Artificial Organs

SANGUINATE (PEGylated Carboxyhemoglobin Bovine): Mechanism of Action and Clinical Update

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Abstract: Historically, blood substitutes were under development that would provide oxygen carrying capacity as well as fluid replacement for both trauma and surgical indications. Their development was halted by the inability of the products to deliver therapeutic amounts of oxygen targeted to hypoxic tissue as well as from the inherent toxicity of the molecules. This led to the concept of an oxygen therapeutic that would be targeted for indications caused by anemia/ischemia/hypoxia but would not exhibit the toxicity that plagued earlier products. The complex pathophysiology of diseases such as sickle cell and hemorrhagic stroke not only has hypoxia as a pivotal event but also includes inflammation and vasoconstriction that perpetuate the oxygen deprivation. There is a need for an effective therapeutic that addresses the multiple events of inflammation and oxygen deprivation. SANGUINATE acts as a dual mode carbon monoxide (CO) and oxygen delivery therapeutic. SANGUINATE is designed not only to treat hypoxia but also to act on concurrent pathologies such as inflammation and reperfusion injury. This expands the potential therapeutic utility of SANGUINATE beyond anemia into indications such as early brain injury and delayed kidney graft function, where inflammation plays a pivotal pathological role as well as in indications such as sickle cell disease where the inflammation and hypoxia contribute to the development of comorbidities such as vaso-occlusive crisis. Clinical trials in multiple indications are underway. **Key Words:** Oxygen carrier—Clinical trial—Hypoxia.

A new generation of oxygen-delivering therapeutics is under development following the failure of early hemoglobin-based oxygen carriers to demonstrate safety and/or clinical benefit in randomized controlled trials (1). Rather than being developed as blood substitutes, these newer products are specifically intended to be oxygen-delivery therapeutics for use in conditions in which tissue oxygenation is compromised. Both hemoglobin- and perfluorocarbon-based oxygen carriers are being developed for indications such as lower limb ischemia, stroke, kidney transplantation, cancer, wound healing, and sepsis.

One oxygen-delivery therapeutic product in development is SANGUINATE (PEGylated carboxyhemoglobin bovine). SANGUINATE is a gas-transfer agent designed to deliver carbon monoxide (CO) as well as oxygen. It is stable and no autoxidation has been observed at packaged conditions under refrigeration (4°C) for 4 years and at room temperature for 2 years. Methemoglobin levels have not been seen to increase in vivo. Increases in methemoglobin levels were not seen following administration in toxicology studies in multiple animal models. Results from a Phase I study demonstrated a safety profile that supported further clinical development (2). Initial indications chosen for clinical evaluation are vaso-occlusive crisis (VOC) of sickle cell disease (SCD), delayed cerebral ischemia, delayed graft function (DGF) following organ transplant, and severe anemia in patients when red blood cell (RBC) transfusion is not an option.

SANGUINATE is a polyethylene glycol (PEG) modified form of bovine hemoglobin. The modification of hemoglobin with the appropriate sized polymer, linkage technology, and amino acid site selection is key to both the safety and functionality

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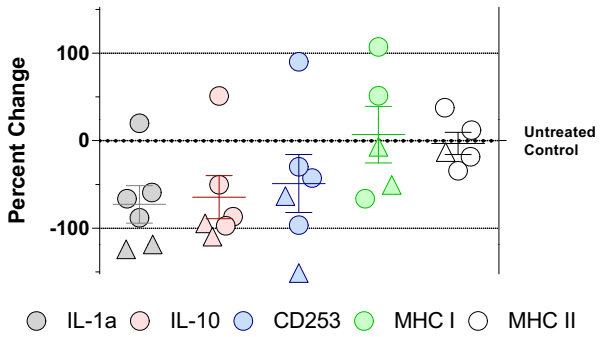

FIG. 1. In vitro down-regulation of inflammatory markers in lipopolysaccharide-challenged whole blood samples.

of this drug. PEG-modification (or PEGylation) is a well-established drug delivery technology that confers beneficial characteristics on protein therapeutics. PEGylation increases the protein's circulating life through steric interference with clearance receptors, preventing extravasation by increasing the molecular size and decreasing immunogenicity (3).

It is well established that endogenous CO produced from heme-oxygenase activity has pleiotropic, cytoprotective, and homeostatic effects including inhibition of apoptosis and inflammation, as well as reduction of oxidative stress and vasodilatory activity (4). Exogenous CO, delivered in vivo via CO-releasing molecules (CORMs), has been shown to promote these effects and to modulate ischemia-reperfusion injury (5,6). Vasoconstriction and inflammation induced by hypoxia or other disease pathologies can result in tissue damage that can exacerbate the effects of already reduced levels of oxygen delivery. The multitude of actions of CO released by SANGUINATE may act to improve tissue oxygenation. Studies in models of focal cerebral ischemia and traumatic brain injury have shown that a top load dose of SANGUINATE inhibited constriction of pial arterioles (7,8). Anti-inflammatory activity has also been confirmed in both in vitro and in vivo studies of SANGUINATE. miRNA levels of lipopolysaccharide-stimulated whole SCD blood samples (Fig. 1) and in blood from a focal cerebral ischemia (middle cerebral artery occlusion) animal model showed decreases in inflammatory cytokine RNA levels (Fig. 2) and improved neurological deficit scores in SANGUINATE-treated groups as compared to control groups (Fig. 2).

CO has been shown to be rapidly released from SANGUINATE within 10 min in vitro and whole blood COHb increased to 5.3 and 4.9% in two rats at 1 min after the transfusion was complete and then decreased exponentially to 1.0 and 1.1%, respectively, by 2 h (6). This CO release then enables the binding and transport of oxygen. SANGUINATE has an average p50 range of 12 mm Hg, and its ability to off-load oxygen in the presence of hypoxic conditions (partial pressure of O_2 approx. 5 mm Hg) has been demonstrated in vitro (Fig. 3). Using phosphorescence quenching microscopy in a rat model of severe hemorrhagic shock, SANGUINATE was shown to significantly increase oxygen levels ($P_{ISF}O_2$) and survival. The results demonstrated that while reestablishing blood pressure was acutely important for post-hemorrhage

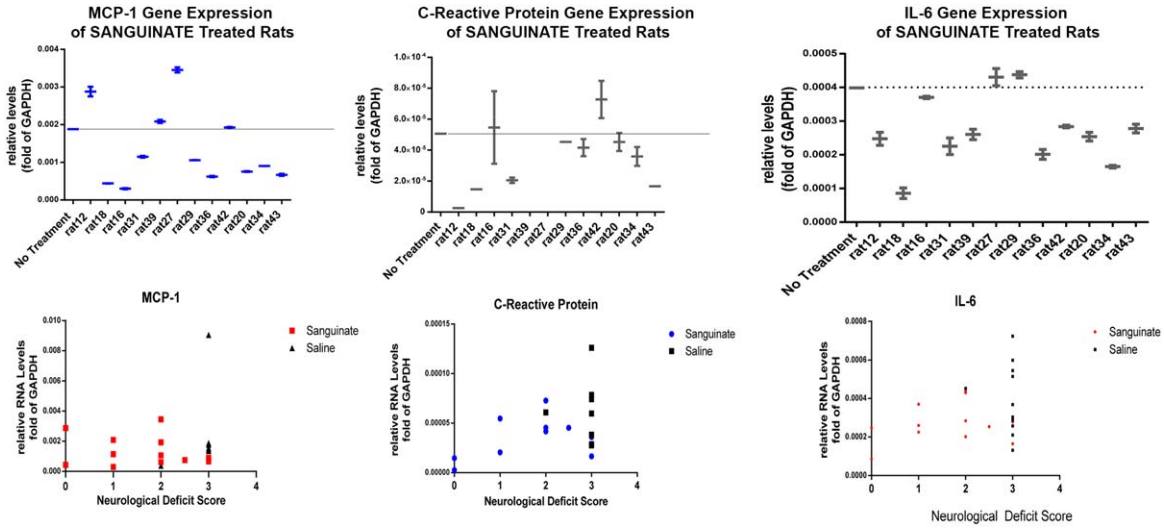

FIG. 2. Reduction of inflammatory markers and neurological deficit scores following induction of focal cerebral ischemia in a transient reversible middle cerebral artery occlusion (MCAO)/reperfusion model in rats.

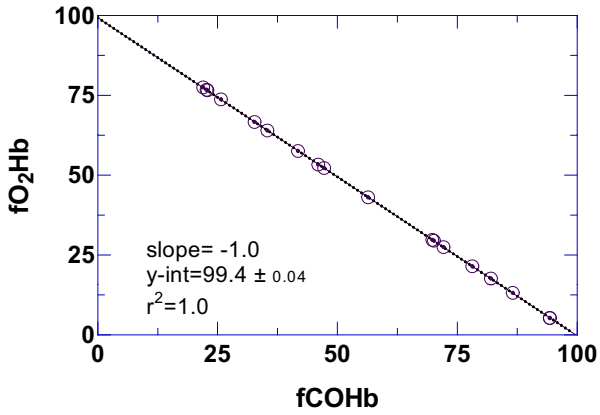

FIG. 3. Conversion of COHb to O_2Hb Form.

survival, restoration of oxygen delivery to peripheral tissues was critical for improving long-term outcomes (publication pending).

As the development of serious adverse events halted the development of earlier hemoglobin-based blood substitutes, animal toxicity studies have been performed with SANGUINATE, revealing no serious adverse physiological, histological, or immunological effects (9). Additionally, safety and pharmacokinetic Phase I clinical studies have been performed in both healthy subjects and stable SCD patients. Dizziness, lethargy, and musculoskeletal adverse events of mild to moderate severity were the most commonly reported adverse events (2). Transient increases in systolic and diastolic blood pressure following an infusion of SANGUINATE have been observed in patients with no clinically meaningful untoward effects (2). This has been attributed to the colloid osmotic (oncotic) pressure of the large and hygroscopic PEGylated protein. Increases in circulating troponin I have also been observed in SCD patients without accompanying evidence of cardiac ischemia (in press). This effect is believed to be due to a cardiac myocyte-stretch phenomenon caused by increased fluid pressure, resulting in a release of troponin from a cytosolic pool (10). Increased tricuspid regurgitant jet velocity (TRV) has also been observed in an SCD patient, but without accompanying clinical signs or symptoms (in press). Clinical studies to date have assessed vital signs, TRV, and electrocardiograph measurements as well as laboratory measures of serum biochemistry, hematology, and urinalysis. At this time, over 200 subjects have received either single or multiple doses of SANGUINATE.

The VOC of SCD is the most common comorbidity of SCD, accounting for the majority of emergency department visits and hospitalizations, and a well-suited target for SANGUINATE. VOC is due to a complex pathology of hemolysis, inflammation, adhesion, and deoxygenation of RBCs that result in the obstruction of microvasculature in organs and muscles (11). Not only does this cause debilitating pain but the subsequent hypoxia can lead to organ damage and may result in more serious complications such as acute chest syndrome (12). The occlusive, adhesive, and fragile properties of sickled RBCs are primarily responsible for the development of the numerous comorbidities associated with SCD. It is only when the fraction of oxygenated sickle hemoglobin (HbS) reaches a sufficient level that reversion to normal cell morphology occurs, restoring vascular perfusion. In vitro experiments have shown a concentration and time-dependent effect of SANGUINATE's ability to deliver both oxygen and CO to sickled RBCs (Fig. 4). Studies using deoxygenated sickle RBCs have shown the ability of SANGUINATE to deliver oxygen and revert the cells to a more normal morphology (Fig. 5). These data suggest that SANGUINATE has the potential to improve sickle cell morphology by reversing sickling, the underlying pathology of SCD co-morbidities. SANGUINATE

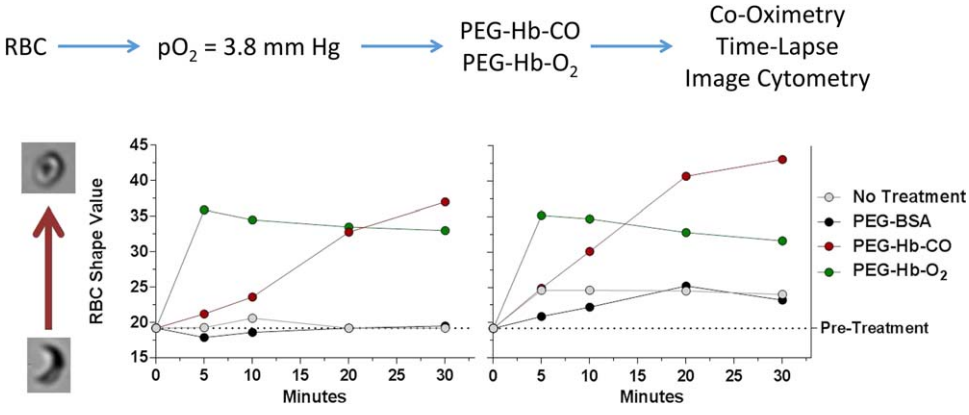

FIG. 4. PEGylated bovine hemoglobin in vitro mediated oxygen and carbon monoxide delivery to sickled SCD RBCs reverses cell shape to normal.

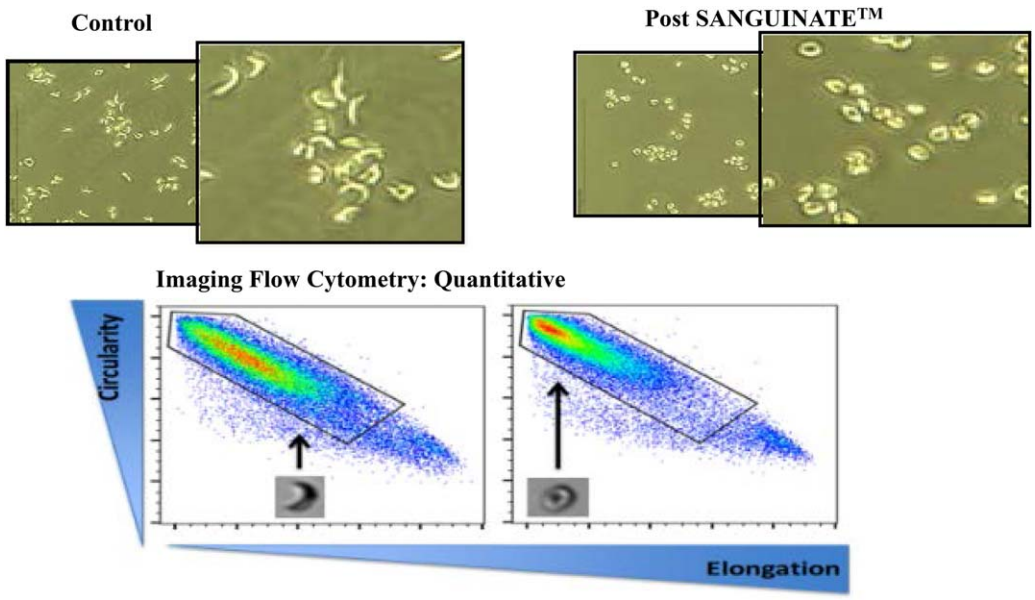

FIG. 5. SANGUINATE transfers oxygen and restores morphology to sickle cells in vitro.

is in a Phase 2 trial for the treatment of VOC. In addition, patients with SCD with acute chest syndrome (13) and hyperhemolysis who were unable to receive blood transfusions have been treated with SANGUINATE under the compassionate use and Phase I severe anemia protocols (see below).

Delayed cerebral ischemia (DCI) is the occurrence of focal neurological impairment (such as hemiparesis, aphasia, apraxia, hemianopia, or neglect), or a decrease of at least two points on the Glasgow Coma Scale (14), and is a sporadic and frequently lethal adverse effect of subarachnoid hemorrhage. A Phase 2, open-label study of single escalating-doses has been completed and was designed to assess the effect of SANGUINATE in patients at risk of DCI after aneurysmal subarachnoid hemorrhage as well as assess the impact of SANGUINATE on brain oxygenation and blood flow as measured by positron emission tomography. No serious adverse event concerns have been reported in this study.

A third indication under investigation is DGF which is a common sequelae following kidney transplantation. DGF is a form of acute renal injury resulting in increased early organ dysfunction (i.e., poor urine output) and decreased long-term organ survival. Poor functioning of the kidney necessitates the use of dialysis until the kidney recovers and resumes normal functioning. It is estimated that DGF occurs in approximately 20–50% of all deceased donor kidney transplantations. Ischemia-reperfusion injury is the primary causative factor triggering damage to renal cells, particularly vascular endothelial and tubular epithelial cells, and initiation of a pro-inflammatory state (15). Enrollment has been completed in the Phase 2 study of SANGUINATE for the reduction of occurrence of DGF in recipients of a donation after brain death kidney transplant.

An open-label Phase 1 safety study of SANGUINATE infusion in patients with acute severe anemia who are unable to receive RBC transfusion has also been initiated. Severe hypoxia due to low hemoglobin levels causes significant morbidity and mortality. This is of great concern for patients who cannot receive blood transfusions due to personal (e.g., religious) or medical (e.g., hyperhemolysis) reasons, or simply due to the lack of availability of safe blood. SANGUINATE has been used in 62 patients under the Food and Drug Adminstration expanded access emergency Investigational New Drug (eIND) program. Patients received single or multiple (up to 5) units of the product. All patients provided with timely emergency treatment with SANGUINATE were reported by the investigator to show clear signs of improved function that were temporally associated with infusion, despite continued life-threatening low hemoglobin levels. Improvements in cerebrovascular oximetry, cerebral blood flow, pulmonary infiltrates, renal function, and serum biochemistry (bilirubin, liver function, lactate dehydrogenase) were reported in various patients. While severe anemia persisted for all of these

patients, it appears that the life-threatening hypoxia did not, suggesting that SANGUINATE may find a role as a "bridge therapeutic" to promote tissue oxygenation until recovery of the patient's hemodynamic or anemic status.

CONCLUSIONS

SANGUINATE represents the next generation of oxygen delivering agents. The four indications in clinical development have hypoxia and inflammation as major factors in the disease pathology. Reversing hypoxia should have a corresponding impact on clinical symptoms and outcomes. As hypoxia plays a fundamental role in a wide variety of disorders and diseases, it can be anticipated that oxygen carriers may become a pivotal therapeutic approach in disease management.

Conflict of Interest: None

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