

Reliability of allergy skin testing

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ABSTRACT

Background: Percutaneous allergen skin testing remains an established benchmark for diagnosing atopic disease. The reliability of skin testing depends greatly on the performance of allergen extracts used, methods used, and the presence of antihistamine medications.

Objective: To determine the differential effect of cetirizine on 2 different concentrations of histamine control solution and 5 common allergens used for percutaneous skin testing.

Methods: Twelve individuals underwent skin testing with histamine (1 and 6 mg/mL), control diluent, and 5 common aeroallergens. Wheal and flare measurements were measured in a masked fashion by a single operator. Cetirizine was administered for 4 consecutive days to determine the effect on both histamine and allergen wheal and flare responses.

Results: A total of 384 skin tests were performed on 12 volunteers. Cetirizine began to suppress wheal and flare responses at 1 hour ($P < .05$), with maximum suppression at day 5 ($P < .05$). Wheal and flare responses returned to greater than 90% baseline within 4 days of not taking cetirizine. Suppression was more apparent with 1 vs 6 mg/mL of histamine (62% vs 33%). Four of the 12 individuals taking cetirizine had a positive skin test result using 6 mg/mL of histamine control when the 1-mg/mL histamine test result was negative. Importantly, twice as many individuals had false-negative allergen responses using 6 mg/mL of histamine vs the 1 mg/mL as a positive control, although this finding did not reach statistical significance.

Conclusion: The use of a 6-mg/mL histamine control for some percutaneous skin test devices may result in more false-negative allergen responses because of the inability to detect the presence of antihistamines.

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Introduction

Percutaneous skin prick tests (SPTs) are the most widely used investigation to determine the presence of allergic disease, such as asthma, allergic rhinitis, and food allergy.¹ SPTs are considered extremely safe, with less than 1 of 100,000 patients having a systemic reaction and only 1 death in reported history.^{2–5} SPTs are extremely sensitive and specific when good technique is used.^{1,6} Several groups have previously reported analytical sensitivity of greater than 95% and specificity of greater than 98% for devices using histamine to elicit a response.^{7–9} It is well known that the reliability and reproducibility of skin prick testing depends greatly on technician experience and the specific methods used.^{6,10,11} Similarly, the quality of allergenic extracts and selection of control reagents used for testing are critical.

Two different concentrations of histamine-positive control reagents are commonly used as reference material for skin prick testing, namely, 6-mg/mL base (10 mg/mL of histamine dihydrochloride) and 1-mg/mL base (2.75 mg/mL of histamine phosphate). Both are approved for use in humans by the US Food and Drug Administration, but the justification for this is unclear. Few studies address the rationale for selecting a histamine concentration of 1 vs 6 mg/mL for skin testing, but both are used in clinical practice.

Because the reliability of skin prick testing for allergens depends on the comparative accuracy of the positive and negative control reagents selected and the implications of a false-positive control result may lead to incorrect interpretation of allergen responses, it is important to address this question. In the case of drug or food allergies, misinterpretation may have serious consequences. Although the effect of oral antihistamine medications on SPTs is well established, the differential effect on the relative concentrations of control reagents has not been determined.

The focus of this study was to evaluate the suppressive effect of oral antihistamine medications on SPTs when 2 different positive control reagents are used. In particular, we sought to determine the differential effect of cetirizine on the wheal and flare responses using 2 concentrations of positive control reagent (1 and 6 mg/mL of histamine) and the effect on allergen responses.

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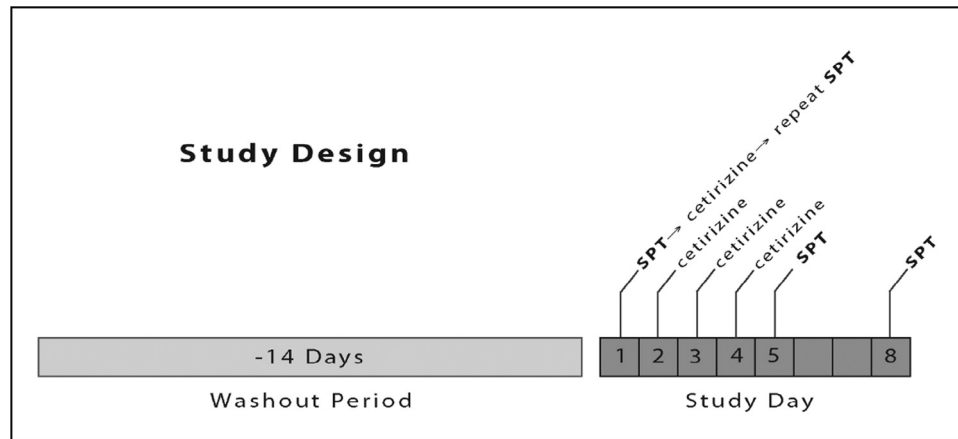


Figure 1. Study design. SPT, skin prick test.

Methods

Study Design

A prospective study of the suppressive effect of oral antihistamine medications on SPTs was conducted with approval by the Johns Hopkins Institutional Review Board. SPTs were performed using Multi-test PC (Lincoln Diagnostics, Decatur, Illinois) with 5 aeroallergens (*Dermatophagoides farinae*, timothy, ragweed, oak, cat), histamine (1 and 6 mg/mL), and control diluent. Cetirizine was administered for 4 consecutive days to determine the effect on both histamine and allergen wheal and flare responses (Fig 1).

Study Participants

Twelve volunteers aged 18 to 65 years were included in the study. Nine of the 12 volunteers were considered allergic defined by history of symptoms of rhinoconjunctivitis and positive SPT results to aeroallergens. A total of 384 SPTs were performed (32 per individual). The primary exclusion criteria included severe concurrent illness, uncontrolled asthma, extensive eczema, urticaria, dermatographism, and pregnancy. Individuals with antihistamine, tricyclic antidepressant, clonidine, or sleeping aid use within the previous 14 days, as well as the use of topical corticosteroids, immunomodulatory drugs, or long-term use of oral steroids, were also excluded.

Devices and Reagents

The skin test devices and reagents chosen for testing included several commonly used items available in the United States. All skin test devices and reagents have been approved by the US Food and Drug Administration and were used with standard manufacturer recommended practices: Multi-Test PC skin test device, 6 mg/mL of histamine diluent (Hollister-Stier, Spokane, Washington), 1 mg/mL of histamine diluent (ALK-Abello, Horsholm, Denmark), and 5 aeroallergens, which were selected on the basis of their common availability and use in our clinics (Hollister-Stier; Greer Laboratories, Lenoir, North Carolina; and ALK), and cetirizine (Pfizer, New York, New York). All individuals received the same interventions, and the same reagents were used throughout the study.

Skin Test Protocol

Manufacturer recommendations were followed in the application of each extract. All individuals underwent a 14-day washout period during which they abstained from taking any agents that can

suppress the SPT (Fig 1). On the initial study day, all individuals underwent testing on the forearm with histamine (1 and 6 mg/mL), control diluent, and 5 common aeroallergens (*D farinae*, timothy, ragweed, oak, and cat) using Multi-Test PC followed by taking a 10-mg cetirizine tablet by mouth. After waiting for 1 hour, the SPT was performed again to the same reagents. The individuals continued taking 10 mg of cetirizine for 3 more days. On day 5, the individuals abstained from taking cetirizine and underwent another SPT. After a total of 1 week (on day 8), all individuals returned for another SPT to evaluate the effect of residual cetirizine.

After 15 minutes, the wheal and flare response were recorded by circling with a skin-safe marker and transferring to paper using a strip of micropore tape. At a later date, the tape transfer markings were measured by a masked technician.

A result was considered positive with a maximum wheal diameter that was at least 3 mm and simultaneously at least 2 mm more than the negative control. An allergen test result was considered false negative when it met criteria for positivity at baseline testing (day 1) but was measured to be negative after cetirizine suppression on day 5. A 6-mg/mL histamine response was considered false positive after cetirizine suppression on day 5 if the 1-mg/mL histamine response was appropriately negative at the same time point.

Statistical Analysis

An a priori 2-tailed power analysis determined that 12 individuals were needed to evaluate a 1-mm difference in the mean wheal responses ($\alpha = .05$, 85% power). A 2-tailed, matched *t* test on mean wheal and flare measurements for the 2 concentrations of histamine on each day were performed. A Fisher exact test was used to compare the allergen false-negative rate between the 1-mg/mL and 6-mg/mL histamine control reagents.

Results

Twelve individuals aged 18 to 65 years with or without a history of allergic disease underwent a total of 384 SPTs (32 per individual). Cetirizine began to suppress wheal and flare responses at 1 hour ($P < .001$), with maximum suppression at day 5 ($P < .001$). Wheal and flare responses returned to more than 90% baseline within 4 days of not taking cetirizine. At day 5, suppression was more apparent with 1 vs 6 mg/mL of histamine (60% vs 38%) (Fig 2).

Four of the 12 individuals taking cetirizine had a positive SPT result using the 6-mg/mL histamine control when the 1-mg/mL histamine test result was negative (Table 1). Importantly, 6 of the 9 allergic individuals had false-negative allergen responses using

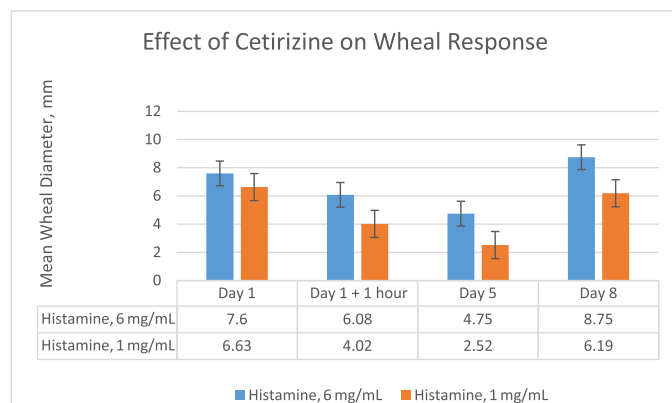


Figure 2. Effect of cetirizine on the 1 and 6 mg/mL of histamine response days 1 through 8 measured as the mean induration diameter. Error bars represent SEM. Comparisons were made with a 2-tailed matched *t* test between the mean wheal diameters for 1 and 6 mg/mL on days 1, 1 + 1 hour, 5, and 8. Asterisk indicates overall $P < .001$.

6 mg/mL of histamine as a positive control (66%) compared with 3 of the 9 allergic individuals having false-negative allergen responses with 1 mg/mL of histamine (33%). Overall, there were twice as many false-negative allergen responses with 6 mg/mL compared with 1 mg/mL histamine, but this finding did not reach statistical significance because nonallergic individuals were also included in the study ($P = .34$).

Discussion

We recently reported that some skin prick test devices do not always yield reliable positive control reactions using 1 mg/mL of histamine.¹¹ Indeed, some manufacturers recommend using a higher concentration of control reagent to more reliably produce a robust positive reaction. However, this practice may lead to false-negative allergen responses in certain situations. Wheals may be larger using 6 mg/mL of histamine; however, in some settings, there may be a risk of failing to detect the presence of residual antihistamine medication because the positive control reagent is too potent. As a result, clinicians may be tempted to declare an allergen test result as

negative when in fact the test result would have been positive on another day in the absence of medication that may be suppressing the wheal and flare.

Large wheals may be easy to read and provide clinician's reassurance that skin prick tests were placed properly. However, overexaggerated response to histamine placement may lead to misinterpretation of allergen testing. In the case of severe asthma or food and drug allergy, a false-negative SPT result may have serious consequences. Patients experiencing the effects of residual antihistamines may not be identified if unnecessarily high concentrations of positive control reagents are used.

Our study found 6 individuals who initially tested positive to an allergen at baseline testing, but those same allergens were falsely identified as negative because of the residual effects of medication superimposed on the higher-strength control reagent of 6 mg/mL of histamine. Three individuals were identified who had false-negative results with 1-mg/mL histamine reagents. Overall, using the 6-mg/mL histamine control almost doubles the false-negative rate compared with the rate of 1 mg/mL of histamine. This finding did not reach statistical significance because the study was not powered to detect allergen responses and not all individuals tested were allergic. Further studies should formally address this question.

Our results may only be applied to the specific SPT device used in the study, namely, the Multi-Test PC supplied by Lincoln Diagnostics. However, one would assume that the best choice of histamine control to use with skin test devices from any manufacturer would be the minimum concentration required to yield high sensitivity and specificity. We previously published a report that found excellent analytical sensitivity and specificity for 9 of 10 different devices evaluated from 5 manufacturers using 1 mg/mL of histamine.⁷ Using these devices, nothing would be gained by increasing the concentration of histamine used above 1 mg/mL. On the contrary, higher histamine concentration may lead to misinterpretation of allergens tested. On the basis of our analysis, we believe that the US Food and Drug Administration recommendations for allowing the use of 6 mg/mL of histamine should be reevaluated. At the very least, practitioners should use caution interpreting negative allergen SPT results if the 6-mg/mL histamine control results in a borderline positive reaction. If a borderline positive reaction occurs with the 6-mg/mL histamine test, we

Table 1
Wheal Diameters of the Histamine Controls and Allergens for Days 1 and 5^a

Component	Wheal diameter by patient No., mm											
	1	2	3	4	5	6	7	8	9	10	11	12
Day 1												
Saline	0	1.75	0	1.5	1	2.5	0	0	0	0	0	4
Histamine, 6 mg/mL	7	6.5	8.75	8	6.5	8.5	6.5	8.5	7	8.75	5.75	9.5
<i>Dermatophagoides farinae</i>	1.5	2.5	0	5	2.5	4.5	13	6.5	6	19.5	14	15.5
Timothy	2	2.5	0	9	0	4	12	8.5	0	5.5	2	5
Ragweed	1.5	2	7	1.5	0	4.5	0	0	0	1	3.25	3.5
Oak	1.5	2.5	0	1	0	6	2	13	4	5	3	4
Cat	0	0	0	9	0	5.5	9	10	4	5	3	4
Histamine, 1 mg/mL	5.75	5	7.5	6	6.5	10	7	5.25	5.5	7	6.75	7.25
Day 5												
Saline	0	0	0	0	0	0	0	0	0	0	0	2.5
Histamine, 6 mg/mL	0	3*	5*	6.5	2.5	5*	4.5	6.75	6.25	4.5*	4	8
<i>Dermatophagoides farinae</i>	0	0	0	3	1.5	3	6.5	1†	4.5	9.25	11.5	12.5
Timothy	0	0	0	7	0	3	6.5	9	0	0†	3.25	3
Ragweed	0	0	0†	0	0	0	0	0	0	0	0†	0
Oak	0	0	0	0	0	4.5	0	11	0†	0†	2	3.5
Cat	0	1	0	6.5	0	0†	5.25	5.75	0†	6.5	2.75†	5.5
Histamine, 1 mg/mL	0	0	0	5.25	0	0	5.25	3.5	4	2.75	4	5.5

^aA result was considered positive with a wheal of at least 3 mm and 2 mm more than the negative control. Allergen results were considered false negative (†) when it met criteria for positivity at baseline (day 1) but was measured to be negative on day 5. The 6 mg/mL of histamine test result was considered false positive (*) when it met criteria for positivity on day 5, yet the 1 mg/mL of histamine test result was appropriately negative at the same time point.

recommend a reassessment of the medication history to ensure that all medications with antihistamine properties have been reported. Indeed, sensitivity to important food allergens or the opportunity to treat allergic airway disease with immunotherapy may be lost in some cases.

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