Novel E-Selectin Antagonist GMI-1271 Decreases Venous Thrombosis without Increased Bleeding Potential in a Mouse Model

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Introduction

Selectins function in venous thrombosis presumably by binding and activating immune cells to initiate the coagulation cascade. E-selectin (CD62E) is known to bind and activate both monocytes and neutrophils. GMI-1271 is a small molecule antagonist that specifically inhibits E-selectin and is rationally designed to mimic the bioactive conformation of the sialyl-Lex carbohydrate ligand. Here we determine whether specific inhibition of E-selectin is sufficient to inhibit acute venous thrombosis and associated inflammatory events in both prophylactic and treatment protocols without causing the broader effects of increased bleeding time.

Methods

Male C57BL/6J mice underwent our electrolytic inferior vena cava (IVC) model to produce a non-occlusive thrombosis via electrical stimulation (250 µAmp). Animals were divided into prophylactic or treatment groups. Both groups included the following: non-thrombosed animals (TC, no surgery or drug), 2 Day sham (needle inside the IVC and no current or drug), 2 Day CTR (current and no drug), 2 Day GMI-1271 (10mg/kg IP BID), and LMWH (Lovenox®, 6mg/kg SQ QD). Animals were divided into prophylactic or treatment groups. Mice in the prophylactic group were dosed one day pre-thrombus induction through day 1. Animals in the treatment groups received the first dose of the drug following thrombus induction on day 1. Mice were euthanized 2 days post-thrombosis for tissue harvest and blood collection for the following evaluations: thrombus weight; vein wall inflammatory cell counts per high power field; vein wall-thrombus histology; and intra-thrombus polymorphonuclear cell (PMN) counts. A separate group of mice received IV administration of compounds for tail bleeding time evaluation (seconds).

Results

GMI-1271 Significantly Decreases Venous Thrombus Weight

Treatment with GMI-1271 decreased venous thrombus formation in a dose-dependent manner with significant inhibition at 10mg/kg (P=0.0271). Treatment with LMWH significantly decreased thrombus formation 2 day post induction at 6mg/kg (P=0.0203) [Figure 1]. All mice pre-treated prophylactically with GMI-1271 or LMWH followed the same pattern of decreasing thrombus weight 2 days post injury (P<0.05).

Results

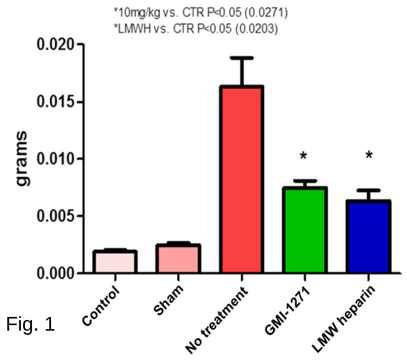

Fig. 1

Vein Wall Morphometrics and Histology

Treatment: Only treatment with GMI-1271 significantly (P<0.05) decreased vein wall monocyte extravasation compared to controls (Fig. 2).

Prophylaxis: GMI-1271 and LMWH prophylaxis significantly decreased vein wall PMN extravasation 2 days post thrombosis (P=0.027 and P=0.007 respectively). (Fig. 3) Prophylaxis with GMI-1271 and LMWH also significantly decreased vein wall monocyte extravasation at the same time point (P<0.01).

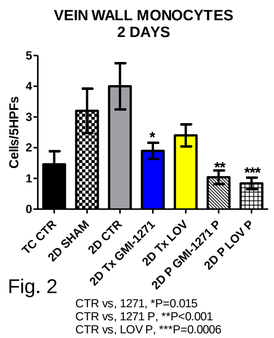

Fig. 2

CTR vs, 1271, *P=0.015
CTR vs, 1271 P, **P<0.001
CTR vs, LOV P, ***P=0.0006

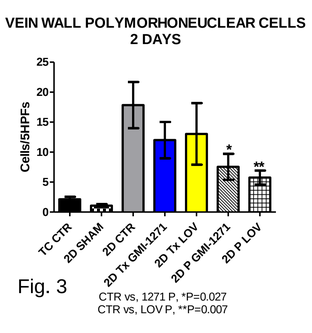

Fig. 3

CTR vs, 1271 P, *P=0.027
CTR vs, LOV P, **P=0.007

Results

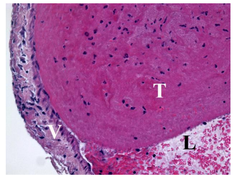

Fig.4 Almost totally occlusive 2 day venous thrombus (T) with flow in a small lumen (L). Mag. 40X.

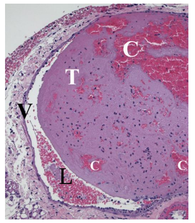

Fig.5 Thrombus (T) containing multiple flowing Channels (C) and a large Lumen (L). Mag. 40X

Intra-Thrombus PMN Counts: GMI-1271 prophylactic therapy significantly decreases intra-thrombus cell counts versus control animal (14.5±3.7 vs. 37.4±4.7 PMNs/HPF, P=0.009), and these animals had decreased venous thrombus burden. Of interest, only mice receiving GMI-1271 therapy visually have more intra-thrombus vascular channels compared to control animals and mice receiving LMWH therapy (Figures 4 and 5).

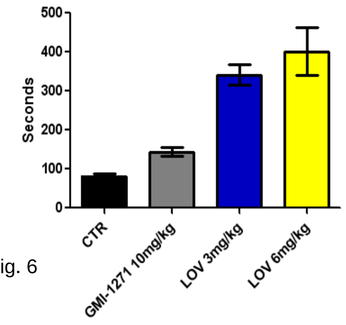

Fig. 6

E-selectin Inhibition with GMI-1271 Does Not Increase Bleeding Potential (Figure 6)

LMWH at 6 mg/kg dose significantly elevated tail bleeding times in mice versus controls (341±27, 491±60 vs. 82±6 seconds, P<0.01). GMI-1271 (10mg/kg, IV) had significantly lower tail bleeding times compared to an IV dose of LMWH (6mg/kg, P<0.01).

Discussion

Thrombogenesis is initiated by the recognition and binding of carbohydrate ligands on monocytes and neutrophils to selectins expressed on vascular endothelial cells. Once bound to E-selectin, these cells become activated and kick off the coagulation cascade by releasing tissue factor (TF) through the shedding of TF filled microparticles.(1). This mechanism is supported by studies demonstrating significant reduction in thrombosis weight (2) and intra-thrombus fibrin deposition (3) in mice genetically lacking E-selectin. Further support comes from clinical studies showing that patients with a polymorphism for E-selectin (S128R) which is more active, have a 4-fold greater risk for recurrent thrombosis after therapy (4). GMI-1271 is a small molecule glycomimetic antagonist that was rationally designed to specifically inhibit E-selectin. In the current study, we have demonstrated that GMI-1271 is effective in decreasing venous thrombosis and bleeding risk when compared to the current standard of-care, low molecular weight heparin (LMW).

Summary

GMI 1271 inhibits venous thrombosis and significantly decreases thrombus weight. GMI 1271 proposes a much lower risk of patients having bleeding complications. Vascular channels exclusively present in thrombi from mice receiving GMI-1271 therapy may aide in thrombus resolution which is currently under investigation. Delayed inflammatory cell recruitment of all cell types into the vein wall post thrombus induction indicates a possible decrease in leukocyte activation. This data suggest that inhibition of E-selectin is sufficient to inhibit venous thrombosis without an increased bleeding risk and the small molecule E-selectin specific antagonist GMI-1271 is a viable therapeutic candidate for venous thrombosis treatment and prophylaxis.

References

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