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ACUTE TOXICITY STUDY OF RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (LBD-005) IN RATS

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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 Sprague-Dawley rats, 4 weeks old, by the oral, subcutaneous and intravenous routes of administration. LBD-005 in the acute toxicity study in the rats was not considered to induce any toxicological effect on the rats in mortalities, clinical findings, body weights and gross findings. It is suggested that LD_{50} values in rats would be >48 mg/kg in the oral route and >12 mg/kg in the subcutaneous or intravenous route.*
Key words: *granulocyte macrophage colony-stimulating factor, acute toxicity study, rats.*

INTRODUCTION

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 controled 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 23, 000 (Jain, 1986). It stimulates proliferation of granulocyte, monocyte, and eosinophil colonies and may be required for early differentiation of erythroid cells (Metcalf, 1986; Donohue *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's have 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).

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 forms of GM-CSF, are still continuing, with varying degrees of success (Goldstone and Khwaja, 1990; Lieschke *et al.*, 1989).

Because of its potential to enhance the function of the hematopoietic system, the 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 and intravenous routes of administration.

MATERIALS AND METHODS

Test 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, Taejon, Korea).

The vehicle, phosphate buffered saline (pH 7.2) was supplied from Lucky R & D Center, Biotechnology.

Animals and Maintenance

Both sexes of specific-pathogen-free (SPF) Sprague-Dawley rats were obtained at 4 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 material under the barrier-sustained animal room maintained at a temperature of 23± 3°C, a relative huminity of 50± 10% and illumination cycle of 12 hours light and 12 hours dark (light during 07 : 00-19 : 00). The rats were housed in stainless-steel wire cages (220x410x200 mm). Standard rat and mouse pellets (Jeil Feed Co., Ltd., Taejon, Korea) sterilized by gamma-irradiation at dose of 2 Mrad and tap water sterilized by an ultra-violet sterilizer were fed *ab libitum*.

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

Experimental Procedure

1) Oral route

The rats received 0, 3, 6, 12, 24 or 48 mg LBD-005/kg of body weight (BW) as a single oral dose in a volume of phosphate buffered saline (pH=7.2) equivalent to 20 ml/kg of BW after fasting overnight.

2) Subcutaneous and intravenous route

The rats received 0, 0.75, 1.5, 3, 6, or 12 mg LBD-005/kg of BW a single subcutaneous or intravenous dose in a volume of phosphate buffered saline (pH 7.2) equivalent to 5 ml/kg of BW. It was expected that no mortality could be occurred at the maximum dose level from the preliminary study but 6 dose levels

were selected according to the result of discussion with the sponsor.

3) 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 intravenously dosed animals.

4) Body weight

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

5) 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.

6) 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 1, body weights in Table 2, and gross findings in Table 3.

Table 1. Mortalities and LD_{50}'s of male and female rats after a single administration of LBD-005

Route	Dose (mg/kg)	Final mortality		LD50 (mg/kg)	
		Male	Female	Male	Female
p.o.	0	0/5	0/5	>48	>48
	3	0/5	0/5		
	6	0/5	0/5		
	12	0/5	0/5		
	24	0/5	0/5		
	48	0/5	0/5		
s.c.	0	0/5	0/5	>12	>12
	0.75	0/5	0/5		
	1.5	0/5	0/5		
	3	0/5	0/5		
	6	0/5	0/5		
	12	0/5	0/5		
i.v.	0	0/5	0/5	>12	>12
	0.75	0/5	0/5		
	1.5	0/5	0/5		
	3	0/5	0/5		
	6	0/5	0/5		
	12	0/5	0/5		

Table 2. Body weights of male and female rats after a single administration of LBD-005

(Mean± S.D. : g)

Route	Sex	Days after treatment	Dose (mg/kg) 0	3	6	12	24	48
p.o.	Male	0	132.3± 8.3 (5)[a]	133.0± 8.0 (5)	133.8± 3.9 (5)	134.1± 7.9 (5)	131.6± 5.7 (5)	133.8± 9.3 (5)
		1	147.1± 8.9 (5)	149.9± 7.6 (5)	149.4± 4.2 (5)	149.8± 9.8 (5)	150.7± 6.0 (5)	149.9± 10.9 (5)
		3	174.3± 8.3 (5)	175.1± 7.6 (5)	175.0± 5.1 (5)	174.6± 10.0 (5)	176.8± 7.0 (5)	175.3± 11.2 (5)
		7	213.5± 9.5 (5)	212.3± 11.4 (5)	213.1± 6.2 (5)	217.5± 13.0 (5)	215.6± 7.9 (5)	214.1± 14.8 (5)
		14	276.0± 15.4 (5)	276.3± 13.6 (5)	277.6± 9.0 (5)	286.2± 11.2 (5)	279.2± 9.6 (5)	278.4± 14.0 (5)
	Female	0	110.2± 7.3 (5)	108.0± 6.8 (5)	109.2± 7.0 (5)	112.1± 3.1 (5)	111.7± 5.9 (5)	112.1± 5.6 (5)
		1	126.2± 9.4 (5)	120.6± 8.4 (5)	126.0± 11.5 (5)	128.4± 6.0 (5)	124.7± 9.9 (5)	128.1± 7.8 (5)
		3	140.2± 11.2 (5)	136.0± 8.3 (5)	140.0± 12.0 (5)	142.1± 8.6 (5)	139.5± 10.1 (5)	141.6± 9.0 (5)
		7	157.5± 14.2 (5)	154.6± 9.3 (5)	161.0± 12.6(5)	161.3± 7.7 (5)	158.5± 13.2 (5)	158.1± 10.3 (5)
		14	185.2± 16.9 (5)	180.0± 8.2 (5)	184.8± 13.7 (5)	189.2± 8.2 (5)	183.8± 14.3 (5)	184.8± 10.7 (5)

Route	Sex	Days after treatment	Dose (mg/kg) 0	0.75	1.5	3	6	12
s.c.	Male	0	135.8± 10.9 (5)	137.2± 9.4 (5)	136.3 12.6 (5)	136.0± 10.0 (5)	136.3± 14.2 (5)	138.8± 12.8 (5)
		1	143.4± 10.5 (5)	146.5± 9.3 (5)	144.8± 12.9 (5)	145.2± 11.0 (5)	146.6± 15.4 (5)	146.4± 11.0 (5)
		3	159.9± 10.7 (5)	162.6± 10.6 (5)	161.9± 14.5 (5)	163.1± 11.1 (5)	163.3± 16.9 (5)	165.4± 14.2 (5)
		7	191.4± 12.8 (5)	193.4± 11.2 (5)	194.4± 13.5 (5)	193.5± 12.7 (5)	194.8± 19.8 (5)	195.6± 15.0 (5)
	Female	0	116.3± 11.0 (5)	118.2+ 5.3 (5)	117.3± 9.3 (5)	118.4± 8.3 (5)	118.8± 15.3 (5)	118.5± 5.5 (5)
		1	120.9± 10.8 (5)	125.3± 9.7 (5)	123.2± 8.9 (5)	123.3± 6.4 (5)	123.5± 14.5 (5)	120.9± 5.5 (5)
		3	132.8± 9.2 (5)	133.6± 7.1 (5)	132.3± 9.0 (5)	131.5± 6.7 (5)	134.1± 13.7 (5)	131.6± 3.6 (5)
		7	148.6± 7.7 (5)	150.2± 6.6 (5)	151.3± 8.6 (5)	146.6± 7.0 (5)	152.3± 16.1 (5)	146.7± 3.4 (5)

Table 2. Continued

Route	Sex	Days after treatment	Dose (mg/kg) 0	0.75	1.5	3	6	12
i.v.	Male	0	147.0± 16.0 (5)	147.4± 14.3 (5)	147.6± 17.1 (5)	147.3± 10.3 (5)	150.5± 13.0 (5)	151.0± 4.4 (5)
		1	156.1± 14.9 (5)	154.2± 13.9 (5)	154.6± 19.8 (5)	153.0± 10.1 (5)	155.2± 17.1 (5)	153.9± 5.0 (5)
		3	164.4± 15.6 (5)	159.9± 15.0 (5)	159.8± 21.7 (5)	163.1± 13.6 (5)	172,4± 14.6 (5)	172.7± 7.4 (5)
		7	206.8± 16.7 (5)	204.9± 21.6 (5)	201.3± 27.7 (5)	203.6± 14.1 (5)	203.6± 18.1 (5)	206.9± 9.3 (5)
	Female	0	126.0± 5.6 (5)	129.0± 11.4 (5)	128.4± 5.4 (5)	129.5± 13.9 (5)	128.0± 6.8 (5)	124.8± 7.9 (5)
		1	130.7± 7.9 (5)	132.1± 10.8 (5)	130.1± 6.6 (5)	131.7± 14.2 (5)	130.7± 7.3 (5)	127.7± 6.9 (5)
		3	141.9± 5.9 (5)	139.7± 15.8 (5)	141.7± 5.5 (5)	141.5± 14.5 (5)	131.9± 7.3 (5)	135.0± 6.5 (5)
		7	157.9± 5.4 (5)	154.5± 16.9 (5)	158.5± 8.5 (5)	159.2± 15.8 (5)	155.5± 7.9 (5)	151.4± 6.8 (5)

[a] No. of animals examined.

*Significantly different from control value at $p<0.05$ (Student's t-test).

Mortalities

There was no dead animal observed in all groups. Therefore, the LD_{50} value in rats was >48 mg/kg in the orally administered group and >12 mg/kg in the subcutaneously and intravenously administered groups.

Clinical Findings

No abnormality was clinically seen in all groups.

Body Weights

No significant difference was statistically observed in body weights between the treated and the control groups in both orally and subcutaneously administered animals. Female rats of the 6 mg/kg group had statistically significant ($p<0.05$) decreased body weights 3 days after intravenous dosing.

Gross Findings

1) Oral route

The thymus had dark red spots in 1 male from the control group, 1 female from the 3 mg/kg and 1 female from the 48 mg/kg group. Distension of the renal pelvis with urine was observed in 1 female from the 48 mg/kg group. The uterus was distended with clear fluid in 1 female from the 3 mg/kg group. Congestion of the ovary was observed in 1 female from the 6 mg/kg group.

2) Subcutaneous route

The spleen revealed mild atrophy in 1 male from the 1.5 mg/kg group. The thymus showed locally extensive dark red spots in 1 male from the 3 mg/kg group.

Table 3. Gross findings of male and female rats after a single administration of LBD-005

Route	Sex	Fate	Dose (mg/kg)									
			0		3		6		12		24		48	
			ts	fd	ts	fd	ts	fd	ts	fd	ts	fd	ts	fd
		No. of animals Male	5	0	5	0	5	0	5	0	5	0	5	0
		Female	5	0	5	0	5	0	5	0	5	0	5	0
p.o.	Male	NAD	4		5		5		5		5		5	
		Thymus : dark red spots	1											
	Female	NAD	5		3		4		5		5		3	
		Thymus : dark red spots			1								1	
		Kidney(pelvis) : distension with urine											1	
		Uterus : retention of body fluid			1									
		Ovary : congestion					1							

Route	Sex	Fate	Dose (mg/kg)									
			0		0.75		1.5		3		6		12	
			ts	fd	ts	fd	ts	fd	ts	fd	ts	fd	ts	fd
		No. of animals Male	5	0	5	0	5	0	5	0	5	0	5	0
		Female	5	0	5	0	5	0	5	0	5	0	5	0
s.c.	Male	NAD	5		5		4		3		5		5	
		Spleen : atrophy					1							
		Thymus : dark red spot							1					
		Salivary gland : congestion							1					
	Female	NAD	4		5		4		5		4		4	
		Liver : yellowish brown color	1											
		Ovary : congestion					1				1		1	
		Uterus : retention of body fluid					1							
i.v.	Male	NAD	5		5		1		5		5		5	
		Seminal vesicle : atrophy					4							
	Female	NAD	4		5		5		5		4		5	
		Kidney : grayish brown	1											

ts : terminal sacrifice, fd : found dead, NAD : no abnormality detected.

Yellowish brown discoloration of the liver was observed in 1 female from the control group. Moderate to severe congestion was noted in the ovary of 1 female from each of the 1.5, 6 and 12 mg/kg group. The uterus was distended with clear fluid in 1 female from the 1.5 mg/kg group.

3) Intravenous route

There was mild atrophy of the seminal vesicle observed in 4 males from the

1.5 mg/kg group. Grayish brown discoloration of the kidney was observed in 1 female from the control group.

DISCUSSION

In general, no toxicological effect due to the administration of the test material the observed in mortalities, clinical findings and body weights in all groups in the present study. Although female rats of the 6 mg/kg intravenous group showed decrease of body weight, it was not considered to be related with the dose because it had no dose-dependency and recovered 7 days after administration.

A few gross findings were noted in various organs. 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 histopathology was not performed, the absence of remakable dose dependent abnormalities of gross findings and other parameters was enough to indicate that the recombinant GM-CSF (LBD-005) in the study is not considered to induce any toxocological effect on rats in mortalities, clinical findings, body weights and gross necropsy findins. It is suggested that LD_{50} values in rats would be >48 mg/kg in orally administered group and >12 mg/kg in subcutaneously and intravenously administered groups. The LD_{50} in subcutaneous route is above 1,200 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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