Chapter 58
PEGylated Bovine Carboxyhemoglobin (SANGUINATE™): Results of Clinical Safety Testing and Use in Patients

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Abstract Oxygen transfer agents have long been sought as a means to treat hypoxia caused by congenital or acquired conditions. Hemoglobin-based oxygen carriers were in clinical development as blood substitutes, but development was halted due to the finding of significant vasoactivity. Rather than develop a blood substitute, a product for indications characterized by hypoxia is in development. PEGylated bovine carboxyhemoglobin (SANGUINATE™) is both a carbon monoxide releasing molecule and an oxygen transfer agent. It is comprised of three functional components that act to inhibit vasoconstriction, reduce inflammation and optimize the delivery of oxygen. SANGUINATE has the potential to reduce or prevent the effects of ischemia by inhibiting vasoconstriction and re-oxygenating tissue. Phase 1 safety trials in healthy volunteers were completed in 2013. SANGUINATE was shown to be safe and well tolerated with no serious adverse effects. Phase Ib studies have been completed in stable patients with Sickle Cell Disease. SANGUINATE has also been administered to two patients under emergency use protocols. Both patients exhibited improved status following treatment with SANGUINATE.

Keywords SANGUINATE • Clinical • Safety • Hypoxia • Ischemia

1 Introduction

The holy grail of developing a clinical substitute for blood transfusions was halted when serious adverse side effects such as, cardiac, gastrointestinal, hepatic, pancreatic, central nervous system, and renal damage with a concomitant increase in mortality were observed in some trials. The adverse events were attributed to nitric oxide (NO) scavenging and auto-regulatory vasoactivity resulting in vasoconstriction, hypertension and heme-mediated oxidative damage [1, 2]. Many of the trial

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designs for these products were hampered by its use in severely injured trauma or surgical patients, thereby making any analysis of safety and efficacy difficult.

While the development of blood substitutes has been largely abandoned by the biopharmaceutical industry, there is progress in the development of oxygen transfer agents (OTAs). These products are not blood substitutes but rather facilitate the transfer of oxygen to hypoxic tissue. OTAs are intended as therapeutics and will be developed in specific target indications that permit clearer analysis of toxicity as well as efficacy.

Despite the toxicity issues seen when used as a blood substitute, hemoglobin still remains the ideal transport molecule for oxygen. Therefore, the development has focused on optimizing the molecular design to modulate the hemoglobin affinity for oxygen (p50), reduce NO scavenging, increase the resistance to auto and chemically-induced oxidation and possess the appropriate viscosity/colloidal osmotic pressure characteristics for circulatory use.

While a number of approaches have been used to develop the optimal OTA, many attempts could not surmount the hurdles in optimizing p50 and reducing NO scavenging. For example, in patients undergoing primary hip arthroplasty, MP40X showed minimal effects on blood pressure changes, but had higher rates of adverse events, including elevation of liver enzymes and troponin levels [3]. In a Phase 2b trial in trauma patients, it failed to meet its primary endpoint [4]. A follow-on product MP4CO has been abandoned as well. This experience illustrates the importance of appropriate molecular design, choice of targeted indication and clear clinical endpoints.

SANGUINATE (PEGylated bovine carboxyhemoglobin) has been designed to avoid the problems of NO scavenging and auto-oxidation while optimizing oncotic pressure and p50. It acts both as a CO releasing molecule and an O_2 transfer agent. Its components act to prevent the toxicity and work synergistically to enhance its therapeutic potential.

SANGUINATE is produced through the polyethylene glycol modification (PEGylation) of surface lysine residues on purified bovine Hb with 5000 molecular weight PG-succinimidyl carbonate followed by carboxylation. PEGylation is a well-established drug delivery technology. Due its hydrophilic nature, PEG increases the effective molecular size of hemoglobin and thereby inhibits the interaction of haemoglobin with vascular endothelium [5]. PEG-hemoglobins have shown decreased vasoactivity as compared to acellular hemoglobin. PEG increases oncotic pressure which is known to impact blood flow dynamics and induce the production of vasodilatory NO by vascular wall shear stress activity [6]. PEGylation also has the properties of extending circulating half-life and decreasing immunogenicity of protein therapeutics [7].

Bovine hemoglobin (bHb) has several advantages over its human counterpart. The oxygen affinity (p50) of PEG modified bHb (p50 = 7–16 mmHg) is superior to PEG modified human derived hemoglobin products (p50 = 4) [8]. Since the goal is to maximize oxygen delivery to oxygen-deprived tissues, utilizing PEG-bHb, with

a higher p50, offers therapeutic advantages over a non-bovine source. Also, as the p50 of PEG-bHb lies between that of the red blood cell (p50 = 26) and hypoxic tissue, it acts as a conduit to transfer the oxygen from the blood cells to the tissue. In addition, bHb requires only plasma chloride for stability, unlike human hemoglobin which is dependent upon 2,3diphosphoglycerate to remain in its active tetrameric form.

CO is an essential component to the therapeutic effect of SANGUINATE. CO reduces auto-oxidation of hemoglobin and improves shelf-life such that very little methemoglobin is formed, even after storage at room temperature for up to 18 months. More importantly, CO has therapeutic activity that complements the oxygen transfer role of hemoglobin. It is an endogenous regulatory molecule that plays a key role in regulating vascular muscle tone and reducing platelet aggregation. Delivery of exogenous CO has shown potent protection in numerous experimental models of inflammation, sepsis and hemorrhagic shock [9]. Potent anti-inflammatory activity associated with down-regulation of proinflammatory has been reported as a result of exposure to CO [10]. As such, the CO released from SANGUINATE can prevent the vasoconstriction that was observed with early hemoglobin-based products and contributes a potential therapeutic effect. In animal studies, CO was shown to be rapidly released by SANGUINATE within minutes of transfusion with a whole blood COHb peak level of 5 % that declined to baseline levels over a 2 h period [11].

To address toxicity and safety concerns of its use as a single or repeating dose therapeutic, eight toxicology preclinical studies were performed to obtain regulatory approval for the Phase I study [12]. Parameters evaluated for the assessment of toxicity included clinical observations, body weights, ophthalmic observations, food consumption, hematology, clinical chemistry, coagulation, urinalysis, and organ evaluation (44 different tissues examined including brain, heart, liver, spleen, kidney) and histopathology. Additional assessments included in some of the studies were special histopathology staining, toxicokinetics, functional observations, immunogenicity, and cardiovascular measurements. There were no adverse effects identified for any dose and, therefore, a (no) observed adverse effects level (NOEL) could not be determined even at dosage levels of 1200 mg/kg (monkey), 1600 mg/kg (pig) and 2400 mg/kg (rat). To evaluate whether the bovine protein would induce antibody formation, 6 and 9 month repeat dosing studies were performed in rats and pigs. The presence of IgG antibodies directed against SANGUINATE was measured in serum samples by the ELISA method. The data indicated that SANGUINATE did not induce an immunogenic response.

For assessment of its therapeutic effects, SANGUINATE was tested in ischemic animal models. Diabetic mouse models were also tested, as these demonstrate high oxidative stress. In animal models of stroke and myocardial infarction, SANGUINATE was effective in significantly reducing the area of infarct as compared to controls [11–13]. Studies in animal models of focal cerebral ischemia demonstrated that SANGUINATE inhibited vasoconstriction [13]. In diabetic mice, administration of SANGUINATE improved blood flow recovery and capillary density in ischemic muscle tissue after femoral artery ligation [11]. Data from

an ischemic/reperfusion myocardial model in diabetic mice indicated that SANGUINATE treatment reduced oxidative stress during the early phase of reperfusion, indicating that SANGUINATE is effective in protecting against reperfusion injury [12]. SANGUINATE has also been shown to reduce neurological deficits in rat models of focal cerebral ischemia [11]. This demonstrates SANGUINATE acts to halt the hypoxic cascade and protect the cells and tissues surrounding the area of insult. Collectively, these studies support an ongoing investigation of SANGUINATE in a wide variety of indications where an OTA is needed.

SANGUINATE has been evaluated in a randomized Phase I single-blind placebo-controlled study in 24 healthy volunteers. Three cohorts of 8 [6 receiving SANGUINATE (40 mg/ml, 2 receiving saline as a placebo comparator)] in an ascending dose study received 80, 120 or 160 mg/kg. There were no clinically meaningful safety findings following the single intravenous infusion in all dose groups. In particular, there were no significant signs of systemic or pulmonary hypertension. There was an observed trend toward increased blood pressure (both systolic and diastolic) in subjects administered SANGUINATE that was not seen in placebo subjects. These changes were transient and returned to baseline by 72 h after the infusion was completed. Mean increases did not reach the level of arterial hypertension. There was no clear dose- proportionality suggesting that the increase in blood pressure was likely due to the oncotic effect of SANGUINATE. Pharmacokinetic analysis found all parameters measured (Cmax, tmax, AUC, 0-last, AUC0-inf, kel, t1/2) had a dose related response. The circulating half-life (t1/2) ranged from 7.9 to 13.8 h [14].

Due to its multiple mechanisms of action, SANGUINATE has potential use in a broad range of indications. The initial indication is for the treatment of vaso-occlusive crisis in patients with sickle cell disease (SCD). This is one of several SCD comorbidities which include stroke, leg ulcers, priapism and acute chest syndrome. The underlying pathophysiology is caused by ischemia and hemolysis resulting in extensive inflammation, reactive oxygen species formation and up-regulation of adhesion molecules.

Two patients with hemoglobinopathies that could not or would not receive blood transfusion received SANGUINATE under emergency INDs. Despite persistently low hemoglobin levels due to hemolytic manifestations of sickle cell disease, improvements in symptoms were seen in both patients with no evidence of drug-related adverse events following the administration of SANGUINATE. Conclusions regarding the efficacy of SANGUINATE cannot be drawn from these cases as they are not part of a controlled clinical trial. In each case, however, the attending physician attributed the improvement in the patient's symptoms to the treatment with SANGUINATE.

The first patient, a 61-year-old female, refused blood transfusion due to religious reasons and was admitted to the ICU with hemoglobin level of 6.5 g/dL. She was in near-comatose condition and unresponsive to voice with a pre-infusion brainoxygen saturation level of 48 %. One 500 mL bag of SANGUINATE 40 mg/mL (=290 mg/kg dose based on the recorded 69 kg mass) was administered by 2-h intravenous infusion. No acute toxicity was noted. The next morning, the

patient continued to be tachypneic, but was more alert with improved brain oxygenation (EQUANOX™ Oximeter System) increasing from the high 40s to low 50s to the mid to upper 60s. The patient nodded/shook head appropriately to questions. The next day, the patient experienced acute respiratory failure and had a hemoglobin level of 2.9 and a second infusion of SANGUINATE was administered. There were no acute toxicities noted post-infusion. The patient showed poor response to therapy, with severe hypotension and negligible renal function. The family requested discontinuation of respiratory support. The site investigator credited the patient's improvement in status to the infusion with SANGUINATE, and assessed the event of death as not related to study drug [15].

The second patient was 23 years of age and was admitted to the ICU presenting with respiratory distress (PO_2 54.3, sPO_2 88.4 %, pH 7.48, PCO_2 38.2, hemoglobin 4.6) secondary to acute chest syndrome. Patient was offered transfusions and, despite risk of death, refused due to religious beliefs. A total of three doses of SANGUINATE were administered (Day 1, Day 2 and Day 8) and the patient was extubated successfully on Day 10. Over the course of treatment, the patient had improved cardiac, pulmonary and renal function. A measure of inflammation (C-reactive protein) also improved substantially. Most of the patient's laboratory values were restored to within the normal range, despite the continued severe anemia demonstrated by the extremely low hemoglobin and hematocrit levels. Despite her persistently low hemoglobin of 3.1, she reported improvement in her dyspnea upon extubation. Her initial transcranial Doppler (TCD) revealed hyperemia with high blood flow velocities that normalized following SANGUINATE infusion. Following the third administration at approximately 18 h post-dose, the TCD revealed a statistically significant ($p < 0.001$, with ANOVA and post-hoc Bonferroni tests) velocity reduction in both MCAs, which correlated with the patient's clinical improvement [16].

2 Conclusion

From the knowledge elucidated from the development of blood substitutes, the design of an OTA would require higher O_2 affinity, a viscosity similar to blood, increased oncotic pressure as compared to blood and long plasma retention [2]. The use of a single approach (i.e. PEGylation) would be insufficient to address all these issues. SANGUINATE has a mechanistic profile that addresses the issues of acellular hemoglobin toxicity and adds functionality that goes beyond oxygen transfer. Nonclinical studies have shown that SANGUINATE can play a key role in the inhibition of vasoconstriction, reduction in infarct volume and impact on oxidative stress. In vitro work has demonstrated that SANGUINATE is able to repetitively bind and transfer oxygen to deoxygenated cells (manuscript pending). The increase in cerebral oximetry readings in patients treated under eINDs without an accompanying increase in hemoglobin levels also suggests that this product is transferring oxygen.

A Human safety study has demonstrated that SANGUINATE produces no serious adverse events in humans. These results are consistent with preclinical data that no NOEL could be determined, even at the highest doses tested. A Phase Ib study in stable SCD patients has reported no serious adverse events at the lower dose and analysis of the higher dose findings will be completed shortly.

Because SANGUINATE has multiple modes of action that result in the inhibition of vasoconstriction and promotion of plasma expansion, oxygenation of hypoxic tissue, reduction of infarct volume and potential anti-inflammatory, its potential use is broad, ranging from treatment of hemoglobinopathies to stroke and chronic wounds. All these indications have a pathophysiology that results from initiation of the ischemic cascade and the ensuing damaging inflammation, apoptosis and necrosis. SANGUINATE may be able to act upon different steps in the cascade to reduce or prevent cellular death and tissue injury.

At this time, a Phase II study for the use of SANGUINATE for the reduction or prevention of delayed cerebral ischemia following subarachnoid hemorrhage has been approved by the FDA. A Phase II trial in SCD patients with vaso-occlusive crisis has been filed and other indications are under consideration. Careful selection of the initial indications and clinical endpoints for regulatory approval are the next step in the development of this product.

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