

A STUDY ON THE RECOMBINANT HUMAN INTERFERON α A (LBD-007) FOR PRIMARY EYE AND SKIN IRRITATION IN RABBITS

Jong-Il Park, Sung-Hoon Kim, Sang-Seop Han and Jung-Koo Roh

Toxicology Research Center, Korea Research Institute of Chemical
Technology P.O. Box 9, Daedeog-Danji, Daejeon, 305-606, Korea

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ABSTRACT: *LBD-007, a newly developed recombinant human interferon α A, was tested for primary eye and skin irritation in male New Zealand White rabbits.*

In the primary eye irritation test, 0.1 ml of a solution of LBD-007 was instilled into the eye. In rinsing group, the eye was washed with water 30 seconds after instillation. No reaction was observed at the cornea, iris and conjunctivae by LBD-007.

In the primary skin irritation test, LBD-007 was applied to the back of rabbits for 24 hours. Primary irritation index was "0" in test and control sites of all animals.

Thus LBD-007 was evaluated as a non-irritant on the basis of the criteria of Draize et al., (1944).

Key words: *LBD-007, recombinant human interferon α A, primary eye and skin irritation test, rabbit*

INTRODUCTION

Interferons are immunologically active and biological response modifier in the human beings and animals. In case of recombinant products, the identity and purity have to be defined with the natural active agent (Hohbach and Koss, 1987), Interferon (IFN) α is widely used for the treatment of viral infections and malignancies (George and Sidney, 1972). It is very difficult to extract and purify large enough volume of IFN α from a natural resource for commercial purposes. This problem has been solved by Lucky R & D Center, Biotechnology (Daejeon, Korea) through the development of a recombinant form of IFN α A, LBD-007, was obtained by means of genetic engineering from yeast (*Saccharomyces cerevisiae*) in large quantities.

As a part of toxicological screening of test agent LBD-007, primary eye and skin irritation of New Zealand White rabbits were studied. These studies were per-

formed to assess the potential sources of ocular and cutaneous irritation and carried out according to the Guidelines for Eye and Skin Irritation Studies for Safety Evaluation of Drugs for Human Use (National Institute of Safety Research, Korea, 1988).

MATERIALS AND METHODS

Test Substances

LBD-007 was supplied by the Lucky R & D Center, Biotechnology (84, Jang-Dong, Yousung-Koo, Daejeon, Korea) with a titer of 1.2 mg/ml, pH of 7.4 and osmotic pressure of 281 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 purchased from Sam-Yuk Experimental Animal Breeding Center (Hwasung, Korea) and were quarantined and acclimatized for 2 weeks.

The animals were housed in metal cage (42×50×30 cm), fed pellet diet for rabbit (Purina Korea Co. Kunsan, Korea) and given the sterilized water *ad libitum*.

The animals were maintained on a constant 12 hr light: dark cycle with temperature and humidity kept at $23 \pm 3^\circ\text{C}$ and $50 \pm 10\%$, respectively.

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 Eye Irritation Test

Nine healthy rabbits were used for the test. LBD-007 was placed into conjunctival sac of the left eye. The right eye was served as a blank. The eyes of the 3 animals, rinsing group, were washed with water 30 seconds after instillation and the animals, Non-rinsing group, were not. The eyes were examined and the grade of ocular reaction was recorded at 1, 24, 48, 72, 96 hr and then 7 days after the treatment of LBD-007. Corneal opacity, iris and conjunctival redness, edema, secreta were classified according to the Draize score (Draize *et al.*, 1944).

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-007 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

The result of primary eye irritation test was shown in Table 1. No remarkable

Table 1. Primary eye irritation scores in rabbits applied with LBD-007

		Group	T1(Non-rinsing)					T2(Rinsing)				
		Animal No.	1	2	3	4	5	6	7	8	9	
Cornea	Degree of opacity (A)	1*	0	0	0	0	0	0	0	0	0	
		24	0	0	0	0	0	0	0	0	0	
		48	0	0	0	0	0	0	0	0	0	
		72	0	0	0	0	0	0	0	0	0	
		168	0	0	0	0	0	0	0	0	0	
	Diffuse areas of opacity (B)	1	0	0	0	0	0	0	0	0	0	
		24	0	0	0	0	0	0	0	0	0	
		48	0	0	0	0	0	0	0	0	0	
		72	0	0	0	0	0	0	0	0	0	
		96	0	0	0	0	0	0	0	0	0	
		168	0	0	0	0	0	0	0	0	0	
		Iris (C)	1	0	0	0	0	0	0	0	0	0
			24	0	0	0	0	0	0	0	0	0
			48	0	0	0	0	0	0	0	0	0
72	0		0	0	0	0	0	0	0	0		
96	0		0	0	0	0	0	0	0	0		
168	0		0	0	0	0	0	0	0	0		
Conjunctivae	Redness (D)	1*	0	0	0	0	0	0	0	0	0	
		24	0	0	0	0	0	0	0	0	0	
		48	0	0	0	0	0	0	0	0	0	
		72	0	0	0	0	0	0	0	0	0	
		96	0	0	0	0	0	0	0	0	0	
		168	0	0	0	0	0	0	0	0	0	
	Edema (E)	1*	0	0	0	0	0	0	0	0	0	
		24	0	0	0	0	0	0	0	0	0	
		48	0	0	0	0	0	0	0	0	0	
		72	0	0	0	0	0	0	0	0	0	
		96	0	0	0	0	0	0	0	0	0	
		168	0	0	0	0	0	0	0	0	0	
	Secreta (F)	1*	0	0	0	0	0	0	0	0	0	
		24	0	0	0	0	0	0	0	0	0	
		48	0	0	0	0	0	0	0	0	0	
		72	0	0	0	0	0	0	0	0	0	
		96	0	0	0	0	0	0	0	0	0	
		168	0	0	0	0	0	0	0	0	0	
	Score ^{a)}	1*	0	0	0	0	0	0	0	0	0	
		24	0	0	0	0	0	0	0	0	0	
		48	0	0	0	0	0	0	0	0	0	
		72	0	0	0	0	0	0	0	0	0	
		96	0	0	0	0	0	0	0	0	0	
		168	0	0	0	0	0	0	0	0	0	

^{a)}: $(A \times B \times 5) + (C \times 5) + 2(D + E + F)$

*: Observation time (hrs)

Table 2. Primary skin irritation scores in rabbits applied with LBD-007

Sites		Control site								Test site							
Change		Erythema & Eschar				Edema				Erythema & Eschar				Edema			
Phase ^{aj} (hrs.)		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. ^{bj}		0								0							

^{aj}: Time after topical application, 0 is the time before topical application.

^{bj}: P.I.I.: Primary Irritation Index = Σ Mean score/4

changes in cornea, iris and conjunctivae were observed between rinsing and non-rinsing group with LBD-007. The Primary Irritation Index (P.I.I.) of LBD-007 following the Draize method (1944) was scored as "0".

The result of primary skin irritation test was shown in Table 2. No treatment-related changes in erythema, eschar and edema of skin were observed in both intact and abraded skin treated with LBD-007. The Primary Irritation Index (P.I.I.) of LBD-007 following the Draize method (1944) was scored as "0".

DISCUSSION

The present studies were carried out to examine LBD-007 (recombinant human interferon αA) possessed or for the effects of primary eye and skin irritation in New Zealand White rabbits.

In the primary eye irritation test, there were no substance-related changes in the cornea, iris and conjunctivae of rinsing and non-rinsing groups.

In the primary skin irritation test, no treatment-related changes in erythema, eschar and edema of skin were examined between intact and abraded skin.

From the results mentioned above, it might be considered the LBD-007 has none of primary eye and skin irritation; The Primary Irritation Index (P.I.I.) of LBD-007 following the Draize method was scored as "0" under the present experimental condition.

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