THE SOLUBLE GUANYLATE CYCLASE STIMULATOR OLINCIGUAT ATTENUATES LEUKOCYTE/ENDOTHELIAL CELL INTERACTIONS IN BERKELEY SCD MICE

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BACKGROUND

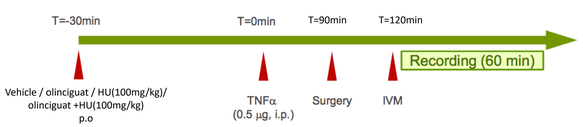
Patients with hemolytic anemias such as sickle cell disease (SCD) have reduced nitric oxide (NO) bioavailability (1). NO insufficiency results in suppression of NO-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) signaling pathway and has been implicated in vasoconstriction, intravascular inflammation, and formation of heterocellular vaso-occlusive aggregates in the venous microcirculation of SCD patients. In mouse models of SCD, cGMP amplifying agents, including NO donors and inhibitors of phosphodiesterase 9 (PDE9), reduce heterotypic adhesive interactions mediated by sickled red blood cells, leukocytes, platelets and activated endothelial cells (2, 3). Co-treatment of PDE9 inhibitors with hydroxyurea (HU) augments anti-inflammatory and anti-adhesive effects in these models (2, 3).

OBJECTIVES

In response to NO binding, soluble guanylate cyclase (sGC) catalyzes synthesis of cGMP from GTP. Therefore, we evaluated the effect of the sGC stimulator olinciguat on leukocyte-endothelial cell interactions in a mouse model of vaso-occlusive crisis (VOC).

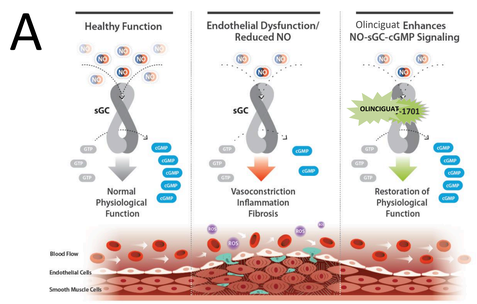
MATERIALS & METHODS

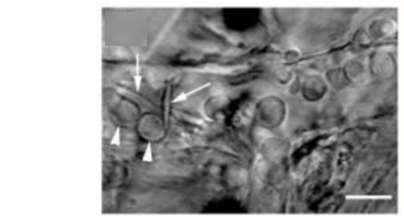
- The effect of olinciguat in the absence or presence of HU on leukocyte-endothelial cell interactions was studied in chimeric C57BL/6 mice engrafted with the bone marrow from Berkeley SCD mice.

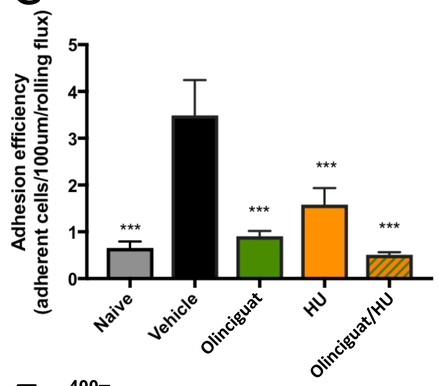


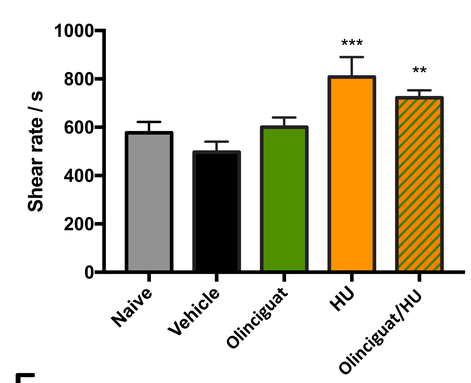
- Leukocyte-endothelial cell interactions in postcapillary venules of mouse cremaster muscle were analyzed by intravital microscopy
- A flow rate and centerline blood shear rate in each venule was measured using optical Doppler velocimeter.

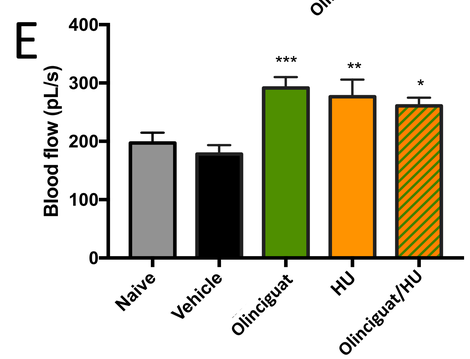
OLINCIGUAT IMPROVED VASO-OCCLUSION PARAMETERS IN SCD MICE











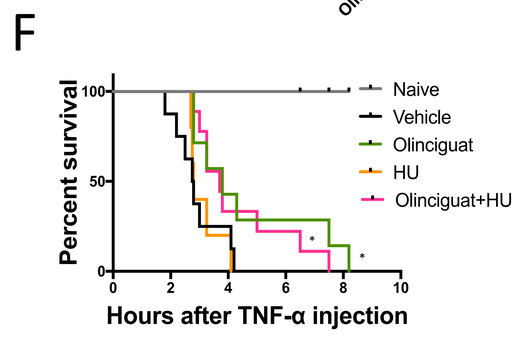


Figure legend: (A) Simplified cartoon mechanisms on how Olinciguat promote NO-sGC-cGMP signaling pathway to restore physiological function of endothelial cells. (B) a real introvital microscopy video clip demonstrates how leukocytes (indicated by arrow head) attach to the vessel, and interact with sickled RBC (indicated by arrow) to induce vaso-occlusion. (C) Leukocytes adhesion efficiency represents number of adherent leukocytes per 100um of video image divided by number of rolling leukocytes passing each vessel per minute. (D) shear rate (γ) was calculated based on Poiseuille's law for a Newtonian fluid, $\gamma = 2.12\ (8V_{mean})/D_v$, where D_v is the venule diameter, V_{mean} is estimated as $V_{RBC}/1.6$, and 2.12 is a median empirical correction factor obtained from actual velocity profiles measured in microvessels in vivo. (E) Blood flow rate (Q) was calculated as $Q = V_{mean}\pi d^2/4$, where d is venule diameter and is expressed as nL/sec. (F) SS mice survival after TNF-α injection
* P<0.05 compared to Vehicle group; ** P<0.01 compared to Vehicle group;
***P<0.001 compared to Vehicle group;

SUMMARY

- Animals pretreated with TNFα had significantly higher adhesion efficiency of leukocytes to the vascular wall compared to non-treated, naïve animals
- Adhesion efficiency of leukocytes was lower in mice pretreated with either olinciguat or HU than in vehicle-treated mice.
- Adhesion efficiency of leukocytes to activated endothelium was lowest in mice treated with both olinciguat and HU.
- Blood flow in TNFα-challenged mice that were pretreated with olinciguat, HU, or their combination was greater than vehicle-treated controls.
- Relative to vehicle-treated mice, blood shear rates were only enhanced in HU and combination treated animals.
- Finally, mice treated with olinciguat alone or in combination with HU survived longer (hours) than mice in the vehicle group.

CONCLUSION

- Our data indicate that in a TNFα-induced model of vaso-occlusion, pretreatment with the sGC stimulator olinciguat improved blood flow and attenuated leukocyte endothelial cell interactions in the venous microcirculation of sickle cell mice.
- In addition, treatment of olinciguat significantly extended survival of SCD mice in a lethal vaso-occlusion model induced by TNFα injection and surgical trauma.

REFERENCES

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3. Jasuja R., et al., Blood 2014 124(21):2694