Hyperbranched Polyglycerol Significantly Improves Therapeutic Index of Small Molecular Weight Iron Chelator

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Introduction: The majority of drugs approved for clinical use are of the low molecular weight (MW) class. As a result, they are rapidly cleared from circulation, often before reaching the target site in sufficient amounts. Additionally, small MW drugs can freely enter healthy, non target areas and exert toxic effects. This is the major challenge associated with treating transfusion-dependent patients with patients with inherited and acquired refractory anemias such as sickle-cell and thalassemia. These patients are frequently treated with small molecular iron chelators like desferrioxamine (DFO)[4]. However, due to its short half-life of 20 minutes, DFO must be administered subcutaneously for 8-12 hours a day, 5-7 times per week. Additionally, DFO is highly toxic and can cause vision impairment and bone deformities. Given that the toxicity and half-life of drug molecules are strongly influenced by molecular weight (MW), **we hypothesized that***: A biocompatible macromolecular chelator with iron binding groups attached will reduce the systemic toxicity of DFO, and enhance its capacity to mobilize iron thereby increasing their therapeutic efficiency*[5-6].

Materials and Methods: HPG-DFO (figure 1) was generated by conjugating HPGs of various molecular weights (MW) to varying numbers of DFO molecules using Schiff-base chemistry. The influence of HPG MW and DFO density on DFO iron binding of HPG-DFO was determined using Isothermal Titration Calorimetry (ITC) and UV-visible spectroscopy. The safety of HPG-DFO was evaluated by examining blood coagulation, platelet activation and complement activation. The effect of HPG-DFO on cell viability was evaluated in human umbilical vein endothelial cells (HUVECs). Tolerance was studied by injecting up to 1000 mg/kg of HPG-DFO in mice and monitoring body weight, lactate dehydrogenase levels and organs morphology at 14 days. Tritium labeled HPG-DFO was used to determine the circulation t1/2 in mice. Iron excretion efficacy of HPG-DFO compared to DFO was tested in iron overloaded mice by treating the animals with 150 mg/kg DFO or DFO equivalent. The iron content of organs, urine and feces was analyzed to determine the

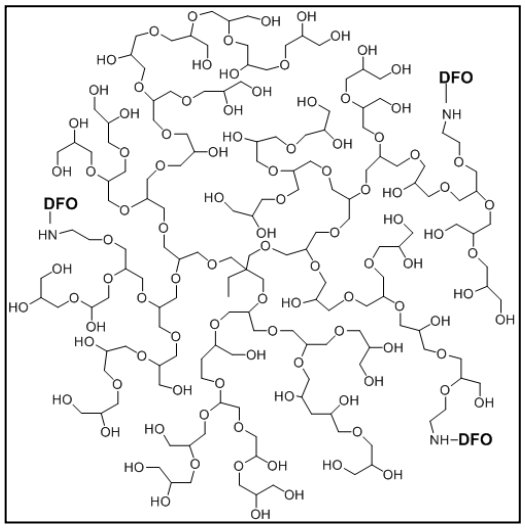

Figure 1: The structure of HPG-DFO

amount of iron excreted. Histological examination of the organs was performed using the Prussian blue stain.

Results: ITC and UV analysis demonstrated that the conjugation of DFO to HPG did not alter the iron chelating properties of DFO (figure 2). HPG-DFO did not activate platelets, coagulation or the complement system. *In vitro* cell tolerance studies showed there was no increase in toxicity compared to the DFO molecule after polymer conjugation.

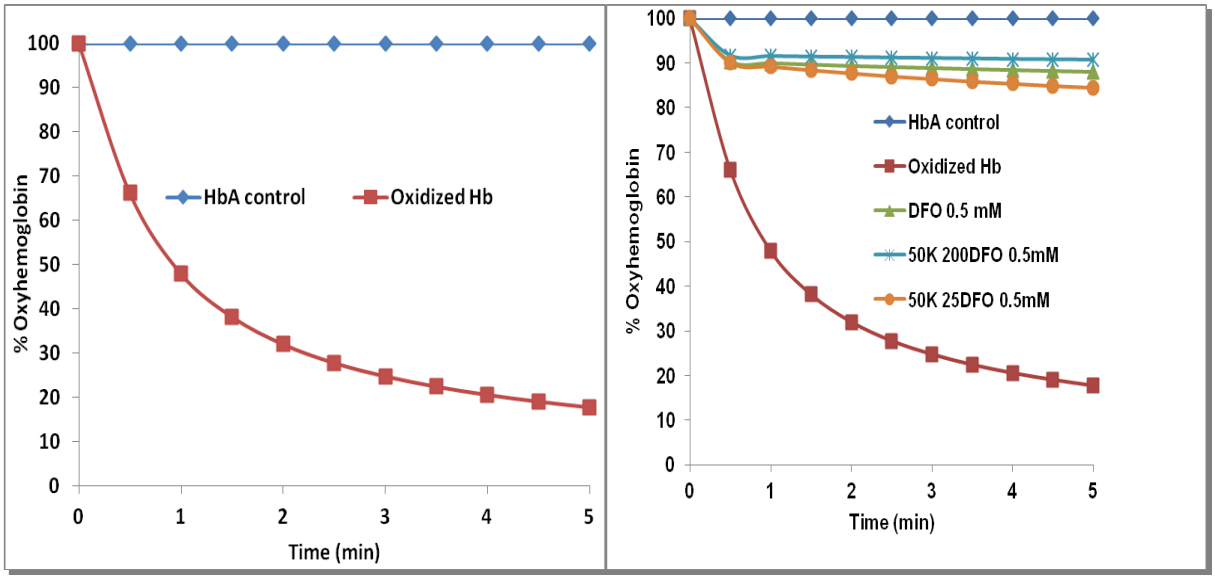

Figure 2: Hemoglobin oxidation. 50 kDa HPG with 50 and 200 DFO attached are effective at reversing Fe^{3+} mediated oxidation of hemoglobin a (HbA) at 0.5 mM. Conjugates were similar to small MW DFO in their chelating properties and were able to maintain oxyhemoglobin levels above 90%.

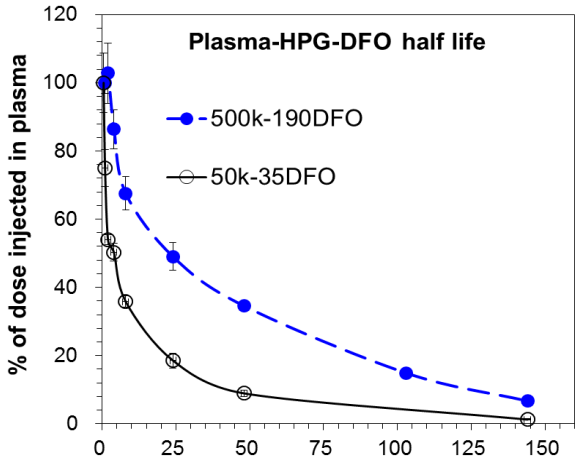

Figure 1: The plasma half-life of 2 tritium-labeled HPG-DFO conjugates after

Mice tolerance studies showed that the HPG-DFO conjugates were non toxic up to the maximum injected dose of 1000 mg/kg. The t1/2 of HPG-DFO in normal mice was 16 h for the 50 kDa conjugate and 44 h for the 500 kDa conjugate (figure 3). The iron excretion efficiency as measured by urinary iron of the 50kDa HPG-DFO in iron overloaded mice showed significant improvement compared to placebo ($p=0.002$) and DFO treated mice ($p=0.0011$).

Discussion: HPG-DFO shows a significant increase in t ½ which may result in the maintenance of much higher plasma concentrations and more effective iron removal. This approach has the potential to significantly decrease the number and duration of injections required to offload significant amounts of organ and total body iron in transfusion dependent patients. HPG-DFO appears to be a very promising drug conjugate for iron chelation therapy.