LETTER TO THE EDITOR

Subcutaneous injection of IHP-102 prevents lung vaso-occlusion in sickle cell disease mice

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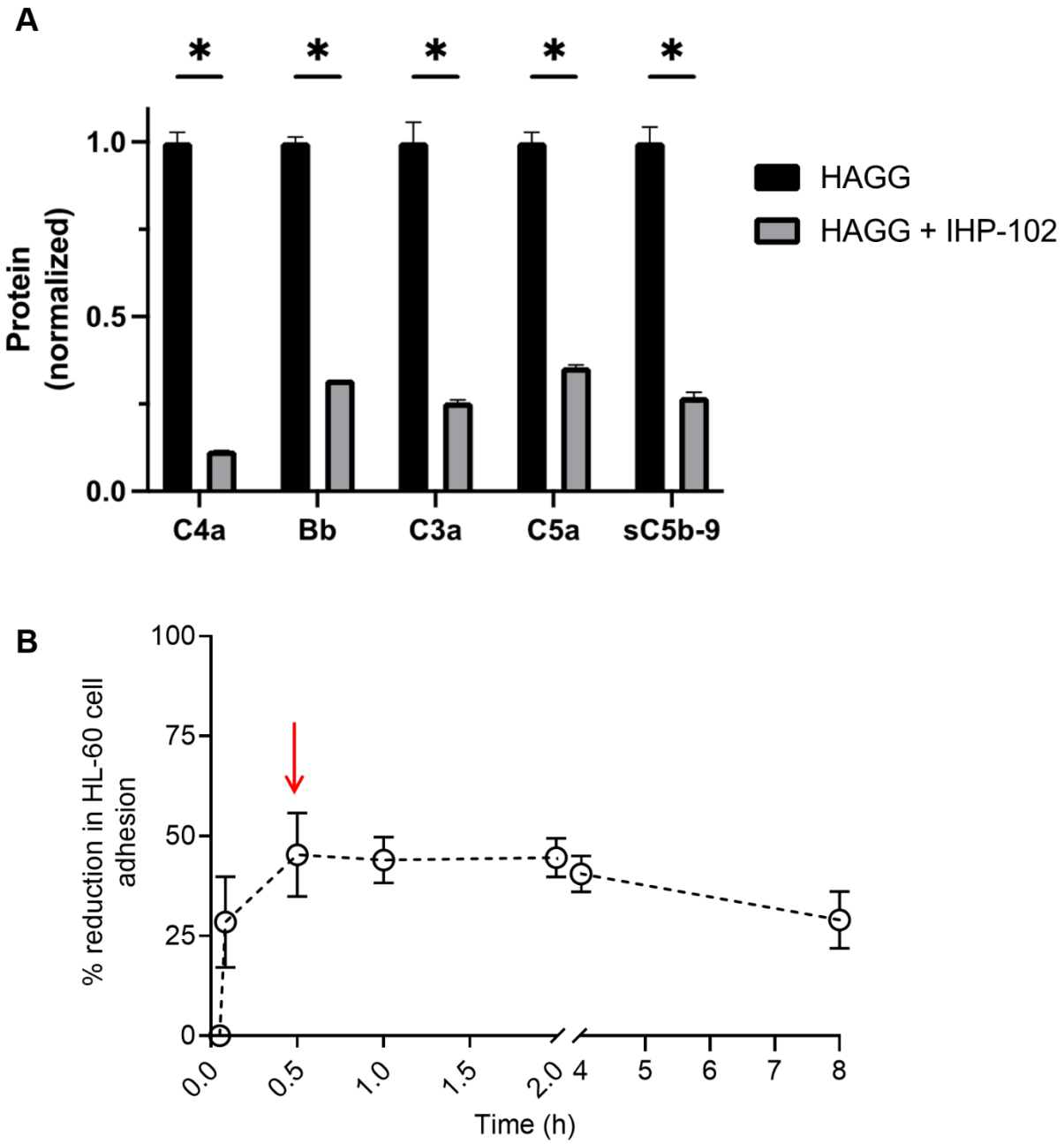
ONLINE SUPPLEMENTARY INFORMATION



Supplementary Figure S1. (**A**) To investigate the anti-complement activity of IHP-102, we measured the production of complement protein fragments in human serum activated by the classical pathway. Serum was collected from three healthy donors, complement was activated by adding heat aggregated gamma globulin (HAGG) followed by incubation at 37° C for 30 min and then storage at -80°C until further use in ELISA (Quidel or Pharminogen) for detection of

complement fragments. Pre-treatment with IHP-102 (2 μg/ml) inhibited the production of the complement fragments C4a, Bb, C3a, C5a, and sC5b-9 by more than 70%. Samples were run in duplicate and shown as Mean ± SD. Means compared using two-way t-tests (* $p < 0.05$). **(B)** The pharmacodynamic profile of IHP-102 was investigated in rats to establish bioavailability and serum P-selectin inhibition potential following subcutaneous (SQ) administration of IHP-102. Sprague Dawley rats (2 males and 2 females) purchased from Charles River (Wilmington, MA) were administered a single SQ dose of IHP-102 (30 mg/kg) at 0 h. Serum was collected at different time points. Serum samples (5% serum) were analyzed for P-selectin inhibition using a HL-60 cell/P-selectin binding assay. Briefly, P-selectin-Fc chimera was bound to a 96-well plate coated with Protein A. HL-60 cells ($2x10^5$ cells/well) labeled with 5-chloromethylfluorescein diacetate (CMFDA; Thermo Fisher Scientific Inc) were added to the wells and allowed to adhere for 1 h at room temperature. After 1 h, unbound cells were washed and bound cells were lysed with 1% Triton-X solution, and fluorescence measured at 480/520 nm to quantify the % reduction in HL-60 cell adhesion. Data shown as Mean ± SE. P-selectin blocking activity was detectable in serum within 30 min of SQ administration (red arrow) and persisted through 8 h.

LEGENDS FOR SUPPLEMENTARY MOVIES

Supplementary Movie S1: Aggregates (white ovals) of neutrophils (red) and platelets (green) occluding the pulmonary arteriole in the lung of an SCD mouse subcutaneously administered saline, 30 min prior to intravenous challenge with 10 μmol/kg oxy-Hb. Pulmonary microcirculation (purple). Scale bar 20μm. Arrow-flow direction. 1x original acquisition rate.

Supplementary Movie S2: Neutrophils (red) and platelets (green) trafficking down the pulmonary arteriole and into the capillaries, suggestive of the absence of lung vaso-occlusion in an SCD mouse subcutaneously administered 30 mg/kg IHP-102, 30 min prior to intravenous challenge with 10 μmol/kg oxy-Hb. Pulmonary microcirculation (purple). Scale bar 20 μm. Arrow-flow direction. 1x original acquisition rate.