Effects of Selectin Antagonist GMI-1070 on the Activation State of Leukocytes in Sickle Cell Patients not in Crisis

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Abstract #34174

It is hypothesized that activated leukocytes play key roles in sickle cell vaso-occlusion by adhering to inflamed venules and capturing circulating platelets and sickle red blood cells. GMI-1070 is a small molecule selectin antagonist which was recently reported to reverse acute vascular occlusion in a humanized sickle cell disease (SCD) mouse model (Chang et al, Blood 2010) presumably by inhibiting E-selectin and its effects on downstream signaling of leukocyte activation. Sickle cell patients express elevated levels of soluble E-selectin (Kato et al, Brit J Haem 2005) activated polymorphonuclear neutrophils (PMN) (Lum et al Amer J Hem 2004) and platelet/monocyte aggregates (PMA) (Wun et al Clin Lab Haem 2002). In this study, the activation state of leukocytes from whole blood samples of sickle cell patients not in crisis before and after infusion of GMI-1070 was evaluated *ex vivo*. Isolated PMN from normal, healthy volunteers were strongly activated by binding soluble E-selectin/hIg *in vitro* as determined by a 7-fold increase of the integrin MAC1 (CD11b) and an 8-fold increase in expression of the high affinity form of CD18 detected by antibody 327C. Addition of GMI-1070 completely blocked upregulation of MAC1 and 327C at 50µg/ml and showed pronounced inhibition (79% MAC1; 75% 327C) at 10µg/ml. These in vitro concentrations are consistent with blood levels of GMI-1070 found in sickle cell patients 4 and 8 hours after dosing. A phase 1/2 study was conducted on 10 adult subjects with SCD at steady state. GMI-1070 was given IV at 20mg/kg as a loading dose and at 10 hours a final dose of 10mg/kg was given. Blood samples were drawn from these adults pre-infusion and at 8, 24, and 48 hours after the initial infusion. In some subjects, a blood sample was also drawn at 4 hours post infusion. Activation of PMN's in whole blood samples from subjects was assessed by upregulation of MAC-1, expression of the high affinity CD18 and the loss of CD62L due to shedding of L-selectin determined by flow cytometric analysis of cell surface labeling with fluorescently conjugated antibodies. Of 4 subjects tested, 3 showed increased surface expression of L-selectin, 3 showed decreased expression of MAC-1, and 2 showed decreased expression of high affinity CD11b at the first time point tested (4 or 8hr) after dosing with GMI-1070 suggesting an inhibition of PMN activation in these patients. A functional consequence of monocyte activation is the formation of platelet/monocyte aggregates due to expression of high affinity integrins. Platelet-monocytes aggregates (PMA) in blood were detected using anti-CD11c for monocytes and anti-CD41a for platelets. Treatment of samples with lipopolysaccharide (LPS) was used for positive controls. Intracellular IL-1β was used as a marker of activated monocytes. In 5 patients out of 6 tested with this assay, PMA in the subject's blood were decreased at the first time point after dosing (8hr). These results are consistent with an effect of GMI-1070 on inhibition of activation given its IC_{50} value for E-selectin (4.3µM), the blood concentration in subjects after dosing, and the serum half life (7.7hr) in steady state sickle cell adults.

Introduction

Individuals with sickle cell disease are known to contain elevated levels of platelet-monocyte aggregates (PMA) in their bloodstream which also play a role in formation of occlusions during crisis. PMAs result from interactions with activated leukocytes. Sickle cell patients also contain elevated levels of soluble E-selectin in their blood which correlates with mortality. E-selectin is also known to activate leukocytes through ligation and signaling. Here we analyze the effects of a selectin antagonist GMI-1070 on the activation state of leukocyte in adults with SCD.

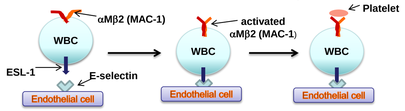

Fig.1 Schematic representation of the activation of leukocytes and their cell surface integrins mediated by ligation with E-selectin.

Methods

In vitro assays

Isolated human neutrophils were activated by incubation with soluble recombinant human E-selectin/Fc chimera (10 ug/ml, 15 min. at 37^{0}C) and subsequently crosslinked by incubation with goat anti-human (H+L) F(ab')2 fragment (100 ug/ml, 15 min at 37^{0}C) in the presence of GMI-1070. Neutrophils incubated with IL-8 (5 nM) were used as a positive control for activation. Negative controls consist of incubation with noncrosslinked E-selectin/Fc chimera (100ug/ml) or goat anti-human antibody (X-linker) alone or with no treatment (NS) .

Integrin activation was detected by labeling with 327C-Alexa 488, which binds specifically to the activated extended conformation of the beta-chain of β_2 integrins. Upregulation of the β_2 integrin Mac-1 (CD11b/CD18) was detected by labeling the alpha chain of Mac-1 integrins using anti-human CD11b-PE.

Phase 1/2 clinical study

Adults with sickle cell disease at steady state were enrolled in an open label Phase 1/2 study. GMI-1070 was administered in two IV doses with the first dose delivering 20 mg/kg followed by a second dose 10 hours later at 10 mg/kg. Blood samples are taken just prior to the first dose (t = 0) for establishing baseline values, followed by additional samples being taken 8, 24 and 48 hrs later.

Results

GMI-1070 Inhibits E-selectin-mediated Expression of Activated Mac-1 as determined by mAb 327C

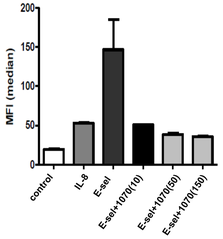

Fig. 2 Multivalent binding of E-selectin activates the β_2 integrin (Mac-1) expression on neutrophils as detected by antibody 327C. Selectin antagonist GMI-1070 significantly inhibits activation down to 10 ug/ml. Incubation with IL-8 provides a positive control.

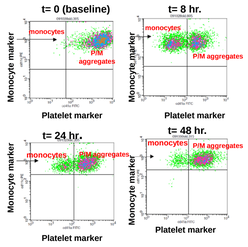

Fig. 4 FACs analysis of blood samples from Patient 106 during treatment with GMI-1070. cells are labeled with anti-CD11c FITC (monocytes) and anti-CD41c PE (platelets). The highest blood concentration of GMI-1070 at 8 hrs. (see abstract #1632) correlates with the appearance of monocytes not in aggregates.

GMI-1070 Inhibits the E-selectin-mediated Upregulation of the β_2 Integrin, Mac-1

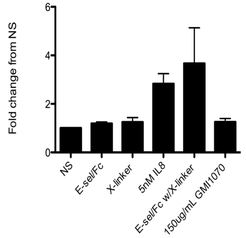

Fig. 3 Multivalent binding of E-selectin induces enhanced expression of Mac-1 which is inhibited by GMI-1070 at 150 ug/ml. Multivalent presentation is important as free E-sel/Fc chimera and the cross-linking secondary antibody (X-linker) alone do not promote expression. IL-8 provides a positive control for neutrophil activation.

Platelet-Monocyte Aggregates in Patients after Dosing with GMI-1070

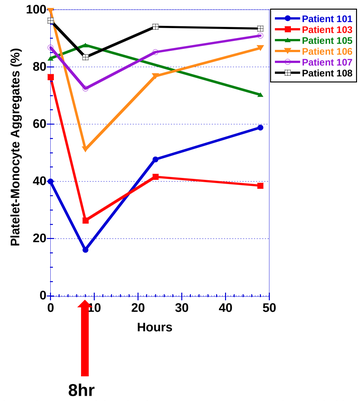

Fig. 5 Summary of the platelet-monocyte aggregates (PMAs) in blood of 6 adults with sickle cell disease in steady state during treatment with GMI-1070. The lowest appearance of PMAs correlates with the highest blood level of GMI-1070 at 8 hours post dosing (see abstract #1632) in 5 out of 6 treated adults. The serum half life of GMI-1070 is between 7 and 8 hours.

L-selectin expression

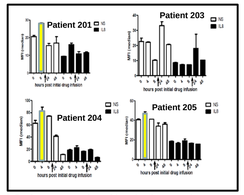

Fig. 6 Surface expression of L-selectin on patients neutrophils was determined by anti-CD62L. Three of 4 patients show highest expression of L-selectin just after dosing with GMI-1070 (yellow). Activated neutrophils shed L-selectin. Closed bars represent these Neutrophils activated in vitro with IL-8.

Activated Mac-1 expression

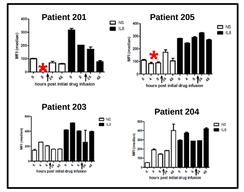

Fig. 7 Surface expression of activated Mac-1 was detected using antibody 327C. In 2 of 4 patients expression of activated Mac-1 on neutrophils is lower after dosing with GMI-1070 (open bars). Closed bars represent these neutrophils activated in vitro with IL-8.

Conclusions

GMI-1070 significantly inhibited E-selectin-mediated activation of PMN's in vitro as determined by expression of the integrin MAC-1 and high affinity CD18 at 10ug/ml. Similar concentrations of GMI-1070 in sickle cell subjects' blood at 4 and 8 hours after dosing also resulted in a lowered activation state of PMN's identified by reduced expression of cell surface integrin molecules as well as the inhibition of shedding of L-selectin in some cases. A more functional measure of leukocyte activation is the aggregation of platelets on monocyte cell surfaces. In 5 of 6 subjects tested, GMI-1070 reduced PMAs 8 hours after dosing. Thus, GMI-1070 not only inhibits E-selectin, but also blocks the expression of downstream integrin adhesion molecules that together play crucial roles in vaso-occlusion by promoting the adhesion to platelets and erythrocytes in the formation of occlusions that block blood flow. The effects of GMI-1070 on the activation state of leukocytes via the inhibition of functional adhesion molecules in steady state sickle cell subjects supports the further evaluation of treatment with GMI-1070 during vaso-occlusive episodes.

Conflict of Interest Disclosures: SI Simon, S Chase, SK Larkin, F Kuypers, L Styles and T. Wun performed these studies under contract with GMI. H Thackray and JL Magnani are employees of GMI.

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