

851 Inhibition of E-Selectin Inflammatory Function by the Glycomimetic GMI-1070

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Introduction: E-selectin expression by endothelium plays dual roles in inflammation by supporting slow rolling and subsequently eliciting integrin activation and arrest of leukocytes. This process may be important for immune surveillance. In a previous mouse model of sickle cell disease, E-selectin mediated outside-in signaling results in upregulated leukocyte Mac-1 and increased red cell capture, exacerbating vaso-occlusion. This process was attenuated by infusion of GMI-1070, a novel synthetic small molecule pan-selectin antagonist. GMI-1070 is now in Phase II clinical trial to determine efficacy in treatment of vaso-occlusive crisis (VOC) of sickle cell disease (SCD). Here, we studied its dose dependent effects in SCD subjects not in VOC on neutrophil activation and its effects on E-selectin mediated β_2 integrin activation and rolling and arrest in shear flow.

Methods: Samples were obtained from 4 SCD subjects not in VOC enrolled in a Phase I study of the effects of GMI-1070, a pan-selectin antagonist that preferentially inhibits E-selectin. An intravenous (IV) loading dose of 20 mg/kg was followed 10 hours later by a dose of 10 mg/kg. Samples were drawn before the loading dose, then 4 and 8 hours after the initial infusion. Polymorphonuclear Neutrophil (PMN) activation was analyzed in whole blood and isolated cell assays. Monoclonal antibodies and fluorescent-activated cell sorting (FACS) were used to assay expression of CD11b/CD18 (Mac-1), CD62L (L-selectin), and the high affinity active conformation of CD18 (327C) as markers of neutrophil activation. E-selectin mediated activation of CD18 was achieved in isolated PMN by incubating with E-selectin-IgG and goat antibody F(ab')₂ fragment to crosslink E-selectin-IgG PMN activation was assessed by surface expression of high affinity CD18 by the mAb 327C and FACS in the presence of various concentrations of GMI-1070. PMN rolling and arrest on an inflammatory substrate was quantified using a *lab on a chip* assay. Whole blood or isolated PMN were perfused through a microfluidic flow chamber at a shear stress of 2 dynes/cm². The flow chamber had immobilized E-selectin and ICAM-1 to support PMN rolling and adhesion. Video recordings of PMN interacting with this substrate were taken to quantify the number of rolling versus arrested cells and to measure rolling velocity in the presence of GMI-1070.

Results: An inverse relationship between the serum concentration of GMI-1070 and either activated CD18 or upregulated CD11b was observed. In the flow chamber assays, 6 of 7 samples showed GMI-1070 diminished PMN arrest that also correlated with diminished integrin activation. Incubation of PMN with GMI-1070 blocked CD18 activation in response to E-selectin-IgG cross-linking, with an IC_{50} of 0.5 mM. PMN rolling and arrest was measured following shearing of isolated PMN on E-selectin and ICAM-1 using a *lab on a chip* assay. The mean rolling velocity of 2 mm/sec was increased to 6 mm/sec at a GMI-1070 concentration of 20 mM, with an IC_{50} of 5.5 mM for the increase in rolling velocity. In contrast, an IC_{50} = 0.8 mM was required to antagonize the fraction converting from rolling to arrest under shear flow.

Summary and Conclusions: There was a dose dependent inhibitory effect on PMN activation after IV administration of GMI-1070 as measured in *ex vivo* whole blood samples in a small cohort of SCD subjects at steady state. A systematic study of the activity of GMI-1070 on isolated PMN revealed two distinct patterns of inhibition. At low concentrations (IC_{50} = 0.5mM), GMI-1070 effectively blocked the capacity of E-selectin to activate CD18, in response to cross-linked ligands, and mediate arrest. Only at higher concentrations (IC_{50} = 5.5mM) did we observe a significant alteration in the capacity of GMI-1070 to increase the rolling velocity and frequency of capture on a substrate of E-selectin under fluid shear stress. This differential capacity to alter function reveals that slow rolling, which is mediated by recognition of a number of sialylated ligands on the surface of PMN can be distinguished from the processes associated with the transition to arrest. Thus, treatment with GMI-1070 at doses that efficiently block vascular occlusion may spare some PMN rolling and immune surveillance function.

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