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

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It is hypothesized that activated leukocytes play key roles in sickle cell vaso-occlusion by adhering to inflamed, necrotic 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 PMA stimulation of leukocyte-endothelial cell (EC) adhesion signaling. In vitro, GMI-1070 also completely blocks the upregulation of Mac-1 (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 MAC-1 and 327C at 50μg/ml and showed pronounced inhibition (75% MAC1, 75% 327C) at 10μg/ml. These in vitro concentrations are consistent with blood levels of GMI-1070 found in sickle cell patients tested, 3 showed increased surface expression of L-selectin, 3 showed decreased expression of L-selectin (see abstract) for establishing baseline values, and at 24, 48, and 48 hours after dosing also resulted in a lowered activation state of PMN’s identified by reduced expression of downstream integrin adhesion molecules that together play crucial roles in vaso-occlusion by promoting platelet-monocyte aggregates.

Methods

In vitro assays

Isolated human neutrophils were activated by incubation with soluble recombinant human E-selectin/Fc chimera (10 ng/ml), IL-8 (10 ng/ml). Incubation with IL-8 provides a positive control. Multivalent binding of E-selectin activates its IC50 value for E-selectin (4.3 μM), and its Fc fragment (100μg/ml). E-selectin/Fc chimera (100ug/ml) or goat anti-human antibody (X-linker) alone or with no treatment (NS) . In vitro concentrations are consistent with blood levels of GMI-1070 found in sickle cell patients tested, 3 showed increased surface expression of L-selectin, 3 showed decreased expression of L-selectin (see abstract).

Results

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

Activated Mac-1 expression

Activated neutrophils shed L-selectin. Closed bars represent these neutrophils activated in vitro with IL-8. Mac-1 on neutrophils is lower after dosing with GMI-1070 (open bars). A functional consequence of monocyte activation is the formation of platelet-monocyte aggregates due to expression of high affinity integrins. Platelet-monocyte aggregates (PMA) in blood were detected using anti-CD11b for monocytes and anti-CD41A for platelets. Treatment of samples with propidium iodide (PI) 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 a trend of GMI-1070 on inhibition of activation as its IC50 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.

Conclusions

GMI-1070 significantly inhibited E-selectin-mediated activation of PMA’s in vivo as determined by expression of the integrin MAC-1 (CD11b). GMI-1070 administration during vaso-occlusive episodes, particularly those lasting 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 was observed 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 of platelets and monocytes to the formation of occlusions that lead to sickle cell vaso-occlusion.

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.