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EXPERIMENTAL STUDY ON THE MELANOGENIC EFFECT OF SOME COSMETICS

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Synopsis

Pigmented cosmetic dermatitis composes a major sort of undesirable reactions induced by cosmetics, but the melanogenic effect of cosmetics can scarcely be detected with patch tests in human. In the present study 10 cosmetic products most frequently used by the patients with pigmented cosmetic dermatitis and 8 ingredients contained in the cosmetics were painted onto separate areas of the dorsal flank of blownish guinea pigs following the method reported by Imokawa et al, then skin specimens were removed, the paraffin embeded sections were stained with H. E., Schmorl's reaction was used to show melanin and Bacher, Praver and Thatcher's dopa-oxidase reaction was used to study the melanogenic effect of the cosmetics and the ingredients. The results showed that all of the 10 cosmetics significantly increased the number of cells contianing melanin in the epiderml basal layer of the guinea pigs, while among the 8 ingredients only 5 (carmine, parabens, limocitrim, resin and lanolin) revealed this kind of effect. This suggests that the guinea pig model, being sensitive in response to the melanogenic activity of cosmetics on the one hand and reacting differentially to various kinds of ingredients on the other might be used in assaying the melnogenic effect of cosmetic products. The existence and the degree of the histological inflammatory reaction induced by the tested materials did not go parallel to those of hyperpigmentation, suggesting that cosmetic inducing hyperpigmentation may not be simply a sequence of dermatitis. Therefore in order to solve the problem of cosmetic hyperpigmentation, it should not only avoid the sensitizers, but also the melanogenic agents contained in the cosmetic products.

Riassunto

La dermatite pigmentata da cosmetici costituisce uno dei maggiori effetti indesiderati causati dai cosmetici, ma l'effetto melanogenico dei cosmetici può difficilmente essere rivelato da patch test condotti sugli uomini.
Nel presente studio 10 prodotti cosmetici usati più frequentemente da pazienti con dermatiti pigmentate da cosmetici e 8 principi attivi contenuti nei cosmetici sono stati applicati su aree separate del fianco dorsale di porcellini d'Indìa marroni seguento il metodo di Imokawa et al. Sono stati poi

prelevati campioni di pelle, le sezioni paraffinate sono state colorate con H.E. ed è stata utilizzata la reazione di Schmorl per evidenziare la melanina, mentre la reazione del dopa-ossidasi di Bacher, Praver e Thatcher è stata utilizzata per studiare l'effetto melanogenetico dei prodotti cosmetici e dei principi attivi. I risultati hanno mostrato che tutti e dieci i cosmetici hanno aumentato significativamente il numero di cellule contenenti melanina nello strato basale dell'epidermide dei porcellini d'India, mentre tra i principi attivi solo 5 (il carminio, il parabene, la limocitrina, la resina e la lanolina) hanno causato questo tipo di effetto.

Questo suggerisce che il modello del porcellino d'India, essendo da una parte sensibile all'attività melanogenetica dei cosmetici e reagendo, dall'altra, in modo differenziato ai vari tipi di principi attivi, può essere utilizzato per analizzare l'effetto melanogenetico dei prodotti cosmetici.

Il fatto che la presenza ed il grado delle reazioni infiammatorie istologiche causate dal materiale studiato non si siano manifestate parallelamente ai fenomeni di iperpigmentazione suggeriscono che l'iperpigmentazione può non essere una semplice conseguenza delle dermatiti.

Quindi per risolvere il problema dell'iperpigmentazione da cosmetici, si dovrebbero non solo evitare gli agenti sensibilizzanti, ma anche gli agenti melanogenetici contenuti nei prodotti cosmetici.

INTRODUCTION

Pigmented cosmetic dermatitis composes a major sort of undesiderable reactions induced by cosmetics, but the melanogenic effect of cosmetics can scarcely be detected with patch tests in human. Following the method reported by Imokawa et al (1-2), we studied the melanogenic effect of 10 cosmetic products most frequently used by the patients with pigmented cosmetic dermatitis and 8 ingredients contained in the cosmetics.

MATERIALS AND METHODS

Animals. All experiments were performed on healthy brownish guinea pigs weighed about 400g, provided by the Experimental Animal Center of Dalian Medical University.

Cosmetic products. Based upon the data of clinical observation, 10 cosmetic products, C_1-C_{10} in code name, most frequently used by the patients suffering from facial hyperpigmentation were selected for experiments.

Ingredients of cosmetics. Eight chemical ingredients were selected from among the raw materials of the cosmetic products for experiments, including 7% aromatic mixture, 1% cinnamic aldehyde, 5% carmine, 3% limocitrin, 1% parabens, 0.1% merthiolate, 30% lanolin and 5% nickel sulfate, numbered as M1-M8. White vaselin was used as vehicle.

Experiments on the melanogenic effect of cosmetic products. Ten brownish guinea pigs were used in the experiments. Hair was removed by clipping and shaving from 11 separated areas sized 1x2 cm on the dorsal flank of each animal. The 10 cosmetic products were painted onto these areas separately twice a day and the 11th area served as blank control.

After 28 days, skin specimens were removed and processed for paraffin embeded sections. Three observations were accomplished. (1) H. E. staining routine microscopy. Histological changes of the specimens were observed and scored following "The Procedures and Methods for Safety Evaluation of Cosmetic Products" issued by the Minister of Public Health, PRC and the mean of scores in each group was calculated. (2) Number of cells containing melanin granules. The sections were stained with Schmorl's method (3) and observed under optical microscope with high magnifying objective. The numbers of cells containing melanin granules per 300 basal cells were counted, the mean and standard deviation of each group were calculated and the data were analysed statistically with variance analysis. (3) Number of dopa-positive cells. The sections were stained with Bacher Praver and Thatcher's dopa-oxidase reaction (3) The numbers of dopa-positive cells per 100 basal cells were counted, the mean and standard deviation of each group were calculated and the data were analysed statistically with rank sum test, all observations were performed in a single-blind way and rechecked 2 weeks later to confirm their reproducibility.

Experiments on the melanogenic effect of cosmetic ingredients. Experiments were performed in 10 brownish guinea pigs, following the methods employed in the product experiments.

RESULTS

Cosmetic product experiments. The results are showed in Table I. The sites treated with all of the 10 cosmetic products showed various degrees of histological changes of dermatitis, expressed mainly as epidermal intracellular edema and acanthosis and in addition, epidermal intercellular edema, intraepidermal vesicle, dermal capillary dilation and inflammatory infiltrates. The values of the score mean of the histological changes in the tested groups can be ranged sequentially as C_3, C_6, C_2, C_9, C_1, C_8, C_5, C_4, C_{10} and C_7 in a decreasing order. Application of the 10 tested products led to a significant increase in the number of melanin containing cells

in the basal layer of guinea pig epidermis. According to the mean values of these number, the tested groups can be ranged as C_5, C_{10}, C_2, C_6, C_8, C_3, C_4, C_7, C_1 and C_9 in a decreasing order. Among the 10 tested cosmetic products, C_6 and C_5 induced an extremely significant increase ($p<0.01$) in the number of epidermal dopa-positive cells while C_3, C_2 and C_{10} induced an significant ($p<0.05$) increase.

Cosmetic ingredient experiments. The results are showed in Table II. All of the ingredients tested induced various degrees of histological changes of dermatitis, resembling those induced by the tested cosmetic products. According to the mean values of the scores of the histological changes, the tested groups can be ranged in decreasing order as M_1, M_8, M_4, M_7, M_2, M_5, M_3 and M_6. Among the 8 ingredients tested, M_3, M_5 and M_4 induced an extremely significant ($p<0.01$) increase in the number of melanin containing cells in the guinea pig epidermal basal layer while M_8 and M_7 induced a significant increase ($p<0.05$). Both of M_5 and M_3 induced an extremely significant increase ($p<0.01$) in the number of dopa-positive cells in guinea pig epidermis.

DISCUSSION

A number of animal models including black-haired, light-skinned C-57BL black mice (4), DBA/2 mice (5) and hairless mice (6) have been used for testing the effects of UV and chemicals on melanogenesis, according to the reports of Imokawa et al (1) (2), brownish guinea pig skin, which has a distribution of melanocytes and melanosomes similar to that found in human and a pigmentation pattern resembling mongoloid, has been found to be a readily reproducible experimental model more suitable for studying the melanogenic effect of UV and chemicals than routinely used mice. In the present study, all of the 10 cosmetic products frequently used by patients suffering from facial pigmented cosmetic dermatitis induced an increase in the melanin containing cells in the epidermis of brownish guinea

Table I

Dermatitis inducing and melanogenic effects of 10 cosmetic products on dorsal skin of brownish guinea pigs (n=10)

Cosmetic groups	Histological changes scores ($\overline{X}$)	*Cell number/100 Basal cells ($\overline{X}\pm s$)* Melanin granules containing	Dopa-positive
C_1	2	55.6±30.5*	6.2±4.2
C_2	2.6	80.7±17.1*	8.5±3.6**
C_3	3.2	76.1±27.2*	8.8±3.5**
C_4	1.8	67.3±24.9*	4.1±2.4
C_5	1.9	88.6±12.3*	9.9±4.0*
C_6	3.1	79.1±23*	10.2±6.7*
C_7	1.5	59.6±31.2*	6.6±5.0
C_8	2.0	76.7±27.6*	5.2±5.0
C_9	2.2	42.3±31**	5.1±6.0
C_{10}	1.6	88.0±11.6*	7.7±5.2**
C_0	0	13.5±27	1.8±3.0

*Compared with Co: * $p<0.01$, ** $p<0.05$*

pig, suggesting that the melanogenic effects of cosmetics are quite detectable by using the animal as a model. Furthermore, among the 8 tested cosmetic ingredients, only 5 revealed various degrees of melanogenic effect and only 3 of the 5 ingredients revealed an extremely significant effect, suggesting that the experimental model can be used to differentiate between cosmetics in terms of their effects on melanogenesis. Thus we deem that using the brownish guinea pig model to assay the melanogenic activities of cosmetic products and ingredients may be available.

It is ordinarily held that the hyperpigmentation in pigmented cosmetic dermatitis is a sequence of dermatitis which may be too mild to be recognized clinically (1) (7). Our experiments showed that all of the cosmetic products and ingredients revealing melanogenic effects did induce histological changes of dermatitis, supporting the view that hyperpigmentation is a sequence of inflammation. However, results of our experiments showed that the dermatitis inducing effect of the tested substances did not appear in parallel with their melanogenic effect. Even more, some chemicals inducing significant histologic changes of dermatitis did not inerease the number of melanin containing cells in epidermis. The results of our experiments support the view that the pigmentation inducing abilities of allergic contact dermatitis caused by various substances differ from each other (1) (2). Thus a kind of substance which induces contact allergy by no means concomitantly induces hyperpigmentation and vice versa. In this sense, avoidance of all discovered contact sensitizer cannot completely exclude the possibility of a hyperpigmentation caused by certain sorts of hypermelanosis inducer. The "Allergen Control System" in Japan, which was developed based upon the results of investigations on contact allergens, completely or almost completely cured 75% of 165 patients with pigmented cosmetic dermatitis, but still there were uneffetive cases, suggesting that further study on the melanogenic effect of cosmetics may be helpful in resolution of the problem (8).

Table II

Dermatitis inducing and melanogenic effects of 8 cosmetic ingredients on dorsal skin of brownish guinea pigs (n=10)

Cosmetic group	Histological changes scores ($\bar{X}$)	*Cell number/100 Basal cells ($\bar{X} \pm s$)* Melanin granules containing	Dopa-positive
M_1	2.7	23.0±23.0	3.1±3.0
M_2	1.0	35.5±25.5	3.3±0.95
M_3	0.9	71.5±26.0*	5.9±2.5*
M_4	1.2	55.4±32.4*	4.8±3.3
M_5	1.0	65.6±30.8*	6.8±3.2*
M_6	0.7	32.8±34	3.7±3.0
M_7	1.2	48.5±39.0**	4.6±3.9
M_8	1.7	51.7±37.3**	3.5±2.8
M_0	0	9.2±12	1.4±1.2

*Compared with Mo: * $p<0.01$, ** $p<0.05$*

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