# The Effect of Glatiramer Acetate Treatment on Mitochondrial Fission/Fusion in EAE

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## INTRODUCTION

The approved disease modifying therapies (DMTs) for multiple sclerosis (MS) primarily target inflammation rather than neurodegeneration even though the latter is more closely linked to disability.

Some MS DMTs, such as glatiramer acetate (GA), may reduce neurodegeneration in MS, but the mechanism for that effect is not fully understood.

Mitochondrial dysfunction appears to play a key role in other neurodegenerative conditions and could play a role in MS (1,2).

One indicator of mitochondrial stress and dysfunction is changes in mitochondrial fission and fusion.

The balance of mitochondria dynamics (fusion/fission events and changes in mitochondrial subcellular distribution) is controlled by several dynamin-family GTP-binding proteins.

Dynamin-linked proteins (Drp/DNM) are required for mitochondrial fission along with fission1 (Fis1) (3, 4). On the other hand, three proteins are associated with mitochondrial fusion namely mitofusin 1 (MF-1), mitofusin2 (MF-2) and optic atrophy 1 (Opa1) (5,6,7).

## OBJECTIVE

❖**The aim of the present study is to investigate the effect of GA treatment on mitochondrial stress as evidenced by changes in fission and fusion.**

## METHODS

**Induction of EAE:** EAE was induced in 10 weeks old C57 BL/6J mice with 200 µg of myelin oligodendrocyte glycoprotein 35–55 ($MOG_{35-55}$) peptide (Biomer Technology, CA, USA) in an equal volume of complete Freund's adjuvant containing Mycobacterium tuberculosis H37RA (Difco,, MI, USA). On days 0 and 2, a total of 200 ng of pertussis toxin (SIGMA) was injected i.p.

**Clinical Scoring:** Mice were examined everyday for signs of EAE and were graded on a 0-5 scale of increasing severity: 0- No abnormality, 1-Floppy tail, 2-Floppy tail with moderate hind limb weakness, 3-Severe hind limb paralysis, 4- complete hind limb paralysis and 5- Death.

**Drug:** GA 150 µg/mouse/day was injected subcutaneously everyday following onset of symptoms(Score >1). Mice were euthanized after 20 days of GA treatment.

**Pathological Analysis of spinal cord specimens:** 7 µm thick paraffin embedded spinal cord sections were stained with hematoxylin and eosin (H&E) to detect inflammatory infiltrates in EAE and slides were stained for Luxol Fast Blue to examine demyelination following standard protocols.

**Immunohistochemistry:** Immunohistochemistry was performed using VECTASTAIN Elite ABC Kits (#PK-6100 (Vector Laboratories, Burlingame, CA) following standard protocols.

**Immunofluorescence:** Immunofluorescence was performed on paraffin embedded tissue sections following standard protocols.

**Statistical Analysis:** Statistical analyses were performed by Prism software (GraphPad, San Diego, CA). Data are provided as mean ± SEM. In all experiments, a *P* value of <0.05 was defined as statistically significant.

## RESULTS

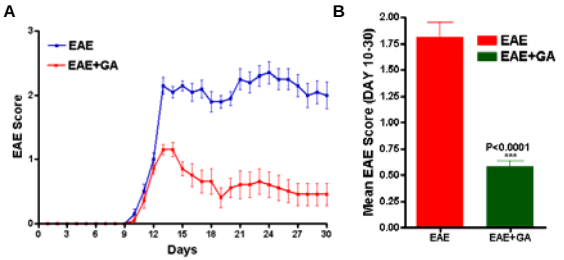



**FIGURE 1: Glatiramer Acetate suppresses clinical severity of EAE.** **(A)** Clinical disease scores in EAE and EAE+GA animals over a 30-d period. **(B)** Mean score for all animals in each group over during active phase of the disease (Day 10-30). The mean score was significantly lower in EAE+GA mice compared with WT EAE mice; t-test. Mice were euthanized on Day 30 and pathological analysis was done on lumbar sections of spinal cord.

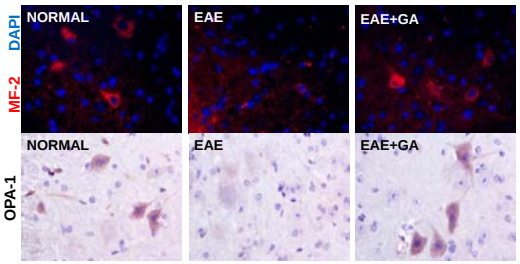
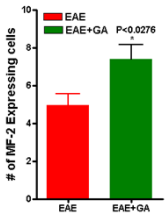



**Figure 4: Glatiramer Acetate treated EAE mice show increased mitochondrial Fusion in the spinal cord.** Sections stained with antibody against Mitofusin-2 (MF-2) showed a significant increase (P<0.0276) in expression of MF-2 (RED) in EAE+GA mice compared to EAE mice. Sections stained with antibody against OPA-1 (represents Fusion) showed an increase in expression of OPA-1 in EAE+GA mice compared to EAE mice. Original magnification: X 400 . t-test, N=4/group



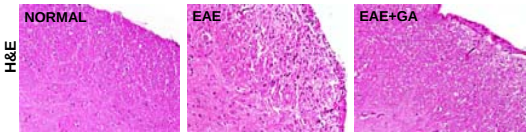




**Figure 2: Glatiramer Acetate treated EAE mice show decreased inflammation in the spinal cord.** Fewer inflammatory pockets and inflammatory cells are seen in EAE+GA mice compared with EAE mice. Normal mice show no inflammatory infiltrates. Original magnification: X 200

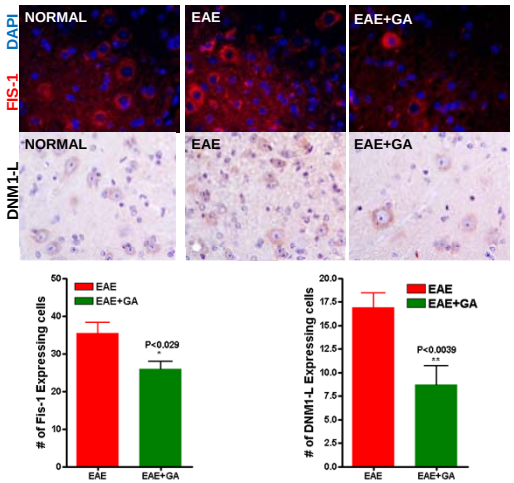



**Figure 5: Glatiramer Acetate treated EAE mice show decreased mitochondrial Fission in the spinal cord**. Sections stained with antibody against Fission 1 (Fis-1) showed a significant decrease (P<0.029) in expression of Fis-1 (RED) in EAE+GA mice compared to EAE mice. Sections stained with antibody against DNM1-L (represents Fission) showed a significant decrease (P<0.0039) in expression of DNM1-L in EAE+GA mice compared to EAE mice. Original magnification: X 400 . t-test; N=4/group

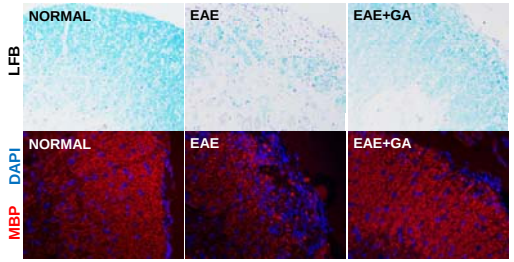



**Figure 3: Glatiramer Acetate treated EAE mice show decreased demyelination in the spinal cord**. Luxol Fast Blue (LFB) was used to stain Myelin in the spinal cord (Upper Panel). Demyelination was decreased in GA treated EAE mice compared to untreated EAE mice. Normal mice do not show any demyelination. Sections stained with antibody against Myelin Basic protein (MBP) (Lower panel) showed decreased myelin (RED) in EAE compared to EAE+GA. Also, decreased myelin corresponds to increased inflammation. (Blue). Original magnification: X 200

## Conclusions:

- ✓ In this study, we show that GA treatment actively attenuates the symptoms of disease progression in EAE.
- ✓ Our data also demonstrate that GA treatment suppresses inflammation and demyelination in EAE.
- ✓ Our study showed that GA treatment increases mitochondrial fusion (MF-2 and OPA-1) and decreases fission (Fis1 and DNM1-L), thereby playing a crucial role in regulating mitochondrial dynamics.
- ✓ These discoveries highlight the importance of organelle reorganization in neuronal cells, thus promoting exploration of impact of mitochondrial dynamics with special reference to GA treatment in MS.

**References:**

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**Grant Support:**

❖ TEVA Pharmaceuticals(Investigator Sponsored Study).