Siponimod Demonstrates Pro-remyelination Effects in the Mouse Cuprizone-Intoxication Model

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Introduction

- Siponimod is the first oral disease-modifying therapy shown to reduce disability progression, cognitive worsening, and total brain volume loss versus placebo in SPMS patients[1]
- Recent clinical observations, using MRI to assess the MTR and T2-weighted signal intensity (T2-WSI), revealed pro-myelination effects for siponimod[2]
- Hence, siponimod appears to be an ideal pharmacological tool to validate/invalidate preclinical mechanistic models for the translational study of remyelination processes

Objective

- To assess the relevance of the cuprizone-intoxication model for studying the pro-remyelination effects of siponimod

Methods

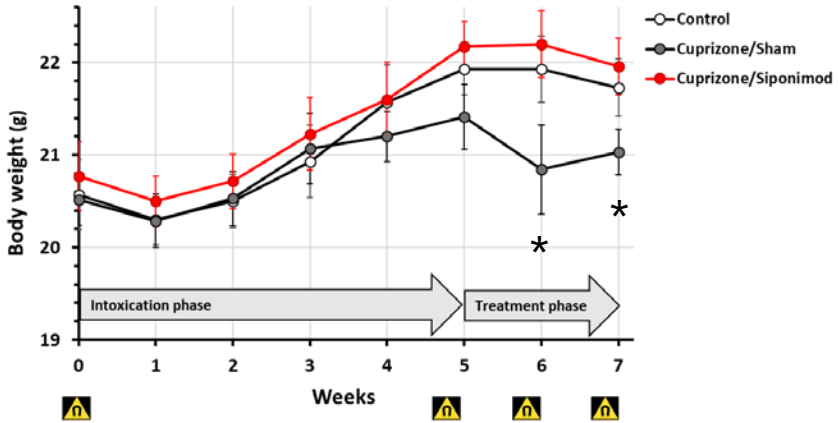
- Typically, cuprizone administration in mice (5 weeks; 0.2% in diet – intoxication phase) produces robust signs of demyelination in the brain caudal CC, visualized by MRI (MTR and T2-WSI)
- Upon stopping cuprizone administration, slow spontaneous remyelination can be observed by quantitative immuno-histochemistry (qIHC) for myelin density (Luxol Fast Blue staining [LFB]) and oligodendrocyte numbers (GST-π+ cells) in brain sections, and can lead to a change in the MRI parameters
- This study compared the level of CC-myelination in:
  - Control mice (non-intoxicated)
  - Cuprizone-intoxicated mice subsequently treated with siponimod at the end of intoxication (via a drug-loaded diet [10 mg/kg of food])
  - Cuprizone-intoxicated mice subsequently treated with drug-free diet (sham) at the end of intoxication

CC, corpus callosum; GSTπ+, glutathione S-transferase pi immunoreactive; MRI, magnetic resonance imaging; MTR, magnetization transfer ratio

1. Kappos L, et al. *Lancet*. 2018;391:1263–1273; 2. Arnold DL et al, presented at *ACTRIMS-ECTRIMS* 2020. P0587

Results

Longitudinal body weight changes



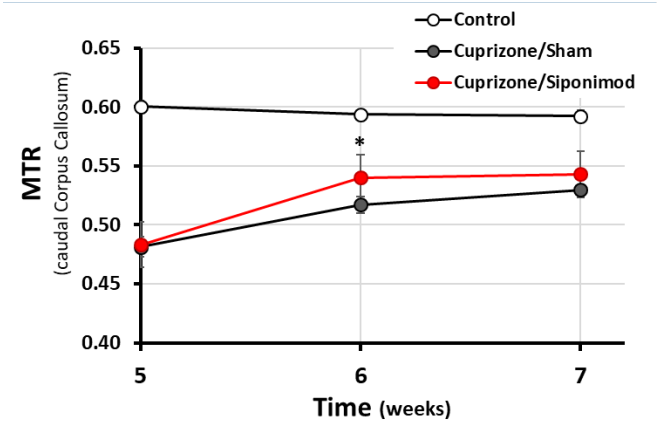
- During the cuprizone-intoxication phase, no particular health issue was detected and similar longitudinal bodyweight changes were observed in the three study groups

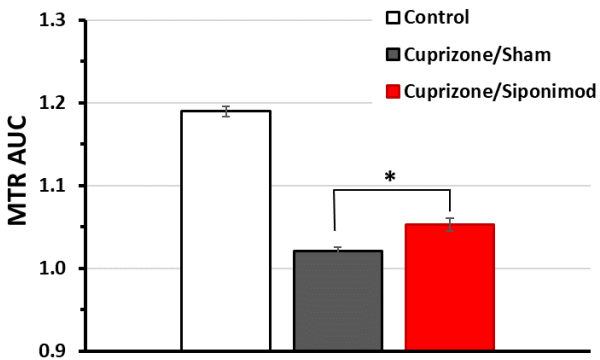
- At the end of the intoxication phase, mean body weight was similar for all 3 groups:
  - 21.9±0.4, 21.4±0.4 g, and 22.2±0.3 g for the control, cuprizone-sham, and cuprizone-siponimod groups, respectively
- Over the following 2 weeks, the mice that received drug-free pellets (cuprizone-sham group) showed a slight but significant loss in bodyweight versus controls
- This was not observed in mice receiving siponimod-loaded pellets (cuprizone-siponimod group):
  - In this group, the mean siponimod concentrations measured in blood and brain homogenates were within the expected ranges; i.e. 0.4±0.0 µM and 2.7±0.7 nmol/g (equivalent to 2.7±0.7 µM), confirming the success of the treatment

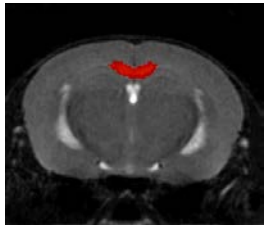
**P<0.05*

Results

Longitudinal MRI monitoring for MTR changes in the caudal CC







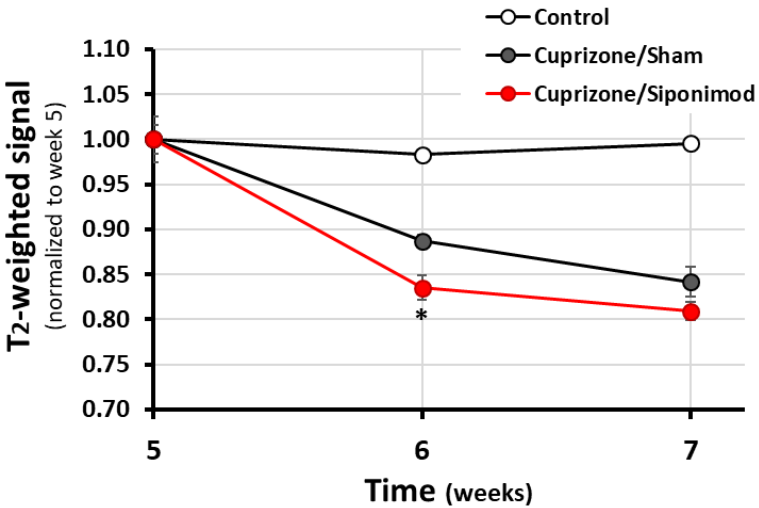
Area in red, marks the caudal CC

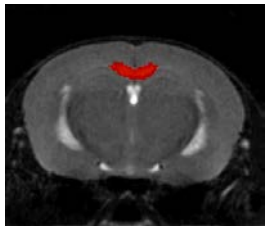
- After 5 weeks of cuprizone diet, all intoxicated mice showed marked demyelination within their caudal CC, as indicated by a ~25% reduction in mean MTR versus controls
- Upon cuprizone washout, sham mice showed spontaneous remyelination, as revealed by MTR recovery of ~40% and ~52% at Week 6 and Week 7, respectively
- Siponimod treatment significantly accentuated the MTR recovery to ~59% and 63% at Week 6 and Week 7, respectively
  - This suggests a ~20% increase in remyelination versus sham mice (as indicated by AUC analysis; $p<0.05$)

*$P<0.05$; AUC, area under the curve; CC, corpus callosum; MRI, magnetic resonance imaging; MTR, magnetization transfer ratio

Results

Longitudinal MRI monitoring of the T2-weighted signal in the caudal CC





Area in red, marks the caudal CC

- After 5 weeks of cuprizone diet, the marked demyelination observed within the caudal CC of intoxicated mice was associated with a significant 24% increase in the T2-weighted signal
- At Week 5, the T2-weighted signal was normalized for all mice (represented by the value of 1.00 on the Y-axis opposite)
- In control (non-intoxicated) mice, no significant changes in T2-weighted signal were observed at Week 6 and 7
- In the cuprizone-sham group (intoxicated drug-free treated mice), the T2-weighted signal was reduced by ~11% and 18% at Week 6 and Week 7, respectively, in line with the spontaneous remyelination indicated by the MTR readout
- In the cuprizone-siponimod group, this reduction in the T2-weighted signal reached ~18% and 23% at Week 6 and Week 7, respectively, suggesting a ~30% increase in recovery vs sham mice ($p<0.05$)
- These results were confirmed by terminal quantitative immunochemistry for changes in myelin density (LFB staining) and in numbers of oligodendrocyte (GSTπ+ cells) in the CC

*$P<0.05$; AUC, area under the curve; CC, corpus callosum; GSTπ+, glutathione S-transferase pi immunoreactive; LFB, luxol fast blue; MTR, magnetization transfer ratio; vs, versus

Conclusions

- Following cuprizone intoxication, and relative to sham-fed mice, siponimod treatment accelerated spontaneous remyelination
- Monitoring periods longer than 2 weeks are required to achieve a full recovery
- **The present data suggest the mouse cuprizone-intoxication to be a relevant mechanistic model for exploring siponimod-sensitive remyelination processes**

Affiliations

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Disclosures

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The final responsibility for the content lies with the authors.

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