# Single-Stranded Oligonucleotide Aptamer Binding to P-Selectin Inhibits Adhesion of Sickle Red Blood Cells And Leukocytes to Endothelial Cells in Sickle Cell Disease Model Mice: Novel Therapeutics for Vaso-Occlusive Episodes

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**BACKGROUND**

Adhesive interactions between circulating red blood cells (RBC), leukocytes, and endothelial cells in post-capillary venules have been implicated as a contributing factor in the pathogenesis of vaso-occlusion, the major cause of morbidity and mortality associated with sickle cell disease (SCD). An inherited hematologic disease caused by a single amino acid substitution in the β-globin chain of hemoglobin.

Endothelial cell P-selectin, a member of the selectin family of cell adhesion molecules, plays a key role in leukocyte recruitment as well as in the adhesion of sickle RBC (SRC) to the endothelium. The expression of P-selectin is elevated in SCD and the interaction of P-selectin with its ligand is likely to contribute to impairment of the microvascular flow and thereby to the development of post-capillary vaso-occlusive abnormalities. 

Aptamers, short single-stranded oligonucleotides that fold into complex 3 D structures and bind to ligands, have been developed for a wide range of therapeutic targets. Although and P-selectin aptamers have been shown to inhibit leukocyte rolling in vivo and in vitro, studies in mouse models for inflammation, anti-adhesion activity of anti-P-selectin aptamers has never been evaluated in SCD.

**Objectives**

To determine in vivo whether the anti-P-selectin aptamer ARC5690 can inhibit adhesion of SRC and leukocytes to vascular endothelial cells in the bone marrow microvasculature of SCD model mice.

**Materials and Methods**

**Mouse model:** Knockout transgenic (K-T) mouse model of SCD deleter for the murine α and β-globin genes and transgenic transgenic of the human α-, β-, and γ-globin gene sequences.2

**Blood cell isolation and labeling:** RBC from donor K-T SCD mice were washed and labeled with 7-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF). Leukocytes were labeled in vivo with PE-tail anti-mouse CD45 antibody.

**Aptamer:** Anti-P-selectin aptamer ARC5690 and scrambled aptamer ARC5694 were supplied by Archemix Corp. (Cambridge, MA).

**Experimental design:** Saline (0.9%, NaCl, 10 μg/ml bwe), ARC5690 (20 mg/kg bwe in saline), and ARC5694 (20 mg/kg bwe in saline) were injected IP. Two and a half hours after injection, mice were subjected to 1 hour of hypoxia (12% O2) followed by one hour reoxygenation at room air. Anti-P-selectin antibody (positive control) was infused just before intravital experiments.

**Surgical procedure:** Mice were anesthetized with ketamine/xylazine (0.1 mg/kg, 0.15 mg/kg bw, IP), and a tracheotomy and connected to a small animal respirator. Polycarbonate catheter (PE-10) for injection of fluorescent sickle RBC and antibodies and monitoring blood pressure was inserted into the right common carotid artery and placed in the aortic arch. Spley was incised in the midst to expose tracheal-carotid artery. Temperature was constantly monitored.

**Intervention video microscopy:** Observations of skull bone marrow microvascular network were conducted using an intravital microscope equipped with water-immersion objectives, SIT camera, a time-base generator and SYCAM digital videocassette recorder (Figures 1 and 2). Images were recorded and analyzed by playback of VideoScope in 4-6 frame/s using image Pro-Plus 5.3 software imaging.

**RESULTS**

**Figure 2. Experimental setup:**

(a) microscopic stage; (b) ventilation; (c) temperature controller; (d) blood pressure recorder.

**Figure 3. Velocity (A) and adhesion (B) of BCECF-labeled sickle RBC in the skull bone marrow microvasculature of K-T SCD mice after pretreatment with saline (control), ARC5690, ARC5694 or anti-P-selectin antibody. Values are mean ± standard error (SE) obtained from 3 to 5 mice in each group p<0.01 compared to saline treated group.

**Figure 4. Leukocyte flow dynamics in the skull bone marrow microvasculature of K-T SCD mice after pretreatment with saline, ARC5693, ARC5694, or anti-P-selectin antibody: (A) Leukocyte velocity; (B) Leukocyte rolling flux; (C) Leukocyte adhesion. Adhesion values are mean ± SE. SE obtained from 3 to 5 mice in each group p<0.01 compared to saline treated group.

1. ARC5690, at the dose 20 mg/kg bw significantly reduced adhesion of sickle RBC to the endothelium in two-fold and carrying a 30× increase in SCD model of SCD. (p<0.001). Decreases in RBC adhesion correlated with improved blood flow in microcirculation as shown by an increase in RBC velocity in ARC5690-treated sickle mice compared to the saline-treated group.

2. At 20 mg/kg bw, ARC5690 induced a four-fold decrease in leukocyte rolling flux and five-fold decrease in leukocyte adhesion in K-T mouse model of SCD compared to the saline-treated group.

3. SCD mice treated with ARC5690 demonstrated trends of a lower mortality rate through the surgical procedures and intravital experiments compared with mice injected with saline (43.3% ARC5690 vs 33.3% saline), suggesting a protective effect during surgical and hypoxic stress in this animal model.

**CONCLUSION**

Our study demonstrates significant anti-adhesive activities of ARC5690 which reduces the adhesion of SRC and leukocytes to the vascular endothelium in the mouse model of SCD. ARC5690 may represent a novel therapeutic strategy that can be used to treat vaso-occlusive episodes in SCD.

**REFERENCES**


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