Red blood cell nitric oxide and sickle cell anemia - potential and pitfalls

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Aim: Sickle cell disease (SCD) is a primary rheologic disease with increased erythrocyte (RBC) stiffness. RBC nitric oxide (NO) production is controversial with regard to origin and importance. Multiple pathways for NO production proposed within RBC, one of which being an analogue of endothelial nitric oxide synthase that is shear activated. Hemoglobin is thought to be a nitrite reductase. Is shear activated RBC NO production different between SCD and Healthy RBC?

Methods: RBC from 8 healthy and 7 SCD were washed and incubated with nitrite, a NOS inhibitor L-NAME or both. They were then incubated with NO sensitive fluorescent marker DAF-FM. The RBC were then placed in a poly L-lysine coated flow chamber and exposed to 0.5Pa shear stress. Analysis was done on a single cell basis (ImageJ 1.49s).

Results: There was similar increase in RBC DAF fluorescence under shear stress in healthy and SCD, percent change 19.3% vs 22.0% (P=0.60). There was no significant change in the rate of increased DAF fluorescence upon shear activation; the mean slope difference pre to post flow initiation was -70369±53519.2 u/min (P=0.21) with no difference between healthy and SCD. Incubation with L-NAME or nitrate did not alter the change in fluorescence.

Conclusions: RBC produces NO as measured by DAF fluorescence; however, there is minimal shear activated change on a cell-by-cell basis and no significant increase with the addition of nitrite under oxygenated and deoxygenated conditions.