Crizanlizumab Reduces Endothelial Adhesion of Red Blood Cells from SCD Patients in Standardized Microfluidic Platform: in vitro Technology Assessment

#P30

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INTRODUCTION

Crizanlizumab (ADAKVEO®, Novartis, Switzerland) a monoclonal P-selectin blocking antibody designed to disrupt the blood cells and endothelium interactions in sickle cell disease (SCD) patients, which leads to reduction of vaso-occlusive crises, the major manifestation of the disease. We report the novel microfluidic platform (endothelium-on-a-chip), to test the effects of Crizanlizumab on the adhesion of red blood cells (RBCs) to perfusion-cultured, acutely and chronically activated human endothelial cells (ECs).

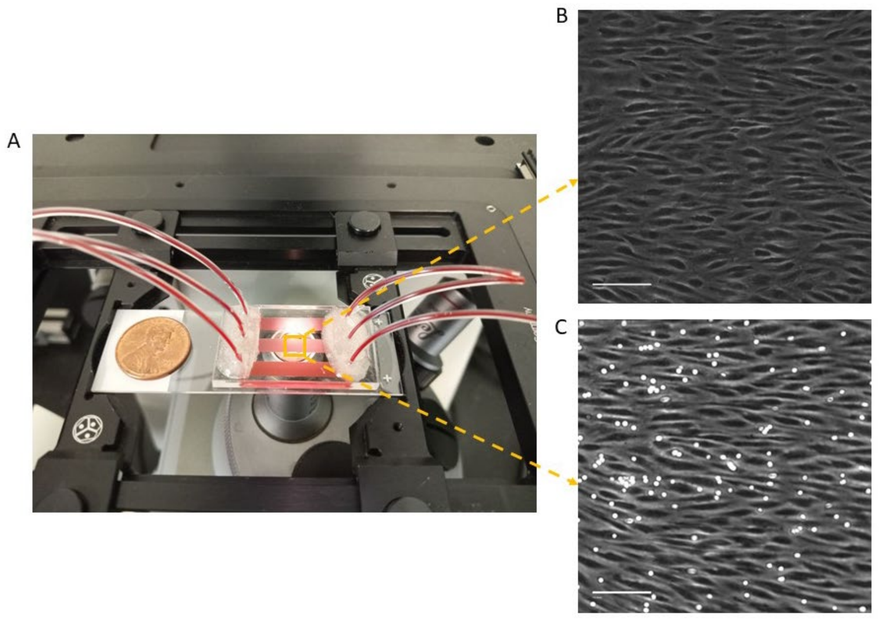


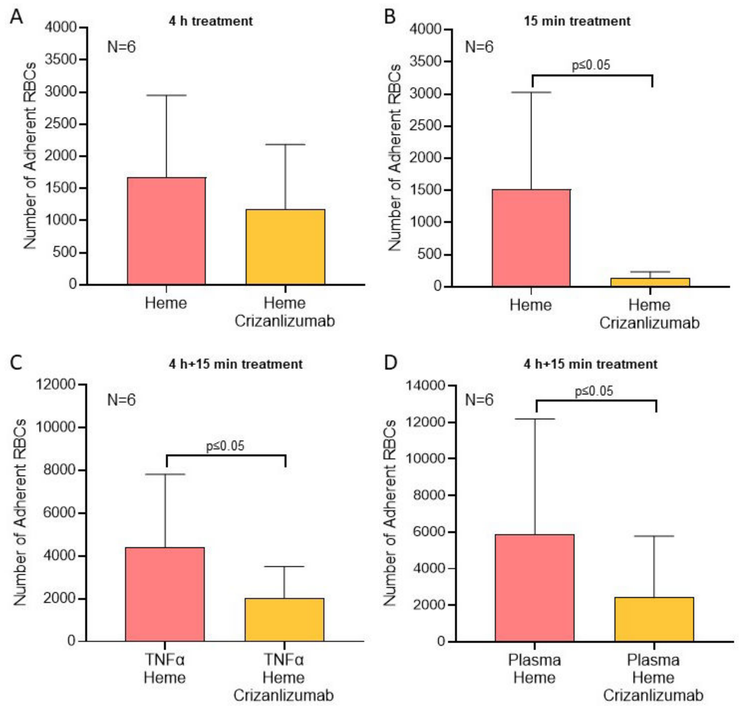
Figure 1. (A) Endothelium-on-a-chip set-up. **(B)** The phase-contrast image of HUVEC demonstrate a confluent layer of cells aligned with flow. **(C)** RBCs adhesion to Plasma and Heme activated HUVECs. Microscope images at 10X. Scale bare 100um

MATERIALS & METHODS

Whole blood samples collected from SCD subjects (n=21), 20 HbSS and 1 HbSC in EDTA and sodium citrate. RBCs were isolated via centrifugation from whole blood and resuspended in basal cell culture medium (EBM; Lonza, Morristown, NJ, USA) at a hematocrit of 20% with 10 mM of HEPES. Human umbilical vein endothelial cells (HUVECs; Lonza, Morristown, NJ, USA) were cultured within the microfluidic platform channels at 15 dyne/cm2 for at least 48 hours prior to experiments. To mimic chronic pre-activation in SCD HUVECs were pretreated for 4 hours with heme (40 μM), TNFα (20 ng/ml) or 50% plasma of SCD patients in basal media, +/- 100 μg/ml Crizanlizumab followed by injection of blood samples through the microfluidic channels. For acute EC activation, blood samples were supplemented with 40 μM heme +/- 100 μg/ml Crizanlizumab and injected through the microfluidic channels for 15 minutes. Thereafter, non-adherent RBCs were rinsed via washing solution with or without Crizanlizumab and the remaining RBCs were quantified based on previous published methods. Paired t-test was used to calculate statistical significance.

RESULTS AND DISCUSSION

The inhibitory effects of Crizanlizumab on blood cell adhesion to ECs was linked to the type and duration of EC activation (fig.2). Crizanlizumab slightly reduced RBC adhesion to 4-hours heme activated ECs (1170±413vs1671±522 p>0.05). Reduction of RBC adhesion due to Crizanlizumab treatment was significant in 15-minutes heme activated ECs (135±40 vs 1513±617, p≤0.05). The effect of Crizanlizumab on decreasing RBC adhesion was significant when ECs were pre-activated by TNF-α (4404±1393 vs 2016±609, p≤0.05) or subjects' autologous plasma for 4 hours followed by a 15-minute heme activation (5876±2579 vs 2397±1381p≤0.05).



Fifure2: Effects of Crizanlizumab on RBC adhesion levels to HUVECs activated for **(A)** 4-hours with heme **(B)** 15-minutes with heme **(C)** 4-hours with TNF-α and 15-minutes with heme, **(D)** 4-hours with plasma and 15-minutes with heme. Error bars represent the standard error of the mean.

DISCUSSION

In accordance with the label of Crizanlizumab to reduce VOC, application of microfluidic platform associated with Crizanlizumab use displayed a reduced RBC adhesion to acutely heme-activated EC. Effects were preserved in presence or absence of chronic TNFα or autologous plasma pre-activation. Endothelium-on-a-chip microfluidic platform has been shown as reliable in monitoring patient response to anti-adhesive therapies in SCD.

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