Abstract # 2211

A NOVEL GLYCOMIMETIC COMPOUND (GMI-1757) WITH DUAL FUNCTIONAL ANTAGONISM TO E-SELECTIN AND GALECTIN-3 DEMONSTRATES INHIBITION OF THROMBUS FORMATION IN AN INFERIOR VENA CAVA MODEL

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Background and Introduction

E-selectin functions in venous thrombosis by binding and activating host cells to initiate the coagulation cascade. The E-selectin antagonist, Uproleselan (GMI-1271), has been shown to attenuate thrombus formation following electrical stimulation in a preclinical inferior vena cava (IVC) model without significantly affecting hemostasis (Culmer DL et al. Thromb Haemost. 117:1171-1181, 2017). A recent study has reported that galectin-3 (gal-3) and gal-3 binding protein are associated with murine thrombogenesis where they interact at the thrombus-vein wall interface, and that gal-3 may be contributing to thrombosis through proinflammatory mechanisms (Elise P et al. Blood 125:1813-1821, 2015). Collectively these studies suggest that pharmacologic targeting of both E-selectin and gal-3 function may afford a new class of therapeutics for treatment of venous thrombosis. In the present studies, we report on the activity of a novel glycomimetic compound with dual functional antagonism of E-selectin and gal-3, and demonstrate its anti-thrombotic activity in an IVC model.

Results

Figure 1. GMI-1757 Antagonizes E-selectin and Galectin-3 Interactions

GMI-1757 (dual E-selectin/galectin-3 antagonist) was assessed for inhibition of sialyl LeX binding to immobilized E-selectin (top panel) and of galectin-3 binding to immobilized Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ (bottom panel). IC50's (μM) were determined and summarized in Table 1.

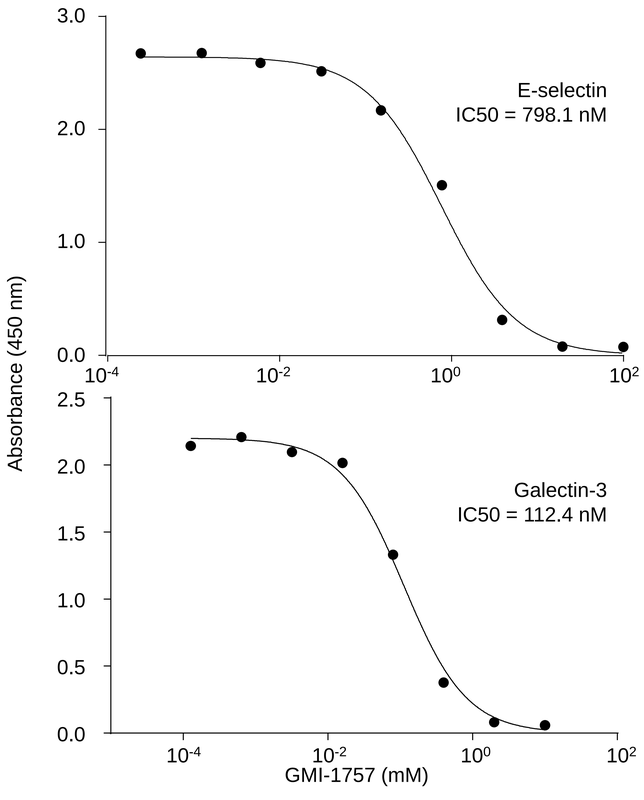
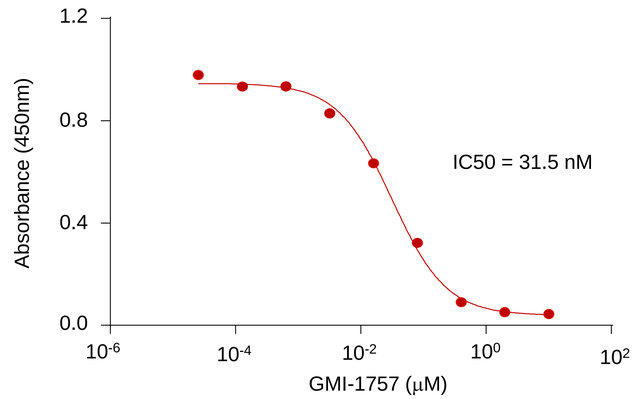


Figure 2. GMI-1757 Inhibits Galectin-3 Binding to Galectin-3 Binding Protein

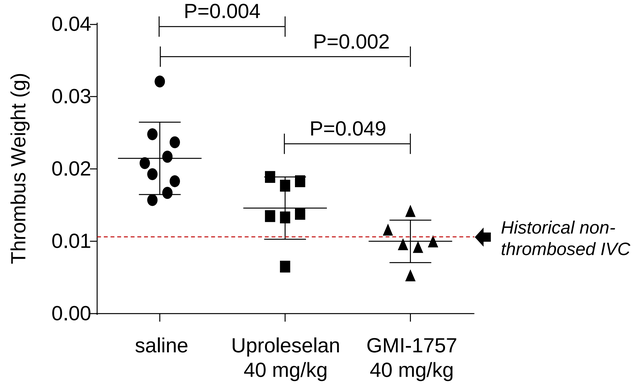
GMI-1757 was assessed for the ability to inhibit binding of galectin-3 to galectin-3 binding protein using an ELISA format. Aliquots of a 0.5 μg/mL solution of galectin-3 (IBL America IBATGP0414) were incubated with various concentrations of GMI-1757 and the mixtures were added to the wells of a 96-well plate that were coated with 1 μg/mL galectin-3 binding protein (R&D Catalog # 2226-GAB). After a two hour incubation at room temperature the wells were washed and the amount of bound galectin-3 was determine with an anti-galectin-3 HRP-conjugated antibody (R&D, Catalog # 893752). The IC50 of GMI-1757 for galectin-3/galectin-3 binding protein interaction was determined.



Summary (Figure 1 & 2): GMI-1757 exerts dual antagonistic interactions between E-selectin/E-selectin ligand(s) and galectin-3/galectin-3 ligand(s). The IC50 of GMI-1757 for galectin-3/galectin-3 binding protein was 31.5 nM. Additional studies using surface plasmon resonance revealed K_D values of GMI-1757 for E-selectin and galectin-3 to be 276 ± 98 nM and 61 ± 4 nM, respectively.

Figure 3. GMI-1757 Inhibits Thrombosis in a Mouse Inferior Vena Cava Model

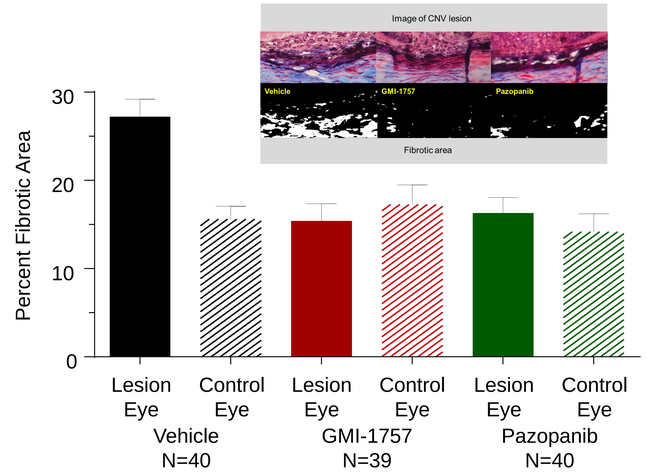
Male C57BL/6J mice underwent an electrolytic inferior vena cava (IVC) model to produce a non-occlusive thrombosis via electrical stimulation (250 μAmp). Animals were divided into three cohorts (N = 6 to 9 mice/group) and treated with: Cohort 1- saline (0.2 mL/20g SC); Cohort 2- Uproleselan (40 mg/kg IP); and Cohort 3- GMI-1757 (40 mg/kg IP). Mice received a single injection of saline or drug following thrombus induction on day 1. Mice were euthanized 2 days post-thrombosis for tissue harvest and thrombus weight was determined.



Summary: Intraperitoneal administration of GMI-1757 inhibited thrombus formation in an IVC model to a greater extent than Uproleselan (P=0.049). The greater inhibition of thrombus formation in this model may be the consequence of dual E-selectin and galectin-3 antagonism.

Figure 4. GMI-1757 Inhibits Fibrosis in a Rat Model of Choroidal Neovascularization

The purpose of this study was to evaluate the efficacy of Glycomimetics Compound GMI-1757 in the treatment of laser-induced choroidal neovascularization (CNV). It was anticipated that GMI-1757 is efficacious against fibrosis, which is a hallmark of this model. CNV lesions were induced by laser treatment in male, 8 week old Brown Norway rats on Day 0 (4 lesions/eye). Animals were divided into three cohorts (N = 10 rats/group) and treated with: Cohort 1- vehicle (5 μL/eye IVT); Cohort 2- GMI-1757 (35 μg/eye IVT); and Cohort 3- Panzopanib (100 mg/kg PO). Treatment with Vehicle and GMI-1757 began 1 week post laser induction and were given weekly for 2 weeks. Treatment with Panzopanib began 3 days prior to laser induction and were given twice-a-day for 17 days. Seven days after the final GMI-1757 treatment, lasered eyes were dissected, fixed in Davidson's solution, stained by Masson's trichrome. The central sections of each lesion were identified, digitized, and analyzed for fibrosis using a custom ImageJ macro based on a published algorithm.



Summary: In comparison to the non-CNV eye, intravitreal administration of GMI-1757 led to a complete inhibition of laser-induced fibrosis.

Conclusion

An innovative dual antagonist of E-selectin and galectin-3, GMI-1757, has been produced that demonstrates marked attenuation of thrombus formation in a murine IVC model. GMI-1757 is also shown to inhibit the development of fibrosis in a rat CNV model. Mechanism-of-action studies continue to be pursued to fully understand the impact of E-selectin and galectin-3 inhibition in this model and other models where disease progression is dependent on both inflammation and fibrosis.

References

1. Culmer DL et al. E-selectin inhibition with GMI-1271 decreases venous thrombosis without profoundly affecting tail vein bleeding in a mouse model. Thromb Haemost. 117:1171-1181, 2017
2. Elise P et al. The role of galectin-3 and galectin-3–binding protein in venous thrombosis. Blood 125:1813-1821, 2015

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