P1429: In vitro comparison of the effects of Inclacumab vs Crizanlizumab on reducing Endothelial Adhesion of Red Blood Cell in Standardized Microfluidic platform

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INTRODUCTION

Crizanlizumab is a recent FDA approved humanized IgG2 anti-P-selectin antibody to decrease the frequency of vaso-occlusive crisis VOCs in Sickle cell disease (SCD) patients. Inclacumab is a full human IgG4 monoclonal antibody that selectively targets P-selectin. Anti-cell adhesion effects of Inclacumab were first reported in patients with cardiovascular disease. Two phase 3 clinical trials are evaluating the efficacy of Inclacumab in SCD patients to reduce VOCs (NCT04935879 and NCT04927247)

OBJECTIVE

The objective of the study is to compare in-vitro efficacy of the Inclacumab vs Crizanlizumab on reducing SCD red blood cells (RBCs) adhesion to perfusion-cultured, acutely and chronically activated human endothelial cells by using endothelium-on-a-chip microfluidic platform.

METHODS

Whole blood samples from SCD subjects (n=12), HbSS were collected in EDTA at University Hospital Cleveland Medical Center, Cleveland, OH, USA under the Institutional Review Board approved protocol (IRB 05-14-07C). Informed consent was obtained from all study participants. Following previous published protocol(1-3), RBCs were isolated after centrifugation of the 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 endothelialized microfluidic channels at 15 dyne/cm2 for at least 48-72 hours prior to experiments. For acute short-term activation, blood samples were supplemented with 40 µM heme +/- 100 µg/ml and Inclacumab or Crizanlizumab and injected through the microfluidic channels for 15 minutes. For long term chronic treatment HUVECs were incubated for 4 hours with heme (40 µM), in basal media and HEPES, followed by injection of blood samples through the microfluidic channels +/- 100 µg/ml Inclacumab or Crizanlizumab (both compounds were provided by Global Blood Therapeutics, Inc., a wholly owned subsidiary of Pfizer Inc. South San Francisco, CA, USA). Non-adherent RBCs were removed using washing solution with or without compounds and phase-contrast images of the remaining RBCs were acquired with an inverted microscope (DMi8 Leica Microsystems Inc. Deerfield, IL, USA) and quantified based on previous published methods. Paired t-test was used to calculate statistical significance.

RESULTS

Acute short-term heme activation induces high level of RBCs adhesion. Both compounds showed a reduction of the number of adherent RBCs to the activated heme, the effect of Inclacumab results statistically significant different compared to untreated RBCs and treated with Crizanlizumab (Figure 1A). Long term chronic heme activation results in a lower level of RBCs adhesion, both compounds showed a reduction in RBCs adhesion to activated HUVECs. Even though not statically significant with the limited number of patient samples tested, Inclacumab demonstrates a more evident effect compared to Crizanlizumab on lowering RBCs adhesion levels

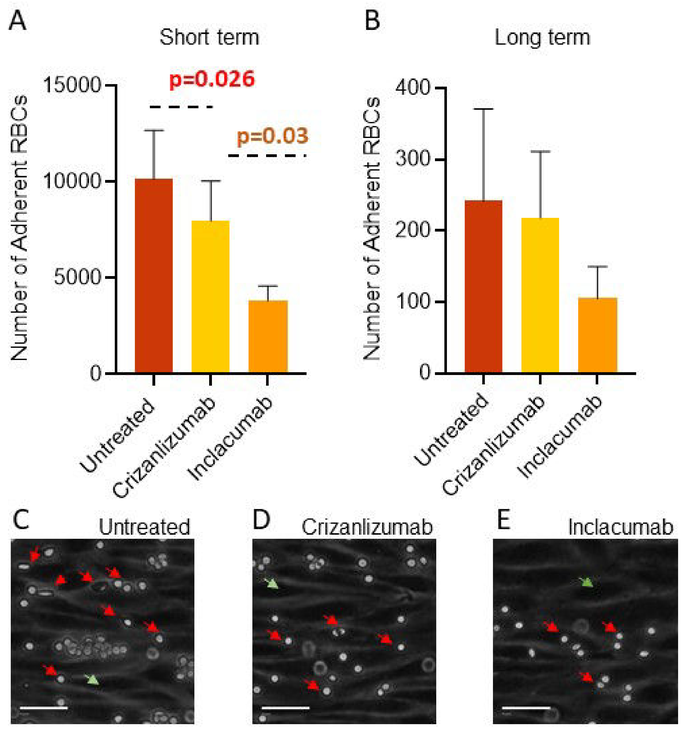

Fig 1. (A) Inclacumab treatment of short-term acute heme-activated HUVECs resulted in significantly reduced RBC adhesion levels compared to untreated control, and Crizanlizumab (N=6). **(B)** Inclacumab showed higher effect compared to Crizanlizumab in lowering the RBC adhesion levels of long-term chronic heme-activated HUVECs even though not statistically significant (N=6). Microscope 10X phase-contrast images of untreated sample **(C)** treated with Crizanlizumab 100ug/ml **(D)** treated with Inclacumab 100ug/ml **(E).** Red arrows show RBCs and green arrows show HUVECs. p-values were based on paired t-test. Error bars represent the standard error of the mean (SEM). Scale bare 50um

CONCLUSION

Inclacumab reduces the levels of RBCs adhesion to chronic and acute heme activated HUVECs compared to untreated samples and treated with Crizanlizumab, using in vitro microfluidic system. Endothelium-on-a-chip microfluidic platform is a novel system that can be used as companion in new drug efficacy study and could be used to monitor patient response to anti-adhesive therapies in SCD.

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