

ORIGINAL ARTICLE

Study of the usefulness of patch testing and use test to predict the safety of commercial topical drugs

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ABSTRACT

Patch testing (PT) can be used to identify allergens and irritants responsible for contact allergic and irritant dermatitis, respectively. However, the reproducibility of PT and correlation between PT and use test has not been fully evaluated. The aim of the present study was to examine the reproducibility of PT and its usefulness in assessing the safety of topical drugs. A total of 55 topical drugs were applied to the backs of two groups of subjects for either 24 or 48 h, and skin irritant reactions were graded at 2 and 24 h after patch removal. For the repeat open application test, six topical drugs with different irritation scores were applied to the arms of two groups of subjects twice daily for 3 weeks, and local symptoms were recorded. The mean irritation scores were similar between the two PT groups. The percentage of subjects positive for symptoms provoked by the use tests was similar between the two groups. The mean irritation scores 24 h after patch removal correlated with the skin symptoms provoked by the use test. PT was reproducible and the results correlated with the use test results. PT is a useful method for evaluating the safety of commercial topical drugs.

Key words: mean irritation score, patch test, repeat open application test, reproducibility of results, topical drugs.

INTRODUCTION

The ingredients contained in over-the-counter (OTC) topical drugs in Japan are regulated. Safety data regarding local irritation are not required in applications for new generic topical drugs if the ingredients conform to the standards set by the Ministry of Health, Labor and Welfare of the Japan Government.¹ Therefore, each drug manufacturer is responsible for performing safety evaluations of topical products. The type of assessment method is important in evaluating the cutaneous safety of OTC topical drugs.

The cutaneous safety of topical drugs must be tested on human subjects, and it is desirable to use the repeat open application test that reflects the clinical use of the drug. However, the test is time-consuming and may be stressful for the subjects. Therefore, patch testing (PT), a diagnostic method used to identify the cause of allergic contact dermatitis, has been used to assess the cutaneous safety of topical agents.²

In assessing skin irritation, skin reactions are rated and the mean skin irritation score is calculated.^{2–5} Assessment of skin irritation by PT is usually performed on a limited number of subjects (~20–40) and the results may vary widely due to genetic and/or environmental differences.

Studies on evaluation of skin irritation by PT have focused mainly on variability in the visual interpretation of PT results between observers or institutions,^{6–16} and comparisons between PT and *in vitro* skin irritation assays or animal data.^{17–22} The reproducibility of PT reactions in the evaluation of topical drugs has not been assessed. Assessment of skin irritation by the use test has not been fully examined. Furthermore, the relationship between the results of PT and use test in testing for skin irritation remains unclear, despite reports comparing the two methods.^{23–25}

In order to examine the reliability of PT in assessing the safety of topical drugs, we evaluated the reproducibility of PT and use test, and the relationship between PT and repeated open application test results in this study.

METHODS

Study design

This study was approved by the Institutional Review Board of Fujita Health University and the HUMA R&D Testing Review Board. Written informed consent was obtained from all participants. PT of 55 topical drugs commercially available in Japan was conducted on a total of 59 healthy individuals who were divided into two groups of 29 and 30 prior to testing. The skin

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reactions were rated and the mean skin irritation scores were calculated to evaluate the reproducibility between the two groups. Six of the 55 topical drugs with different skin irritation scores were applied to the arms of 52 healthy individuals for 3 weeks to simulate clinical use of the topical drugs, and the results were compared with those of PT.

PT

Subjects. A total of 59 volunteers were recruited for the PT study and were divided into two groups. Study 1 consisted of 29 subjects including four men and 25 women with an age range of 19–64 years who were living in the Aichi region in January 2011, while study 2 consisted of 30 subjects including four men and 26 women with an age range of 21–69 years who were living in either Aichi or Gifu regions in June 2011. The inclusion criteria were normal back skin and no oral or topical anti-allergic and corticosteroid drug use.

PT materials. Patch testing was conducted using 55 commercially available topical drugs, including topical antipruritic drugs, in Japan (13 creams for insect bites, 12 liquids for insect bites, 13 topical drugs for miliaria of the vulva, 17 topical drugs for dry skin) and seven control substances consisting of five skin irritants and two non-irritants (0.1% sodium lauryl sulfate [SLS] solution, 0.2% SLS, 2.0% sodium laurate solution, 0.1% benzalkonium chloride solution, 5.0% polyoxyethylene [$n = 10$] oleyl ether solution, distilled water, white petrolatum).^{5,11,26} The concentrations of the skin irritants are likely to cause irritation.⁵ The nature of the sample was not revealed until completion of the study.

PT. Using Finn Chambers on Scanpor tape (Smart Practice Japan, Yokohama, Japan), the test drugs were applied to subjects' backs and sealed for 24 (study 1) or 48 h (study 2). A total of 20 mg of ointment, cream or lotion was placed on an aluminum cup with a diameter of 8 mm. If the test drug was a liquid, a Finn Chamber filter paper disc was soaked in 15 μ L of the sample. Patches had 10–12 chambers and the application site varied among individuals. The chambers were removed after 24 (study 1) or 48 h (study 2) after application, and the skin reactions at 2 and 24 h after chamber removal were assessed by a single observer blinded to the test drug. One

Table 1. Skin reaction scoring criteria

Score	Reaction
0	No reaction
1	Noticeable erythema or erythema $\leq 50\%$ of the patch area
2	Minimal to moderate erythema or erythema $> 50\%$ of the patch area
3	Distinct erythema
4	Erythema with papular or edematous reaction
5	Erythema with vesicular reaction
6	Corrosive reaction (bullae formation, necrosis)

Table 2. Concentrations of active ingredients (%)

Drug number	No. 1 (gel)	No. 2 (lotion)	No. 3 (liquid)	No. 4 (liquid)	No. 5 (cream)	No. 6 (liquid)
Prednisolone acetate	0.125	2	2	2 (hydrochloride)	2 (hydrochloride)	2 (hydrochloride)
Lidocaine hydrochloride	3	1	1 (hydrochloride)	2 (hydrochloride)	0.5	
Diphenhydramine		10	10		0.2	
Urea					0.5	
Tocopheryl acetate		0.5		0.1		
Glycyrrhetic acid		0.2		5		
L-menthol	3	0.5		1		
DL-camphor					Isopropylmethylphenol (0.1)	Panthenol (1)
Others	Chlorpheniramine maleate (1) Benzethonium chloride (0.1) Glycol salicylate (2)	Crotamiton (5)				

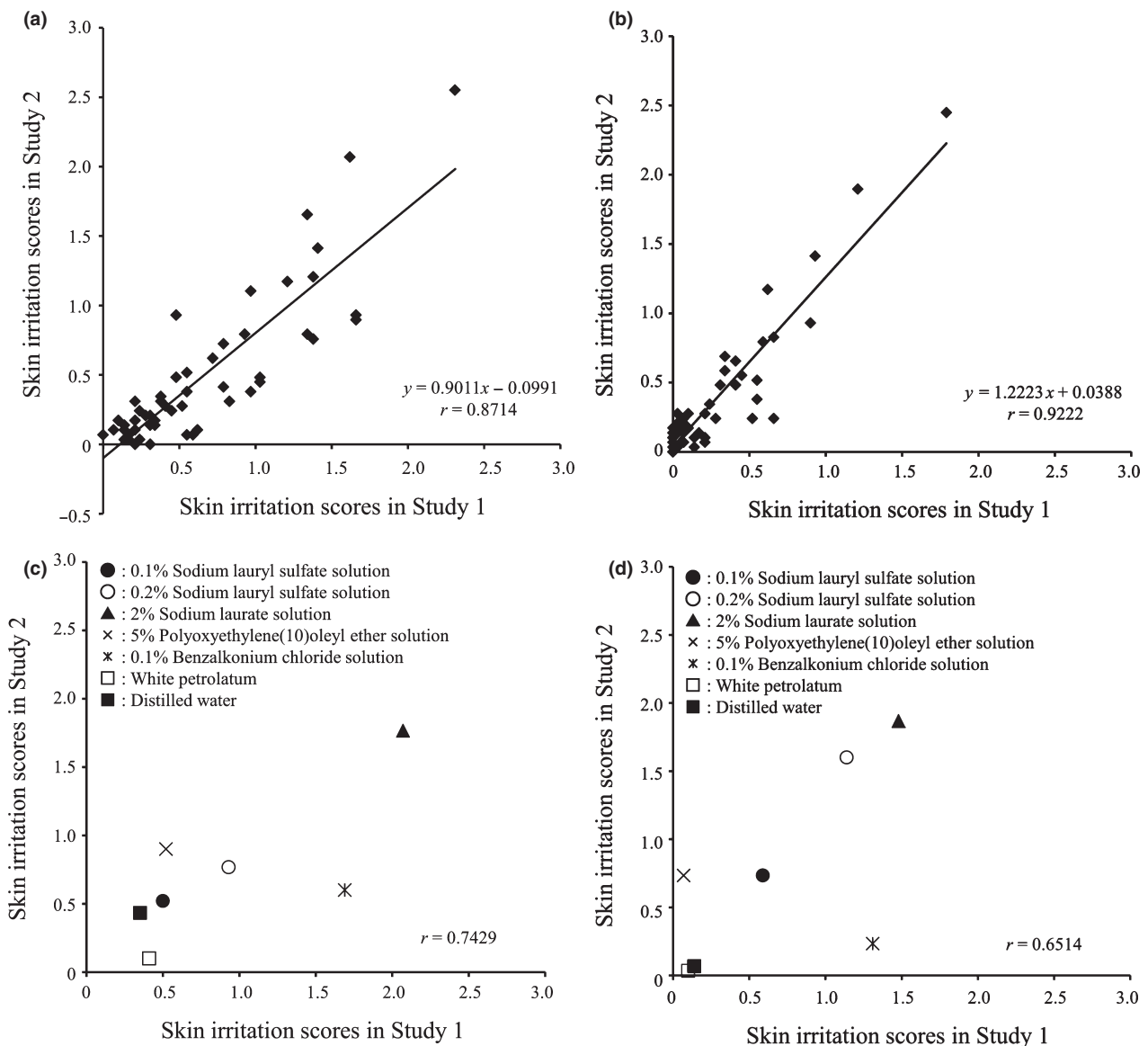


Figure 1. (a) Correlation between the mean skin irritation scores at 2 h after patch removal after 24 h (study 1) and 48 h (study 2) of drug exposure. (b) Correlation between the mean skin irritation scores at 24 h after patch removal after 24 h (study 1) and 48 h (study 2) of drug exposure. (c) Correlation between the mean skin irritation scores at 2 h after patch removal after 24 h (study 1) and 48 h (study 2) of exposure to control substances. (d) Correlation between the mean skin irritation scores at 24 h after patch removal after 24 h (study 1) and 48 h (study 2) of exposure to control substances.

dermatologist inspected the sites at 2 h after removal in study 1, and at 2 and 24 h in study 2. Another dermatologist inspected the sites at 24 h after removal in study 1. They were trained and gave ratings according to the criteria²⁷ for skin irritation of the skin irritation research group of the Japanese Society for Contact Dermatitis, as shown in Table 1.

Use test

Subjects. A total of 52 healthy subjects were recruited for the use test study and were divided into two groups. Study 3

consisted of 10 men and 12 women with an age range of 24–60 years who were living in the Toyama region in September 2011. Study 4 consisted of 12 men and 18 women with an age range of 21–58 years who were living in Tokyo, Kanagawa, Chiba or Saitama region in December 2013.

Test materials. Six topical drugs (Table 2) with the following mean irritation scores 24 h after patch removal were selected: 1.5 or more ($n = 1$); less than 0.2 ($n = 1$); and 0.2–1.4 ($n = 4$).

Table 3. Number and type of skin symptoms in study 3 and study 4

Drug No.	No. 1		No. 2		No. 3		No. 4		No. 5		No. 6	
	1.79/2.45		0.34/0.59		0.52/0.24		0.62/1.17		0.66/0.83		0/0.10	
Mean skin irritation score 24 h after patch removal (study 1/study 2)	Study 3	Study 4	Study 3	Study 4	Study 3	Study 4	Study 3	Study 4	Study 3	Study 4	Study 3	Study 4
Scaling	0	0	0	0	2	0	0	0	1	0	0	0
Erythema	6	0	1	0	0	0	2	0	1	0	1	0
Redness (transient)	4	1	12	0	12	0	8	0	9	1	7	0
Papule	1	3	2	3	0	2	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0
Soreness	2	1	4	0	1	0	4	0	0	0	1	0
Heat	0	0	0	0	0	0	1	1	0	0	0	0
Itchiness	3	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous: Skin peeling-like symptom (dry cream)	4	5	1	1	0	0	0	0	0	0	0	0
Miscellaneous: Coolness	0	0	0	3	0	0	0	0	0	0	0	0
Miscellaneous: Pigmentation	1	0	0	0	1	0	0	0	0	0	0	0
Total no. of symptoms observed	21	10	20	7	16	2	15	1	11	1	9	0
Total no. of symptoms observed excluding dried cream	17	5	19	6	16	2	15	1	11	1	9	0
Total no. of symptoms observed excluding transient redness	17	4	8	6	4	2	7	1	2	0	2	0
No. of subjects positive for symptoms provoked by the drug (% of total 22 [study 3/30 [study 4] subjects])	10 (45.5)	8 (26.7)	9 (40.9)	3 (10)	5 (22.7)	1 (3.3)	5 (22.7)	1 (3.3)	2 (9.1)	1 (3.3)	2 (9.1)	0
No. of subjects with objective symptoms (% of total 22 [study 3/30 [study 4] subjects])	6 (27.3)	3 (10)	6 (27.3)	2 (6.7)	4 (18.2)	1 (3.3)	4 (18.2)	0	2 (9.1)	1 (3.3)	2 (9.1)	0
No. of subjects with subjective symptoms (% of total 22 [study 3/30 [study 4] subjects])	4 (18.2)	5 (16.7)	4 (18.2)	2 (6.7)	1 (4.5)	0	2 (9.1)	1 (3.3)	0	0	1 (4.5)	0

Method. The test drugs were applied to one site on both upper arms and to two sites on both forearms (total of six application sites) twice daily (morning and after bathing in the evening) for 3 weeks.

Evaluation. Subjects were asked to rate objective symptoms (scaling, erythema, papules and edema) and subjective symptoms (soreness, heat and itchiness) on a scale of 0–3 (3, severe; 2, moderate; 1, mild; and 0, none). They were also asked to record the presence of any other symptoms. When the symptom score was either 1 or 2, they were required to send pictures of the cutaneous symptoms to the observer and they were provided instructions regarding continuation of the test and treatment. We instructed the participants to stop the test prior to assessment by the observer if the symptom score was more than 2 or if they suffered another adverse event. In addition, the observer or test administrator inspected the participant's arm when the test was finished.

Statistical analysis

The irritation scores between study 1 and study 2 at 2 and 24 h after patch removal, and the percentage of objective symptoms between study 3 and study 4 were compared using Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Reproducibility of PT

The distribution of the irritation scores for test drugs and control substances in study 1 and study 2 is shown in Figure 1. The irritation scores of each test drug at 2 (Fig. 1a) or 24 h (Fig. 1b) after patch removal in both studies were similar (Fig. 1a, $y = 0.9011x - 0.0991$, $r = 0.8714$, $P < 0.01$; Fig. 1b, $y = 1.2223x + 0.0388$, $r = 0.9222$, $P < 0.01$). Moreover, the mean scores were similar regardless of the duration of drug exposure.

The mean irritation scores ranged 0.03–0.43 for the negative control drugs and 0.07–2.07 for positive controls; a clear difference between the negative and positive controls in the range of values was observed (Fig. 1c, $r = 0.7429$; Fig. 1d, $r = 0.6514$). The mean irritation scores of 0.2% SLS solution in study 1 and study 2 were 0.77 and 0.93 at 2 h after patch removal, and 1.14 and 1.60 at 24 h after patch removal, respectively. The mean irritation scores of 0.1% SLS solution in study 1 and study 2 were 0.50 and 0.52 at 2 h after patch removal, and 0.59 and 0.73 at 24 h after patch removal, respectively. The results of the SLS PT suggest that the skin irritation scores are concentration-dependent.

Use test with twice daily application to the arm for 3 weeks

Table 3 show the mean skin irritation scores 24 h after drug patch removal, the number of individuals who developed skin symptoms or abnormal sensation, and the nature of the skin manifestations in study 3 and study 4. The distribution of the percentage of subjects positive for symptoms provoked by the

test drugs in study 3 and study 4 is shown in Figure 2 (Fig. 2a, $r = 0.8414$, $P < 0.05$; Fig. 2b, $r = 0.7651$; Fig. 2c, $r = 0.8510$, $P < 0.05$). The percentage of positive symptoms was similar between the two groups.

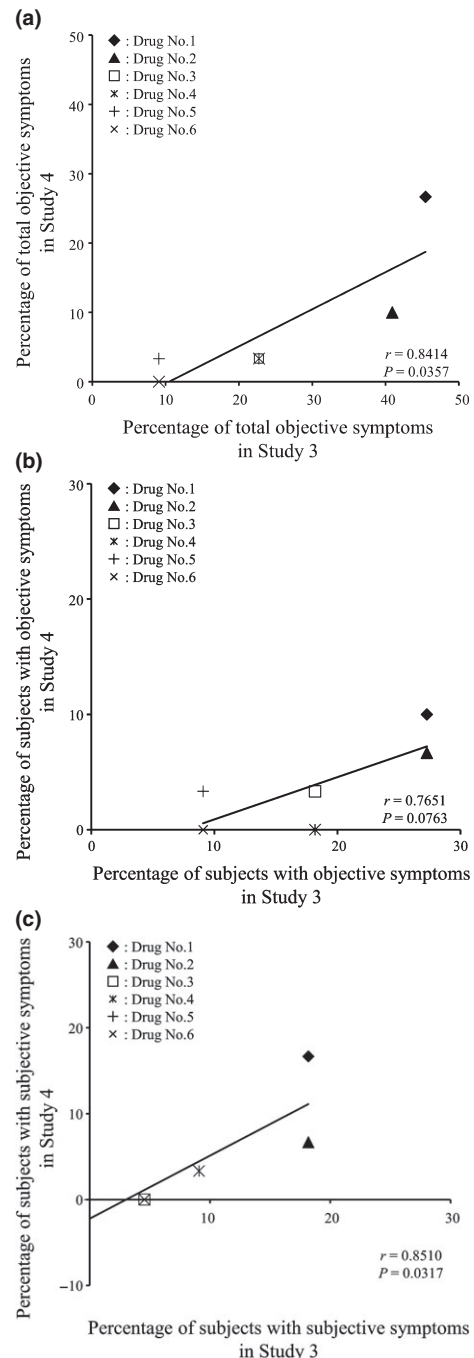


Figure 2. Correlation between the number of subjects and symptoms in study 3 and study 4. (a) Percentage of total objective symptoms. (b) Percentage of subjects with objective symptoms. (c) Percentage of subjects with subjective symptoms.

With respect to the nature of the symptoms, redness was observed most often, which was described as transient (disappearing within 0.5–1 h) and associated with an elevation of body temperature due to exercise, drinking and high ambient temperature. Drug no. 1 and no. 2 resulted in skin peeling-like

features, which might have been due to hardening of the topical drug.

During the application test, one subject was suspected to have an allergic reaction to test drugs no. 1 and 2, and the application of these drugs was discontinued on day 2. Another

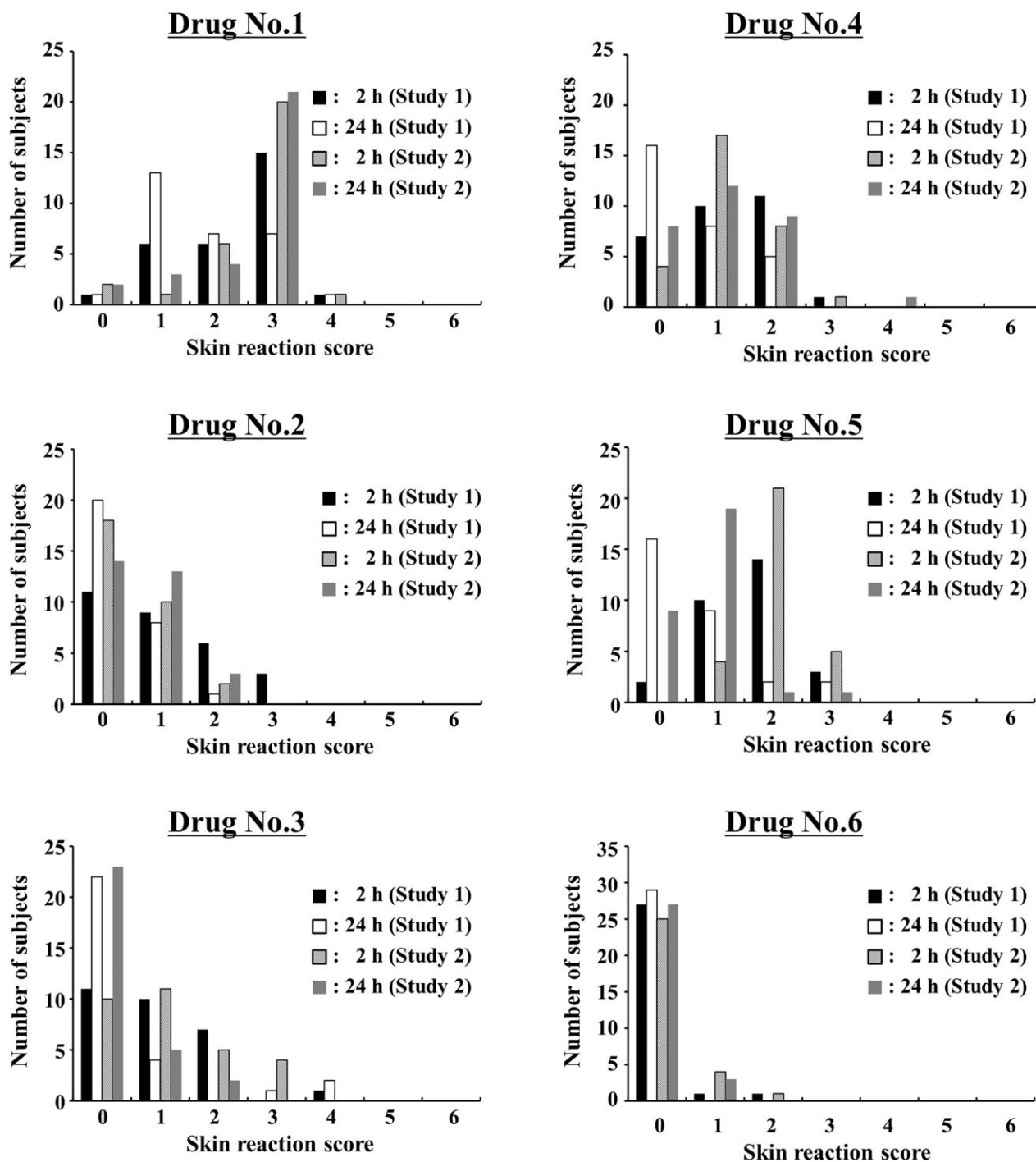


Figure 3. Patch testing irritation scores at 2 and 24 h after patch removal for each test drug in study 1 and study 2.

subject complained of itchiness on day 12 of application of test drug no. 1, and it was subsequently discontinued on day 14 because it was difficult to continue the test (study 3). One subject had a papule on day 8 of application of test drugs no. 1 and no. 2, and they were discontinued on day 9. Another subject had a papule on day 6 of application of test drugs no. 1, no. 2 and no. 3, and they were discontinued on day 7 (study 4).

Relationship between the results of PT and the use test with twice daily application for 3 weeks

Figure 3 shows the PT results of six topical drugs. Specifically, the skin reaction scores 2 and 24 h after patch removal are shown. Over half of the subjects had a score of 3 after exposure to drug no. 1 (mean irritation score 24 h after patch removal, ≥ 1.5). Most subjects had a score of 0 after exposure to drug no. 6 (mean irritation score 24 h after patch removal, < 0.2). The scores of drugs no. 2–5 (mean irritation scores 24 h after patch removal, 0.2–1.4) varied from 0 to 4. When the test drugs were classified according to their skin irritation scores, it was found that the number of subjects with skin symptoms increased as the mean score rose, suggesting that the skin irritation scores correlate with skin symptoms (Fig. 4).

Table 4 shows the use test results including the number of subjects with objective or subjective symptoms, reaction score and assessment periods. Objective or subjective symptoms were documented from days 1–21. The total symptom scores of each drug, excluding transient redness, increased as the mean score rose.

DISCUSSION

Patch testing has been used to evaluate the skin irritancy of cosmetics, quasi-drugs and topical drugs. However, detailed evaluation of the reproducibility of this method or the significance of its results in relation to skin irritation on clinical application of the drug has not been examined.^{23–25}

In this study, participants were selected at random who had normal back skin and on inspection. Biological assessments of barrier function such as transepidermal water loss, skin hydration and pH were not performed, and menstrual cycle was not considered in the selection criteria. PT of 55 topical drugs and seven control substances was performed with either a 24- or 48-h patch application (study 1 and 2, respectively) on two groups of subjects in January (study 1) and in June (study 2). The mean skin irritation scores^{2–5} were similar in both studies, suggesting that the PT skin irritation score is reliable, even for a group of approximately 30 individuals with different genetic backgrounds and living environments.

Furthermore, the skin irritation score of the positive and negative control drugs were clearly different, and PT using different concentrations of SLS solutions revealed that the skin irritation score was concentration-dependent. As the skin reaction after 24 h of exposure was similar to that after 48 h of exposure, the 24-h closed test is considered sufficient for testing skin irritancy, and is also more convenient for the patient

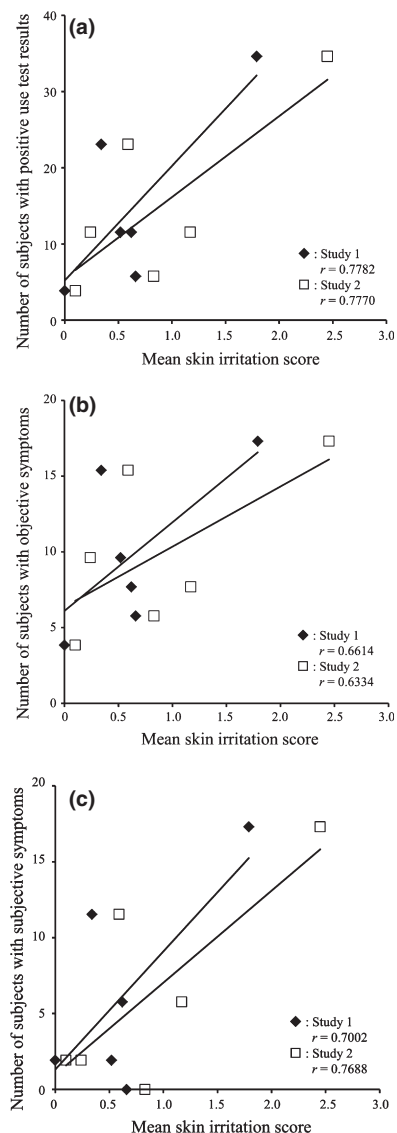


Figure 4. Relationship between patch testing-derived mean skin irritation scores at 24 h after patch removal and repeated open application test results. (a) Number of subjects with positive use test results. (b) Number of subjects with objective symptoms. (c) Number of subjects with subjective symptoms.

and examiner.⁵ The above findings suggest that PT can identify skin irritancy of topical drugs and that PT results reflect the intensity of irritant reactions. Thus, this study confirms the reliability of PT.

It is important that the cutaneous safety of topical drugs is tested on human subjects, and it is desirable to use the repeat open application test that reflects the clinical use of the drug. In this study, six topical drugs with different irritation scores were applied to the arms of two groups of 22–30 subjects, and symptoms were documented from days 1–21. It was found that the number of positive symptoms was similar between the

Table 4. Use test results: number of subjects with objective and subjective reactions and their scores

Drug no.	Assessment period	Scaling			Erythema			Redness (transient)				Papule			Soreness			Heat			Itchiness		
		0	1	2	0	1	2	0	1	2	3	0	1	2	0	1	2	0	1	2	0	1	2
1	Days 1–7		–		51	0	1	47	5	0	0	49	2	1	49	0	3	–			52	0	0
	Days 8–14		–		50	2	0	52	0	0	0	51	1	0	52	0	0	–			49	0	3
	Days 15–21		–		49	3	0	52	0	0	0	52	0	0	52	0	0	–			52	0	0
	Total score		–			7		5				5			6			–			6		
2	Days 1–7		–		51	0	1	45	6	1	0	49	2	1	49	2	1	–			–		
	Days 8–14		–		52	0	0	50	1	1	0	50	1	1	51	1	0	–			–		
	Days 15–21		–		52	0	0	49	3	0	0	52	0	0	52	0	0	–			–		
	Total score		–			2		16				7			5			–			–		
3	Days 1–7	52	0	0	–			45	6	1	0	50	2	0	51	1	0	–			–		
	Days 8–14	52	0	0	–			50	2	0	0	52	0	0	52	0	0	–			–		
	Days 15–21	50	2	0	–			49	3	0	0	52	0	0	52	0	0	–			–		
	Total score		2		–			13				2			1			–			–		
4	Days 1–7	–			52	0	0	48	3	1	0	–			–			52	0	0	–		
	Days 8–14	–			52	0	0	47	1	4	0	–			–			51	0	1	–		
	Days 15–21	–			50	2	0	51	1	0	0	–			–			51	1	0	–		
	Total score	–				2		15				–			–			3			–		
5	Days 1–7	52	0	0	51	1	0	47	3	2	0	–			–			–			–		
	Days 8–14	52	0	0	52	0	0	49	2	0	1	–			–			–			–		
	Days 15–21	51	1	0	51	1	0	50	1	0	1	–			–			–			–		
	Total score		1			2		14				–			–			–			–		
6	Days 1–7	–			51	1	0	48	3	1		–			51	1	0	–			–		
	Days 8–14	–			52	0	0	50	1	1		–			52	0	0	–			–		
	Days 15–21	–			52	0	0	51	1	0		–			52	0	0	–			–		
	Total score	–				1		9				–			1			–			–		

two groups, and the number of subjects with skin symptoms increased as the mean score rose. Furthermore, total symptom scores, excluding transient redness, increased as the mean skin irritation scores rose.

Skin irritation was observed despite the application of anti-inflammatory drugs including steroid and those with anti-inflammatory ingredients in PT and use test. Therefore, PT and use test can detect skin irritation without being affected by anti-inflammatory effects. Application of drugs including steroid resulted in a bluish tinge to the skin at 2 h after chamber removal in PT. Compared with the PT skin irritation scores 2 h after patch removal, the scores at 24 h after patch removal showed a higher correlation with the number of subjects who developed skin symptoms in the use test (data not shown). It has been reported that skin irritant reactions were strongest at 24 h after patch removal and tended to be weaker at 48 h after patch removal.⁵ These observations suggest that 24 h after patch removal is an optimal time point for reading the results of PT for potential skin irritants. Drug no. 1 had a high mean skin irritation score on PT (scores, >3), a higher number of discontinued subjects and was associated with skin symptoms, while drug no. 6 had a low PT score associated with few skin symptoms. Other drugs were associated with skin symptoms and had to be discontinued in several subjects, possibly caused by PT reactions with scores of 3. Further study is needed to clarify the significance of PT and skin irritation scores in determining skin safety.

The transient redness appeared when the body temperature rose and disappeared after 0.5–1 h, and was not perceived by patients as irritative. Presumably, it was caused by transient vasodilation without inflammatory cell infiltration.^{28,29} The total score of objective symptoms excluding transient redness also increased with the skin irritation mean score, suggesting that the skin irritation scores correlate with skin symptoms (Table 4).

Drugs no. 1 (gel) and no. 2 (lotion) resulted in skin peeling-like features but no erythema or itchiness were observed (Table 3). This was thought to be due to hardening of the topical drugs rather than inflammation. The above-mentioned drugs, those with high PT scores or use test scores, those that provoked symptoms, and those that induced skin peeling-like symptoms require further study to determine whether these are related to skin irritation.

The above-mentioned observations suggest that PT is useful in predicting the safety of topical drugs in addition to the repeated open application test that simulates the clinical use of the test drug. In this study, the participants had normal skin on inspection; when we study the relationship between normal skin and sensitive skin, we should examine biological assessment of barrier function. As transient redness was observed in the repeated open application test in this study, the erythema in PT is thought to reflect vasodilation combined with an irritant reaction with an inflammatory cell infiltrate, especially in commercially available drugs containing active ingredients.

In conclusion, the mean skin irritation score derived from PT is a reproducible system for evaluating the safety of topical drugs and accurately reflects the severity of skin irritation. Also, the skin irritation score of PT closely correlated with the number of skin symptoms observed in the repeated open application test that simulated the clinical use of the drug. PT is a useful method for predicting the safety of topical drugs.

CONFLICT OF INTEREST: The authors have no conflict of interest.

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