Case Report

JVC Journal of Veterinary Clinics

pISSN 1598-298X • eISSN 2384-0749
J Vet Clin 2024;41:165-169
https://doi.org/10.17555/jvc.2024.41.3.165

Transient Non-Regenerative Anemia in a Dog with Granulomatous Meningoencephalitis Following Leflunomide Treatment

Ga-Hyun Lim[1,†]
Ju-Hyun An[2,†]
Su-Min Park[1]
Jeong-Hwa Lee[1]
Ye-In Oh[3]
Kyoung-Won Seo[1]
Hwa-Young Youn[1,*]

[1]Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Science, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea
[2]Department of Veterinary Emergency and Critical Care Medicine, College of Veterinary Medicine, Kangwon National University, Chuncheon 24341, Korea
[3]Department of Veterinary Internal Medicine, College of Veterinary Medicine, Kyungpook National University, Daegu 41566, Korea

[†]Ga-Hyun Lim and Ju-Hyun An contributed equally to this work.

*Correspondence: hyyoun@snu.ac.kr

ORCID
Ga-Hyun Lim: https://orcid.org/0000-0001-7019-6246
Ju-Hyun An: https://orcid.org/0000-0002-3756-9482
Su-Min Park: https://orcid.org/0000-0002-1709-7000
Jeong-Hwa Lee: https://orcid.org/0000-0003-1716-8152
Ye-In Oh: https://orcid.org/0000-0001-8082-2458
Kyoung-Won Seo: https://orcid.org/0000-0002-1561-3278
Hwa-Young Youn: https://orcid.org/0000-0002-0283-1348

Abstract A 10-year-old, spayed female Maltese dog was tentatively diagnosed with granulomatous meningoencephalitis (GME) on magnetic resonance imaging. The meningoencephalitis was classified as aseptic GME because cerebral fluid analysis did not reveal an infectious aetiology. Two months after leflunomide treatment (Arava; Sanofi; 4 mg/kg/day), the patient developed non-regenerative, macrocytic, and normochromic anemia. As the patient's anaemia began after the administration of leflunomide, and other differentials for anaemia had been ruled out, the leflunomide was determined to be the cause and this treatment ended. After 15 days, the anaemia resolved spontaneously. This is the first report of reversible aplastic anaemia following treatment with leflunomide in a canine patient with GME.

Key words anemia, canine, drug adverse event, leflunomide, nonregenerative.

Received May 27, 2024 / Revised June 12, 2024 / Accepted June 12, 2024

Introduction

Leflunomide may be an effective treatment for immune-mediated disorders in dogs that do not respond well to standard therapy or have adverse effects to glucocorticoid administration (1). Though the occurence of adverse effects to leflunomide are rare they can, as reported in human medicine, include diarrhea, dermatitis, hepatotoxcity, pneumonia, neurotoxicity, and myelosuppression (2,13). In veterinary medicine, the adverse effects of leflunomide have rarely been reported (10). Therefore, further studies are needed in order to ensure the safety of this mediccation in canine patients both now and in the future. To the best of our knowledge, this is the first report of leflunomide-induced aplastic anaemia in a canine patient with granulomatous meningoencephalitis (GME).

Case Report

A 10-year-old, female spayed Maltese dog was presented to veterinary hospital with a two-day history of visual impairment. After performing an ophthalmologic examination, a magnetic resonance imaging (MRI) scan, and a cerebrospinal fluid (CSF) analysis, she was tentatively diagnosed with aseptic ocular GME. She was started on prednisolone (1 mg/kg, every 12 hours) and cyclosporine (3 mg/kg, every 12 hours) therapies and two weeks after initial treatment, she regained her vision. However, four months after initial treatment, her visual impairments occurred again. A repeat MRI was performed, and a new suspected inflammatory lesion was confirmed in the right optic nerve. Due to the presence of the new lesion, we decided to change her immunosuppressive therapy from cyclosporine to cytosine arabinoside (50 mg/m^2 every 12 hours for 2 days repeated every 3 weeks). Even after three cycles of cytosine arabinoside treatment, her vision had not improved. Fifteen months after the first visit, a new immunosuppressant drug, leflunomide (Arava; Sanofi; 4 mg/kg/day), was added to her therapy, and cytosine arabinoside was discontinued. At this time, A complete blood count (CBC) analysis showed the following findings: white blood cell count (WBC), 19.57 $\times$ 10^3/μL (reference range: 5.05-16.76 $\times$ 10^3/μL); haemoglobin (Hb), 13.5 g/dL (reference range: 13.1-20.5 g/dL); haematocrit (HCT) concentration, 41.3% (reference range: 37.3-61.7%); red blood cell (RBC) count, 5,580 $\times$ 10^3/μL (reference range: 5,700-8,800 $\times$ 10^3/μL); mean corpuscular volume (MCV), 73.9 fL (reference range: 58.8-71.2 fL); mean corpuscular haemoglobin (MCH), 24.2 pg (reference range: 20.5-24.2 pg); mean corpuscular haemoglobin concentration (MCHC), 32.7 g/dL (reference range: 31.0-36.2 g/dL); red cell distribution width (RDW), 13.7% (reference range: 11.9-14.5%) and platelet count, 117.7 $\times$ 10^3/μL (reference range: 731-400 $\times$ 10^3/μL). A follow-up examination two months after starting leflunomide treatment showed that the patient's oral mucosa was pale. On physical examination, the patient's vital signs were as follows: heart rate, 162 beats/minute; systolic blood pressure, 130 mmHg; respiratory rate, 24 breaths/minute; and body temperature, 39.6 ℃. A CBC analysis showed the following findings: WBC, 14.19 $\times$ 10^3/μL; Hb, 7.8 g/dL; HCT, 24.3%; RBC, 3,250 $\times$ 10^3/μL; MCV, 74.8 fL; MCH, 23.9 pg; MCHC, 32.0 g/dL; RDW, 14.7%; platelet count, 73.1 $\times$ 10^3/μL; and reticulocyte count, 16.7 $\times$ 10^3/μL (reference range: 0-60 $\times$ 10^3/μL). Evidence of haemolysis could not be confirmed on a blood smear. Despite performing further diagnostic tests, including serum biochemistry and an abdominal ultrasound examination, the cause of the anaemia could not be identified. Additionally, general examination revealed no evidence of chronic hemorrhage such as GI hemorrhage in this patient. An iron-based supplement (Fercobsang®, Vétaquinol SA, Lure, France; 1 ml/dose) was initially prescribed for a non-regenerative anaemia, but there was no improvement in the anaemia after 6 days of administration. A repeat complete blood count revealed the following values: packed cell volume (PCV), 21.1%; Hb, 6.8 g/dL, MCV, 74.8 fL; MCH, 32.0 pg; RDW, 15.6%; and reticulocyte count, 15.3 $\times$ 10^3/μL. Although aplastic anaemia has not been reported in veterinary medicine, it has been reported following leflunomide treatment in human rheumatoid arthritis patients (10). To correct the anemia, the dose of leflunomide was reduced for 6 days and then the treatment was stopped (Fig. 1). Despite discontinuation of leflunomide treatment, the patient's anaemia persisted, but her reticulocyte count improved (PCV, 21.4%; and reticulocyte count, 136.6 $\times$ 10^3/μL) (Fig. 1). Six days after leflunomide treatment ended, the patient's anaemia improved (with a PCV of 36.6%) and 11 days after that, her PCV was within the normal range (a PCV of 44.6%) (Fig. 1).

Discussion

Leflunomide is an isoxazole derivative that inhibits dihydro-orotate dehydrogenase (DHODH), which is the rate-limiting enzyme in the de novo synthesis of pyrimidines, and inhibits effector T cell proliferation (12). Due to its anti-inflammatory properties, leflunomide has been used as a therapeutic agent for various immune-mediated diseases including rheumatoid arthritis (3,10).

In human medicine, the most commonly reported adverse effects of leflunomide include diarrhoea, nausea, hepatotoxicity, hypertension, and hair loss (7). According to one report,

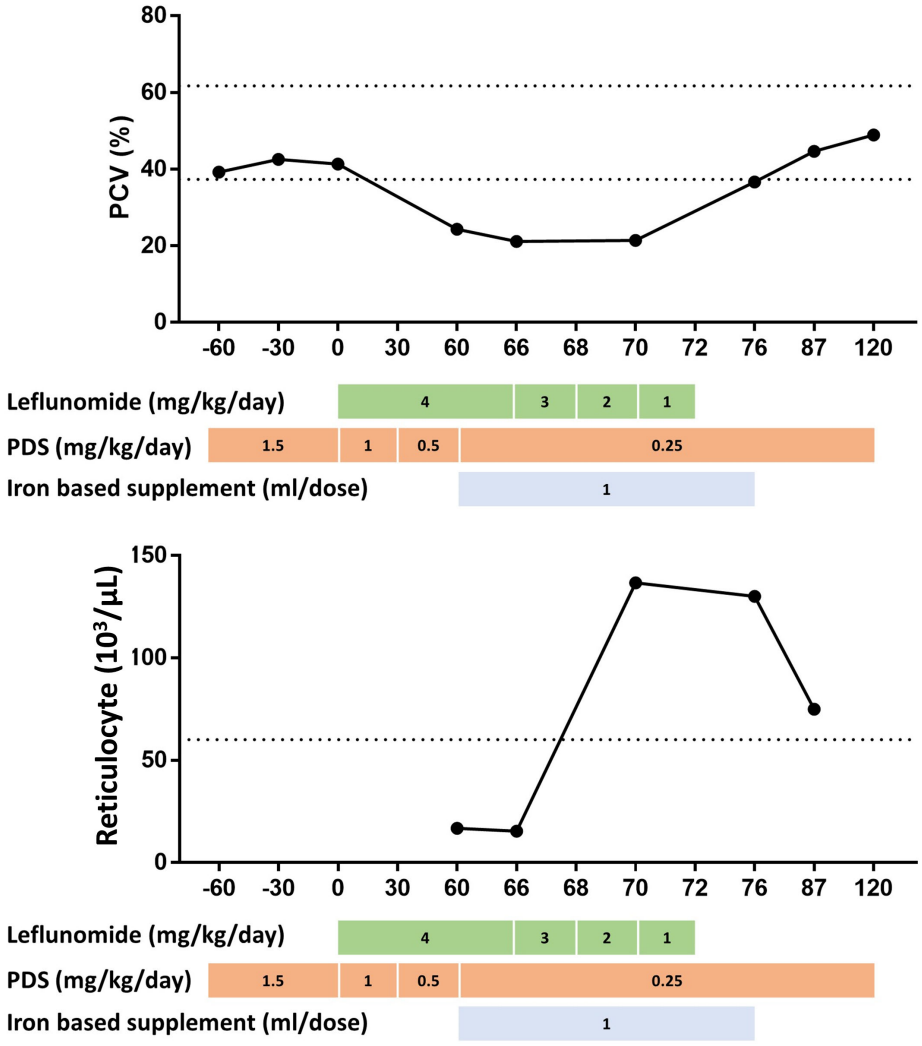

Fig. 1. Medication dosage, PCV and reticulocyte before and after leflunomide treatment. Graph showing dosage of leflunomide, PDS and Iron based supplement, PCV, reticulocyte value from the time of admission with leflunomide to the period before and after treatment.

leflunomide was discontinued within one year in 67% of cases due to adverse drug reactions. Additionally, several cases of leflunomide induced anaemia have been reported in the human medical literature although the underlying mechanism is still unknown. Wüsthof et al reported that a 32-year-old woman diagnosed with rheumatoid arthritis developed severe aplastic anaemia after taking leflunomide for 60 days, although her arthritis improved (14). Toyokawa et al reported that patients with arthritis developed thrombocytopenia, lymphopenia, and macrocytic anaemia after taking leflunomide (11). Although further studies are needed to determine the mechanism, it is known that leflunomide-induced anaemia is caused by bone marrow failure due to impaired pyrimidine biosynthesis, and in most cases, the anaemia may be accompanied by pancytopenia (1,9). Adverse events that may be associated with leflunomide administration in veterinary medicine include diarrhoea, lethargy, unexplained bleeding, thrombocytopenia, and increased liver enzyme activity (10). However, currently, there are no case reports of non-regenerative anaemia after leflunomide administration.

In this case, reticulocyte numbers were determined using a cationic dye (Oxazine 750) and a light-scatter technique on the ADVIA 2120i (Siemens Healthineers, Erlangen, Germany) that quality assessment was performed weekly previous described (6). The presence of anemia and regeneration was determined based on previous study for dogs (red blood cell count $<5.7 \times 10^6/\mu L$, hematocrit <37%, hemoglobin <12.9 g/dL, reticulocyte $>80 \times 10^3/\mu L$) (6). A general examination revealed no evidence of chronic bleeding, and the patient did not have non-regenerative, microcytic, and hypochromic anemia, which is typical of iron deficiency anemia. Instead, two months after leflunomide administration, the patient presented with non-regenerative, macrocytic, and normochromic anemia. Bone marrow examination was not performed because the owner of the patient did not provide consent

for the procedure. However, inhibitors of pyrimidine DNA synthesis often cause drug-induced aplastic macrocytic anaemia, similar to that caused by anticancer drugs (5,8). In addition, no other cause of anaemia was observed; moreover, since the anaemia improved after discontinuation of leflunomide, it is highly likely that the patient in this report had anaemia secondary to leflunomide. According to a previous case report, leflunomide-induced anaemia improved after discontinuation of leflunomide without the need for additional drug therapies (4). Based on these reports, we discontinued leflunomide treatment, and the patient's PCV returned to the normal reference interval 15 days later.

Unfortunately, our case was unable to determine whether the anemia that occurred was a dose-dependent side effect of leflunomide. In this patient, leflunomide was prescribed at a dose of 4 mg/kg/day (3). According to a previous study, the dose causing leflunomide adverse effects was also investigated. Significant dose differences were found between dogs with adverse events (median: 2.9 mg/kg/day; range: 1.8-3.6 mg/kg/day) and dogs without adverse events (median: 1.6 mg/kg/day; range: 0.8-4.3 mg/kg/day) (10).

To the best of our knowledge, this is the first case report of reversible aplastic anaemia following treatment with leflunomide in dog patient with meningoencephalitis. Based this case, the rare incidence of non-regenerative, macrocytic, normochromic, non-haemolytic anaemia following leflunomide treatment should be considered. Our case highlights the importance of recognizing leflunomide as a drug that can induce aplastic (non-regenerative) anaemia. In addition, if an adverse reaction to leflunomide is suspected, therapy should be discontinued immediately and, if necessary, symptomatic treatment should be started.

Conclusions

We report transient aplastic anemia in a dog with granulomatous meningoencephalitis following leflunomide treatment. This well-documented case of aplastic anaemia associated with leflunomide may help inform the veterinary and medical communities about the potential complications linked to this medication.

Author contributions

GHL and JHA drafted the initial manuscript. GHL, JHA, SMP, JHL, YIO, KWS acquired, analyzed and interpreted the data; GHL, JHA, HYY revised the article; and HYY approved the final version of the article prior to submission. All authors have read and approved the manuscript.

Acknowledgements

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2023-00240708).

Conflicts of Interest

The authors have no conflicting interests.

References

1. Chan J, Sanders DC, Du L, Pillans PI. Leflunomide-associated pancytopenia with or without methotrexate. Ann Pharmacother 2004; 38: 1206-1211.
2. Chan V, Tett SE. How is leflunomide prescribed and used in Australia? Analysis of prescribing and adverse effect reporting. Pharmacoepidemiol Drug Saf 2006; 15: 485-493.
3. Colopy SA, Baker TA, Muir P. Efficacy of leflunomide for treatment of immune-mediated polyarthritis in dogs: 14 cases (2006-2008). J Am Vet Med Assoc 2010; 236: 312-318.
4. Fukushima K, Eguchi N, Ohno K, Kanemoto H, Takahashi M, Igarashi H, et al. Efficacy of leflunomide for treatment of refractory inflammatory colorectal polyps in 15 miniature dachshunds. J Vet Med Sci 2016; 78: 265-269.
5. Hesdorffer CS, Longo DL. Drug-induced megaloblastic anemia. N Engl J Med 2015; 373: 1649-1658.
6. Jung JH, Yang Y, Seo D, Cho S, Choi G, Kim Y. Clinical utility of immature reticulocyte fraction for identifying early red blood cell regeneration in anemic dogs. J Vet Intern Med 2023; 37: 484-489.
7. Koller G, Cusnir I, Hall J, Ye C. Reversible alopecia areata: a little known side effect of leflunomide. Clin Rheumatol 2019; 38: 2015-2016.
8. Mintzer DM, Billet SN, Chmielewski L. Drug-induced hematologic syndromes. Adv Hematol 2009; 2009: 495863.
9. Park SY, Back SY, Park SW, Kim JH, Son CN, Cho SK, et al. Hemolytic anemia in a patient with rheumatoid arthritis treated with leflunomide. J Korean Soc Clin Pharmacol Ther 2008; 16: 87-91.
10. Sato M, Veir JK, Legare M, Lappin MR. A retrospective study on the safety and efficacy of leflunomide in dogs. J Vet Intern Med 2017; 31: 1502-1507.
11. Toyokawa Y, Kingetsu I, Yasuda C, Yasuda J, Yoshida K, Kurosaka D, et al. Pancytopenia, including macrocytic anemia, associated with leflunomide in a rheumatoid arthritis patient. Mod Rheumatol 2007; 17: 436-440.
12. van der Heijden EH, Hartgring SA, Kruize AA, Radstake TR, van Roon JA. Additive immunosuppressive effect of leflunomide and hydroxychloroquine supports rationale for combination therapy

for Sjögren's syndrome. Expert Rev Clin Immunol 2019; 15: 801-808.
13. van Riel PL, Smolen JS, Emery P, Kalden JR, Dougados M, Strand CV, et al. Leflunomide: a manageable safety profile. J Rheumatol 2004; 71: 21-24.
14. Wüsthof M, Smirnova A, Bacher U, Kröger N, Zander AR, Schuch G, et al. Severe aplastic anaemia following leflunomide therapy. Rheumatology (Oxford) 2010; 49: 1016-1017.