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# Central Versus Peripheral Drug Exposure Ratio, A Key Parameter for Therapeutic Efficacy of S1P Receptor Modulators in SPMS

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## Introduction

- Siponimod is the first and only oral S1P receptor (S1PR) modulator shown to reduce disability progression, decline in cognitive processing speed and total brain/grey matter volume loss in patients with secondary progressive multiple sclerosis (SPMS)[1,2,3]
- Preclinical models show that siponimod penetrates the central nervous system (CNS) and suggest that efficacy on processes such as remyelination follow a bell-shaped dose response for S1PR modulators in the CNS, with a loss of S1PR modulator efficacy at supramaximal CNS drug exposures (>2–3 mM in brain homogenate)[4]

## Objective

- To understand how S1PR modulators enter the CNS, what determines their central efficacy and what differentiates siponimod from other S1PR modulators

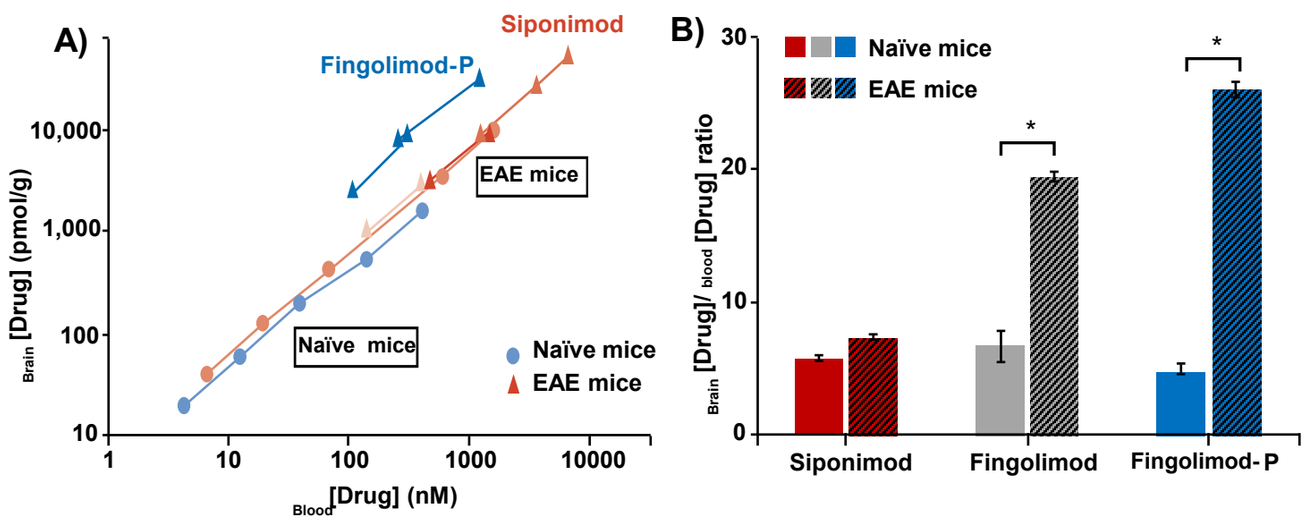
## Methods

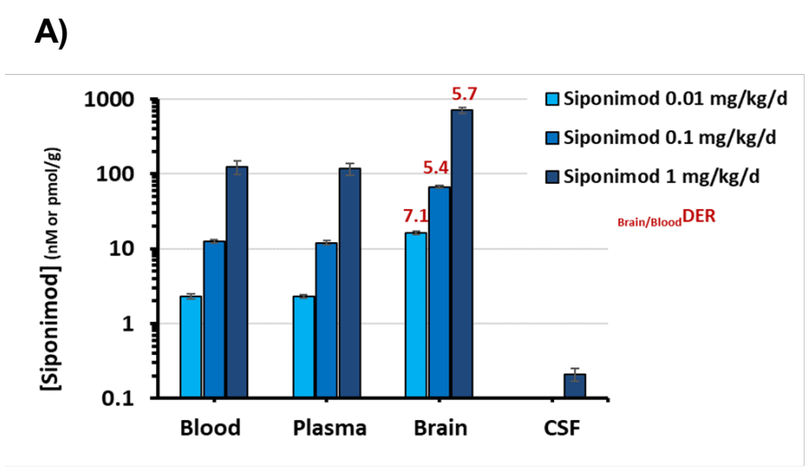
- Pharmacological profiles for S1PR modulators were reviewed and put in perspective with recent preclinical pharmacokinetic/pharmacodynamic results

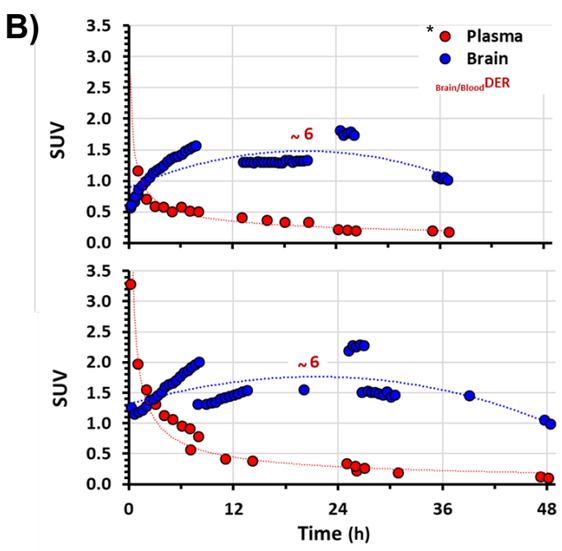
## Results

- Cellular assays indicate that siponimod is not a substrate for active efflux transporters (**Table 1**), suggesting that CNS penetration is driven by high lipophilicity ($logD_{7.4}$ = 2.6) and passive membrane diffusion
- In healthy siponimod-treated mice, dose-proportional steady-state total drug levels in brain tissues and blood were observed, with a CNS-to-blood drug exposure ratio ($_{CNS/Blood}$DER) of around 6 (**Figure 1**); similar results are observed in healthy rats and non-human primates (**Figure 2**)
- In healthy mice, fingolimod and its active metabolite (Fingolimod-P) achieved comparable dose-proportional drug exposures in brain and blood, with a $_{CNS/Blood}$DER around 6 (**Figure 1**), regardless of a higher lipophilicity ($logD_{7.4}$ = 4.5)
- Although not corrected for protein binding (≥99.7%), these observations suggest that CNS transport of fingolimod is regulated via active mechanisms; this is supported by the fact that fingolimod is a well-established substrate for P-glycoprotein (P-gp)[5], an ATP-dependent efflux transporter acting at the blood-brain barrier to protect the CNS from exogenous molecules (**Table 1**)
- Importantly, in mice subjected to experimental autoimmune encephalomyelitis (EAE), the resulting disease is associated with a marked increase in fingolimod CNS penetration ($_{CNS/Blood}$DER >20) (**Figure 1**) and reduced P-gp activity; in contrast, siponimod was found not to be a P-gp substrate and to maintain a $_{CNS/Blood}$DER around 6 in EAE mice (**Figure 1**)
- Ozanimod is known to be a weak P-gp substrate with a $_{CNS/Blood}$DER of 10–16 in healthy rodents while the impact of EAE has not been described

**Figure 1: A) Dose-proportional steady-state siponimod levels in blood and brain homogenates from healthy and EAE mice fed for 10 days with siponimod- or fingolimod-loaded food pellets at various concentrations (0.1 to 200 mg per kg of food); for both treatments, maximal efficacy against EAE development is obtained at the food-loading dose of 10 mg/kg (i.e., CNS/Blood levels of ~3/0.5 mM and ~5/0.2 mM for siponimod and fingolimod-P, respectively)**
**B) Mean $_{CNS/Blood}$DER of siponimod, fingolimod and fingolimod-P in naïve vs EAE mice**




CSF, cerebrospinal fluid; SPECT, single-photon emission computed tomography; SUV, standard uptake value

**Figure 2: A) Dose-dependent siponimod levels in rats that were treated orally for 7 days with siponimod at 0.01, 0.1, and 1 mg/kg/day (n=3/group; blood, plasma, and CSF concentrations are expressed in nM and brain levels in pmol/g [equivalent to nM])**
**B) Quantitative SPECT imaging for assessing the levels of siponimod analog ([123I]MS565) in *rhesus macaques* (n=2) after one single IV bolus injection (decay-corrected time–activity curves expressed as SUV). In both cases, drug washout from the plasma started immediately upon injection and was nearly complete (>90%) within 24 h. In contrast, peak brain uptake occurred within 18–24 h post injection, with a $_{CNS/Blood}$ DER of about 6**

**Table 1: A) Inhibitory potency (IC50 values) of siponimod on efflux pumps in cellular assays (CaCO2 cells transfected with a particular human efflux pump); B) Uptake characteristics of siponimod**

| A<br>Human efflux pump | Multidrug-resistance protein 1 (MDR1/P-gp / ABCB1) IC50 (µM) | Breast cancer resistant protein (BCRP / MXR / ABCG2) IC50 (µM) | Bil salt export pump (BSEEP / ABCB11) IC50 (µM) | Multidurg-resistance protein 2 (MRP2 / ABCC2) IC50 (µM) |
|---|---|---|---|---|
| | No inhibition observed up to: | | | |
| Siponimod | 25 mM | 25 mM | 40 mM | 50 mM |

| B | $CL_m$[a] (µL/min/mg) | $K_{c.max}$[b] (µL/min/mg) | $V_m$[c] (pmol/min/mg) | $K_{m.ap}$[d] (µL/min/mg) | Possible transporter(s) |
|---|---|---|---|---|---|
| Siponimod | ~ 3.5 | na | na | na | na |

na=not applicable
a) Estimated nonspecific (passive) uptake clearance based on "non-corrected" uptake data (n=6); b) Estimated maximal carrier-mediated (saturable) uptake clearance; c) Maximum transporter uptake velocity/rate (pmol/min/mg protein); d) Proposed based on the inhibition profile observed in the presence of Fumitremorgin C (MXR inhibitor) and Cyclosporine A (MDR1 inhibitor)

## Conclusions

- **Siponimod has a P-gp independent $_{CNS/Blood}$DER ~6, across animal species**
- **Considering the bell-shaped dose-response curve for central efficacy of S1PR modulators, $_{CNS/Blood}$DER appears to be a potentially critical differentiator vs other S1P modulators that enables a dual mechanism of action**
- **This hypothesis helps to explain siponimod's therapeutic benefit in both the periphery (on inflammatory measures) and in the CNS (on measures of myelin content and tissue integrity), as observed in clinical studies of SPMS patients**

## References

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