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A Study of Recombinant Human Interferon β (LB00013) for Primary Eye and Skin Irritation in Rabbits

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ABSTRACT : *LB00013, a newly developed recombinant human Interferon β, 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 LB00013 was instilled into the eye. In the rinsing group, the eye was washed with water at 30 seconds after instillation. No reaction was produced at the cornea, iris and conjunctivae by LB00013. In the primary skin irritation test, LB00013 was applied to the back of rabbits for 24 hours. Primary irritation index was "0" in test and control sites of all animals. Thus LB00013 was evaluated as a non-irritant on the basis of the criteria of Draize et al. (1944).*

Key Words : *LB00013, Recombinant human interferon β, Primary eye and skin irritation test, Rabbit*

I. INTRODUCTION

Interferons are immunologically active, biological response modifier in the human being and animals. In case of recombinant products, the identity and purity have to be defined with the naturally active agent (Hohbach and Koss, 1987). Interferon (IFN) β is widely used for the treatment of viral infections and malignancies. It is very difficult to extract and purify large volumes of IFN β as a natural product for commercial purposes. This problem has been solved by the development of a recombinant form of IFN β. LB00013 was obtained by means of genetic engineering from *E. coli* in large quantities.

As a part of toxicological screening of test agent LB 00013, primary eye and skin irritation of New Zealand White rabbits was studied. These studies were performed to assess the potential sources of ocular and cutaneous irritation and carried out according to the Guidelines for Eye and Skin Irritation Studies in Safety Evaluation of Drugs for Human Use (National Institute of Safety Research, Korea, 1994).

II. MATERIALS AND METHODS

1. Test Substances

LB00013 was supplied by the LG Chemical Ltd., Research Park (104-1, Moonji-Dong, Yusung-Ku, Taejon, Korea) with a titer of 0.57 mg/ml, pH 5.5. Phosphate buffered saline (pH 5.5) was used as a vehicle.

2. Animals and Environmental Conditions

Male New Zealand White rabbits (2..0~3.0 kg) were purchased from Sam-Yuk Experimental Animal Breeding Center (O-San, Kyunggi-Do, Korea), quarantined, and acclimatized for 2 weeks.

The animals were housed in metal cages (420× 500×300 mm), fed pellet diet for rabbit (Purina Korea Co.), and given sterilized water *ad libitum*.

The animals were maintained on a constant 12 hr light/dark cycle with temperature and humidity kept at 23±3°C and 50±10%, respectively.

3. Primary Eye Irritation Test

Nine healthy rabbits were used for the test. LB 00013 was placed into conjunctival sac of the left eye. The right eye was served as a blank. The eyes of 3 animals in the rinsing group were washed with water 30 seconds after instillation, whereas

Table 1. Scale of weighted scores for grading the severity of ocular lesions

I. Cornea	
A. Opacity-Degree of Density	
(area which is most dense is taken for reading)	
Scattered or diffuse area-details of iris clearly visible	1
Easily discernible translucent areas. details of iris slightly obscured	2
Opalescent areas. no details of iris visible, size of pupil barely discernible	3
Opaque. iris invisible	4
B. Area of Cornea Involved	
One quarter (or less) but not zero	1
Greater than one quarter-less than one-half	2
Greater than one one-half less than three quarters	3
Greater than three quarters up to whole area	4
Score equals A×B×5	Total maximum=80
II. Iris	
A. Values	
Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any one or all of these)	2
Score equals A×5	Total possible maximum=10
III. Conjunctivae	
A. Redness (refers to palpebral conjunctivae only)	
Vessels definitely injected above normal	1
More diffuse deeper crimson red individual vessels not easily discernible	2
Diffuse beefy red	3
B. Chemosis	
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
C. Discharge	
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3
Score (A + B + C)×2	Total maximum=20
The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctivae.	

Draize *et al.* (1944).

Table 2. Evaluation of skin reactions

A. Erythema and Eschar Formation		
Very slight erythema (barely perceptible)		1
Well defined erythema		2
Moderate to severe erythema		3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)		4
Total possible erythema score		4
B. Edema formation		
Very slight edema (barely perceptible)		1
Slight edema (edges of area well defined by definite raising)		2
Moderate edema (area raised approximately 1 mm)		3
Severe edema (raised more than 1 mm and extending beyond area of exposure)		4
Total possible edema score		4
Total possible score for primary irritation		8
Primary irritation index (PII):	P.I.I. < 2	mild irritant
	2 ≤ P.I.I. < 5	moderate irritant
	5 ≤ P.I.I.	severe irritant

Draize *et al.* (1944).

the other animals in the 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 7 days after treatment of LB00013. Corneal opacity, erythema, chemosis, secreta and iritis were classified according to the Draize score (Draize *et al.*, 1944, Table 1).

4. Primary Skin Irritation Test

The test was performed by using a patch test technique on the abraded and intact skin of the six healthy rabbits, clipped free of hair. 0.5 ml of LB00013 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, Table 2).

II. RESULTS

The result of primary eye irritation test was shown in Table 3. No remarkable changes in cor-

Table 3. Primary eye irritation scores in rabbits applied with LB00013

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
		96	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 4. Primary skin irritation scores in rabbits applied with LB00013

Sites		Control site								Test site							
Change		Erythema & Eschar				Edema				Erythema & Eschar				Edema			
Phases (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
Animal 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
Mean Score		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣMean Score		0				0				0				0			
Total		0								0							
P.I.I.[b]		0								0							

[a] Time after topical application, 0 is the time before topical application.
[b] P.I.I.; Primary Irritation Index=ΣMean Score/4.

nea, iris and conjunctivae were observed between rinsing and non-rinsing group with LB00013. The Primary Irritation Index (P.I.I) following the Draize method (1944) was "0" (Table 3).

The result of primary skin irritation test was shown in Table 4. No treatment-related changes in erythema, eschar and edema of skin were observed in both intact and abraded skin treated with LB 00013. The Primary Irritation Index (P.I.I.) following the Draize method (1944) was "0" (Table 4).

III. DISCUSSION

Due to the development of recombinant technology, many biological products which exist in body too few to be isolated can be produced in large quantities. These products include growth homones, interferons, interleukins, and so forth. Though their endogeneity, many countries including the United States of America strictly require the preclinical data on their toxicity for approving in market, because the endogenous proteins may induce many undesirable toxicity when administered systematically. For example, endogenous proteins may also induce severe toxicity by induction of non-target receptor, systemic exposure to a protein that should be present only in small quantities at target sites, and chronic receptor induction (Galbraith, 1987). From these concerns, the preclinical data on local irritation is also required for approval, even though the product is perfectly identical protein.

The present studies were carried out to examine whether or not LB00013 (recombinant human interferon β) possesses a property to cause primary eye and skin irritation in New Zealand White rabbits.

In the primary eye irritation test, there was 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 observed between intact and abraded skin.

From the results mentioned above, it might be considered that the LB00013 has none of primary eye and skin irritation. The Primary Irritation Index (P.I.I.) of LB00013 following the Draize method was "0" under the present experimental condition.

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