Direct Soluble Guanylate Cyclase Targeting Improves Endothelial Dysfunction in a Mouse Model of Sickle Cell Disease

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Abstract

Background: Endothelial dysfunction caused by chronic intravascular hemolysis is a hallmark of sickle cell disease (SCD) affecting multiple organs, including the lung. A common complication of SCD is Pulmonary Arterial Hypertension (PAH) which is associated with early mortality.

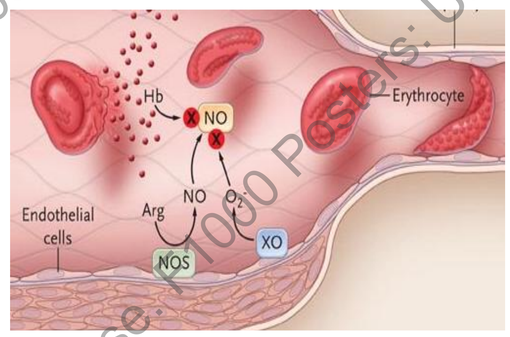
Hypothesis: Direct soluble guanylate cyclase (sGC) targeting may bypass nitric oxide (NO) scavenging and improve endothelial dysfunction.

Methods: Using an established animal model of SCD we investigated a novel soluble guanylate cyclase (sGC) targeting in the pathobiology of global vascular dysfunction. Age matched wild type mice were used as controls. SCD mice were treated with sGC activator chow (17mg/kg/d; BAY 54-6544, structurally similar to 58-2667 x HCl (cinaciguat)) or placebo for 30 days. Following the treatment period, mice underwent invasive cardiac interrogation of the right ventricle (RV), and the thoracodorsal artery (TDA) was harvested for pressure myography.

Results: We found that SCD mice had an increased baseline tone and increased response to PE as compared with controls. SCD mice treated with sGC activator for 30 days showed improved baseline tone and improved constriction response to PE. We found that SCD mice have PAH, which is acutely reversed after exposure to 30 days of sGC activator.

Conclusions: The transgenic sickle mouse develops significant vascular dysfunction with age. Novel targeting of smooth muscle cell sGC represents a novel therapeutic approach for global vascular dysfunction leading to PAH in SCD and other hemolytic disease processes.

Introduction



Intravascular hemolysis leads to decreased nitric oxide bioavailability, reactive oxygen species formation and endothelial dysfunction.
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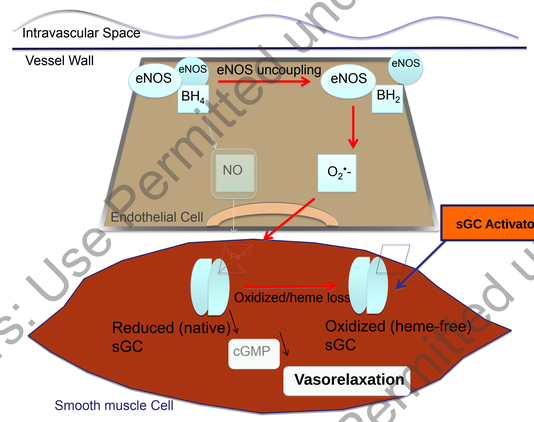
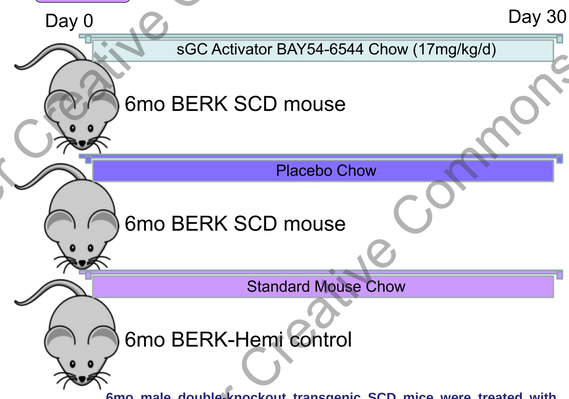
Hypothesis



Direct targeting of smooth muscle cell soluble guanylate cyclase as a method to bypass the scavenging of nitric oxide that occurs in sickle cell disease can improve endothelial dysfunction and treat pulmonary arterial hypertension.

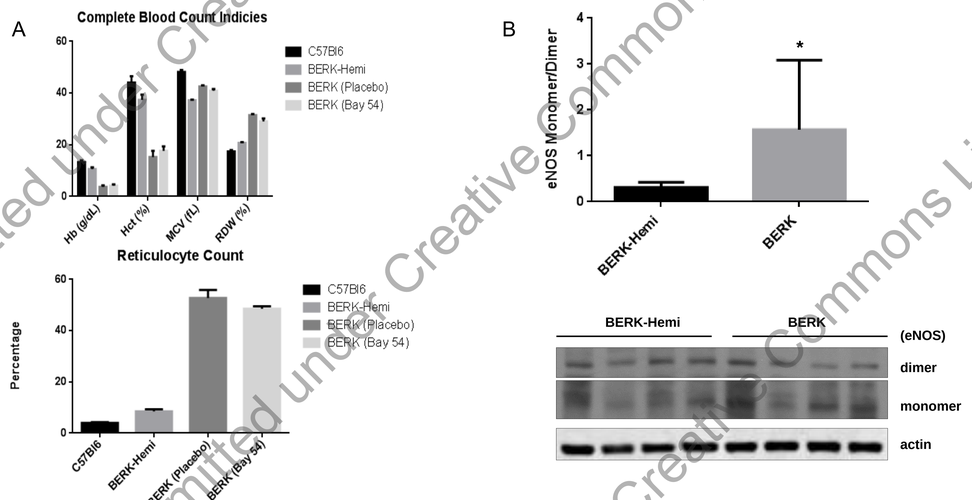
Methods



6mo male double-knockout transgenic SCD mice were treated with sGC Activator or Placebo for 30days, and compared with age matched hemizygous transgenic littermate controls. After 1mo the mice underwent cardio-phenotype interrogation and their TDA was harvested for myographic studies.

Result 1

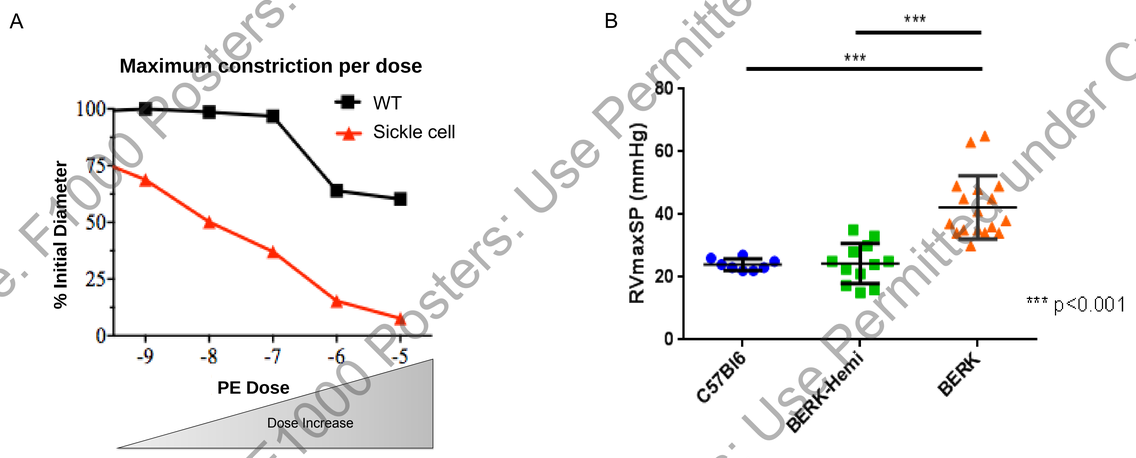
Sickle cell mice have severe hemolytic anemia and evidence of eNOS uncoupling



***A*: Hematological indices obtained from blood of SCD and control mice at time of sacrifice. *B*: representative Western blots of endothelial nitric oxide synthase (eNOS monomer and dimer ratios (*top*) with eNOS and β-actin (*bottom*)). Total proteins were obtained from whole lung lysate of SCD and control mice, and were subjected to low-temperature SDS-PAGE (LT-PAGE) to assess eNOS dimers and monomers. The expression of β-actin was used as a loading control. *Significant increase compared with control mice, $P < 0.05$.**

Result 2

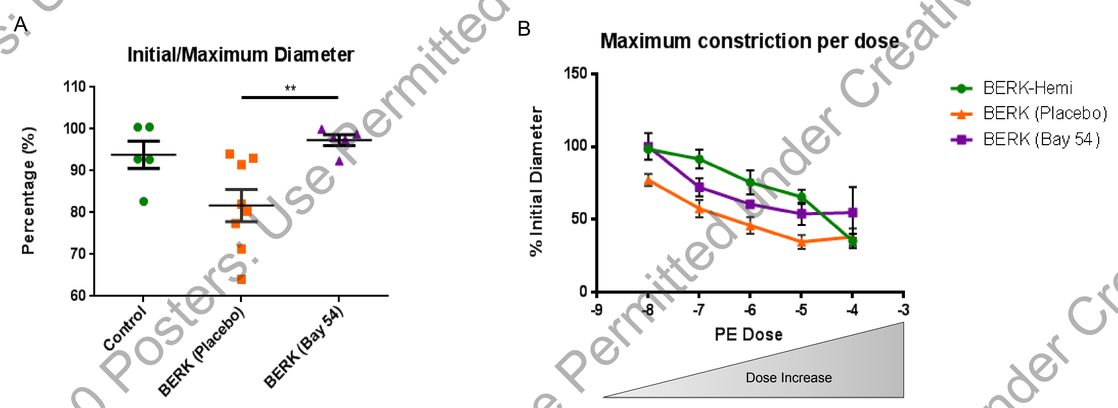
Sickle cell mice have endothelial dysfunction and develop pulmonary arterial hypertension with age



***(A)* ~225 um of thoracodorsal artery was harvested from SCD mice and controls and a dose response to phenylephrine was performed. There is a major constriction in the sickle mice compared to the control. (*B*) 6mo male mice underwent closed chest micro heart catheterization using Transonic 3.5mm 1.2Fr catheter and lox2 software for analysis. SCD mice have statistically significant elevation of RVmaxSP as evidence of PAH compared with controls. ***Significant increase compared with control mice, $P < 0.0001$.**

Result 3

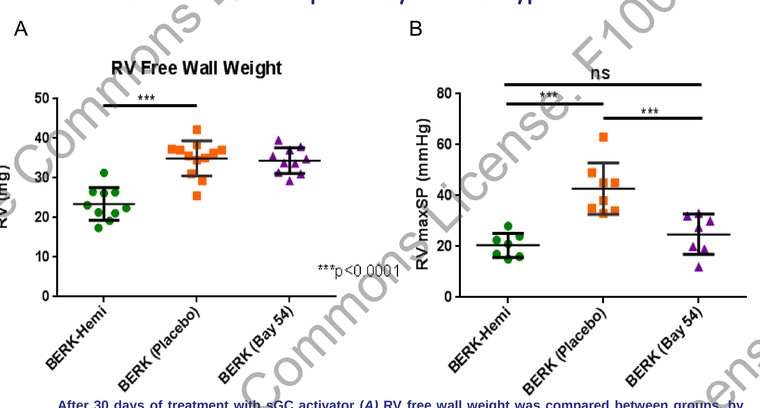
Sickle cell disease mice treated with sGC activator Bay-54 have improved endothelial function



(A) ~225 um of thoracodorsal artery was harvested from SCD mice and controls. Basal tone in SCD mice treated with 30 days of sGC activator was normalized compared with controls. **Significant increase compared with placebo SCD mice, $P < 0.005$. (*B*) A concentration response curve was performed using phenylephrine. There is a major constriction in the sickle mice compared to the controls. SCD mice treated for 30days with sGC activator had near normalization of constriction response to phenylephrine.

Result 4

Sickle cell mice exposed to sGC activator Bay 54 have acute resolution of their pulmonary arterial hypertension



After 30 days of treatment with sGC activator (*A*) RV free wall weight was compared between groups, by dissection of heart to separate RV from LV + Septum. SCD mice have evidence of RV remodeling secondary to PAH, which was unchanged treatment v placebo. (*B*) Using closed chest micro heart catheterization we see acute normalization of RVmaxSP in treated SCD mice compared with placebo. ***Significant decrease compared with placebo SCD mice, $P < 0.0001$.

Summary

① sGC activators improve endothelial dysfunction bypassing nitric oxide scavenging
② This mouse model of sickle cell disease demonstrates pulmonary arterial hypertension and RV remodeling secondary to oxidative stressors
③ Direct sGC targeting may function as a new therapeutic agent for diseases with poor NO bioavailability
④ Long term therapy may have potential to reverse RV remodeling seen in hemolytic disease associated PAH

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