Red Blood Cell Age Distribution and Metabolic Features in Hereditary Spherocytosis, Hereditary Xerocytosis and Congenital Dyserythropoietic Anemia type II

Baseline Results of the Exploratory Analysis from the Satisfy Study

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AIM

To characterize baseline red blood cell (RBC) age distribution and selected metabolic properties in HS, HX and CDAII patients.

INTRODUCTION

- Hereditary spherocytosis (HS), hereditary xerocytosis (HX) and congenital dyserythropoietic anemia type II (CDAII) are rare hereditary red blood cell disorders characterized by hemolytic anemia.
- Pre-clinical evidence indicates impaired metabolism in RBC membranopathies, providing a rationale for PK-activation in these diseases, currently being evaluated in a phase 2 trial (SATISFY, NCT05935202).
- Mitapivat, a pyruvate kinase (PK) activator, enhances glycolysis, thereby increasing cellular ATP levels.
- As PK-activity is RBC age dependent, investigating metabolic features of RBC subsets may potentially provide further information on the mechanism of action of PK activation in these diseases.

METHODS

The SATISFY study evaluates safety and efficacy of mitapivat in HS, HX and CDAII patients. This exploratory analysis aims to provide further understanding of metabolic and functional RBC properties and the effects of mitapivat treatment.

RBC subsets

RBCs were separated according to density (age) into 4 fractions (F1-F4) by percoll gradient centrifugation.

- F1 contains the least dense (youngest) RBCs.
- F4 contains the densest (oldest) RBCs.

Read-out techniques

- PK activity: Spectrophotometrically
- ATP/2,3-DPG: Liquid Chromatography coupled with High-Resolution Mass Spectrometry (LC-HRMS)
- p50 (hemoglobin oxygen affinity): Hemox Analyzer

RESULTS

Inclusion

- 16 HS, 4 HX and 4 CDAII patients were included.

RBC density (age) distribution showed distinct patterns per patient group (Fig. 1).

- F1 was the minor fraction in all patient groups (1-2% of RBC).
- The densest fraction was most pronounced in HS patients (24 ± 13%, mean ± SD).

Selected metabolic properties

PK activity showed an RBC age-dependent decline in all groups.

- This was most pronounced in **HS**. PK-activity decreased from 14.9 U/gHb (F2) to 6.9 U/gHb (F4) (p<0.0001) (Fig. 2A).
- **HX** and **CDAII** showed a less pronounced decrease for F2 to F4: from 9.8 to 7.7 U/gHb in HX (non-significant) and 11.7 to 6.3 U/gHb in CDAII (p=0.004) (Fig. 3A and 3B).

ATP levels were lowest in F1 and similar for F2 to F4 in **HS** (Fig. 2B), **HX** and **CDAII** (not shown), without statistically significant differences between fractions.

2,3-DPG levels decreased from F1 to F4 in all groups.

- This decrease was most pronounced in **HS** (from 3598 to 1864 mg/L RBCs, p<0.001) (Fig. 2C).
- To a lesser degree, similar decreases for both **HX** (2780 to 1986 mg/L RBC, p=0.150) and **CDAII** (3105 to 1903 mg/L RBCs, p=0.011) were observed (Fig. 3C and Fig. 3D).

p50 values decreased from F1 to F4 in **HS** (Fig. 2D), **HX** and **CDAII** (not shown).

- 2,3-DPG was strongly correlated with p50 across all disease groups (Pearson correlation coefficient for HS r=0.9299, p<0.001, HX, r=0.9065, p =0.002, CDAII, r = 0.8844, p <0.001)

Figure 1 – Red blood cell (RBC) age distribution in HS, HX and CDAII.

(A) Representative percoll gradient separation of RBCs from HS, HX and CDAII patients.

(B) Cumulative distribution of RBC subsets in HS, HX and CDAII patients included in this study (mean ± SD).
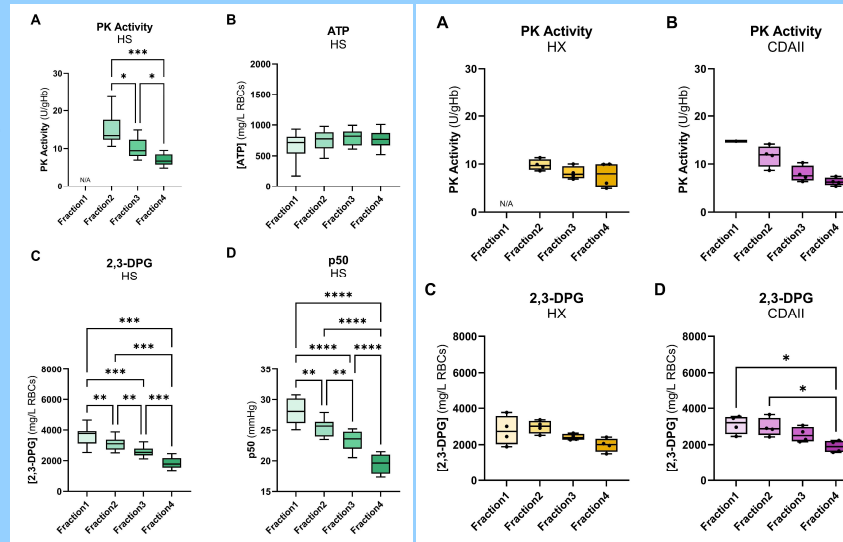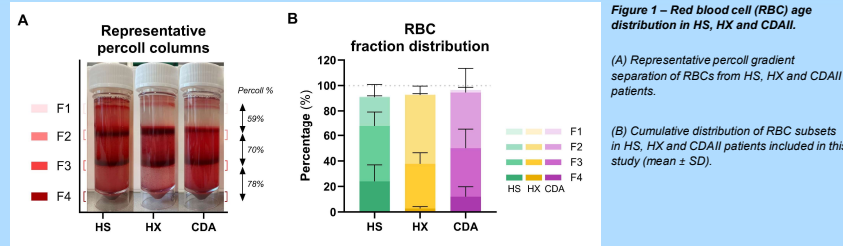

Figure 2 – Selected red blood cell (RBC) metabolic features at baseline in HS. (A) Pyruvate Kinase Activity. (B) Adenosine Triphosphate levels. (C) 2,3-diphosphoglycerate levels. (D) p50 Hemoglobin oxygen affinity. N/A: Not available.

Figure 3 – Selected red blood cell (RBC) metabolic features at baseline in HX and CDAII. (A-B) Pyruvate Kinase Activity. (C-D) 2,3-Diphosphoglycerate levels. N/A: Not available.

As appropriate either one-way ANOVA or Kruskal-Wallis is performed to determine statistical significance, and correction for multiple testing is applied. *P<0.05, **P<0.01, *** P<0.001

CONCLUSION

- We observed an RBC age dependent decrease in PK activity and 2,3-DPG levels, with the latter showing a strong correlation with p50.
- Distinct differences in RBC density (age) distribution were observed across disease groups.
- HS patients had a significant larger high density (old) RBC fraction, with the lowest PK activity, compared to HX and CDA.
- Differences in RBC fractions at baseline across these disease states may further contribute to the understanding of mitapivat's clinical benefit in RBC membranopathies and CDAII, currently being evaluated in the SATISFY trial (**EHA Abstract S297**).

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