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Mitapivat and Rapamycin Increase Adenosine Triphosphate Production and Maintain Platelet Function in Cold-Stored Whole Blood.

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Introduction

- Whole blood is a critically important cellular therapy for the treatment of trauma and coagulopathy. However, the quality of blood stored at 1–6°C is affected by biochemical and structural changes collectively termed the "storage lesion" induced by increases in oxidative stress and depletion of adenosine triphosphate (ATP)[1,2]. This lesion often affects cell viability leading to rapid clearance of transfused blood cells.
- Thus, blood ATP production and preservation are critical for cellular viability and the maintenance of hemostatic functions.
- Here, we investigate the potential of mitapivat and rapamycin to enhance ATP production and preservation in whole blood during cold storage.

Objectives

- Demonstrate how supplementing blood storage media with mitapivat or rapamycin enhances and sustains ATP levels throughout storage duration.
- Demonstrate how ATP production is accompanied by reduction in oxidative stress.
- Demonstrate that mitapivat or rapamycin supplementation does not negatively impact blood coagulation and hemostatic function.

We hypothesized that supplementation of blood with the following molecules, mitapivat, rapamycin, dexamethasone, or sericin, would enhance metabolic ATP levels needed for cellular viability without impairing platelet function throughout 14 days cold storage.

Methods

- Whole blood from five healthy volunteers was supplemented with either rapamycin (25 nM), mitapivat (10 µM), dexamethasone (50 nM), sericin (150 µg) or vehicle control (DMSO) and stored at 4°C for 14 days.
- Analysis of stored blood samples was performed on days 1 and 14.
- Whole blood ATP levels were determined using CellTiter Glo reagent on GloMax Discovery microplate reader.
- Complete blood count (CBC) was obtained using Advia 2120i. Blood glucose and lactate levels were measured using an i-STAT device.
- Platelet activation was determined by flow cytometer.
- Blood coagulation and platelet aggregation were analyzed using ROTEM and Light Transmission Aggregometry respectively.

Results

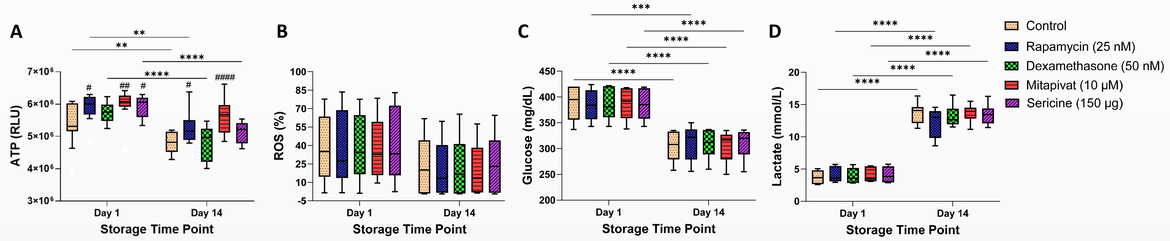

Figure 1. Analysis of ATP Production and Metabolic Profile: Mitapivat and rapamycin-treated blood samples on Days 1 and 14 showed significantly higher ATP levels compared to same day controls. While sericin showed slight increase in ATP levels on Day 1 compared to control, dexamethasone on the other hand did not show any effect at either time points. ATP reduces in all blood samples including control up to Day 14 except for mitapivat-treated blood (A). No change in ROS between Day 1 and 14 (B). Glucose (C), and lactate (D) levels were unchanged between controls and drug-treated samples on Day 1 and Day 14, which displayed decreased glucose and increased lactate levels with increasing storage duration. Values represent means ± SD with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ of Day 1 compared to Day 14 storage for all samples, and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$ of drug treated samples versus same-day control.

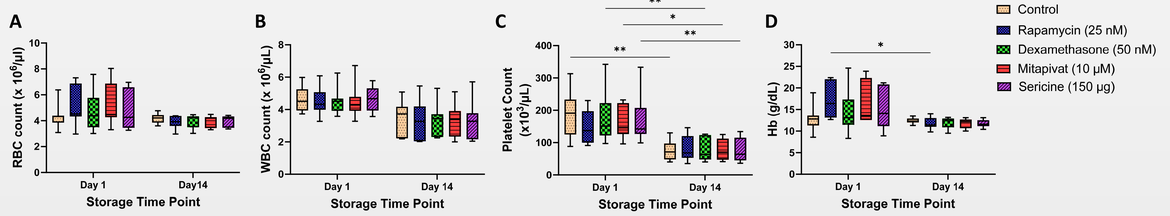

Figure 2. Complete Blood count and Hematologic parameters: No significant differences in red blood cells (RBC), white blood cells (WBC) and platelets count were observed between drug-treated samples and same-day controls at either storage time point, or between Day 1 and Day 14 for any treatment (A and B). Platelet count on Day 14 declined compared to Day 1 across all samples except for rapamycin treated samples (C). Hemoglobin (Hb) levels decrease with increase in storage time in rapamycin and mitapivat-treated blood (D). Values represent means ± SD with $^{*}p < 0.05$, $^{**}p < 0.01$ of Day 1 compared to Day 14 of all samples.

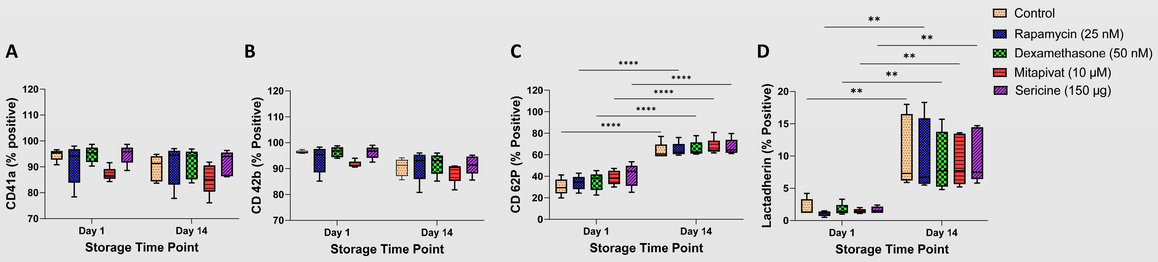

Figure 3. Flow cytometry analysis of platelet activation markers and apoptosis: Platelet membrane glycoproteins, GP-IIb (A) and GPIbα (B), remained unchanged between controls and drug-treated samples of same day storage for Day 1 or Day 14. Similarly, no significant changes were observed when samples of Day 1 storage were compared to Day 14 storage. P-selectin surface expression (C) and Phosphatidylserine (PS) exposure (D) did not differ between control and drug-treated samples of either Day 1 or Day 14 but significantly increased on storage Day 14 compared to Day 1. Values represent means ± SD with $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$ of Day 1 compared to Day 14 storage for all samples.

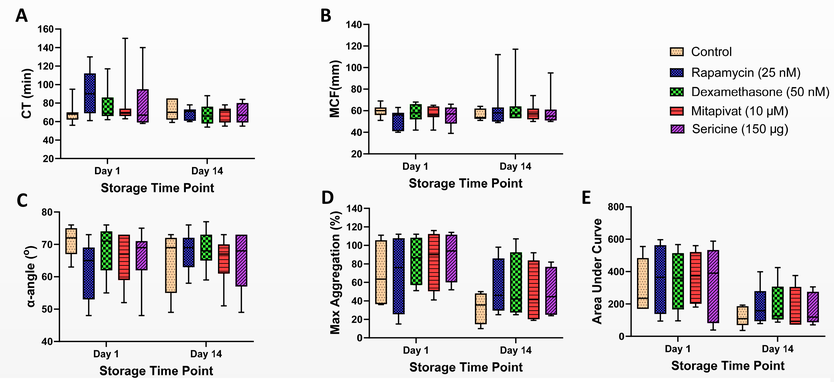

Figure 4 : Coagulation & Aggregation: No significant differences in Clot Time (CT) (A), Mean Clot Firmness (MCF) (B), and α-angle (C) were observed between drug-treated samples versus same day control or between storage Day 1 versus Day 14 samples. All drug-treated samples except rapamycin on Day 1 demonstrated an increase in aggregation compared to control, with mitapivat showing a 22.9 %, dexamethasone 22.6 %, and sericin 24.8% increases over control (D). Similarly on Day 14, rapamycin, dexamethasone, mitapivat, and sericin showed 64.1 %, 64.9%, 48.1% and 48.8 % increases, respectively, over control in the dual agonist-induced aggregation, though these increases in aggregation compared to controls were not statistically significant. A similar trend is observed with the Area Under Curve (E).

Conclusion

While ATP is vital for cell metabolism, its depletion during storage can compromise blood component quality. Our preliminary 14-day data suggest that adding mitapivat or rapamycin to storage media may sustain ATP levels; however, larger-scale studies extending to ≥35 days are necessary to confirm these findings and evaluate product safety and efficacy in trauma models.

References

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Acknowledgements

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Statements

This study was conducted under a protocol reviewed and approved by a United States Army Institute of Surgical Research (USAISR's) Research Regulatory Compliance Division.

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