Enhancing mesenteric lymphatic transport to target the sphingosine-1-phosphate receptor

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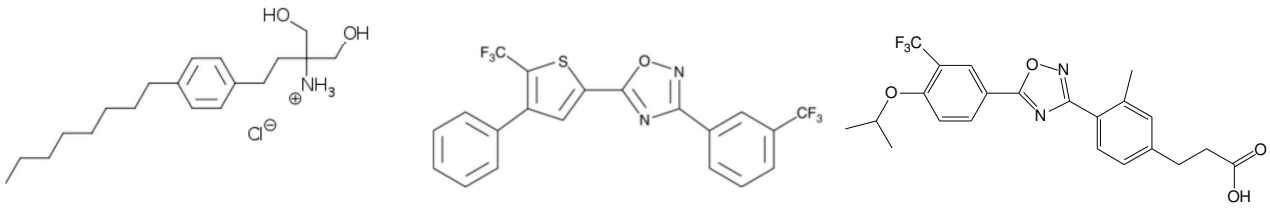
Introduction: Endogenous sphingosine-1-phosphate (S1P) stimulates lymphocyte egress from lymph nodes via activation of S1P1 receptors on lymphocytes and the lymphatic endothelium. Administration of S1P receptor modulators (S1PRMs) can block lymphocyte egress from lymph nodes, resulting in a reversible systemic lymphopenia. Fingolimod is an S1PRM in current clinical use for the treatment of multiple sclerosis. However, finglomod has a number of off-target toxicities. Here we investigate the potential to enhance the activity and reduce the systemic toxicity of immunomodulators such as fingolimod by targeting drug delivery to lymphocytes in the mesenteric lymphatics via drug incorporation into intestinal lipid transport pathways. This strategy is attractive for S1PRMs due to their site of action. We therefore compared the lymphatic transport and receptor binding affinities of fingolimod with SEW2871 (a more lipophilic S1PRM and, therefore, one with a greater likelihood of lymphatic transport) in order to investigate the pharmacodynamic (PD) benefit of targeting the delivery of S1PRM to the lymphatics.

Method: Fingolimod and SEW2871 were administered orally or intraduodenally to rats in lymph-directing lipid formulations or lipid free formulations. Drug concentrations in the mesenteric lymph and systemic blood (HPLC-MS) and systemic leukocyte, lymphocyte and neutrophil counts were subsequently measured over time after drug dosing. These data were compared with *in vitro* S1P1 binding affinity data for fingolimod and SEW2871. To further confirm the relationship between lipophilicity, potency and lymphatic transprt, a SEW2871 analogue was synthesised where the lipophilic $–CF_3$ aryl substitution on the head group of SEW2871 was changed to a more polar –COOH group (Figure 1), with the expectation that this would reduce lymphatic transport, but may enhance receptor binding

Results: The lymphatic transport of fingolimod was negligible. In contrast, the lymphatic transport of SEW2871 was extremely high (>40% dose), and unusually, was independent of the formulation with which it was co-administered. Systemic lymphopenia was dose-dependent after oral administration of SEW2871 and occurred at doses as low as 1.5-10 mg/kg. This was surprising given that the effective dose of fingolimod in rats is only approximately 10 times lower (0.05-0.5 mg/kg) and yet SEW2871 has a 1000 fold lower EC_{50} at the S1P1 receptor. The data suggest that lymphatic transport may promote the activity of S1PRMs. As expected the –COOH substitution significantly increased S1P1 receptor affinity (>1000 times) but completely diminished lymphatic transport (<1% total dose).

Conclusion: The *in vivo* activity of SEW2871 (lymphopenia) is far greater than might be predicted based on *in vitro* receptor binding affinity at S1P1. The current data suggest that this may reflect improved delivery to the site of action (lymphatics and lymph nodes) via promotion of lymphatic transport. These findings highlight the potential to improve PD outcomes for S1PRMs through strategies that seek to optimize both the receptor binding of S1PRMs and to promote access to sites of action in the lymphatics. Future studies will further refine the structure-transport-activity relationships for SEW2871 analogues and better investigate the mechanism of lymphatic transport of SEW 2871.



Fingolimod **SEW2871** **SEW2871 -COOH analogue**

Figure 1. Chemical structures of fingolimod, SEW2871 and the –COOH analogue analogue