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Acute Toxicity Study of Recombinant Human Interferon αA (LBD-007) in ICR Mice

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Abstract—The acute toxicity of a recombinant human interferon αA (code name: LBD-007) was evaluated in both sexes of ICR mice, 5 weeks old, by the oral, subcutaneous and intravenous routes of administration. Based on the results, LBD-007 was not considered to induce any toxic effect on the mice in mortalities, clinical findigs, body weights and gross findings. It is suggested that LD_{50} values in mice would be $>48\times10^8$ IU/kg in the oral, subcutaneous or intravenous routes.

Keywords □ recombinant human interferon αA, acute toxicity study, mice.

Since the discovery of interferon (IFN) by Isaacs and Lindenmann (1957) many studies have shown that IFN behaves as a macrophage activating factor, decreases division of tumor cells and has antiviral activity.

At present, only a few drugs have been approved by the FDA, U.S.A., for the therapy of viral infection in humans. IFN α has proved to be effective as an antiviral as well as an anticancer agent. It is allowed to treat hairy cell leukemia, Kaposi's sarcoma, blood cancer, multiple myeloma and chronic myeloid leukemia. In recent years it has been used as a medicine for hepatitis type B and C.

Recognizing the importance of human IFN α for the treatment of many diseases, Lucky R & D Center, Biotechnology (Daejeon, Korea) successfully produced a recombinant human IFN α(αA) from *Saccharomyces cerevisae*, which has high purity and high titer, by using a genetic manipulation.

The purpose of this study was to obtain the acute toxicity data on LBD-007 by the oral, subcutaneous or intravenous administration.

Materials and Methods

Materials

Recombinant human IFN αA (LBD-007, Lot No. AI 001) with a titer of 4.8×10^8 IU/ml and pH 6.9~7.3 was produced and supplied from Lucky R & D Center, Biotechnology (84, Jang-Dong, Yosung-Koo, Daejeon, Korea). Phosphate buffered saline (pH 7.4) was used for the vehicle.

The test substance was diluted with the vehicle just prior to the administration.

Animals and Maintenance

Both sexes of specific-pathogen-free (SPF) ICR mice were obtained at 4 weeks of age from the Laboratory of Animal Breeding, Korea Research Institute of Chemical Technology. The mice were housed under the barrier-sustained condition in a well-ventilated animal room maintained at a temperature of 23± 3℃, a relative humidity of 50± 10% and illumination cycle of 12-hr light/dark with light from 07 : 00 to 19 : 00 hr. They were acclimatized for about 1 week prior to administration of the test substance. The male and female mice were seperated and housed five to a cage in stainless-steel wire mesh, suspended cages (175×240×145 mm). Standard rat and mouse pellets (Jeil Feed Co., Ltd,. Daejeon, Korea) sterilized by gamma-irradiation at dose of 2 Mrad and tap water sterilized by an ultra-violet sterilizer were given *ad libitum*.

Ninety male and ninety female mice were devided into three groups according to the dosing routes. In each group, thirty male and thirty female mice were

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devided into 6 groups according to the dose levels.

Experimental Procedure

Oral, subcutaneous or intravenous dosing: The mice received 0, 3, 6, 12, 24 or 48×10^8 IU LBD-007/kg of body weight (BW) as a single oral, subcutaneous or intravenous dose in a volume of phosphate buffered saline equivalent to 10 ml/kg of BW. The mice dosed orally had been fasted overnight before the oral administration. The mice of control group (0 IU/kg) received only the vehicle.

It was expected that no mortality would be occurred at a maximum dose level from the preliminary study but 6 dose levels were selected according to the result of discussion with the sponsor.

Clinical observation: Clinical observations and death checks were made daily for 14 days in the orally dosed animals and for 7 days in the subcutaneously or intravenously dosed animals.

Body weight: Body weights were determined 0, 1, 3, 7 and 14 days after administration of the test materials in the orally dosed mice and 0, 1, 3 and 7 days after administration of the test materials in the subcutaneously or intravenously dosed mice.

Necropsy: At the termination of the study, a complete necropsy was performed on all surviving animals following ether anesthesia and bloodletting. All tissues and organs were grossly checked for abnormalities.

Statistical analysis: The LD_{50} was not caculated because there was no dead animal during the study. The body weights were analyzed using Student's t-test.

Results

Mortalities and LD_{50} are shown in Table I, clinical findings in Table II, body weights in Table III and gross findings in Table IV.

Table I. Mortalities and LD_{50} values of male and female mice after a single administration of LBD-007

Route	Dose ($\times10^8$ IU/kg)	Final mortality		LD_{50} ($\times10^8$ IU/kg)	
		Male	Female	Male	Female
P.O	0	0/5	0/5		
	3	0/5	0/5		
	6	0/5	0/5	>48	>48
	12	0/5	0/5		
	24	0/5	0/5		
	48	0/5	0/5		
S.C.	0	0/5	0/5		
	3	0/5	0/5		
	6	0/5	0/5	>48	>48
	12	0/5	0/5		
	24	0/5	0/5		
	48	0/5	0/5		
I.V.	0	0/5	0/5		
	3	0/5	0/5		
I.V.	6	0/5	0/5	>48	>48
	12	0/5	0/5		
	24	0/5	0/5		
	48	0/5	0/5		

Table II. Clinical findings of mice after a single administration of LBD-007

Route	Dose ($\times10^8$ IU/kg)	Findings	Hours after treatment						Days after treatment							
			1	2	3	4	5	6	1	2	3	4	5	6	7	8~14
P.O	0	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	3	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	6	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	12	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	24	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	48	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
S.C.	0	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	3	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	6	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	12	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	24	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	48	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
I.V.	0	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	3	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	6	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	12	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	24	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
	48	NAD	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	

NAD: No abnormality detected.

Table III. Body weights (g) of male and female mice after a single administration of LBD-007

Route	Sex	Days after treatment	Dose ($\times 10^8$ IU/kg) 0	3	6	12	24	48
P.O.	Male	0	24.4± 2.0(5)[a]	23.3± 1.5(5)	23.3± 1.2(5)	22.3± 1.0(5)	23.9± 1.7(5)	23.7± 1.4(5)
		1	27.4± 2.8(5)	27.0± 1.7(5)	27.0± 0.9(5)	25.8± 1.9(5)	27.2± 2.5(5)	26.5± 1.8(5)
		3	29.1± 2.5(5)	28.5± 2.1(5)	28.7± 1.1(5)	27.4± 2.0(5)	28.7± 2.3(5)	28.1± 1.2(5)
		7	31.5± 2.6(5)	30.7± 1.6(5)	30.9± 1.5(5)	29.4± 1.8(5)	30.5± 2.4(5)	31.0± 1.4(5)
		14	34.8± 2.3(5)	33.0± 2.0(5)	33.8± 2.2(5)	31.2± 2.0(5)	32.7± 2.4(5)	33.9± 1.9(5)
	Female	0	19.3± 1.5(5)	18.9± 1.6(5)	19.5± 0.9(5)	19.2± 0.9(5)	19.8± 1.2(5)	19.2± 0.9(5)
		1	22.0± 1.5(5)	21.7± 2.1(5)	21.8± 1.2(5)	21.8± 1.3(5)	21.8± 1.2(5)	21.6± 1.4(5)
		3	22.6± 1.9(5)	21.4± 1.9(5)	22.3± 2.0(5)	22.3± 1.5(5)	22.6± 1.2(5)	21.9± 1.1(5)
		7	24.0± 1.8(5)	23.1± 2.2(5)	24.0± 1.7(5)	23.7± 1.6(5)	23.9± 1.0(5)	23.3± 0.8(5)
		14	25.6± 1.7(5)	24.4± 2.2(5)	25.8± 2.1(5)	24.6± 2.1(5)	25.0± 1.3(5)	26.2± 1.3(5)
S.C.	Male	0	28.8± 1.0(5)	29.0± 1.4(5)	28.3± 1.0(5)	29.0± 1.3(5)	28.9± 1.8(5)	28.7± 1.7(5)
		1	29.3± 1.7(5)	29.1± 1.6(5)	28.6± 0.9(5)	29.2± 1.2(5)	29.2± 1.7(5)	29.0± 1.3(5)
		3	29.6± 1.8(5)	29.3± 1.6(5)	29.2± 0.9(5)	29.9± 1.2(5)	29.8± 1.9(5)	29.8± 2.1(5)
		7	31.7± 2.3(5)	31.2± 2.1(5)	31.6± 0.6(5)	32.1± 1.1(5)	31.9± 1.8(5)	31.4± 2.1(5)
	Female	0	22.1± 0.9(5)	22.5± 1.5(5)	22.5± 1.3(5)	22.7± 1.4(5)	22.2± 1.4(5)	22.5± 0.7(5)
		1	21.7± 0.9(5)	23.0± 1.2(5)	22.6± 1.4(5)	22.9± 1.5(5)	22.1± 1.1(5)	22.9± 0.6(5)
		3	22.5± 1.0(5)	23.0± 1.5(5)	23.0± 0.8(5)	24.1± 1.9(5)	23.3± 0.8(5)	23.8± 0.6(5)
		7	23.3± 0.9(5)	23.7± 1.8(5)	23.7± 1.4(5)	25.2± 2.2(5)	24.5± 0.9(5)	24.6± 0.6(5)
I.V.	Male	0	27.0± 1.5(5)	26.7± 1.9(5)	27.4± 1.3(5)	27.2± 1.3(5)	26.6± 1.7(5)	26.4± 1.2(5)
		1	27.2± 1.6(5)	27.1± 2.1(5)	27.8± 1.4(5)	27.5± 1.2(5)	27.6± 1.7(5)	26.9± 1.1(5)
		3	28.3± 1.9(5)	27.9± 2.3(5)	28.3± 1.6(5)	27.9± 1.4(5)	28.3± 1.7(5)	27.8± 1.5(5)
		7	29.2± 2.3(5)	28.6± 2.6(5)	29.8± 1.6(5)	28.9± 1.6(5)	29.3± 1.5(5)	29.0± 1.7(5)
	Female	0	21.5± 2.1(5)	22.1± 0.9(5)	20.6± 1.4(5)	21.8± 0.6(5)	21.6± 0.7(5)	21.7± 0.7(5)
		1	21.8± 2.1(5)	22.6± 1.2(5)	21.1± 1.3(5)	21.7± 0.8(5)	21.9± 0.7(5)	22.1± 1.0(5)
		3	21.5± 1.8(5)	22.8± 1.0(5)	21.3± 1.4(5)	22.4± 1.1(5)	22.3± 0.6(5)	22.2± 1.0(5)
		7	22.3± 1.7(5)	23.3± 1.3(5)	22.0± 1.1(5)	22.9± 0.8(5)	22.4± 0.9(5)	22.7± 1.2(5)

Values are Mean± S.D.
[a]No. of animals examined.

Table IV. Gross findings of male and female mice after a single administration of LBD-007

Route	Sex		Dose ($\times 10^8$ IU/kg) 0	3	6	12	24	48
		No. of animals Male	5	5	5	5	5	5
		Female	5	5	5	5	5	5
P.O.	Male	NAD	5	5	5	5	4	5
		Kidney: multiple cysts					1	
	Female	NAD	5	5	5	4	5	5
		Lung: congestion, moderate edema				1		
S.C.	Male	NAD	5	5	5	5	5	5
		Small intestine : congestion						
	Female	NAD	4	5	4	3	5	4
		Cystic ovary	1			1		
		Uterus: retention of body fluid			1	1		1
I.V.	Male	NAD	5	5	5	4	4	5
		Lung: dark red spots				1		
		Stomach: filled with gas					1	
	Female	NAD	5	5	5	5	5	5

NAD: No abnormality detected.

Mortalities

There was no dead animal observed in all groups. Therefore, the LD_{50} value in mice was $>48\times10^8$ IU/kg in the orally, the subcutaneously or the intraveously administered groups.

Clinical Findings

No abnormality was clinically seen in all groups.

Body Weights

No significant difference was statistically observed in the body weights between the treated and the control groups.

Gross Findings

Oral route : Multiple cysts of the kidney was observed in 1 male mouse from the 24×10^8 IU/kg group. Congestion and moderate edema of the lung was observed in 1 female mouse from the 12×10^8 IU/kg group.

Subcutaneous route : Congestion of the small intestine was observed in 1 male mouse from the 24×10^8 IU/kg group. The cystic ovary was observed in 1 female mouse from each of the 0 and 12×10^8 IU/kg groups. The uterus was filled with body fluid in 1

female mouse from each of the 6, 12 and 48×10^8 IU/kg group.

Intravenous route : Dark red spots of the lung were observed in 1 male mouse from the 12×10^8 IU/kg group. The stomach was filled with gas in 1 male mouse from the 24×10^8 IU/kg group.

Discussion

For evaluation of the acute oral, subcutaneous and intravenous toxicity of the recombinant human IFN αA (LBD-007), this compound was administered into ICR mice at dose levels of 0, 3, 6, 12, 24 or 48×10^8 IU/kg in the oral, subcutaneous or intravenous route.

In general, no toxic effect was observed in mortalities, clinical findings and body weights in all the groups of this study. A few gross findings were noted in various organs. However, they did not seem to be regarded as treatment-related changes, because the incidence of each change was very low and was not related with the dose level.

Many clinical trials have been studied since the recombinant IFN has been produced commercially. From the results it is suggested that the toxicity of the recombinant IFN include nausea, vomiting, fever, fatigue and anorexia (Edmonson *et al.*, 1988; Klein and Weinhouse, 1986; Thivolet *et al.*, 1990; Walder, 1990). However no sign in relation to these clinical findings was observed in this study.

Although histopathology was not performed, the absence of remarkable dose dependent abnormalities of gross findings and other parameters was enough to indicate that a recombinant human IFN αA (LBD-007) in this acute toxicity study dose not induce any toxic effect on mice in mortalities, clinical findings, body weights and gross necropsy findings. It is suggested that LD_{50} values in mice would be $>48\times10^8$ IU/kg in the oral, subcutaneous or intravenous routes, which is about eighty thousand times the assumed human clinical dose.

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