

GMI-1070: A Small Pan-Selectin Antagonist That Inhibits Leukocyte Adhesion and Migration in Multiple Disease Models *in vivo*



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Rational design of glycomimetic inhibitors based on the bioactive conformation of functional carbohydrates provides new therapeutic opportunities for the development of small molecule drugs with improved activity, pharmacokinetics, and bioavailability relative to native ligands. In designing more active glycomimetic selectin inhibitors, the low enthalpy (ΔH°) of the reaction is compensated by pre-forming the bioactive conformation thereby improving the entropy (ΔS°), known as SH compensation. Modifications of the molecule that also stabilize the core structure, further improve ΔS° and binding activity. To improve ΔH° , second site interactions were explored. To address the requirements for P and L-selectins, interactions were combined for both carbohydrate and sulfate-binding domains to produce heterobifunctional molecules. By stabilizing the bioactive core and exploring second site molecular interactions, we have produced a family of pan-selectin antagonists, one of which (GMI-1070) is now in development and scale-up synthesis as a lead compound. GMI-1070 is a potent inhibitor of E, P, and L-selectins *in vitro* and inhibits E and P-selectin-mediated leukocyte adhesion to endothelial monolayers under flow conditions. More importantly, GMI-1070 is active in several animal models of diseases requiring leukocyte adhesion and migration such as: a delayed-type hypersensitivity (DTH) response, cardiac ischemia/reperfusion injury, and vaso-occlusive crisis in sickle cell disease. GMI-1070 significantly inhibited infarct size in an ischemia/reperfusion cardiac injury model in rats. A single dose at 10mg/kg gave maximal inhibition. The effects of GMI-1070 on T-cell migration was studied in a DTH model. Mice were sensitized with oxazolone on the abdomen and then, 7 days later were challenged on the ear. Donor T-cells from a different cohort of similarly sensitized mice were fluorescently labeled and injected into the test cohorts at the time of administration of GMI-1070. Three hours following injection, migration of fluorescent T-cells to the challenged area was determined. Selectin-dependent T-cell migration in the DTH response was completely eliminated with a dose of 10 mg/kg of GMI-1070, suggesting the potential clinical application in diseases involving skin homing T-cells such as graft vs. host disease (GVHD) after bone marrow transplantation, and other inflammatory skin diseases. We have also shown that acute myelogenous leukemia (AML) cells adhere to endothelial cells under flow in a selectin-dependent mechanism suggesting that graft vs. leukemia as well as graft vs. host may be affected by treatment with GMI-1070. GMI-1070 was also tested in a model of vaso-occlusive crisis in sickle cell disease using Berkeley sickle cell mice. Blood flow was restored to normal values and adhesion of sickle red blood cells to adherent leukocytes was essentially eliminated as determined by intravital microscopy using a dose of 20 mg/kg administered at the time of elicitation of the vaso-occlusive challenge (TNF α) and at the start of the intravital microscopy (70 minutes later). Based on the encouraging results in these disease models, we are advancing the clinical development of GMI-1070 into Phase 1 studies to support indications which include the treatment of sickle cell patients in vaso-occlusive crisis.

Results

GMI 1070 Inhibition of PMN Rolling on HUVECs

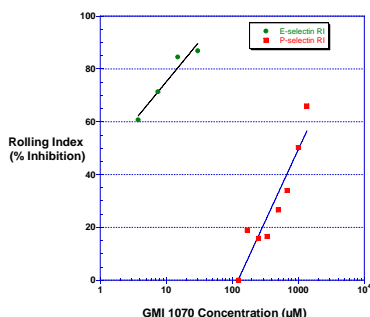


Figure 1. A parallel plate flow chamber is used to study the rolling and adhesion of human neutrophils under the shear forces of normal blood flow on stimulated monolayers of human endothelial cells isolated from the umbilical vein (HUVECs). Data are obtained by digital image analysis. To induce the expression of E-selectin, endothelial monolayers are stimulated with TNF α (30 μ g/ml) for 3 hours. For P-selectin expression, monolayers are stimulated with a combination of IL-4 and histamine. A suspension of neutrophils (106 cells/ml) containing GMI-1070 is perfused through the flow chamber at a shear rate corresponding to a wall shear stress of 0.9 dynes/cm².
Results: GMI-1070 is a pan-selectin inhibitor that inhibits both E and P-selectin mediated rolling and adhesion of neutrophils on endothelial monolayers under flow. GMI-1070 is a more effective antagonist for E-selectin.

Results

Human Leukemic Cells (AML) Rolling on HUVECs Expressing Selectins

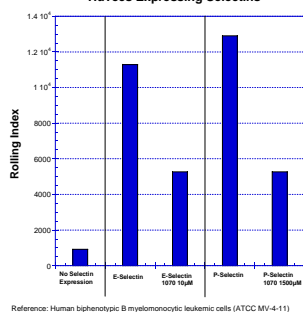


Figure 2. GMI-1070 inhibits the rolling and adhesion of acute myelogenous leukemic cells (ATCC: MV-4-11) on stimulated human endothelium (HUVECs). Monolayers of HUVECs are stimulated as described above to express either E-selectin or P-selectin. Flow chambers are then fixed to the monolayers and AML cell are perfused at a flow rate of 0.9 dynes/cm². Data are obtained by digital image analysis of videomicroscopy.
Results: GMI-1070 inhibits both E and P-selectin-mediated rolling and adhesion of AML cells to activated human endothelial monolayers under the shear forces of normal blood flow.

GMI-1070 Significantly Inhibits Infarct Size in a Rat Model of Myocardial Infarction

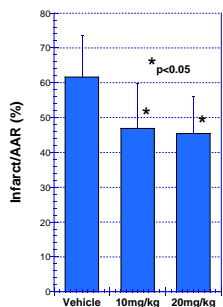


Figure 3. Effects of GMI-1070 on acute myocardial injury in the rat post ischemia/reperfusion injury. Blood flow to the heart is occluded by ligation of the left anterior descending coronary artery for 45 minutes. Reperfusion is allowed in the presence of test compounds and the infarcted area is measured after 4 hours of recovery.
Results: Doses of either 10 or 20 mg/kg of GMI-1070 afforded significant protection of area at risk within the heart from infarct damage.

Results

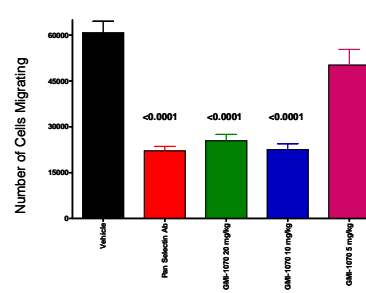


Figure 4. Effects of GMI-1070 on T-cell migration in a delayed-type hypersensitivity reaction in sensitized mice. Donor mice are sensitized with oxazolone (2%) by intradermal injections on the back. On day 6, T-cells are isolated from the donor mice and labeled with a fluorescent dye (CFSE). Recipient mice are also sensitized with oxazolone and at day 7 are challenged by this antigen on the ear and injected with fluorescent T-cells from the donor mice along with GMI-1070, a mixture of monoclonal antibodies to E, P and L-selectins (positive control) or vehicle alone (negative control). After 4 hr, migrating T-cells to the challenged ear are determined by fluorescence intensity.
Results: GMI-1070 is a potent inhibitor of T-cell migration in a DTH response, displaying complete selectin-mediated migration at a dose of 10mg/kg.

Results

GMI-1070 Reduces Adherence of SSRBCs to Leukocytes in Sickle Cell Mice during Vaso-occlusion *in vivo*

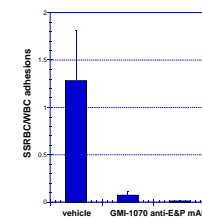


Figure 7. The effects of GMI-1070 on the adhesion of sickle red blood cells to leukocytes immobilized on the endothelium is determined by intravital microscopy of sickle cell disease mice undergoing vaso-occlusion (VOC) according to the protocol outlined in figure 5. Either GMI-1070 or a mixture of antibodies against E- and P-selectins practically eliminates adhesion among these cells types.

Summary

GMI-1070 is rationally-designed heterobifunctional molecule directed to interact with both the carbohydrate and sulfate binding domains, thereby inhibiting all three selectins, E, P, and L. In assays designed to measure inhibition of selectin dependent neutrophil adhesion to activated endothelial monolayers, GMI-1070 demonstrated most potent activity for E-selectin. The selectins are a family of adhesion molecules that have been implicated in many different disease states requiring cell adhesion, extravasation and migration. Examples include inflammation, cancer metastasis, ischemia/reperfusion injury and vaso-occlusion. All of these disease states have been modeled in laboratory animals and representative experiments are presented in the poster. In all models dependent on the function of the selectins, GMI-1070 has demonstrated a statistically significant effect.

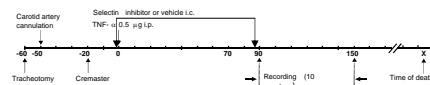


Figure 5. Timeline of animal preparation and experimentation to determine effects of test compounds on vaso-occlusion induced in sickle cell mice by TNF α as determined by intravital microscopy.

GMI-1070 Normalizes Blood Flow in Sickle Cell Mice in Vaso-occlusive Crisis *in vivo*

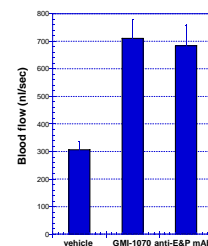


Figure 6. The effects of GMI-1070 on blood flow in the sickle cell disease mouse during an induced vaso-occlusive event. Sickle cell disease mice developed by bone marrow transplantation from Berkeley mice are treated according to the protocol in figure 5. In control mice, where no drug, but only vehicle is injected, blood flow is very slow and indicative of the sickle cell disease state after stimulation. Either GMI-1070 or a mixture of antibodies against E and P-selectin have dramatic effects by restoring blood flow to a velocity observed in normal mice.

Discussion

GMI-1070 demonstrates significant activity in several different animal models of disease. One of the most direct involvements of selectin adhesion molecules is in the vaso-occlusive crisis (VOC) of sickle cell disease patients. Based on encouraging results, we have formulated GMI-1070 for i.v. dosing of acute VOC in a hospital setting. Toxicology studies in both mice and monkeys have shown no adverse effects of the compound. Chemical synthesis of GMI-1070 is undergoing scale-up under GMP conditions with clinical trials anticipated to start in 2Q of 2008.

