Sevuparin Blocks Sickle Blood Cell Adhesion and Sickle Leukocyte Rolling on Immobilized L-Selectin in a Dose-Dependent Manner

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BACKGROUND

The cause and continuation of vaso-occlusion in sickle cell disease (SCD) are fueled by the sickle-red blood cell interactions with multiple cell populations (Okpala et al. 2002). These interactions promote inflammation, obstruct the vasculature, and injure the endothelium, leading to end organ injury. Recent studies have identified multiple cellular components and molecular factors that contribute to the pathophysiology of SCD (Zhang et al. 2016). It is likely that a multi-targeted approach for addressing SCD vaso-occlusion will be required to achieve the best clinical outcome. Sevuparin (DF02), a novel drug in Phase II clinical development for acute treatment of vaso-occlusive crises in SCD (NCT02515858), is a polysaccharide that blocks abnormal adhesion and normalizes obstructed blood flow. In vitro and in vivo studies have shown potent anti-adhesive effects with a multimodal mechanism of action blocking the key adhesion receptors P-selectin, L-selectin, thrombospondin, von Willebrand factor and fibrinogen (Telen et al. 2016).

OBJECTIVES

The objective was to study the mechanism of sevuparin’s anti-adhesive effects under physiologic flow conditions using a standardized microfluidic flow-based adhesion assay.

METHODS

Peripheral blood was obtained from patients with homozygous SS SCD (15-25yrs, n=12) in steady state or crises as indicated (Table 1). Informed consent, or assent when indicated, was obtained in accordance with the Declaration of Helsinki. The protocol was approved by the IRB at Wayne State University. Whole blood and isolated WBC adhesive properties were measured during simulated blood flow as previously described by White et al. 2014. Briefly, whole blood adhesion was measured using a standardized Flow Adhesion™ assay (1 dyne/cm², 1.67 Hz to FCAM-1 and cultured human endothelial umbilical vein cells (HUVECs) stimulated with TNF-α and Histamine). Isolated leukocyte rolling density (cells/mm²), rolling flux (%), and rolling velocity (μm/sec) was assessed using a standardized Flow Dynamic Adhesion™ assay to immobilized L-selectin at 1 dyne/cm² (Functional Fluidics, Detroit MI).

RESULTS

Sevuparin acts in a multicellular manner, blocking both sickle whole blood adhesion and L-selectin-mediated rolling adhesion of sickle-leukocytes, as well as interacting with yet another key adhesion receptor; FCAM-1. This further adds to sevuparin’s multimodal action and its potential clinical benefits in treating the complex mechanisms manifested in vaso-occlusion and encourages exploration of applying sevuparin treatment at home for early symptoms of a painful episode.

REFERENCES

2. Okpala, Iheanyi, et al. "Pathways involved in the pathogenesis of sickle cell disease:

CONCLUSIONS

Sevuparin contains the best combination of activity in vitro through multiple mechanisms of action in microfluidic flow assays. These results encourage further investigation of applying sevuparin treatment at home for early symptoms of a painful episode.

Table 1. Patient demographic for the samples analyzed in Figure 1 and figure 2. SS: at steady state; VOC: during vaso-occlusive crises.