AATEX **14**, Special Issue, 415-416
Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences
August 21-25, 2007, Tokyo, Japan

Study on the prediction of human lip irritation from cosmetics materials using HeLa-MTT assay

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Abstract
The irritation of cosmetics and their ingredients on lips has mostly been estimated by using human lips. However, human tests are a heavy burden on subjects, making it difficult to evaluate multiple samples. Therefore, it was examined whether the HeLa-MTT assay, which is used as a substitution method for eye irritation test, could be used as a method for evaluating the irritation on lips.

Oils and waxes that are well used in lipsticks were selected as samples. For evaluating them, HeLa-MTT assay and human test were performed. The results were compared and a weak correlation was observed between the result from HeLa-MTT assay and the result from human test. This result suggests a possibility that a lip irritation from materials can be predicted by the HeLa-MTT assay.

Keywords: irritation, lip, cosmetic ingredients, HeLa-MTT assay

Introduction

Animal testing, human patch test and their alternatives are well known procedures in evaluating skin irritation for cosmetics and their ingredients. On the other hand, there are few methods to estimate irritation of cosmetics and their ingredients on mucous membrane of the lip, which is in junction with skin and mucous membrane, and most have been estimated by using human lip. However, human tests are a heavy burden to subjects, making it difficult to evaluate many samples.

In this study, we examined whether the HeLa-MTT assay, an alternative method of the Draize eye irritation test (animal testing), could be used as a method of evaluating the irritation on the mucous membrane of lips. First, we performed HeLa-MTT assay, and for the sample which couldn't be evaluated by the assay, cytotoxicity assay using 3-dimentional epidermal model was performed. After that, human test was carried out. The results were compared with each other. In consequence, a possibility that a lip irritation from materials can be predicted by the HeLa-MTT assay was suggested.

Materials & methods

• Samples

Oils and waxes that are frequently used in lipsticks were tested.

• HeLa-MTT assay

HeLa-MTT assay was performed as previously described (Chiba K. *et al.*, 1999). DMSO was used as dispersant, and only the samples with a good dispersion in Eagle's MEM medium were examined. For each sample, 4 exposure doses were tested (maximum dose was 0.5% (DMSO was 2%)), and the concentration score for 50% cell viability (50% effective concentration; EC_{50}) was obtained.

• Human test

Approximately 20 subjects were exposed to each sample for 2 consecutive days. 100% samples were applied twice a day (morning and noon) on their lips. The stimulus on lips was evaluated subjectively, and its occurrence rate was calculated. At the same time, the moisture content of the stratum corneum and water transpiration from lip were measured. However, only the subjective evaluation was adopted in this study since the two measurements above had no correlation with the subjective evaluation.

• 3-dimensional cultured human epidermal model assay.

LabCyte™ (air-lift cultured for 5days) supplied by Japan Tissue Engineering Co. Ltd., Aichi, Japan was used. This model had few stratum corneum layers because it was air-lift cultured for only 5 days (shorter period than normal model). After samples were applied on the models at the concentration of 100%, they were cultured for 72 hours. The cytotoxicity of the samples was estimated by MTT assay, and

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Table 1. Tested samples
Oils and waxes that are frequently used in lipsticks were selected as samples.

	INCI NAME	MAIN COMPONENT(MORECULAR WEIGHT)	VISCOSITY(mPa・S,25℃)
1	POLYGLYCERYL-2 TRIISOSTEARATE	POLYGLYCERYL-2 TRIISOSTEARATE(966)	7050
2	DIETHOXYETHYL SUCCINATE	DIETHOXYETHYL SUCCINATE(262.3)	10
3	ETHYL MACADAMIATE	ETHYL OLEATE(310.5)	10
4	CHOLESTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE	CHOLESTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE(UNKNOWN)	100000
5	SQUALANE	SQUALANE(422.8)	25
6	PENTAERYTHRITYL TETRAETHYLHEXANOATE	PENTAERYTHRITYL TETRAETHYLHEXANOATE(640.9)	50
7	OCTYLDODECYL MYRISTATE	OCTYLDODECYL MYRISTATE(508.9)	50
8	TRIISOSTEARIN	GLYCELYL TRIISOSTEARATE(891.5)	50
9	PETROLATUM	PETROLATUM(UNKNOWN)	22500
10	MACADAMIA TERNIFOLIA SEED OIL	MACADAMIA TERNIFOLIA SEED OIL(UNKNOWN)	40
11	ETHYLHEXYL PALMITATE	ETHYLHEXYL PALMITATE(508.9)	10
12	HYDROGENATED POLYISOBUTENE	HYDROGENATED POLYISOBUTENE(1000)	14450
13	TRIETHYLHEXYL TRIMELLITATE	TRIETHYLHEXYL TRIMELLITATE(320)	40
14	PENTAERYTHRITYL TETRAETHYLHEXANOATE	PENTAERYTHRITYL TETRAETHYLHEXANOATE(640.9)	40
15	CETYL ETHYLHEXANOATE	CETYL 2-ETHYLHEXANATE(368.6)	50
16	DIISOSTEARYL MALATE	DIISOSTEARYL MALATE(639.1)	1000
17	ISONONYL ISONONANOATE	ISONONYL ISONONATE(284.5)	50
18	HEXYLDECYL ISOSTEARATE	2-HEXYLDECYL ISOSTEARATE(508.9)	50
19	DIHEPTYLUNDECYL ADIPATE	2-HEPTYLUNDECYL ADIPATE(651.1)	50
20	ISOTRIDECYL ISONONANOATE	ISOTRIDECYL ISONONANOATE(340.6)	50
21	TRIETHYLHEXANOIN	TRI2-ETHYLHEXANOIN(470.7)	50
22	DIISOPROPYL DIMER DILINOLEATE	DIISOPROPYL DIMER DILINOLEATE(UNKNOWN)	50
23	ISOCETYL STEARATE	ISOCETYL STEARATE(508.9)	50
24	POLYGLYCERYL-2 ISOSTEARATE	POLYGLYCERYL-2 ISOSTEARATE(433)	1600
25	OCTYLDODECYL MYRISTATE	OCTYLDODECYL MYRISTATE(508.9)	25

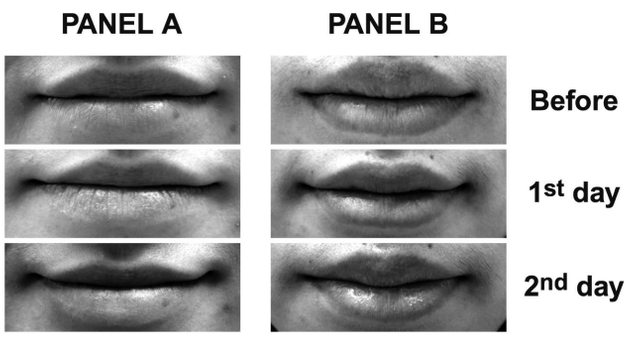

Fig. 1. Dried lips caused by sample

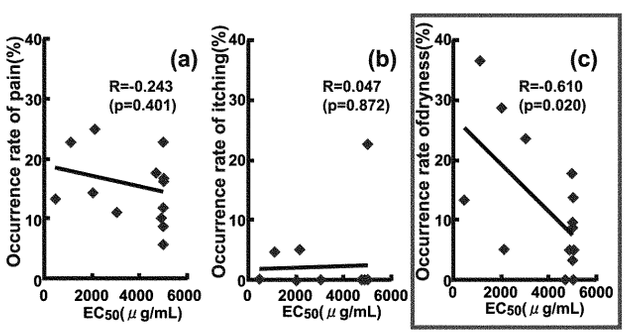

Fig. 2. The relation between the result of HeLa-MTT assay and the result of human test

the viability of the model was calculated by being compared with non-treated model.

Results

In human test, the moisture content of the stratum corneum and the water transpiration from lip had no correlation with the subjective evaluation. Therefore the influences of samples on the lips were evaluated subjectively. As a result, pain, itching and dryness of the lips were observed. Fig. 1 shows the lips of the panelists who felt dryness on their lips. When compared with the "before" picture, the dryness of lips can be observed on both 1st and 2nd day pictures.

The relations between the result of HeLa-MTT assay and the result of human test are shown in Fig. 2. There was a correlation between EC_{50} (HeLa-MTT assay) and occurrence rate of dryness (human test) (c), although there was no correlation between EC_{50}(HeLa-MTT assay) and the occurrence rate of pain or itching (human test) (a,b).

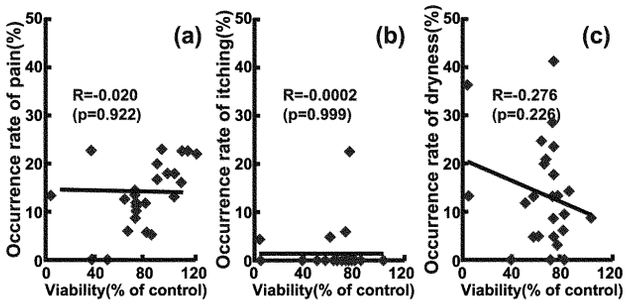

Fig. 3. The relation between the result of 3D epidermal model assay and the result of human test

The relations between the result of 3D epidermal model assay and the result of human test are shown in Fig. 3. There was no correlation between the viability after 72h (3D epidermal model assay) and the occurrence rate of pain or itching or dryness on lips (human test) (a,b,c).

Discussion

A significant correlation between cytotoxicity (EC_{50}) evaluated by HeLa-MTT assay and occurrence rate of dryness obtained by human test was observed, although there was no correlation between cytotoxicity (viability) evaluated by 3D epidermal model assay and occurrence rate of dryness obtained by human test. This shows that the difference between toxicity on single-layered cell and toxicity on multi-layered cell may have a relation with the dryness on lips.

Additional physical parameter like permeability might be necessary for a better prediction of the dryness of the lips caused by cosmetics and their ingredients because there was a weak correlation between the result of HeLa-MTT assay (EC_{50}) and human test (occurrence rate of dryness) ($R=-0.610$, $p<0.05$). In addition, it might be necessary to find an objective parameter that reflects subjective evaluations by human subjects, because subjective evaluations comparatively have a wide dispersion of data.

Reference

Chiba et al., *Toxicol in Vitro*, 13, 189~198(1998)