HIGH-DENSITY LIPOPROTEIN INHIBITS VWF-INDUCED MICROVASCULAR THROMBOSIS IN MICE

N. Rhoads[1], R. Adili[1], J. Harris[1], J. Chen[1], JA. López[1], DW. Chung[1]

1. Bloodworks Research Institute, Seattle, WA. USA

INTRODUCTION

Von Willebrand factor (VWF) is a multimeric glycoprotein stored in platelet a-granules and endothelial Weibel Palade bodies. Upon endothelial activation VWF is released and self-associates into large multimers to facilitate platelet aggregation at the site of vascular injury. The metalloprotease ADAMTS13 cleaves VWF multimers to regulate thrombosis and prevent excessive platelet-VWF thrombi. ADAMTS13 deficiency leads to increased VWF-mediated microvascular thrombosis and eventual organ ischemia. We recently reported high-density lipoprotein (HDL) decreases VWF self-association in vitro indicating HDL may have an antithrombotic effect through VWF.

AIM

We used intravital microscopy to determine the effect of HDL on VWF in vivo as a potential therapy for thrombosis in cardiovascular diseases.

METHODS

- Microvascular thrombosis induced by calcium ionophore in ADAMTS13$^{-/-}$ mice pre-treated with HDL (100mg/kg)
- Laser injury induced cremaster arteriole thrombosis model in wild type mice pre-treated with HDL
- $FeCl_3$-induced carotid artery thrombosis model in wild type mice pre-treated with HDL (100mg/kg)
- Recombinant VWF challenge model in ADAMTS13$^{-/-}$ mice observed up to 90 minutes in cremaster microvasculature

RESULTS

HDL pretreatment prevented the thrombotic response, decreased the number of emboli and size of the thrombi in the calcium ionophore induced microvascular thrombosis model. rVWF infusion increased platelet adhesion to VWF multimers and microvascular occlusion. HDL pretreatment attenuated platelet adhesion, VWF string formation, and vessel occlusion. In the cremaster arteriole thrombosis model HDL pretreatment decreased platelet accumulation and fibrin formation in response to laser injury. HDL pretreatment delayed vessel occlusion time in the $FeCl_3$-induced carotid artery thrombosis model.

HDL PRETREATMENT ATTENUATES VWF SELF-ASSOCIATION, PLATELET ACCUMULATION, AND PLATELET-ULVWF MICROVASCULAR THROMBOSIS

Figure 1: Effect of HDL in rVWF induced microvascular thrombosis

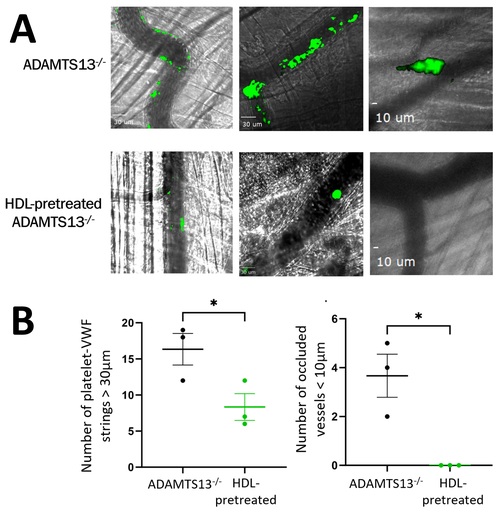

Figure 1: HDL pretreatment attenuates VWF self-association, platelet accumulation, and platelet-ULVWF microvascular thrombosis in the rVWF challenge model (A) ADAMTS13-deficient mice have increased VWF-platelet microvascular thrombosis following rVWF challenge (2000U/kg) which was attenuated by HDL pretreatment (100mg/kg) as observed in the cremaster microvasculature. Platelets are fluorescently labeled green. **(B)** HDL pretreatment significantly decreases the number of platelet-VWF strings and the number of occluded vessels in ADAMTS13-deficient mice after rVWF challenge.

Figure 2: Effect of HDL in ionophore-induced endothelial released ULVWF-mediated thrombosis

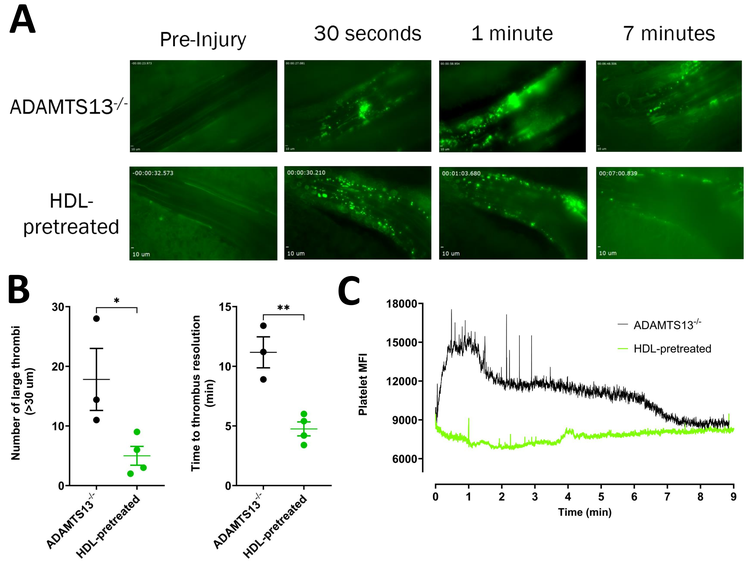

Figure 2: HDL pretreatment attenuated endothelial released ULVWF-mediated thrombosis in mesenteric venules of ADAMTS13-deficient mice (A) ADAMTS13-deficient mice have increased ULVWF-thrombus formation following topical application of calcium ionophore. HDL pretreatment (100mg/kg) attenuates the severity and size of thrombus formation. Platelets are fluorescently labeled green. **(B)** HDL pretreatment significantly decreases the number of large thrombi and the time to thrombus resolution in ADAMTS13-deficient mice. **(C)** This representative trace of the platelet MFI demonstrates the decrease in thrombus formation with HDL pretreatment.

HDL PRETREATMENT DELAYS VESSEL OCCLUSION TIME IN THE CAROTID ARTERY THROMBOSIS MODEL

Figure 3: Effect of HDL in $FeCl_3$-induced carotid artery thrombosis model

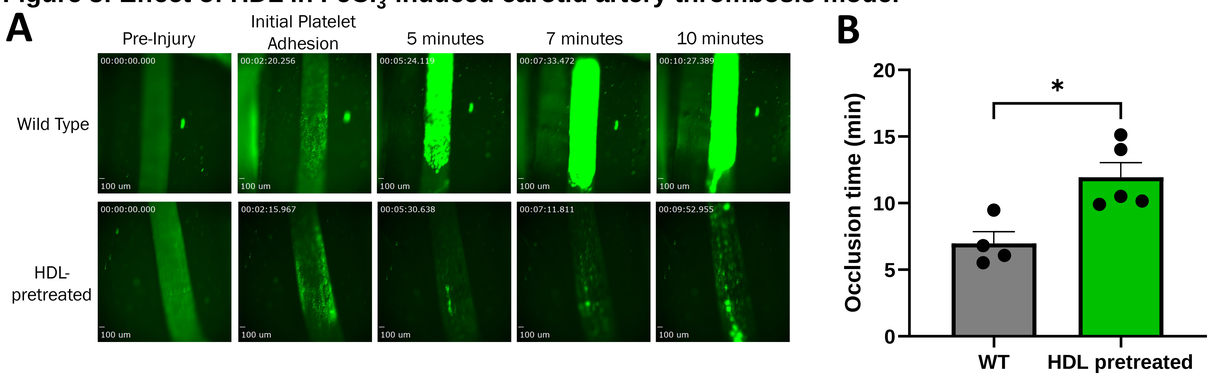

Figure 3: HDL pretreatment delays vessel occlusion time in WT mice in the carotid artery thrombosis model. (A) Representative images demonstrating the delayed occlusion time in WT mice. Green is fluorescently labeled platelets. **(B)** HDL pretreatment significantly delays the occlusion time in WT mice after $FeCl_3$-induced thrombosis.

HDL PRETREATMENT REDUCES MICROVASCULAR THROMBOSIS

Figure 4: Effect of HDL in laser-induced cremaster arteriole thrombosis model

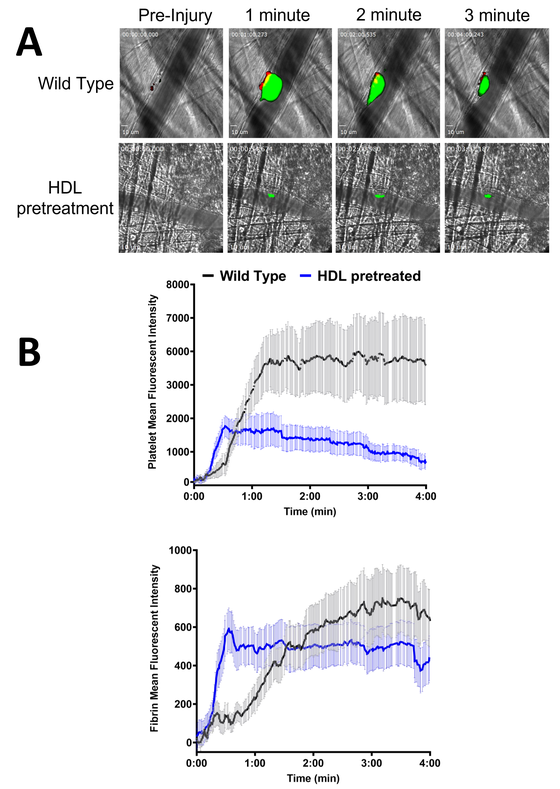

Figure 4: HDL pretreatment reduces platelet accumulation and fibrin formation at the site of injury in the cremaster arteriole laser-injury thrombosis model. (A) Representative image showing decreased platelet (green) accumulation and fibrin (red) formation in HDL pretreated mice after laser injury in the cremaster arterioles. **(B)** HDL pretreatment decreases platelet accumulation and fibrin formation in WT mice.

CONCLUSIONS

Our study indicates HDL has a significant role in decreasing the formation of VWF-mediated macro and microvascular thrombi

ACKNOWLEDGEMENTS

Dr. López: R35 HL145262
Dr. Adili: Bloodworks Start up Research Fund