Pharmacology Discovery Services

A Eurofins Discovery Partner Lab

Myelin Oligodendrocyte Glycoprotein (MOG_{35-55})-Induced Experimental Autoimmune Encephalomyelitis (EAE) Model in Mice

Multiple sclerosis (MS) is an autoimmune disorder mediated by an immune attack of autoreactive T cells directed at the myelin sheath of nerves. This leads to a progressively degenerating disorder of the central nervous system (CNS), producing inflammation and neurodegeneration.

Experimental autoimmune encephalomyelitis (EAE) is the animal model of MS and can be induced by various encephalitogenic peptides, including oligodendrocyte glycoprotein (MOG). EAE induced by MOG_{35-55} or MOG_{1-125} represents the chronic progressive form of MS. Our scientists have successfully validated the MOG_{35-55}-induced EAE in female C57BL/6 mice in our AAALAC-accredited laboratory. Our validated model utilizes Fingolimod, a modulator of sphingosine-1-phosphate (S1P) receptors, approved to treat MS and shown to be effective in MOG-EAE model[1,2].

Procedure Summary

EAE Induction and Administration

Groups of five (5) sham (no treatment) or ten (10) female C57BL/6 mice are immunized for EAE induction according to the standard procedure below. Hooke kit #EK-2110 is used for antigen emulsion injection (MOG_{33-35}) at two sites per mouse (0.1 mL/site, SC) on Day 0. Pertussis toxin (PTX, 0.1 mL) is injected intraperitoneally (IP) on Day 0 and Day 1.

Vehicle or Fingolimod (0.1, 0.3, 1, or 3 mg/kg) is administered by oral gavage (PO) once daily for 21 days, from Day 0 to Day 20.

Study Outline and Endpoint Measurement

The study outline is shown in Figure 1.

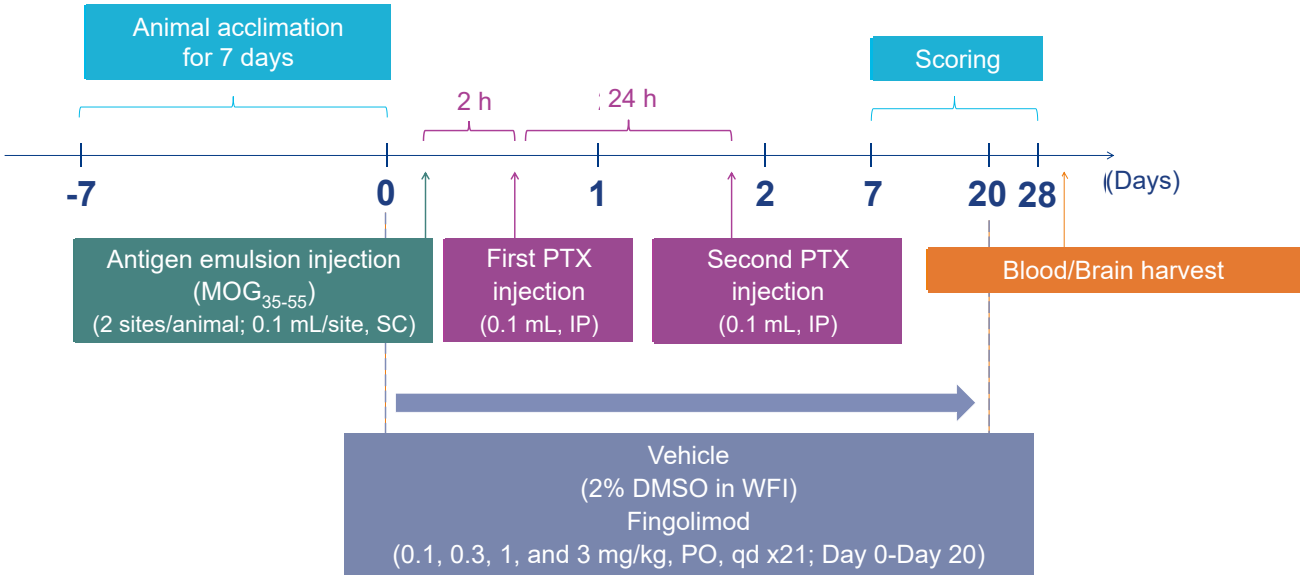

Figure 1. Outline of MOG-EAE study in mice.

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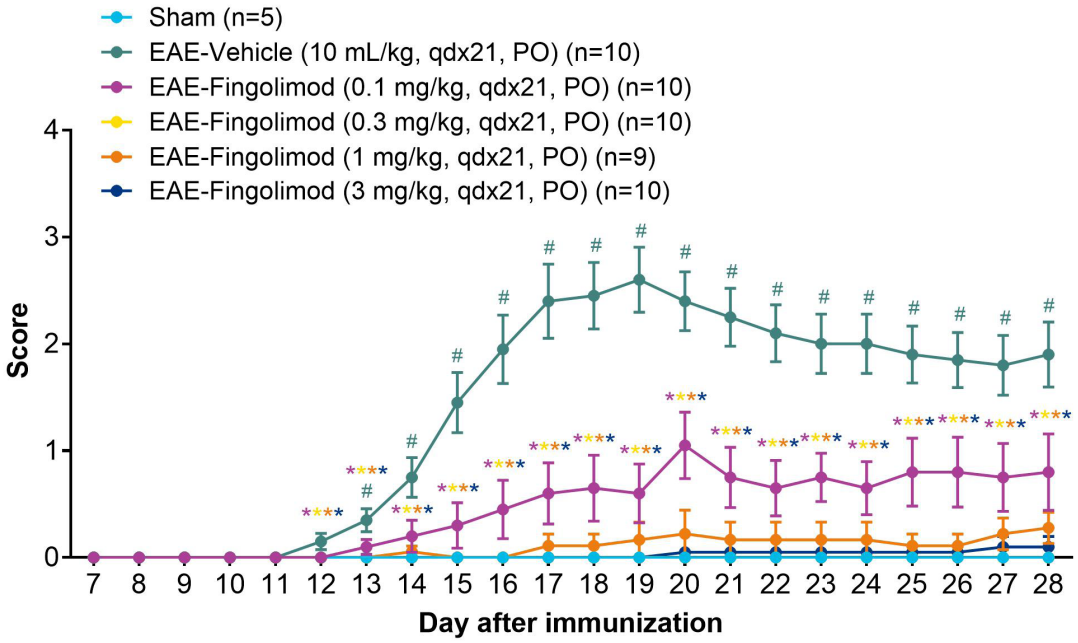
The body weight is recorded daily. The clinical scoring is performed daily starting from Day 7 post-immunization, according to the standard scoring criteria for EAE[1,2]. Behavioral testing, including Rotarod can also be conducted at weekly intervals.

At termination on Day 29, blood is collected by cardiac puncture for hematology, and some blood can be processed into serum for biomarker measurement. Brain is harvested for histopathological evaluation. Spleen and draining lymph nodes, as well as spinal cord, can also be collected for additional analyses such as total cell and differential counts, fluorescence-activated cell sorting (FACS), and immunohistochemistry (IHC).

The animals will be humanely euthanized during the study if they are moribund or meet the criteria for euthanasia based on the clinical endpoints.

Results and Discussions

Consistent with literature reports, the clinical signs of disease became evident around Day 12 post-immunization in the vehicle-treated animals. The signs worsened over time, peaked around Day 19, and then stayed progressive after a partial recovery (Figure 2) to at least Day 60[3]. Fingolimod was administered at different dose levels (0.1, 0.3, 1, or 3 mg/kg), given orally (PO) prophylactically starting on Day 0, and produced a dose-dependent suppression of clinical scores from Day 12 to Day 28 (Figure 2), resulting in both delayed onset of EAE and reduced mean maximum score (MMS). The reduction in body weight in vehicle-treated animals was also dose-dependently improved from Day 15 to Day 29 by Fingolimod treatment (not shown).



#p < 0.05, vs. sham control; unpaired Student's *t-test*;
**p* < 0.05, vs. vehicle control; one-way ANOVA followed by Dunnett's test.

Figure 2. Daily clinical score (Day 7 to Day 28).

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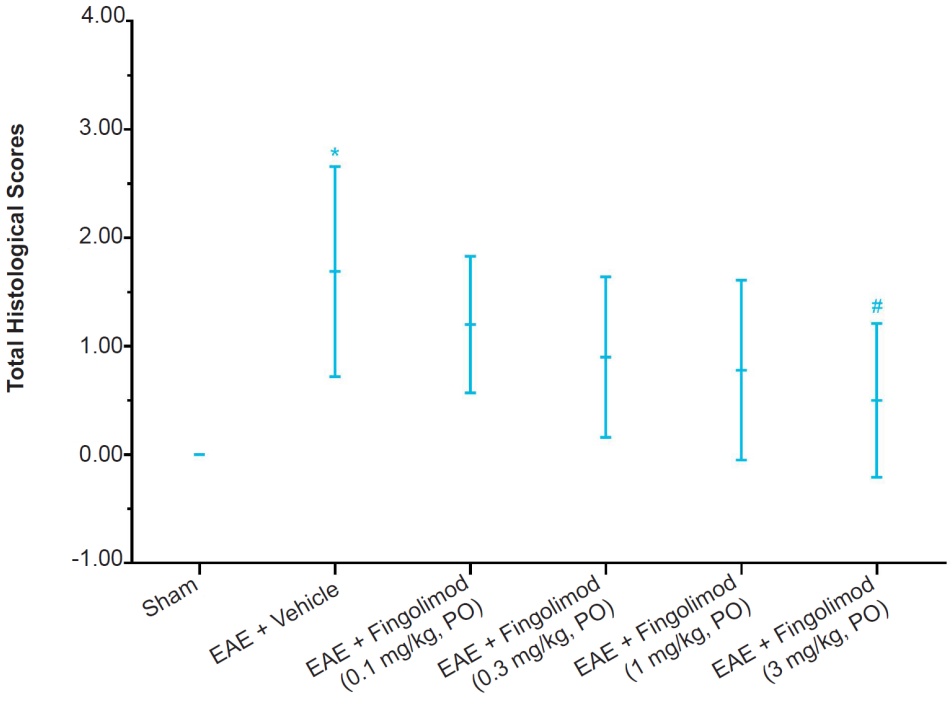
Histopathological evaluation of the brain collected from vehicle-treated animals revealed severe focal inflammatory cell infiltration and focal necrosis when compared to the sham control group (Figure 3). Fingolimod treatment dose-dependently reduced the histopathological scores, producing a statistically significant effect at 3 mg/kg (Figure 3).

In summary, our PDS science team successfully developed the EAE model induced by MOG_{33-35} in C57BL/6 mice. We also validated the model using an approved and clinically effective drug Fingolimod, which was shown to be effective in treating EAE disease in a dose-dependent manner. The MOG-EAE model can be used to study the (auto)immune mechanisms involved in the initiation and development of MS and as the first-pass screening assay for testing the therapeutical potentials of new MS drug candidates.



*$p < 0.05$, Vehicle (Group 2) vs. No treatment (Group 1); unpaired Student's *t-test*;
#$p < 0\ 05$, Treated vs Vehicle (Group 2); one-way ANOVA followed by Dunnett's test.

Figure 3. Histological scores of brains collected on Day 29.

References

1. Bonfiglio T, *et al.*, Prophylactic versus Therapeutic Fingolimod: Restoration of Presynaptic Defects in Mice Suffering from Experimental Autoimmune Encephalomyelitis. *PLoS One.* 2017 Jan 26;12(1):e0170825. doi: 10.1371/journal.pone.0170825. PMID: 28125677; PMCID: PMC5268435. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5268435/

2. Rossi S, *et al.*, Oral fingolimod rescues the functional deficits of synapses in experimental autoimmune encephalomyelitis. *Br J Pharmacol.* 2012 Feb;165(4):861-9. doi: 10.1111/j.1476-5381.2011.01579.x. PMID: 21740406; PMCID: PMC3312484. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3312484/

3. Aharoni R, *et al.*, Neurogenesis and neuroprotection induced by peripheral immunomodulatory treatment of experimental autoimmune encephalomyelitis. *J Neurosci.* 2005 Sep 7;25(36):8217-28. doi: 10.1523/JNEUROSCI.1859-05.2005. PMID: 16148229; PMCID: PMC6725544. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6725544/

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