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 crisis in SCD (NCT02515838), 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 fibronectin (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/cm^2, 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/mm^2), rolling flux (%), and rolling velocity (μm/sec) was assessed using a standardized Flow Dynamic Adhesion™ assay to immobilized L-selectin at 1 dyne/cm^2 (Functional Fluidics, Detroit MI).

Patients	On Hydroxyurea	Visit	Age	Sex	CRP (mg/L)	Hct (%)	HbF (%)	LDH (units/L)	Platelates (K/cumm)	Reticulocytes (%)	White blood cells (K/cumm)
Pt1	yes	SS	25	M	4,44	28,4	20,3	519	377	9,2	9,8
Pt2	no	SS	17	M	<2,90	22,8	1,5	571	372	9	7
Pt3	yes	VOC	20	M	12,2	30	10,1	512	359	11	9,2
Pt4	no	SS	23	F	7,24	22,8	8,7	383	272	10	13,2
Pt5	no	SS	28	M	6,59	24,7	10,2	673	363	11,3	9,7
Pt6	no	SS	18	F	2,98	30,1	33,3	344	104	6,6	5,6
Pt7	yes	SS	15	M	8,29	24,6	10,7	672	386	16	9,8
Pt8	yes	SS	18	F	<2,90	25,2	12,2	736	355	15	8,6
Pt9	yes	VOC	20	M	<2,90	30,3	10,4	429	406	9	8,7
Pt10	no	VOC	25	F	14,3	16,4	2,2	950	299	7	12,2
Pt11	no	SS	18	M	<2,90	24,1	1,5	544	360	7	6,5
Pt12	no	VOC	18	F	<2,90	30,8	33,6	471	117	8,4	5,2

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

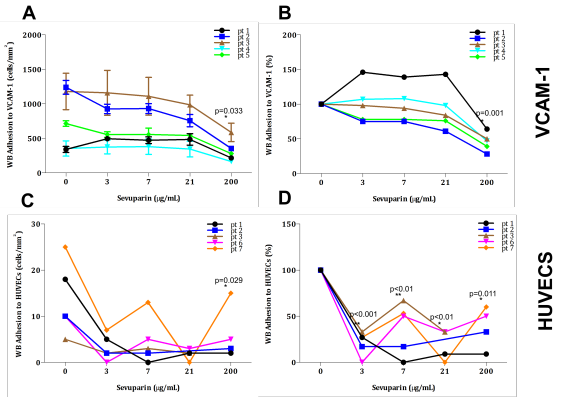

Figure 1. Sevuparin dose response of sickle whole blood Flow Adhesion™ (FF-FA) to **VCAM-1**: Absolute adhesion (A), % change from baseline (B), and **HUVECs**: absolute adhesion (C), % change from baseline (D). Individual patients (1-7) are indicated by a unique colored symbol connected by lines to demonstrate dose-response. Statistically significant comparisons (p<0.05) are indicated by "*" and p-values.

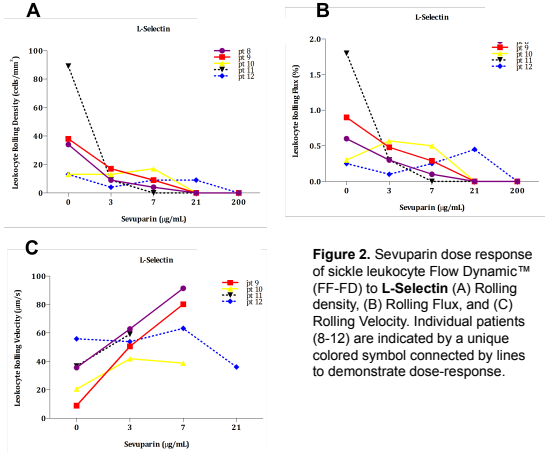

Figure 2. Sevuparin dose response of sickle leukocyte Flow Dynamic™ (FF-FD) to **L-Selectin** (A) Rolling density, (B) Rolling Flux, and (C) Rolling Velocity. Individual patients (8-12) are indicated by a unique colored symbol connected by lines to demonstrate dose-response.

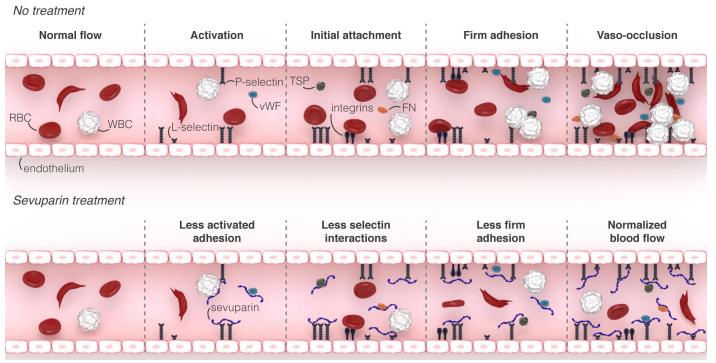

Figure 3. Potential multimodal mechanism of sevuparin action (Figure from Telen et al. 2016).

RESULTS

- Sevuparin (3 µg/mL) inhibits sickle whole blood adhesion to HUVECs (p<0.001)
- Sevuparin (200 µg/mL) inhibits sickle whole blood adhesion to VCAM-1 (p=0.033, absolute, p=0.001, % baseline)
- Each patient sample demonstrated a reduction in adhesion after sevuparin treatment
- Sevuparin reduced sickle leukocyte rolling cell density, rolling cell percentage and increased average rolling velocity on L-selectin.

CONCLUSIONS

Sevuparin acts in a multicellular manner, blocking both sickle whole blood firm 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 and encourages exploration of applying sevuparin treatment at home for early symptoms of a painful episode.

REFERENCES

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Modus Therapeutics is a Drug development Company in phase II clinical development. Functional Fluidics is a Life Science Product development company.