PRIMARY DERMAL AND EYE IRRITABILITY TESTS OF HYDROPHOBICALLY MODIFIED HYDROXYPROPYL METHYLCELLULOSE IN RABBITS

Sakae OBARA, Hiroaki MUTO, Hiroyasu KOKUBO, Naoto ICHIKAWA*,
Mikiko KAWANABE* and Osamu TANAKA*

Specialty Chemicals Research Center, Shin-Etsu Chemical Co., Ltd.
28-1 Nishifukushima, Kubiki-mura, Nakakubiki-gun, Niigata 942, Japan

*Bio-Medical Research Laboratories Co., Ltd.

3079, Susugaya, Kiyokawa-mura, Aiko-gun, Kanagawa 243-01, Japan

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Abstract.....Primary dermal and eye irritation tests of hydrophobically modified hydroxypropyl methylcellulose (HM-HPMC, hydroxypropyl methylcellulose modified with stearyl glycidylether), a new cellulose derivative used as a thickener for topical pharmaceuticals and cosmetics, were conducted in rabbits. A dispersion of HM-HPMC (3%) was applied to intact and abraded skins and reactions were observed. A very slight erythema was observed in both skins and this polymer was categorized as a "mild irritant". In the eye irritation test, with a dispersion of the same concentration, it was categorized as "marginal" in unrinsed eyes and "negative" in rinsed eyes.

Key words : Hydrophobically modified hydroxypropyl methylcellulose, primary, dermal, eye, irritability, rabbit.

INTRODUCTION

Hydrophobically modified hydroxypropyl methylcellulose (HM-HPMC) is a thickener developed for topical pharmaceuticals and cosmetics. Single-dose toxicological studies have indicated that the polymer has essentially no toxic effects in rats following oral administration or dermal application at doses up to 900 mg/kg (Obara et al., 1992). In order to establish the safety of the polymer for its proposed applications to topical pharmaceuticals and cosmetics, its dermal and eye irritability in rabbits was investigated.

Correspondence : Sakae OBARA at the above address.

MATERIALS AND METHODS

Experimental animals :

Female Japanese white rabbits weighing 1.75–2.10 kg were purchased from Ichikawaya (Tokyo) and acclimated for 8 days before use. Experimental room temperature was maintained at $24 \pm 2^\circ\text{C}$ and relative humidity at $55 \pm 10\%$. The room air was ventilated 13 times per hr and there was a 12 hr/12 hr light-dark cycle. The animals were given laboratory chow (RC-4, Oriental Yeast Co., Tokyo) and filtered tap water *ad libitum*.

Dose level :

HM-HPMC does not dissolve completely in water but when dispersed in water, the particles are hydrated and the viscosity increases. Thus the concentration that can be given by a syringe is limited by the viscosity. Considering this limitation, 3% dispersion was used as the dosage form.

Preparation of dosage form :

HM-HPMC powder was initially dispersed in hot water at 70°C to prevent lumping. The dispersion was stirred for 20 min, then the beaker was put in a water bath which was maintained at 30°C . The stirring was continued until particle hydration was completed. The temperature was kept at 30°C until administration to avoid a viscosity increase. The dispersion was administered immediately after preparation.

Primary dermal irritation test :

The study was conducted in accordance with the Federal Register (1973). Six healthy rabbits were assigned to the study. On the day before application, the back area of each rabbit was shaved. The area was divided into 4 quadrants ; two as intact skin and the other two as abraded skin. The abraded skin was prepared by stripping with adhesive tape. Half gram of the HM-HPMC dispersion was put on a piece of lint and attached to each test area. The area was covered with a rubber sheet which was fixed in place with adhesive tape, and finally an elastic bandage was applied. The application was continued for 24 hrs, then the treated skin was exposed and gently washed with lukewarm water. The skin reactions were recorded at 24, 48, 72, 96 and 168 hrs after the application and scored according to Draize (1959). The primary irritation index (PII) was calculated from the scores at 24 and 72 hrs.

Primary eye irritation test :

This study was conducted in accordance with the guideline of the Environmental Protection Agency (1978). Nine healthy rabbits were divided into two groups ; six rabbits in which the eyes were rinsed and other 3 in which they were not. For every rabbit, 0.1 ml of the dispersion was placed in a cup formed by pulling the lower lid of the left eye and the lids were held together for a few seconds. The right eye served as a control. After the application, the eye reaction was observed at 1, 3, 6, 24, 48, 72, 96, and 168 hrs according to Draize (1959).

RESULTS

Primary dermal irritation test :

The evaluations of skin reaction are shown in Table 1. For the intact skin, very slight erythema (1 point) was observed at 2 quadrants in two rabbits at 24 hrs after removal of the occlusion. No other reactions were observed in the intact area. In the case of the abraded skin, very slight erythema was observed at 24 hrs at 4 quadrants of three rabbits, at 24 and 48 hrs at 6 quadrants of five rabbits, and at 24, 48, and 72 hrs at 2 quadrants of two rabbits. No other reactions were observed during the test. Mean evaluation scores for intact skin at 24, 48, 72, 96, and 168 hrs after removal of the occlusion were 0.167, 0.000, 0.000, 0.000, and 0.000, respectively, while for abraded skin, the scores were 1.000, 0.667, 0.167, 0.000 and 0.000. PII calculated from the values at 24 and 72 hrs was 0.333. In accordance with the evaluation of Draize (1959), the PII score places the test compound into one of three categories depending on the severity of response : mild irritant (0–2) ; moderate irritant (2–5) ; and severe irritant (5–8). The obtained PII values indicate that HM-HPMC can be categorized as a “mild irritant” under the conditions of this study.

Primary eye irritation test :

The evaluations of eye reaction are shown in Table 2. In all rabbits in the eye-unrinsed group, the eyelids remained closed for 6 hrs. Moreover, tears and conjunctival redness appeared from 1 hr to 6 hrs after the application. In the eye-rinsed group, lid closing was observed in one rabbit immediately after the application and in other one rabbit at 6 hrs. Lacrimation was observed in two rabbits at 6 hrs. Redness of conjunctiva was observed in one rabbit from 1 to 6 hrs. A corneal wound was found in one rabbit at 168 hrs after application. The evaluations are shown in Table 3. The maximum scores were 6.00 ± 0.00 for the unrinsed group and 2.67 ± 1.33 for the rinsed group. The maximum was recorded at 6 hrs after the application in both groups. In the unrinsed group, HM-HPMC was categorized as “marginal” and in the rinsed group, as “negative”.

Table 1. Skin reaction to HM-HPMC (Score according to Draize (1959)).

Animal No.	Test Area	24 hrs		48 hrs		72 hrs		96 hrs		168 hrs	
		Erythema and Eschar	Edema	Erythema and Eschar	Edema	Erythema and Eschar	Edema	Erythema and Eschar	Edema	Erythema and Eschar	Edema
1	1 intact	0	0	0	0	0	0	0	0	0	0
	2 abraded	1	0	1	0	0	0	0	0	0	0
	3 abraded	1	0	1	0	0	0	0	0	0	0
	4 intact	1	0	0	0	0	0	0	0	0	0
2	1 intact	0	0	0	0	0	0	0	0	0	0
	2 abraded	1	0	0	0	0	0	0	0	0	0
	3 abraded	1	0	1	0	0	0	0	0	0	0
	4 intact	0	0	0	0	0	0	0	0	0	0
3	1 intact	0	0	0	0	0	0	0	0	0	0
	2 abraded	1	0	1	0	0	0	0	0	0	0
	3 abraded	1	0	0	0	0	0	0	0	0	0
	4 intact	0	0	0	0	0	0	0	0	0	0
4	1 intact	0	0	0	0	0	0	0	0	0	0
	2 abraded	1	0	1	0	1	0	0	0	0	0
	3 abraded	1	0	1	0	0	0	0	0	0	0
	4 intact	1	0	0	0	0	0	0	0	0	0
5	1 intact	0	0	0	0	0	0	0	0	0	0
	2 abraded	1	0	1	0	0	0	0	0	0	0
	3 abraded	1	0	1	0	0	0	0	0	0	0
	4 intact	0	0	0	0	0	0	0	0	0	0
6	1 intact	0	0	0	0	0	0	0	0	0	0
	2 abraded	1	0	0	0	0	0	0	0	0	0
	3 abraded	1	0	0	0	0	0	0	0	0	0
	4 intact	0	0	0	0	0	0	0	0	0	0
Mean Score	intact	0.167		0.000		0.000		0.000		0.000	
	abraded	1.000		0.667		0.167		0.000		0.000	

Dermal and eye irritability of HM-HPMC

Table 2a. Eye reaction to HM-HPMC in eye-unrinsed group (Score according to Draize (1959)).

Animal No.		1										2										3									
		hr																													
Eye Reaction	hr	1	3	6	24	48	72	96	168	1	3	6	24	48	72	96	168	1	3	6	24	48	72	96	168						
		Opacity(A1)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
Cornea	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		Area(B1)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		A1×B1×5	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		Value (A2)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		A2×5	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		Redness(A3)	R	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0					
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		Chemosis(B3)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		Discharge(C3)	R	0	2	2	0	0	0	0	0	0	1	2	0	0	0	0	0	2	2	0	0	0	0	0					
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		(A3+B3+C3) ×2	R	2	4	6	0	0	0	0	0	0	2	6	0	0	0	0	2	6	0	0	0	0	0	0					
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
		Total Score	R	2	4	6	0	0	0	0	0	0	2	6	0	0	0	0	2	6	0	0	0	0	0	0					
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
			R	2	4	6	0	0	0	0	0	0	2	6	0	0	0	0	2	6	0	0	0	0	0	0					

Table 2b. Eye reaction to HM-HPMC in eye-unrinsed group (Score according to Draize (1959)).

Animal No. <div>hr</div>		4										5										6												
		1	3	6	24	48	72	96	168	1	3	6	24	48	72	96	168	1	3	6	24	48	72	96	168	1	3	6	24	48	72	96	168	
Cornea	Eye Reaction	Opacity(A1)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Area(B1)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Iris	A1×B1×5	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Value (A2)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Conjunctiva	A2×5	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Redness(A3)	R	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Conjunctiva	Chemosis(B3)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Discharge(C3)	R	0	2	2	0	0	0	0	0	1	2	2	0	0	0	0	0	1	2	2	0	0	0	0	0	1	2	2	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total Score	(A3+B3+C3)×2	R	2	6	6	0	0	0	0	0	4	6	6	0	0	0	0	0	2	6	6	0	0	0	0	0	2	6	6	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Total Score	R	2	6	6	0	0	0	0	0	4	6	6	0	0	0	0	0	2	6	6	0	0	0	0	0	2	6	6	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table 2c. Eye reaction to HM-HPMC in eye-rinsed group (Score according to Draize (1959)).

Animal No.		7										8										9											
		1	3	6	24	48	72	96	168	1	3	6	24	48	72	96	168	1	3	6	24	48	72	96	168								
Cornea	Eye Reaction	hr																															
		Opacity(A1)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Area(B1)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Iris	A1×B1×5	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Value (A2)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	A2×5	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Redness(A3)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	Chemosis(B3)	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Discharge(C3)	R	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(A3+B3+C3) ×2	R	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total Score	R	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table 3. Primary eye irritation score after application of HM-HPMC
(Mean $\pm$ S. E.).

Group	Site	n	hrs							
			1	3	6	24	48	72	96	168
Unrinsed eye	R	6	2.00 ± 0.52	5.00 ± 0.68	6.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	L	6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Rinsed eye	R	3	0.67 ± 0.67	0.67 ± 0.67	2.67 ± 1.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	L	3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

DISCUSSION

In the dermal irritation test, only very slight erythema was observed after application of HM-HPMC to both abraded skin and intact skin. Although the compound was categorized as a mild irritant, the PII values are so low that the compound almost fall into the "non-irritant" category. Dermal irritation tests for other aqueous cellulose derivatives have been reviewed (Cosmetic Toiletry and Fragrance Association, 1986). Primary skin irritation tests have been conducted on hydroxyethyl cellulose (2% solution), hydroxypropyl cellulose (2%), methylcellulose (2%) and carboxymethyl cellulose (1, 4 and 10%) and the PII values ranged from 0.04 to 0.21, indicating that these polymers are mild irritants. Hydroxypropyl methylcellulose moistened with water produced only a slight redness on rabbit skin after occlusive application for 3 days. Thus, HM-HPMC had a similar effect on the skin to other cellulosic polymers.

In the eye irritation test, lid closing was observed in some rabbits, which could be due to the high viscosity of this polymer dispersion. The corneal wound which was observed in one rabbit after 168 hrs could have been caused by an accident. Ocular irritation tests for other cellulose compounds were also reviewed by the Cosmetic Toiletry and Fragrance Association (1986). A 2% solution of hydroxyethyl cellulose scored 2.5 at 1 hr in rinsed eyes of rabbits, and the irritation was cleared by 24 hr; 0.5 and 1.0% solutions of hydroxypropyl cellulose scored 0 on eyes of rabbits. A 0.1 mg solid sample of HPMC caused slight conjunctival irritation in both rinsed and unrinsed eyes, but the eyes were healed within 48 hrs. The review concludes that solutions of the cellulose derivatives are not irritant, but

the solid form may cause slight transient irritation to eyes. In the present study, the dosage form was a dispersion in which the particles were hydrated, so the dispersion had a high viscosity and was like a paste. The observed reaction may have been due to the particles in the dispersion. In unrinsed eye, the compound was categorized as a "marginal" irritant. But, since every reaction was mild and transient, it is suggested that HM-HPMC can be regarded as effectively non-irritant for rabbit eyes. In human use, it is suggested that the eyes should be quickly washed with water after exposure to this compound.

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