Comparison of fingolimod and nod-like receptor protein 3 inflammasome inhibitor treatment on disease progression in experimental autoimmune encephalomyelitis model

PS03-2198

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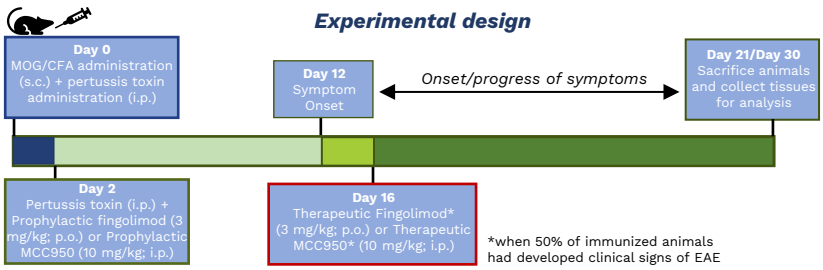
INTRODUCTION

Experimental autoimmune encephalomyelitis (EAE) is a representative animal model of multiple sclerosis (MS) that exhibits the hallmarks of the human disease, such as central nervous system (CNS)-related histopathological findings, motor deficits and neuroinflammation. The immunomodulator fingolimod, a sphingosine 1-phosphate (S1P) modulator, is the first oral drug approved for the MS treatment and has been widely used in the EAE model. Fingolimod crosses the blood-brain barrier and prevents the egress of lymphocytes to the CNS. The involvement of the nod-like receptor protein 3 (NLRP3) inflammasome in the onset and development of MS has been demonstrated in animal models of EAE, making it a potential therapeutic target for MS treatment.

This study aimed to evaluate the efficacy of fingolimod treatment, administered in both prolonged and shortened regimens, and the selective NLRP3 inflammasome inhibitor (MCC950) on disease progression, motor function and levels of key biomarkers in the mouse model of EAE.

METHODS

C57BL/6J female mice (6-8 weeks old) were immunised with myelin oligodendrocyte glycoprotein (MOG) peptide (35-55) emulsified in complete Freund's adjuvant on day 0 (s.c.) and injected with pertussis toxin on days 0 and 2 (i.p.). Control mice were administered vehicle (PBS) at the same time points. Clinical scoring was performed daily by two independent investigators in a blinded manner. EAE scores were evaluated as follows: 0.5, paralysis up to half of the tail; 1, complete tail paralysis; 2, complete tail paralysis and slight difficulty in gait; 3, complete tail paralysis and severe difficulty in gait; 3.5, paralysis of one hindlimb; 4, paralysis of both hindlimbs; 5, hindlimb and forelimb paralysis. The mice were randomly divided into experimental groups (n=8-14).



RESULTS

Fingolimod Treatment (30 Day)

Naive vs EAE vehicle: *p<0.05, **p<0.01, ***p<0.001
Naive vs EAE therapeutic: *p<0.05, **p<0.01, ***p<0.001
EAE prophylactic vs EAE vehicle: #p<0.05, ##p<0.01
EAE prophylactic vs EAE therapeutic: #p<0.05, ##p<0.01, ###p<0.001
EAE vehicle vs EAE therapeutic: &p<0.05

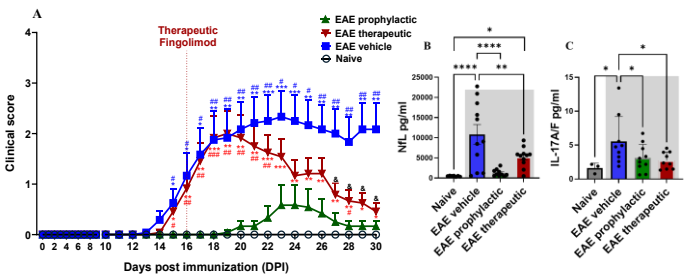


Fig. 1. (A) Clinical EAE scores are represented as mean ± SEM. All significant differences are shown in the graph above and calculated using Two-way ANOVA followed by Fisher's LSD test. Naïve: n=8, EAE Vehicle: n=12, EAE prophylactic: n=12, EAE therapeutic: n=12.
(B) NfL and (C) IL-17 A/F in plasma were quantified using the Mesoscale discovery platform. All data are presented as mean ±SEM and were analysed by One-way ANOVA with Fisher's LSD test post hoc. NfL: Naïve: n=7, EAE Vehicle: n=9, EAE prophylactic: n=10, EAE treatment: n=11. IL-17 A/F: Naïve: n=3, EAE Vehicle: n=8, EAE prophylactic n=9, EAE treatment: n=10.

Prolonged (14-day) therapeutic fingolimod administration resulted in a significant reduction in the clinical outcome of immunised mice. Concurrent with the improvement in clinical symptoms the levels of key plasma biomarkers were altered (NfL & IL-17A/F).

MCC950 Treatment (21 Day)

Naive vs EAE vehicle: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
Naive vs EAE therapeutic: *p<0.05
Naive vs EAE prophylactic: *p<0.05, **p<0.01
EAE prophylactic vs EAE vehicle: #p<0.05

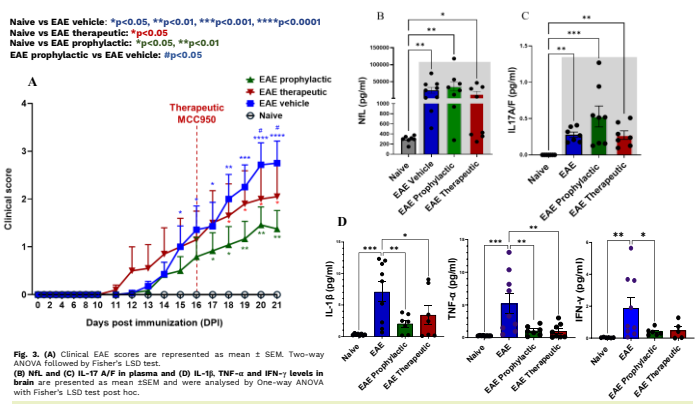


Fig. 3. (A) Clinical EAE scores are represented as mean ± SEM. Two-way ANOVA followed by Fisher's LSD test.
(B) NfL and (C) IL-17 A/F in plasma and (D) IL-1β, TNF-α and IFN-γ levels in brain are presented as mean ±SEM and were analysed by One-way ANOVA with Fisher's LSD test post hoc.

Prophylactic MCC950 treatment led to a reduction in pro-inflammatory cytokine levels in the CNS and an improvement in clinical scores in contrast to therapeutic (5-day) MCC950 treatment.

Fingolimod Treatment (21 Day)

Naive vs EAE vehicle: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
Naive vs EAE therapeutic: *p<0.05, **p<0.01
EAE prophylactic vs EAE vehicle: #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001
EAE prophylactic vs EAE therapeutic: #p<0.05, ##p<0.01

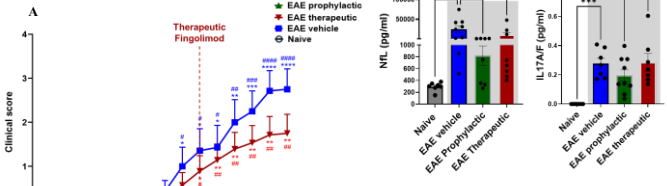
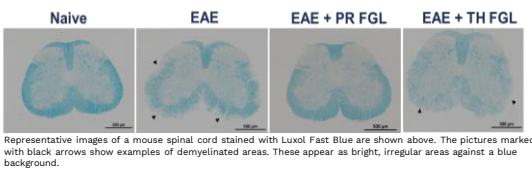


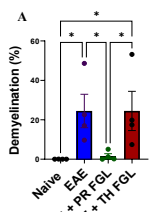
Fig. 2. (A) Clinical EAE scores are represented as mean ± SEM. All significant differences are shown in the graph above and calculated using Two-way ANOVA followed by Fisher's LSD test. Naïve: n=12, EAE Vehicle: n=14, EAE prophylactic: n=13, EAE therapeutic: n=14.
(B) NfL and (C) IL-17 A/F in plasma were quantified using the Mesoscale discovery platform. All data are presented as mean ± SEM and were analysed by Kruskal-Wallis with Uncorrected Dunn's test post hoc. NfL: Naïve: n=7, EAE Vehicle: n=9, EAE prophylactic: n=8, EAE treatment: n=8. IL-17 A/F: Naïve: n=7, EAE Vehicle: n=7, EAE prophylactic n=9, EAE treatment: n=7.

Shortened (5-day) therapeutic fingolimod administration did not improve the clinical outcome or reduce the levels of key biomarkers (NfL and IL-17A/F) in immunized mice.

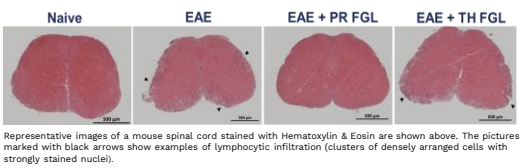
Spinal Cord Demyelination



Representative images of a mouse spinal cord stained with Luxol Fast Blue are shown above. The pictures marked with black arrows show examples of demyelinated areas. These appear as bright, irregular areas against a blue background.
PR FGL – Prophylactic Fingolimod ; **TH FGL** – Therapeutic Fingolimod



Spinal Cord Inflammatory Cell Infiltration



Representative images of a mouse spinal cord stained with Hematoxylin & Eosin are shown above. The pictures marked with black arrows show examples of lymphocytic infiltration (clusters of densely arranged cells with strongly stained nuclei).

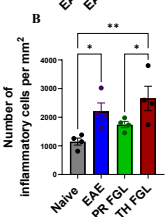


Fig. 4. (A) Demyelination (%) and (B) Number of inflammatory cells per mm² were analysed by One-way ANOVA test with Fisher's LSD test post hoc. All data are presented as **mean ±SEM**. Outliers were removed using ROUT (Q=5%), n=4 per group, *p<0.05; **p<0.01.

The degrees of demyelination and inflammatory cell infiltration were elevated in EAE-vehicle and EAE–therapeutic fingolimod-treated mice. Prophylactic fingolimod treatment effectively prevented spinal cord demyelination but did not inhibit inflammatory cell infiltration.

CONCLUSIONS

- **The present study demonstrates the efficacy of both fingolimod and MCC950 administration on clinical severity in the mouse EAE model, depending on the duration and regimen of treatment.**
- **Prolonged (14-day) fingolimod therapy significantly improved clinical outcomes and key biomarker levels in mice, in contrast to the shortened (5-day) treatment regimen.**
- **The results highlight the visible link between clinical outcomes, levels of key biomarkers and spinal cord histology in the mouse EAE model.**

The authors declare no conflict of interest