SCD Model Systems

Effects of Pan-Selectin Antagonist GMI-1070 on the Treatment of Vaso-Occlusion in Sickle Cell Mice

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Abstract

Acute vaso-occlusion (VOC) in patients with sickle cell disease (SCD) induces intense pain arising from organ damage and is the major cause of morbidity and mortality. Hypoxia and abnormal sickle red blood cells (RBC) induce inflammatory mediators and activation of the vascular endothelium leading to the recruitment of adherent leukocytes and sickle RBC followed by aggregates that eventually occlude blood flow. Previous studies have implicated the critical roles of cell adhesion molecules E- and P-selectins by using intravital microscopy in SCD mice (Berkeley strain) with altered genetic backgrounds (SCD transplanted in recipients lacking E-and P-selectins), or antibodies against endothelial selectins, or small molecules directed against the selectins. Here, we designed a treatment protocol for this SCD mouse model, in which a small molecule pan-selectin antagonist (GMI-1070) is administered to sickle cell mice late in the process of established vaso-occlusion in order to test the effects of GMI-1070 in a more clinically relevant model. GMI-1070 is a small molecule pan-selectin antagonist designed on the bioactive conformation of the carbohydrate ligand and inhibits leukocyte adhesion to activated endothelium in vitro with particularly strong activity against E-selectin (IC50 = 3.4 μM). Berkeley SCD mice were generated by bone marrow transplantation into lethally irradiated C57BL/6 male mice and the fully engrafted (100% donor RBC chimerism) mice were used for intravital microscopy experiments. VOC events were induced by injection with TNF-α at time 0 and the formation of occlusions were allowed to proceed as long as possible just prior to the death of the control mice. GMI-1070 (20 mg/kg) or vehicle (PBS pH 7.4) were administered at t = 110 min. Post-capillary and collecting venules in the cremaster muscle were analyzed for effects on an established VOC event. Under these conditions, GMI-1070 significantly increased the microcirculatory blood flow to levels observed in non-sickle cell mice (vehicle: 237 ± 15 nL/sec; GMI-1070: 533 ± 58 nL/sec; p<0.0001). The recruitment of adherent leukocytes to the vascular endothelium was also significantly reduced (vehicle: 2235 ± 156; GMI-1070: 1270 ±
203 cells/mm$^2$; $p=0.0013$), and there were significant and dramatic reductions in the capture of sickle red blood cells to adherent leukocytes (vehicle: $0.68 \pm 0.27$; GMI-1070: $0.03 \pm 0.01$ interactions/WBC, min. 100ml; $p=0.0003$). Mice began to succumb to VOC within 2.5 hours after injection of TNF-$\alpha$ and surgical trauma which continued until all of the control SCD mice died. Administration of GMI-1070 prevented the death of half of the treated mice within the timeframe of the experiment and extended the median survival of mice from 5 hours (control, vehicle-treated) to greater than 9 hours for the GMI-1070- treated SCD mice ($p = 0.0067$). These studies show that GMI-1070 can significantly and dramatically improve the condition and survival of the animals with a severe VOC even when dosed well after the initiating challenge. Thus these data strongly support the use of GMI-1070 for the treatment of patients in acute vaso-occlusive crisis. GMI-1070 is currently in a Phase I clinical trial.