

Intradermal reactivity to two concentrations of pollen extracts in atopic dogs

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Background – Intradermal test (IDT) is used widely for determination of allergens to include in allergen-specific immunotherapy (AIT). The optimal concentration of allergen solutions used for testing has not been established in atopic dogs. Inadequate concentrations could lead to falsely negative test results and lack of inclusion of important allergens in AIT.

Hypothesis/Objectives – To compare IDT reactivity to two concentrations of five pollen extracts and determine the effect on ASIT prescription.

Animals – Atopic dogs ($n = 67$) had IDT results including all of the allergens of interest.

Methods and materials – Medical records from a veterinary teaching hospital were reviewed for all atopic dogs undergoing IDT over a three year period. In addition to the hospital's standard IDT concentration of 1,000 PNU/mL for pollen allergens, five pollen allergens of higher concentration (3,000–8,000 PNU/mL) had been added at the beginning of that period. The IDT scores for the standard test concentration of each of the five allergens were compared to the scores for the higher concentration.

Results – Positive reactions occurred much more commonly to the higher test concentration for each of the paired allergens ($P < 0.05$, chi-square). The AIT formulation was different in 58% of the dogs from what it would have been without the higher concentration allergens.

Conclusions and clinical importance – The current standard IDT concentration of 1,000 PNU/mL for pollen allergens is likely too low, possibly resulting in inaccurate AIT formulations. This might explain treatment failures and the incorrect diagnosis of atopic-like dermatitis. Further work is needed to establish optimal allergen test concentrations.

Introduction

Intradermal testing (IDT) is the gold standard for determination of allergen-specific hypersensitivity in dogs with atopic dermatitis (AD).¹ The results are used to support the diagnosis of atopic dermatitis and determine which allergens to include in allergen-specific immunotherapy (AIT).^{1,2} Intradermal testing has been used in dogs with clinical signs compatible with AD for >70 years.^{3–5} There are many variables that can affect test results, producing false positive or false negative reactions.⁶ Causes of false positive reactions include dermatographism, irritating or overly concentrated test extracts, traumatic technique and nonimmunological histamine release. False negative reactions can result from drug interference, anergy, testing out of season, systemic disease and other factors inherent to the dog, mishandled or outdated allergens, and improper injection technique. One particularly

intriguing cause of false negative reactions is insufficient concentration of the allergen extracts used for testing.

Optimal test concentrations of allergenic extracts used in IDT have not been established in dogs. The concentration used for IDT should be based on establishment of each allergen's irritant threshold concentration (ITC) via serial dilutions in both healthy and atopic dogs.² Early investigators extrapolated from concentrations used in people, diluting the extracts for IDT based on the concentrations which evoked a positive prick test in people.^{4,5,7} Over time weight per volume (w/v) pollen extract concentrations were generally replaced by PNU/mL to denote extract potency, although other units have been used. This shift makes it difficult to compare studies over time.^{8,9}

Veterinary allergen extracts for pollens are generally provided in PNU/mL.^{10,11} The first reports of PNU/mL for IDT in allergic dogs recommended 1,000–1,500 PNU/mL for pollen allergens.^{4,5,12–14} Those investigators based that recommendation on personal observation. The first attempt to determine ITCs for pollen allergens in healthy dogs established that 1,000 PNU/mL is likely too low for most allergens.¹⁵ Although 1,750 PNU/mL was recommended as likely more appropriate, for most pollen allergens it was shown that the ITC was higher than 1,750 PNU/mL. A subsequent study established ITCs for

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pollen allergens ranging from 3,000 to 8,000 PNU/mL, where the ITC could be established.¹⁶ A comparison of IDT reactivity in atopic dogs showed a significantly greater number of positive reactions to higher concentrations of certain pollen extracts.¹⁷

Despite these recommendations, major veterinary allergen extract manufacturers and textbooks still recommend 1,000 PNU/mL as the appropriate test concentration for the majority of pollen allergens.^{6,10,11} Based on an informal survey of colleagues by the author, many veterinary clinicians use 1,000 PNU/mL for pollen allergen concentration for IDT.

The objective of this study was to review IDT results for atopic dogs to compare reactivity to two concentrations of five pollen extracts and determine the effect on the AIT prescription.

Methods and materials

The medical records of a veterinary teaching hospital were searched for dogs undergoing IDT. These dogs had met clinical criteria for the diagnosis of AD; other causes of pruritic skin disease, such as external parasites or secondary infections, had been ruled out or were controlled.¹⁸ An elimination diet trial, with a commercial prescription hydrolyzed protein diet, had been performed in dogs with nonseasonal pruritus to rule out cutaneous adverse food reaction.

Intradermal testing was performed with the hospital's standard set of 50 allergens (Stallergenes Greer; Lenoir, NC, USA). In 2015, five pollen allergens at higher concentration than used in the standard set of allergens had been added based on recommended irritant threshold concentrations (Table 1).^{1,16} One of these higher-concentration pollen allergens, Kentucky blue grass/June grass, was part of a multi-allergen mix (seven grass) used at 1,000 PNU/mL in the standard set.

Results of IDT were tabulated and compared for the five allergens at two different concentrations. A positive test result was defined as a subjective score of ≥ 2 , on a scale of 0–4. Chi-square test was used to determine if the number of positive results at the higher concentration was significantly different from the number of positive tests at the lower concentration. A *P*-value of <0.05 was considered statistically significant.

Results

There were IDT results for 67 dogs for all allergens in question at the two concentrations. Breed, age and sex information is shown in Table 2. Table 3 shows the number of dogs with positive scores to each concentration of allergen. The number of dogs with a negative score to the lower concentration and a positive to the higher concentration ranged from 37 to 57% depending on the allergen. When the cut-off for a positive test was increased from 2 to 3, then those results changed to 15–27%, again

depending on the allergen. Without the higher test concentrations in the IDT set, 57% of atopic dogs would have received a different AIT formulation. Twenty-seven of the dogs (40%) had all negative results to the lower concentrations and at least one positive to the higher concentration. Six of the dogs (9%) had no positive results on the entire IDT.

Discussion

The findings in this project are consistent with published data from nonatopic dogs indicating that 1,000 PNU/mL for pollen allergens is likely too low to produce reliable positive results. A much higher proportion of atopic dogs were positive to the higher concentration allergens, which led to more allergens for inclusion in AIT.

Two studies have compared pollen allergen ITCs in healthy dogs between two major veterinary allergen extract suppliers; attempting to further clarify ITCs in healthy dogs using protein analysis and statistical modelling.^{19,20} Both studies showed that for most of the pollen allergens tested, the ITC was $>1,000$ PNU/mL when it could be established, although results greatly varied.

Allergen-specific immunotherapy is the only actual treatment for AD in dogs.²¹ With the large number of variables that can affect IDT results, it is important that appropriate concentrations of allergens are used for testing in order to reduce the possibility of false negative results and consequently influencing allergen choices for AIT.

The lack of any positive result in six dogs could be explained by any of the known causes of false negative IDT reactions.⁶ Those results also could be true negatives, indicating that the dogs are either not atopic and another cause of pruritus was missed in the diagnostic investigation or that they fall into the category of atopic-like dermatitis.

The lack of objective measurements for the results in the present study is a possible confounding factor as subjective scoring results can be clinician-dependent.^{19,22} There were multiple clinicians, including trainees, who performed IDTs during the period in question. They might have had different interpretations of positive results. Because of variations in record-keeping, it was not possible to determine which clinician interpreted which IDT for all dogs, so no analysis was performed. Given the retrospective clinical nature of this study, there is the possibility of bias in IDT interpretation. All clinicians interpreting IDTs knew that there were five higher concentration allergens at the end of the test.

Table 1. Pollen allergens and concentrations used for intradermal testing in atopic dogs

Allergen	Standard concentration (PNU/mL)	High concentration (PNU/mL)
Kentucky Blue grass/June (<i>Poa pratensis</i>) seven grass mix (meadow fescue, Kentucky blue/June, orchard, redtop, perennial rye, sweet vernal, Timothy)	1,000 (seven grass mix)	8,000 (Kentucky blue grass/June grass)
Ragweed mix (giant/short) <i>Ambrosia trifida</i> , <i>Ambrosia artemisiifolia</i>	1,000	8,000
Cocklebur (<i>Xanthium atrumarium</i>)	1,000	7,000
Pigweed mix (<i>Amaranthus hybridus</i> , <i>Amaranthus palmeri</i> , <i>Amaranthus retroflexus</i>)	1,000	3,000
Sheep sorrel/yellow dock mix (<i>Rumex acetosella</i> / <i>Rumex crispus</i>)	1,000	5,000

Table 2. Demographic information for 67 atopic dogs with intradermal test results

Breed distribution	Age range	Sex distribution
17 of 67 (25%) Labrador retriever or cross	1–10 years	35 of 67 (52%) castrated male
7 of 67 (10%) Golden retriever or cross		4 of 67 (6%) male
4 of 67 (6%) Bulldog (type not indicated), French bulldog or cross		26 of 67 (39%) spayed female
4 of 67 (6%) German shepherd dog or cross		2 of 67 (3%) female
4 of 67 (6%) Pitbull, pit bull terrier, American Staffordshire terrier		
3 of 67 (4%) Boxer or cross		
3 of 67 (4%) Boston terrier or cross		
Two or fewer of 17 other breeds		

Table 3. Number of 67 atopic dogs with positive intradermal test results to two concentrations of pollen allergen extracts

N = 67, Positive (2–4)	Low concentration (1,000 PNU/mL)	High concentration (varied)
Ragweed	12 (18%)	45 (67%)
Cocklebur	7 (10%)	44 (66%)
Pigweed mix	14 (21%)	40 (60%)
Sorrel/dock mix	16 (24%)	40 (60%)
Grass	9 (13%)	47 (70%)

$P < 0.05$ for all allergens.

Another source of variation in this study is the allergen extracts themselves. Allergen extract variability between batches, even those with the same PNU/mL concentration, has been demonstrated.²⁰ It is possible that some extract solutions contained more actual allergen than others, leading to more positive results with those particular solutions. Because of the retrospective nature of this study, it was not possible to determine whether there were more positive results in association with different extract batches.

It is possible that the positive results in this study could be attributed to irritant reactions and not true IgE-mediated reactivity. Although irritant reactions are mentioned by many sources, there is no agreed-upon definition or description of an irritant reaction in an atopic dog. Many dogs did not have positive results to the higher concentration allergens and those that did have positive results were not uniformly positive to the higher concentrations. There also was a varying degree of positivity to the higher concentration, which suggests that the reactions were not irritant reactions. Because these data were collected, the IDT set used in the author's practice has been changed so that all pollen allergens are tested at a higher concentration and there are varying patterns of reactivity, including dogs with no positive results at all. This would suggest that the allergen extract concentrations used in this study are not strong irritants in atopic dogs.

Lastly, another consideration in interpreting these data is the comparison of reactivity to an allergen mixture with reactivity to a single allergen from that mixture. It has been recommended that single house dust mite extracts are used instead of mixtures because single allergens appear more accurate.²³ It is possible that the grass pollen mixture used in the present study did not have sufficient concentration of any single allergen to reliably produce a positive result and that more dogs would have had positive results to 1,000 PNU/mL of Kentucky blue grass as a single allergen rather than the grass mixture. However, considering the grass family pollen cross-reactivity, comparison of reactivity between a mixture and a

single allergen is not unwarranted.²⁴ Given the expense of allergen extracts, knowledge of a single allergen's reactivity compared to a mixture of related allergens, might enable clinicians to reduce the number of extracts used. It would be interesting to compare reactivity to an allergen mixture at varying concentrations to each of the individual components at varying concentrations.

In summary, the present study shows increased IDT reactivity in atopic dogs to higher concentrations of pollen allergens. Although it is not possible to determine the optimal test concentration for each allergen in the present study, the data are compatible with results from nonallergic dogs. Considering the long history of IDT in dogs, it is clear that much more work needs to be done in order to determine optimal allergen extract test concentrations. Improvement in testing should improve specificity of AIT prescriptions and presumably response to treatment.

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Résumé

Contexte – Les tests intradermiques (IDT) sont largement utilisés pour déterminer les allergènes à inclure dans une immunothérapie spécifique (AIT). La concentration optimale des solutions d'allergènes utilisées pour les tests n'a pas été établie chez le chien atopique. Les concentrations inadéquates pourraient mener à des tests faux négatifs et le défaut d'inclusion d'allergènes importants pour AIT.

Hypothèses/Objectifs – Comparer la réactivité des IDT à deux concentrations de cinq extraits d'allergènes de pollens et déterminer l'effet sur la prescription d'ASIT.

Sujets – Des chiens atopiques (n = 67) avec des résultats d'IDT incluant tous les allergènes d'intérêt.

Matériel et méthode – Les données médicales d'un hôpital d'enseignement vétérinaire ont été revues pour tous les chiens atopiques ayant eu des IDT sur une période de trois ans. En plus des concentrations standard d'IDT de 1000 PNU/mL pour les pollens, cinq pollens de plus haute concentration (3,000–8,000 PNU/mL) ont été ajoutés au début de la période. Les scores d'IDT pour la concentration test standard de chacun des cinq allergènes ont été comparés aux scores pour les plus hautes concentrations.

Résultats – Les réactions positives se sont produites plus fréquemment aux concentrations tests plus élevées pour chacun des allergènes pairés ($P < 0.05$, chi-square). La formulation d'AIT était différente de ce que ça aurait été sans les concentrations les plus hautes d'allergènes pour 58% des chiens.

Conclusions et importance clinique – La concentration standard d'IDT actuelle de 1 000 PNU/mL pour les pollens semble trop faible, ce qui pourrait résulter en des formulations inadéquates de AIT. Ceci expliquerait les échecs de traitement et les erreurs de diagnostic des atopique-like. D'autres études sont nécessaires pour déterminer les concentrations optimales d'allergènes.

Resumen

Introducción – la prueba intradérmica (IDT) se usa frecuentemente para la determinación de alérgenos que se deben incluir en la inmunoterapia específica para alérgenos (AIT). La concentración óptima de soluciones de alérgenos utilizadas para las pruebas no se ha establecido en perros atópicos. Concentraciones inadecuadas podrían conducir a resultados falsos negativos y la falta de inclusión de alérgenos importantes en AIT.

Hipótesis/Objetivos – comparar la reactividad IDT con dos concentraciones de cinco extractos de polen y determinar el efecto sobre la prescripción en ASIT.

Animales – perros atópicos (n = 67) tuvieron resultados de positivos IDT, incluidos todos los alérgenos de interés.

Métodos y materiales – se revisaron los historiales clínicos de un hospital de enseñanza veterinaria de todos los perros atópicos sometidos a IDT durante un período de tres años. Además de la concentración de IDT estándar del hospital de 1,000 PNU/mL para alérgenos de polen, al principio de ese período se agregaron cinco alérgenos de polen a mayor concentración (3,000–8,000 PNU/mL). Las puntuaciones IDT para la concentración de prueba estándar de cada uno de los cinco alérgenos se compararon con las puntuaciones para la concentración más alta.

Resultados – reacciones positivas ocurrieron con mucha mayor frecuencia a la concentración más alta

para cada uno de los alérgenos pareados ($P < 0,05$, chi-cuadrado). La formulación de AIT fue diferente en el 58% de los perros de lo que hubiera sido sin los alérgenos de mayor concentración.

Conclusiones e importancia clínica – la concentración de IDT estándar actual de 1,000 PNU/mL para los alérgenos de polen es probablemente demasiado baja, lo que posiblemente resulte en formulaciones de AIT inexactas. Esto podría explicar los fracasos del tratamiento y el diagnóstico incorrecto de dermatitis atópica. Se necesita más trabajo para establecer concentraciones óptimas de prueba de alérgenos.

Zusammenfassung

Hintergrund – Der Intradermaltest (IDT) wird weitgehend zur Bestimmung von Allergenen, die bei der Allergen-spezifischen Immuntherapie (AIT) inkludiert werden, verwendet. Die optimale Konzentration von Allergenlösungen, die zum Testen Verwendung findet, ist bei atopischen Hunden noch nicht bewiesen. Unzureichende Konzentrationen können zu falsch negativen Testergebnissen führen und dazu, dass wichtige Allergen nicht in der AIT inkludiert werden.

Hypothese/Ziele – Der Vergleich einer IDT Reaktivität auf zwei Konzentrationen von fünf Pollenextrakten und die Feststellung, welchen Einfluss das auf die ASIT Verschreibung hat.

Tiere – Atopische Hunde ($n = 67$) zeigten IDT Ergebnisse, die alle Allergene beinhalteten, die von Interesse sind.

Methoden und Materialien – Es wurden die medizinischen Daten aus Veterinärkliniken von allen atopischen Hunden durchgesehen, die über einen Zeitraum von drei Jahren einem IDT unterzogen wurden. Zusätzlich zu den Standard IDT Konzentrationen der Kliniken von 1.000 PNU/mL für Pollenallergene, wurden fünf Pollenallergene höherer Konzentration (3.000-8.000 PNU/mL) am Anfang dieses Zeitraums hinzugefügt. Die IDT Werte für die Standard Testkonzentrationen eines jeden der fünf Allergene wurden mit den Werten für die höheren Konzentrationen verglichen.

Ergebnisse – Positive Reaktionen traten viel häufiger bei den höheren Testkonzentrationen eines jeden gepaarten Allergens auf ($P < 0,05$, chi-square). Die AIT Formulierung unterschied sich bei 58% der Hunde von einer Formulierung ohne die höheren Allergenkonzentrationen.

Schlussfolgerungen und klinische Bedeutung – Die momentanen Standard IDT Konzentrationen für 1.000 PNU/mL für Pollenallergene ist vermutlich zu niedrig, was in einer falschen AIT Formulierung resultieren könnte. Das könnte Therapiefehlschläge erklären und eine inkorrekte Diagnose einer atopic-like Dermatitis. Es sind weitere Forschungsarbeiten nötig, um optimale Allergentestkonzentrationen zu etablieren.

要約

背景 – 皮内試験(IDT)は、アレルギー特異的免疫療法(AIT)に含まれ、アレルギーの決定に広く使用されている。テストに使用するアレルギー溶液の最適濃度は、アトピー犬においては確立されていない。不適当なアレルギー溶液濃度は試験結果を偽陰性にし、アレルギー特異的免疫療法を実施する際に重要なアレルギーの包含の欠如を引き起こす可能性がある。

仮説/目的 – 本研究の目的は、5種類の花粉抽出物に対し、2つの濃度によってIDT反応性を比較し、ASIT処方に対する効果を決定することである。

被験動物 – 関連したすべてのアレルギーを含むIDT結果を有していたアトピー犬($n = 67$)。

材料と方法 – 3年間にわたってIDTを実施しているすべてのアトピー犬について、獣医教育病院の医療記録をレビューした。花粉アレルギーに対する病院の標準IDT濃度1,000 PNU / mLに加え、試験期間初期に5種類の高濃度花粉アレルギー(3,000~8,000 PNU / mL)を追加した。5種類のアレルギーについて、それぞれの標準テスト濃度IDTスコアと、より高濃度IDTスコアを比較した。

結果 – 各アレルギーのテスト濃度が高くなると、共通して陽性反応はるかに多く発生した($P < 0.05$ 、カイ二乗検定)。AIT調査は、高濃度アレルギーなしでは58%の犬で異なった。

結論と臨床的重要性 – 現在の花粉アレルギー標準IDT濃度1,000 PNU / mLは低すぎる可能性が高く、AIT調査が不正確になる可能性がある。これは、治療失敗およびアトピー様皮膚炎の誤った診断を説明する可能性がある。最適なアレルギー試験濃度を確立するには、さらなる研究が必要である。

摘要

背景 – 皮内試験(IDT)被广泛用于检测过敏原,根据结果再进行过敏原特异性免疫治疗(AIT)。用于测试异位性犬的过敏原溶液,其最佳浓度尚未确定。浓度不足可能导致检测结果呈假阴性,并且使AIT缺失重要过敏原。

假设/目的 – 比较五种花粉提取物两种浓度的IDT反应性,确定其对ASIT配方的影响。

动物 – 有IDT结果的异位性犬($n=67$),试验包括所有相关过敏原。

方法和材料 – 家兽医教学医院三年期间所有接受IDT治疗的异位性犬,回顾他们的病例记录。除了医院对花粉过敏原的IDT标准浓度为1000 PNU/mL外,初期还添加了5种浓度更高的花粉过敏原(3000 - 8000 PNU/mL)。将五种过敏原标准浓度的IDT评分与较高浓度的IDT评分进行比较。

结果 – 每对过敏原检测浓度越高,阳性反应越常见($P < 0.05$, 卡方检验)。根据标准浓度过敏原结果制定的AIT过敏原成分,比高浓度的少58%。

结论和临床意义 – 目前花粉过敏源的IDT标准浓度为1000 PNU/mL,可能太低,从而导致AIT的过敏原成分不准确。这可以解释治疗失败和误诊为异位性皮炎的原因。需要进一步确定最佳的过敏原测试浓度。

Resumo

Contexto – O intradérmico (IDT) é amplamente utilizado para a determinação dos alérgenos a serem incluídos na imunoterapia alérgeno-específica (AIT). A concentração ótima de soluções de alérgenos utilizadas nos testes ainda não foi estabelecida em cães atópicos. Concentrações inadequadas podem levar a resultados falso negativos e à não inclusão de alérgenos importantes na AIT.

Hipótese/objetivos – Comparar a reatividade do IDT em duas concentrações de cinco extratos de pólen e determinar o efeito na prescrição de AIT.

Animais – Sessenta e sete cães atópicos que possuíam resultados de teste alérgico incluindo todos os alérgenos de interesse.

Métodos e materiais – Foram revisados os prontuários de todos os cães atópicos que foram submetidos ao IDT atendidos em um hospital veterinário escola em um período de três anos. Além da concentração de 1.000 PNU/mL que é utilizada como padrão no hospital para alérgenos de pólen, foram adicionadas no início daquele período cinco concentrações mais fortes de alérgenos de pólen (3.000-8.000 PNU/mL). Os escores de IDT para a concentração padrão do teste de cada alérgeno foram comparados aos escores das concentrações mais altas.

Resultados – Reações positivas ocorreram muito mais comumente na concentração mais altas para cada alérgeno pareado ($P < 0.05$, chi-quadrado). A formulação de AIT foi diferente do que seria sem os alérgenos em alta concentração em 58% dos cães.

Conclusão e importância clínica – A concentração padrão de 1.000 PNU/mL para alérgenos de pólen é provavelmente muito baixa, resultando em formulações de AIT imprecisas. Isto pode explicar as falhas terapêuticas e o diagnóstico incorreto na dermatite atópica-like. São necessários mais estudos para se estabelecer concentrações alergênicas ideais.