AKP-11 - A novel S1P1 agonist with favorable safety profile attenuates experimental autoimmune encephalomyelitis in rat model of Multiple Sclerosis

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Abstract

Sphingosine-1-phosphate receptor 1 (S1P1) mediated regulation of lymphocyte egress from lymph organs is recognized as the mechanism of FTY720 (Fingolimod, Gilenya) efficacy in relapse-remitting forms of multiple sclerosis (RRMS). In this study we describe a novel S1P1 agonist, AKP-11, most generation of S1P1 agonists with immunomodulatory activities in cell culture model and for therapeutic efficacy against an animal model of MS, i.e., experimental autoimmune encephalomyelitis (EAE) but without the adverse effects observed with FTY720. Like FTY720, AKP-11 bound to S1P1 is internalized and activates intracellular AKT and ERK1/2 cellular signaling pathways. In contrast to FTY720, AKP-11 mediated S1P1 downregulation is independent of sphingosine kinase activity indicating it to be a direct agonist of S1P1. The S1P1 loss and inhibition of lymphocyte egress by FTY720 leads to lymphopenia. In comparison with FTY720, oral administration of AKP-11 caused milder and reversible lymphopenia while providing a similar degree of therapeutic efficacy in the EAE animal model. Consistent with the observed reversible lymphopenia with AKP-11, the S1P1 agonist AKP-11 reduced total lymphocytes from observed cell counts in comparison with control animals. Consistent with previous observations, FTY720 treatment is associated with adverse effects of bradycardia and lung edema in rodents, whereas AKP-11 treatment had undetectable effects on bradycardia and reduced lung water as compared to FTY720. Taken together, the data suggest that AKP-11 treatment cause milder and reversible lymphopenia with milder adverse effects while maintaining therapeutic efficacy similar to that observed with FTY720, thus indicating therapeutic potential of AKP-11 for treatment of MS and related autoimmune disorders.

Objectives

To investigate two S1P1 agonists (FTY720 and AKP-11) in rat model of EAE for clinical efficacy and their adverse effects in lymphopenia, bradycardia and lung vascular permeability.

Methods

EAE induced rats treated with vehicle or FTY720 (1mg/kg) or AKP-11 (1.3mg/kg) were scored for clinical disease manifestation. Peripheral lymphocyte count was measured for different groups of lymphocytes as well as for reversal of lymphopenia following drug termination. Lungs were investigated for lung vascular permeability was examined by Evan’s blue dye method and basic heart rate and blood pressure were investigated by non-invasive tail-cuff method. Spinal cord sections were analyzed by Lacto-indigo blue (LFB) staining for lymphocyte morphology and infiltration. For mechanism of action, viable S1P1 expressing CHO cell line was used to determine AKP-11 or FTY720 induced S1P1 internalization, turnover and cell signaling activities.

Results

AKP-11 and FTY720 treatments attenuate EAE disease in the Lewis rat

(A) AKP-11 and FTY720 treatments attenuate disease in Lewis rats. S1P1 receptor expression is upregulated in the disease state. Therefore, we will examine the possibility of therapeutic efficacy of AKP-11 and FTY720 to upregulate the expression of S1P1 in the disease state.

AKP-11 withdrawal increases cell surface expression of S1P1 receptor

(A) Control and EAE animals were treated with AKP-11 (3 or 3.3mg/kg) or FTY720 (3mg/kg) for 14 days starting onset of clinical disease progression. Spinal cord tissue was fixed and infiltration of mononuclear cells was examined by H&E staining and for demonstration of T-cell. AKP-11 was performed. (B) Infiltrated CD4 cells in the spinal cord were analyzed by RT-PCR. (C) Western blotting for AKP-11 and FTY720-treated mice was performed. (D) EAE and SCI models were subjected to FTY720 or AKP-11.

Conclusion

Both FTY720 and AKP-11 provide efficacy against clinical disease of EAE and reduction in disease activity score.

Both FTY720 and AKP-11, as S1P1 agonists induce similar cellular mechanisms such as inhibition of proinflammatory cytokines and ERK1/2 and AKT signaling pathways.

Both FTY720 and AKP-11 provide efficacy against EAE via inhibition of S1P1 mediated lymphocytes and hence decreased infiltration of activated immune cells into the CNS. However, lymphopenia induced by AKP-11 was milder and transient (quickly reversible) as compared to the one induced by FTY720.

Accordingly, FTY720 treatment cause serious adverse effects whereas AKP-11 treatment produced much milder adverse effects of bradycardia and lung vascular leakiness when compared to FTY720.

These findings indicate that AKP-11 mediated S1P1 agonist activity may be of therapeutic value for MS and other immune mediated disorders with better safety profile.

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