Siponimod treatment of chronic EAE induces an altered microglial phenotype

Short title: Effects of siponimod treatment in chronic EAE

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Microglial activation represents a prominent feature of progressive disease in multiple sclerosis (MS) and is linked to neurodegenerative processes. The sphingosine-1-phosphate receptor modulator siponimod is a CNS-penetrating drug approved for active secondary progressive MS.

In this study, the impact of siponimod treatment on the phenotype of microglia was investigated in experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis. EAE was induced in wild type C57BL/6 mice aged 8-12 weeks with MOG35-55 peptide emulsified in CFA and with pertussis toxin. Siponimod treatment was started 20 days post immunization, after the peak of disease was reached. Mice were fed ad libitum with vehicle food or food containing 3 mg, 10 mg or 30 mg siponimod/kg. After 2 months of treatment, mice were sacrificed; brain and spinal cord were isolated either for histological analysis or ex vivo analysis of microglia using flow cytometry. Additionally, peripheral immune cell compartments (blood, lymph nodes and spleen) were characterized with flow cytometry.

Therapeutic siponimod treatment lead to a disease amelioration associated with reduced demyelination, a prominent downregulation of microglial MHC class II expression and a reduced CNS infiltration of CD3+ cells. A significant reduction of T cells in all peripheral immune cell compartments was observed. Clinical disease severity correlated with the degree of CNS T cell infiltration. Furthermore, siponimod induced a moderate downregulation of PD-L1 and CD69 in microglia. Only minor or no effects were observed in the microglial expression of CX3CR1 and CD200R, molecules involved in the interaction of microglia with neurons.

Siponimod treatment in chronic EAE changed the phenotype of microglia with a downregulation of markers associated with microglial activity and antigen presentation. The correlation of CNS T cell infiltration and disease severity indicated that this effect was at least partly mediated by the inhibition of T lymphocyte migration to the CNS.

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