PEG-COHB MEDIATED CO AND OXYGEN TRANSFER TO HYPOXIC RBCS PREVENT, SLOW, AND/OR REVERSE SICKLING IN VITRO: IMPLICATIONS FOR CLINICAL UTILITY IN SCD

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Sickle Cell disease(SCD) is characterized by HbS polymerization upon deoxygenation that can promote occlusion of small blood vessels that is often associated with increased blood viscosity and circulatory inflammation. SANGUINATE(SG) was designed as a novel therapeutic agent to initially release carbon monoxide(CO) and then transfer O2 to hypoxic tissue and cells. Delivery of either CO or O2 to hypoxic, sickled red blood cells(RBCs) should return cells to a more normal cell morphology and help reestablish normal blood flow. To examine these potential therapeutic effects, in vitro studies examined the effects of time and dose of SG to not only reverse but also prevent or delay sickling by transferring CO as well as facilitate atmospheric O2 transfer to the sickled RBCs. Reversal-of-sickling studies were conducted by deoxygenating RBCs from healthy(control) and SCD volunteers then by treatment with either carboxylated SG(SG-CO), oxygenated SG(SG-O2) or PEG-BSA for 2 hrs. For prevention of sickling studies, fully oxygenated RBC suspensions were treated with SG and then subjected to hypoxia for 3 hrs. Time-dose effects were quantified by area under the curve(AUC) analysis. O2-transfer studies were conducted by treating hypoxic, sickled RBCs to increasing concentrations of SG and raising the pO2 from 3.8mm to 40mm. 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. SG-mediated delivery of either CO or O2 can unsickle hypoxic SCD RBCs. Controls exhibited gas exchange similar to SCD RBCs. Sickle reversion time-course studies showed differential kinetics between the CO and O2 capacity to cause unsickling. AUC analysis at 20 mins demonstrated that both CO and O2 reversed sickling by 41% and 42%, respectively. SG-O2 was able to exert substantial unsickling by 5 mins, where SG-CO showed a delayed more pronounced effect peaking approximately 20 to 40 mins post-treatment. When fully oxygenated SCD RBCs were pretreated with SG prior to oxygenation, sickling was inhibited with an IC50 of 2.5±0.6 mg per mL in deoxygenated saline(PBS). In addition, treatment concentrations below IC50 values had increased time-dose AUC values indicating that although, not completely inhibited, sickling was delayed. Oxygen transfer facilitation studies indicated that SG increased the rate of unsickling as measured by AUC by 50% and 15% at 4 and 2 mg per mL, respectively. These levels are within the expected therapeutic dosage of SG. The occlusive and fragile properties of sickled RBCs are responsible for the development of the numerous comorbidities associated with SCD. It is only when the fraction of oxygenated or carboxylated HbS reaches a sufficient level that reversion to normal cell morphology occurs restoring vascular perfusion. These experiments showed a concentration and time-dependent effect of SG ability to deliver both O2 and CO to sickled RBC. These data suggested that SG has the potential to improve sickle cell morphology by reversing sickling; the underlying pathology of SCD comorbidities. This MOA supports the potential of SG in treating acute SCD comorbidities. SG is currently in phase 2 trials in Central and South America for VOC that may include the presence of other comorbidities such as acute chest syndrome.