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

Marina Lindgren, PhD*; Jennell White, PhD*; Ke Liu, PhD*; Lena Jendeberg, PhD*; and Patrick C. Hines, MD, PhD2.
1Modus Therapeutics AB, Stockholm, Sweden; 2Functional Fluidics, Detroit, MI, US

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 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 crisis in SCD (NCT02515838), is a polysaccharide that blocks abnormal adhesion and normalizes obstructed blood flow. In vivo and in vitro 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 fibronectin (Telen et al. 2016).

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 VCAM-1 and cultured human endothelial umbilical vein cells (HUVECs) stimulated with TNF-α and Histamine). Isolated leukocyte rolling density (cells/mm²), rolling flux (µm/sec), and rolling velocity (µm/sec) was assessed using a standardized Flow Dynamic™ assay (1 dyne/cm², 1.67 Hz to VCAM-1 and cultured human endothelial umbilical vein cells (HUVECs) stimulated with TNF-α and Histamine). Isolated leukocyte rolling density (cells/mm²), rolling flux (µm/sec) and rolling velocity (µm/sec) was assessed using a standardized Flow Dynamic™ assay (1 dyne/cm², 1.67 Hz to VCAM-1 and cultured human endothelial umbilical vein cells (HUVECs) stimulated with TNF-α and Histamine).

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.

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; VCAM-1. This further adds to sevuparin’s multimodal action and its potential clinical benefits in treating the complex mechanisms manifested in vaso-occlusion. In addition, it encourages exploration of applying sevuparin treatment at home for early symptoms of a painful episode as a complement to the on-going acute treatment clinical study.

CONCLUSIONS

REFERENCES


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