IL-31-induced pruritus in dogs: a novel experimental model to evaluate anti-pruritic effects of canine therapeutics

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Hypothesis/Objectives – The objectives were to characterize an IL-31-induced pruritus model by evaluating the efficacy of prednisolone, dexamethasone and oclacitinib, and to compare the speed of anti-pruritic effects of oclacitinib against those of prednisolone and dexamethasone.

Animals – Purpose-bred beagle dogs were used in all studies.

Methods – Randomized, blinded, placebo-controlled studies were designed to evaluate and compare the anti-pruritic properties of prednisolone, dexamethasone and oclacitinib following a single intravenous injection of recombinant canine IL-31. Video surveillance was used to monitor and score pruritic behaviours in study animals.

Results – Prednisolone [0.5 mg/kg, per os (p.o.)] reduced IL-31-induced pruritus when given 10 h prior to observation. When the time interval between drug treatment and observation was shortened to 1 h, dexamethasone (0.2 mg/kg, intramuscular) but not prednisolone (0.25 or 0.5 mg/kg, p.o.) reduced IL-31-induced pruritus. Oclacitinib (0.4 mg/kg, p.o.) reduced pruritus when given 1, 6, 11 and 16 h prior to the observation period, and the anti-pruritic activity of oclacitinib was greater when compared to prednisolone and dexamethasone at all time points assessed.

Conclusion and clinical importance – The efficacy of prednisolone, dexamethasone and oclacitinib in the IL-31-induced pruritus model gives confidence that this may be a relevant model for acute pruritus associated with allergic dermatitis including AD and can be used to evaluate novel compounds or formulations.

Introduction

Pruritus is a common complaint in dogs with allergic skin disease and represents a key clinical feature in the diagnostic tree for atopic dermatitis (AD). Long term pruritus can significantly affect the quality of life for affected dogs and their owners; therefore, treatments that can significantly and rapidly reduce pruritus are in great demand. To evaluate the efficacy of potential novel therapeutics, a variety of laboratory-based canine models of allergy have been developed such as dogs sensitized to allergens (e.g. house dust mites, fleas), or spontaneous canine models expressing the receptor to drive clinical signs associated with AD. In order to address some of these concerns, we were interested in developing a canine model of acute pruritus. Findings from murine, human and canine studies suggest that IL-31 cytokine can be produced from T cells in the skin after allergen exposure or exposure to bacterial antigens. This cytokine, in turn, may directly activate peripheral nerves expressing the IL-31 receptor to induce pruritic behaviours and activate additional cells expressing the receptor to drive clinical signs associated with AD. Based on these data, we developed a canine model of pruritus which employed canine IL-31 as the pruritogenic agent. The objectives of our studies were to (i) validate the model by evaluating drugs such as prednisolone, dexamethasone and oclacitinib, used in clinical practice and known to rapidly reduce pruritus in naturally occurring canine atopic dermatitis and (ii) to compare the speed of anti-pruritic effects of oclacitinib to those of prednisolone and dexamethasone.

Materials and methods

Animals and feeding procedures

Experiments were performed in purpose-bred beagle dogs (neutered males, spayed and intact females, ranging in age from 1 to 8 years old.) Mean weights for males in each study ranged between 12.0 and 22 kg.
19.9 kg, and mean weights for females in each study ranged between 7.4 and 14.9 kg. Mean body weights among the different treatment groups within a study did not vary beyond 20%. Beagle dogs were obtained from either Marshall BioResources, North Rose, NY, USA, or Ridgian Farms Inc, Mt. Horeb, WI, USA, and were maintained and used as part of an in-house colony whose pruritic behaviours to recombinant canine IL-31 (cIL-31) were extensively characterized. All animal procedures were performed following Institutional Animal Care and Use Committee guidance to assure compliance with the US Animal Welfare Act Regulations, Title 9, Code of Federal Regulations Parts 1, 2 and 3, and with the Guide for the Care and Use of Laboratory Animals, issued by the Institute for Laboratory Animal Research Committee of Life Sciences, National Academy Press (Washington DC, 1996). Water and a limited diet of Purina Lab diet #5007 were available.

Test drugs

Oral capsules containing active ingredients were made within five percent of the targeted dose. Oral doses of placebo hydroxypropyl methylcellulose (HPMC) capsules (Capsugel; Peapack, NJ, USA) were filled with microcrystalline cellulose (Acivell PH, FMC Corporation; Philadelphia, PA, USA). Prednisolone, (Prednis Tab® 5 mg tablets, Lloyd Inc.; Shenandoah, IA, USA) or oclacitinib (Zoetis; Kalamazoo, MI, USA) were delivered to the dogs via HPMC capsules back-filled with cellulose. Intramuscular (i.m.) injection of dexamethasone (DexaJext® Dexamethasone Solution 2 mg/mL, Butler Schein Animal Health; Dublin, OH, USA) or placebo injections containing 500 mg/mL polyethylene glycol 400, 9 mg/mL benzyl alcohol, 1.8 mg/mL methyyparaben and 0.2 mg/mL propylparaben, adjusted to pH 4.9 were given.

Video surveillance and pruritus monitoring

On each scheduled day of pruritus measurements, dogs were transferred to video rooms and placed in free-standing, single housed pens (approximately 90 cm x 180 cm), each equipped with ceiling-mounted cameras (Multicam Digital Surveillance System, RMISS Inc.; Wilmington, DE, USA) that digitally recorded the animals for real-time observation and/or viewing of recordings via computer links. Animals were acclimated ≥1 h prior to initiation of any video observation period for pruritus assessment. For each observation period, four dogs were evaluated for 2 h in real time using split-screen monitors by one observer. Video observers were scientists trained to observe and score pruritic behaviours in dogs. There was one observer for every four dogs, and each observer watched and scored their four dogs for the duration of the study. Observers were blinded to treatment. Categorical “yes/no” decisions were made at discrete 1 min intervals with regard to whether at least one pruritic behaviour was displayed by the study animals. Displays of pruritic behaviour such as licking/scratching of paws, flank and/or anal regions, scratching of flanks or neck, floor pawing, head-shaking and scooting of their bottom across the cage flooring were registered with a “yes” response. The cumulative number of “yes” determinations made within each observation period provided the pruritus score.

Induction of pruritus

Recombinant canine IL-31 was produced as described.20 To induce pruritus, a single intravenous (i.v.) injection of recombinant cIL-31 was given at doses ranging from 1.5 to 1.75 mg/kg approximately 20–40 min before the video observation period began. All IL-31 treatments were prepared in sterile phosphate buffered saline without calcium chloride and magnesium chloride under aseptic conditions within 30 min of scheduled dosing.

Statistical evaluation

Placement of animals to rooms and pens was done according to a statistically generated allotment plan using SAS software v9.2. (SAS; Cary, NC, USA). All hypothesis testing was done at the 10% significance level. Pruritus scores (for 1 min intervals over the 2 h observation period) were analysed using a general linear mixed model. The model included the fixed effect of treatment and random effects of batch, block within batch and error. Least-square means were used as estimates of the treatment means and standard errors; 90% confidence intervals were calculated. Treatment differences were assessed using Fisher’s protected least significant difference test.

Study design

Five randomized, blinded, placebo-controlled studies were designed. Treatment groups consisted of eight animals, and pruritic behaviours were observed and quantitated over a 2 h observation window in every study. IL-31 was given 20 min before each observation window to induce pruritus except when noted below.

Repeat dose study with oral prednisolone

Two different treatment groups were included. Dogs were administered either prednisolone (0.5 mg/kg, p.o.) or placebo, p.o., twice daily, every 12 h, for a total of 7 days. Pruritic behaviours were observed and quantified on study Day 0, 10–12 h after dogs were administered their first dose, and again on Day 6, 10–12 h after the last dose was administered (Figure 1a).

Duration of action study after single injection of dexamethasone

Three different treatment groups were included. Dogs were given either a single injection of placebo, i.m., 10 h prior to the observation period or a single injection of dexamethasone (0.2 mg/kg, i.m.) 1 or 10 h prior to the observation window for pruritus (Figure 2a).

Duration of action study after single dose of oclacitinib

Four different treatment groups were included. Dogs were given either a single dose of placebo, p.o., 6 h prior to the observation period provided the pruritus score.

Study design

Five randomized, blinded, placebo-controlled studies were designed. Treatment groups consisted of eight animals, and pruritic behaviours were observed and quantitated over a 2 h observation window in every study. IL-31 was given 20 min before each observation window to induce pruritus except when noted below.

Repeat dose study with oral prednisolone

Two different treatment groups were included. Dogs were administered either prednisolone (0.5 mg/kg, p.o.) or placebo, p.o., twice daily, every 12 h, for a total of 7 days. Pruritic behaviours were observed and quantified on study Day 0, 10–12 h after dogs were administered their first dose, and again on Day 6, 10–12 h after the last dose was administered (Figure 1a).

Duration of action study after single injection of dexamethasone

Three different treatment groups were included. Dogs were given either a single injection of placebo, i.m., 10 h prior to the observation period or a single injection of dexamethasone (0.2 mg/kg, i.m.) 1 or 10 h prior to the observation window for pruritus (Figure 2a).

Duration of action study after single dose of oclacitinib

Four different treatment groups were included. Dogs were given either a single dose of placebo, p.o., 6 h prior to the observation period provided the pruritus score.
period or a single dose of oclacitinib (0.4 mg/kg, p.o.) 6, 11 or 16 h prior to the observation window (Figure 3a).

**Speed of onset comparison study of oclacitinib and prednisolone**

Four treatment groups were included. Dogs were given either a single oral dose of placebo capsule, oclacitinib (0.4 mg/kg, p.o.) or prednisolone (at a dose of either 0.25 mg/kg, p.o., or 0.5 mg/kg, p.o.) 1 h prior to the observation period; IL-31 was given 40 min prior to the observation period to induce pruritus (Figure 4a).

**Results**

**Repeat dose study with oral prednisolone**

Prednisolone reduced pruritic behaviours compared to placebo after a single dose and after repeat dosing (Figure 1). On Day 0, the least-square mean (LSM) pruritus score ± SE for the dogs treated with prednisolone was 43 ± 7 versus 59 ± 7 for placebo (P = 0.0656). Following 7 days of twice daily dosing of prednisolone, the LSM pruritus score continued to be reduced compared to the placebo (49 ± 7 versus 85 ± 7 for placebo; P = 0.0003).

**Duration of action study after single injection of dexamethasone**

Dexamethasone reduced pruritic behaviours in beagle dogs after a single injection when given 10 h prior to the assessment window (P = 0.0150). However, dexamethasone did not reduce pruritic behaviours when injected 1 h prior to the assessment window (P > 0.1) (Figure 2). LSM pruritus scores ± SE for the treatment groups were 54 ± 21 (placebo), 52 ± 21 (dexamethasone given 1 h prior) and 30 ± 22 (dexamethasone given 10 h prior).
Oclacitinib reduced pruritus compared to placebo during the 1–3 h post-dosing window (P = 0.0202), whereas oral prednisolone evaluated at either dose did not (Figure 4). Additionally, the reduction in pruritus was greater in the oclacitinib-treated animals than in those treated with 0.25 mg/kg prednisolone (P = 0.0101) or 0.5 mg/kg prednisolone (P = 0.0240; Figure 4). LSM pruritus scores ± SE for the different treatment groups were 57 ± 12 (placebo), 16 ± 13 (oclacitinib 0.4 mg/kg, p.o.), 58 ± 11 (prednisolone 0.25, mg/kg p.o.) and 48 ± 8 (prednisolone 0.5 mg/kg, p.o.).

**Speed of onset comparison study of oclacitinib and dexamethasone**

Oclacitinib reduced pruritus compared to the placebo group (P < 0.0001). Dexamethasone also reduced pruritus compared to placebo (P = 0.0650). However, the reduction in pruritus was greater with oclacitinib when compared to dexamethasone (P < 0.0001). LSM pruritus scores ± SE for the different treatment groups were 75 ± 7 (placebo), 10 ± 2 (oclacitinib) and 55 ± 8 (dexamethasone), and illustrated in Figure 5.

**Discussion**

An IL-31-induced canine model of pruritus was developed to (i) recapitulate key pathways involved in pruritus due to allergy, (ii) assess acute anti-pruritic responses of novel therapeutics and (iii) benchmark novel agents or formulations against current therapies used by veterinarians in clinical practice. Canine IL-31 was chosen as the pruritogenic agent because of its demonstrated ability to induce pruritus in dogs and due to its presence in dogs with allergic skin conditions including AD. Administration of cIL-31 routinely produced a robust but acute pruritic response in normal beagle dogs 20–40 min after infusion, allowing for pruritus to be assessed over an observation window as short as 2 h. Dogs usually returned to baseline levels by 24 h (data not shown), allowing for dogs to be re-used in subsequent studies. Effects of repeat exposure to cIL-31 were not studied extensively, but an increase in pruritus scores were seen after the second exposure to cIL-31 in the Repeat dose study with oral prednisolone (Figure 1), in which cIL-31 was given to dogs twice within 1 week. It is unclear whether this increase represented a real biological change or whether it was variation in the model; however, cIL-31 evaluations in mice have demonstrated that IL-31 can induce the expression of IL-31 receptor A and oncostatin M receptor beta in dorsal root ganglia after repeated administration and increase long-lasting scratching. The ability to rapidly and reproducibly induce pruritus in normal animals after a single cIL-31 injection allows for any laboratory beagle dog to potentially be used in studies. The downside to an acute pruritus model is that other endpoints such as skin lesions, erythema, or biomarker analyses such as leukocyte, cytokine or mRNA changes do not make sense to monitor, as skin lesions do not develop. Therefore, this model may be best used as an initial assessment of agents for acute pruritus before evaluation in more complex models where changes associated with allergen sensitization occur naturally, and chronic changes such as immune dysregulation and skin barrier changes can be evaluated clinically or at the cellular and molecular level.

Standard therapies used to control pruritus in allergic skin diseases were effective in reducing pruritus in this model, building confidence that IL-31-induced pruritus may be a relevant model for pruritic allergic skin diseases. Specifically, oral prednisolone, injectable dexamethasone and oral oclacitinib were capable of reducing IL-31-induced pruritus. Oclacitinib consistently demonstrated rapid anti-pruritic effects 1–3 h post-dosing in all three studies performed. Oral prednisolone reduced pruritus 10–12 h post-dosing, and injectable dexamethasone reduced pruritus as quickly as 1–3 h post-injection in one study, but responses were variable possibly due to variability in drug bioavailability or pruritic responses in the animal model. A third possibility could be that the null hypothesis may have been incorrectly rejected in one of the studies due to the use of less stringent statistical criteria. Hypothesis testing was done at the 10% significance level due to the acceptance of a higher risk of type I errors for nonclinical studies.
By incorporating objective and quantitative scoring of pruritus, differentiation among drugs could be seen in this model. For example, a single oral dose of oclacitinib demonstrated a faster onset of action than oral prednisolone and produced a greater suppression of pruritus compared to prednisolone or injectable dexamethasone. These findings could be due to differences in pharmacokinetic properties of the drugs, as oclacitinib is shown to have rapid absorption as demonstrated by a $t_{\text{max}}$ of 0.9–1.2 h.$^{27}$ Although the $t_{\text{max}}$ has not been reported for prednisolone, drops in eosinophil cell counts in dogs can be detected around 4–6 h after dosing (PrednisTab™ Freedom of Information Summary, Nov 8, 1991). Alternatively, differential responses in the model could reflect differences in how the drugs work, mechanistically. Oclacitinib inhibits the function of the IL-31 cytokine by inhibiting Janus kinase activity directly downstream of the IL-31 receptor,$^{28}$ whereas glucocorticoids bind an intracellular glucocorticoid receptor in target tissues that then translocates to the nucleus, where the hormone-receptor complex binds specific DNA sequences to alter gene transcription.$^{29}$ Many of these corticosteroid-responsive genes are involved in decreasing inflammatory mediators. The need to induce gene transcription changes before inhibiting IL-31 function could be contributing to the differences in speed of onset or magnitude of response. Additionally, this study only evaluated two different types of glucocorticoids at commonly used dose levels and formulations; however, there are numerous alternative formulations and dose regimens that can be used based on the needs of the dog that may show a different speed of onset of anti-pruritic activity.$^{30–32}$ Nevertheless, this model has the potential to detect differential responses between different formulations, doses, regimens or therapies with different mechanisms of action.

In summary, the IL-31 pruritus model was able to detect the efficacy of standard anti-pruritus therapies such as glucocorticoids and oclacitinib and to quantitate differences in efficacy responses between them. These findings indicate that the IL-31-induced itch model in dogs represents a potential in vivo assessment that could be used to evaluate novel anti-pruritus compounds or formulations for dogs and to benchmark them against standard therapies used in clinical practice.

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Zusammenfassung

Hintergrund – Juckreiz ist ein charakteristisches Symptom allergischer Erkrankungen, wie der atopischen Dermatitis (AD) des Hundes. IL-31 ist ein Zytokin, welches im Serum von einigen Hunden mit AD gefunden wurde und Kratzverhalten bei Laborbeagles auslösen konnte.

Hypothese/Ziele – Die Ziele dieser Studie waren die Darstellung eines durch IL-31 induzierten Juckreizmodells zur Evaluierung von Prednisolon, Dexamethason und Oclacitinib, und ein Vergleich der Geschwindigkeit der juckreizstillenden Wirkung von Oclacitinib im Vergleich zu Prednisolon und Dexamethason.

Tiere – Für diese Studie wurden für diesen Zweck gezüchtete Beagles verwendet.

Methoden – Die randomisierten, geblindeten und Plazebo-kontrollierten Studien wurden entworfen, um die juckreizstillende Wirkung von Prednisolon, Dexamethason und Oclacitinib nach einer einzigen intravenösen Injektion von rekombinantem caninen IL-31 zu evaluieren und zu vergleichen. Videountersuchung wurde verwendet, um das Juckreizverhalten bei den Versuchstieren zu beobachten und zu bewerten.

Ergebnisse – Prednisolon [0,5 mg/kg, per os (p.o.)] reduzierte die IL-31 induzierten Juckreiz, wenn es 10 h vor der Beobachtung gegeben wurde. Wenn die Zeitspanne zwischen IL-31 und Juckreiz gemessen werden, reduzierte Dexamethason [0,2 mg/kg, intramuskulär], aber nicht Prednisolon [0,25 oder 0,5 mg/kg, p.o] den IL-31 induzierten Juckreiz. Oclacitinib [0,4 mg/kg, p.o.] reduzierte den Juckreiz bei Gabe von 1, 6, 11 und 16 h vor der Beobachtungsperiode und die juckreizstillende Wirkung von Oclacitinib war zu allen verglichenen Zeiten größer als jene von Prednisolon und Dexamethason.

Schlussfolgerungen und klinische Bedeutung – Die Wirkung von Prednisolon, Dexamethason und Oclacitinib im IL-31 induzierten Juckreizmodell bestätigt, dass es sich hier um ein relevantes Modell für akuten Juckreiz, welcher mit allergischer Dermatitis inklusive AD einhergeht, handelt und dass es verwendet werden kann, um neue Wirkstoffe oder Formulierungen zu evaluieren.