

A STUDY ON THE RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (LBD-005) FOR PRIMARY SKIN IRRITATION IN RABBITS

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ABSTRACT: *LBD-005, a newly developed recombinant granulocyte-macrophage colony stimulating factor, was tested for primary skin irritation in male New Zealand White rabbits. In the primary skin irritation test, LBD-005 was applied to intact and abraded skins for 24 hours. Primary irritation index was "0" in test and control sites of all animals; thus LBD-005 was evaluated as a non-irritant on the basis of the criteria of Draize et al. (1944).*

Key Words: *LBD-005, Recombinant Granulocyte-Macrophage Colony Stimulating Factor, Skin irritation test, Rabbits.*

INTRODUCTION

Granulocyte-macrophage colony stimulating factor (GM-CSF) has been shown not only to stimulate the proliferation of granulocyte and macrophage colonies (Burgess and Metcalf, 1980; Sieff *et al.*, 1985; Metcalf *et al.*, 1986) but also to regulate some of the functional activities of stem cells (Metcalf *et al.*, 1986). This has stimulated a great deal of research into the possible relationships of GM-CSF in combating myeloid leukemias and other leucocyte deficiency disease (Gasson, 1984).

It is very difficult to extract and purify large enough volumes of GM-CSF as a natural product for commercial purpose. This problem has been solved by Lucky R & D Center, Biotechnology (Daejeon, Korea) through the development of a recombinant form of GM-CSF (LBD-005).

As a part of toxicological screening of test agent LBD-005, the primary skin irritation in New Zealand White rabbits was studied. The study was performed to assess the potential sources of cutaneous irritation and carried out according to the Guidelines for Skin Irritation Studies for Safety Evaluation of Drugs for

Human Use (National Institute of Safety Research Korea, 1988).

MATERIALS AND METHODS

Test Substances

LBD-005 was supplied by the Lucky R & D Center, Biotechnology (Taejon, Korea) with a titer of 2.4 mg/ml, pH of 7.5 and osmotic pressure of 313 mOsm. Phosphate buffered saline (pH 7.4) was used as a vehicle.

Animals and Environmental Conditions

Male New Zealand White rabbits (2.0~3.0 kg) were supplied by Sam-Yuk Experimental Animal Breeding Center (Hwasung, Korea) and the animals were acclimatized for 2 weeks prior to the start of the study.

During the study the animals were housed in metal cage (420×500×300 mm), fed pallet diet for rabbit (Purina Korea Co., Kunsan, Korea) and given the sterilized water *ad libitum*.

The room was illuminated by fluorescent tubes at the 200~300 lux and controlled a 12 hour light/dark cycle (07 : 00~19 : 00). The room was maintained at the temperature of 23±3°C with a relative humidity of 50±10%.

The studies were done according to GLP and inspected by the QAU of TRC /KRICT (Toxicology Research Center, Korea Research Institute of Chemical Technology).

Primary Skin Irritation Test

The test was measured by a patch test technique on the abraded and intact skin of the six healthy rabbits, clipped free of hair. 0.5 ml of LBD-005 was inserted under a square patch such as surgical gauzed measuring 2.5 cm by 2.5 cm and two single layers thick. The entire trunk of the animals was wrapped with non-toxic adhesive tape for the 24 hour period of exposure. After 24 hours of exposure, the patches were removed and the resulting reactions were evaluated 24 and 72 hr on the basis of the Draize score (Draize *et al.*, 1944).

RESULTS AND DISCUSSION

The present study was carried out to examine if LBD-005 (recombinant granulocyte-macrophage colony stimulating factor) possesses primary skin irritation in New Zealand White rabbits.

No remarkable changes in clinical signs and body weight change were observed.

Table 1. Mortality and clinical signs in rabbits applied with LBD-005

Sex	No. of animals examined	Clinical signs	Mortality
Male	6	NAD*	0/6

*NAD : No abnormalities detected.

Table 2. Body weight changes in rabbits applied with LBD-005

Sex	Animal No.	Day after administration			Weight gains
		0 day	24 hrs	72 hrs	
Male	1	2497.0	2520.7	2647.3	+150.3
	2	2355.2	2376.4	2382.2	+ 27.0
	3	2541.5	2406.5	2614.7	+ 73.2
	4	2635.2	2570.0	2681.9	+ 46.7
	5	2634.6	2443.2	2649.3	+ 14.7
	6	2613.2	2499.4	2655.9	+ 42.7
	Mean	2546.1	2649.4	2605.2	59.1
S.D.	108.8	73.4	111.3	48.9	
N	6	6	6	6	6

Table 3. Primary skin irritation scores in rabbits applied with LBD-005

Sites		Control site				Test site			
Change		Erythema & Eschar		Edema		Erythema& Eschar		Edema	
Phase (hrs.) ^a		Intact	Abraded	Intact	Abraded	Intact	Abraded	Intact	Abraded
		24 72	24 72	24 72	24 72	24 72	24 72	24 72	24 72
Ani. No.	Sex								
1	♂	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2	♂	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
3	♂	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
4	♂	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
5	♂	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
6	♂	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Total score		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Mean score		0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
P.I.I. ^b		0				0			

^aTime after topical application.

^bP.I.I. : Primary Irritation Index = Σ Mean score/4.

Also, no mortality was seen in all animals (Table 1 and 2). The result for the primary skin irritation test of LBD-005, there were no treatment-related changes in erythema, eschar and edema of skin examined between intact and abraded skin treated with LBD-005 (Table 3).

From the results mentioned above, it might be considered that the LBD-005 has no primary skin irritation; The primary irritation index (P.I.I.) of LBD-005 following the Draize method was scored as "0" under the present experimental condition.

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