

Dose-dependent pruritogenic and inflammatory effects of intradermal injections of histamine, compound 48/80 and anti-canine IgE in healthy dogs

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Background – Scratching behaviours associated with intradermal (i.d.) injection of pruritogens such as histamine and compound 48/80 into the skin of mice and humans is the commonly used model to advance itch research and drug development. The predictive validity of this model is poorly documented in dogs.

Objectives – To evaluate the dose-dependent effects of pruritogenic substances, each with a different mechanism of action, in healthy dogs.

Animals – Ten healthy laboratory beagles.

Methods and materials – All dogs were video-recorded for 30 min post-injection (mpi) of i.d. goat anti-canine IgE (4 and 25 µg/site), histamine and compound 48/80 (50, 100, 200, 400 µg/site); two buffered saline injections served as controls. Two blinded investigators reviewed the pruritic behaviours of all video recordings. Global wheal scores were evaluated at 30 min by a blinded investigator.

Results – All dogs showed wheal and erythema at the pruritogen injection site; global wheal scores at 30 min of each substance significantly increased at all concentrations compared to control ($P \leq 0.05$). A blinded evaluation revealed that all pruritogens induced mild acute pruritic behaviours at the site of injection. There was no injection site pain seen in any dog. Compared to controls, injections of pruritogens did not significantly affect the pruritic seconds or occurrence of pruritic episodes for any of the substances.

Conclusions and clinical significance – These preliminary results suggest that i.d. injections of the studied pruritogens can induce cutaneous wheal and flare response in healthy dogs; but inconsistencies occur in the induction of itch, even with the different concentrations of pruritogens.

Introduction

Pruritus is defined as a sensation that provokes a desire to scratch. Atopic dermatitis, scabies, cutaneous T-cell lymphoma, and bacterial and yeast infections cause pruritus as an important sign in humans and dogs.^{1,2} Therefore, it is reasonable to investigate in dogs the same substances and mediators of pruritus known to induce pruritus in healthy humans. Identifying canine models of pathological itching is essential for pharmacological evaluation of the antipruritic efficacy of more specific and potentially safer therapies to treat itch as a common and unpleasant sensation.

Scratching behaviours associated with intradermal (i.d.) injection of pruritogens such as histamine and compound 48/80 into the skin of mice and humans is the commonly used model to advance itch research and drug development.^{3–7} The predictive validity of this model is poorly documented in dogs. Immunoglobulin-E mediated cutaneous reactions grossly and histologically resemble changes

seen in naturally occurring allergic dermatitis in dogs;⁸ however, this model has not been evaluated for induction of pruritus in dogs.

The primary goals of this study were to determine the dose-dependent effects of i.d. injections of pruritogenic substances, histamine, compound 48/80 and goat anti-canine IgE, on pruritic behaviour and cutaneous inflammatory reactions in healthy dogs.

Methods and materials

Study population

Ten clinically healthy male castrated research beagle dogs with no previous history of pruritus or skin disease were included in this study. The dogs ranged in age between 1 and 2 years and were housed in a university setting under standard conditions. The dogs had not received any medications for at least eight weeks before enrolment. All aspects of the study were approved and conducted in accordance with the Institutional Animal Care and Use Committee (IACUC).

Substances for intervention

Increasing concentrations of the following known human and mice pruritogenic substances were injected i.d. to evaluate the pruritic response in dogs: histamine (Sigma-Aldrich, St Louis, MO, USA) and compound 48/80 (Sigma-Aldrich) at 50, 100, 200 and 400 µg/

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site i.d., goat anti-canine IgE (AHP946, Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 4 and 25 µg/site i.d. (based on the IgG concentration of the polyclonal antibody product) and phosphate buffered saline (Sigma/Aldrich) served as a negative control. Buffered saline was used for all dilutions of the pruritogens. The pruritogen concentrations were selected to mirror studies in humans and mice where the same substances were evaluated for pruritus induction.³⁻⁷

Study protocol

All dogs were examined by the investigators and confirmed healthy and free of any dermatological or systemic disease 5 days before the first study day. A small (about 10 × 10 cm) area for injection of the substances was clipped on the right and left lateral thoracic region; all injection site clippings were performed ≥48 h before each injection in order to avoid any occurrence of localized skin reaction or irritation in concurrence with the administration and evaluation of the pruritogens and control substances.

All injections were given i.d. with two separate rounds of i.d. buffered saline as a negative control; buffered saline was used to determine if the i.d. injection of a known nonpruritogenic substance would elicit a pruritic response.⁹ The order of injections was randomized using statistical computer software (PRISM 8.0; GraphPad Software, La Jolla, CA, USA). Each injection was given as a single intervention, and a washout period between 72 h and 7 days was utilized between injections.⁹ To avoid residual inflammation from the previous injection, injections were rotated to alternate side between the left and right sides of the thorax; the injection site was moved cranial and caudal as well as dorsal and ventral on the thorax.⁹ The protocol of injections followed the form: injection on left thorax, washout period, injection on right thorax, washout period, injection on left thorax, and so on.

Dogs were acclimatized to their kennels and recording video equipment for at least one week prior to recording. All dogs were video-recorded for 30 min post-injection (mpi) after each injection using GoPro Hero Session (GoPro, Inc., San Mateo, CA, USA) high-definition, waterproof cameras; the cameras were placed in the kennels to assure that each dog was fully visible for the duration of the recording period. All injections, as well as the video recordings, were performed at the same time of the day without the investigators presence during the filming. All dogs were single-housed in their kennels during the study. All toys were removed immediately before recording, and only food and water were available at the time of injections.

Assessment of pruritus

Localized pruritic behaviour measurements in areas of intradermal injections were completed through the creation of a canine ethogram for itch behaviour (Table S1). Behaviours were defined in the written definition and with video example for observers to objectively quantify the presence of pruritic behaviour. Behaviours quantified included licking, biting/chewing, scratching, head shaking, scooting, rolling,

pawing and rubbing, whereas flinching from the site of injection or vocalization was considered to be indicative of pain.⁹ Two trained investigators reviewed all video recordings and were blinded to the substances administered until all data were analysed. Investigators evaluated the number of pruritic episodes, the type of pruritic behaviour, the duration of each pruritic episode and the time relative to injection that the behaviours occurred for each dog.

Assessment of global wheal score

As described previously, a global wheal score (GWS) was evaluated at 30 mpi.^{10,11} The average diameter (*D*) of wheals was measured in two orthogonal directions in millimetres. Erythema (*E*) and induration (firmness, *F*) were then assigned a score from 1 to 3 (1, no erythema/flaccid wheal; 2, weak erythema/moderate induration; 3, strong erythema/firm induration).^{10,11} The same investigator evaluated the wheal and erythema formation and was blinded as to which substance had been administered until all data were collected. Global wheal score was calculated as $GWS = D \times E \times F$.

Statistical analysis

The evaluation of the pruritic effects and GWSs was compared between the vehicle (buffered saline) and different pruritogens. Statistical comparison was performed by computer software (Prism 8.0; GraphPad Software). Data were evaluated for normality with a D'Agostino and Pearson omnibus normality test. Depending on the normality test outcome, parametric repeated measures ANOVA or the nonparametric alternative, the Friedman test, was utilized. All tests were considered significant at $P \leq 0.05$; Bonferroni or Dunn's correction test was used for multiple comparisons between all groups of values depending on the parametric versus nonparametric test.

Results

Global wheal scores

Intradermal injections of histamine and anti-canine-IgE resulted in a positive wheal and erythema reactions in all dogs (Figure 1a,c); global wheal scores at 30 mpi of each substance significantly increased at all concentrations compared to the control (Figure 2a,c; repeated measures ANOVA, $F_{(3,318, 29.87)} = 47.50$, $P \leq 0.05$). The GWSs induced by all of the test concentrations of compound 48/80 were significantly increased relative to control (Figures 1b, 2b). In addition, the development of wheals and erythema induced by compound 48/80 at 400 µg/site was significantly greater than that generated by concentrations of 50 µg/site and 100 µg/site in all of the dogs (Figure 2b; repeated measures ANOVA; 50 versus 400 µg/site and 100 versus 400 µg/site, $P \leq 0.05$, respectively).

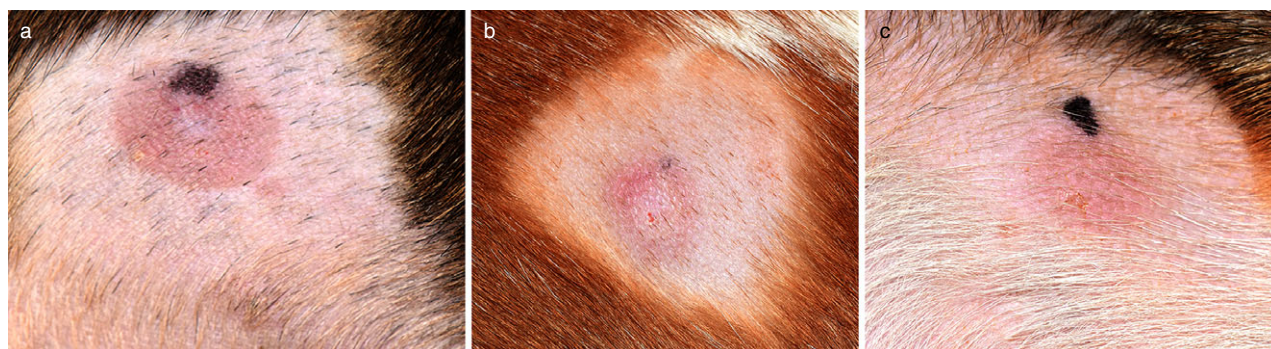


Figure 1. Clinical images of wheal and flare responses of tested pruritogens.

Intradermal injections of histamine (50 µg/site), compound 48/80 (100 µg/site) and anticanine-IgE (4 µg/site) resulted in a positive wheal and erythema reactions in all dogs.

Itch behaviours

No episodes of behaviour considered to be pain-related were observed in any of the dogs. A blinded evaluation revealed that histamine and anti-canine-IgE injections induced dose-dependent mild acute pruritic behaviours at the site of injection in seven of 10 and four of 10 dogs, respectively, when compared to the saline control (Figure 3a,c,d,f). However, these differences were not statistically significant for the pruritic seconds or episodes for either substance, histamine [Figure 3a,d; repeated

measures ANOVA, $F_{(1.696, 15.26)} = 2.212$, seconds at 400 μg , $P = 0.24$; $F_{(3.498, 31.48)} = 1.593$, episodes at 400 μg , $P = 0.08$] or anti-canine-IgE (Figure 3c,f; repeated measures ANOVA, seconds at 25 μg , $P = 0.21$; episodes at 25 μg , $P = 0.31$).

The compound 48/80 injections at 50, 100, 200 and 400 $\mu\text{g}/\text{site}$ produced mild pruritic behaviours in four of 10 dogs and a dose-dependent increase in pruritic behaviour in only three of 10 dogs (Figure 3b,e). However, none of the compound 48/80 concentrations achieved

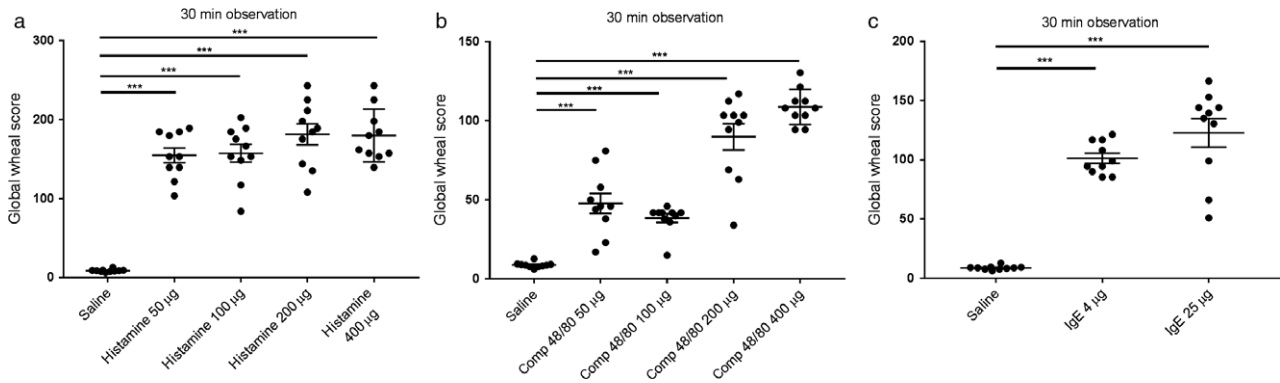


Figure 2. Global wheal scores (GWS) of wheal and flare responses of tested pruritogens.

Global wheal scores 30 min post-injection of each pruritogen substance [(a) histamine, (b) compound 48/80 (comp 48/80) and (c) anticanine-IgE (IgE)] significantly increased at all concentrations compared to control. Data for control intradermal saline injections are presented as a median of two score evaluations. *** $P < 0.001$.

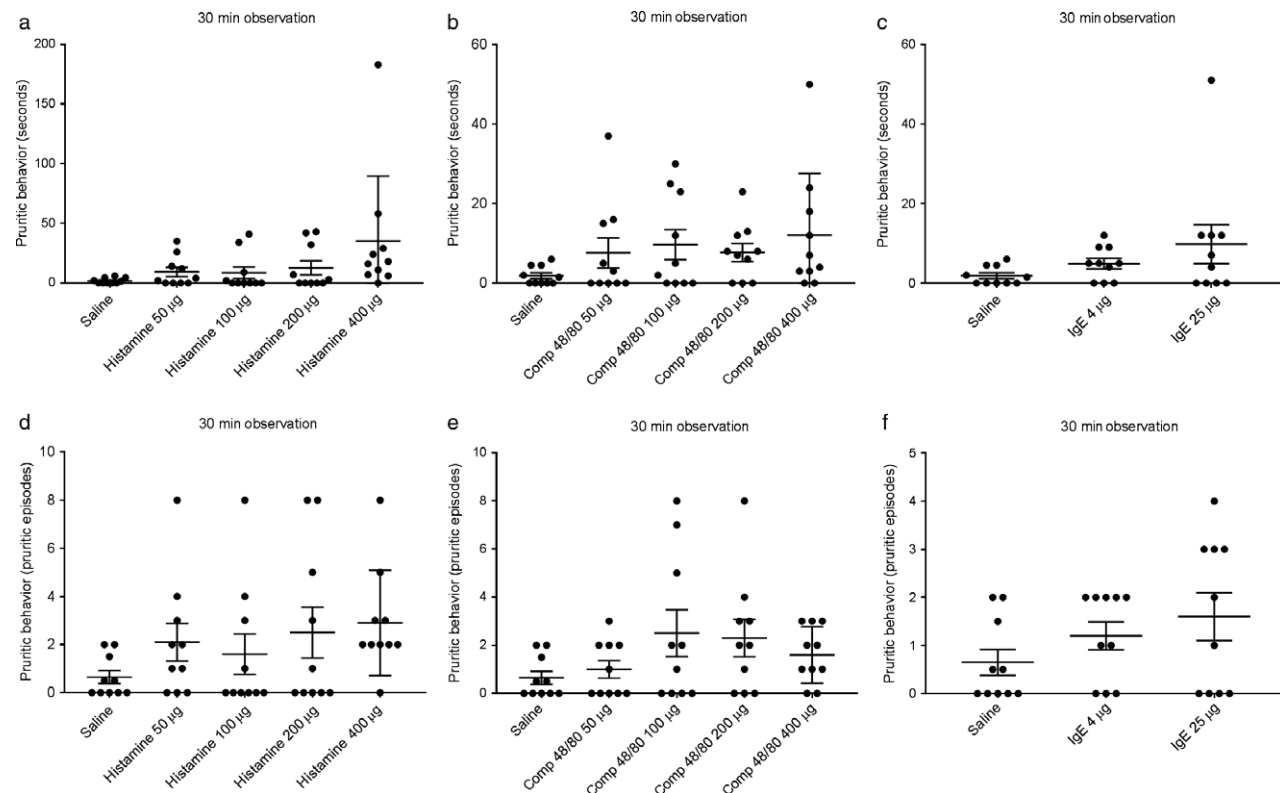


Figure 3. Evaluations of acute pruritic behaviours of pruritic seconds and episodes after intradermal injections of tested pruritogens.

Histamine (a,d) and anticanine-IgE (IgE, c,f) injections induced dose-dependent mild acute pruritic behaviours at the site of injection in seven of 10 and four of 10 dogs, respectively, when compared to the saline control. However, these differences were not statistically significant for the pruritic duration or number of pruritic episodes for either substance, histamine or anticanine-IgE. The compound 48/80 (comp 48/80; b,e) injections at 100, 200 and 400 $\mu\text{g}/\text{site}$ produced mild pruritic behaviours in four of 10 dogs and a dose-dependent increase in pruritic behaviour in only three of 10 dogs. However, none of the compound 48/80 concentrations achieved significant increases in pruritus when compared to the saline control.

significant increases in pruritus when compared to saline control (Friedman test, seconds at 400 µg, $P = 0.18$; episodes at 400 µg, $P = 0.26$).

Discussion

In this study, none of the investigated pruritogens was able to elicit statistically significant pruritic behaviour when injected i.d. in healthy dogs. This finding is unlikely to be related to lack of pruritogen-induced cutaneous inflammation or the concentrations of tested pruritogens. Positive wheal reactions were observed in all dogs included in this study, and the injected pruritogen concentrations were equivalent to those used in humans and mice for the investigations of pruritus.^{3–7} Furthermore, our recording period of 30 min is identical to the recordings utilized in murine itch evaluation⁶ and was 10 min longer than a previous canine study⁹ which investigated several i.d. pruritogens.

Acute itch models, which are commonly induced by i.d. injections of pruritogens in humans and mice, are rarely investigated in dogs. Histamine is the most commonly studied pruritogen; histamine injection into the skin of humans and mice evokes itching sensation which can be quantified and used as an experimental measure of itch.^{3–6} Histamine binds to and activates the histamine H1 receptor, a G-protein coupled receptor (GPCR), on peripheral sensory neurons and dorsal root ganglia; the inward (depolarizing) ionic current in neurons secondary to H1 receptor activation involves opening transient receptor potential vanilloid-1 (TRPV1) receptor as well.^{12–15} In contrast to humans and mice, dogs develop wheal and erythema reactions after i.d. injections of histamine at 50 µg/site; however, none of the dogs demonstrated strong pruritic behaviour in previous studies when a single histamine concentration was tested.^{9,16} In our study, there was mild acute pruritic behaviour with increasing i.d. concentrations of histamine, but similar to previous canine studies,^{9,16} no significant differences compared to control were observed.

Immunoglobulin-E mediated reactions, induced by i.d. injection of anti-canine-IgE antibodies, are characterized by wheal and flare responses which histologically resemble changes seen in naturally occurring allergic dermatitis in dogs.⁸ Intradermal injection of anti-canine-IgE in normal dogs results in a rapid biphasic influx of inflammatory cells with early influx of predominantly activated eosinophils, mast cells and neutrophils, whereas mainly T-lymphocytes and dendritic cells predominate at 24 h.⁸ This screening model is used for studying the anti-inflammatory effect of anti-allergic drugs before entering clinical trials;^{10,11} however, to the best of the authors' knowledge, such a model has not been investigated for induction of acute pruritic behaviour. The injection of anti-canine-IgE produced strong wheal and flare responses in all dogs; however, only mild increases in acute pruritic behaviours were observed.

The Mas-related G-protein-coupled receptors (Mrgprs), implicated in allergic inflammation and itch, are expressed on peripheral terminals of sensory neurons and mast cells.¹⁷ Compound 48/80 induces mast cell degranulation and provokes itch by activating Mas-related G protein-

coupled receptor $\times 2$ (MRGPR $\times 2$) and MrgprB2 on neurons in humans and mice, respectively.^{4,17–19} Interestingly, compound 48/80 induces similar numbers of scratching bouts in wild-type and mast cell-deficient mice, which suggests that compound 48/80 provokes itch independent of mast cell degranulation and the subsequent histamine release.²⁰ A comparison of the Mrgprs repertoires in dog and human revealed that dogs have MRGPR $\times 2$ and MRGPRD.²¹ Intradermal injection of compound 48/80 provokes immediate wheal and flare reactions in dogs and is considered a test system to evaluate drug efficacy in an immediate type hypersensitivity, such as glucocorticoids.^{16,22,23} The i.d. injections of compound 48/80 at all concentrations induced wheal and flare reactions in dogs tested in this study; however, in contrast to human and mice reports,^{4,6,7} there was no significant increase in pruritic behaviours in the dogs.

The main limitation of our study is the small number of healthy dogs evaluated. Future attempts to experimentally induce itch in healthy dogs should assess pruritic behaviours using a larger number of dogs and different locations (e.g. neck versus thorax). The standard model of itch in the mouse has measured scratching in response to pruritic stimuli delivered to the cheek or the nape of the neck, which has not been evaluated in the dog.⁶

In conclusion, the studied pruritogens (histamine, anti-canine-IgE and compound 48/80) induced the wheal and erythema reactions in all dogs. However, none of the substances produced significant pruritus when injected i.d. in clinically healthy dogs.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Ethogram itch behaviour definitions specific to intradermal injection (IDT) site of pruritogen used to record site specific itch behaviours per minute during video analysis by independent trained viewers.

Résumé

Contexte – Les comportements de grattage associés à une injection intradermique (i.d.) de pruritogènes tels que l’histamine et composé 48/80 dans la peau de souris et d’homme est un modèle fréquemment utilisé pour avancer dans la recherche sur le prurit et le développement de traitements. La valeur prédictive de ce modèle est peu documentée chez le chien.

Objectifs – Evaluer les effets dose-dépendants des substances pruritogènes, chacun avec un mode d’action différent, chez les chiens sains.

Sujets – Dix beagles de laboratoire, sains.

Matériel et méthode – Tous les chiens ont été enregistrés par vidéo pendant 30 minutes après l’injection (mpi) d’IgE anti-canin de chèvre i.d. (4 et 25 µg/site), histamine et composé 48/80 (50, 100, 200, 400 µg/site); deux injections de solution saline servant de contrôle. Deux investigateurs en aveugle ont revu les comportements de prurit de tous les enregistrements vidéo. Les scores globaux de plaques ont été évalués à 30 mpi par un investigateur en aveugle.

Résultats – Tous les chiens ont montré de l’érythème et des plaques au site d’injection du pruritogène; les scores globaux de plaque à 30 mpi de chaque substance étaient augmentés pour toutes les concentrations, comparé au contrôle ($P \leq 0.05$). Une évaluation en aveugle a révélé que tous les pruritogènes induisaient des comportements de prurit modérés au site d’injection. Aucune douleur au site d’injection n’a été observée pour aucun des chiens. En comparaison avec les contrôles, les injections de pruritogènes n’ont pas significativement modifié les secondes de prurit ou le développement d’épisodes de prurit quelque soit la substance.

Conclusions et signification clinique – Ces résultats préliminaires suggèrent que les injections i.d. des pruritogènes étudiés peuvent induire de plaques cutanées et une réponse aiguë chez les chiens sains; mais les inégalités sont observées dans le développement du grattage, même avec les différentes concentrations de pruritogènes.

Resumen

Introducción – el comportamiento de rascado asociado con la inyección intradérmica (i.d.) de pruritógenos como la histamina y el compuesto 48/80 en la piel de ratones y humanos es un modelo comúnmente utilizado para avanzar en la investigación del picor y el desarrollo de fármacos. La validez predictiva de este modelo está mal documentada en perros.

Objetivos – Evaluar los efectos dependientes de dosis de sustancias pruritogénicas, cada una con un mecanismo de acción diferente, en perros sanos.

Animales – Diez Beagles de laboratorio sanos.

Métodos y materiales – todos los perros se grabaron en video durante 30 minutos después de la inyección (mpi) intradérmica de IgE anti-canina de cabra (4 y 25 µg/sitio), histamina y compuesto 48/80 (50, 100, 200, 400 µg/sitio); dos inyecciones de solución salina tamponada sirvieron como controles. Dos investigadores ciegos revisaron los comportamientos indicativos de prurito de todas las grabaciones de video.

Las puntuaciones globales de los habones fueron evaluados a 30 mpi por un investigador a ciegas.

Resultados – todos los perros mostraron habones y eritema en el sitio de inyección del pruritígeno; las puntuaciones globales de los habones a 30 mpi de cada sustancia aumentaron significativamente en todas las concentraciones en comparación con el control ($P \leq 0,05$). Una evaluación a ciegas reveló que todos los pruritógenos inducían conductas indicativas de picor agudas leves en el lugar de la inyección. No se observó dolor en el lugar de la inyección en ningún perro. En comparación con los controles, las inyecciones de pruritógenos no afectaron significativamente los segundos de prurito ni la aparición de episodios de prurito en ninguna de las sustancias.

Conclusiones y significación clínica – estos resultados preliminares sugieren que las inyecciones i.d. de los pruritógenos estudiados pueden inducir una respuesta cutánea de habones y eritema en perros sanos; pero se producen inconsistencias en la inducción del picor, incluso con las diferentes concentraciones de pruritógenos.

Zusammenfassung

Hintergrund – Kratzverhalten in Zusammenhang mit einer intradermalen (i.d.) Injektion von Pruritogenen wie Histaminen und Compound 48/80 in die Haut von Mäusen und Menschen ist ein häufig verwendetes Modell, um die Forschung über den Juckreiz und die Entwicklung von Medikamenten voranzubringen. Die prädiktive Validität dieses Modells ist bei Hunden noch wenig beschrieben.

Ziele – Eine Evaluierung der Dosis-abhängigen Wirkungsweisen von pruritogenen Substanzen, mit einer jeweils anderen Wirkungsweise, bei gesunden Hunden.

Tiere – Zehn gesunde Beagles aus dem Versuchslabor.

Methoden und Materialien – Alle Hunde wurden für 30 Minuten nach i.d. Injektion von Ziegen Anti-canine IgE (4 und 25 µg/Hautstelle), Histamin und Compound 48/80 (50, 100, 200, 400 µg/Hautstelle) über Video aufgenommen; zwei Injektionen mit gepufferter Kochsalzlösung dienten als Kontrollen. Zwei geblindete UntersucherInnen sahen das Juckreizverhalten auf allen Videoaufnahmen durch. Es wurde ein Global Wheal Score nach 30 mpi durch einen geblindeten Untersucher erfasst.

Ergebnisse – Alle Hunde zeigten eine Quaddelbildung und Erythem an der Injektionsstelle des Pruritogens; der Global Wheal Score einer jeden Substanz nahm 30 mpi in allen Konzentrationen im Vergleich zu den Kontrollen signifikant zu ($P \leq 0,05$). Eine geblindete Evaluierung zeigte, dass alle Pruritogene ein mildes akut juckendes Verhalten an der Injektionsstelle auslösten. Bei keinem der Hunde konnte an der Injektionsstelle ein Schmerz festgestellt werden. Im Vergleich zu den Kontrollen beeinflussten die Injektionen der pruritogenen Substanzen für keine derselben die Anzahl der Juckreizsekunden oder das Auftreten von Juckreizepisoden.

Schlussfolgerungen und klinische Bedeutung – Diese vorläufigen Ergebnisse zeigen, dass i.d. Injektionen der untersuchten Pruritogene kutane Quaddeln und Schübe bei gesunden Hunden auslösen können; allerdings treten Uneinheitlichkeiten bei der Auslösung des Juckreizes selbst mit den unterschiedlichen Konzentrationen der Pruritogene auf.

要約

背景 – マウスおよび人の皮膚へのヒスタミンおよびcompound 48/80などの掻痒剤の皮内注射(i.d.)に関連する引っ掻き行動は、掻痒の研究および薬物開発を進めるために一般的に使用されるモデルである。このモデルの予測的妥当性は、犬ではあまり文書化されていない。

目的 – 本研究の目的は、それぞれ異なる作用機序を有する掻痒物質の用量依存的効果を健常犬において評価することである。

被験動物 – 健常実験用ビーグル犬10頭。

材料および方法 – 全ての犬にヤギ抗イヌIgE (4 and 25 lg/部位)、ヒスタミンおよびcompound 48/80 (50, 100, 200, 400 lg/部位)の皮内注射を実施し、注射後30分間(mpi)ビデオ記録した。2つの緩衝食塩水注射を対照として用いた。2人の盲検化された研究者がすべてのビデオ録画の掻痒行動をレビューした。全体的な膨疹スコアを、盲検化された研究者が注射後30分後に評価した。

結果 – すべての犬は、掻痒物質の注射部位に膨疹と紅斑を示した。各物質の注射30分後の全体的膨疹スコアは、対照と比較して全濃度で有意に増加した($P \leq 0.05$)。盲検評価により、全ての掻痒物質が注射部位に軽度の急性掻痒行動を誘発したことが明らかになった。注射部位の疼痛はどの犬にも見られなかった。対照と比較して、掻痒物質の注射は、いずれの物質についても掻痒時間または掻痒行動の発生に有意な影響を及ぼさなかった。

結論と臨床的重要性 – これらの予備結果は、本試験における掻痒物質の皮内注射が、健常犬において皮膚の膨疹および発赤反応を誘発する可能性があることを示唆している。しかし、掻痒の誘発には、たとえ濃度が異なる掻痒剤を用いても矛盾が生じた。

摘要

背景 – 向小鼠或人皮肤皮内注射(i.d.)如组胺和化合物48/80这类致痒素,从而引起的抓挠行为,一般是开展瘙痒研究和药物开发的常用模型。这种模型在犬中的预测有效性报道很少。

目的 – 评估不同致痒素物质对健康犬的作用机制,及剂量依赖性效果。

动物 — 十只健康的比格实验犬。

方法和材料 — 所有犬在山羊抗犬IgE(4和25μg/位点)、组胺和复合物48/80(50、100、200、400μg/位点)注射30分钟后(mpi)进行视频记录;两次缓冲盐水注射作为对照。两名调查者盲法审查了所有视频录像的瘙痒行为,由盲法调查者在30mpi给总体风团评分。

结果 — 所有犬在致痒素注射部位均出现风团和红斑;与对照组相比,各物质在30mpi时的总体风团评分在所有浓度下均显著增加($P \leq 0.05$)。盲法评估显示,所有致痒素在注射部位均可诱导轻度急性瘙痒行为;所有犬注射部位均未见疼痛。与对照组相比,注射任何致痒素都无法显著制造瘙痒的短暂或持续发作。

结论和临床意义 — 这些初步研究结果表明,皮内注射致痒素可诱导出健康犬的皮肤风团及潮红反应;但即使致痒素浓度再高,都无法诱发瘙痒。

Resumo

Contexto — A manifestação de comportamentos de prurido após injeção intradérmica (i.d.) de pruritógenos como a histamina e o composto 48/80 na pele de camundongos e humanos é o modelo comumente utilizado no avanço de pesquisas de prurido e desenvolvimento de fármacos. O valor preditivo deste modelo é pouco documentado em cães.

Objetivos — Avaliar os efeitos dose-dependentes de substâncias pruritogênicas, cada uma com um mecanismo de ação, em cães saudáveis.

Animais — Dez beagles de laboratório saudáveis.

Métodos e materiais — Todos os cães foram gravados em vídeo por 30 minutos após injeção (mpi) i.d. de IgE caprino anti-canino (4 e 25 μg/local), histamina e composto 48/80 (50, 100, 200, 400 μg/local); duas injeções de salina tamponada serviram de controles. Dois investigadores cegos revisaram os comportamentos de prurido de todos os vídeos gravados. Escores globais de pápula foram avaliados em 30 mpi por um investigador cego.

Resultados — Todos os cães demonstraram formação de pápula e eritema no local de injeção do pruritógeno; os escores globais de pápula em 30 mpi de cada substância aumentou em todas as concentrações comparado ao controle ($P \leq 0.05$). A avaliação cega revelou que todos os pruritógenos induziram comportamentos de prurido agudos discretos no local da injeção. Não foi observada dor no local de injeção em nenhum cão. Comparado aos controles, as injeções de pruritógenos não afetaram significativamente os segundos pruriginosos ou a ocorrência de episódios de prurido para nenhuma das substâncias.

Conclusões e significância clínica — Estes resultados preliminares sugerem que injeções i.d. dos pruritógenos estudados podem induzir resposta de pápula e eritema em cães saudáveis; mas podem ocorrer inconsistências na indução do prurido, mesmo que em diferentes concentrações de pruritógenos.

Supporting Information

Figure S1. Ethogram itch behavior definitions specific to intradermal injection (IDT) site of pruritogen used to record site specific itch behaviors per minute during video analysis by independent trained viewers.

Localized Itch Behaviors (to IDT site)	Definition
Term	
Site Licking	Tongue contacts the skin and fur, and the head makes a motion with the neck muscles to move the tongue along the specific site of injection. Lasts greater than one second.
Site Chewing/Biting	Mouth contacts the specific site of injection and a chewing motion is visible, utilizing the masseter muscles and visibly moving the temporal muscles of the head. Sometimes this motion involves a rocking motion of the entire body. Lasts greater than one second.
Site Scratching	Toes and claws from the limb (usually the hind limb) are utilized by the limb muscles to drag the claws along the site of injection multiple times. Lasts greater than one second.
Site Rubbing	The site of injection on the body is moved along the ground, either in a back and forth motion or just one direction, for greater than 2 seconds. Other muscles of the body are utilized to achieve this motion (intention). Behavior is like scooting, except instead of the rectum contacting the ground it is another part of the body (in this case the injection site)
Site Rolling	Entire body in lateral recumbency and the ventral abdomen is twisted up towards the air. The roll can then twist the body entirely to the other side from which lateral recumbency began or may move back to the side it started. This behavior is often, but not necessarily, associated with other itch behaviors. For this behavior to qualify as a site roll, the injection site must contact the ground at some point during the displayed behavior.