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# Canine Experimental Autoimmune Encephalomyelitis: Potential Therapeutic Effect of Fingolimod

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**ABSTRACT**

***Background*:** Meningoencephalitis of unknown etiology (MUE) is a representative sterile inflammatory disease of the central nervous system (CNS) in dogs. The treatment involves the use of immunosuppressive therapies. Despite intensive immunosuppressive therapy, the survival time of MUE remains short. Fingolimod is a novel immunomodulatory drug primarily used to treat human neuroinflammatory diseases such as multiple sclerosis. However, fingolimod has not yet been used in veterinary medicine. Therefore, this study aimed to identify the potential therapeutic effect of fingolimod as an alternative treatment agent for MUE in dogs, using a canine experimental autoimmune encephalomyelitis (EAE) model.
***Materials, Methods & Results*:** The canine EAE model was induced using brain tissues obtained from client-owned dog. Eight grams of forebrain tissue were homogenized in an ice bath for 5 min with phosphate-buffered saline. The resulting suspension was emulsified with an equal amount of Freund's complete adjuvant. A laboratory Beagle dog was subcutaneously injected with the homogenate in the axillary and inguinal regions (a total of 4 sites) under sedation. The dog received a booster injection 7 days later using the same procedure as the 1st injection. After 25 days of the 1st injection, the dog showed decreased activity and hyporexia. When a magnetic resonance imaging (MRI) examination was performed to determine whether the inflammatory lesion was induced, a lesion in the left white matter of the frontal lobe was identified as hyperintense in T2-weighted and fluid-attenuated inversion recovery images and hypointense to isointense in T1-weighted images. Additionally, cerebrospinal fluid (CSF) analyses revealed a slight increase in total protein concentration and severe mononuclear pleocytosis. The EAE dog was prescribed oral fingolimod [0.05 mg/kg once daily]. At 14 and 28 days post-fingolimod therapy, assessments of clinical signs and 2nd and 3rd MRIs were performed to evaluate therapeutic effectiveness. The clinical signs and most lesions were no longer observed. CSF analysis results were also normal at 14 and 28 days after the commencement of fingolimod therapy.
***Discussion*:** The canine EAE model and MUE share several key similarities, making the EAE model a valuable tool for studying MUE. Both conditions are characterized by immune-mediated inflammation of the CNS, leading to demyelination and neurological deficits. The treatment approaches for both conditions often involve the use of immunosuppressive therapies to control inflammation and prevent further neurological damage. These similarities in pathogenesis, clinical presentation, and therapeutic strategies emphasize the relevance of the EAE model in understanding the mechanisms underlying MUE and in developing effective treatments for MUE. Fingolimod was known to be well tolerated and effective, causing a swift decrease in peripheral lymphocytes without any adverse effects at oral doses ranging from 0.01 mg/kg to 0.1 mg/kg in dogs. Despite being a single case, this study evaluated fingolimod as a novel immunomodulatory agent for MUE. When applied for 4 weeks in a canine EAE model, fingolimod revealed a remarkable therapeutic effect by showing recovery of clinical signs, resolution of MRI lesions, and normalization of abnormal CSF findings. Therefore, fingolimod has shown potential as an alternative novel treatment agent for dogs with MUE.

**Keywords:** dog, EAE, fingolimod, treatment, immunomodulatory agent, meningoencephalitis, MUE, S1PR.

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## INTRODUCTION

Meningoencephalitis of unknown etiology (MUE) is a representative idiopathic sterile inflammatory disease of the central nervous system (CNS) in dogs. MUE is associated with genetic and immune-mediated processes, but it most likely has a multifactorial pathogenesis [2]. MUE is pathophysiologically associated with T cell-mediated inflammation [10]. Therefore, it is considered a naturally occurring type of multiple sclerosis, which is a chronic demyelinating disease in humans.

The treatment of MUE primarily involves the immunosuppression to mitigate CNS inflammation [4]. The standard approach includes high-dose glucocorticoids to rapidly reduce inflammation. To minimize the adverse effects associated with long-term steroid use, additional immunosuppressive agents are commonly employed. Despite intensive immunosuppressive therapy, the median survival time is short: 28-357 days with glucocorticoids alone and 240-590 days with a combination of glucocorticoids and other immunosuppressants [4]. Thus, there is a need for alternative medications to replace conventional treatment agents.

Fingolimod is an oral medication used to treat relapsing-remitting multiple sclerosis [1]. It is classified as a sphingosine-1-phosphate receptor (S1PR) modulator [9]. Fingolimod functions by binding to S1PRs on lymphocytes, preventing them from leaving the lymph nodes. This action reduces the number of lymphocytes in peripheral blood circulation, thereby limiting their ability to cause inflammation and damage in the CNS.

The objective of this study was to evaluate the therapeutic effect of fingolimod in a dog with neuroinflammation using a canine experimental autoimmune encephalomyelitis (EAE) model.

## MATERIALS AND METHODS

### *Induction of experimental autoimmune encephalomyelitis*

Brain tissue obtained from 1 client-owned dog was used. EAE was induced following the method described in a previous study [8]. The brain sample used in the present study was stored at -80°C after necropsy. Eight grams of forebrain tissue were homogenized in an ice bath for 5 min with phosphate-buffered saline (4 mL). The resulting suspension was emulsified with an equal amount of complete Freund's adjuvant[1]; each mL of complete Freund's adjuvant contained 1 mg of *Mycobacterium tuberculosis* (H37Ra, ATCC 25177), heat-killed and dried, 0.85 mL paraffin oil, and 0.15 mL mannide monooleate. A laboratory Beagle dog was subcutaneously injected with the homogenate [0.20 mL/kg] in the axillary and inguinal regions (a total of 4 sites) under sedation with alfaxalone[2] [3 mg/kg, intravenous]. The dog received a booster injection 7 days later using the same procedure as the 1st injection.

### *Fingolimod treatment*

The canine EAE model was treated with fingolimod[3] [0.05 mg/kg, PO, q24h] for 4 weeks. To evaluate the therapeutic effect, assessments of clinical signs, magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF) analysis were performed again 2 and 4 weeks after the commencement of treatment. Additionally, a complete blood count was performed to evaluate the decrease in the number of peripheral lymphocytes, which is a pharmacodynamic effect of fingolimod.

## RESULTS

### *Induction of a canine EAE model*

The EAE dog showed decreased activity and hyporexia 25 days after the 1st immunization. When an MRI examination was performed to determine whether the inflammatory lesion was induced, the lesion in the left white matter of the frontal lobe was identified as hyperintense in T2-weighted and fluid attenuated inversion recovery images and hypointense to isointense in T1-weighted images (Figure 1A-C). However, contrast enhancement was not observed (Figure 1D). CSF analyses were also performed, revealing slightly elevated total protein levels and mononuclear pleocytosis [lymphocytes 50%, monocytes 50%] (Table 1).

### *Therapeutic effect of fingolimod*

At 14 and 28 days post-fingolimod therapy, assessments of clinical signs and 2nd and 3rd MRIs were performed to evaluate therapeutic effectiveness. The clinical signs and most lesions were no longer observed (Figure 1E-L). CSF analysis results were also normal at 14 and 28 days after the commencement of fingolimod therapy, and the number of peripheral lymphocytes decreased at 14 and 28 days compared to pre-treatment (Table 1).

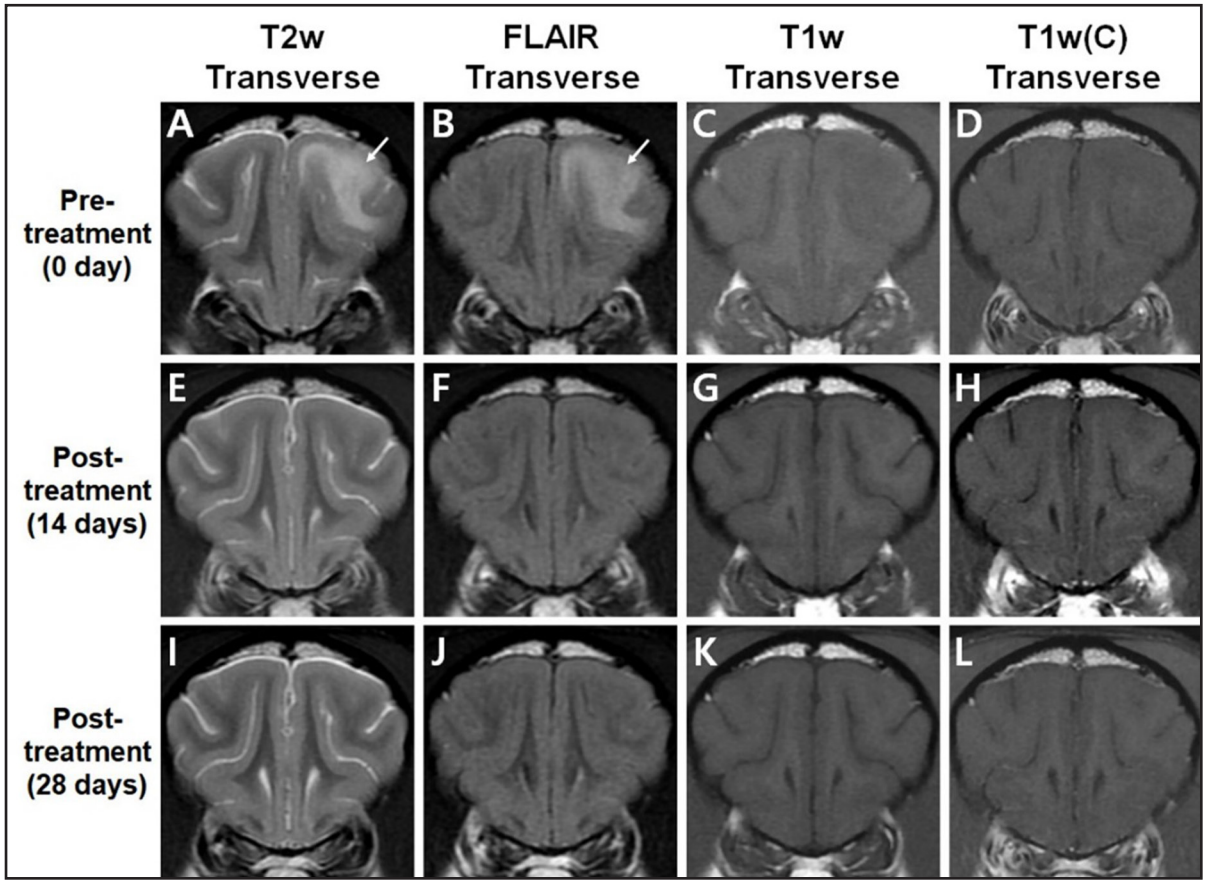


**Figure 1.** Change of MRI characteristics of a canine EAE model. A-D: The 1st MRI was acquired on the 1st neurologic sign (25 days after immunization; 0 day post-treatment). A lesion in the left white matter of the frontal lobe was identified as hyperintense (arrow) in T2w and FLAIR images and hypointense to isointense in T1w images. However, contrast enhancement was not observed. E-H: The 2nd MRI was acquired 14 days after the commencement of fingolimod therapy. The intracranial lesion observed in the first MRI was no longer identified. I-L: The 3rd MRI was acquired 28 days after the commencement of fingolimod therapy. Similarly, the intracranial lesion observed in the 1st MRI was no longer identified. [FLAIR: fluid-attenuated inversion recovery; MRI: magnetic resonance imaging; T1w: T1-weighted; T1w(C): post-contrast T1-weighted; T2w: T2-weighted].

**Table 1.** Changes in findings of CSF and lymphocytes count after fingolimod treatment in a canine EAE model**.**

| | | Pre-treatment (0 day) | Post-treatment (14 days) | Post-treatment (28 days) | Reference range |
|---|---|---|---|---|---|
| CSF | TP (mg/dL) | 30 | 10 | 10 | < 25 |
| | TNCC (cells/µL) | 120 | 2.2 | 0 | <5 |
| | Cytology | L 50% M 50% | - | - | - |
| CBC | Lymphocytes ($10^3$/ µL) | 2.26 | 0.32 | 0.36 | 1.05 – 5.10 |

CBC: complete blood count; CSF: cerebrospinal fluid; EAE: experimental autoimmune encephalomyelitis; L: lymphocytes; M: monocytes; TNCC: total nucleated cell count; TP: total protein.

## DISCUSSION

Despite being a single case, this study evaluated fingolimod as a novel immunomodulatory agent for MUE. When applied for four weeks in a canine EAE model, fingolimod revealed a remarkable therapeutic effect by showing recovery of clinical signs, resolution of MRI lesions, and normalization of abnormal CSF findings. Therefore, fingolimod has

shown potential as an alternative novel treatment agent for dogs with MUE.

The canine EAE model and MUE share several key similarities, making the EAE model a valuable tool for studying MUE. Both conditions are characterized by immune-mediated inflammation of the CNS [8], leading to demyelination and neurological deficits. In EAE, the disease is induced by the introduction of CNS antigens, resulting in an autoimmune response that closely mimics the pathophysiological processes observed in MUE [3]. Histopathologically, both conditions exhibit perivascular cuffing, infiltration of mononuclear cells, and demyelination, which are hallmark features of autoimmune CNS disorders. Additionally, the treatment approaches for both conditions often involve the use of immunosuppressive therapies to control inflammation and prevent further neurological damage. These similarities in pathogenesis, clinical presentation, and therapeutic strategies emphasize the relevance of the EAE model in understanding the mechanisms underlying MUE and in developing effective treatments for MUE.

Fingolimod has shown significant therapeutic effects in the treatment of relapsing-remitting multiple sclerosis, a chronic neuroinflammatory disease [1]. Multiple sclerosis is characterized by the immune system attacking the CNS, leading to demyelination and neurodegeneration. Fingolimod acts as a S1PR modulator, sequestering lymphocytes in lymph nodes, thereby preventing them from entering the CNS and contributing to inflammatory damage [9]. In patients with relapsing-remitting multiple sclerosis, fingolimod has been proven to reduce the frequency of relapses, slow the progression of disability, and decrease the number of active lesions in the brain and spinal cord, as seen on MRI scans [6]. By limiting the infiltration of autoreactive lymphocytes into the CNS, fingolimod helps preserve the integrity of myelin, the protective sheath around nerve fibers, and thus maintains better neural function [5].

In preclinical studies, fingolimod has demonstrated significant therapeutic effects in various neuroinflammatory and neurodegenerative conditions using both mouse and rat model [7,13]. In mouse models of EAE, a widely accepted model for multiple sclerosis, fingolimod effectively reduced clinical symptoms, inflammation, and demyelination by modulating lymphocyte trafficking via S1PR [7]. Similarly, in rat models of ischemic stroke, fingolimod reduced infarct volume and improved neurological outcomes by increased repair of microvascular injury and enhancing neuronal survival [13]. These findings suggest that fingolimod's dual action as an immunomodulator and neuroprotective agent makes it a promising candidate for treating CNS disorders beyond multiple sclerosis, potentially extending its utility to stroke, spinal cord injuries, and neurodegenerative diseases. These preclinical outcomes pave the way for further exploration of fingolimod's therapeutic scope in veterinary medicine.

Research on fingolimod in dogs has demonstrated promising therapeutic potential, particularly for treating immune-mediated conditions such as MUE [11,12]. Previous studies have shown that S1PR1, the main target receptor of fingolimod, is expressed in leukocytes, and the expression of S1PR1 on endothelial cells, astrocytes, oligodendrocytes, and neurons is increased in inflammatory brain lesions [12]. Additionally, fingolimod was well tolerated and effective, causing a swift decrease in peripheral lymphocytes, neutrophils, and eosinophils without any adverse effects at oral doses ranging from 0.01 mg/kg to 0.1 mg/kg [11]. Clinically, this translated to improvements in neurological signs and the resolution of lesions and abnormal CSF findings in a canine EAE model. Overall, results on fingolimod in a canine EAE model suggest it is a promising therapeutic option for managing MUE in dogs, paving the way for further studies and potential clinical applications in veterinary medicine.

The main limitation of this study is that the efficacy of fingolimod was experimentally evaluated using only a single canine EAE model. Therefore, further studies using a larger sample of canine EAE models are necessary before applying fingolimod to dogs with MUE.

## CONCLUSION

In conclusion, fingolimod showed an improvement in neuroinflammation in a canine EAE model by demonstrating resolution of clinical signs, MRI lesions, and abnormal CSF findings. Therefore, fingolimod can be used as an alternative treatment agent in dogs with MUE.

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***Ethical approval*.** This study received approval from the Institutional Animal Care and Use Committee (CBNUA-1466-20-01) at the Laboratory Animal Research Center, Chungbuk National University.

***Declaration of interest*.** The authors declare no conflicts of interest. The authors are solely responsible for the content and writing of the article.

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