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Mitigating Oxidative Stress via Targeted Reduction of Reactive oxygen species and Inflammatory Cytokines in Cold Stored Blood

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Introduction

- While whole blood remains a superior cellular therapy for trauma-induced coagulopathy, its efficacy is compromised by biochemical and structural changes collectively termed the "storage lesion"[1] which occur during prolonged storage at 1–6°C.
- As stored blood ages, oxidative stress caused by overwhelming increases of reactive oxygen species (ROS) and pro-inflammatory cytokines could affect viability leading to post-transfusion clearance of blood cells.
- Mitigating oxidative stress in stored blood is critical to preserving cellular viability and robust hemostatic function.
- Rapamycin and dexamethasone are among pharmacologic compounds that independently suppress ROS and pro-inflammatory markers.[2]
- In this study, we examined the potential of these molecules to mitigate cold storage-induced oxidative stress, specifically through reduction of ROS and pro-inflammatory markers over 21 days cold storage of whole blood.

Objectives

- Demonstrate how suppression of reactive oxygen species improves quality of stored blood.
- Recognize how reduction of pro-inflammatory cytokine levels in stored blood could be better achieved by a cocktail of rapamycin and dexamethasone supplementation
- Evaluate how dose optimization strategy yields the most effective dose sufficient to achieve results of oxidative stress reduction.

Methods

- Whole blood from healthy volunteers was supplemented with varying concentrations of rapamycin (10 nM, 25 nM, and 50 nM) and dexamethasone (25 nM, 50 nM, and 100 nM).
- A combination of Rapamycin/Dexamethasone was also tested at the following concentrations: 10nM/25nM, 25nM/50nM, and 50nM/100nM, respectively.
- Samples were stored at 4°C and sampled for analysis on days 1, 12, and 21.
- Whole blood ROS levels were determined using flow cytometry with CellRox reagents.
- Pro-inflammatory cytokines levels were measured by multiplex ELISA only after 21 days of storage, and
- Platelet ectodomain shedding was determined using flow cytometry and analyzed by surface expression of GPIbα (integrin αIIb) binding to CD41a antibodies.

Results

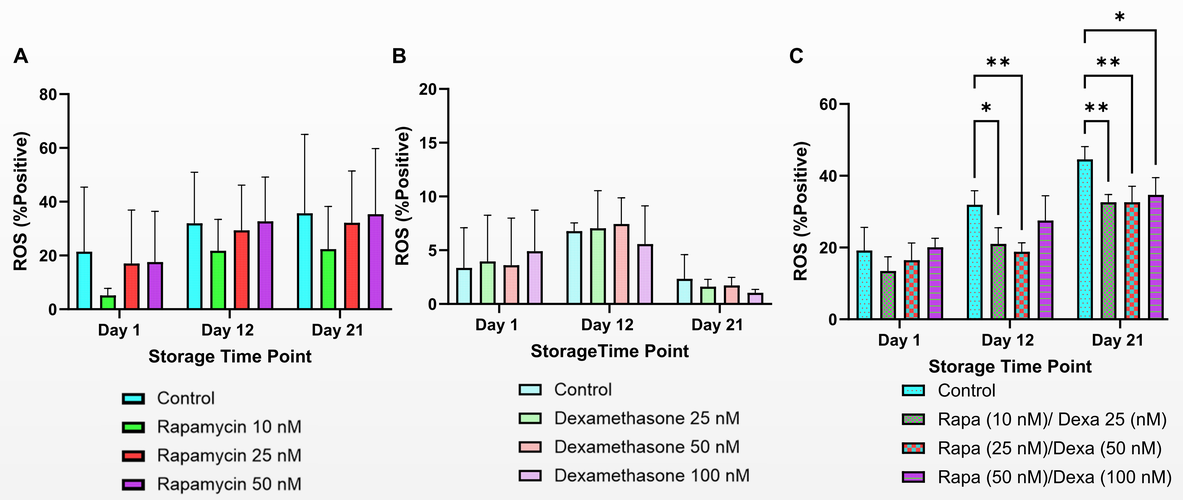

Figure 1: Effects of Drugs on reactive oxygen species (ROS) accumulation: Treatment with rapamycin or dexamethasone alone did not achieve significant reduction of ROS or pro-inflammatory cytokines over 21 days (A and B). A synergistic effect was observed by rapamycin/dexamethasone at 10/25 nM and 25/50 nM doses which significantly reduced ROS levels on Day 12 (P = 0.016 and P = 0.0037) compared to controls (C). On Day 21 all three doses of the combined therapy 10/25 nM, 25/50 nM, and 50/100 nM significantly reduced ROS levels (P = 0.0082, P = 0.0084, and P = 0.0306) compared to controls (C). Values represent means ± SD with n = 4 donors, *P < 0.05, **P < 0.01 and P < 0.001

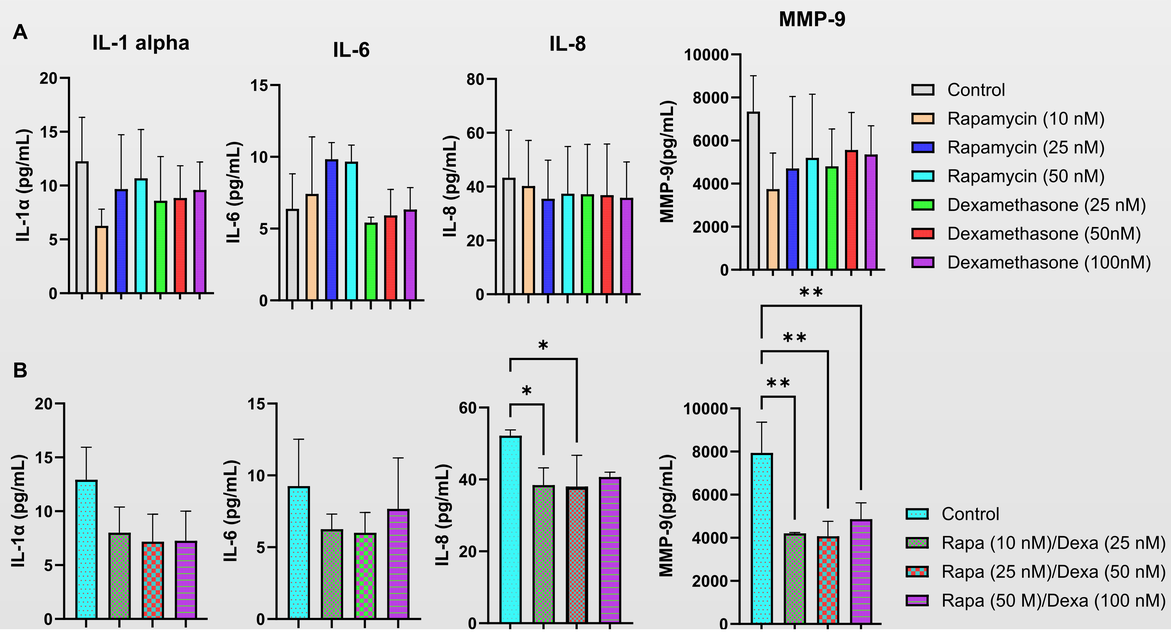

Figure 2. Effects of drugs on pro-inflammatory cytokines level: Individual drugs application did not have any significant reduction of any of the pro-inflammatory cytokines tested (A). Whereas two different doses of combined therapy (10/25 nM and 25/50 nM) effectively suppressed pro-inflammatory cytokine (IL-8) levels on day 21(P = 0.0265 and P = 0.0229), all three doses of the drug combination 10/25 nM, 25/50 nM, and 50/100 nM significantly suppressed MMP-9 (P = 0.0021, P = 0.0017, and P = 0.0067) (B). Values represent means ± SD with n = 4 donors, *P < 0.05, **P < 0.01 and P < 0.001

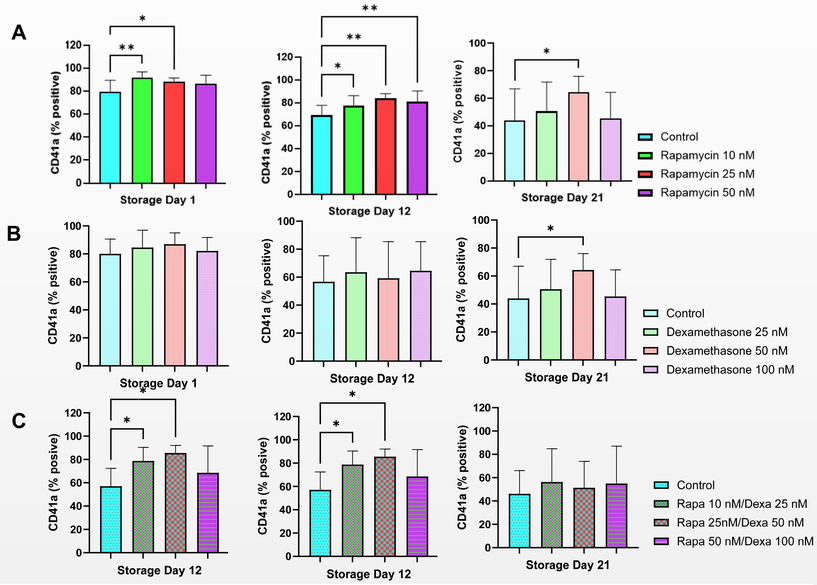

Figure 3. Rapamycin 10 nM and 25 nM (A), and two cocktail concentrations (B and C) respectively preserved GPIbα (integrin αIIb) compared to control on Day 1 through day 12 of storage. Values represent means ± SD with n = 4, *P < 0.05, **P < 0.01.

Conclusion

By inhibiting ROS and pro-inflammatory cytokine accumulation while reducing platelet membrane shedding, rapamycin in synergy with dexamethasone show significant promise in mitigating structural damage to red blood cells and platelets in storage. Future research will focus on validating the long-term efficacy of the drugs and assessing the impact on blood oxygen transport, coagulation, and hemostasis.

References

1. Yoshida et al. Red blood cell storage lesion: causes and potential clinical consequences. Blood Transfus 2019; 17: 27-52
2. Zhao et al. Autophagy ameliorates reactive oxygen species-induced platelet storage lesion. Oxidative Medicine and Cellular Longevity. 2022: 1898844. doi: 10.1155/2022/1898844.

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Statements

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

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