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) for 30 days. Following the treatment period, mice underwent intracardiac 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 elicitation 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.

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) dimer and monomer ratios (first lane SCD, second lane WT). Total proteins were obtained from whole lung tissue of SCD and control mice, and were subjected to low-temperature SDS-PAGE and Western Blotting. The expression of β-actin was used as a loading control. **Significant increase compared with control mice.

Result 2

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

(A) -225 μm of thoracodorsal artery was harvested from SCD mice and controls. PE dose in SCD mice treated with 30 days of sGC activator was normalized compared with controls. (B) A concentration response curve was performed using phenylephrine. There is a major constriction in the sickle mice compared to the controls. SCD mice facing treatment with sGC activator had near normalization of constriction compared to phenylephrine.

Result 3

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

(A) Initial/maximum diameter of endothelial cell showing a reduction in diameter in SCD mice treated with sGC activator Bay-54 compared to Placebo Chow. (B) Maximum constriction per dose in sickle cell mice treated with sGC activator Bay-54 compared to Placebo Chow. ***Significant decrease compared with placebo SCD mice, P < 0.001.

Result 4

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

(A) -225 μm of thoracodorsal artery was harvested from SCD mice and controls. PE dose in SCD mice treated with 30 days of sGC activator was normalized compared with controls. (B) A concentration response curve was performed using phenylephrine. There is a major constriction in the sickle mice compared to the controls. SCD mice facing treatment with sGC activator had near normalization of constriction compared to phenylephrine.

Methods

Day 0

6mo BERK SCD mouse

Placebo Chow

6mo BERK SCD mouse

Standard Mouse Chow

6mo BERK-Hem control

Sickle cell mice were treated with sGC activator for 30 days, and compared with age matched hemizygous sickle cell mice. After time the mice were euthanized and their TDA was harvested for myographic studies.

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 remodelling 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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