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Effects of different base agents on prediction of skin irritation by sodium lauryl sulfate using patch testing and repeated application test

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ABSTRACT

Animal testing for cosmetics was banned in the European Union (EU) in 2013; therefore, human tests to predict and ensure skin safety such as the patch test or usage test are now in demand in Japan as well as in the EU. In order to investigate the effects of different bases on the findings of tests to predict skin irritation, we performed patch testing (PT) and the repeated application test (RAT) using sodium lauryl sulfate (SLS), a well-known irritant, dissolved in 6 different base agents to examine the effects of these bases on skin irritation by SLS. The bases for PT were distilled water, 50% ethanol, 100% ethanol, a gel containing 50% ethanol, white petrolatum, and hydrophilic cream. The concentrations of SLS were 0.2% and 0.5%. Twelve different base combinations were applied to the normal back skin of 19 individuals for 24 h. RAT was performed with distilled water, 50% ethanol, 100% ethanol, a gel containing 50% ethanol, white petrolatum, and hydrophilic cream containing SLS at concentrations of 0.2%, 2%, and 5%, being applied to the arms of the same PT subjects. The test preparation of each base was applied at the same site, with 0.2% SLS being used in the first week, 2% SLS in the following week, and 5% SLS in the final week. The results of PT revealed that skin irritation scores varied when SLS at the same concentration was dissolved in a different base. The results of RAT showed that although skin irritation appeared with every base at a concentration of 5%, the positive rate was approximately the same. In conclusion, our results suggest that skin irritation elicited in PT depends on the base, while in RAT, it does not depend on the type of base employed.

1. Introduction

Patch testing (PT) and the usage test are among the methods available for predicting skin irritation (Sugai, 1977; Basketter et al., 1997; Clemmensen et al., 2008; Fartasch et al., 2012; Löffler et al., 2007; Slotosch et al., 2007). Since animal testing for cosmetics is banned in the European Union (EU), human tests such as PT or the usage test to predict and ensure skin safety are now in demand in Japan as well as in the EU. We previously reported a strong correlation between the findings of PT and those of the repeated application test (RAT) to predict skin irritation caused by commercially available Japanese topical drug formulations (Horita et al., 2014, 2015). Most studies on skin irritation by individual chemicals in a drug formulation have used SLS as an irritant and employed aqueous solutions (Smith et al., 2002; Wilhelm et al., 1990). However, these chemicals are frequently used in products dissolved in creams, ethanol, gels, and other types of solvents such as cosmetics or external medicines. Therefore, a clearer understanding of the effects of different bases on the results of tests to predict skin irritation is important. Previous studies examined the effects of the duration and concentration of SLS solution on skin irritation in PT (Aramaki et al., 2001); however, the relationship between the findings of PT and RAT for individual chemicals has not yet been elucidated in humans.

The present study investigated the effects of different base agents on the prediction of SLS using PT and RAT. We selected the major irritant SLS, prepared various formulations by dissolving SLS

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Table 1
Test design.

Test	Number of subjects	Test site	Test period	Test material	Base					
					Distilled water	50% ethanol	100% ethanol	A gel containing 50% ethanol	White petrolatum	Hydrophilic cream
PT	19	Normal back skin	24 h closed	0.2% SLS	◆	◆	◆	◆	◆	◆
				0.5% SLS	◆	◆	◆	◆	◆	◆
				Negative control	◆	◆	◆	◆	◆	◆
RAT		Normal both arms	1. Days 1–7	0.2% SLS	◆	◆	◆	◆	◆	◆
			2. Days 8–14	2% SLS	◆	◆	◆	◆	◆	◆
			3. Days 15–21	5% SLS	◆	◆	◆	◆	◆	◆

in a number of different bases, and evaluated them using PT and RAT (Judge et al., 1996; Kanto et al., 2013; Tupker et al., 1997). PT and RAT are performed under different application conditions, either by closed application or open use. In order to investigate the relationship between these conditions and the appearance of skin irritation, we dissolved different concentrations of SLS in different bases and used these for RAT.

2. Materials and methods

2.1. PT study design and subjects

This study was approved by the Institutional Review Board of HUMA R&D Co. (HM01120040104). Written consent for voluntary participation in the study was obtained from each subject prior to enrolment. PT was conducted on 19 participants (3 men, 16 women; age range, 23–69 years) in September 2014. Inclusion criteria for the test were that the participant had normal back skin and was not using any anti-allergic or steroid medication. Table 1 shows the design for PT and RAT. Twelve different SLS base combinations were tested with PT applied to the normal back skin of 19 individuals. RAT used distilled water, 50% ethanol, 100% ethanol, a gel containing 50% ethanol, white petrolatum, and hydrophilic cream containing SLS at concentrations of 0.2%, 2%, and 5% applied to normal skin at a total of five locations on both arms of the same 19 PT subjects. Each test preparation of each base was applied at the same site, with 0.2% SLS used for the first week, 2% SLS in the following week, and 5% SLS in the final week.

2.2. PT materials

Test materials were 0.2% SLS and 0.5% SLS, with each concentration being dissolved in the six bases. Table 1 shows each base and the concentration applied. Table 2 shows the composition of each base. Materials: SLS was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Bases: We used distilled water, ethanol solution, an ethanol-containing gel, white petrolatum, and hydrophilic cream (Japanese Pharmacopoeia). If the irritant did not fully dissolve in the test formulation during its preparation, this formulation was not applied to the skin. Hydrophilic cream was prepared by mixing aqueous- and oil-phase components together. Depending on physicochemical properties, the irritant was added to either the aqueous or oil phase, and the cream was prepared once the irritant was confirmed to have dissolved. The concentrations of skin irritants used were previously indicated to cause irritation (Kanto et al., 2013). This study was double-blinded, with the nature of the sample not revealed to the subject or investigator until study completion.

2.3. Patch test

Test materials were sealed to the back skin using aluminum chambers (Finn Chamber on Scanpor; Smart Practice Japan, Yokohama, Japan). Regarding the gel containing 50% ethanol, white petrolatum, and hydrophilic cream, 20 mg of the product was placed directly on the aluminum plate. Regarding distilled water and ethanol solution, 15 μL of the product was applied to

Table 2
Compositions of bases.

Base	Distilled water	50% ethanol	100% ethanol	A gel containing 50% ethanol	White petrolatum	Hydrophilic cream
Test materials	0–5 g	0–5 g	0–5 g	0–5 g	0–5 g	0–5 g
Distilled water	Adequate amount	Adequate amount	–	Adequate amount	–	Adequate amount
Ethanol	–	50 g	Adequate amount	50 g	–	–
White petrolatum	–	–	–	–	Adequate amount	25 g
Hydroxypropyl methylcellulose	–	–	–	1.5 g	–	–
Stearyl alcohol	–	–	–	–	–	20 g
Propylene glycol	–	–	–	–	–	12 g
Polyoxyethylene hydrogenated castor oil 60	–	–	–	–	–	4 g
Glyceryl monostearate	–	–	–	–	–	1 g
Methyl parahydroxybenzoate	–	–	–	–	–	0.1 g
Propyl parahydroxybenzoate	–	–	–	–	–	0.1 g
Total	100 g	100 g	100 g	100 g	100 g	100 g