表1　原因製品別の症例内訳（化粧品）

	原因製品	2010年	2011年	2012年	合計
1	染毛剤	75	85	101	261
2	化粧水	63	86	86	235
3	洗顔料	58	90	79	227
4	シャンプー	54	90	142	286
5	美容液	53	79	128	260
6	クリーム	38	54	53	145
7	ファンデーション	22	47	42	111
8	口紅	30	37	31	98
9	日焼け止め	26	41	40	107
10	乳液	27	40	47	114
11	化粧下地	8	19	34	61
12	トリートメント	6	12	19	37
13	コンディショナー			12	12
14	アイライナー	4	13	6	23
15	整髪料	6	11	11	28
16	アイシャドウ	6	6	12	24
17	マスカラ	4	7	11	22
18	リンス	5	4	5	14
19	頬紅	1	8	2	11
20	パック	1	5	7	13
21	クレンジング	5		57	62
22	ネイル用品	2	3	3	8
23	ボディソープ	5		11	16
24	リップクリーム	3	2	18	23
25	養毛料	5			5
26	アイブロウ	1	3		4
27	アロマオイル	1	3	6	10
28	パーマ剤	4		3	7
29	ハンドクリーム	1	3	3	7
30	おしろい	1	2	2	5
31	ひげそり用化粧品	2	1		3
32	スプレー	1	1		2
33	マッサージクリーム		2		2
34	まつ毛化粧料	2			2
35	美白剤	2			2
36	アートメイク		1		1
37	アイメイクリムーバー	1			1
38	コンシーラー		1		1
39	ベビーオイル	1			1
40	ムース		1		1
41	香水		1	2	3
42	植物エキス	1			1
43	制汗剤		1		1
44	全身ローション		1		1
45	美容オイル		1		1
46	保湿ゲル	1		9	10
47	不明	12	17	19	48
	化粧品合計	538	778	1,001	2,317

品へ処方する段階で，3）市販する現場で，4）消費者の実際の使用時，5）皮膚障害の発症時，6）障害情報の収集，共有と活用，そのすべてのチェックポイントを総合的に考えなければ確保できない．

ここで述べた2つの事例は，医師からの情報を企業がいち早く活かして行動すれば早く解決に繋がったことを示唆している．安全で安心な質の高い Made in Japan を自国および世界に出していくには，医師が市販後の皮膚等への健康被害を早期に把握し，医療者，企業，行政に情報提供し，そして国民に周知し情報を共有活用する産学官の情報ネットワークが必要である．筆者等は2014年5月からネットワーク「**化粧品等皮膚安全性症例情報ネット**」の本格始動を開始する．厚生労働省は4月1日より化粧品・医薬部外品についても，治療が30日以上必要な症例は，メーカーから厚労省への症例報告義務を課し市販後の化粧品・医薬部外品の健康被害情報の収集に乗り出している．この**化粧品等皮膚安全性症例情報ネット**は2014年度厚生労働省食品医薬品局安全対策課の指定研究として3年間の2年目の年を迎える．有益なネットワーク構築をめざす．

文　献

1) 高山かおる，横関博雄，松永佳世子，他：接触皮膚炎診療ガイドライン日皮会誌，119：1757－1793，2009.
2) 千貫祐子，森田栄伸：最近話題の皮膚疾患　旧「茶のしずく石鹸」中の加水分解小麦により感作されたFDEIA．臨床皮膚科，66：8－11，2012.
3) 矢上晶子，松永佳世子：加水分解コムギ含有石鹸によるコムギアレルギーの疫学と社会的意義．アレルギー・免疫，20：224－232，2013.
4) Fukutomi Y, Itagaki Y, Taniguchi M, et al : Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise induced anaphylaxis. J Allergy Clin Immunol 127 : 531－533, 2011.
5) Chinuki Y, Morita E : Wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat protein in soap. Allergol Int, 61 : 529－537, 2012.
6) Hiragun M, Ishii K, Hiragun T, et al : The sensitivity and clinical course of patients with wheat-dependent exercise-induced anaphylaxis sensitized to hydrolyzed wheat protein in facial soap - secondary publication. Allergol Int, 62 : 351－358, 2013.

JAPANESE DERMATOLOGICAL ASSOCIATION
THE JOURNAL OF DERMATOLOGY

Possible allergic contact dermatitis with reticulate postinflammatory pigmentation caused by hydroquinone

Dear Editor,

Hydroquinone is one of the most prevalent skin-lightening agents. It functions by inhibiting enzymatic oxidation of tyrosine and phenol oxidases.[1] It shows sensitizing potential.[2]

A 50-year-old woman visited us with pruritic erythema with reticulate pigmentation on the face. Four months prior, pruritic erythematous macules had developed around the lip and the nasal cavity where she had used a skin-lightening product. A topical ointment containing methylprednisolone and fradiomycin sulfate had been applied. After improvement, the affected lesions had turned into hyperpigmentation. The patient had been recommended to use another skin-lightening product containing 3% of hydroquinone. The next day of application, pruritic erythema had developed. The patient had stopped using it, but the lesion turned into erythema with pigmentation. Physical examination revealed slightly pruritic erythema with reticulate pigmentation around the lip and the nasal cavity (Fig. 1a). Dermoscopy showed reticulated (arrow) and dotted (dotted arrow) hyperpigmentation and sting-like structures (bold arrow) (Fig. 1b). We did not take a biopsy specimen.

The first patch testing (International Contact Dermatitis Research Group criteria; Finn Chambers on Scanpor tape; Epitest, Tuusula, Finland) was done with the secondary used skin-lightening product as is. It showed a positive reaction to the product at D2 (+) and D4 (+).

The ingredients, provided by the manufacturer, were prepared for second patch testing as follows: the hydroquinone 3% pet., 1,2-hexanediol 1% pet., stearate/glycolate glyceryl 1% pet., royal jelly 1% pet., soybean seed extract 1% pet., polysorbate-60 5% pet., allantoin 0.5% aq. and disodium glycyrrhizinate 1% pet. Second patch testing showed a positive reaction to hydroquinone 3% pet. at D2 (+), D4 (+) (Fig. 1c) and D12 (+) (Fig. 1d), and postinflammatory reaction at D19.

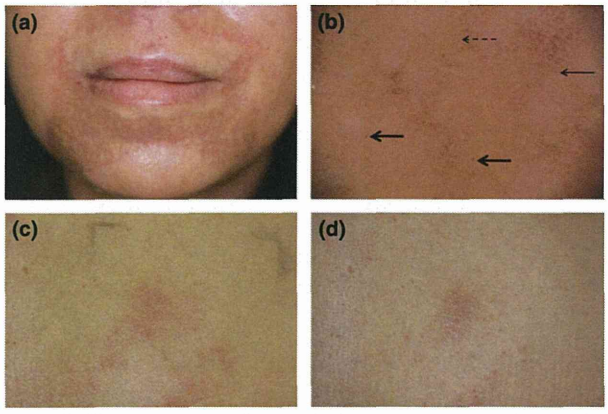

Figure 1. (a) Slightly pruritic erythema with reticulate pigmentation around the lip and the nasal cavity. (b) Dermoscopy showed reticulated (arrow) and dotted (dotted arrow) hyperpigmentation and sting-like structures (bold arrows). (c,d) A positive patch test reaction to hydroquinone 3% pet. at (c) D4 and (d) D12.

Referring to the clinical course, we surmised that delayed-type allergy to hydroquinone was sensitized during using the initially used skin-lightening product and that recurrence as allergic contact dermatitis was provoked by the secondary used one.

Hydroquinone can induce exogenous ochronosis when overused.[3] Irritant contact dermatitis can be caused dose-dependently, usually at a concentration of more than 4%.[4] In this case, we speculated that allergic contact dermatitis accelerated topical toxicity of hydroquinone itself and induced reticulate postinflammatory pigmentation. The patch tested site at D19 showed diffuse pigmentation. Ultraviolet exposure may influence reticulate pigmentation on the face.

Nath and Thappa reported the frequency of allergic contact sensitivity to hydroquinone as 4% in 25 patch-tested patients with allergic or pigmented contact dermatitis from the same bland cosmetics.[5] Allergic contact dermatitis from hydroquinone is not common. We further need to patch test with hydroquinone 0.01%, 0.1% and 1% pet. in our patient to confirm distinct allergic sensitivity, although we could not perform the third patch testing.

Exogenous ochronosis is characterized by yellow-brown, banana-shaped pigment fibers in the dermis.[3] Mishra *et al.*[3] reported multiple thin, short arciform structures as a dermoscopic figure of ochronosis. We speculated that sting-like structures in our case would be associated with ochronosis.

We need to accumulate case series of allergic contact dermatitis with particular adverse reaction caused by skin-lightening agents for the safety of customers.

CONFLICT OF INTEREST: No funding was received and no conflicts of interest declared.

Megumi TATEBAYASHI,[1] Naoki OISO,[1] Tamae WADA,[2] Kayoko SUZUKI,[3] Kayoko MATSUNAGA,[4] Akira KAWADA[1]

[1]*Department of Dermatology, Kinki University Faculty of Medicine,* [2]*Department of Dermatology, PL Hospital, Osaka,* [3]*Department of Dermatology, Kariya Toyota General Hospital and* [4]*Department of Dermatology, Fujita Health University School of Medicine, Aichi, Japan*

doi: 10.1111/1346-8138.12526

Correspondence: Megumi Tatebayashi, M.D., Department of Dermatology, Kinki University Faculty of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan. Email: mhama@med.kindai.ac.jp

REFERENCES

1 Draelos ZD. Skin lightening preparations and the hydroquinone controversy. *Dermatol Ther* 2007; **20**: 308–313.
2 To-o K, Nomura K, Sugimoto K, Nishimura T, Kuriki T, Itoh M. Experimental study on cross-reactivity of α-arbutin toward *p*-phenylenediamine and hydroquinone in guinea pigs. *J Dermatol* 2010; **37**: 455–462.
3 Mishra SN, Dhurat RS, Deshpande DJ, Nayak CS. Diagnostic utility of dermatoscopy in hydroquinone-induced exogenous ochronosis. *Int J Dermatol* 2013; **52**: 413–417.
4 Kligman AM, Willis I. A new formula for the depigmenting human skin. *Arch Dermatol* 1975; **111**: 40–48.
5 Nath AK, Thappa DM. Kumkum-induced dermatitis: an analysis of 46 cases. *Clin Exp Dermatol* 2007; **32**: 385–387.

Immunoglobulin G4-related disease in a psoriasis vulgaris patient treated with ustekinumab

Dear Editor,

Immunoglobulin (Ig)G4-related disease (IgG4-RD) is a new proposed disease characterized by elevated serum IgG4 levels and the infiltration of IgG4-positive cells.[1] To our knowledge, the current case is the first one of IgG4-RD in a patient of psoriasis vulgaris treated with ustekinumab.

A 71-year-old man had a 20-year history of severe psoriasis vulgaris. Although he had been treated using a topical steroid and phototherapy, the therapeutic efficacy was limited. He was admitted to our hospital in January 2011. Although infliximab therapy was effective, it was stopped for 1 month because of the incidence of bacterial pneumonia. In March 2012, his skin condition became worse (Psoriasis Area and Severity Index [PASI], 37.9). At that time, blood examination showed the following results: white cell count, 8100/μL; C-reactive protein, 0.06 mg/dL; urea nitrogen, 16.9 mg/dL; and creatinine, 0.94 mg/dL. Whole-body computed tomography (CT) showed aorta dissection and coronary artery calcification. Thus, ustekinumab was administrated at weeks 0 and 4 and every 12 weeks (45 mg per s.c. injection).

At 14 months after initiation of ustekinumab therapy, renal function became slowly worse (serum urea nitrogen, 20.4 mg/dL; serum creatinine, 1.73 mg/dL) although his psoriatic lesion improved (PASI, 3.6). Whole-body plain CT revealed mediastinal lymphoadenopathy, retroperitoneal fibrosis and bilateral hydronephrosis (Fig. 1). Additional laboratory studies revealed the following values: white cell count, 6800/μL; C-reactive protein, 2.03 mg/dL; serum IgG, 3714 mg/dL (normal range, 800–1600); and serum IgG4, 311 mg/dL (normal, <105). These results indicated that he had IgG4-related retroperitoneal fibrosis. Although contrast enhanced CT-guided biopsy was essential for definite diagnosis, it could not be conducted because of his kidney dysfunction. Thus, he was clinically diagnosed as having IgG4-RD. We discontinued ustekinumab and started oral prednisolone therapy (1.0 mg/kg per day; 60 mg/day). Three months later, serum IgG4 levels had increased, and renal dysfunction and retroperitoneal fibrosis had largely improved. Currently, the patient takes oral prednisolone (2.5 mg/day) and cyclosporin (100 mg/day) because of recurrence of psoriasis without the exacerbation of IgG4-RD.

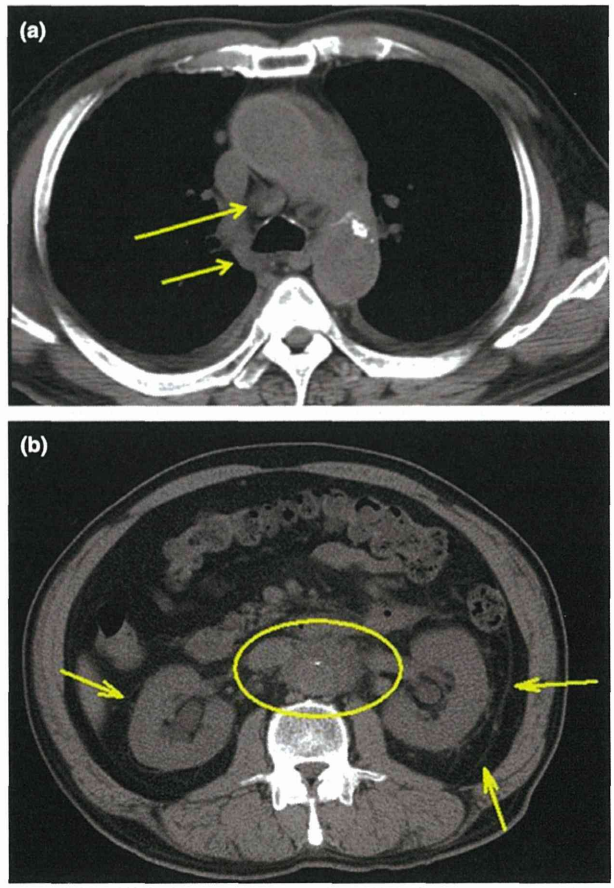

Figure 1. Computed tomography showed (a) mediastinal lymphoadenopathy (arrows) and (b) retroperitoneal fibrosis (circle) and its secondary bilateral hydronephrosis (arrows).

Correspondence: Ikko Kajihara, M.D., Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan. Email: kajiderma@gmail.com

Allergology International. 2014;63:243-249
DOI: 10.2332/allergolint.13-OA-0618

ORIGINAL ARTICLE

A New Reliable Method for Detecting Specific IgE Antibodies in the Patients with Immediate Type Wheat Allergy due to Hydrolyzed Wheat Protein: Correlation of Its Titer and Clinical Severity

Masashi Nakamura[1,2], Akiko Yagami[1], Kazuhiro Hara[2], Akiyo Sano[1], Tsukane Kobayashi[1], Michiko Aihara[3], Michihiro Hide[4], Yuko Chinuki[5], Eishin Morita[5], Reiko Teshima[6] and Kayoko Matsunaga[1]

ABSTRACT

Background: Immediate-type wheat allergy caused by a specific hydrolyzed wheat protein (HWP-IWA), Glupearl 19S (GP19S), typically develops food-dependent exercise-induced anaphylaxis (FDEIA), but is different from conventional FDEIA, or simple wheat allergy in many aspects. The skin prick test (SPT) is considered to be the most effective method for diagnosis of HWP-IWA. As SPT is a relatively qualitative method, we developed quantitative and high-throughput test method for HWP-IWA.

Methods: An enzyme-linked immunosorbent assay (ELISA)-based GP19S-specific IgE assay was tested using sera from 14 HWP-IWA and five conventional wheat-dependent exercise-induced anaphylaxis (CO-WDEIA) patients, as well as five healthy subjects. Then a validation study at five different institutions was carried out using sera from 10 HWP-IWA and five CO-WDEIA patients, as well as five healthy subjects different from the previous studies.

Results: The mean unit values converted from measured absorbance of ELISA were 68.3, 1.3 and 1.1 respectively. Furthermore, the validation study revealed reproducible results across all five institutions, with the standard deviation (SD) being 0.3-0.4 for the healthy group, 0.2-0.6 for the CO-WDEIA group, and 3.8-9.6 for HWP-IWA group except for one case. One case of HWP-IWA was excluded from analysis due to the high SD of 53.3 units, indicating that samples with a unit value > 100.0 will affect inter-laboratory reproducibility.

Conclusions: Our findings suggest that the ELISA-based GP19S-specific IgE assay can be used to test HWP-IWA using venous blood samples, except for those with a unit value > 100.0.

KEY WORDS

enzyme-linked immunosorbent assay, Glupearl 19S, hydrolyzed wheat protein, immediate-type wheat allergy, test method

[1]Department of Dermatology, Fujita Health University School of Medicine, [2]General Research and Development Institute, Hoyu Co., Ltd., Aichi, [3]Department of Environmental-Immunodermatology, Yokohama City University Graduate School of Medicine, Kanagawa, [4]Department of Dermatology, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima, [5]Department of Dermatology, Shimane University Faculty of Medicine, Shimane and [6]National Institute of Health Sciences, Tokyo, Japan.

Conflict of interest: MH received honoraria and research funding from Sanofi, GlaxoSmithKline, Tanabe-Mitsubishi. Other authors have no conflict of interest.

Correspondence: Kayoko Matsunaga, MD, PhD, Department of Dermatology, Fujita Health University School of Medicine, 1–98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi 470–1192, Japan.
Email: kamatsu@fujita-hu.ac.jp

Received 20 August 2013. Accepted for publication 15 December 2013.

INTRODUCTION

Many cases of Immediate-type allergy after wheat intake have been observed in Japanese consumers using cosmetics that contain hydrolyzed wheat protein (HWP), HWP-IWA, who had used "Cha no Shizuku" soap (sold by Yuuka, Fukuoka, Japan) that contained Glupearl 19S (GP19S), a substance manufactured by Katayama Chemical Industries, Osaka, Japan. HWP-IWA patients, but not conventional wheat allergy patients, react to GP19S. Therefore, GP19S hypersensitivity is essential for diagnosis of HWP-IWA.[1,2]

HWP is a cosmetic ingredient specified in the Japanese Standards of Quasi-drug Ingredients, and which is a collective term for water-soluble materials that are produced by hydrolyzing wheat glutens with acid, alkali, enzymes or other substances. Cases of HWP allergy have been reported in Western nations; notably, cases are fewer and less severe than those in Japan.[3-6] In order to address the problem of HWP allergy, the "Special Committee for the Safety of Protein Hydrolysates in Cosmetics" was organized by the Japanese Society of Allergology to study the epidemiology, pathogenesis, and establish diagnostic criteria, among other activities.

HWP-IWA is different from conventional wheat allergy. In contrast to the onset of conventional wheat allergy in children, HWP-related allergy arise in adults with a history of cosmetic use.[7] Although both conventional and HWP-related wheat allergy in adults can cause wheat-dependent exercise-induced anaphylaxis (WDEIA), unlike conventional WDEIA (CO-WDEIA), HWP-IWA is not mediated by ω-5 gliadin.[8]

Currently, the skin prick test (SPT) by GP19S is considered the most useful method in diagnosing HWP-IWA.[7] It would be advantageous to develop a quantitative, high-throughput method for the laboratory diagnosis of HWP-IWA that gives consistent results across different institutions. For this purpose, we evaluated the utility of GP19S-specific IgE antibody detection by enzyme-linked immunosorbent assay (ELISA) for the diagnosis of HWP-IWA using sera from healthy individuals and from patients with CO-WDEIA or HWP-IWA. Five institutions were involved in this study to assess the reliability of this method.

METHODS

SUBJECTS

The HWP-IWA group consisted of 24 patients diagnosed with immediate-type wheat allergy induced by GP19S according to the diagnostic criteria by Special Committee for the Safety of Protein Hydrolysates in Cosmetics; CO-WDEIA group consisted of 10 patients with conventional WDEIA; and the healthy control group consisted of 10 individuals without wheat allergy (Table 1, 2). HWP-IWA patient 1 to 14, CO-WDEIA patient and healthy control 1 to 5 were used for utility evaluation of ELISA-based GP19S-specific IgE assay. HWP-IWA patient 15 to 24, CO-WDEIA patient and healthy control 6 to 10 were used for validation this method. Patients 1 to 14 in the HWP-IWA group were classified into the following four grades of severity based on symptoms after wheat ingestion: grade 1, eyelid swelling, symptoms limited to the face and nasal mucosa; grade 2, generalized urticaria in addition to grade 1 symptoms; grade 3, systemic symptoms (e.g. dyspnea, diarrhea) in addition to dermal swelling; and grade 4, anaphylactic shock. The presence of specific serum IgE was determined using ImmunoCAP (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). Sensitivity to GP19S was evaluated using SPT.

ELISA-BASED GP19S-SPECIFIC IgE ASSAY

GP19S (Katayama Chemical Industries) at 1 mg/ml dissolved in phosphate-buffered saline (PBS) was centrifuged and the supernatant was recovered (GP19S solution). Next, 100 µl of GP19S solution was added to each well of a Nunc MaxiSorp flat bottom 96-well plate (Thermo Fisher Scientific, Waltham, MA, USA), and the plate was sealed and left overnight at 4°C. The plate was blocked with 1% skim milk/PBS with 0.1% Tween 20 (PBS-T) for 1 hour at room temperature, after which 100 µl patients' sera diluted to 20% in 1% skim milk/PBS-T were added to the wells, followed by a further incubation for 1 hour at room temperature. The plate was then washed with 1% skim milk/PBS-T. A total of 100 µl of 0.1 µg/ml anti-human IgE-HRP conjugate (KPL, Gaithersburg, MD, USA) in 1% skim milk/PBS-T was added to the wells, and the plate was incubated for 1 hour at room temperature. The plate was washed, and the colorimetric reaction was developed by adding 1-Step Ultra TMB-ELISA (Thermo Fisher Scientific) and incubated for 15 min at room temperature. The reaction was stopped by adding 2 M H_2SO_4. Absorbance was measured by multi-plate optic densitometories, VersaMax (Molecular Devices, Sunnyvale, CA, USA), with a wavelength of 450 nm.

CONVERSION OF ABSORBANCE INTO "UNIT" VALUES

Serum taken from HWP-IWA patient 5 was chosen as the standard. Serial dilution was performed using 1% skim milk/PBS-T, starting at 40 times dilution, with subsequent doubling of the dilution factor up to 5120 times dilution. The GP19S-specific IgE ELISA was performed as described above. To create a curve for the conversion of absorbance values to "unit" values, the absorbance of the 40 times-diluted serum was defined as that corresponding to 100.0 units, with the absorbance of the 80 times-diluted serum as 50.0 units, and that of the 640 times-diluted serum as 6.3 units, and so forth, such that the absorbance at each dilution factor corresponds to a "unit" value. For each

Table 1 Clinical characteristics of the patients with HWP-IWA

ID	Age	Sex	Past allergic history	Severity	CAP-FEIA						GP19S Skin prick test
					wheat-sIgE		gluten-sIgE		ω-5 gliadin-sIgE		Threshold for positive prick reaction (%)
					(UA/mL)	(Class)	(UA/mL)	(Class)	(UA/mL)	(Class)	
HWP-IWA 1	47	F	Pollinosis	4	4.52	3	7.16	3	0.34>	0	0.001
HWP-IWA 2	38	F	Pollinosis, Graves disease	4	4.1	3	4.71	3	0.34>	0	0.0001
HWP-IWA 3	43	F	Non	4	2.21	2	1.94	2	0.34>	0	0.01
HWP-IWA 4	18	F	Atopic dermatitis, Asthma, Pollinosis	4	2.28	2	5.37	3	0.71	2	0.01
HWP-IWA 5	45	F	Atopic dermatitis (Childhood), Rhinitis, Pollinosis	4	25.1	4	57.3	5	0.68	1	0.001
HWP-IWA 6	61	F	Pollinosis	3	0.72	2	0.98	2	0.34>	0	0.001
HWP-IWA 7	62	F	Non	4	4.44	3	6.41	3	0.34>	0	0.001
HWP-IWA 8	33	F	Pollinosis	3	<0.35	0	<0.35	0	0.34>	0	0.01
HWP-IWA 9	44	F	Non	3	0.35	1	0.73	2	0.34>	0	0.001
HWP-IWA 10	49	F	Rhinitis	2	1.08	2	1.53	2	0.34>	0	0.001
HWP-IWA 11	43	F	Non	3	3.6	3	7.89	3	1.29	2	0.001
HWP-IWA 12	37	F	Non	2	0.67	1	1.41	2	0.34>	0	0.001
HWP-IWA 13	63	F	Contact dermatitis	1	<0.35	0	0.56	1	0.34>	0	0.01
HWP-IWA 14	30	F	Rhinitis, Pollinosis, Metal allergy	1	0.45	1	0.75	2	0.34>	0	0.10

sIgE, specific IgE.

Severity: 1: eyelid swelling, symptoms limited to face and nasal mucosa; 2: generalized urticaria besides Stage 1 symptoms; 3: general symptoms in addition to dermal disorders (diarrhea, dyspnea, etc.); 4: anaphylactic shock.

GP19S Skin prick test : GP19S was diluted to 100 μg/ml in sterile physiologic saline (PS) and then made into solutions at concentration from 0.00001% to 0.1%. Reactions were read at 15 min, a wheal at least half the size of that caused by histamine dihydrochloride (10 mg/ml) or 3 mm was considered a positive reaction.

serum, a "unit" value was obtained from the measured absorbance with 5 times-diluted serum samples according to this curve.

CORRELATION BETWEEN LABORATORY VALUES AND CLINICAL SEVERITY

Pearson's correlation coefficient (Pearson's product-moment correlation coefficient, represented by the letter r), between grades of severity and the following test values were calculated: wheat-specific IgE (UA/mL), gluten-specific IgE (UA/mL), ω-5 gliadin-specific IgE (UA/mL), GP19S SPT positive concentration (%), and GP19S-specific IgE (unit).

VALIDATION STUDY OF THE ELISA-BASED GP19S-SPECIFIC IgE ASSAY AT FIVE INSTITUTIONS

In order to validate and determine the inter-laboratory reproducibility of the ELISA-based GP19S-specific IgE Assay, the method was performed at five institutions affiliated with members of the Special Committee for the Safety of Protein Hydrolysates in Cosmetics. A manual was complied and distributed prior to the study to ensure common understanding of the technique and to allow discussion of uncertainties. All participating institutions used the same reagents and consumables that were prepared by members of Fujita Health University School of Medicine. The microplate reader for absorbance detection and other laboratory equipment were prepared by each institution. ELISA was performed using sera from 10 HWP-IWA and five CO-WDEIA patients, as well as five healthy subjects. Each sample was tested in duplicates to obtain absorbance and unit values. The absorbance and unit values obtained by all five institutions were examined to determine the validity of the test conditions. The standard deviation (SD) of absorbance and unit values was calculated to assess inter-laboratory reproducibility.

ETHICAL CONSIDERATION

This study was approved by the Ethics Committee of Fujita Health University (No. 11-210). Venous blood samples were collected with patients' informed consent.

RESULTS

ELISA-BASED GP19S-SPECIFIC IgE ASSAY

The range of measured absorbance was 0.01-0.09 optic density (OD) (mean, 0.04 OD) for the healthy control group (n = 5), 0.00-0.11 OD (mean, 0.05 OD) for the CO-WDEIA group (n = 5), and 0.21-3.92 OD

Table 2 Laboratory findings of the sera from patients and controls in the validation study

ID	Total IgE	CAP-FEIA						GP19S Skin prick test
		wheat-sIgE		gluten-sIgE		ω-5 gliadin-sIgE		
	(U/ml)	(UA/mL)	(Class)	(UA/mL)	(Class)	(UA/mL)	(Class)	
Healthy 6	8.16	0.34>	0	0.34>	0	0.34>	0	Nagative
Healthy 7	138	0.34>	0	0.34>	0	0.34>	0	Nagative
Healthy 8	NT	NT	NT	NT	NT	NT	NT	NT
Healthy 9	NT	NT	NT	NT	NT	NT	NT	NT
Healthy 10	NT	NT	NT	NT	NT	NT	NT	NT
CO-WDEIA 6	NT	0.57	1	2.65	2	0.34>	0	Nagative
CO-WDEIA 7	NT	2.37	2	1.19	2	9.73	3	Nagative
CO-WDEIA 8	148	0.46	1	2.51	2	13.3	3	Nagative
CO-WDEIA 9	NT	3.36	3	1.48	2	NT	NT	Nagative
CO-WDEIA 10	NT	0.55	1	3.85	3	9.39	3	Nagative
HWP-IWA 15	3650	8.91	3	NT	NT	NT	NT	Positive
HWP-IWA 16	36	0.34>	0	0.39	1	0.34>	0	Positive
HWP-IWA 17	101	0.85	2	2.91	2	0.34>	0	NT
HWP-IWA 18	285	0.77	2	1.84	2	0.34>	0	Positive
HWP-IWA 19	82	4.25	3	7.18	3	0.34>	0	Positive
HWP-IWA 20	738	13.1	3	24.3	4	0.34>	0	Positive
HWP-IWA 21	148	4.44	3	6.41	3	0.34>	0	Positive
HWP-IWA 22	442	0.54	1	1.23	2	0.34>	0	Positive
HWP-IWA 23	2343	3.55	3	4.27	3	0.34>	0	Positive
HWP-IWA 24	67	0.4	1	0.6	1	0.34>	0	Positive

NT, Not tested; sIgE, specific IgE.

Skin prick test: Reactions were read at 15 min, a wheal at least half the size of that caused by histamine dihydrochloride (10 mg/ml) or 3 mm was considered a positive reaction.

(mean, 2.10 OD) for the HWP-IWA group (n = 14). The absorbance values of the healthy and CO-WDEIA groups were relatively low and no marked difference was observed between the two groups. On the other hand, a wide range of absorbance values were observed in the HWP-IWA group, and were markedly different from those in the healthy and CO-WDEIA groups (Table 3).

CONVERSION OF ABSORBANCE INTO "UNIT" VALUES

Serial dilution of the serum sample resulted in a decrease in the measured absorbance, producing a standard curve. After assigning arbitrary "unit" values to the measured absorbance at each dilution factor, the following unit values were obtained from the measured absorbance: a range of 0.2-2.1 (mean, 1.1) for the healthy group, 0.0-2.5 (mean, 1.3) for the CO-WDEIA group, and 5.2-115.5 (mean, 59.5) for the HWP-IWA group (Table 3).

CORRELATION BETWEEN LABORATORY VALUES AND CLINICAL SEVERITY

The correlation coefficients of clinical severity and wheat-, gluten- and ω-5 gliadin-specific IgE antibodies were 0.43, 0.36 and 0.24, respectively. The correlation coefficient of clinical severity and GP19S SPT positive concentration was -0.53, which is high enough for quantitative diagnosis of HWP-IWA, but not high enough to indicate a correlation with severity. The correlation coefficient of severity and GP19S-specific IgE was 0.76, which was higher than all other parameters (Table 4).

VALIDATION STUDY OF ELISA-BASED GP19S-SPECIFIC IgE ASSAY AT FIVE INSTITUTIONS

The results for GP19S-specific IgE were obtained by each institution. Notably, the absorbance and unit values were low in the healthy and CO-WDEIA groups but high in the HWP-IWA group across all institutions. All samples were tested in duplicates and similar absorbance and unit values were obtained. The respective SD for absorbance and unit values were 0.02-0.05 OD and 0.3-0.4 in the healthy group, and 0.03-0.04 OD and 0.2-0.6 in the CO-WDEIA group. In the HWP-IWA group, the SD ranged from 0.19-0.31 OD and 3.8-9.6 for HWP-IWA 16-24, and it was as high as 0.93 OD and 53.3 for HWP-IWA 15. It was observed that the SD became higher as the GP19S-specific IgE level increased. We consider that a high inter-laboratory reproducibility is achieved only when the "unit" value is below 100.0 (Table 5).

Table 3 Results of the ELISA-based GP19S-specific IgE assay

ID	Absorbance	Unit
Healthy 1	0.01	0.2
Healthy 2	0.01	0.2
Healthy 3	0.05	1.2
Healthy 4	0.07	1.6
Healthy 5	0.09	2.1
CO-WDEIA 1	0.11	2.5
CO-WDEIA 2	0.06	1.4
CO-WDEIA 3	0.00	0.0
CO-WDEIA 4	0.03	0.7
CO-WDEIA 5	0.07	1.7
HWP-IWA 1	3.92	115.5
HWP-IWA 2	3.89	114.5
HWP-IWA 3	2.60	71.3
HWP-IWA 4	3.89	114.5
HWP-IWA 5	3.89	114.5
HWP-IWA 6	3.60	104.0
HWP-IWA 7	2.54	69.7
HWP-IWA 8	0.28	6.9
HWP-IWA 9	0.43	10.8
HWP-IWA 10	1.49	39.0
HWP-IWA 11	1.20	30.9
HWP-IWA 12	1.10	28.3
HWP-IWA 13	0.35	8.7
HWP-IWA 14	0.21	5.2

Absorbance, absorbance at 450 nm.

Table 4 Correlation between clinical severity and laboratory findings

	r	*p*
wheat-specific IgE (UA/mL)	0.43	0.12
gluten-specific IgE (UA/mL)	0.36	0.20
ω-5 gliadin-specific IgE (UA/mL)	0.24	0.41
GP19S SPT positive concentration (%)	-0.53	0.052
GP19S-specific IgE (unit)	0.76	0.0015

Positive concentration, threshold for positive prick reaction.
Correlation coefficient *r* were calculated by Pearson's product-moment correlation coefficient.

Table 5 Combined ELISA results from the five institutions

ID	Absorbance		Unit	
	Mean	SD	Mean	SD
Healthy 6	0.08	0.05	0.8	0.4
Healthy 7	0.07	0.04	0.7	0.3
Healthy 8	0.07	0.03	0.6	0.3
Healthy 9	0.08	0.02	0.8	0.3
Healthy 10	0.08	0.05	0.8	0.3
CO-WDEIA 6	0.11	0.03	1.5	0.5
CO-WDEIA 7	0.10	0.03	1.3	0.5
CO-WDEIA 8	0.08	0.03	0.8	0.2
CO-WDEIA 9	0.08	0.03	0.9	0.2
CO-WDEIA 10	0.10	0.04	1.2	0.6
HWP-IWA 15	4.36	0.93	154.1	53.3
HWP-IWA 16	3.22	0.31	97.0	9.2
HWP-IWA 17	3.06	0.23	90.1	9.6
HWP-IWA 18	3.10	0.19	92.0	8.8
HWP-IWA 19	2.60	0.28	72.0	5.9
HWP-IWA 20	2.59	0.30	71.8	5.7
HWP-IWA 21	2.38	0.24	63.6	6.1
HWP-IWA 22	1.55	0.25	36.7	3.8
HWP-IWA 23	1.73	0.24	42.3	3.9
HWP-IWA 24	1.32	0.24	30.1	4.5

Absorbance, absorbance at 450 nm.

DISCUSSION

In Japan, approximately 4.7 million people bought 46.7 million cakes of "Cha no Shizuku" soap that contained GP19S. According to an epidemiological study released online by the Japanese Society of Allergology on 20 November 2013, there were 2026 cases of HWP-IWA, of which 95.9% were females mainly in their 40s. About half of these cases experienced anaphylactic symptoms, of which half experienced anaphylactic shock. Many of these patients developed WDEIA suddenly, manifesting as eyelid edema after eating wheat-containing food, even though no symptoms appeared while using the soap.[7] This phenomenon was also noted in patients in our study (Table 1). Unsuspectingly, patients with oral wheat allergy continued to use the offending soap, highlighting the possibility of a large number of patients who are unaware of their condition.

As a result of sensitization to GP19S contained in the soap, the produced IgE cross-reacts with orally ingested wheat protein.[8] SPT using GP19S is considered a fast and sensitive method for the diagnosis of HWP-IWA; the condition is ruled out if the SPT using 0.1% GP19S solution is negative. However, some patients decline SPT, which causes discomfort and can induce a severe allergic reaction. In addition to SPT, the Special Committee for the Safety of Protein Hydrolysates in Cosmetics recommends other immunological methods such as dot blotting, ELISA, Western blotting (patient is considered HWP-IWA-positive if GP19S-specific IgE is detected in the blood), or basophil activation test that uses GP19S as the antigen (a positive result suggests HWP-IWA).

Reports exist regarding the diagnosis of wheat allergy using various immunological methods. Western blotting for GP19S using serum IgE antibody has been employed at many institutions.[1,2,8] Using patients' basophils, Hiragun *et al.* conducted the histamine release test[9] and Chinuki *et al.* performed the

CD203c expression-based basophil activation test.[10] Nakamura *et al.* described the EXiLE (IgE Cross linking-induced Luciferase Expression) method, which uses a rat mast cell line expressing human IgE antibody receptors.[11] Though useful, the abovementioned methods have drawbacks. Quantitative evaluation is difficult in Western blotting. Basophil-based tests and the EXiLE method are not widely available, and samples cannot be preserved in the former.

Continuing investigation into HWP-IWA will be required to assess the incidence, natural clinical course, allergenicity, treatment, and appropriate patient education. We believe that the most important issue was the development of a quantitative, high-throughput, GP19S-specific IgE diagnostic test that can provide consistent results at any institution. For these reasons, we tested an ELISA-based assay that would satisfy these conditions.

First, we compared the results of GP19S-specific IgE measurement by ELISA between healthy controls (five subjects), CO-WDEIA (five patients) and HWP-IWA patients (14 patients). Under the described test conditions, the measured absorbance was high in the HWP-IWA group but low in the healthy and CO-WDEIA groups, suggesting that ELISA is effective for the diagnosis of HWP-IWA (Table 3). Using the absorbance results from one patient's serum sample (HWP-IWA 5), "unit" values were assigned to absorbance values in order to improve inter-test and inter-laboratory reproducibility. Using one patient's serum as a standard is disadvantageous, as the created standard cannot be reproduced at other institutions. Therefore, dilution series using sera from other HWP-IWA patients were performed to determine whether the resulting curves are comparable to that of HWP-IWA 5; similar curves were obtained in each case (data not shown). This suggests that it is unnecessary to use the sample of HWP-IWA 5 as the standard if the relative concentration of antibodies against GP19S to HWP-IWA 5 can be determined.

The ELISA results differed widely among HWP-IWA patients (1 to 14); therefore, we examined the correlation between clinical severity and laboratory values to analyze the nature of these differences. GP19S-specific IgE had a higher degree of correlation with severity than gluten and wheat (Table 4), suggesting that it may be predictive of symptoms experienced by HWP-IWA patients. We believe that such data will be useful in gauging the effect of treatment and for patient education. The ELISA-based GP 19S-specific IgE assay provides quantitative results that are meaningful in predicting disease severity.

In the validation study conducted at five different institutions, repeat tests demonstrated reproducible results, with the HWP-IWA group consistently showing higher values. This suggests that the test conditions were appropriate. However, it was noted that one sample (HWP-IWA 15) had a high SD of 53.3, which was different from the SD of the HWP-IWA (<9.6), CO-WDEIA (<0.6) and healthy groups (<0.4) (Table 5). The absorbance of HWP-IWA 15 sample exceeded the maximum measurable limit of the microplate reader at each institution, thus causing the large SD. To ensure inter-laboratory reproducibility, we practically set the maximum "unit" value at 100.0.

A total of 10 healthy subjects, 10 CO-WDEIA and 24 HWP-IWA patients were examined in the present study. The respective maximum unit values in the healthy and CO-WDEIA groups were 2.1 and 2.5 units, while the minimum unit value in the HWP-IWA group was 5.2 units. Therefore, taking between 2.5 to 5.2 the cut-off value, the diagnostic criterion was set as follows: <3.0, negative; 3.0-5.0, suspected; >5.0, positive. Results based on this criterion were consistent with available SPT results. Cases (HWP-IWA 8, 16 and 24) with negative ImmunoCAP results (class 2 and above were considered positive) were positive based on this criterion. These observations suggest that our ELISA-based GP19S-specific IgE assay is a sensitive diagnostic method.

Our findings suggest that the ELISA-based GP19S-specific IgE assay is a useful quantitative and high-throughput method for the diagnosis of HWP-IWA. This method enables the examination and diagnosis of cases nationwide using a laboratory-based, measurable criterion. Hiragun *et al.* studied wheat- and gluten-specific IgE antibodies with CAP-FEIA, and glutenin-specific IgE with histamine-release test, concluding that HWP-IWA may get better over time.[9] We believe that the results of the ELISA-based GP19S-specific IgE assay accurately reflect the clinical situation. We envisage that this method will be useful not only for patients and doctors, but also for medical researchers and cosmetics makers. Many challenges remain regarding this disease, but we expect our test method to advance the diagnosis of this condition.

ACKNOWLEDGEMENTS

This study is part of the "Study on Quasi Drug/Cosmetics Additives Safety" conducted by Teshima Group under the sponsorship of Pharmaceutical and Medical Device Regulatory Science General Research Project of the Ministry of Health, Labour and Welfare of Japan.

REFERENCES

1. Fukutomi Y, Itagaki Y, Taniguchi M *et al.* Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2011;**127**:531-3.
2. Chinuki Y, Kaneko S, Sakieda K, Murata S, Yoshida Y, Morita E. A case of wheat-dependent exercise-induced anaphylaxis sensitized with hydrolysed wheat protein in a soap. *Contact Dermatitis* 2011;**65**:55-7.
3. Hann S, Hughes M, Stone N. Allergic contact dermatitis to hydrolyzed wheat protein in a cosmetic cream. *Contact*

Dermatitis 2007;**56**:119-20.
4. Laurière M, Pecquet C, Bouchez-Mahiout I *et al.* Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities. *Contact Dermatitis* 2006;**54**: 283-9.
5. Pecquet C, Lauriere M, Huet S, Leynadier F. Is the application of cosmetics containing protein-derived products safe? *Contact Dermatitis* 2002;**46**:123.
6. Niinimäki A, Niinimäki M, Mäkinen-Kiljunen S, Hannuksela M. Contact urticaria from protein hydrolysates in hair conditioners. *Allergy* 1998;**53**:1078-82.
7. Information provided by the Japanese Society of Allergology. http://www.jsaweb.jp/modules/news_topics/index.php?page=article&storyid=114 (in Japanese).
8. Chinuki Y, Morita E. Wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat protein in soap. *Allergol Int* 2012;**61**:529-37.
9. Hiragun M, Ishii K, Hiragun T *et al.* [The sensitivity and clinical course of patients with wheat-dependent exercise-induced anaphylaxis sensitized to hydrolyzed wheat protein in facial soap]. *Arerugi* 2011;**60**:1630-40 (in Japanese).
10. Chinuki Y, Kaneko S, Dekio I *et al.* CD203c expression based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2012;**129**:1404-6.
11. Nakamura R, Nakamura R, Adachi R, Itagaki Y, Fukutomi Y, Teshima R. Evaluation of allergenicity of acid-hydrolyzed wheat protein using an in vitro elicitation test. *Int Arch Allergy Immunol* 2012;**160**:259-64.

JAPANESE DERMATOLOGICAL ASSOCIATION
THE JOURNAL OF DERMATOLOGY

doi: 10.1111/1346-8138.12505

Journal of Dermatology 2014; **41**: 505–513

ORIGINAL ARTICLE

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

Kotomi HORITA,[1,2] Chiaki TANOUE,[2] Mitsue YASOSHIMA,[2] Takao OHTANI,[1,3] Kayoko MATSUNAGA[1]

[1]*Department of Dermatology, Fujita Health University School of Medicine, Toyoake,* [2]*Research Laboratory, Ikedamohando, Nakaniikawa, and* [3]*Department of Dermatology, Kariya Orthopedic Hospital, Kariya, Japan*

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

Correspondence: Kotomi Horita, Department of Dermatology, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi 470-1192, Japan. Email: horita.kt@ikedamohando.co.jp
Received 27 January 2014; accepted 29 March 2014.

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 μL 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 ≤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					
Lidocaine hydrochloride	3	2	2			
Diphenhydramine		1	1 (hydrochloride)	2 (hydrochloride)	2 (hydrochloride)	2 (hydrochloride)
Urea		10	10			
Tocopheryl acetate		0.5			0.5	
Glycyrrhetinic acid		0.2		0.1	0.2	
L-menthol	3	0.5		5	0.5	
DL-camphor				1		
Others	Chlorpheniramine maleate (1) Benzethonium chloride (0.1) Glycol salicylate (2)	Crotamiton (5)			Isopropylmethylphenol (0.1)	Panthenol (1)

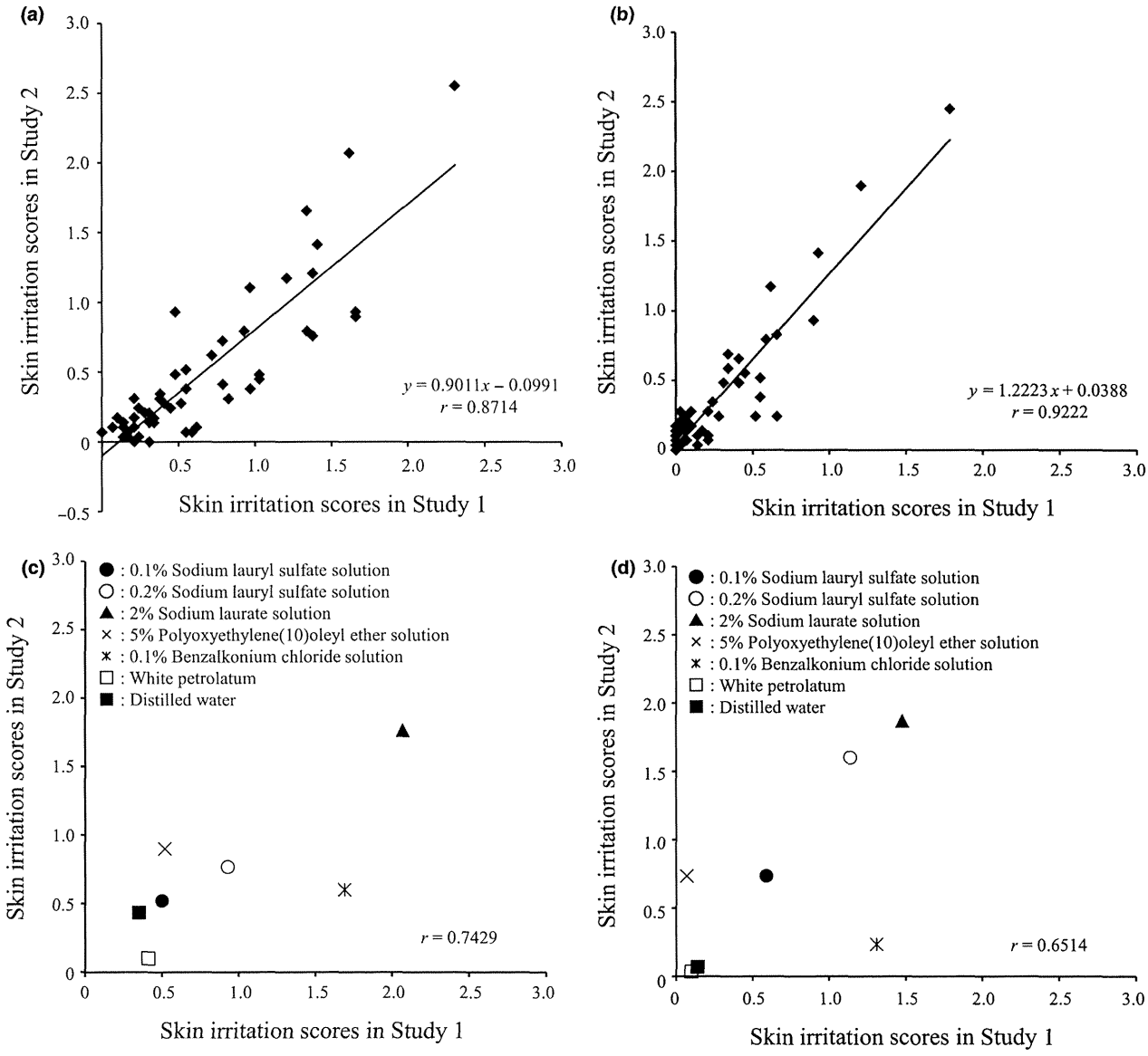

Figure 1. (a) Correlation between the mean skin irritation scores at 2 h after patch removal after 24 h (study 1) and 48 h (study 2) of drug exposure. (b) Correlation between the mean skin irritation scores at 24 h after patch removal after 24 h (study 1) and 48 h (study 2) of drug exposure. (c) Correlation 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 criteria[27] 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	
Mean skin irritation score 24 h after patch removal (study 1/study 2)	1.79/2.45		0.34/0.59		0.52/0.24		0.62/1.17		0.66/0.83		0/0.10	
	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.

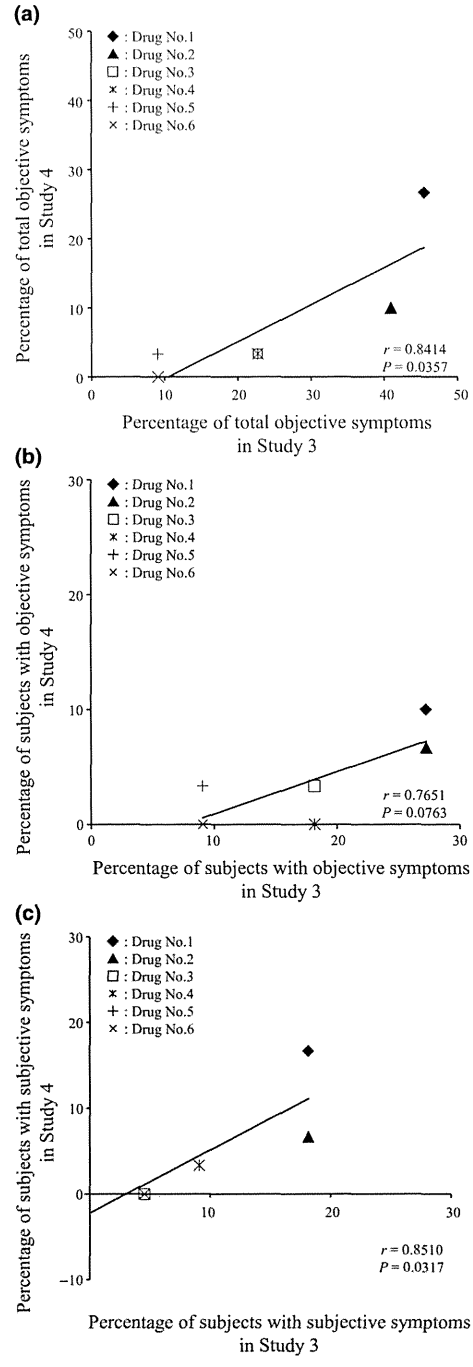

Figure 2. Correlation between the number of subjects and symptoms in study 3 and study 4. (a) Percentage of total objective symptoms. (b) Percentage of subjects with objective symptoms. (c) Percentage of subjects with subjective symptoms.

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

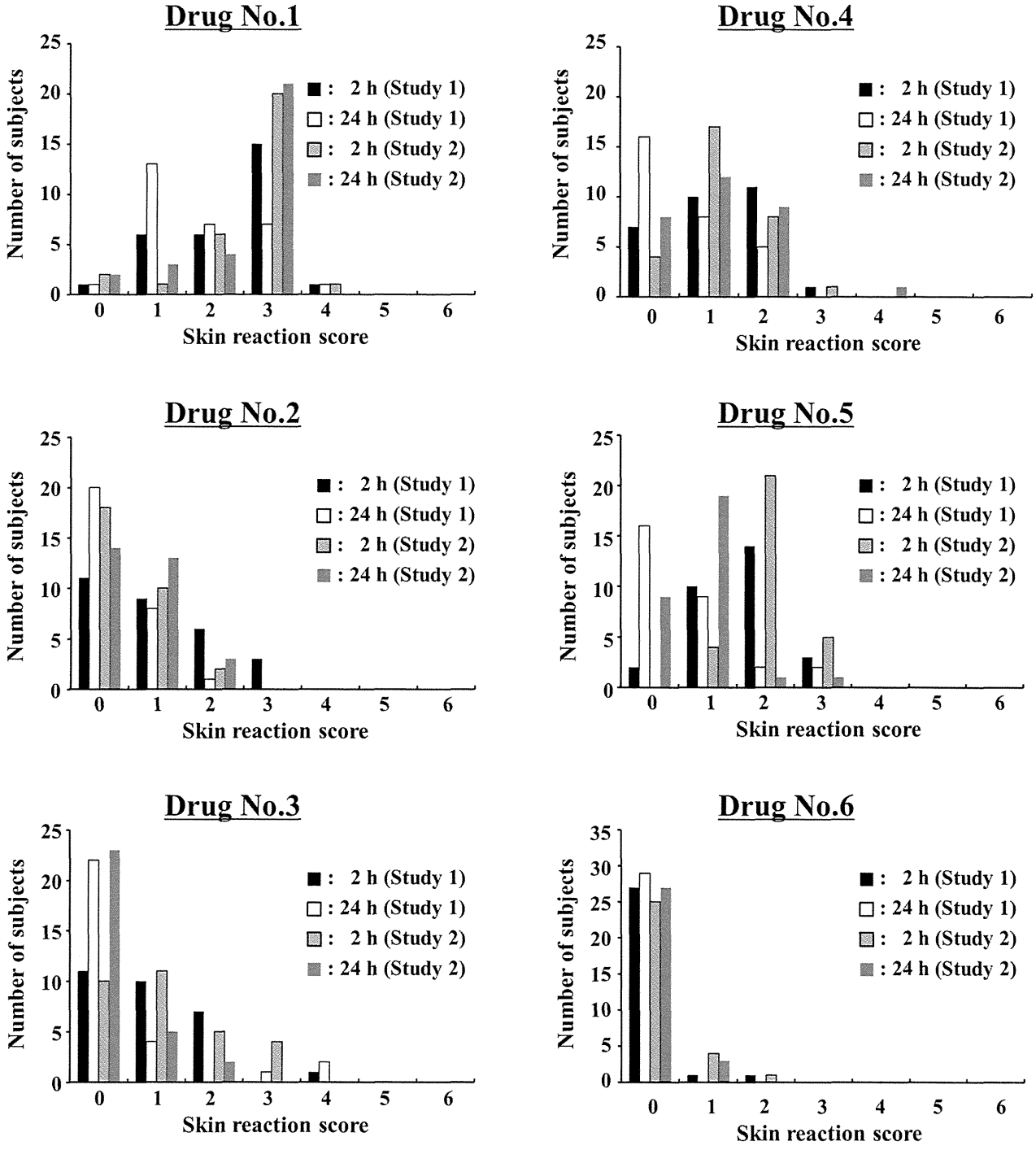

Figure 3. Patch testing irritation scores at 2 and 24 h after patch removal for each test drug in study 1 and study 2.

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

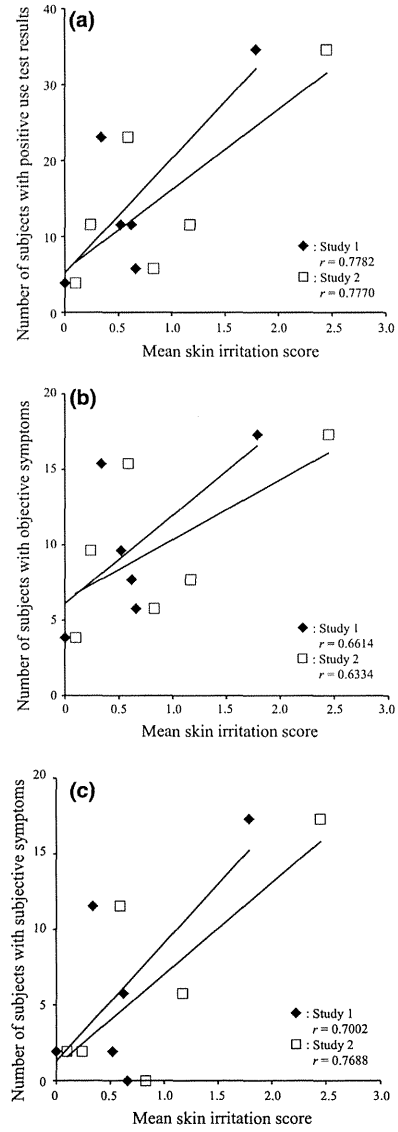

Figure 4. Relationship between patch testing-derived mean skin irritation scores at 24 h after patch removal and repeated open application test results. (a) Number of subjects with positive use test results. (b) Number of subjects with objective symptoms. (c) Number of subjects with subjective symptoms.

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

		Scaling			Erythema			Redness (transient)				Papule			Soreness			Heat			Itchiness		
Drug no.	Assessment period	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.

REFERENCES

1 Society of Regulatory Science of Medical Products. Drug Approval and Licensing Procedure, 2010.
2 Kawamura T, Shoji S, Masuda T *et al.* Basic studies on the standardization of patch test. *Jpn J Dermatol* 1970; **80**: 301–314 (in Japanese).
3 Sugai T. Contact dermatitis and patch test. *Skin Res* 1977; **19**: 210–222.
4 Sugai T. Delayed irritation ratio in prophetic patch tests. *Skin Res* 1985; **27**: 793–803.
5 Kanto H, Washizaki K, Ito M *et al.* Optimal patch application time in the evaluation of skin irritation. *J Dermatol* 2013; **40**: 363–369.
6 Basketter DA, Reynolds F, Rowson M, Talbot C, Whittle E. Visual assessment of human skin irritation: a sensitive and reproducible tool. *Contact Dermatitis* 1997; **37**: 218–220.
7 Mehling A, Chkarnat C, Degwert J *et al.* Interlaboratory studies with a proposed patch test design to evaluate the irritation potential of surfactants. *Contact Dermatitis* 2010; **62**: 157–164.
8 Basketter DA, Griffiths HA. A study of the relationship between susceptibility to skin stinging and skin irritation. *Contact Dermatitis* 1993; **29**: 185–188.
9 Basketter DA, Chamberlain M, Griffiths HA, Rowson M, Whittle E, York M. The classification of skin irritants by human patch test. *Food Chem Toxicol* 1997; **35**: 845–852.
10 York M, Basketter DA, Cuthbert JA, Neilson L. Skin irritation testing in man for hazard assessment–evaluation of four patch systems. *Hum Exp Toxicol* 1995; **14**: 729–734.
11 Tupker RA, Willis C, Berardesca E *et al.* Guidelines on sodium lauryl sulfate (SLS) exposure tests. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1997; **37**: 53–69.
12 Foy V, Weinkauf R, Whittle E, Basketter DA. Ethnic variation in the skin irritation response. *Contact Dermatitis* 2001; **45**: 346–349.
13 Basketter DA, Miettinen J, Lahti A. Acute irritant reactivity to sodium lauryl sulfate in atopics and non-atopics. *Contact Dermatitis* 1998; **38**: 253–257.
14 Basketter DA, York M, McFadden JP, Robinson MK. Determination of skin irritation potential in the human 4-h patch test. *Contact Dermatitis* 2004; **51**: 1–4.
15 Brasch J, Becker D, Effendy I. Reproducibility of irritant patch test reactions to sodium lauryl sulfate in a double-blind placebo-controlled randomized study using clinical scoring: results from a study group of the German Contact Dermatitis Research Group (Deutsche Kontaktallergie-Gruppe, DKG). *Contact Dermatitis* 1999; **41**: 150–155.
16 Basketter DA, Griffiths HA, Wang XM, Wilhelm KP, McFadden J. Individual, ethnic and seasonal variability in irritant susceptibility of skin: the implications for a predictive human patch test. *Contact Dermatitis* 1996; **35**: 208–213.
17 Basketter DA, Scholes EW, Kimber I. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. *Food Chem Toxicol* 1994; **6**: 543–547.
18 Whittle E, Basketter DA. The *in vitro* skin corrosivity test. Development of method using human skin. *Toxicol In Vitro* 1993; **7**: 265–268.
19 Whittle E, Basketter DA. The *in vitro* skin corrosivity test. Comparison of *in vitro* human skin with *in vivo* data. *Toxicol In Vitro* 1993; **7**: 269–273.
20 Basketter DA, Selbie E, Scholes EW, Lees D, Kimber I, Botham PA. Results with OECD recommended positive control sensitizers in the maximization, Buehler and local lymph node assays. *Food Chem Toxicol* 1993; **31**: 63–67.
21 Botham PA, Basketter DA, Maurer T, Mueller D, Potokar M, Bontinck WJ. Skin sensitization–a critical review of predictive test methods in animals and man. *Food Chem Toxicol* 1991; **29**: 275–286.
22 Tornier C, Rosdy M, Maibach HI. *In vitro* skin irritation testing on reconstituted human epidermis: reproducibility for 50 chemicals tested with two protocols. *Toxicol In Vitro* 2006; **20**: 401–416.
23 Basketter D, Gilpin G, Kuhn M, Lawrence D, Reynolds F, Whittle E. Patch tests versus use tests in skin irritation risk assessment. *Contact Dermatitis* 1998; **39**: 252–256.
24 Roggeband R, Helmlinger G, Smith I, Wilhelm KP, Ryan CA, Gerberick GF. A skin sensitization safety assessment of a new bleach activator technology in detergent applications. *Contact Dermatitis* 2002; **46**: 211–219.
25 Zaghi D, Maibach HI. Quantitative relationships between patch test reactivity and use test reactivity: an overview. *Cutan Ocul Toxicol* 2008; **27**: 241–248.
26 Judge MR, Griffiths HA, Basketter DA, White IR, Rycroft RJ, McFadden JP. Variation in response of human skin to irritant challenge. *Contact Dermatitis* 1996; **34**: 115–117.
27 Kawai K. Proposal of a new standard for skin irritation reaction in patch testing. *Vis Dermatol* 2004; **3**: 74–81 (in Japanese).
28 Nickoloff BJ. Immunologic reactions triggered during irritant contact dermatitis. *Am J Contact Dermat* 1998; **9**: 107–110.
29 Tabata N, Tagami H, Kligman AM. A twenty-four-hour occlusive exposure to 1% sodium lauryl sulfate induces a unique histopathologic inflammatory response in the xerotic skin of atopic dermatitis patients. *Acta Derm Venereol* 1998; **78**: 244–247.