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A Retrospective Study on the Safety and Efficacy of Leflunomide in Dogs

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Background: Little clinical information is available concerning the use of leflunomide in dogs with immune-mediated diseases.

Objectives: To report the safety and efficacy of leflunomide for the treatment of naturally occurring immune-mediated diseases in dogs.

Animals: Ninety-two dogs treated with leflunomide for management of suspected immune-mediated diseases.

Methods: Retrospective medical record review from Jan 1995 to Dec 2014. Data that were extracted from the medical records included signalment, body weight, underlying indication for leflunomide, dosage of leflunomide, treatment duration, concurrent medications, treatment response, and adverse events.

Results: Adverse events that could be related to leflunomide administration included diarrhea (3 of 92, 3.3%), lethargy (2 of 92, 2.2%), unexplained hemorrhage (3 of 92, 3.3%), thrombocytopenia (2 of 31, 6.5%), and increased liver enzyme activities (1 of 16, 6.3%). Significant dose differences between dogs with adverse events (n = 11; median, 2.9 mg/kg/d; range, 1.8–3.6 mg/kg/d) and dogs without adverse events (n = 81; median, 1.6 mg/kg/d; range, 0.8–4.3 mg/kg/d) were found ($P < 0.001$). Treatment response could be evaluated in 17 dogs. Of these 17 dogs, 12 dogs (70.5%) had an apparent positive response to the use of leflunomide. There was no significant difference ($P = 0.22$) in dosages between dogs that responded to leflunomide (n = 12; median, 1.9 mg/kg/d; range, 1.0–3.5 mg/kg/d) and those that did not respond (n = 5; median, 1.7 mg/kg/d; range, 1.0–2.0 mg/kg/d).

Conclusions and Clinical Importance: Results suggest that the starting dosage of leflunomide should be 2 mg/kg/d rather than the currently suggested dosage of 3–4 mg/kg/d.

Key words: Adverse effects; Canine; Immunosuppressant; Toxicity.

Leflunomide is an immunomodulatory agent that inhibits pyrimidine synthesis and leads to decreases in DNA and RNA synthesis and lymphocyte proliferation.[1] It was introduced for treatment of rheumatoid arthritis in humans and also has been used to treat other immune-mediated diseases.[2–4] The most common adverse events associated with leflunomide use in humans are nausea, diarrhea, headaches, skin rashes, and alopecia.[5,6] In addition, increased liver enzyme activities have been reported in 2–13% of patients treated with leflunomide.[6] More severe adverse effects reported in humans include epidermal necrosis, myelosuppression, and interstitial lung disease, although they are not common.[5,6] Discontinuation of leflunomide because of adverse events has been reported in 16–70% of patients.[2,5,7]

Leflunomide has been used in canine renal transplant models, and addition of leflunomide to immunosuppressive regimens to prevent allograft rejection resulted in improved allograft survival times when compared to monotherapy.[8–10] There are only 3 published studies reporting leflunomide use in dogs with naturally occurring immune-mediated or inflammatory diseases.[11–13] In these studies, the median starting dosage of leflunomide was 3–4 mg/kg/d,[11–13] because severe adverse effects including anemia and anorexia have been observed at dosages >4 mg/kg/d in an experimental study of dogs.[14] At a dosage of 3–4 mg/kg/d, leflunomide was effective at controlling several immune-mediated or inflammatory diseases with positive response rates reported of 80–93%.[11–13] The incidence rates of adverse events varied from 7 to 73%. Hematologic disorders or hepatotoxicity associated with administration of leflunomide at

Abbreviations:

ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
BSA	body surface area
CH	cutaneous histiocytosis
CSU-VTH	Colorado State University Veterinary Teaching Hospital
IBD	inflammatory bowel disease
IMHA	immune-mediated hemolytic anemia
IMNP	immune-mediated neutropenia
IMPA	immune-mediated polyarthritis
IMTP	immune-mediated thrombocytopenia
PT	prothrombin time

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the current suggested dosage of 3–4 mg/kg/d was not described, but limited follow-up data were available for dogs in these studies.[11–13] The primary purpose of this retrospective study was to evaluate the safety and efficacy of leflunomide in a larger number of dogs with preexisting immune-mediated or inflammatory diseases.

Materials and Methods

Case selection and Medical Records Review

Medical records of dogs that were prescribed leflunomide at the Colorado State University Veterinary Teaching Hospital (CSU-VTH) were identified by computerized medical record review for the 20-year time period between January 1995 and December 2014. Dogs were excluded from the study if they had inadequate follow-up to determine the efficacy of or adverse events associated with leflunomide. Data extracted from the medical records included signalment, body weight, body surface area (BSA), underlying indication for leflunomide, starting and tapering dosage of leflunomide, treatment duration, concurrent medications, treatment response, and adverse events.

Leflunomide Adverse Events

Clinical adverse events possibly attributable to leflunomide, including lethargy, anorexia, vomiting, diarrhea, coughing, bleeding, and other events, were recorded. Hematologic disorders associated with leflunomide were evaluated in dogs with nonhematologic diseases if CBC data both before and after starting leflunomide were available. Anemia, neutropenia, and thrombocytopenia were defined as PCV <36%, neutrophil count <3000/μL, and platelet count <200,000/μL, respectively. Hepatotoxicity associated with leflunomide was evaluated in dogs with alanine aminotransferase (ALT) activity, alkaline phosphatase (ALP) activity or both before and after starting leflunomide. To be included in the hepatotoxicity assessment group, dogs must have not been either receiving glucocorticoids or on a stable or decreasing dosage of glucocorticoids when leflunomide was added to the treatment. Hepatotoxicosis was defined as a new increase in ALT or ALP activity higher than the upper limit of the reference range or a 2-fold increase compared to the baseline if baseline ALT or ALP activity was above the reference range.

Response to Leflunomide Treatment

Dogs that received only leflunomide or had no change in prescribed immunosuppressive drugs when leflunomide was added to the treatment were evaluated for the efficacy of leflunomide. Dogs with immune-mediated polyarthritis (IMPA) were assessed for response to treatment on the basis of clinical signs of IMPA and physical examination findings[12] and were categorized as having complete response, partial response, or no response as previously defined.[12] Dogs with immune-mediated thrombocytopenia (IMTP) were assessed for response to treatment based on platelet counts. Dogs were classified as having complete response if the platelet count normalized (platelets ≥200,000/μL) after starting leflunomide. Dogs were classified as having a partial response if the platelet count increased by >50,000/μL compared to the baseline before starting leflunomide but was still lower than the reference range (platelets <200,000/μL). Dogs were classified as having no response if the platelet count increased by <50,000/μL compared to the baseline before starting leflunomide. A dog with cutaneous histiocytosis (CH) was assessed for treatment response based on the gross appearance of the skin lesions as assessed by the primary clinician.

Statistical Analyses

A Mann-Whitney *U*-test and Fisher's exact test were used for continuous variables (age, dosage, and body weight) and categorical variables (breed and sex), respectively. All analyses were performed using a commercial software package.[a]

Results

Animals

One hundred and thirty-seven medical records of dogs prescribed leflunomide at CSU-VTH from 1995 to 2014 were screened for the study. Of these, 45 dogs were excluded because of no follow-up (n = 39) or euthanasia or death immediately after the diagnosis (n = 6). Therefore, 92 dogs were identified for further analyses. Of these 92 dogs, 37 were neutered males, 50 were neutered females, 1 was a sexually intact male, and 4 were sexually intact females. Thirty-two breed classifications were included in this study: mixed breed (n = 24), Labrador Retriever (n = 5), Shih Tzu (n = 4), German Shepherd (n = 4), Miniature Dachshund (n = 4), Beagle (n = 3), Boxer (n = 3), and <3 dogs of 26 other breeds (n = 45). The median age, body weight, and body surface area (BSA) of the dogs at the time of starting leflunomide were 7 years (range 1–14 years), 23.7 kg (range 4.8–71.5 kg), and 0.85 m^2 (range 0.29–1.76 m^2), respectively. Underlying diseases that prompted leflunomide use were IMPA (n = 42), IMTP (n = 17), immune-mediated hemolytic anemia (IMHA; n = 17), inflammatory bowel disease (IBD; n = 6), IMHA and IMTP (n = 4), pancytopenia (n = 2), vasculitis (n = 1), immune-mediated neutropenia (IMNP; n = 1), uveitis (n = 1), and CH (n = 1; Table 1).

Leflunomide Use

The median starting dosage of leflunomide was 1.7 mg/kg/d (range 0.8–4.3 mg/kg/d). The median duration of treatment with leflunomide was 23.5 weeks (range 1–208 weeks; Table 1). Leflunomide was used as first-line adjunctive therapy in 44 dogs (47.8%), second-line adjunctive therapy in 37 dogs (40.2%), first-line single therapy in 7 dogs (7.6%), and second-line single therapy in 4 dogs (4.4%). The immunosuppressants being concurrently administered when leflunomide was added to the treatment protocol were prednisolone alone (n = 71), prednisolone and mycophenolate (n = 5), prednisolone and cyclosporine (n = 2), cyclosporine alone (n = 2), and prednisolone and azathioprine (n = 1). Leflunomide was discontinued during the observation period in 47 dogs (50.5%) because of remission of the underlying disease (n = 24, 51.1%), euthanasia or death because of the severity of the underlying diseases (n = 12, 25.5%), adverse effects of leflunomide (n = 6, 12.8%), and perceived lack of response to leflunomide (n = 5, 10.6%).

Adverse Events

Clinical adverse events possibly attributable to leflunomide were observed in 8 of 92 dogs (8.7%).

Table 1. Demographic data for 92 dogs with leflunomide. Data are reported as medians with ranges.

Leflunomide dosage	1.7 mg/kg/d (0.8–4.3)
Leflunomide duration	23.5 weeks (1–208)
Age	7 years (1–14)
Body weight	23.7 kg (4.8–71.5)
Body surface are	0.85 m^2 (0.29–1.76)
Sex	
Male neutered	n = 37
Male intact	n = 1
Female spayed	n = 50
Female intact	n = 4
Breed	
Mixed breed	n = 24
Labrador Retriever	n = 5
Shih Tzu	n = 4
German Shepherd	n = 4
Miniature Dachshund	n = 4
Beagle	n = 3
Boxer	n = 3
Other purebred	n = 45
Underlying diseases	
IMPA	n = 42
IMTP	n = 17
IMHA	n = 17
IBD	n = 6
IMHA/IMTP	n = 4
Pancytopenia	n = 2
Vasculitis	n = 1
IMNP	n = 1
Uveitis	n = 1
CH	n = 1

Acute diarrhea starting within a week of beginning leflunomide was reported in 3 dogs (3.3%, 3 dogs with IMHA). All 3 dogs also were being treated with prednisolone and low-dose aspirin or clopidogrel. Leflunomide was discontinued in 1 dog, and the dose was reduced by 30 and 50% in 2 dogs. The diarrhea resolved within 3–7 days after the discontinuation or dose reduction of leflunomide. Lethargy was reported in 2 dogs (2.1%, 1 dog with IBD and 1 dog with pancytopenia) within a week of starting leflunomide. Both dogs were concurrently receiving prednisolone and the dog with pancytopenia also was receiving cyclosporine at the time lethargy was noted. Leflunomide was discontinued in the 2 dogs, and lethargy resolved within 2–3 days. Unexplained hemorrhage was reported in 3 dogs (3.3%, 1 dog with CH, 1 dog with IMPA, and 1 dog with IMHA) within 6–20 weeks after starting leflunomide. The dog with CH acutely developed severe hematochezia necessitating hospitalization 20 weeks after starting leflunomide. The dog was not receiving any other medications concurrently before the hematochezia developed. On presentation, the packed cell volume (PCV) was 17% with increased reticulocytes (87,000/μL) and decreased serum albumin (2.2 g/dL) and globulin (1.4 g/dL) concentrations. A CBC, serum biochemistry, prothrombin time (PT), activated partial thromboplastin time (APTT), basal cortisol concentration, thoracic radiographs, and abdominal ultrasound examination did not identify a cause for the severe hematochezia. The dog was euthanized as a result of owner financial constraints and the severity of clinical signs. Necropsy and histopathology identified suppurative inflammation in the small intestine, but no obvious etiology for hematochezia was found. The dog with IMPA developed hematochezia and ecchymosis 6 weeks after starting prednisolone and leflunomide. Diagnostic testing included CBC, serum biochemistry and PT/APTT, which were within normal limits. Clinical signs resolved within 3 days after discontinuing leflunomide. The dog with IMHA developed hematochezia and hematuria 13 weeks after starting leflunomide. The dog also was receiving prednisolone, mycophenolate, and clopidogrel at the time hematochezia and hematuria developed. Diagnostic testing including CBC, serum biochemistry, urinalysis, PT/APTT, and abdominal ultrasound examination did not identify a cause of bleeding. Leflunomide and clopidogrel were discontinued, and the clinical signs resolved within 5 days of discontinuation. The clopidogrel was restarted 3 weeks after resolution of clinical signs, and the dog never again had the same adverse effects.

Thirty-one dogs (26 dogs with IMPA and 5 dogs with IBD) met inclusion criteria to evaluate for potential hematologic adverse effects of leflunomide. The median duration between baseline (pre-leflunomide) and the first CBC was 2 weeks (range, 1–24 weeks). No dogs developed anemia or neutropenia at the first CBC. Two of 31 dogs (6.5%) developed mild thrombocytopenia (156,000/μL and 182,000/μL) at reevaluations 2 weeks after starting leflunomide. Platelet counts were back to within the reference interval after a 50% reduction of leflunomide dose. A second CBC was evaluated in 22 dogs, and the median duration between baseline and the second CBC was 40 weeks (range, 10–208 weeks). No evidence of hematologic disorders was observed in any of the second CBCs.

Sixteen dogs (9 dogs with IMPA, 6 dogs with IMTP, and 1 dog with IMHA) met inclusion criteria to evaluate for potential hepatotoxicity of leflunomide. The median duration between baseline and the first liver enzyme evaluation was 2 weeks (range, 2–21 weeks). Of these 16 dogs, 1 dog developed increases in both ALT (164 IU/L; reference range, 10–90 IU/L) and ALP (1064 IU/L; reference range, 15–140 IU/L) activities at the reevaluation 2 weeks after starting leflunomide. The ALT activity was back to within the reference range and the ALP activity was decreased to 421 IU/L after a 50% reduction of leflunomide dose. The ALT and ALP activities were evaluated a second time in 11 dogs and 6 dogs, respectively. The median duration between baseline and the second liver enzyme evaluation was 20 weeks (range, 6–42 weeks). No results met the criteria described for evidence of hepatotoxicity in any of those dogs.

There were no significant differences in age, sex, breed, body weight, and BSA between dogs with adverse events of leflunomide (n = 11) and dogs without adverse events (n = 81; Table 2). Significant dose differences between dogs with adverse events (n = 11; median, 2.9 mg/kg/d; range, 1.8–3.6 mg/kg/d) and dogs without

Table 2. Differences between dogs with adverse events and dogs without adverse events associated with the use of leflunomide. Data are reported as medians with ranges.

	Adverse events (n = 11)	No adverse events (n = 81)	*P*
Age (years)	9 (3–14)	7 (1–14)	0.31
Body weight (kg)	27 (8–44.0)	23.4 (4.8–71.5)	0.92
BSA (m^2)	0.92 (0.32–1.27)	0.84 (0.29–1.76)	0.44
Sex	Male (n = 6)	Male (n = 32)	0.53
	Female (n = 5)	Female (n = 49)	
Breed	Mixed breed (n = 5)	Mixed breed (n = 19)	0.23
	Purebreds (n = 6)	Purebreds (n = 62)	
Leflunomide dosage (mg/kg/d)	2.9 (1.8–3.6)	1.6 (0.8–4.3)	<0.001

adverse events (n = 81; median, 1.6 mg/kg/d; range, 0.8–4.3 mg/kg/d) were found ($P < 0.001$; Table 2).

Response to Leflunomide Treatment

The treatment response could be evaluated in 9 dogs with IMPA, 7 dogs with IMTP, and 1 dog with CH. All of the 9 dogs with IMPA, 1 dog with IMTP, and 1 dog with CH received leflunomide without administration of concurrent immunosuppressants. Six of the dogs with IMTP received leflunomide with concurrent immunosuppressant drugs (prednisolone alone, n = 3; prednisolone and mycophenolate, n = 1; prednisolone and cyclosporine, n = 1; prednisolone and azathioprine, n = 1), the doses of which were unchanged for at least the last 3 weeks when leflunomide was added to the treatment. Of the 9 dogs with IMPA, 7 dogs had a positive response (complete response, 5 dogs; partial response, 2 dogs) within 1–2 weeks after starting leflunomide. Of the 7 dogs with IMTP, 4 dogs had a complete response to leflunomide reported within 1–3 weeks after starting leflunomide, whereas 3 dogs had no response. Complete resolution of the multiple skin lesions was reported in the dog with CH at a recheck 3 months after starting leflunomide. In total, 12 dogs (70.5%; 7 dogs with IMPA, 4 dogs with IMTP, and 1 dog with CH) had an apparent positive response to the use of leflunomide. Of the 11 dogs that had leflunomide monotherapy, 9 dogs (81.8%) had a positive response. There was no significant difference ($P = 0.22$) in dosages between dogs that responded to leflunomide (n = 12; median, 1.9 mg/kg/d; range, 1.0–3.5 mg/kg/d) and those that did not respond (n = 5; median, 1.7 mg/kg/d; range, 1.0–2.0 mg/kg/d).

Discussion

In our study, 11 dogs with naturally occurring immune-mediated diseases had adverse events possibly attributable to the use of leflunomide, and all of these dogs underwent either a dose reduction or discontinuation of leflunomide because of the adverse effects. The median starting dosage of leflunomide was significantly higher at 2.9 mg/kg/d in dogs with adverse events than the dosage of 1.6 mg/kg/d in dogs without recorded adverse events. The median dosage of leflunomide in dogs that had an apparent positive response to leflunomide was 1.9 mg/kg/d, and the positive response rate (70.5% in total, 81.8% in leflunomide monotherapy) was comparable with previous reports.[11–13] On the basis of these findings, a lower starting dosage of leflunomide at 2 mg/kg/d as compared with the currently suggested dosage of 3–4 mg/kg/d[11,12] is recommended to decrease the risk of adverse events without eliminating the benefits of the drug.

The observed clinical adverse events seen in the dogs in our study included diarrhea and lethargy, which also were reported in previous studies.[11–13] Unexplained hemorrhage during treatment with leflunomide in 3 dogs was first reported in our study. Although the diarrhea and lethargy were reported shortly after starting leflunomide (1 week), unexplained hemorrhage occurred at a relatively later time (6, 13 and 20 weeks after starting leflunomide). In these 3 dogs, platelet counts were not at levels associated with spontaneous bleeding and PT/APTT were within normal limits, suggesting that a possible primary hemostatic disorder such as vascular anomaly or platelet dysfunction could be a cause of bleeding. In humans, unexplained hemorrhage with ecchymosis has been reported with leflunomide therapy, although it was very uncommon and the mechanism of the toxicity was not fully understood.[15] Further studies are needed to elucidate the mechanism of hemorrhage.

In previous studies, the frequency of hematologic disorders and hepatotoxicity associated with leflunomide was not fully investigated in dogs because a follow-up CBC and serum biochemistry were performed for only a small number of dogs.[11–13] In our study, the frequency of hepatotoxicity and hematologic disorders as evidenced by increases in liver enzyme activity or cytopenia was 6.3 and 6.5%, respectively. The mild increases in ALT and ALP activities and thrombocytopenia occurred within 2 weeks after starting leflunomide and resolved after dose reduction. Hepatic or hematologic toxicity associated with leflunomide was considered a rare event in dogs, and no abnormalities were reported in the second CBC and liver enzyme reevaluations in our study.

A higher incident rate of adverse effects during the use of leflunomide was recently reported in 15 Miniature Dachshunds with inflammatory colorectal polyps[13] compared to the rate in our study and the previous 2 studies.[11,12] Of the 15 Miniature Dachshunds, 11 dogs (73.3%) had adverse events including lethargy,

hematologic disorders, and increases in liver enzyme activities,[13] indicating that Miniature Dachshunds or small dogs may be predisposed to the adverse effects of leflunomide. However, no breed tendency to adverse events was observed in our study. There were also no significant differences in age, sex, body weight, and BSA between dogs with adverse events and those with no adverse events.

In humans, polymorphisms of cytochrome P450 are linked to an increase in leflunomide toxicity.[5,16,17] A further study to investigate the relationship between breeds or genetic polymorphisms and leflunomide toxicity in dogs is needed.

Several limitations should be considered when interpreting our results. Because of the nature of our retrospective study, reevaluations for physical examination or blood tests were not performed at regular time points. Therefore, the actual time of onset of toxicity or efficacy of leflunomide could not be precisely defined. Another potential confounding factor in our study was the concurrent administration of other medications in many of the dogs. Concurrent medications may have contributed to the adverse events or response to treatment, although we attempted to avoid these factors by setting strict inclusion criteria. In addition, serum concentrations of teriflunomide, which is one of the active metabolites of leflunomide, were not measured in all the dogs in our study. In people, serum concentrations of teriflunomide <16 μg/mL are associated with poor response to therapy.[18] The serum concentration of teriflunomide may be an objective marker to evaluate the efficacy or toxicity of leflunomide in dogs, although a specific cutoff for teriflunomide serum concentrations in dogs needs to be investigated, because it has been reported previously that the pharmacokinetics between healthy humans and dogs are different.[19] Finally, the number of dogs was relatively small, especially in the assessment of hepatotoxicity and response to leflunomide treatment. The majority of dogs included in our study were treated concurrently with glucocorticoids. As we included only the dogs receiving leflunomide monotherapy in the treatment response analysis, the group was significantly decreased in number. Also, only IMPA, IMTP and CH cases were available to assess treatment response in our study. It was conceivable that the primary clinicians were able to use leflunomide monotherapy or add leflunomide to the treatment with no change in other immunosuppressive drugs more readily for these diseases because dogs with these disorders were considered more clinically stable compared to those with other diseases such as IMHA. As noted, we did not detect a significant difference in doses of leflunomide administered to dogs that responded to therapy. We therefore suggest that an increase in dosage does not improve response to therapy, but it should be noted that the small number of dogs in our study means that the lack of significance could simply be due to a lack of power (type II error). A larger, prospective study with standardized follow-up criteria is warranted to determine the safety and efficacy of leflunomide in dogs with immune-mediated diseases.

In summary, an initial starting dosage of leflunomide at 2 mg/kg/d could be safer and provide similar efficacy for treatment in dogs with immune-mediated diseases compared to the current suggested dose at 3–4 mg/kg/d. In patients that fail to respond, a dosage increase up to 4 mg/kg/d can be considered if no adverse effects are observed. Discontinuation of leflunomide may be indicated if unexplained hemorrhage occurs. Based on the dogs in our study, resolution of bleeding may be expected 3–5 days after discontinuation of the drug.

Footnote

[a] Statmate3, ATMS, Tokyo, Japan

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Conflict of Interest Declaration: The authors declare no conflict of interest.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

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