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Comparison of different local and imported histamine concentrations used as a skin prick test positive control

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Abstract

Background: The skin prick test (SPT) is a valid and approved tool that is used to diagnose atopic diseases. The SPT is accurate, safe, simple and inexpensive. However, the histamine concentration used as a positive control in the SPT varies among centers.

Objective: To compare SPT results using different concentrations of locally-prepared and imported histamine solutions.

Method: This prospective, randomized, double-blind, self-controlled study was performed in healthy adult volunteers. The SPT was performed using 4 concentrations of histamine solutions (imported, 1 mg/mL; locally-prepared, 1, 5 and 10 mg/mL). Locally-prepared histamine positive controls were prepared from histamine biphosphate monohydrate using sterile technique.

Results: Seventy-five adult volunteers (mean age, 36 years) were included in the study. Eight volunteers were male and 9 had a history of atopy. Mean wheal diameter (MWD) for imported histamine was 3.49 mm for a concentration of 1 mg/mL, and that of locally-prepared histamine was 2.94 mm, 5.05 mm and 5.52 mm for concentrations of 1, 5 and 10 mg/mL histamine, respectively. Negative SPT results (MWD <3 mm) were found in 11 subjects (14.7%) who received imported histamine and 26 subjects (34.7%) who received the locally-prepared histamine at concentration of 1 mg/mL. All subjects tested with locally-prepared histamine at concentrations of 5 and 10 mg/mL had a MWD > 3 mm.

Conclusions: Locally-prepared histamine base at concentrations of 5 and 10 mg/mL yielded better positive results than both imported and locally-prepared histamine at a concentration of 1 mg/mL.

Keywords: Skin prick test, histamine concentration, skin test positive control, locally-prepared histamine, imported histamine

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Introduction

Atopic diseases are IgE-mediated diseases. An atopic disease diagnosis is made based on a history of symptoms, physical examination and laboratory tests that support an IgE-mediated mechanism. Tests that are approved and valid to use for patients with atopic diseases are measurement of specific IgE levels and skin test results. The skin prick test (SPT) is accurate, simple and easy to perform; it also provides rapid results and is economical for diagnosing IgE-mediated diseases. The SPT is also safe,[1] and the incidence of mild adverse reactions from SPT is usually not more than 0.04%.[2] Severe side effects from SPT are also rare,[3] and no fatalities have been reported from the aeroallergen SPT.[1-4] A previous study in 5,879 Thai patients showed no adverse systemic reactions from 82,306 skin prick tests.[5] Positive and negative controls should be used to interpret SPT results. The positive control should have a mean wheal diameter (MWD) of more than 3 mm while the negative control should give a MWD of less than 3 mm. MWD of specific allergens that are equal to or larger than the histamine MWD are considered to represent a moderate-to-high degree of sensitization. However, there is still not enough information on the best histamine concentration

to use as a positive control for the SPT. The available concentrations of histamine control are 1 and 10 mg/mL.[6-7] In Thailand, the concentration used as a SPT positive control varies among centers. The available concentration of imported histamine solution is 1 mg/mL. At Siriraj Hospital, Mahidol University, the concentrations of locally-prepared histamine solution used in the Department of Pediatrics and the Department of Otorhinolaryngology are 10 and 2.5 mg/mL, respectively. Determining the best concentration of histamine solution for a SPT positive control will allow more accurate interpretation of SPT results.

The aim of this study is to compare SPT results among different concentrations of locally-prepared (1, 5 and 10 mg/mL) and imported (1 mg/mL) histamine solutions.

Methods

This prospective, randomized, double-blind, self-controlled study was performed in adult volunteers. Subjects with acute asthma exacerbation, skin diseases, chronic disease (such as autoimmune diseases, immune deficiency and cancer) or those who were pregnant were excluded. Antihistamine, systemic corticosteroid (≥ 20 mg/day) and topical corticosteroid use was discontinued for at least 7 days before testing. This study was approved by the Institutional Ethics Committee, Siriraj Hospital, Mahidol University. Written informed consent was obtained from all subjects before enrollment. The study was registered with ClinicalTrials.gov (NCT02561429).

Preparation of local histamine positive control solution

Local histamine positive controls (Greater Pharma Manufacturing Co., Ltd. Bangkok, Thailand) were prepared using sterile technique in a laminar air flow hood. Histamine biphosphate monohydrate powder (0.2926, 1.4628 and 2.9256 g) was added to 0.40 g phenol (GR for analysis ACS, Reag. PhEur), 70 g glycerin (99%) and 100 ml water for injection to make histamine (base) control at concentrations of 1, 5 and 10 mg/mL, respectively. The histamine concentration in the preparations was double-checked, stored in a quarantined cold room at 2-8°C and protected from light until used. Imported histamine (base) concentration is 1 mg/mL (ALK Laboratories, Port Washington, New York).

SPT was performed by an experienced technician in a room with resuscitation equipment, and a blood lancet (Vitrex Medical A/S, Herlev, Denmark) was used to prick the skin on the subjects' upper back. The 4 histamine concentrations (imported, 1 mg/mL; locally-prepared, 1, 5 and 10 mg/mL) were tested in each subject. Each SPT was separated by 5 cm. Wheals and flares were recorded 10 minutes after testing. The wheal and flare outlines were transferred to a case record form using the transpore technique. The MWDs (longest diameter plus the perpendicular diameter, divided by 2) were calculated. A reaction was considered positive when the MWD was at least 3 mm. For safety, all subjects were observed for at least 30 minutes after the SPT, and any adverse effects were recorded. The subjects with a MWD of more than 10 mm received a non-sedating antihistamine immediately and their observation time was increased to 2 hours or until the MWD decreased.

Body mass index (BMI) was classified as normal (BMI = 18.5-24.9), underweight (BMI <18.5), overweight (BMI > 25-29.9) and obese (BMI ≥ 30).[8]

Statistical analysis

The data were analyzed using SPSS software version 18 (SPSS Inc., Chicago, IL, USA). Demographic data are presented as the mean and standard deviation (SD) for continuous data. Categorical data are presented as the number and percentages. The mean differences and 95% confidence interval (95% CI) of the wheal diameter between histamine concentrations (1, 5, 10 mg/mL) were analyzed using a paired t-test. McNemar's test was used to compare positive test results (defined as a wheal diameter ≥ 3.0 mm) at different concentrations. Correlation between the wheal diameters from different concentrations was assessed using Pearson's correlation coefficient. A p-value of < 0.05 was considered statistically significant.

Results

This study was a prospective, randomized, double-blind, self-controlled study in 75 adult volunteers. The demographic characteristics of the study population are shown in **Table 1**. The overall mean age was 36 years, and 8 subjects (10.7%) were male. A history of atopy was found in 9 subjects, which was mostly allergic rhinitis. No statistical difference was found between age and BMI of the study subjects in the group with atopy (n=9) and the group without atopy (n=66).

Imported histamine (1 mg/mL) showed negative results (MWD <3 mm) in 11 subjects (14.7%) while locally-prepared solutions (1 mg/mL) showed negative result in 26 subjects (34.7%). No skin reaction (MWD = 0 mm) was found in 2 subjects tested with the imported histamine and in 10 subjects tested with the locally-prepared histamine (1 mg/mL; **Table 2**). All subjects tested with locally-prepared histamine at concentrations of 5 and 10 mg/mL showed a positive SPT result (≥ 3 mm MWD). MWD for the imported histamine was 3.49 mm for a concentration of 1 mg/mL, and that of locally-prepared histamine was 2.94 mm, 5.05 mm and 5.52 mm for concentrations of 1, 5 and 10 mg/mL, respectively (**Figure 1**). MWDs for locally-prepared histamine solution concentrations of 5 and 10 mg/mL and for imported histamine

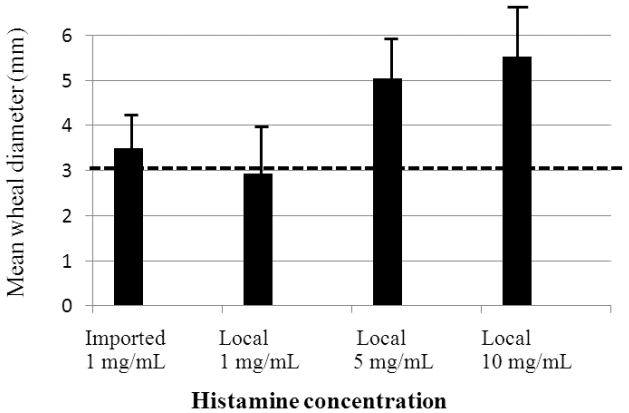

Figure 1. Skin prick test mean wheal diameter distribution at concentrations of 1, 5 and 10 mg/mL. (The dash line = cut point mean wheal diameter; 3 mm.)

Table 1. Subject demographics

Characteristics	n (%)
Total	75 (100)
Age (years) mean (min-max)	36 (20-59)
Sex	
Male	8 (10.7)
Body mass index	23.8 (15.6-36.4)
History of atopy	9 (12.0)
Allergic rhinitis	8 (10.7)
Asthma	1 (1.3)
Family history of atopy	12 (16)
Smoking	4 (5.3)
Drank alcohol	6 (8)

Table 2. Skin prick test mean wheal diameter from different histamine solution concentrations.

	Imported histamine	Locally-prepared histamine		
	1 mg/mL	1 mg/mL	5 mg/mL	10 mg/mL
MWD* (mm) ± SD	3.49±0.99	2.94±1.44	5.05±0.98	5.52±1.16
Minimum-maximum	0-5.50	0-6.00	3.00-7.50	3.00-9.00
MWD ≥ 3 mm (n, %)	64 (85.3)	49 (65.3)	75 (100)	75 (100)
MWD < 3 mm (n, %)	11 (14.7)	26 (34.7)	0 (0)	0 (0)
MWD = 0 mm (n, %)	2 (2.7)	10 (13.3)	0 (0)	0 (0)

* MWD, mean wheal diameter

Table 3. Skin prick test mean wheal diameters, SD and 95%CI from different histamine solution concentrations compared with body mass index.

Histamine	BMI	n	Mean wheal diameter (mm)	SD	95%CI interval for mean	p
Imported (1 mg/mL)	<18.5	5	3.70	1.24	2.2-5.19	0.598
	18.5-24.9	44	3.56	0.92	3.28-3.84	
	25.0-29.9	16	3.40	0.78	2.99-3.82	
	≥ 30	10	3.20	1.46	2.15-4.24	
	Total	75	3.49	0.99	3.26-3.71	
Local (1 mg/mL)	<18.5	5	2.30	2.11	-0.32-4.92	0.712
	18.5-24.9	44	3.06	1.37	2.66-3.49	
	25.0-29.9	16	3.00	1.33	2.29-3.71	
	≥ 30	10	2.60	1.63	1.43-3.77	
	Total	75	2.94	1.44	2.61-3.27	
Local (5 mg/mL)	<18.5	5	5.70	0.91	4.57-6.83	0.101
	18.5-24.9	44	5.06	1.05	4.75-5.39	
	25.0-29.9	16	4.63	0.83	4.18-5.07	
	≥ 30	10	5.36	0.67	4.87-5.83	
	Total	75	5.05	0.98	4.83-5.28	
Local (10 mg/mL)	<18.5	5	5.30	0.91	4.17-6.43	0.333
	18.5-24.9	44	5.43	1.15	5.08-5.78	
	25.0-29.9	16	5.44	0.87	4.97-5.90	
	≥ 30	10	6.15	1.63	4.98-7.32	
	Total	75	5.52	1.16	5.25-5.79	

solution at a concentration of 1 mg/mL were significantly larger than MWDs of the locally-prepared solution at a concentration of 1 mg/mL (p = 0.001, 0.001 and 0.004, respectively). The maximum MWD (9 mm) was observed in a SPT administered using locally-prepared histamine at a concentration of 10 mg/mL.

MWD between locally-prepared histamine at concentrations of 1 and 5 mg/mL and between concentrations of 1 and 10 mg/mL showed significant paired-sample correlations ($p<0.05$), but the correlations were weak (0.251 and 0.347, respectively).

The difference between the median MWD for locally-prepared histamine at a concentration of 5 and 10 mg/mL was 1 mm. The difference between the median MWD for locally-prepared histamine and imported histamine, both at a concentration of 1 mg/mL, was also 1 mm. The MWDs for

Table 4. Skin prick test mean wheal diameters and SD from different concentrations of histamine solution in atopic (n=9) and non-atopic (n=66) subjects.

Histamine	Atopic status	Mean wheal diameter (mm)	SD	p
Imported (1 mg/mL)	Atopy	3.49	1.04	0.141
	Non-atopy	3.44	0.46	
Local (1 mg/mL)	Atopy	2.89	1.42	0.556
	Non-atopy	3.27	1.64	
Local (5 mg/mL)	Atopy	5.01	1.01	0.225
	Non-atopy	5.33	0.71	
Local (10 mg/mL)	Atopy	5.50	1.12	0.193
	Non-atopy	5.67	1.50	

locally-prepared histamine at a concentration of 5 and 10 mg/mL were >1.5 mm larger than the MWD for imported histamine at a concentration of 1 mg/mL.

Mean flare diameter (MFD) using the imported histamine solution (1 mg/mL) was 13.92±9.53 mm. MFD using the locally-prepared histamine at concentration of 1, 5 and 10 mg/mL was 12.97±8.52, 23.35±6.63 and 24.51±5.87 mm, respectively. Maximum MFD (36.5 mm) was found for the SPT using locally-prepared histamine at a concentration of 10 mg/mL.

According to the BMI classification, 58.7% of subjects had a normal body weight, 21.3% were overweight, 13.3% were obese and 6.7% were underweight. There was no statistically significant correlation between subject BMI and MWD for all histamine concentrations (**Table 3**). There was a difference between the MWD for SPTs administered with histamine at different concentrations in subjects who had a history of atopy and those who did not, but this difference was not significant ($p>0.05$; **Table 4**).

Our study showed no systemic adverse reactions related to the histamine SPT. Mild-to-moderate pruritus at the wheal area was the only observed local reaction.

Discussion

SPT is widely used to diagnose IgE-mediated allergic diseases such as allergic rhinitis, asthma, atopic dermatitis and food allergy. SPT is reliable, simple, inexpensive and safe. Adverse reactions are usually mild and local, and generalized reactions are rare. There was a report of generalized reactions in young infants who had a positive SPT in response to fresh foods.[9] The overall rate of generalized reactions was 521 per 100,000 tested children and 6,522 per 100,000 tested infants less than 6 months of age.[9]

Anaphylaxis and death have been reported as a result of intracutaneous tests.[9] Two adults with previous anaphylactic reactions in response to kiwi fruit and fish ingestion had an anaphylactic reaction after a SPT for the same foods.[10] One adult fatality was confirmed after a SPT with 90 commercial food allergens.[10] A systemic allergic reaction to food SPTs occurs in less than 0.02% of tests.[4]

Histamine solution is most commonly used as a standardized positive control in SPT. A proper understanding of histamine skin reactivity is essential for the interpretation of SPT results, and reactivity to histamine differs among individuals. Many studies have evaluated the correlation among sex, parental allergy history, parental smoking status, allergic sensitization and number of sensitized allergens with histamine skin reactivity. Multivariate analysis found no correlation among these factors[11], but some studies reported that these factors influenced histamine reactivity in the SPT.

Our study was performed in adult volunteers to eliminate the age effect on histamine reactivity. Previous studies reported hyporeactivity to the histamine SPT in infancy and an increase in histamine skin reactivity with age.[7,12] MWD in the histamine SPT significantly increases from infancy to childhood. The reactivity is stable in adults 20-50 years of age and then decreases significantly, to reach a plateau after 60 years of age.[13]

A recent study has shown that histamine skin reactivity increases with elevated BMI in Korean children.[14] This may be explained by obesity inducing physiological changes, e.g. increased sweat gland activity, high blood pressure and physiological temperature-regulating system activity.[15] Increased blood flow in the skin in obese individuals could be a reason for the increased histamine reactivity.[15] However, we did not find a correlation between BMI and the MWD of the histamine positive control in this study.

There was no significant difference between MWD in adults with and without a history of atopy in our study. This was supported by a previous study, which showed that skin tests with histamine were similar in atopic and non-atopic individuals[13] and the number of sensitized allergens was not associated with histamine skin reactivity.[11] In contrast, few studies showed that histamine skin reactivity increases with allergic sensitization.[12,16] The more positive allergen SPTs, the bigger the histamine wheal.[16] One study showed a significant difference in histamine wheal size between non-sensitized compared with multiple-allergen sensitized children.[17] The only other factors associated with significantly larger histamine wheal sizes were a history of eczema and a history that suggests asthma.[17]

The distance between each test, bodily area of the test, device using for the test and extract concentration used for the test may influence the results of the histamine positive control and the allergen SPT.[18-19] A previous study showed that the distance between 2 prick tests should be ≥ 2 cm.[18] In our study, we performed the SPT with 5 cm between each skin prick to prevent interaction between the tests. We found that the largest MWD was 9 mm and the largest MFD was 36.5 mm, so a space of 5 cm between the tests was appropriate for evaluation of both SPT wheal and flare results. Several previous studies showed that the upper back is more reactive than the forearm,[20-21] so we performed the SPT on the upper back. The histamine positive control concentrations used over the past few decades have been between 1 and 10 mg/mL histamine base. A European study in 1993 used a 10-fold increase of histamine concentrations (from 1 to 10 mg/mL), which resulted in a doubling of the histamine reaction and increased the MWD from 4 to 7 mm.[19,22] Because of the limited wheal area and the low reproducibility with 1 mg/mL histamine, the European Academy of Allergy and Clinical Immunology (EAACI) recommended a change from 1 mg/mL histamine to 10 mg/mL histamine as an international positive reference.[22]

However, the reference histamine concentration as a positive control for SPT still varies from 1 to 10 mg/mL. The imported histamine (ALK Laboratories, Port Washington, New York) concentration was 1 mg/mL. We found negative results (MWD <3 mm) for the histamine SPT in 11 subjects (14.7%) for imported histamine at 1 mg/mL, and negative results in 26 subjects (34.7%) for the locally-prepared solution at 1 mg/mL. No skin reaction (MWD = 0 mm) was observed in 2 subjects tested with the imported histamine at 1 mg/mL and in 10 subjects tested with locally-prepared histamine at 1 mg/mL. All subjects tested with locally-prepared histamine at concentrations of 5 and 10 mg/mL showed positive SPT results (≥ 3 mm MWD). Histamine at a concentration of 10 mg/mL

showed the largest MWD without any serious side effects. Our results are consistent with those of previous studies[19,20,22], which showed that 1 mg/mL histamine is not the best concentration to use for a SPT positive control.

Based on our study results, we recommend that histamine at a concentration of between 5 and 10 mg/mL should be used as a positive control for the SPT, especially in young children because their skin is less reactive to histamine than adults. The EAACI recommends that the positive control, i.e. the histamine prick, should only be used to demonstrate that the skin can react.[22] A recent study recommended that 10 mg/mL histamine HCl (6 mg/mL histamine base) should be used to reduce the influence of differences in techniques among assistants and centers.[23] The same study also showed that the SPT wheal results must be correlated with skin sensitivity that is measured using histamine in all research, if the size of wheals are to be used as an outcome measure.[23]

Conclusion

Locally-prepared histamine base at concentrations of 5 and 10 mg/mL yielded better positive results than both imported and locally-prepared histamine at a concentration of 1 mg/mL.

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