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Acute Toxicity Study of Recombinant Granulocyte-Macrophage Colony Stimulating Factor (LBD-005) in ICR mice

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Abstract—The acute toxicity of a recombinant granulocyte-macrophage colony stimulating factor (code name: LBD-005) was evaluated in both sexes of ICR mice, 5~6 weeks old, by the oral, subcutaneous and intravenous routes of administration. Based on the results of the acute toxicity study, LBD-005 was not considered to induce any toxic effect on the mice in mortalities, clinical findings, body weights and gross findings. It is suggested that LD_{50} values in mice would be >48 mg/kg in the oral route and >24 mg/kg in the subcutaneous or intravenous route.

Keywords □ granulocyte-macrophage colony stimulating factor, acute toxicity study, mice.

Hematopoiesis during postnatal life in human occurs principally in the bone marrow, producing all kinds of blood cells via complicated and various phases. The hematopoiesis is controlled by a variety of endogenous and exogenous factors.

Granulocyte macrophage colony-stimulating factor (GM-CSF) is one of the factors which is a multispecific glycoprotein with a molecular weight of about 22,000 (Gasson *et al.*, 1984). It stimulates proliferation and differentiation of granulocyte and monocyte progenitors and may be required for early differentiation of erythroid cells (Metcalf, 1986; Donaohue *et al.*, 1986; Burgess *et al.*, 1987). Although native GM-CSF has been purified to homogeneity from a human cell line (Gasson *et al.*, 1984) and murine lung conditioned medium (Burgess *et al.*, 1985), it is difficult to produce a large amount of the factor enough to supply the demand. Therefore, biologically active, recombinant GM-CSF has been purified from COS cells in both the human and murine (Wong *et al.*, 1985; Gough *et al*, 1984), yeast (Park *et al.*, 1986; Miyajima *et al.*, 1986) and *Escherichia coli* (DeLamarter *et al.*, 1985; Burgess *et al.*, 1987).

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There were a great deal of researches into the possible relationships of GM-CSF in combating myeloid leukemias and other leukocyte deficiency diseases (Gasson *et al.*, 1984; Barlogie *et al.*, 1990). Human clinical trials, using different recombinant form of GM-CSF, are still continuing, with varing degrees of success (Goldstone and Khwaja, 1990; Lieschke *et al.*, 1989).

Because of its potenital to enhance the function of the hematopoietic system, recombinant GM-CSF is considered a candidate for the treatment of myelogenous disease.

The purpose of this study was to obtain the acute toxicity data on LBD-005 by the oral, subcutaneous or intravenous routes of administration.

Materials and Methods

Materials

Recombinant GM-CSF (LBD-005) with a protein content of 2.4 mg/ml (w/v) and pH 7.3 was produced and supplied from Lucky R & D Center, Biotechnology (84, Jang-Dong, Yousung-Koo, Daejeon, Korea).

The vehicle, phosphate buffered saline (pH 7.4) was supplied from Lucky R & D Center, Biotechnology.

Animals and Maintenance

Both sexes of specific-pathogen-free (SPF) ICR mice were obtained at 4~5 weeks of age from the Laboratory of Animal Breeding, Korea Research Institute of Chemical Technology. They were acclimatized for about 1 week prior to administration of the test materials under the barrier-sustained 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. The mice were housed in stainless-steel wire 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 ultraviolet sterilizer were fed *ad libitum*.

Ninety male and ninety female mice were divided into three groups according to routes. In each route, thirty male and thirty female mice were divided into 6 groups according to the dose levels.

Experimental Procedure

Oral route : The mice received 0, 3, 6, 12, 24 and 48 mg LBD-005/kg of body weight (BW) as a single oral dose in a volume of phosphate buffered saline (pH=7.4) equivalent to 20 ml/kg of BW after fasting overnight.

Subcutaneous or intravenous route : The mice received 0, 1.5, 3, 6, 12 and 24 mg LBD-005/kg of BW as a single subcutaneous or intravenous dose in a volume of phosphate buffered saline (pH=7.4) equivalent to 10 ml/kg of BW.

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 and in-

Table I. Motalities and LD_{50} values of male and female mice after a single administration of LBD-005

Route	Dose (mg/kg)	Final mortality		LD_{50} (mg/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		
	1.5	0/5	0/5		
	3	0/5	0/5	>24	>24
	6	0/5	0/5		
	12	0/5	0/5		
	24	0/5	0/5		
I.V.	0	0/5	0/5		
	1.5	0/5	0/5		
	3	0/5	0/5	>24	>24
	6	0/5	0/5		
	12	0/5	0/5		
	24	0/5	0/5		

Tabe II. Clinical findings of mice after a single administration of LBD-005

Route	Dose (×mg/kg)	Findings	Hours after treatment						Days after treatmet							
			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	
	1.5	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	
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	
	1.5	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	

NAD : No abnormality detected.

Table III. Body weights(g) of male and female mice after a single administration of LBD-005

Route	Sex	Days after treatment	Dose (mg/kg)					
			0	3 (1.5)[b]	6 (3)	12 (6)	24 (12)	48 (24)
P.O.	Male	0	30.2± 1.0(5)[a]	30.8± 0.7(5)	31.4± 1.5(5)	30.7± 2.0(5)	30.5± 2.0(5)	30.8± 1.3(5)
		1	31.3± 1.3(5)	31.3± 1.1(5)	32.5± 2.4(5)	32.4± 3.1(5)	31.5± 1.9(5)	31.9± 1.6(5)
		3	32.6± 1.4(5)	32.2± 1.3(5)	33.5± 2.0(5)	32.0± 3.2(5)	32.8± 1.9(5)	32.9± 1.6(5)
		7	34.3± 1.7(5)	33.1± 1.3(5)	35.4± 2.4(5)	35.6± 3.2(5)	35.2± 2.1(5)	34.9± 1.8(5)
		14	35.8± 2.2(5)	34.7± 1.5(5)	37.3± 2.7(5)	37.8± 3.5(5)	37.7± 1.9(5)	36.2± 1.1(5)
	Female	0	22.8± 1.4(5)	23.5± 2.0(5)	22.9± 1.4(5)	22.2± 1.4(5)	23.2± 2.0(5)	23.3± 1.0(5)
		1	22.8± 1.6(5)	24.4± 1.7(5)	23.6± 1.8(5)	23.2± 1.4(5)	23.7± 2.1(5)	23.9± 1.2(5)
		3	24.0± 1.7(5)	25.5± 2.1(5)	24.0± 1.9(5)	23.4± 1.3(5)	23.8± 2.4(5)	24.4± 1.0(5)
		7	24.3± 2.0(5)	26.2± 2.1(5)	24.7± 1.9(5)	23.8± 1.0(5)	25.1± 2.5(5)	25.4± 1.3(5)
		14	26.8± 2.0(5)	27.7± 2.6(5)	25.7± 2.7(5)	25.5± 1.4(5)	25.8± 2.3(5)	27.0± 1.4(5)
S.C.	Male	0	28.9± 2.1(5)	28.8± 2.4(5)	28.7± 1.4(5)	28.8± 3.1(5)	28.6± 3.0(5)	27.9± 2.3(5)
		1	28.9± 2.2(5)	29.1± 3.2(5)	28.8± 1.5(5)	29.0± 3.2(5)	29.0± 3.2(5)	28.8± 2.1(5)
		3	29.4± 2.0(5)	29.6± 3.0(5)	29.3± 1.6(5)	29.5± 3.1(5)	29.6± 2.3(5)	28.7± 1.8(5)
		7	31.9± 2.0(5)	31.3± 2.8(5)	31.4± 1.5(5)	31.7± 3.0(5)	32.0± 2.6(5)	31.3± 1.9(5)
	Female	0	22.9± 1.4(5)	22.8± 1.3(5)	22.2± 0.8(5)	22.7± 1.1(5)	22.1± 1.7(5)	23.0± 1.8(5)
		1	23.0± 1.5(5)	22.3± 1.1(5)	22.4± 1.8(5)	23.2± 0.7(5)	22.6± 1.3(5)	22.8± 1.8(5)
		3	23.3± 1.8(5)	22.0± 1.2(5)	22.6± 0.6(5)	23.2± 0.9(5)	22.9± 1.2(5)	32.1± 1.6(5)
		7	24.0± 1.6(5)	23.0± 1.6(5)	23.4± 0.6(5)	24.1± 0.9(5)	23.4± 1.4(5)	24.4± 1.7(5)
I.V.	Male	0	28.7± 1.7(5)	28.7± 2.1(5)	28.7± 1.8(5)	28.4± 1.4(5)	28.5± 1.6(5)	27.9± 1.2(5)
		1	29.1± 1.9(5)	29.2± 1.9(5)	29.6± 1.7(5)	29.7± 1.6(5)	29.4± 1.6(5)	28.9± 1.2(5)
		3	29.7± 1.5(5)	30.2± 1.7(5)	31.1± 1.9(5)	30.2± 1.3(5)	29.9± 2.1(5)	30.1± 1.2(5)
		7	31.1± 1.6(5)	31.7± 2.3(5)	32.3± 2.0(5)	31.9± 1.5(5)	31.2± 2.2(5)	32.2± 1.5(5)
	Female	0	22.8± 1.3(5)	22.2± 1.2(5)	22.2± 1.6(5)	21.8± 0.9(5)	21.7± 1.3(5)	22.2± 0.7(5)
		1	22.9± 1.7(5)	23.1± 1.1(5)	23.3± 2.0(5)	22.9± 0.9(5)	22.5± 1.1(5)	23.6± 0.3(5)
		3	23.4± 1.9(5)	23.2± 1.2(5)	23.4± 1.4(5)	23.6± 1.0(5)	22.8± 1.5(5)	23.5± 0.6(5)
		7	24.0± 1.5(5)	23.7± 0.8(5)	24.4± 1.3(5)	24.4± 1.2(5)	23.5± 0.9(5)	24.5± 0.6(5)

Values are Mean± S.D.
[a]: No. of animals examined.
[b]: Dose of S.C. or I.V.

travenously 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 animals and 0, 1, 3, and 7 days after administration of the test materials in the subcutaneously or intravenously dosed animals.

Necropsy : At the termination of the study, all surviving animals were necropsied following ether anesthesia and bloodletting. All tissues and organs were checked for abnormalities.

Statistical analysis : The LD_{50} was not calculated because there was no death during the study. 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

Table IV. Gross findings of male and female mice after a single administration of LBD-005

Route	Sex		Dose (mg/kg)					
		P.O.	0	3	6	12	24	48
		S.C. or I.V.	0	1.5	3	6	12	24
		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	5	5
	Female	NAD	5	5	5	5	5	5
S.C.	Male	NAD	5	4	5	4	5	5
		Lung: congestion		1				
		Kidey: hypertrophy				1		
	Female	NAD	5	5	5	4	5	5
		Kidney: grayish yellow color				1		
I.V.	Male	NAD	5	5	5	5	5	5
	Female	NAD	5	4	5	5	5	5
		Lung: bright red color		1				

NAD: No abnormality detected.

gross findings in Table IV.

Mortalities

There was no dead animal observed in all groups. Therefore, the LD_{50} value in mice was >48 mg/kg in the orally administered group and >24 mg/kg in the subcutaneously and intravenously administered groups.

Clinical Findings

No abnormality was clinically seen in all groups.

Body Weights

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

Gross Findings

Oral route : No abnomality was observed in all animals.

Subcutaneous route : Congestion of the lung was observed in 1 male mouse from the 1.5 mg/kg group. Hypertrophy of the kidney was observed in 1 male mouse from the 6 mg/kg group. Grayish yellow discoloration of the kidney was observed in 1 female mouse from the 6 mg/kg group.

Intravenous route : Bright red discoloration of the lung was observed in 1 female mouse from the 1.5 mg/kg group.

Discussion

In general, no toxic effect due to the administration of the test materials was observed in mortalities, clinical findings and body weights in all animals of this study. A few gross findings were noted in the lung and the kidney. However, they could not be regarded as treatment-related changes, because the incidence of each change was very low and was not related with the dose level.

It has been shown that toxicities of GM-CSF in clinical trials are chills, rigors, high fever and bronchospasm (Thompson *et al.*, 1989). No sign in relation to these clinical findings was observed in this study.

Although histopathologic examination was not performed, the absence of remarkable dose dependent abnormalities of gross findings and other parameters was enough to indicate that a recombinant GM-CSF (LBD-005) 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 mg/kg in the oral route and >24 mg/kg in the subcutaneous or intravenous route. The LD_{50} in subcutaneous route is above 2,400 to 4,000 times as large as a predicted clinical dose of 3 to 10 μg/kg (Thompson *et al.*, 1989).

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