

The anti-inflammatory effect of topical tofacitinib on immediate and late-phase cutaneous allergic reactions in dogs: a placebo-controlled pilot study

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Background – Topical Janus kinase (JAK) inhibition is a promising therapeutic target for several inflammatory skin diseases of humans.

Objectives – To evaluate the anti-inflammatory effect of tofacitinib, a JAK 1/3 inhibitor, on immediate and late-phase skin reactions in dogs.

Animals – Five healthy laboratory beagle dogs.

Methods – Topical tofacitinib (total daily dosage: 0.5 mg/cm²) or its gel vehicle were applied on either the left or right lateral thorax of each dog for eight days. Three days before application and after eight days of topical treatment, intradermal injections of histamine and anticanine-IgE antibodies were performed on both sides; they were evaluated by an investigator blinded to the interventions.

Results – The tofacitinib gel was well-tolerated; one dog developed mild erythema at Day 5 that resolved by the next application. Treatment with tofacitinib reduced histamine and anticanine-IgE global wheal scores (one-way ANOVA, $P \leq 0.005$ for both) compared to baseline; there was no significant difference for the vehicle placebo (histamine; $P = 0.163$; IgE, $P = 0.223$). Late-phase reactions (LPRs) were markedly, but not significantly reduced after tofacitinib treatment ($P = 0.071$). A blinded histological evaluation of 6 h-anti-IgE-associated LPRs revealed a significant reduction in the total leucocyte superficial dermal cellularity ($P = 0.022$), as well as eosinophil ($P = 0.022$) and mast cell ($P = 0.022$) counts at tofacitinib-treated sides compared with pretreatment values. Post-treatment complete blood counts and serum chemistry profiles did not show relevant tofacitinib-induced changes.

Conclusions – Our observations suggest that topical tofacitinib exerts an inhibitory effect on activated canine skin-emigrating immune cells; this drug should be investigated further as a topical immunosuppressive drug in dogs.

Introduction

Canine atopic dermatitis (cAD) is a highly pruritic and inflammatory skin disease; it is estimated that that atopic dermatitis (AD) affects 10% of the canine population with a majority of cases demonstrating IgE antibodies directed at environmental allergens.¹ Treatment of severe and chronic cAD cases can be challenging; topical agents, including emollients and glucocorticoids, are the mainstay of AD therapy.¹ Systemic immunosuppressants such as

glucocorticoids, ciclosporin and oclacitinib are used for long-term control of refractory cases with recurrent flares.¹

The discovery of the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signalling pathway opened a new window of opportunity for the treatment of inflammatory skin diseases in humans. The JAK-STAT pathway is an important signalling mechanism for many cytokines and growth factors; JAK 1 and JAK 3 are necessary for signalling for IL-4, IL-13 and IL-31, and are potential targets for the treatment of AD in humans and dogs.^{2,3} Topical tofacitinib citrate, a JAK1/JAK3 inhibitor, has been shown to remarkably reduce the severity of refractory AD in humans, with early onset of effect and good local tolerability.⁴

The primary goals of this study were to determine the immunomodulatory potential of the topical tofacitinib on cutaneous allergic reactions in dogs and to

Abbreviations: GWS, global wheal score; JAK, Janus kinase; LPR, late-phase reaction; PLO, pluronic lecithin organogels; STAT, signal transducer and activator of transcription.

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assess the safety and local tolerability of repeated topical applications.

Materials and Methods

Animals

Five healthy, adult, male neutered beagle dogs (aged 1–2 years) with no previous history of disease were housed in a laboratory setting under standard conditions (20–22°C, 12 h light/dark cycle, water *ad libitum*, food twice daily). The sample size was determined to be sufficient to provide an 80% power to detect a 50% difference between two groups of values (tofacinib versus vehicle) with a standard deviation of 30% at $P = 0.05$ (<http://powersamplesize.com/Calculators/>). All aspects of the study were conducted in accordance with the Institutional Animal Care and Use Committee.

Administration of topical tofacitinib/placebo gels

All dogs were involved in a randomized, blinded and placebo (vehicle)-controlled experiment; topical tofacitinib (total daily dosage: 0.5 mg/cm²) or its gel vehicle (pluronic F-127, lecithin and isopropyl palmitate) were applied on either the left or right lateral thorax of each dog for 8 days. Each dog was randomized using software (Graphpad Prism Inc. 7.0; San Diego, CA, USA) to receive the topical gel formulation within a clipped 4 × 4 cm treatment area on either the left or right thorax. The ointment was applied independently from another investigator who evaluated intradermal reactions, and the randomization sequence for each dog was kept blinded.

Intradermal testing and skin biopsy collection

Dogs were sedated intravenously using medetomidine (Domitor, Pfizer; Exton, PA, USA). Three days before topical application and after 8 days of treatment, intradermal injections of 0.05 mL of phosphate-buffered saline (negative control), histamine phosphate (0.1 mg/mL) and anticanine-IgE polyclonal antibodies (0.08 mg/mL) were administered on both sides of the thorax and evaluated by an investigator blinded to the active treatment site. As described previously,⁴ a global wheal score (GWS) and late-phase reactions (LPRs) were evaluated at 20 min and 6 h, respectively.

In order to evaluate the inhibitory effects of topical tofacitinib formulation on dermal inflammation, one 8 mm punch biopsy of anticanine IgE-injected skin was collected on each lateral thorax from tofacitinib- and vehicle-treated areas 6 h after intradermal injections on days 3 and 8 from all dogs. Five micrometre sections were cut from paraffin blocks and stained with haematoxylin and eosin for examination of inflammatory cells, Luna's stain for eosinophils, a low-pH toluidine blue stain for dermal mast cells and an immunohistochemical method with anti-CD3 rabbit antiserum to enumerate T cells.⁴ A blinded investigator evaluated all slides; the results were expressed as a number of positively staining cells in superficial dermis per 10 consecutive 40× high power fields (HPF), excluding endothelial cells and adnexa.

Local tolerability assessment, complete blood count and serum biochemistry

Local tolerability assessment was performed for each test area by the investigator before and 20 min after the test product application; the presence of erythema, papules and scaling was reported. The systemic safety of repeated topical application was evaluated by measuring complete blood count and serum biochemistry.

Statistical analysis

Results of the clinical evaluation (GWS), as well as cell counts for each time point, were compared with values obtained before treatment (baseline; Day –3) and after topical application of tofacitinib

(Day 8). A nonparametric repeated measures one-way ANOVA (Friedman test; GraphPad Prism 7.0) was performed with a level of significance set at $P < 0.05$.

Results

Intradermal injection of histamine and anti-canine IgE resulted in a positive wheal and erythema reactions on both sides of the thorax in all five dogs before treatment (baseline; Day –3). Treatment with tofacitinib reduced histamine and anti-canine IgE GWS (Figure 1, 2a, b; one-way ANOVA, $P \leq 0.005$ for both) compared to baseline; there was no significant difference for placebo (histamine; $P = 0.163$; IgE, $P = 0.223$). In a similar way, LPRs were markedly, but not significantly, reduced after tofacitinib treatment (Figure 2c; $P = 0.071$).

A blinded histological evaluation of 6 h anti-IgE associated LPRs revealed a significant reduction in total leucocyte superficial dermal cell infiltrate ($P = 0.022$) as well as eosinophil ($P = 0.022$) and mast cell ($P = 0.022$) counts at tofacitinib-treated sides compared with pretreatment values (Figure 3). A difference in dermal neutrophil and lymphocyte numbers was not identified ($P = 0.115$ and $P = 0.227$, respectively). There was no significant difference for any cell count for the vehicle-treated area (dermal cell infiltrate, $P = 0.413$; eosinophils, $P = 0.411$; mast cell, $P = 0.053$).

Results of a complete blood count and serum chemistry profile before and at the end of tofacitinib therapy were unremarkable. The tofacitinib gel was well-tolerated, with one dog developing mild erythema at Day 5 that resolved by the next application; the placebo gel induced mild erythematous papules in two dogs after Day 6 of application.

Discussion

This study demonstrated that topical delivery of tofacitinib, a JAK1 and JAK3 inhibitor,³ exerts inhibitory effects on immediate-histamine and anti-canine IgE-associated skin reactions at directly treated skin sites after 8 days of application. Immunoglobulin E-mediated reactions, induced by intradermal injection of anti-IgE antibodies, grossly and histologically resemble changes seen in naturally occurring allergic dermatitis in humans and dogs;^{4,5} this screening model is proposed for studying the anti-inflammatory effect of anti-allergic drugs before entering clinical trials.⁴

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 are predominant at 24 h.⁴ The engagement of IgE receptors by antigen or anti-IgE antibody triggers a cascade of biochemical signal transduction events, including activation of multiple protein tyrosine kinases, including JAK3 tyrosine kinase.⁶ Several studies demonstrate that JAK3 plays an important role in IgE receptor-mediated mast cell responses and that JAK3 inhibitor reduces degranulation and proinflammatory mast cell mediator release.⁶ In-depth investigations of IgE-mediated skin transcriptome in dogs are lacking. However, upregulated messenger RNA of JAK1 and JAK3 has been identified in pilot

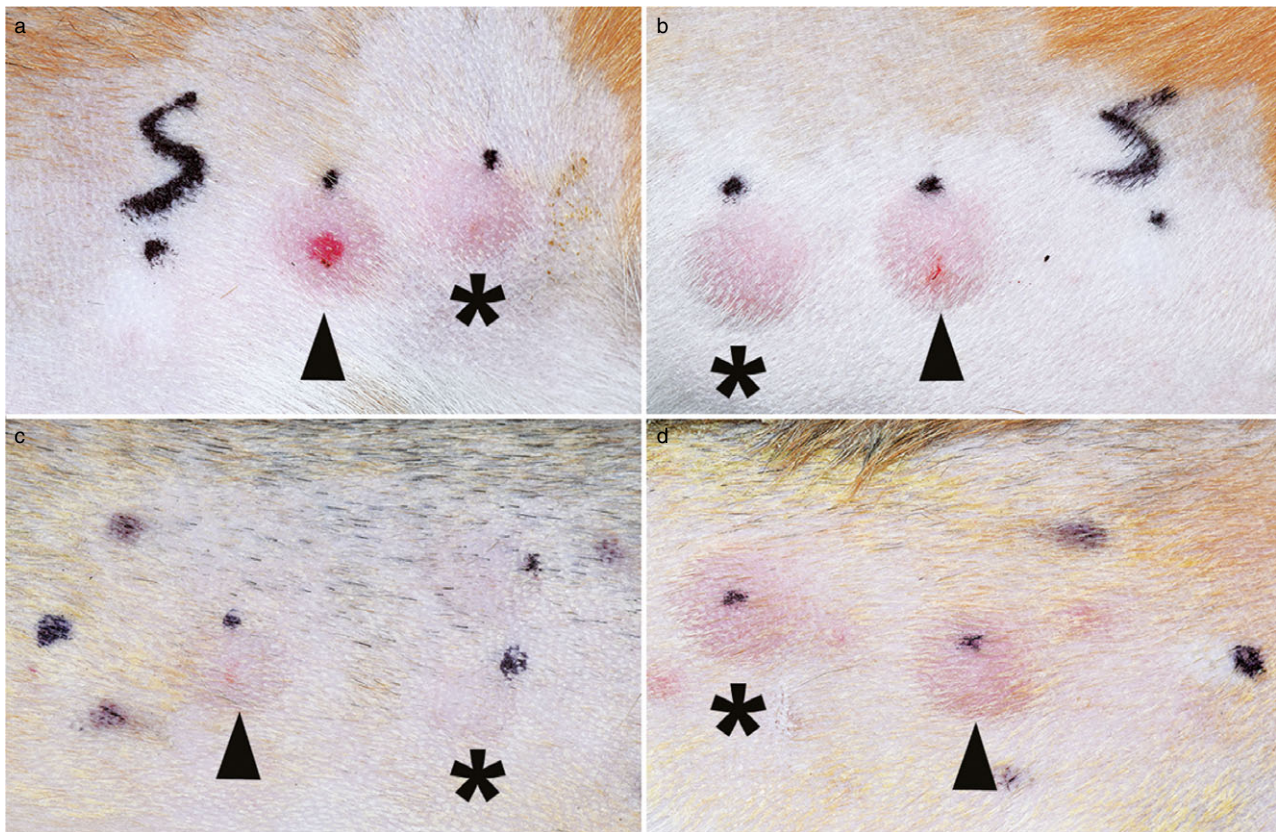


Figure 1. Effects of intradermal injection of histamine (arrowhead) and anti-canine IgE (asterisk) antibodies into healthy canine skin. Intradermal injection of histamine and anti-canine IgE antibodies in the healthy canine skin are followed by an immediate development (20 min) of a raised, oedematous, turgid and erythematous wheal reaction at baseline (a,b). The histamine and anti-IgE cutaneous wheal reactions are significantly reduced after seven days application of tofacitinib gel (c); vehicle-treated areas also reveal mildly reduced reactions (d).

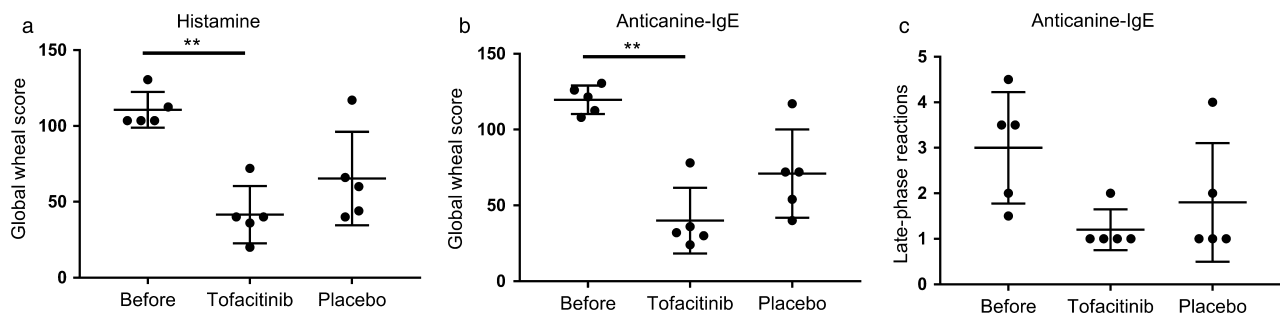


Figure 2. Global wheal scores (GWS) at 20 min after intradermal injections of histamine (a) and anti-canine IgE antibodies (b) in five healthy dogs before treatment (Day -3) and after 8 days of treatment with topical tofacitinib and placebo gel. The anti-canine IgE-mediated late-phase reactions, characterized by erythematous patches, were reduced at tofacitinib- and placebo-treated skin areas. However, there was no significant difference compared to baseline (c). $^{**}P \leq 0.01$.

studies of 6 h anti-IgE-associated canine skin biopsies conducted by the authors (unpublished data). The histological evaluation in this study indicate that topical tofacitinib can reduce superficial dermal cell infiltrate of total leucocytes, eosinophils and mast cells, which is consistent with prior studies that evaluated the anti-inflammatory effect of topical tofacitinib in an allergic mouse dermatitis model.⁷

In the present study, we safely utilized pluronic lecithin organogels (PLO) for topical delivery of tofacitinib. Repeated PLO application suggests that PLO may be beneficial as a delivery vehicle for the local action of diclofenac for osteoarthritis,⁸ and silymarin for AD in

humans.⁹ Studies involving PLO skin application in cats for systemic transdermal absorption have been unsuccessful.¹⁰ An unexpected finding in our study was reduced histamine and anti-canine IgE-induced GWS at the distant placebo-treated thoracic areas; cutaneous diffusion of the active drug from treated to untreated areas or transferring the drug to untreated areas by dog's licking the active product cannot be excluded. Interestingly, the anti-inflammatory evaluation of 0.0584% hydrocortisone aceponate (Cortavance, Virbac SA; Carros, France) spray on canine IgE-mediated reactions also revealed reduced inflammatory cell influx at placebo-treated sites.¹¹

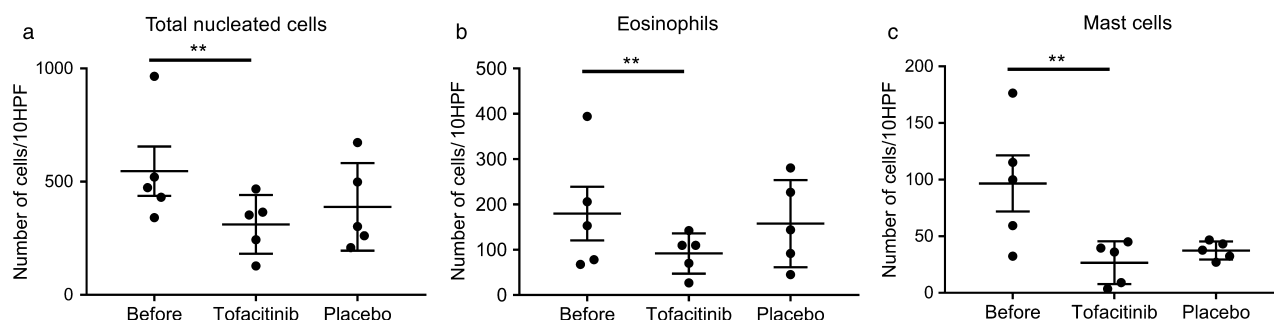


Figure 3. Influence of treatment with topical tofacitinib for 8 days on cell counts in canine skin.

Topical tofacitinib significantly reduced the total dermal cellular infiltrate (a), and eosinophil (b) and dermal mast cell (c) numbers in skin biopsies obtained from anti-canine IgE antiserum-induced late-phase reactions at 6 h. Data represent dermal cell counts/10 high power fields (HPF) from individual dogs. ** $P \leq 0.01$.

The expression of JAK-STAT component in diverse inflammatory skin disease revealed significant upregulation of JAK3 in epidermal and dermal cellular infiltrate followed by JAK1.¹² One of the advantages of topical JAK inhibitors is their structure; they are small molecules which can easily penetrate the epidermal barrier.⁴ Topical tofacitinib (JAK3, JAK1) and ruxolitinib (JAK1) show promising results in the treatment of psoriasis, vitiligo, alopecia areata and AD; the cutaneous atrophy observed during use of topical glucocorticoids was not a concern in these studies.¹² Although dermal thickness was not measured histologically in our study, there was no clinically visible atrophy at any of the topical tofacitinib-treated areas.

In conclusion, the experiments described here demonstrate that the topical tofacitinib exerts inhibitory and anti-inflammatory effects on IgE-mediated skin reactions. Future studies should assess the clinical efficacy and safety of JAK inhibitors through topical delivery in controlled clinical trials of dogs with AD.

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Résumé

Contexte – L'inhibition topique des JAK (Janus Kinase) est une cible thérapeutique prometteuse pour plusieurs maladies inflammatoires cutanées de l'homme.

Objectifs – Evaluer l'effet anti-inflammatoire du tofacitinib, un inhibiteur de JAK 1/3, sur les réactions cutanées immédiates et retardées chez le chien.

Sujets – Cinq chiens beagles de laboratoire en bonne santé.

Méthodes – Le tofacitinib topique (dosage total quotidien : 0.5mg/ cm²) ou son véhicule en gel ont été appliqués pour chaque chien soit sur le thorax latéral gauche soit sur le droit pendant 8 jours. Trois jours avant et 8 jours après le traitement topique, des injections intradermiques d'histamine et d'anticorps IgE anti-canin ont été réalisés sur les deux cotés ; elles étaient évaluées par un investigateur en aveugle.

Résultats – Le gel de tofacitinib a été bien toléré ; un chien a développé un érythème modéré à jour 5 qui s'est résolu avant l'application suivante. Le traitement au tofacitinib a diminué les scores des papules d'histamine et d'IgE-anticanin totaux (one-way ANOVA, $P \leq 0.005$ pour les deux) comparé à la référence ; il n'y avait pas de différence significative pour le placebo (histamine; $P = 0.163$; IgE, $P = 0.223$). Les réactions retardées (LPRs) étaient réduites de façon importante mais non significative après le traitement au

tofacitinib ($P = 0.071$). Une évaluation histopathologique en aveugle des LPRS a révélé une diminution significative de la cellularité leucocytaire totale du derme superficiel ($P = 0.022$), ainsi que les éosinophiles ($P = 0.022$) et les mastocytes ($P = 0.022$) sur les cotés traités au tofacitinib comparés aux valeurs prétraitement. Le bilan sanguin et biochimique post-traitement n'a pas montré de changements induits par le tofacitinib.

Conclusions – Nos observations suggèrent que le tofacitinib topique exerce un effet inhibiteur sur les cellules immunitaires activées, migrées dans la peau du chien : d'autres études sont nécessaires sur ses effets immunosuppresseurs en tant que topique chez le chien.

Resumen

Introducción – la inhibición tópica de Janus kinasa (JAK) es un objetivo terapéutico prometedor para varias enfermedades inflamatorias de la piel en humanos.

Objetivos – Evaluar el efecto antiinflamatorio de tofacitinib, un inhibidor de JAK 1/3, en reacciones cutáneas inmediatas y tardías en perros.

Animales – Cinco perros Beagle de laboratorio sanos.

Métodos – Se aplicó tofacitinib tópico (dosis diaria total: 0,5 mg /cm²) o su vehículo de gel en el tórax lateral izquierdo o derecho de cada perro durante 8 días. Tres días antes de la aplicación y después de 8 días de tratamiento tópico, se realizaron inyecciones intradérmicas de histamina y de anticuerpos frente a IgE canina en ambos lados; fueron evaluados por un investigador que desconocía las intervenciones.

Resultados – El gel de tofacitinib fue bien tolerado; un perro desarrolló eritema leve en el día 5 que se resolvió con la siguiente aplicación. El tratamiento con tofacitinib redujo las puntuaciones globales de vesículas inducidas por histamina y antiIgE canina (ANOVA de una vía, $P \leq 0,005$ para ambos) en comparación con el valor inicial; no hubo diferencias significativas para el vehículo placebo (histamina, $P = 0,163$, IgE, $P = 0,223$). Las reacciones de fase tardía (LPR) se redujeron marcadamente, pero no significativamente, después del tratamiento con tofacitinib ($P = 0,071$). Una evaluación histológica ciega de LPR a las 6 h del tratamiento anti-IgE reveló una reducción significativa en la celularidad dérmica superficial leucocítica total ($P = 0,022$), así como en los recuentos de eosinófilos ($P = 0,022$) y mastocitos ($P = 0,022$) en lados tratados con tofacitinib en comparación con los valores de pretratamiento. Los recuentos sanguíneos completos posteriores al tratamiento y los perfiles de química sérica no mostraron cambios relevantes inducidos por tofacitinib.

Conclusiones – nuestras observaciones sugieren que el tofacitinib tópico ejerce un efecto inhibidor sobre las células inmunitarias caninas activadas que emigran a la piel; este medicamento debe investigarse más a fondo como un fármaco inmunosupresor tópico en perros.

Zusammenfassung

Hintergrund – Die Inhibition der topischen Janus Kinase (JAK) ist ein vielversprechendes therapeutisches Ziel bei einigen entzündlichen Hauterkrankungen des Menschen.

Ziele – Die Evaluierung der entzündungshemmenden Wirkung von Tofacitinib, einem JAK 1/3 Inhibitor, auf die Sofortreaktion und auf die Spätphasenreaktion bei Hunden.

Tiere – Fünf gesunde Labradorbeagle.

Methoden – Tofacitinib (Gesamttagesdosis: 0,5 mg/cm²) oder das Trägergel wurden 8 Tage lang topisch entweder am linken oder am rechten lateralen Thorax eines jeden Hundes aufgetragen. Drei Tage vor der Applikation sowie 8 Tage nach der topischen Behandlung wurden intradermale Injektionen von Histamin und anticaninen-IgE Antikörpern an beiden Seiten durchgeführt; diese wurden von einem Untersucher beurteilt, der gegenüber diesen Interventionen verblindet war.

Ergebnisse – Das Tofacitinibgel wurde gut toleriert; ein Hund entwickelte am Tag 5 ein mildes Erythem, das bis zur nächsten Applikation wieder verschwunden war. Die Behandlung mit Tofacitin reduzierte das Histamin und insgesamt die Quaddeldurchmesser des anticaninen-IgE (Einfache ANOVA, $P \leq 0,005$ für beide) im Vergleich zum Ausgangswert; es bestand kein signifikanter Unterschied zum Trägermedium-Placebo (Histamin; $P = 0,163$; IgE, $P = 0,223$). Spätphasereaktionen (LPRs) waren nach der Tofacitinbehandlung deutlich, jedoch nicht signifikant reduziert ($P = 0,071$). Eine verblindete histologische Evaluierung von 6h-anti-IgE-verknüpfter LPRs zeigte eine signifikante Reduzierung der totalen Leukozytenzahl der oberflächlichen Dermis ($P = 0,022$), sowie der Zahl der Eosinophilen ($P = 0,022$) und der Mastzellen ($P = 0,022$) an den mit Tofacitinib behandelten Körperstellen im Vergleich zu den Werten vor der Behandlung. Das Blutbild und die Serumbiochemie nach der Behandlung zeigte keine relevanten durch Tofacitinib-induzierten Veränderungen.

Schlussfolgerungen – Unsere Beobachtungen zeigen, dass topisch verabreichtes Tofacitinib eine hemmende Wirkung auf aktivierte Immunzellen des Hundes, die aus der Haut auswandern, ausübt; dieser Wirkstoff sollte noch weiter als topisch anwendbarer immunsuppressiver Stoff bei Hunden untersucht werden.

要約

背景 – 外用薬によるヤヌスキナーゼ(JAK)阻害は、ヒトに発症する各種炎症性皮膚疾患に対する治療標的として期待されている。

目的 – 本研究の目的は、JAK1/3阻害剤であるトファシチニブの、犬の即時型および遅発型皮膚反応に対する抗炎症効果を評価することである。

被験動物 – 5頭の健康な実験ビーグル犬。

方法 – 外用トファシチニブ(総一日量:0.5mg/cm²)またはそのゲル基剤を、各犬の左右側胸部のいずれかに8日間塗布した。外用療法を開始する3日前および8日後に、ヒスタミンおよび抗犬IgE抗体を皮内投与した。結果の評価は、治療の詳細を知らされていない一人の研究者が実施した。

結果 – トファシチニブのゲルに対する耐容性は良好であった。1頭の犬が治療開始5日目に軽度の紅斑を発症したが、次の塗布後には改善した。トファシチニブを用いた治療により、ヒスタミンおよび抗犬IgE投与後の膨疹スコアが低下したが(一元配置ANOVA、 $P \leq 0.005$)、プラセボと比較して有意差は認められなかった(ヒスタミン; $P = 0.163$; IgE, $P = 0.223$)。トファシチニブ治療群では遅延型反応(LPR)の改善も認められたが、治療前後で有意差は認められなかった($P = 0.071$)。抗IgEに関連したLPR6時間目における組織学的評価を盲検により行ったところ、トファシチニブによる治療前と比較して、治療後には真皮浅層における全白血球数($P = 0.022$)、好酸球数($P = 0.022$)および肥満細胞数($P = 0.022$)の有意な減少が認められた。治療後の全白血球数および血清化学プロファイルについては、トファシチニブに起因する変化は認められなかった。

結論 – 本研究の成果から、トファシチニブの外用療法により活性化した犬皮膚遊走免疫細胞に対する阻害効果を有することが示唆された。本薬が犬に対する免疫抑制外用薬として適応できるかについては、さらなる研究が必要である。

摘要

背景 – 外用Janus激酶(JAK)抑制剤, 是人数种炎性皮肤病的靶向治疗药。

目的 – 评估托法替尼, 一种JAK1/3抑制剤, 对犬快速和迟发型皮肤反应的抗炎作用。

动物 – 5只健康实验室比格犬。

方法 – 外用托法替尼(每日总剂量:0.5 mg/cm²)或者其凝胶载体, 涂抹于每只实验犬左/右胸前皮肤, 连用8天。在使用托法替尼前3天和使用后8天, 胸腔两侧皮内注射组胺和抗犬IgE抗体; 研究人员对结果进行盲评估。

结果 – 托法替尼凝胶耐受性良好, 其中一只犬在第5天出现轻微红斑, 但在下次用药前消失。与基础值相比, 托法替尼治疗整体降低了组胺和抗犬IgE的风疹评分(ANOVA方差分析, $P \leq 0.005$); 与凝胶载体安慰剂组相比无显著差异(组胺; $P = 0.163$; IgE, $P = 0.223$)。迟发型反应明显, 但在使用托法替尼治疗后无显著降低($P = 0.071$)。组织学盲评估6小时迟发型反应的抗IgE显示: 与前期的药物治疗相比, 使用托法替尼治疗后总白细胞浅表真皮细胞数($P = 0.022$), 以及嗜酸性粒细胞($P = 0.022$)和肥大细胞($P = 0.022$)均有显著减少。治疗后进行全血细胞计数和血液生化分析, 未见托法替尼诱导的变化。

结论 – 观察显示, 外用托法替尼对激活的免疫细胞向犬皮肤移行有抑制作用; 此药作为犬外用免疫抑制剂, 还需进一步研究。

Resumo

A inibição tópica de Janus Kinase (JAK) é uma modalidade terapêutica promissora para diversas dermatopatias inflamatórias em humanos.

Objetivos – Avaliar o efeito anti-inflamatório de tofacitinib, um inibidor de JAK 1/3, em reações cutâneas imediatas e tardias em cães.

Animais – Cinco cães de laboratório, saudáveis, da raça beagle.

Métodos – Aplicou-se tofacitinib tópico (dose diária total: 0,5 mg/cm²) ou o seu veículo gel no tórax lateral direito ou esquerdo de cada cão, por oito dias. Três dias antes e oito dias após o início do tratamento tópico, injeções intradérmicas de histamina e anticorpos anti-IgE canina foram aplicadas em ambos os lados do tórax. As avaliações foram feitas às cegas por um pesquisador que desconhecia as intervenções.

Resultados – O gel de tofacitinib foi bem tolerado; um cão desenvolveu eritema discreto no Dia 5, que se resolveu após a aplicação seguinte. O tratamento com tofacitinib reduziu os escores globais de pápulas de histamina e anticorpo anti-IgE canina (ANOVA simples, $P \leq 0,005$ para ambos) comparado ao tempo zero. Não foram observadas diferenças significativas para o veículo placebo (histamina; $P = 0,163$; IgE, $P = 0,223$). A redução das lesões tardias após a aplicação de tofacitinib foi expressiva, mas não significativa estatisticamente ($P = 0.071$). A avaliação histopatológica às cegas das reações tardias associadas a 6 h-anti-IgE revelou redução significativa na celularidade leucocitária na derme superficial ($P = 0,022$), assim como nas contagens de eosinófilos ($P = 0,022$) e mastócitos ($P = 0,022$) nos lados tratados com tofacitinib, comparado aos valores pré-tratamento. Não foram observadas alterações relevantes no hemograma e nos perfis bioquímicos séricos após tratamento com tofacitinib.

Conclusões – Nossos resultados sugerem que o tofacitinib tópico exerce um efeito inibitório nas células imunes ativadas que migram para a pele. Mais estudos devem ser conduzidos para investigar a ação imunossupressora tópica deste fármaco em cães.