AG-946, a Pyruvate Kinase Activator, Improves PK Properties and Red Blood Cell Metabolism upon Ex Vivo Treatment of RBCs from Patients with Myelodysplastic Syndromes

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Aim

To evaluate red blood cell (RBC) pyruvate kinase (PK) and cellular properties of patients with myelodysplastic syndrome (MDS), and to determine the effect of ex vivo treatment with the PK activator AG-946.

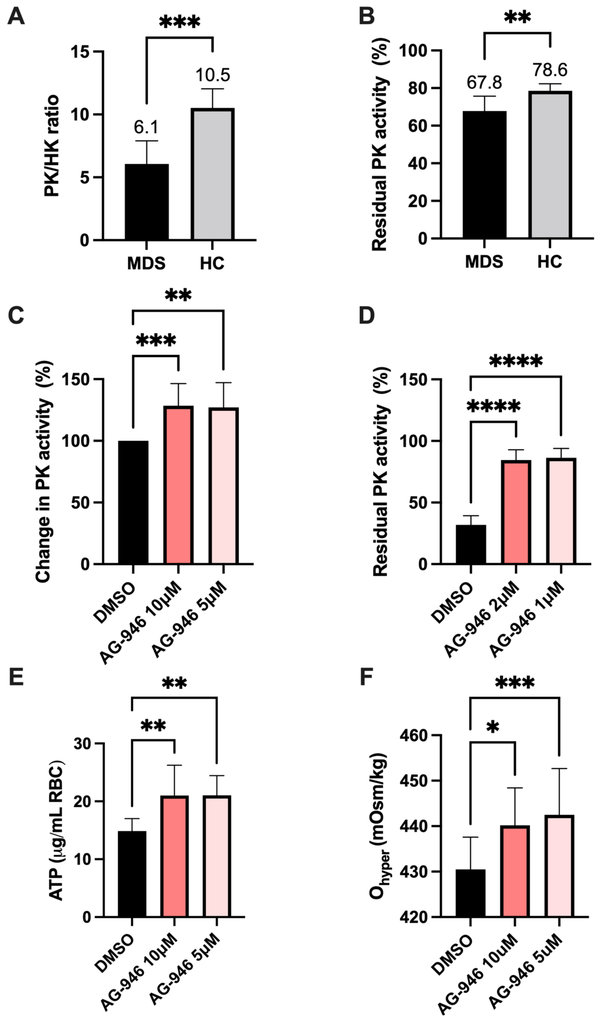
Introduction

- Patients with MDS frequently suffer from anemia, which directly affects their quality of life. Therapeutic options for these patients are limited.
- Decreased activity of RBC PK, a key regulatory enzyme of glycolysis, has been observed previously in MDS patients .
- The PK activator mitapivat has been shown to improve hemoglobin and ineffective erythropoiesis in patients with other forms of anemia (i.e., PK deficiency, thalassemia).
- In light of current advances in PK activation therapies, we studied properties of RBC PK in MDS, as well as the effect of *ex vivo* treatment of MDS RBCs with the PK activator AG-946.

Methods

- Ten low risk MDS patients, non-transfusion dependent, and six healthy controls (HCs) were studied.
- PK activity and thermostability were measured, as well as hexokinase (HK) activity to evaluate PK activity in relation to mean RBC age (PK/HK ratio).
- *Ex vivo* treatment of MDS RBCs with PK activator AG-946 (10µM or 5µM), compared to vehicle control (DMSO). After incubation of 16 hours at 37 °C, the following assays were performed:
  - PK activity (Spectroscopy)
  - ATP levels (LC-MS/MS)
  - Osmotic gradient ektacytometry (Lorrca Maxsis)
- Effect of PK activation on PK thermostability was assessed by incubating RBC lysates (DMSO, AG-946 2µM, AG-946 1µM), after which lysates were incubated at 53 °C for 60 minutes.
- Effect of PK activation on erythroid development was assessed by culturing peripheral blood mononuclear cells in MethoCult™ H4434 medium for 14 days in absence or presence of AG-946 (10µM, 625nM or 0.97nM, DMSO as vehicle control).

Figure 1. PK activity and thermostability are decreased in MDS RBCs and are restored upon *ex vivo* treatment with PK activator AG-946. This improves RBC metabolism as evidenced by increased ATP levels and RBC functionality, evidenced by improved hydration status.



Results

- Mean PK/HK ratio was significantly decreased in MDS compared to HCs (6.1 vs 10.5, **Figure 1A**). Individually, PK/HK ratio varied strongly between patients (range 2.6 - 8.8)
- PK thermostability was decreased at baseline in MDS (residual activity of 67.8% vs 78.6%, **Figure 1B**).
- *Ex vivo* treatment with the PK activator AG-946 led to an increase in both PK activity and residual PK activity after incubation at 53 °C for 60 minutes (**Figure 1C,D**).
  - PK activity: 10µM AG-946, mean increase 29%; 5µM AG-946, 27%.
  - PK thermostability: DMSO, 32% residual activity; 2µM AG-946, 84%; 1µM AG-946, 86%.
- Upon PK activation, ATP levels also significantly increased: DMSO, mean ATP 14.9 µg/mL RBC; 10µM AG-946 21.0 µg/mL RBC; 5µM AG-946 21.1 µg/mL RBC (**Figure 1E**).
- RBC functionality improved upon treatment with AG-946, as reflected by the increase in Ohyper, indicating improved hydration status (10µM AG-946 2.4% increase in Ohyper; 5µM AG-946 2.8%, **Figure 1F**).
- To date, colony forming culture assays have been performed in 4/10 patients. Interestingly, in one of these four there was 36% increase in number of burst forming units-erythroid (non-dose-related) upon treatment with AG-946.

Conclusion

Our findings demonstrate that RBCs from MDS patients show a decrease in PK activity as well as in thermostability. Furthermore, we demonstrate that ex vivo treatment with AG-946 increases PK activity, ATP levels and stabilizes PK. RBC hydration was shown to be improved upon treatment with AG-946. Additional studies will evaluate the effect of AG-946 on erythroid development in MDS.

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