Pharmacodynamic effects of AG-946, a highly potent novel activator of pyruvate kinase, in ex vivo treatment of red blood cells from sickle cell disease patients

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INTRODUCTION

Sickle cell disease (SCD) is a monogenetic red blood cell (RBC) disorder that is characterized by hemolytic anemia and vaso-occlusive crises. Among the many factors that contribute to disease pathophysiology is stiffening and sickling of RBC, which is the direct result of the polymerization of abnormal hemoglobin S. Sickling is one of the core factors that cause vaso-occlusion and sickling is modulated by glycolytic intermediates such as 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP).[1] Previously we showed that RBC pyruvate kinase (PKR), the key regulatory enzyme of glycolysis, is impaired in SCD and that ex vivo treatment with mitapivat, an allosteric activator of PKR, increased enzymatic activity and thermostability, reduced 2,3-DPG levels, decreased p50, and subsequently reduced sickling.[2] Currently, mitapivat is being investigated in phase 1, phase 2, and phase 2/3 trials in patients with SCD (#NCT04000165, EudraCT#2019-003438-18, and NCT05031780).

OBJECTIVE

Recently, AG-946, a novel PK activator, has been developed. Here we investigate the pharmacodynamic effects of AG-946 in ex vivo treatment of RBC from SCD patients in comparison with mitapivat.

METHODS

Buffy coat depleted whole blood obtained from five patients with SCD was incubated for 20-24 hours in absence or presence of mitapivat (100 µM) or AG-946 (1 µM, 5 µM, 50µM). After ex vivo treatment the following assays were carried out:

- Enzymatic activities of PKR and PKR thermostability
- Glycolytic intermediates ATP and 2,3-DPG were measured using LC-MS/MS
- Hemoglobin oxygen affinity (p50)
- RBC sickling was analyzed with the oxygenscan, a method that characterizes individual sickling behavior by oxygen gradient ektacytometry. Individual tendency to sickle is reflected by Point-of-Sickling (PoS) that indicates the specific pO_2 at which RBCs start to sickle during deoxygenation under shear stress.[3]

RESULTS

PKR activity was increased compared to vehicle (DMSO) to a similar extent in the presence of mitapivat or AG-946 (Figure 1A). In addition, PKR thermostability was significantly increased compared to vehicle (mean 22%, SD 6%) in samples treated with mitapivat 100 µM (mean 78%, SD 11%), as well as AG-946 5µM (mean 66%, SD 23%), and AG-946 50 µM (mean 95%, SD 17%, Figure 1B). After incubation with mitapivat or AG-946, 2,3-DPG decreased (Figure 1C), which was further illustrated by the improved ATP/2,3-DPG ratio (Figure 1D).

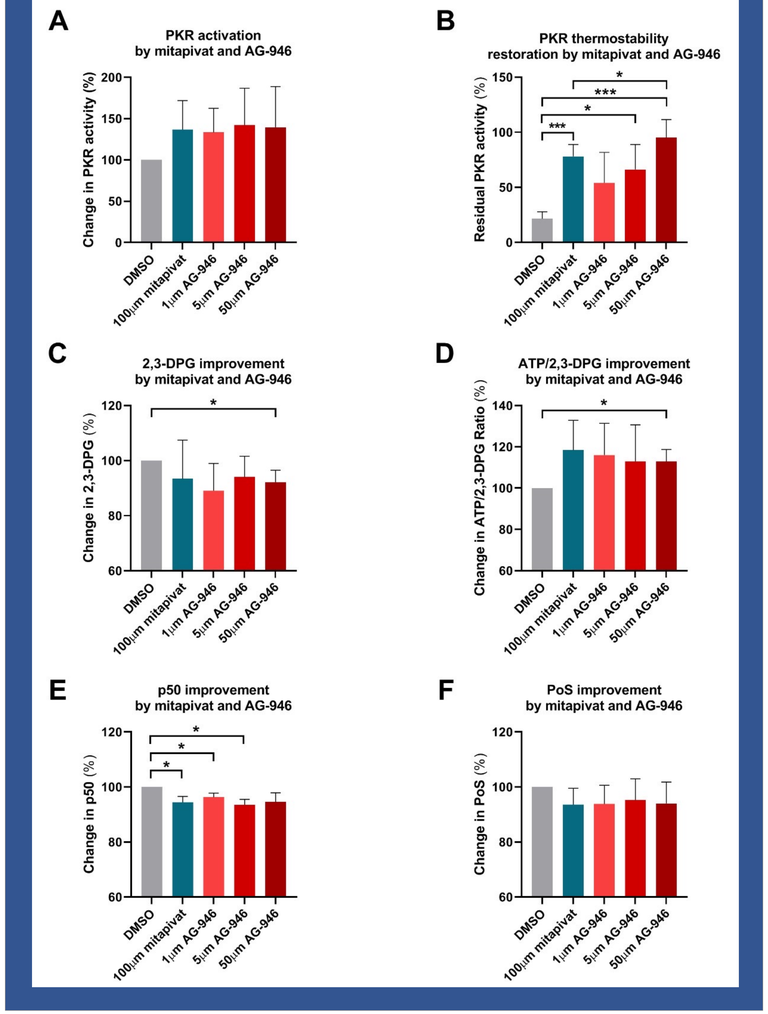

Figure 1. Pharmacodynamic effects of AG-946 in ex vivo treatment of red blood cells (RBC) from sickle cell disease (SCD) patients in comparison with mitapivat*. Buffy coat depleted whole blood was incubated for 20-24 hrs in absence or presence of mitapivat (100 µM, blue bar) or AG-946 (1 µM, 5 µM, 50 µM, orange bars) and compared to vehicle control (DMSO, gray bar). Ex vivo treatment increased pyruvate kinase (PKR) activity to a similar extent for both mitapivat and AG-946 (panel **A**), and significantly increased PKR thermostability (**B**). This was accompanied by a decrease in the levels of 2,3-DPG (**C**) and a comparable improvement in the ATP/2,3-DPG ratio for both mitapivat and AG-946 treated samples (**D**). The metabolic changes were associated with a significant decrease in p50 for both mitapivat and AG-946 treated samples (**E**), and a comparable decrease in PoS which is indicative of a decreased RBC sickling tendency in vitro (**F**). Error bars represent standard deviation. ***p<0.001, *p<0.05*

RESULTS (2)

Accordingly, p50 decreased significantly after incubation with mitapivat 100 µM (mean 95%, SD 2%), as well as AG-946 1 µM (mean 96%, SD 2%), AG-946 5 µM (mean 94%, SD 2%), and AG-946 50 µM (mean 95%, SD 3%, Figure 1E). The improved metabolic status and p50 was accompanied by a decreased PoS compared to vehicle in RBCs treated with mitapivat or AG-946, indicating reduced RBC sickling tendency in vitro (Figure 1F).

CONCLUSION

Ex vivo treatment of SCD RBCs with the novel PK activator AG-946 activates and stabilizes PKR, decreases 2,3-DPG levels, improves the ATP/2,3-DPG ratio, improves p50 and lowers the PoS. These beneficial effects are similar to ex vivo treatment with mitapivat but, importantly, are obtained at much lower concentrations. Taken together, these results are the first in an ex vivo model to demonstrate that the novel PK activator AG-946 has a similar favorable pharmacodynamic profile to mitapivat with enhanced PKR-stabilizing properties and, hence, might represent a potential novel therapeutic option in addition to mitapivat for the treatment of SCD.

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