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Evaluation of Irritating Potential of Newly Developed Toothpaste in the Hamster Oral Mucous Membrane

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ABSTRACT *: Oral mucous membrane test using Syrian hamsters was performed to evaluate the reliability as a model system for the assessment of the potentially irritating substances intended for the mucous membranes, and to determine the irritating potential of a new emulsion-type formulated toothpaste. After test substances were implanted into the cheek pouches of hamsters with diluents (20 mg/Kg) under pentobarbital sodium anesthesia, we made the comparison in irritation between emulsion-type and dispersion-type of triclosan (TCS) formulations in the range of 0.2% to 0.3%. The emulsion-type formulations using non-ionic surfactant showed less mucosal lesion than other commercial toothpastes with 0.3% TCS, or dispersion-type ones. However, no significant difference in irritation was detected between 0.2% and 0.3% TCS. We report that this hamster cheek pouch method could be a reliable approach for the evaluation of slight difference in the irritating potentials of cosmetics and hygiene products intended for the lips or other mucous membranes, and this method showed that the new emulsion-type formulation significantly lowered the TCS-induced toxicity, compared with other commercial toothpastes.*

Key Words *: Hamster, Oral mucous membrane, Irritation test, Toothpaste, Triclosan (TCS)*

I. INTRODUCTION

The irritative properties of cosmetics and hygiene products intended to be applied to the lips or other mucous membranes should be evaluated at the similar local site for the safety assessment. The hamster cheek pouch has been a recognized model for the evaluation of mucosal irritation of products intended to be applied on buccal cavity mucosa since 1960s although there are some physiologic and anatomic differences compared with the human oral mucosa (Ghoshal and Bal, 1990). United States Food and Drug Administration also requires this "hamster cheek pouch irritation test" model for the agents used in the prevention or treatment of oral diseases (Fancher and Calancra, 1968). Because this method is relatively easy for the repeated application of test compounds and the comparative macroscopic observations as well, it has been used for the evaluation of irritating potential of chlorhexidine (Lindhe *et al.*, 1970) and mouth washes (Bernstein and Carlish, 1979).

Both the anatomic characteristics and the fine structure of the hamster cheek pouch epithelium have been thoroughly studied (Ghoshal and Bal, 1990; Veys *et al.*, 1994), and similarities between the oral mucous cells of the hamster and those of the human are presented. Therefore it is suggested to be a good model for the study of absorption from the oral cavity *in vivo* and *in vitro*, and for the safety assessment of agents applied to mucosa (Bourrinet *et al.*, 1995).

Triclosan is a widely accepted antimicrobial ingredient because of the broad-spectrum of its efficacy against microbe (Jones *et al.*, 2000), and also known for its low toxicity in acute, subacute/subchronic, chronic, carcinogenicity, mutagenicity, and teratogenicity (Bhargava and Leonard, 1996). However, in general it has been recognized the triclosan caused the dermal irritations when topically applied and *in vivo* cytotoxicity (Zuckerbraun *et al.*, 1998), although there are some contradictory results such as amelioration of sodium lauryl sulphate (SLS)-induced irritation (Baert and Veys, 1997).

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The aim of this study was to evaluate whether the hamster cheek pouch method is appropriate for the safety assessment of agents applied to mucosa, and to evaluate whether the new emulsion-type toothpaste formulation reduces the irritating potential of triclosan without compromising its anti-microbial activity, compared with the dispersion-type of triclosan.

II. MATERIALS AND METHODS

1. Experimental animals

Conventionally reared male Syrian golden hamsters weighing 80~100 g, were purchased from Samtako Bio Korea. They were housed 5 per cage in a temperature (23±3°C) and humidity (50±10%) controlled room. Lighting was adjusted automatically to give a cycle of 12 hours light and 12 hours dark. Throughout the study, the animals had access *ad libitum* to diet (Purina Korea) and tap water. The water analysis for bacteriologic and chemical contaminants was regularly conducted by local water supply authority. No contaminants were present in diet or water at the levels interfering with the objective of the study. One week, acclimatization period, was allowed before any treatment. Both animal care and protocol for this study were in accordance with IACUC (Institutional Animal Care and Use Committee) and OECD guideline.

2. Characteristics of new formulation

The new formulation was made using modified silica developed by Pacific Corporation in Korea. The modified silica with $-CH_3$ (methyl group) was used to increase the attachment of triclosan (TCS) onto the mucous membrane and the tooth's surface, and to decrease the irritation of TCS because of slow releasing of TCS. The nonionic surfactant for the emulsion was also used to lower the critical micelle concentration, which helps further to reduce the irritation by sodium lauryl sulphate (SLS).

3. Experimental procedure

The assay was performed as previously described with minor modification (Roy and Whishe, 1986).

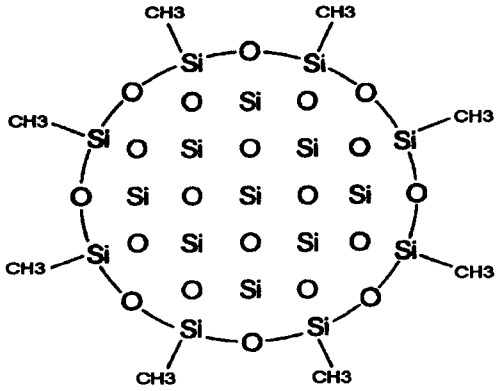

Fig. 1. Structure of modified silica.

Eight groups of 5 male acclimatized animals were treated with the 4 new toothpaste formulations containing TCS (emulsion with 0.3% TCS, emulsion with 0.2% TCS, dispersion with 0.3% TCS and dispersion with 0.2% TCS), formulation without TCS, 2 commercial toothpastes with 0.3% TCS as positive controls, and the physiological saline as negative control.

Each animal's left cheek pouch was everted and cleaned, and 20 ml/kg of test substances as 50% suspension in saline was loosely placed at the bottom of the pouch under pentobarbital sodium anesthesia. All animals were treated with a double-suture technique not to leak the substances, and lasted for 14 days. Hamsters in negative control group were treated with same double-suture without any substances. All animals were observed daily for clinical findings during the study. Individual body weights were recorded before treatment and before necropsy.

On day 15, all hamsters were anesthetized with intraperitoneally administered pentobarbital, then sac-

Table 1. Experimental design

group	test substances[a]	No. of hamster	Volume injected (ml/kg)
1	Formulation without triclosan (TCS)	5	20
2	Emulsion with 0.3% TCS	5	20
3	Emulsion with 0.2% TCS	5	20
4	Dispersion with 0.3% TCS	5	20
5	Dispersion with 0.2% TCS	5	20
6	Positive control 1[b]	5	20
7	Positive control 2[b]	5	20
8	Negative control[c]	5	20

[a]Test substances were prepared as 50% suspension in saline.
[b]Positive controls are the commercial toothpastes on the market.
[c]Negative control was treated with physiological saline.

rificed by exsanguinations. Cheek pouches were withdrawn, excised, laid flat and examined for any signs of irritation or lesions. The scores of the gross and histopathological findings were assessed by the generally used Draize grading system (0, normal; 1, mild; 2, moderate; 3, severe: 4, very severe) (Draize, 1959). All major organs were carefully examined for gross abnormalities and processed for histologic examination in case of suspected findings. After fixation in 10% neutral buffered formalin, the samples were dehydrated, embedded, sectioned at 5 μm, stained with hematoxylin-eosin, and examined for any histopathological lesions.

III. RESULTS AND DISCUSSION

There was no mortality. Decreased the rate of the body weight gain was detected in the hamsters treated with positive control 1 and 2 (significantly different from negative control group, $p < 0.05$). Other groups showed normal body weight gains (data not shown).

Clinically, laceration and granuloma formation were found at cheeks nearby the cheek pouches of all animals in two positive groups at 6 or 7 days after treatment (Table 2). These lesions were thought to be caused by the irritation of triclosan in the toothpaste in- serted into the cheek pouch. There were no other clinical signs except one animal in group 4 showing laceration of cheek.

In the gross necropsy findings of group treated with positive controls (2 commercial toothpastes), laceration of cheek pouch was healed to scar, therefore severe shrinkage of pouch was found (Table 3). These groups showed the severe inflammatory cell infiltration, hemorrhage, epithelial detachment, hyperkeratosis and chronic granulomatous inflammation in histopathological findings (Table 4, Fig. 2).

New formulations developed by Pacific Corporation produced less lesion than positive controls. We thought the decrease of the irritation in the new formulations resulted from the modified silica and nonionic surfac-

Table 2. Clinical findings of male syrian hamster

Group	No. of animals	Findings	No. of animals observed in days after treatment													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	N	5	5	5	5	5	5	5	5	5	5	4	4	4	4
		L											1	1	1	1
5	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	5	N	5	5	5	5	5	5	4	4	3					
		L							1	1	2	5	5	5	5	5
7	5	N	5	5	5	5	5	4	2	1						
		L						1	3	4	5	5	5	5	5	5
8	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

N : Normal, L : Laceration of cheek and granuloma formation.

Table 3. Gross findings of hamster cheek pouch

Group	No. of animals observed	Cheek pouch shrinkage[a]	Desquamation[a]	Congestion[a]	Total grade[c]
1	5	2.3±0.84	1.0±0	1.0±0	1.0
2	5	3.0±0.71	1.4±0.55	0.8±0.44	2.0
3	5	2.6±0.81	1.4±0.55	1.2±0.84	2.0
4	5	3.4±0.56	2.8±0.73	1.0±0	3.0
5	5	2.8±0.73	2.8±0.61	1.6±0.62	2.5
6	5[b]	4.0±0	4.0±0	2.8±0.61	4.0
7	5[b]	4.0±0	4.0±0	3.2±0.75	4.0
8	5	0	0	0	0

Grade : 0, normal; 1, mild; 2, moderate; 3, severe; 4, very severe.
[a]Each lesion was showed by Group mean score ±S.D.
[b]These animals showed very severe lesion of scar and granuloma formation by cheek laceration.
[c]Total grades were synthetically decided by several lesions.

Table 4. Histopathological findings of hamster cheek pouch

Lesions	Group	1	2	3	4	5	6	7	8
	No. of animal	4	5	5	5	5	5	5	5
Inflammatory cell infiltration									
Epithelial layer		2[a]		1	1		5		1*
Lamina propria		1	4	3	3	5	5	5	
Hyperkeratosis		2	3	4	4	4	5	5	
Chronic granulomatous inflammation					4		5	5	
Haemorrhage						4		5	
Epithelial detachment			3	3	4	4		4	
Total grade[b]		1	2	2	3.5	3	4	4	0

Grade : 0, normal; 1, mild; 2, moderate; 3, severe; 4, very severe.
[a]No. of animal with lesion.
[b]Total grades were synthetically decided by several lesions.
*Non-specific lesion by the suture.

tant used in new formulation. The modified silica was thought to decrease irritation of triclosan because of the slower rate of its release from the new formulation.

The formulations of emulsion with triclosan made or produced less lesion than those of dispersion. Since nonionic surfactant used in the emulsion lowered the critical micelle concentration, irritation of SLS seemed to be reduced.

Groups containing triclosan showed more severe lesion than groups without triclosan. It seems that triclosan increased the irritation. However, triclosan contents of 0.2% and 0.3% did not make any significant difference of irritation.

We concluded that new formulation decreased the toxicity induced by triclosan to a remarkable degree, compared with the commercial toothpastes. On the basis of results of this study, the oral mucous membrane test using hamster cheek pouch can be regarded as a good method for the evaluation of the potential irritative properties of cosmetics and hygiene products intended to be applied to the lips or other mucous membranes.

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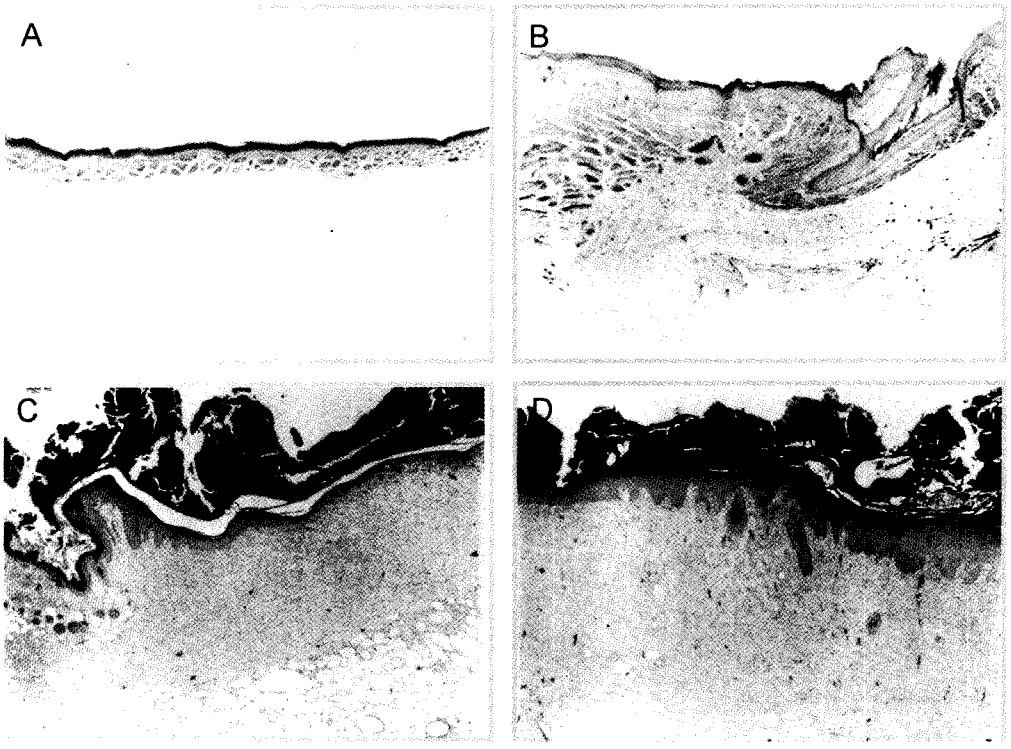

Fig. 2. Histopathological finding of epithelium of the hamster cheek pouch. A, negative control; no significant lesion, Grade 0 (normal). B, treated with formulation by emulsifying with 0.3% TCS; infiltration of inflammatory cells in lamina propria & mild hyperkeratosis, Grade 2 (mild). C, treated with commercial positive control 1; hyperkeratosis, acanthosis, and chronic granulomatous inflammation, Grade 4 (very severe). D, treated with commercial positive control 2; hyperkeratosis, acanthosis, and chronic granulomatous inflammation, Grade 4 (very severe). (H&E stain, ×40).

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