Acta Derm Venereol (Stockh) 1989; 69: 302–307

Cross-sensitization Patterns in Guinea Pigs between Cinnamaldehyde, Cinnamyl Alcohol and Cinnamic Acid

HELLE WEIBEL,[1] JENS HANSEN[1] and KLAUS E. ANDERSEN[2]

[1]*Pharmaceutical Research and Development Department, Pharmacia AS, Hillerød and* [2]*Department of Dermatology, Odense University, Odense, Denmark*

Guinea pig maximization tests (GPMT) were performed with cinnamon substances. There was a certain degree of cross-reactivity between cinnamaldehyde, cinnamyl alcohol and cinnamic acid as animals sensitized to cinnamaldehyde reacted to the challenge with the three substances. Animals sensitized to cinnamyl alcohol reacted to cinnamyl alcohol and cinnamaldehyde, but not to cinnamic acid. Cinnamic acid did not sensitize guinea pigs. Compared to the challenge concentration for cinnamaldehyde, approximately a 15 times higher concentration of cinnamyl alcohol and a 25 times higher concentration of cinnamic acid were required to give positive reactions in animals sensitized to cinnamaldehyde. This could not be explained by differences in permeability properties, as the penetration profiles of the three substances through guinea pig skin in vitro showed permeability coefficients of the same order of magnitude under the test conditions. The study suggests that cinnamaldehyde is the "true" allergen, while cinnamyl alcohol and cinnamic acid are transformed in the skin to cinnamaldehyde, before contact allergic reactions can occur. ***Key words: GPMT, In vitro penetration; "True allergen"; Cinnamaldehyde.***

(Accepted February 20, 1989.)

Acta Derm Venereol (Stockh) 1989; 69: 302–307.

J. Hansen, Pharmaceutical Research and Development Department, Pharmacia AS, Herredsvejen 2, DK-3400 Hillerød, Denmark.

Cinnamaldehyde and cinnamyl alcohol occur in nature in essential oils, and are widely used as perfume chemicals and in flavouring materials (1, 2). They are frequent contact allergens, both included at 1% concentration in the fragrance mixture in the (International Contact Dermatitis Research Group) standard battery. The information on the relative importance of these two allergens is ambiguous. Calnan et al. (3) found cinnamaldehyde sensitivity more common than cinnamyl alcohol sensitivity, when testing 2461 consecutive eczema patients with a mixture containing 2% of each allergen. In a similar study comprising 2700 patients, Santucci et al. (4) found the same sensitization frequency for the two allergens. In contrast, Malten et al. (5) found cinnamic alcohol sensitivity to be more frequent than sensitivity to cinnamaldehyde, 10.5% and 3.7%, respectively. However, cinnamaldehyde was tested in 0.5%, and cinnamyl alcohol in 5%. The North American Contact Dermatitis Group conducted a prospective study, and identified 487 patients with cosmetic induced dermatitis. They also found allergy to cinnamyl alcohol more frequent than to cinnamaldehyde, when tested with 2% of each allergen (6). If these differences reflect different sensitization potentials or a different degree of exposure is not known.

Guinea pig allergy tests with cinnamaldehyde and cinnamyl alcohol have shown different sensitization rates, probably due to different test procedures. For instance, Klecak et al. (7) showed that cinnamyl alcohol as well as cinnamaldehyde were sensitizers in the guinea pig maximization test in contrast to Senma et al. (8) for whom it was not possible to sensitize the animals to cinnamyl alcohol.

In a continuation of studies (9, 10) aiming at providing more detailed knowledge of allergy to cinnamaldehyde and cinnamyl alcohol, guinea pig experiments were undertaken to study the cross-reactivity pattern between cinnamaldehyde, cinnamyl alcohol and cinnamic acid. Further, in vitro penetration experiments were performed to compare the penetration profile of the three chemicals.

MATERIALS AND METHODS

Substances

Induction and challenge were performed with cinnamaldehyde, cinnamyl alcohol and cinnamic acid, all with a purity of more than 98% from Merck. Freund's complete adjuvant (FCA) was purchased from Difco. The vehicles, propylene glycol and ethanol 96% from Merck, were of analytical grade, while dimethylisosorbide was purchased from ICI.

High Performance Liquid Chromatography (HPLC)

The purity of cinnamyl alcohol, cinnamaldehyde and cinnamic acid was determined by the HPLC method as previously described (9). The study showed that cinnamyl alcohol contained 1.2% cinnamaldehyde while no cinnamaldehyde was found in cinnamic acid.

Guinea pig maximization test

The Magnusson & Kligman test described by Wahlberg & Boman (11) was followed. The test includes intradermal induction with FCA on day 0, and topical induction on day 7 followed by challenge on day 21. One group of 20 animals and 10 sham-treated controls were used in each experiment. Munktell filter paper 108014-48 (2×4 cm) from Kebo Lab AS (Denmark) mounted on Leucoflex (Beiersdorf AG) was used for topical induction. Finn Chambers with two matching filters (Epitest Ltd) on Scanpor tape (Norgeplaster AS) were used for challenge.

Animals

Albino female guinea pigs, Ssc:AL, weighing from 250 to 350 g, were purchased from Statens Seruminstitut (Copenhagen, Denmark). They were housed in plastic cages, and kept on a 12-h photoperiod at a room temperature of 20–30°C, and a relative humidity of 45–75%. Food, rabbit pellets (Grøn Kanino, Brogården, Denmark) and water, added vitamin C, were available ad libitum. As bedding was used S8–15 (Brogården, Denmark). The animals were allowed to adapt for one week before testing. Hair was removed by clipping and shaving.

Evaluation of the concentration used for intradermal and topical induction

The induction procedure for cinnamaldehyde was in accordance with the method described by Senma et al. (8).

The concentrations for intradermal induction for the allergens cinnamyl alcohol and cinnamic acid were chosen to be moderately irritant without producing general toxic effects in the animals. For induction propylene glycol was used as a vehicle for the three substances because of the poor solubility in water. Andersen (12) showed that mixing allergens in pro-

Table I. *The frequency of animals giving positive reactions in the cross reactivity study between the cinnamon substances*

	Number of positive animals/animals tested		
Challenge substance	Cinnamaldehyde 5% i.d. 5% e.c.	Cinnamyl alcohol 5% i.d. 25% e.c.	Cinnamic acid 2% i.d. 10% e.c.
Cinnamaldehyde (8 mg/ml ethanol)			
Test	17/20 ($p=0.0001$)	12/19 ($p=0.0039$)	2/20
Control	0/10	0/10	0/10
Cinnamyl alcohol (8 mg/ml ethanol)			
Test	0/20	1/20	0/20
Control	0/10	0/10	0/10
Cinnamyl alcohol (120 mg/ml ethanol)			
Test	15/20 ($p=0.0005$)	9/19 ($p=0.0279$)	0/20
Control	0/10	0/10	0/10
Cinnamic acid (9 mg/ml ethanol)			
Test	0/20	0/20	0/20
Control	0/10	0/10	0/10
Cinnamic acid (120 mg/ml ethanol)			
Test	0/20	0/20	1/20
Control	0/10	0/10	0/10
Cinnamic acid (200 mg/ml dimethylisosorbide)			
Test	7/20 ($p=0.0932$)	0/19	0/20
Control	0/10	0/10	0/10
Ethanol			
Test	0/20		
Control	0/10		
Dimethylisosorbide			
Test	0/20		
Control	0/10		

Table II. *Topical irritancy of the cinnamaldehyde evaluated by the challenge procedure at control animals*

mg/ml ethanol	No. of animals	No. of animals giving positive reactions
Cinnamaldehyde		
2	3	0
4	3	0
8	3	0
15	3	3
30	3	3

pylene glycol with FCA was as effective in sensitizing the animals to chlorocresol as when mixing aqueous allergens with FCA. 100 µl of five concentrations of cinnamyl alcohol (5–25% w/v) and cinnamic acid (1–10% w/v) in propylene glycol were injected intradermally in 5 animals for each allergen. The response was read after 48 and 72 hours.

The topical irritancy of the substances used for induction was studied by a 48-hour closed patch test on the back of animals. The testing was performed one week after pretreatment with Freund's complete adjuvant, to counteract the effect of the excited skin syndrome (13). Five concentrations of cinnamyl alcohol (5–25% w/v) and cinnamic acid (1–10% w/v) in propylene glycol were applied topically on 5 animals for each allergen. The response was read after 48 and 72 hours, and concentrations giving slight irritation were chosen as suitable for topical induction.

Induction procedure

For intradermal sensitization a row of three injections was given on each side of the neck: 1) 0.1 ml of FCA in saline (FCA : saline 50/50 v/v), 2) 0.1 ml of cinnamon substance in propylene glycol; the concentrations chosen were 0.05% and 5% v/v for cinnamaldehyde, 5% w/v for cinnamyl alcohol and 2% w/v cinnamic acid, and 3) 0.1 ml of the test substance in FCA : propylene glycol 1 : 1 v/v. The concentrations of sensitizers were the same as for 2).

In order to enhance the sensitization, sodium lauryl sulfate 10% in petrolatum was massaged into the skin 24 hours before the topical induction. 100 µl of the suspected sensitizer in propylene glycol at a concentration of 0.25% and 5% v/v for cinnamaldehyde, 25% w/v for cinnamyl alcohol, and 10% w/v for cinnamic acid was applied to a 2×4 cm patch of Munktell filter paper (108014-48) mounted on Leucoflex (Beiersdorf AS). The dressing was sealed by Acrylastic (Beiersdorf AS) for 48 hours.

Challenge procedure

On day 21, 24-hours occluded patch tests were performed on the flanks, two tests on each side, using Finn Chambers with two matching filter papers (Epitest Ltd) filled with 30 µl of the test solution mounted on Scanpor (Norgeplaster AS) and sealed by Acrylastic (Beiersdorf AS). The order of the patches applied on the flank of each animal was randomised. The test solutions and the vehicles were patch tested in the same way in the control groups.

The challenge reactions were read blindly 72 h after application. The following grading scale was used: 0 = no visible reaction; 1+ = discrete or patchy erythema; 2+ = moderate and confluent erythema and 3+ = intense erythema and swelling (11). The minimum criteria for a positive patch test was 2+, a moderate and confluent erythema (14).

Controls

The animals in each control group were treated in the same way, concerning the induction and challenge procedure, as the corresponding animals in the test group except that the suspected sensitizer was not administered during the induction period.

Evaluation of the concentration used for challenge

The topical irritancy and the elicitation concentration of various concentrations of cinnamaldehyde (2–30 mg/ml), cinnamyl alcohol (2–120 mg/ml) and cinnamic acid 2–200 (mg/ml) were studied on the flanks by the challenge procedure in 3 controls, pretreated with FCA one week before testing, for each solution.

The animals received the suspected allergens in 96% ethanol and in dimethylisosorbide for the highest concentrations of cinnamic acid to solubilize the substance. Dimethylisosorbide has not been used in GPMT studies earlier, but is known as a non-toxic vehicle showing no irritancy in the guinea pigs (Table I).

The results in Table II show that concentrations of cinnamaldehyde of 16 mg/ml gave irritative reactions, while cinnamyl alcohol and cinnamic acid showed no irritancy for the concentrations tested.

Statistical calculation

χ^2 test for two independent samples was used. A 5% level of significance was adopted.

In vitro penetration procedure

In vitro penetration studies through guinea pig skin were carried out in triple at 30°C using the glass diffusion cell originally developed by Barry (15). After killing the animal, abdominal, dorsal and lateral skin was removed with a scalpel. The hair was removed by clipping and shaving, and the skin was mounted in the glass diffusion cells. On the epidermal side, Finn Chambers with ethanolic solutions of 8 mg/ml cinnamaldehyde and 120 mg/ml cinnamyl alcohol were applied. Cinnamic acid 200 mg/ml was applied in dimethylisosorbide. The substances were allowed to penetrate under occlusion while the lower part of the skin (the dermis side) was in contact with receptor phase, 0.05 M phosphate buffer pH 7.4. At appropriate intervals samples were taken from the receptor phase, and replaced by fresh receptor medium keeping an infinite sink, and analysed by the HPLC procedure. The standard deviation was about 15%.

RESULTS

Cinnamaldehyde sensitized 17 of the 20 animals after challenge with cinnamaldehyde 8 mg/ml (Table I). A simultaneous challenge with cinnamyl alcohol 8 mg/ml and cinnamic acid 9 mg/ml gave no reactions. However, cinnamyl alcohol 120 mg/ml gave positive

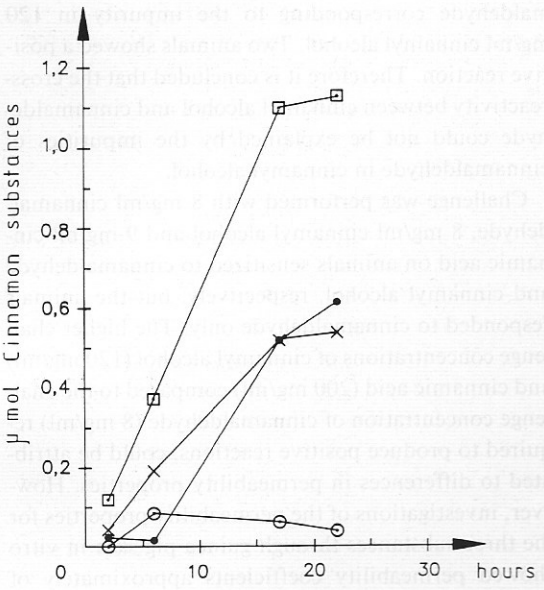

Fig. 1. In vitro percutaneous penetration of cinnamaldehyde through guinea pig skin. □, total amount of penetrated cinnamon substances; ●, cinnamic acid; X, cinnamyl alcohol; 0, cinnamaldehyde.

reactions in 15 of the 20 animals, while cinnamic acid 120 mg/ml did not react. Cinnamic acid in dimethylisosorbide 200 mg/ml gave positive reactions in 7 animals only.

Cinnamyl alcohol sensitized 9 of 19 animals after challenge with cinnamyl alcohol 120 mg/ml. However, 12 animals reacted to a simultaneous challenge with cinnamaldehyde 8 mg/ml, while one reacted to cinnamyl alcohol 8 mg/ml and none to cinnamic acid 9 mg/ml. By challenge with cinnamic acid 120 mg/ml and 200 mg/ml no reactions were seen. As seen from Table I it was not possible to sensitize animals to cinnamic acid, and no positive reactions were observed at the control animals as well as for the vehicles, ethanol and dimethylisosorbide.

Table III. *The frequency of animals giving positive reactions in the evaluation study of the cinnamaldehyde impurity in cinnamyl alcohol*

No. of positive animals/animals tested

Challenge substance	Cinnamaldehyde 5% i.d. 5% e.c.	Cinnamaldehyde 0.05% i.d. 0.25% e.c.	Controls
8 mg/ml	7/10	0/10	0/10
1.5 mg/ml	2/10	0/10	0/10

As cinnamyl alcohol contained 1.2% cinnamaldehyde the GPMT-induction procedure was performed with concentrations of cinnamaldehyde corresponding to the impurity in cinnamyl alcohol. The animals were therefore treated with cinnamaldehyde 0.05% i.d. and 0.25% e.c. corresponding to 5% i.d. and 25% e.c. cinnamyl alcohol. As seen from Table III, it was not possible to sensitize the animals to cinnamaldehyde with the low concentrations 0.05% i.d. and 0.25% e.c. Cinnamaldehyde 8 mg/ml elicited positive reactions in 7 of the 10 animals treated with cinnamaldehyde 5% i.d. and 5% e.c. A simultaneous challenge with 1.5 mg/ml cinnamaldehyde corresponding to the impurity in 120 mg/ml cinnamyl alcohol gave 2 positive reactions (Table III).

The penetration profiles of cinnamaldehyde, cinnamyl alcohol and cinnamic acid through guinea pig skin in vitro as function of time are shown in Figs. 1, 2 and 3. In the experiments where cinnamaldehyde was applied on the epidermal side of the skin, cinnamyl alcohol and cinnamic acid were in addition found in the receptor phase, indicating skin transformation, while in the experiments with cinnamyl alcohol and cinnamic acid only the applied allergens were found in the receptor phase.

The permeability coefficient (Kp) representing the allergen penetration rate is given by Scheuplein & Blank (16):

$$\mathrm{Kp} = dq/dt : A\mathrm{Cv} \qquad (1)$$

where dq/dt is the steady-state rate of penetration or appearance of the solute in the receptor phase μmol/hour. A is the area of the exposed skin 2.25 cm^2 and Cv is the concentration of the solute in the donor phase μmol/ml. From Eq. 1 and the slopes of the linear portions at the plots of Figs. 1, 2 and 3, the permeability coefficients were calculated to be 3.37×10^{-4} cm/h for cinnamyl alcohol, 4.83×10^{-4} cm/h for cinnamaldehyde and 1.11×10^{-4} cm/h for cinnamic acid.

DISCUSSION

The result shows that there is cross-reactivity between cinnamaldehyde and cinnamyl alcohol. Fifteen of 17 cinnamaldehyde sensitized guinea pigs reacted to cinnamyl alcohol, 120 mg/ml. However, none reacted when challenged with cinnamic acid, 120 mg/ml in ethanol. Cross-reactivity between cinnamaldehyde and cinnamic acid was expected and further challenge experiments were carried out, dimethylisosorbide be-

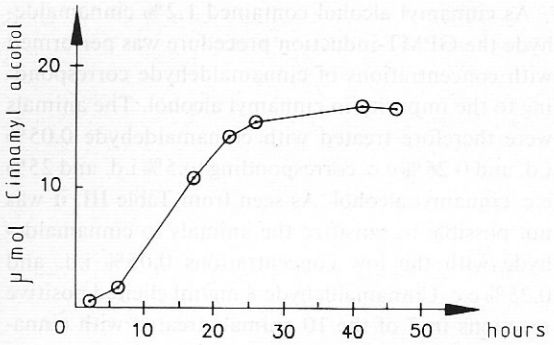

Fig. 2. In vitro percutaneous penetration of cinnamyl alcohol through guinea pig skin.

ing used as a vehicle for cinnamic acid in order to obtain higher challenge concentrations. Seven of the 17 cinnamaldehyde sensitized animals reacted to cinnamic acid 200 mg/ml in dimethylisosorbide, indicating a certain degree of cross-reactivity. This is difficult to evaluate because we have no experience with the use of dimethylisosorbide as a vehicle in sensitization studies.

Cinnamyl alcohol sensitized 9 of 19 animals after challenge with cinnamyl alcohol 120 mg/ml and one reacted to cinnamyl alcohol 8 mg/ml; but 12 reacted to cinnamaldehyde 8 mg/ml, while none reacted to cinnamic acid in the concentrations tested, 8 mg/ml, 120 mg/ml and 200 mg/ml.

Guinea pigs treated with cinnamaldehyde i.d. and e.c. in concentrations corresponding to the impurity of cinnamaldehyde in cinnamyl alcohol did not sensitize the animals (Table III).

Ten animals sensitized to cinnamaldehyde 5% i.d. and 5% e.c. were challenged with 1.5 mg/ml cinnamaldehyde corresponding to the impurity in 120 mg/ml cinnamyl alcohol. Two animals showed a positive reaction. Therefore it is concluded that the cross-reactivity between cinnamyl alcohol and cinnamaldehyde could not be explained by the impurities of cinnamaldehyde in cinnamyl alcohol.

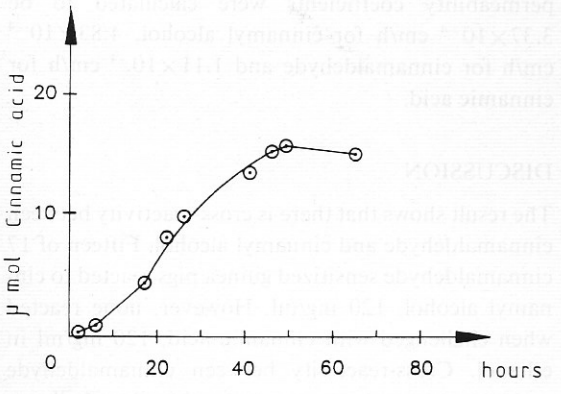

Fig. 3. In vitro percutaneous penetration of cinnamic acid through guinea pig skin.

Challenge was performed with 8 mg/ml cinnamaldehyde, 8 mg/ml cinnamyl alcohol and 9 mg/ml cinnamic acid on animals sensitized to cinnamaldehyde and cinnamyl alcohol, respectively, but the animals responded to cinnamaldehyde only. The higher challenge concentrations of cinnamyl alcohol (120 mg/ml) and cinnamic acid (200 mg/ml) compared to the challenge concentration of cinnamaldehyde (8 mg/ml) required to produce positive reactions, could be attributed to differences in permeability properties. However, investigations of the permeability properties for the three substances through guinea pig skin in vitro showed permeability coefficients approximately of the same order of magnitude under the test conditions.

The higher concentrations of cinnamyl alcohol and cinnamic acid used for challenge suggests that cinnamaldehyde is the hapten, and that cinnamyl alcohol and cinnamic acid are transformed (prohaptens) to cinnamaldehyde in the skin. However, cinnamaldehyde is the only substance of the three investigated which is able to react with protein in vitro, and is therefore expected to be the "true hapten". It is generally agreed that the ability of allergens to bind with protein is important for the development of allergic contact dermatitis, as haptens must bind to some protein receptors on antigen-presenting cells before the reaction can occur (17).

The higher challenge concentrations required for cinnamyl alcohol and cinnamic acid are therefore proposed to be related to a transformation of cinnamyl alcohol to cinnamaldehyde by the enzyme alcohol dehydrogenase and transformation of cinnamic acid to cinnamaldehyde by the enzyme aldehyde dehydrogenase. The equilibrium for the enzymatical reactions are displaced to cinnamyl alcohol and cinnamic acid in vitro (18), and high amounts of cinnamyl alcohol and cinnamic acid may therefore be used to press the equilibriums against cinnamaldehyde. In support of this theory, investigators have found alcohol and aldehyde dehydrogenase in guinea pigs (19) as well as both enzymes being detected in human skin (20, 21). Further absorption and transformation studies are warranted using different vehicles and receptor phases.

The lack of response to cinnamic acid is probably due to a low sensitization potential or a low degree of transformation in the skin. Higher concentrations of cinnamic acid than 2% were too toxic to be used for induction, very big wounds being seen.

REFERENCES

1. Hjorth N. Eczematous allergy to balsams. Thesis. Copenhagen: Munksgaard, 1961: 102–105.
2. Opdyke DLJ. Monographs on fragrance raw materials. Food Cosmet Toxicol 1979; 17: 241–275.
3. Calnan CD, Cronin E, Rycroft RJG. Allergy to perfume ingredients. Contact Dermatitis 1980; 6: 500–501.
4. Santucci B, Cristaudo A, Cannistraci C, Picardo M. Contact dermatitis to fragrances. Contact Dermatitis 1987; 16: 93–95.
5. Malten KE, Ketel W, Nater JP, Liem DH. Reactions in selected patients to 22 fragrance materials. Contact Dermatitis 1984; 11: 1–10.
6. North American Contact Dermatitis Group. Eiermann HJ, Larsen W, Maibach HI, Taylor JS. Prospective study of cosmetic reactions: 1977–1980; J Am Acad Dermatol 1982; 6: 909–917.
7. Klecak G, Geleick H, Frey JR. Screening of fragrance materials for allergenicity in the guinea pig. 1. Comparison of four testing methods. J Soc Cosmet Chem 1977; 28: 53–64.
8. Senma M, Fujiwara N, Sasaki S, Toyama M, Sakaguchi K, Takaoka I. Studies on the cutaneous sensitization reaction of guinea pigs to purified aromatic chemicals. Acta Derm Venereol (Stockh) 1978; 58: 121–124.
9. Weibel H, Hansen J. Penetration of the fragrance compounds, cinnamaldehyde and cinnamyl alcohol, through human skin in vitro. Contact Dermatitis 1989; 20: 167–172.
10. Weibel H, Hansen J. Interaction of cinnamaldehyde—a substance in fragrance—with protein. Contact Dermatitis 1989; 20: 161–166.
11. Wahlberg JE, Boman A. Guinea Pig Maximization Test. In: Andersen KE, Maibach HI, ed. Current problems in dermatology. Basel: Karger, 1985; 14: 59–106.
12. Andersen KE. Guinea Pig Maximization Test: Effect of type of Freund's complete adjuvant emulsion and of challenge site location. Derm Beruf Umwelt 1985; 33: 132–136.
13. Mitchell JC, Maibach HI. The angry back syndrome—the excited skin syndrome. Semin Dermatol 1982; 1: 9–13.
14. Andersen KE, Boman A, Vølund A, Wahlberg JE. Induction of formaldehyde contact sensitivity: Dose response relationship in the Guinea Pig Maximization Test. Acta Derm Venereol (Stockh) 1985; 65: 472–478.
15. Barry BW. Dermatological formulations. New York: Marcel Dekker, 1983.
16. Scheuplein RJ, Blank IH. Permeability of the skin. Physiol Rev 1971; 51: 702–747.
17. Tjernlund U, Scheynius A. Amplified T cell response to PPD by epidermal cell suspensions containing HLA-DR-expressing keratinocytes. Acta Derm Venereol (Stockh) 1988; 68: 57–60.
18. Bergmeyer HU. Methods of enzymatic analysis. New York: Academic Press 1963.
19. Zahlten RN, Nejtek ME, Jacobson JC. Ethanol metabolism in guinea pig: In vivo ethanol elimination, alcohol dehydrogenase distribution, and subcellular localization of acetaldehyde dehydrogenase in liver. Arch Biochem Biophys 1981; 207: 371–379.
20. Wilkin JK, Stewart JH. Substrate specificity of human cutaneous alcohol dehydrogenase and erythema provoked by lower aliphatic alcohols. J Invest Dermatol 1987; 88: 452–454.
21. Goedde HW, Agarwal DP, Harada S. Alcohol metabolizing enzymes: Studies of isozymes in human biopsies and cultured fibroblasts. Clin Genet 1979; 16: 29–33.