

Efficacy of leflunomide for treatment of refractory inflammatory colorectal polyps in 15 Miniature Dachshunds

Kenjiro FUKUSHIMA¹⁾, Nozomi EGUCHI¹⁾, Koichi OHNO^{1)*}, Hideyuki KANEMOTO¹⁾, Masashi TAKAHASHI¹⁾, Hirota IGARASHI¹⁾, Aki OHMI¹⁾, Ko NAKASHIMA¹⁾ and Hajime TSUJIMOTO¹⁾

¹⁾Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

(Received 9 March 2015/Accepted 28 September 2015/Published online in J-STAGE 10 October 2015)

ABSTRACT. Inflammatory colorectal polyp (ICRP), common in miniature dachshunds, presents with hematochezia, tenesmus and mucoid feces. Although an 80% response rate has been reported when treated with prednisolone and cyclosporine, effective treatment is needed for the remaining 20% of ICRP dogs. Leflunomide is an immunosuppressive drug reported as effective in several immune-mediated diseases. In the present study, we retrospectively evaluated the efficacy and adverse effects of leflunomide in 15 ICRP dogs that were refractory to treatment with prednisolone and cyclosporine. Treatment efficacy was assessed by endoscopy, clinical symptoms and rectal palpation. Adverse effects were determined by clinical symptoms and blood testing during follow-up. The leflunomide treatment response rate was 93.3%. The median dosage of leflunomide and the median response time were 3 mg/kg (range: 1.7–4.0 mg/kg) and 35 days (range: 20–119 days), respectively. Adverse effects observed included lethargy (3 dogs), anorexia (1 dog), respiratory symptoms (1 dog), leukocytopenia (2 dogs), thrombocytopenia (1 dog), anemia (1 dog) and liver enzyme elevation (8 dogs). Most of the adverse effects improved with symptomatic treatment and leflunomide discontinuation or dosage reduction. In conclusion, leflunomide treatment is effective in ICRP dogs refractory to treatment with prednisolone and cyclosporine. Because several adverse effects were observed, close monitoring is needed during leflunomide treatment follow-up.

KEY WORDS: canine, gastroenterology, IBD, small animal medicine

doi: 10.1292/jvms.15-0129; *J. Vet. Med. Sci.* 78(2): 265–269, 2016

Inflammatory colorectal polyp (ICRP) is an emerging canine disease, most commonly affecting miniature dachshunds in Japan [10]. Because dogs with ICRP present with persistent hematochezia, tenesmus and mucoid feces, this disease can be quite stressful for the owners as well as their dogs. Polyps are typically found on rectal palpation and are generally round, of varying size, and multiple in most cases. Typical histopathologic features include severe infiltration of inflammatory cells (predominantly neutrophils) and mucosal epithelial proliferation without cellular atypia.

Although the pathogenesis of ICRP in miniature dachshunds remains unknown, several recently published studies reported that dysregulation of innate immune mechanisms and/or overexpression of proinflammatory cytokines, such as IL-17A, IL-8 and IFN γ , are important [7, 11, 13]. These results strongly suggested that ICRP is an immune-mediated disease.

To treat ICRP, combination therapy with prednisolone and cyclosporine is most commonly used and has a reported response rate of 80% [10]. Other reported empirical treatments include rectal pull-through surgery and endoscopic polyp-

ectomy with argon plasma coagulation, but information on treatment outcomes is limited [14].

Leflunomide is an immunosuppressive drug and has been used to treat some immune-mediated diseases in dogs, including polyarthritis, thrombocytopenia, hemolytic anemia and multifocal nonsuppurative encephalitis/meningomyelitis [4, 6]. In humans, common adverse effects of leflunomide include diarrhea, nausea, headaches, alopecia and hypertension [1, 2], and increases in serum liver enzyme activities have been reported in 2.2–19% of patients treated with leflunomide [2]. Severe adverse effects including myelosuppression and interstitial lung disease are reported less frequently [8, 9]. In veterinary medicine, reported adverse effects of leflunomide treatment include anorexia, lethargy, vomiting, anemia and leukocytopenia [4, 6].

Leflunomide is a prodrug, and its metabolite A77-1726, a malononitriloamide, has pharmacological activity. A77-1726 inhibits protein tyrosine kinase and dihydroorotate dehydrogenase, the fourth enzyme in the *de novo* pyrimidine biosynthetic pathway. A77-1726 inhibits T- and B-cell proliferation, suppresses immunoglobulin production, interferes with cell adhesion, inactivates the T lymphocyte receptor and several cytokine receptors, and leads to cell-cycle arrest in proliferating lymphocytes [12].

The purposes of this retrospective study were 1) to determine the efficacy of leflunomide in dogs with ICRP refractory to treatment with prednisolone and cyclosporine and 2) to describe the adverse effects related to leflunomide treatment.

*CORRESPONDENCE TO: OHNO, K., Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. e-mail: aohno@mail.ecc.u-tokyo.ac.jp

©2016 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.

MATERIALS AND METHODS

A retrospective case selection: A retrospective review of medical records from the Veterinary Medical Center at the University of Tokyo (VMC-UT) was conducted from April 2008 to December 2014. Inclusion criteria were: (1) a histopathologic diagnosis of ICRP, (2) lack of response (clinical signs and persistence of rectal masses noted on rectal examination and endoscopy) to conventional treatment with prednisolone and cyclosporine for at least 2 weeks and (3) treatment with leflunomide following conventional treatment.

Medical records review: All dogs received endoscopic biopsy, and histopathological diagnosis of ICRP was confirmed at first presentation. Age, sex, breed and body weight at diagnosis were obtained from the records. Client complaints, complete blood cell count (CBC) and results of serum biochemical analysis before and after leflunomide administration were also noted. Treatment information obtained included duration and dosage of prednisolone and cyclosporine prior to leflunomide administration, leflunomide dosages and concurrent treatments.

Response to leflunomide treatment: Assessment mainly depended on endoscopic gross findings. In case endoscopic evaluation was not applicable, clinical signs (hematochezia, mucoid feces and tenesmus) or rectal palpation was used to evaluate the treatment efficacy. On endoscopic evaluation, if the polypoid lesions had disappeared or reduced in size and/or number, patients were assessed as complete response or partial response, respectively. Clinical signs were assessed as improved or resolved. If the palpable rectal polyps had disappeared or reduced in size, patients were assessed as partial response because rectal palpation potentially underestimates the extent of the polypoid lesions. The period from initiation of leflunomide treatment to any of these responses was recorded as response time.

Complications of leflunomide treatment: Complications of leflunomide treatment were determined on the basis of clinical signs, hematologic and serum biochemical analysis at follow-up. Clinical signs considered adverse effects of leflunomide treatment included anorexia, lethargy and vomiting. Because interstitial lung disease was reported as a severe adverse effect in humans given leflunomide, dogs' respiratory symptoms were also recorded. CBC findings were used to evaluate myelosuppression (leukocytopenia, anemia or thrombocytopenia) before and after leflunomide treatment. Since prednisolone had been administered during prior treatment, liver enzymes were compared before and after treatment with leflunomide. If the value was increased compared to baseline (at initiation of leflunomide treatment), it was considered an adverse effect of leflunomide.

Long-term outcome of leflunomide treatment: To evaluate the long-term outcome of leflunomide treatment, we collected the data of the patients that responded to leflunomide treatment until the end of the study period. Recorded data include the length of follow-up period, medication status and clinical symptoms at the end of the follow-up period.

RESULTS

Cases: Fifteen dogs, all miniature dachshunds, met the inclusion criteria. Their clinical information is summarized in Table 1. Median age was 8.8 years (range; 4.0–13.3 years). Dogs included 12 males (10 castrated) and 3 spayed females. Complaints at initiation of leflunomide treatment included hematochezia (n=12), tenesmus (n=12), mucoid feces (n=10), soft stool (n=10) and anal prolapse (n=2). All dogs had colo-rectal masses found on endoscopy and histopathologically diagnosed as ICRP. Thirteen out of 15 dogs had palpable masses on colo-rectal area.

Median dosages of prednisolone and cyclosporine prior to leflunomide treatment were 1.5 mg/kg (range: 0.7–4.0 mg/kg) once per day and 5 mg/kg (range: 3.4–10 mg/kg) once per day, respectively. Median duration of prior treatment was 70 days (range: 19–199 days). Other treatments given before leflunomide included piroxicam (0.3 mg/kg, 3 dogs), firocoxib (4.7 mg/kg, 1 dog) and endoscopic polypectomy with argon plasma coagulation (2 dogs). Responses to these treatments were insufficient.

Response to leflunomide: Because the 15 dogs in the present study were refractory to primary treatment (prednisolone and cyclosporine), leflunomide was administered as a subsequent medication. The median dosage of leflunomide was 3 mg/kg (range: 1.7–4.0 mg/kg) once per day. Prednisolone (median dosage: 0.9 mg/kg; range: 0.5–1.5 mg/kg) once per day was administered with leflunomide in 14 dogs, of which 2 received cyclosporine (3.4 mg/kg and 5.0 mg/kg) at the same time (Table 1). These concurrent medications were gradually reduced according to response.

Fourteen of the dogs responded to leflunomide treatment (response rate: 93.3%). Response to leflunomide treatment was confirmed by endoscopy in 11 dogs, of which 4 had partial responses (Fig. 1A and 1B) and 7 had complete responses (Fig. 1C and 1D). Another 3 dogs were evaluated as responders based on clinical signs (n=2) and rectal palpation (n=1) (Table 1). Median response time of leflunomide treatment was 35 days (range: 20–119 days). The owner of the single dog that had not responded to leflunomide treatment by day 28 did not continue the leflunomide treatment.

Complications of treatment with leflunomide: Complications related to leflunomide treatment are summarized in Table 2. Lethargy (3 dogs) and anorexia (1 dog) were observed; vomiting did not occur. One dog had respiratory symptoms, including coughing and dyspnea.

Liver enzyme elevation after leflunomide treatment was observed in 8 dogs; all 8 had elevated ALP, and 4 had elevated ALT. In these dogs, median ALP, and ALT values were 901 U/l (range: 241–6,689 U/l) and 120 U/l (range: 90–211 U/l), respectively. Myelosuppression was observed in 3 dogs after administration of leflunomide: 2 dogs developed leukocytopenia (WBC: 1,600/ μ l on day 32 and 4,400/ μ l on day 392), and 1 dog developed anemia (Hct 17% on day 77).

The dog with respiratory symptoms exhibited coughing, dyspnea, lethargy and anorexia on day 32. Blood tests revealed severe leukocytopenia (1,600/ μ l), thrombocytopenia (87,000/ μ l), elevation of C-reactive protein (CRP) (8.6 mg/

Table 1. Clinical information regarding prior treatment and leflunomide treatment of each case

Case No.	Age	Sex	Prior Tx			LEF Tx			
			Medication (mg/kg/day)	Duration (days)	Clinical Symptoms	Medication (mg/kg/day)	Clinical Symptoms (Day)	ES evaluation (Day)	RP evaluation (Day)
1	11.9 y	CM	PSL (0.8), CsA (5.8)	70	HC, MF, TS, SF	LEF (1.7), PSL (0.7)	Improved (35)		
2	11.9 y	SF	PLS (1.1), CsA (7.1)	97	HC, MF, TS	LEF (3.0)	Improved (35)	PR (35)	
3	8.8 y	SF	PSL (4.0), CsA (5.8)	26	HC, MF, TS, SF, AP	LEF (2.8), PSL (1.4)	Resolved (21)	CR (70)	
4	4 y	M	PSL (1.0), CsA (4.9)	21	MF, SF	LEF (4.0), PSL (0.5)	Resolved (35)	CR (35)	
5	13.3 y	CM	PSL (0.7), CsA (3.4)	43	HC, TS, SF	LEF (2.8), PSL (0.7), CsA (3.4)	Resolved (28)	PR (28)	
6	8.8 y	CM	PSL (1.5), CsA (10)	28	HC, MF, TS, SF, AP	LEF (3.1), PSL (1.5)	Improved (21)	CR (21)	
7	8 y	CM	PSL (1.5), CsA (5.0)	51	HC, TS, SF	LEF (2.8), PSL (1.4)	Resolved (33)	CR (33)	
8	10.3 y	CM	PLS (2.0), CsA (5.0)	98	MF, TS, SF	LEF (3.8), PSL (1.0)	Resolved (34)	CR (34)	
9	11.5 y	CM	PSL (1.5), CsA (7.7)	104	HC, TS	LEF (2.9), PSL (0.7)	Improved (35)	CR (70)	
10	7.8 y	CM	PSL (1.4), CsA (7.5)	73	HC, MF, TS, SF	LEF (3.0), PSL (1.4)	Improved (20)		PR (20)
11	9.9 y	SF	PSL (1.5), CsA (5.0)	99	HC, MF	LEF (3.0), PSL (0.7)	Resolved (56)	CR (56)	
12	7.3 y	CM	PSL (1.0), CsA (5.0)	70	HS, TS	LEF (4.0), PSL (0.7)	Improved (78)	PR (78)	
13	8.8 y	M	PSL (2.0), CsA (5.0)	199	HC	LEF (3.6), PSL (2.0)	Resolved (39)		
14	7.4 y	CM	PSL (2.0), CsA (5.6)	119	HC, MF, TS, SF	LEF (4.0), PSL (0.5), CsA (5.0)	Resolved (63)	PR (119)	
15	9.3 y	CM	PSL (1.7), CsA (4.1)	19	HC, MF, TS, SF	LEF (3.3), PSL (1.3)	NR (28)		NR (28)

F: Female, SF: Spayed female, M: Male, CM: Castrated Male, Tx: Treatment, ES: Endoscopy, RP: Rectal palpation, LEF: Leflunomide, PSL: Prednisolone, CsA: Cyclosporine A, HC: Hematochezia, MF: Mucoïd feces, TS: Tenesmus, SF: Soft stool, AP: Anal prolapse, CR: Complete response, PR: Partial response, NR: No response.

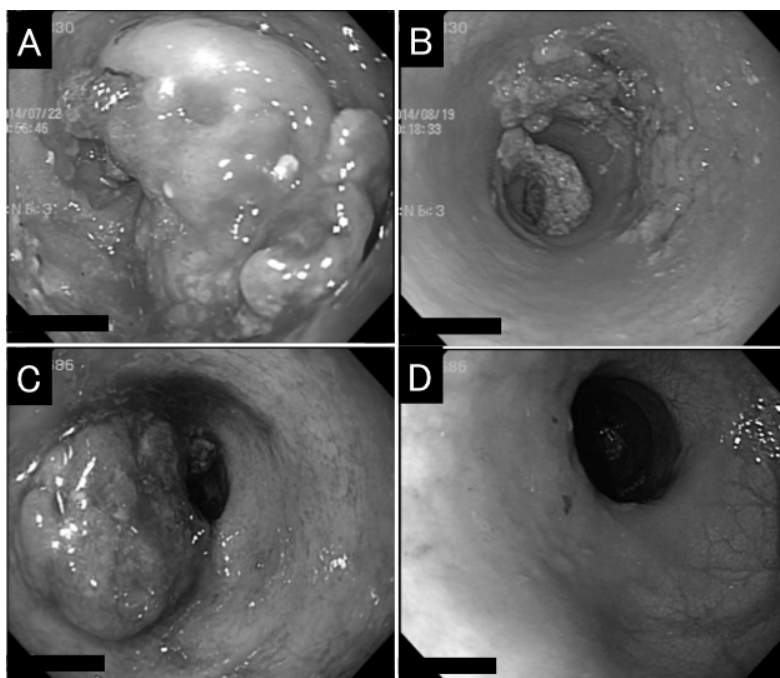


Fig. 1 Efficacy of leflunomide treatment as assessed by endoscopy. (A, B) This dog (Case No. 5), 13.3 years, was a male, castrated miniature dachshund. A large, irregular polyp (A) was observed before initiation of leflunomide treatment. At 28 days after initiation of leflunomide treatment, this polyp's size was clearly decreased (B). Because multiple small polyps remained, this patient was assessed as having a partial response to leflunomide treatment. (C, D) This dog (Case No. 4), 4 years, was a male miniature dachshund. A large polyp was observed (C), along with edema, in colorectal mucosa before treatment with leflunomide. At 35 days after initiation of leflunomide treatment, the polyp had disappeared, and the colorectal edema was improved, with a slightly irregular membrane surface (D). This case was assessed as a complete response to leflunomide treatment.

d/) and prolonged activated prothrombin time (22.6 sec). On radiography, radiographic opacity increased in left and right middle to caudate lobes. Leflunomide was discontinued, and the patient was treated in the hospital with antibiotics,

granulocyte-colony stimulating factor, fluid therapy and low-molecular-weight heparin. This dog recovered within 4 days and was discharged from the hospital.

One dog with liver enzyme elevation was lethargic on

Table 2. Adverse event of leflunomide treatment of each case

Case No.	Adverse event		Remarks
	Clinical symptoms (day)	Blood exam (day)	
1			
2	Lethargy (333)	WBC: 17,100/ml, ALP 732 U/l, CRP: 1.3 mg/dl (333)	Cholecystitis was suspected
3			
4		ALP: 855 U/l (35)	
5		ALP: 6,689 U/l, ALT: 211 U/l (28)	
6		ALP: 775 U/l, ALT: 143 U/l (21)	
7		ALP: 946 U/l (33)	
8		ALP: 2,643 U/l, ALT: 97 U/l (34)	
9	Lethargy (77)	Hct: 17% (77)	
10		ALP: 343 U/l, ALT: 90 U/l (20)	
11		WBC: 4,400 /ml (392)	
12			
13	Lethargy, Anorexia, Dyspnea, Cough (32)	WBC: 1,600 /ml, PLT: 87,000 /ml, CRP: 8.6 mg/dl (32)	Infectious respiratory disease was suspected
14			
15		ALP: 241 U/l (28)	

day 333. Blood examination revealed elevated ALP (732 U/l) and CRP (1.3 mg/dl). Because gallstones were observed on ultrasound, deterioration from bacterial cholecystitis due to immunosuppression was suspected. Leflunomide was stopped, antibiotics were administered orally, and the clinical symptoms improved in about a month.

In the 2 dogs that developed leukocytopenia, discontinuation or dose reduction of leflunomide resulted in WBC recovery. In 1 dog that exhibited lethargy on day 77, blood examination revealed severe anemia (Hct 17%). This patient received a single blood transfusion, and there was no recurrence after discontinuation of leflunomide.

Long-term outcomes of leflunomide treatment: In 14 cases that responded to leflunomide treatment, the data regarding long-term outcomes were available in 13 cases. Median follow-up duration was 423 days (range: 49–1,400 days). Leflunomide was discontinued in 4 dogs that had no clinical symptoms: 1 dog did not receive any medication, and the other 3 dogs were given low-dose prednisolone (0.3–0.5 mg/kg, every three days-every other day). The remaining 9 dogs were treated with tapered doses of leflunomide (1.7–4.0 mg/kg, every three days-once daily) with or without low-dose prednisolone (0.3–1.4 mg/kg, every three days-once daily). Their clinical symptoms related to ICRP either resolved or continued to improve.

DISCUSSION

In this study, we found that 93.3% of dogs with ICRP refractory to treatment with prednisolone and cyclosporine improved with leflunomide treatment.

The efficacy of leflunomide treatment in veterinary medicine has been reported in several immune-mediated diseases, such as polyarthritis, thrombocytopenia, hemolytic anemia and multifocal nonsuppurative encephalitis/meningomyelitis [6]. The rate of response to leflunomide treatment in immune-mediated polyarthritis (IMPA) was reported as 92.9%

in 14 dogs [4]. ICRP and IMPA are similar in that neutrophil infiltration is dominant at the site of the lesions. Overexpression of IL-17A and IL-8 in polyps of ICRP patients has also been reported [11, 13]; these cytokines were considered to be related to neutrophil infiltration in the lesions. In addition, IL-17 has been reported as overexpressed in T lymphocytes in inflammatory bowel disease in humans [5].

Leflunomide inhibits T lymphocyte proliferation and inactivates certain cytokine receptors [11]. These actions may result in regulation of proinflammatory cytokines that leads to decreased neutrophil infiltration in the lesions, conditions that may be essential for development or maintenance of ICRP inflammation.

The metabolite of leflunomide, A77-1726, inhibits activation of nuclear factor-kappa B (NF-kB) in human dendritic cells [15]. Furthermore, it has been reported that dysregulation of some pattern recognition receptors (PRRs) plays an important role in the pathogenesis or aggravation of inflammation in ICRP dogs [7]. Since NF-kB is known as an important signal transduction factor of PRRs, A77-1726 inhibition of this molecule may lead to regulation of the pro-inflammatory pathway of ICRP. Further investigation in this area is needed to clarify the pathogenesis and development of ICRP in dogs.

Of the 13 dogs that responded to leflunomide treatment in the present study, 4 that discontinued leflunomide treatment achieved complete improvement of clinical symptoms, and 9 that received tapered doses of leflunomide achieved partial or complete improvement. These results suggest that leflunomide treatment 1) may lead to complete remission and 2), with reduced dosages, may help to maintain long-term symptomatic improvement.

Liver enzyme elevation was observed in 8 dogs treated with leflunomide. Liver enzymes recovered to baseline in 4 dogs and stabilized in 3; 1 dog lacked further follow-up. Liver enzyme elevation was more frequently observed in the study dogs compared to previously reported IMPA dogs and

humans [2, 4]. Concurrent administration of prednisolone may be responsible for this discrepancy. Although the dosage of prednisolone was not elevated in most of the dogs at leflunomide initiation, combination of these drugs may induce an additive effect on elevation of liver enzyme. In addition, there is a possibility that other medications prior to leflunomide treatment (cyclosporine, piroxicam and firocoxib) may lead to the elevation of liver enzyme. However, the actual mechanism of this discrepancy remains unknown.

Myelosuppression was observed in 3 dogs. Although 2 dogs exhibited severe and 1 exhibited mild leukocytopenia, respectively, all recovered with discontinuation or reduced doses of leflunomide. Although the single dog with severe anemia (Hct 17%) required blood transfusion, anemia did not recur after leflunomide discontinuation. In the 2 dogs with severe myelosuppression, ICRP-related clinical symptoms disappeared completely after recovery from this adverse event.

Because the myelosuppressive and hepatotoxic (hepatic fibrosis) potential of higher dosages of leflunomide (5–10 mg/kg/day) has been reported in rats, close blood count monitoring is essential during leflunomide treatment in ICRP patients [3]. For the early detection of myelosuppression, once a month monitoring of blood count may be safe in the clinical application of leflunomide in dogs.

Infectious respiratory disease was suspected in the single dog that developed respiratory symptoms while receiving leflunomide. Upper respiratory tract infection has been reported as an adverse effect in humans treated with leflunomide [9]. Although interstitial lung disease is thought to be an important adverse effect of leflunomide treatment in human medicine, it was not observed in the present study. Respiratory symptoms should be carefully monitored during the treatment of leflunomide.

This study had several limitations. It was a retrospective study, so the treatment protocol and the assessment of response were not integrated. In addition, this study was lacking a control. A prospective controlled study is needed to confirm the efficacy and the adverse effects of leflunomide treatment more precisely. Additionally, number of patients was small in the present study. Case accumulation is essential to develop stronger evidence for leflunomide treatment of ICRP in dogs.

In conclusion, leflunomide is effective in ICRP dogs refractory to treatment with prednisolone and cyclosporine. Close monitoring is needed during leflunomide treatment to minimize the risk of adverse effects.

REFERENCES

- Alcorn, N., Saunders, S. and Madhok, R. 2009. Benefit-risk assessment of leflunomide: an appraisal of leflunomide in rheumatoid arthritis 10 years after licensing. *Drug Saf.* **32**: 1123–1134. [Medline] [CrossRef]
- Behrens, F., Koehm, M. and Burkhardt, H. 2011. Update 2011: leflunomide in rheumatoid arthritis - strengths and weaknesses. *Curr. Opin. Rheumatol.* **23**: 282–287. [Medline] [CrossRef]
- Bilasy, S. E., Essawy, S. S., Mandour, M. F., Ali, E. A. I. and Zaitone, S. A. 2015. Myelosuppressive and hepatotoxic potential of leflunomide and methotrexate combination in a rat model of rheumatoid arthritis. *Pharmacol. Rep.* **67**: 102–114. [Medline] [CrossRef]
- Colopy, S. A., Baker, T. A. and Muir, P. 2010. Efficacy of leflunomide for treatment of immune-mediated polyarthritis in dogs: 14 cases (2006–2008). *J. Am. Vet. Med. Assoc.* **236**: 312–318. [Medline] [CrossRef]
- Fujino, S., Andoh, A., Bamba, S., Ogawa, A., Hata, K., Araki, Y., Bamba, T. and Fujiyama, Y. 2003. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* **52**: 65–70. [Medline] [CrossRef]
- Gregory, C. R., Stewart, A., Sturges, B., DeManville, T., Cannon, A., Ortega, T., Harb, M. and Morris, R. E. 1998. Leflunomide effectively treats naturally occurring immune-mediated and inflammatory diseases of dogs that are unresponsive to conventional therapy. *Transplant. Proc.* **30**: 4143–4148. [Medline] [CrossRef]
- Igarashi, H., Ohno, K., Maeda, S., Kanemoto, H., Fukushima, K., Uchida, K. and Tsujimoto, H. 2014. Expression profiling of pattern recognition receptors and selected cytokines in miniature dachshunds with inflammatory colorectal polyps. *Vet. Immunol. Immunopathol.* **159**: 1–10. [Medline] [CrossRef]
- Inokuma, S., Sato, T., Sagawa, A., Matsuda, T., Takemura, T., Ohtsuka, T., Saeki, Y., Takeuchi, T. and Sawada, T. 2008. Proposals for leflunomide use to avoid lung injury in patients with rheumatoid arthritis. *Mod. Rheumatol.* **18**: 442–446. [Medline] [CrossRef]
- Kalden, J. R., Schattenkirchner, M., Sörensen, H., Emery, P., Deighton, C., Rozman, B. and Breedveld, F. 2003. The efficacy and safety of leflunomide in patients with active rheumatoid arthritis: a five-year followup study. *Arthritis Rheum.* **48**: 1513–1520. [Medline] [CrossRef]
- Ohmi, A., Tsukamoto, A., Ohno, K., Uchida, K., Nishimura, R., Fukushima, K., Takahashi, M., Nakashima, K., Fujino, Y. and Tsujimoto, H. 2012. A retrospective study of inflammatory colorectal polyps in miniature dachshunds. *J. Vet. Med. Sci.* **74**: 59–64. [Medline] [CrossRef]
- Ohta, H., Takada, K., Torisu, S., Yuki, M., Tamura, Y., Yokoyama, N., Osuga, T., Lim, S. Y., Murakami, M., Sasaki, N., Nakamura, K., Yamasaki, M. and Takiguchi, M. 2013. Expression of CD4⁺ T cell cytokine genes in the colorectal mucosa of inflammatory colorectal polyps in miniature dachshunds. *Vet. Immunol. Immunopathol.* **155**: 259–263. [Medline] [CrossRef]
- Silva, H. T. Jr. and Morris, R. E. 1997. Leflunomide and malononitriloamides. *Expert Opin. Investig. Drugs* **6**: 51–64. [Medline] [CrossRef]
- Tamura, Y., Ohta, H., Torisu, S., Yuki, M., Yokoyama, N., Murakami, M., Lim, S. Y., Osuga, T., Morishita, K., Nakamura, K., Yamasaki, M. and Takiguchi, M. 2013. Markedly increased expression of interleukin-8 in the colorectal mucosa of inflammatory colorectal polyps in miniature dachshunds. *Vet. Immunol. Immunopathol.* **156**: 32–42. [Medline] [CrossRef]
- Tsukamoto, A., Ohno, K., Irie, M., Ohmi, A., Maeda, S., Nakashima, K., Fukushima, K., Fujino, Y., Uchida, K. and Tsujimoto, H. 2012. A case of canine multiple inflammatory colorectal polyps treated by endoscopic polypectomy and argon plasma coagulation. *J. Vet. Med. Sci.* **74**: 503–506. [Medline] [CrossRef]
- Zeyda, M., Stuhlmeier, K. M., Kirsch, B., Watschinger, B., Hörl, W. H., Stulnig, T. M. and Säemann, M. D. 2005. The malononitrilamide FK778 inhibits activation of NF- κ B in human dendritic cells. *Transplant. Proc.* **37**: 1968–1969. [Medline] [CrossRef]