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113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

SR-18292 Induces Fetal Hemoglobin Synthesis and Reduces Disease Pathology in Sickle Cell Mice

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Sickle cell disease (SCD) is a commonly inherited blood disorder, which affects millions around the world. Reactivation of fetal hemoglobin (HbF) synthesis in adult erythroid cells can diminish the severity of many clinical features of the disease. Up-regulation of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) has been shown to induce HbF levels in human primary erythroid progenitor CD34 $^+$ cells. Here we reported the *in vitro* and *in vivo* effects of a small molecular compound, SR-18292, on HbF induction via modulating PGC-1α.

To determine whether SR-18292 can induce HbF in human CD34 $^+$ cells, we performed RT-qPCR, flow cytometry, and western blot to measure the mRNA and protein levels of PGC-1α and γ-globin. We found that mRNA levels of PGC-1α and γ-globin were significantly increased in cells treated with SR-18292 while β-globin mRNA levels were unaffected. Protein levels of γ-globin and the ratio of F-cells were increased in treated cells. Erythroid cellular differentiation markers (CD71/CD235a) and terminal differentiation markers (CD49d/CD233) were used to examine the differentiation status. There was no significant difference in the population of CD71 $^+$CD235a $^+$ cells among all groups. However, SR-18292 treatment increased the percentage of CD49d $^-$CD233 $^+$ cells at 5 or 7.5 μM. These results suggest that SR-18292 at higher concentrations can promote erythroid terminal differentiation.

To evaluate whether the HbF induction by SR-18292 can reduce the SCD disease pathology in SCD mice or not, we intraperitoneally administered SR-18292 to SCD mice. HbF induction was determined by flow cytometric analysis using an anti-HbF antibody. And it was shown that the HbF-high cell population was induced in SCD mice after 4 weeks of treatment. And we used transferrin receptor CD71 and erythroid-specific marker Ter119 to determine whether SR-18292 could affect erythroid differentiation. There was no significant difference in the CD71 $^+$Ter119 $^+$ population after 4 weeks of SR-18192 treatment compared with untreated animals. The mRNA levels of γ-globin and PGC-1α were increased in treated mice. And the increase of PGC-1α and γ-globin protein levels were confirmed by western blot. Reticulocyte is a sign of premature RBC destruction (hemolysis), and the SCD mice usually have high reticulocyte counts (>50%). We measured the reticulocyte in the peripheral blood of SR-18292 treated and untreated SCD mice by flow-cytometric analyses and found that reticulocyte was significantly reduced in SR-18292 treated animals (from 55% to 20%) after 4 weeks of treatment. Complete blood cell counts (CBC) showed that SR-18292 treated mice had slightly elevated numbers of red blood cells (RBCs) and more hemoglobin, but lower counts of white blood cells, lymphocytes, and neutrophils. In addition, the RBC distribution width (RDW) was significantly reduced after SR-18292 treatment, indicating that the size of circulating RBCs was more uniform in treated mice. In addition, erythroid cell morphology was evaluated by blood smears with Wright-Giemsa stain. The number of sickled RBCs were reduced in SCD mice treated with SR-18292 for 4 weeks. This is consistent with our observation that RDW was reduced in SCD mice treated with SR-18292. Furthermore, SR-18292 treated mice had a statistically significant reduction in spleen size and weight in contrast to the spleens of untreated SCD mice. The indices of RBCs were improved in accompany with reduced hemolysis and a higher fraction of circulating mature erythrocytes in treated SCD mice. There were no observable adverse effects on other hematopoietic cell lineages.

We also performed single-cell RNA-seqs in human CD34 $^+$ cells treated with SR-18292 and found that some known HbF repressors (such as BCL11A and ZBTB7A) were decreased in treated groups. Besides that, there were many differentially expressed genes (DEGs), which might be novel HbF regulators. We are verifying those DEGs genes by qPCR and using CRISPR/cas9 to examine their functions in globin regulation.

In summary, our findings suggest that SR-18292 might be a promising therapeutic agent to treat SCD and modulating PGC-1α activity or the signaling pathways that it regulates might benefit therapeutically patients with SCD.

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