Background: In Sickle Cell disease (SCD), a single amino acid substitution in the beta globin chain converts HBA to sickle genotype HHS. This genetic change promotes HbS polymerization upon deoxygenation that can promote occlusion of small blood vessels that is often associated with increased blood viscosity, and circulatory inflammation. PEGylated-carboxyhemoglobin (PEG-COHb; SANGUINATE) was designed as a novel therapeutic agent to initially release carbon monoxide (CO) and then transfer oxygen \( (O_2) \) to hypoxic tissue and cells. Delivery of either CO or/or \( O_2 \) hypoxic, sickled red blood cells (RBCs) should return cells to a more normal cell morphology and help re-establish normal blood flow and rheology. PEG-COHb was shown to mediate transfer of either a CO or \( O_2 \) mixture and restore normal morphology to hypoxic, sickled RBCs in vitro. Studies are now focused on the potential therapeutic implications of delaying or slow sickling, which should maintain normal blood flow through hypoxic microvasculature. Unsickling is expected to be expedited by \( O_2 \) transfer by PEG-COHb. To examine these potential therapeutic effects, current in vitro studies examined the effects of time and dose of PEG-COHb to not only reverse, but also prevent or delay sickling by transferring CO as well as expedite atmospheric \( O_2 \) transfer to the sickled RBCs.

Methods: Reversal of sickling studies were conducted by deoxygenating RBCs from age matched healthy (control) and SCD volunteers in followed by treatment with either PEG-COHb, fully oxygenated PEG-Hb (PEG-OHb) or PEG-BSA for 2 hours. For prevention of sickling studies, fully oxygenated RBC suspensions were treated with increasing amounts of PEG-COHb and then subjected to hypoxia for 3 hours. Time-dose effects were quantified by area under the curve (AUC) analysis. Oxygen transfer studies were conducted by treating hypoxic, sickled RBCs to increasing concentrations of PEG-COHb and raising the \( pO_2 \) from 3.8 mm to 40 mm. In all studies, the fractions of CO-Hb, O2-Hb and reduced Hb were determined by co-oximetry and sickled RBCs were quantified by imaging flow cytometry of fixed RBC specimens.

Results: Sickled RBCs red blood cells from SCD volunteers were treated with bovine PEG-COHb or PEG-OHb and fixed at recurrent intervals and subjected to imaging cytometry. Mean red blood cell shape value (±95% confidence intervals) were plotted as a function of CO and \( rHb \) percentages. Data in dose response curves are fit to 3 parameter logistic curve fit model. EC\(_{50}\) values of CO-Hb (red) and \( rHb \) (blue) with 95% confidence intervals and goodness of fit are shown. Indicated range (x-axis) varies due to individual variability in sickling.

PEG-COHb mediated gas transfer reduces the fraction of deoxygenated sickle cell hemoglobin to prevent sickling.

PEG-COHb acts in a dose-dependent manner to prevent or slow in vitro sickling in a hypoxic atmosphere.

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The Conflict of Interest disclosures for above authors have been satisfied.