Evaluation of purified poloxamer-188 (vepoloxamer) on sickle red blood cell (RBC) adhesion and membrane fragility utilizing microfluidic-based flow adhesion bioassays

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Background Sickle erythrocyte adhesion and membrane fragility contribute to vaso-occlusion and downstream tissue and organ ischemia. Vepoloxamer is an amphipathic triblock copolymer with multi-mechanistic properties believed to improve these pathophysiologic consequences, by sealing damaged cell membranes, inhibiting hydrophobic cellular interactions, and facilitating thrombolysis. Studies have also shown the reduction in both acute vaso-occlusive crisis duration and total opioid analgesic requirements. A phase 3 clinical trial of the effectiveness of vepoloxamer in attenuating acute vaso-occlusive events is currently underway. Because of the significant patient to patient variability in sickle cell disease (SCD) vaso-occlusive phenotype, we evaluated the effects of vepoloxamer on sickle blood to identify individual patients at greater risk of pathologic vascular adhesion, predict if and to what extent vepoloxamer may improve these pathologies, and thus guide its therapeutic use.

Objective This study was to evaluate the effects of vepoloxamer on whole blood adhesive properties and RBC membrane fragility of individual SCD patients.

Methods Blood was obtained from pediatric homozygous sickle cell patients (n = 12) at steady state. The dose response to vepoloxamer was evaluated by measuring whole blood adhesion to vascular cell adhesion molecule (VCAM-1) during physiologic flow conditions. Erythrocyte membrane fragility was measured based on mechanical stress-induced hemolysis at 3 min (Hem_{3min}, %) and 10 min (Hem_{10min}, %).

Results Compared to control, blood samples treated with vepoloxamer (n=12) at 0.1 mg/mL demonstrated an 18% decline of the median number of adherent cells ($p = 0.0015$). At 1.0 mg/mL and 10.0 mg/mL, a 69% and 79% reduction in adherent cells was observed respectively (in both cases, $p < 0.001$). Vepoloxamer also reversed established cell adhesion, and the degree of adhesion reversal varied from patient to patient. In addition, vepoloxamer reduced shear-induced hemolysis compared to untreated blood samples (n = 10). Although these reductions varied patient to patient (e.g. 2.2 to 49.4% for Hem_{3min}), statistical significance was reached at 3 min application of shear stress (Hem_{3min}) ($p = 0.033$).

Conclusion Vepoloxamer reduces whole blood adhesive properties in a dose-dependent manner, and reduces shear-induced hemolysis in a statistically significant manner. This pre-clinical approach to assessing the response of adhesive properties and membrane fragility to vepoloxamer treatment may facilitate selection of patients most likely to benefit from vepoloxamer therapy. Additionally, this approach may provide a mechanism to monitor an individual patient's response to vepoloxamer over time both in clinical studies and in patient therapy.