant leukocyte A interferon: Pharmacokinetics, single-dose tolerance, and biologic effects in cancer patients, *Ann. Intern. Med.*, **96**, 549-556

Herberman, R.B., Ortaldo, J.R., Djey, J.Y., Holden, H.T., Jett, J., Lang, N.P., Rubinstein, M. and Pestka, S. (1980): Role of interferon in regulation of cytotoxicity by natural killer cells and macrophages, *Ann. N.Y. Acad. Sci.*, **350**, 63-71.

Isaccs, A. and Lindenann, J. (1957): Virus interference· I. The interferon, Proc. R. Soc. Lond. (Biol.), B **147**, 258-267.

Kuby, J. (1992): Immunology, W.H. Freemand and Company, New York, p. 258.

Lawn, R.M., Gross, M., Houck, C.M., Franke, A.E., Gray, P.V. and Goeddel, D.V. (1981)· DNA sequence of a major human leukocyte interferon gene, *Proc. Natl. Acad. Sci. USA*, **78(9)**, 5435-5439.

Lindahl, P., Leary, P. and Gresser, I. (1972): Enhancement by interferon of the specific cytotoxicity of sensitized lymphocytes, *Proc. Natl. Acad. Sci. USA*, **69**, 721-725.

Quesada, J.R., Hersh, E.M., Manning, J., Reuben, J., Keating, M., Schnipper, E., Itri, L. and Gutterman, J.U. (1986): Treatment of hairy cell leukemia with recombinant alpha-interferon, *Blood*, **68(2)**, 493-497.

Quesada, J.R. (1989): Role of interferons in the therapy of metastatic renal cell carcinoma, *Urology*, **34(4 Suppl)**, 80-83, 87-96.

Silva, A., Bonavida, B. and Targan, S. (1980): Mode of action of interferon mediated modulation of natural killer cytotoxic activity: recruitment of pre-NK cells and enhanced kinetics of lysis, *J. Immunol.*, **125**, 479-484.

Talpaz, M., Kentarjon, H.M. and McCredie, K.B. (1986): Chronic myelogenous leukemia: Hematologic remission and cytogenic improvement induced by recombinant alpha A interferon. *N. Engl. J. Med.*, **314**, 1065-1069.