Preventing oxidative stress-induced changes in mitochondrial dynamics - Investigation of neuroprotective effects of S1P receptor modulators *ex vivo* using a semi-automated live imaging set-up

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Background

Mitochondrial alterations in neuroinflammation

In multiple sclerosis (MS), **oxidative stress** leads to **mitochondrial alterations**[1,2]. Siponimod and fingolimod are **sphingosine-1-phosphate (S1P) receptor modulators**. Siponimod is approved for treating secondary progressive MS due to its anti-inflammatory and less understood **neuroprotective effects**[3]. Fingolimod was reported to prevent oxidative stress-induced changes in mitochondrial morphology[4].

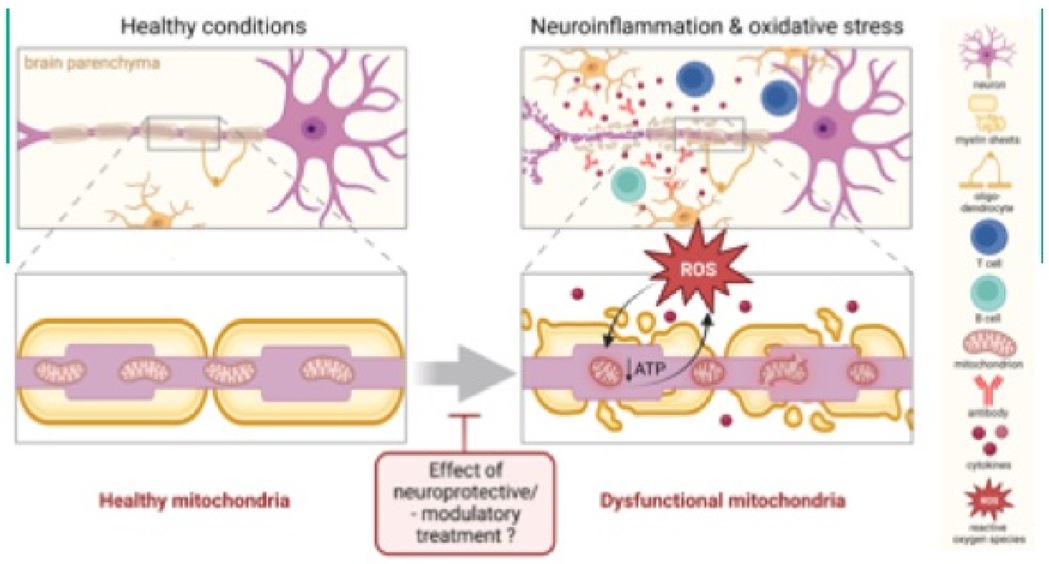

Figure 1. During neuroinflammation, immune cells and activated microglia produce reactive oxygen species that trigger changes in neuronal mitochondrial function and dynamics, contributing to neuroaxonal damage and disease progression in multiple sclerosis[5,6].

Aim

To investigate **siponimod's potential neuroprotective effect** on neuronal mitochondrial dynamics in oxidatively stressed living brain tissue using a **semi-automated image analysis** and to compare it to the effects of fingolimod.

Methods

Mitochondrial live imaging in chronic organotypic hippocampal slice cultures with excellent long-term survival[7]

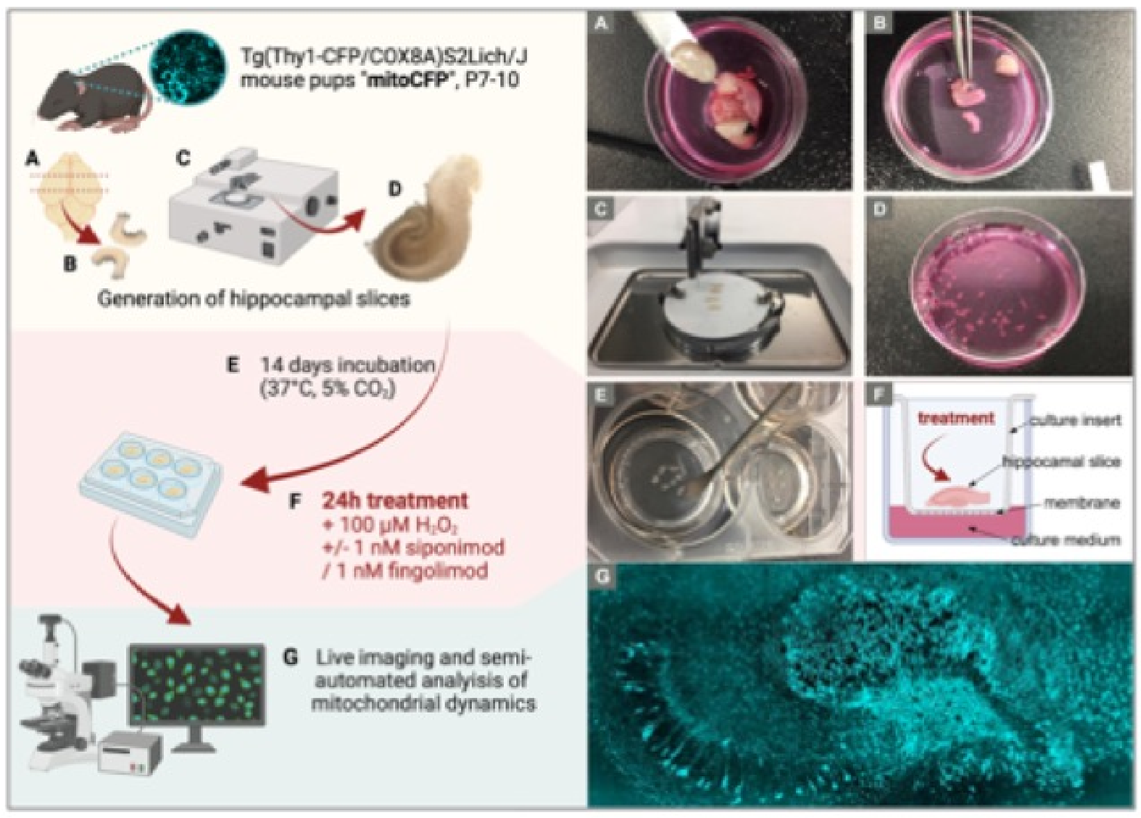

Figure 2. Overview of experimental *ex vivo* set-up – mitoCFP mice. A Coronal separation of mouse brain. **B** Isolation of hippocampi. **C** Slicing of hippocampi using a McIlwain Tissue Chopper. **D** 350 µm thick slices in sterile culture medium. **E** Slice incubation on membrane culture inserts for 14 days. **F** 24h treatment with hydrogen peroxide (H_2O_2) +/- siponimod or fingolimod; untreated slices serve as negative control. **G** Live imaging of fluorescent neuronal mitochondria in 2min time lapses using spinning disc confocal microscopy; semi-automated, randomized analysis of mitochondrial dynamics (12 regions of interest (ROI)/condition, ImageJ software).

Results

Prevention of oxidatively induced changes in mitoch. morphology

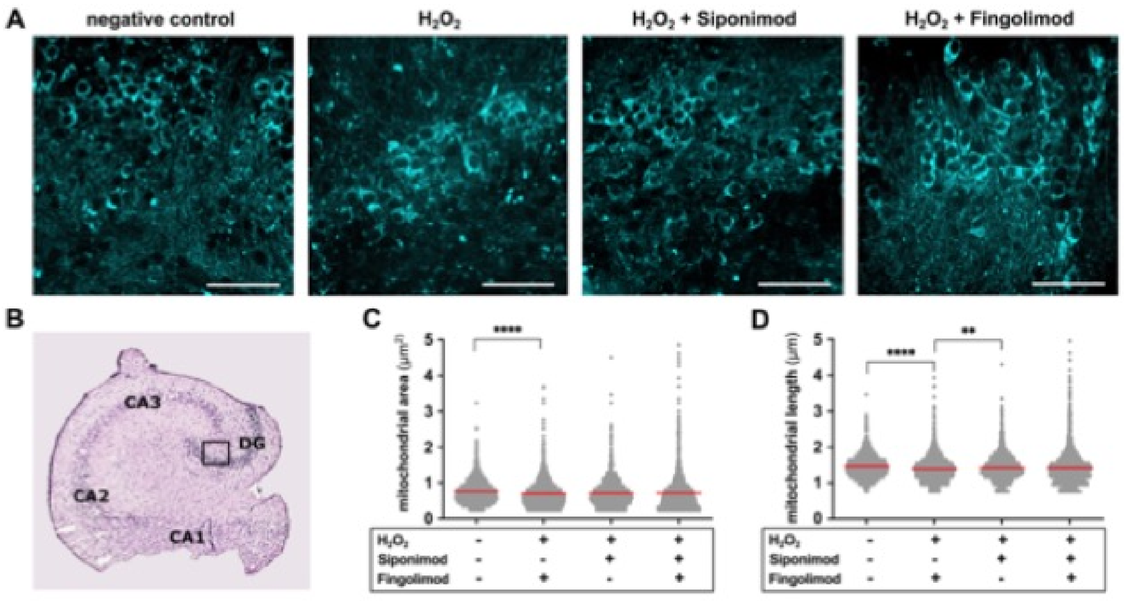

Figure 3. Analysis of mitochondrial area and length. A Differences between conditions are not apparent upon visual inspection. *Scale bars: 50 µm.* **B** H&E stained hippocampal section indicating the dentate gyrus (DG) as ROI for live imaging. **C+D** Semi-automated analysis: under oxidative stress, mitochondria were shorter and smaller compared to the neg. control. Siponimod prevented alterations in mitochondrial length; for fingolimod a similar trend was observed. *Kruskal-Wallis test followed by Dunn's post hoc test. P values < 0.05 were considered significant.*

Prevention of oxidatively induced alterations in mitoch. motility

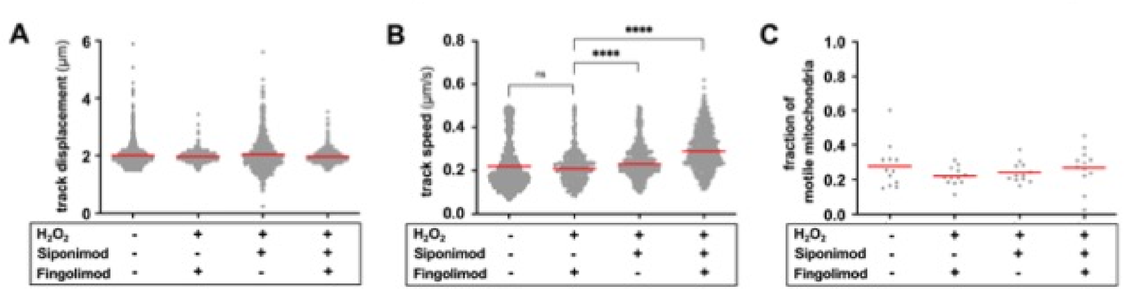

Figure 4. Analysis of mitochondrial track displacement, speed and fraction of motile mitochondria. A Under oxidative stress, mitochondria covered smaller distances compared to neg. controls. Siponimod partly prevented the decrease in track displacement, while fingolimod did not. **B** Both compounds increased track speed, while H_2O_2 alone did not. **C** The fraction of motile mitochondria decreased upon H_2O_2; in slices treated with siponimod or fingolimod, fractions were similar to those of neg. controls. *Kruskal-Wallis test followed by Dunn's post hoc test. P values < 0.05 were considered significant.*

Summary of ROI means from semi-automated image analysis

Parameters (unit)	negative control	H_2O_2	H_2O_2 + Siponimod	H_2O_2 + Fingolimod
mitochondrial length (µm)	1,469	1,400	1,433	1,426
mitochondrial area (µm²)	0,755	0,698	0,711	0,713
track displacement (µm)	2,009	1,968	2,037	1,962
track speed (µm/s)	0,219	0,219	0,244	0,296
fraction of motile mitochondria	0,277	0,220	0,241	0,269
n of motile mitochondria	4693	3600	4328	4325

Conclusion

Our *ex vivo* results confirm previous findings on oxidative stress-induced morphological mitochondrial alterations[1,2].

At 1 nM, **both S1P receptor modulators partially prevented oxidative stress-induced mitochondrial alterations** in living brain tissue *ex vivo*.

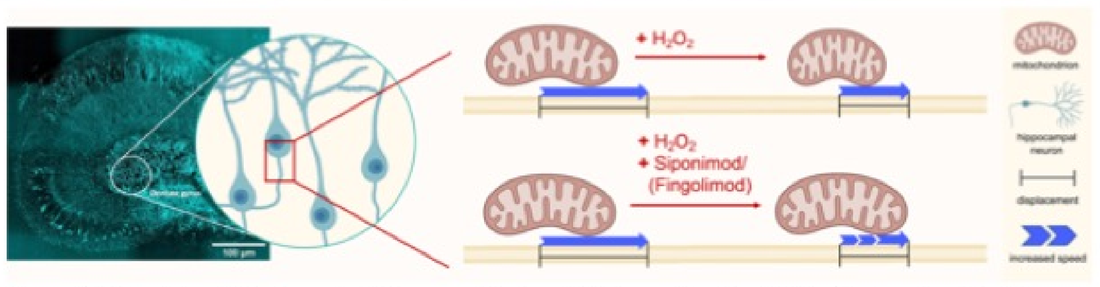

Figure 5. Overview of treatment effects on mitochondrial dynamics. In addition, the fraction of motile mitochondria after treatment with siponimod or fingolimod approached control values.

References

[1]Malla B et al. Teriflunomide preserves peripheral nerve mitochondria from oxidative stress-mediated alterations. *Ther Adv Chronic Dis.* 2020;11:2040622320944773.; [2]Malla B et al. Teriflunomide Preserves Neuronal Activity and Protects Mitochondria in Brain Slices Exposed to Oxidative Stress. *Int J Mol Sci.* 2022;23(3).; [3]Cohan SL et al. The Two Sides of Siponimod: Evidence for Brain and Immune Mechanisms in Multiple Sclerosis. *CNS drugs.* 2022;36(7):703-19.; [4]Martín-Montañez E et al. The S1P mimetic fingolimod phosphate regulates mitochondrial oxidative stress in neuronal cells. *Free Radic Biol Med.* 2019;137:116-30.; [5]Federico A et al. Mitochondria, oxidative stress and neurodegeneration. *Journal of the Neurological Sciences.* 2012;322(1):254-62.; [6]Barcelos IP et al. Mitochondrial Dysfunction and Multiple Sclerosis. *Biology.* 2019;8(2).; [7]Anderhalten L et al. Different Impact of Gadopentetate and Gadobutrol on Inflammation-Promoted Retention and Toxicity of Gadolinium Within the Mouse Brain. *Investigative radiology.* 2022.
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