The Journal of Veterinary Medical Science

NOTE

Internal Medicine

Comparison of the efficacy of cyclosporine and leflunomide in treating inflammatory colorectal polyps in miniature dachshunds

Akiyoshi TANI[1)], Taisuke SENO[2)], Nozomu YOKOYAMA[2)], Taisuke NAKAGAWA[2)], Hirotaka TOMIYASU[1)], Yuko GOTO-KOSHINO[1)], Hajime TSUJIMOTO[1)] and Koichi OHNO[1)]*

[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
[2)]Veterinary Medical Center, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

J. Vet. Med. Sci.
82(4): 437–440, 2020
doi: 10.1292/jvms.19-0560

Received: 11 October 2019
Accepted: 30 January 2020
Advanced Epub:
14 February 2020

ABSTRACT. Inflammatory colorectal polyps (ICRPs) are frequently observed in miniature dachshunds in Japan and treated by prednisolone and immunosuppressive drugs such as cyclosporine and leflunomide. The purpose of this retrospective study was to compare the treatment efficacy, such as response rate, response interval, recurrence rate, and adverse events between cyclosporine and leflunomide. While the response rates were significantly higher in dogs treated with leflunomide, no significant differences were observed in the response interval or recurrence rate. Two of the 11 dogs treated with leflunomide showed hematological or gastrointestinal adverse events, while no dog treated with cyclosporine showed any adverse events. A case-controlled prospective study to compare the treatment efficacy of leflunomide with that of cyclosporine should be conducted.

KEY WORDS: canine, cyclosporine, inflammatory colorectal polyp, leflunomide

Inflammatory colorectal polyps (ICRPs) are frequently found in miniature dachshunds in Japan [7]. ICRPs are generally round and of variable size, are present in multiple numbers, and are located in the colorectal region [7]. The typical symptoms of affected dogs are hematochezia, tenesmus, soft stools, mucoid feces, and, rarely, anal prolapse [3, 7]. The typical histopathological features of ICRPs are a proliferation of the granulomatous tissue, severe infiltration of inflammatory cells of the lamina propria, and mucosal epithelial proliferation without cellular atypia [11].

Treatments for ICRP include prednisolone, non-steroidal anti-inflammatory drugs, immunosuppressive drugs, endoscopic polypectomy with argon plasma coagulation (APC), and rectal pull-through surgery [3, 6, 7, 10]. Cyclosporine, combined with prednisolone, was the first immunosuppressive drug reported as a treatment; 20 of 25 cases (80%) clinically improved with it [7]. Chlorambucil, combined with firocoxib or prednisolone, has also been reported to be effective in treating refractory ICRP, as 4 of 5 dogs responded to this treatment [6]. However, chlorambucil is not available in Japan, and it requires careful handling because it is an anticancer drug. Leflunomide is available in Japan and was found to be effective against cyclosporine-resistant ICRP in our previous study [3]. Although, no study was conducted to compare the treatment efficacy between cyclosporine and leflunomide in ICRP, so far. Thus, the purpose of this study was to compare treatment efficacy of cyclosporine with that of leflunomide.

The medical records at the Veterinary Medical Center, University of Tokyo, from April 2013 to March 2019, were retrospectively reviewed, and interviews were conducted with primary veterinarians when needed. Miniature dachshunds histopathologically diagnosed with ICRP by endoscopic biopsy were included. To compare the treatment efficacy of the 2 immunosuppressive drugs, dogs treated with immunosuppressive drugs at primary veterinarians before diagnosis were excluded. Dogs treated with rectal pull-through surgery, polypectomy and APC after initiation of immunosuppressive treatments were also excluded to evaluate the effects of medical treatments alone. To evaluate the responses to each medication, dogs that could not be followed-up with for more than 1 month were excluded. Dogs diagnosed with colorectal adenocarcinoma were excluded from this study on the day of the diagnosis.

Each record was reviewed for the dog's age, gender, body weight, clinical signs, complete blood cell count (CBC), and blood biochemical analysis at initial examination. Clinical signs including hematochezia, mucoid feces, tenesmus, soft stool, and anal

*Correspondence to: Ohno, K.: aohno@mail.ecc.u-tokyo.ac.jp

prolapse were noted. Treatment response was evaluated based on clinical signs, findings of rectal palpations, or endoscopic appearances. Since objective evaluation based on clinical signs is more difficult than that based on rectal palpations or endoscopic appearances, the disappearance of at least 1 clinical sign that was noted before treatment was evaluated as responded. Rectal palpations and endoscopic appearances were assessed by the size and number of polyps. If the size and/or number of polyps were decreased, the case was evaluated as responded. When either response, which was evaluated from clinical signs, rectal palpations, or endoscopic appearances, was noted on follow-up examinations, the dog was evaluated as "responded." The time from initiation of each treatment to the date (responded date) that any treatment responses was observed was recorded as "response time," and the proportion of responded dogs was recorded as "response rate" as previously described [3]. Doses of each medication were gradually reduced according to the response. Each case was evaluated as "recurred" when clinical signs, rectal palpations, and/or endoscopic appearances worsened and when dosage increases or treatment changes were needed in the follow-up time period. The proportion of dogs in which recurrence was noted was recorded as "recurrence rate", and the period from the responded date to the recurred date was noted as "asymptomatic interval". The prognosis of each patients was investigated until March 2019, which was the end of inclusion in the present study.

All clinical signs and abnormalities in blood CBC and biochemistry values from the beginning of the treatment with each medication were reviewed, and adverse events were evaluated using the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE) [4]. Hepatotoxicity associated with cyclosporine and leflunomide was evaluated from alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities. To evaluate the hepatotoxicity of each drug, the dogs that were not receiving prednisolone or were on a stable or decreasing dosage of prednisolone were added to the evaluation. Hepatotoxicosis was defined as ALT or ALP activities higher than the upper limit of the reference range or a 2-fold increase compared to the baseline, if the baseline ALT or ALP activity was above the reference range, as previously described [9]. When a drug was discontinued, the causes of the discontinuation were noted.

Fisher's exact test was used for comparisons of response rates and recurrence rates. The Mann-Whitney rank-sum test was used for comparison of response time. All statistical analyses were performed with R software (version 3.5.0; available as a free download from https://cran.r-project.org).

Seventy-one dogs were recruited based on medical record review. Of these, 18 dogs were excluded because no immunosuppressive drug was used. In the remaining 53 dogs, 14 dogs were excluded because of prior use of immunosuppressive drugs at primary veterinarians, 8 dogs were excluded because of a short follow-up period, and 6 dogs were excluded because surgical resection (n=3) or polypectomy and APC (n=3) was conducted. As a result, 25 dogs were included for further review. Of these 25 dogs, 12 were male (9 were castrated) and 13 were female (11 were spayed). The median age of dogs was 11.1 years (range, 4–14 years), and the median weight was 5.1 kg (range, 3.6–7.5 kg).

Dogs presented with one or more of the following complaints: hematochezia (n=23), tenesmus (n=20), mucoid feces (n=15), soft stool (n=12), and anal prolapse (n=1). All dogs had colorectal masses and were histopathologically diagnosed as ICRP upon endoscopic examination and biopsy. The median duration of clinical signs until the first immunosuppressive treatment at our facility was 78 days (range, 17–255 days). Until administration of immunosuppressive drugs, dogs were treated with prednisolone (n=20, 0.7–2.2 mg/kg/day), antibiotics (n=16), and piroxicam (n=1).

After administration of immunosuppressive drugs, 16 dogs were treated with prednisolone (1–2.4 mg/kg/day) and cyclosporine (3–10 mg/kg/day), and 8 dogs were treated with prednisolone (0.9–1.3 mg/kg/day) and leflunomide (1.3–2.4 mg/kg/day). One dog was treated with only leflunomide (3 mg/kg/day). As concomitant medications, antibiotics (n=15), antacids (n=11), and hepatoprotective agents (n=7) were used. These concurrent medications were gradually reduced according to the response. During the study period, only one dog, which treated with cyclosporine, developed adenocarcinoma on day 1001 and excluded from the present study on the day of the diagnosis.

The dogs were divided into groups of either cyclosporine-treated (CYA) dogs or leflunomide-treated (LEF) dogs based on the initial choices of immunosuppressive drug. Dogs treated with cyclosporine and without any prior immunosuppressive drugs were assigned to the CYA group, and dogs treated with leflunomide and without any prior immunosuppressive drugs were assigned to the LEF group. As a result, 16 and 9 dogs were assigned to CYA and LEF, respectively. Priory, prednisolone was used in 13 of 16 CYA dogs and 7 of 9 LEF dogs. There was no significant difference in the number of dogs between the 2 groups. In the comparison of CYA and LEF dogs, there were no significant differences in age, sex distribution, body weight, or prior prednisolone treatments (Table 1). A positive response to treatments was noted in 10 of the 16 CYA dogs and in 9 of the 9 LEF dogs, which indicated that LEF dogs had a significantly higher response rate than CYA dogs did (P=0.045) (Table 2). The response time was evaluated in 8

Table 1. The comparison of signalments between cyclosporine-treated (CYA) and leflunomide-treated (LEF) dogs which were able to evaluate treatment efficacies

Signalments	CYA dogs (n=16) (Median (range))	LEF dogs (n=9) (Median (range))	P-value
Age (years)	9.9 (4.0−13.5)	11.1 (7.8−14.0)	0.291
Sex (Male : Female)	8 : 8	4 : 5	1
Body weight (kg)	5.2 (4.1−7.5)	4.5 (3.6−5.9)	0.121
Prior use of prednisolone (used : not used)	13 : 3	7 : 2	1

Table 2. The comparison of treatment efficacies between cyclosporine-treated (CYA) and leflunomide-treated (LEF) dogs which were able to evaluate treatment efficacies

Variables	CYA dogs (n=16) (Median (range))	LEF dogs (n=9) (Median (range))	*P*-value
Response rate	63% (10/16)	100% (9/9)	0.045
Response time (days)	14 (11−35)	28 (7−39)	0.132
Recurrence rate	70% (7/10)	33% (3/9)	0.179

CYA dogs and 9 LEF dogs. The median duration was 14 days (range, 11–35 days) in CYA dogs and 28 days (range, 7–39 days) in LEF dogs, and there was no significant difference in duration (P=0.132). Six of 16 CYA dogs did not respond to cyclosporine treatment. Of these, 5 CYA dogs were subsequently treated with prednisolone (0.5–1.5 mg/kg/day) and leflunomide (3–4 mg/kg/day) and 1 CYA dog was treated with piroxicam.

In the follow-up period, 7 of the 10 CYA dogs and 3 of the 9 LEF dogs once classified as "responded", became "recurred". No significant difference in recurrence rates was observed between the 2 groups (P=0.179). The median asymptomatic intervals were 76 days (range, 28–1,047 days) in CYA dogs and 86 days (range, 45–384 days) in LEF dogs. Three of the 10 CYA dogs and 6 of the 9 LEF dogs showed no recurrence. The median duration from the initiation of treatments to the last follow-up date of those dogs was 358 days (range, 263–1,001 days) in CYA dogs and 178 days (range, 84–264 days) in LEF dogs.

No adverse events related to cyclosporine treatments, based on VCOG-CTCAE [4], were reported. One CYA dog was diagnosed with colorectal adenocarcinoma 1,001 days after the initiation of cyclosporine treatment, and cyclosporine was discontinued.

Adverse events related to leflunomide were noted in 2 LEF dogs. One LEF dog experienced anorexia and vomiting, which was grade 1 on VCOG-CTCAE, 28 days after the initiation of leflunomide (3 mg/kg/day). The leflunomide was discontinued for 7 days, then restarted at the same dosage with no adverse events. The other LEF dog experienced anemia (hematocrit: 15%), which was grade 3 on VCOG-CTCAE, 32 days after the initiation of leflunomide (2 mg/kg/day). The baseline hematocrit value at the date of initiation of leflunomide treatment in that dog was 26%. Although leflunomide was discontinued, that dog's anemia did not resolve until 52 days had passed. Further examinations and follow-up visit were rejected by the owner, therefore the cause of anemia and the prognosis remained unclear. Leflunomide was discontinued in additional 2 dogs because of their complications; 1 was discontinued in favor of treating intestinal lymphoma on day 471 and the other was suspected to have hemangiosarcoma on day 130.

In the present study, we found that dogs treated with leflunomide showed significantly higher response rates than those treated with cyclosporine, and the 2 groups had no significant differences in response time or recurrence rate.

There were no significant differences between CYA dogs and LEF dogs in signalment or prior prednisolone treatment. Although LEF dogs had a significantly higher response rate, the response time was not significantly different between CYA and LEF dogs. Furthermore, no significant difference was observed in the recurrence rates between CYA and LEF dogs. These results indicated that leflunomide could be used as a first-line immunosuppressive treatment of ICRP instead of cyclosporine.

Cyclosporine binds to cytoplasmic cyclophilin, and the cyclosporine-cyclophilin complex blocks the dephosphorylation of the nuclear factor of the activated T cells and decreases the expression of interleukin (IL)-2 and other cytokines, such as IL-3, IL-4, and tumor necrosis factor α [14]. Meanwhile, leflunomide is rapidly converted to its active metabolite, teriflunomide (A77-1726), possibly in the intestine, plasma, and liver [12]. Teriflunomide is a pyrimidine synthesis inhibitor via the reversible selective inhibition of dihydroorotate dehydrogenase, and it inhibits activated B and T cells [12]. And, leflunomide is also reported to inhibit migration of neutrophils and production of inflammatory cytokines in macrophages [2, 5]. These reports suggest that leflunomide have more ubiquitous effect on inflammatory cells than cyclosporine. ICRPs were pathologically reported to be mainly consists of macrophages and neutrophils as well as T and B cell [11]. Therefore, it is reasonable that leflunomide, which targets various inflammatory agent, was superior to cyclosporine, which targets solely T cells. A synergistic effect of leflunomide and cyclosporine was reported in a canine renal transplantation model [13]. The response of dogs treated with leflunomide combined with cyclosporine should be evaluated also in canine ICRP.

Adverse events were noted in 2 LEF dogs. Anorexia and vomiting were also reported in a dog treated with leflunomide for immune-mediated polyarthritis [1]. The dose of leflunomide in that dog was 3 mg/kg/day, which was reported in a previous study to be able to cause adverse events [9]. In that study, the initial starting dose of leflunomide was recommended to be 2 mg/kg/day [9]. While anemia was also reported in a previous study, the anemia resolved when leflunomide was discontinued [3]. The anemia that was found in a LEF dog in this study might not be related to the administration of leflunomide as it did not resolve when leflunomide was discontinued, and other underlying diseases might exist. These results indicated that adverse events of leflunomide are tolerable and that leflunomide can be used to treat ICRP.

One CYA dog was diagnosed as adenocarcinoma in the same region of previous ICRPs when 1,001 days had passed from initiation of cyclosporine treatment. ICRPs were suggested to progress into adenoma and adenocarcinoma during treatments [8]. Therefore, although precise correlation of immunosuppressive drugs and subsequent cancers were not elucidated, careful follow-up is necessarily in ICRPs treated with immunosuppressive drugs.

Some limitations should be considered when interpreting our study. Due to the retrospective nature of this study, treatment protocols and response assessments were not uniform in all cases. Response assessments were relatively subjective since they were assessed from either of clinical signs, rectal palpations, or endoscopic appearances. The number of cases in this study was

relatively small; hence, the results have low statistical power. Therefore, a prospective case-controlled study in a larger population is needed to compare the treatment efficacy of cyclosporine with that of leflunomide.

In summary, leflunomide showed an effect comparable to that of cyclosporine in treating ICRP. Based on the significantly higher response rate, leflunomide might become the first-line immunosuppressive drug used in treating ICRP.

REFERENCES

1. 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]
2. Cutolo, M., Sulli, A., Ghiorzo, P., Pizzorni, C., Craviotto, C. and Villaggio, B. 2003. Anti-inflammatory effects of leflunomide on cultured synovial macrophages from patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **62**: 297–302. [Medline] [CrossRef]
3. Fukushima, K., Eguchi, N., Ohno, K., Kanemoto, H., Takahashi, M., Igarashi, H., Ohmi, A., Nakashima, K. and Tsujimoto, H. 2016. Efficacy of leflunomide for treatment of refractory inflammatory colorectal polyps in 15 Miniature Dachshunds. *J. Vet. Med. Sci.* **78**: 265–269. [Medline] [CrossRef]
4. Group, V. C. O. (Veterinary Co-operative Oncology Group, VCOG) 2004. Veterinary co-operative oncology group-common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.0. *Vet. Comp. Oncol.* **2**: 195–213. [Medline] [CrossRef]
5. Kraan, M. C., de Koster, B. M., Elferink, J. G. R., Post, W. J., Breedveld, F. C. and Tak, P. P. 2000. Inhibition of neutrophil migration soon after initiation of treatment with leflunomide or methotrexate in patients with rheumatoid arthritis: findings in a prospective, randomized, double-blind clinical trial in fifteen patients. *Arthritis Rheum.* **43**: 1488–1495. [Medline] [CrossRef]
6. Murakami, A., Shibahashi, A., Iwasaki, R., Kawabe, M., Murakami, M., Sakai, H. and Mori, T. 2018. Combination chlorambucil/firocoxib or chlorambucil/prednisolone treatment for inflammatory colorectal polyps in miniature dachshunds. *J. Am. Anim. Hosp. Assoc.* **54**: 161–166. [Medline] [CrossRef]
7. 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]
8. Saito, T., Chambers, J. K., Nakashima, K., Uchida, E., Ohno, K., Tsujimoto, H., Uchida, K. and Nakayama, H. 2018. Histopathologic features of colorectal adenoma and adenocarcinoma developing within inflammatory polyps in miniature dachshunds. *Vet. Pathol.* **55**: 654–662. [Medline] [CrossRef]
9. Sato, M., Veir, J. K., Legare, M. and Lappin, M. R. 2017. A retrospective study on the safety and efficacy of leflunomide in dogs. *J. Vet. Intern. Med.* **31**: 1502–1507. [Medline] [CrossRef]
10. 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]
11. Uchida, E., Chambers, J. K., Nakashima, K., Saito, T., Ohno, K., Tsujimoto, H., Nakayama, H. and Uchida, K. 2016. Pathologic features of colorectal inflammatory polyps in miniature dachshunds. *Vet. Pathol.* **53**: 833–839. [Medline] [CrossRef]
12. Viviano, K. R. 2013. Update on immununosuppressive therapies for dogs and cats. *Vet. Clin. North Am. Small Anim. Pract.* **43**: 1149–1170. [Medline] [CrossRef]
13. Waer, M. 1998. Leflunomide, malononitriloamides, and transplantation immunology. *Transplant. Proc.* **12**: 156–164.
14. Whitley, N. T. and Day, M. J. 2011. Immunomodulatory drugs and their application to the management of canine immune-mediated disease. *J. Small Anim. Pract.* **52**: 70–85. [Medline] [CrossRef]