

Amitriptyline Attenuates the Red Blood Cell Storage Lesion in Whole Blood

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Introduction: The red blood cell (RBC) storage lesion collectively describes the biochemical, functional, and morphological changes that occurs to RBCs in stored blood. This lesion increases in severity with increasing duration of storage and is associated with increased morbidity and mortality after transfusion for resuscitation from hemorrhage. Acid sphingomyelinase contributes to this storage lesion by destabilizing the RBC membrane leading to hemolysis and microparticle production. Inhibition of acid sphingomyelinase with amitriptyline has previously been shown to reduce the severity of the RBC storage lesion in packed RBCs (pRBCs). However, the effects of amitriptyline on the RBC storage lesion in whole blood are unknown. We hypothesized that treatment of murine whole blood with amitriptyline reduces the severity of the RBC storage lesion.

Methods: Whole blood from C57BL/6 male mice was collected via cardiac puncture and stored in citrate phosphate double dextrose. Amitriptyline was added at increasing concentrations (2.5, 25, and 250 mM; n=5 for each group) and stored for 10 days at which point the RBC storage lesion was evaluated. RBC size, granularity, eryptosis, band 3 expression, and microparticle concentrations were measured utilizing flow cytometry. Osmotic fragility was assessed by suspending stored RBCs in increasingly hypoosmotic saline solutions. Additionally, cell free hemoglobin was measured.

Results: After 10 days of storage, whole blood treated with 250 mM amitriptyline demonstrated increased RBC size as determined by flow cytometry forward scatter (326,677 v. 307,609, $p < 0.0001$), decreased microparticle concentration (17.36 v. 58.32 microparticles/mL, $p < 0.01$), and decreased cell free hemoglobin (117.2 v. 251.4 mg/dl, $p < 0.01$) compared to control. At physiologic osmolality, the 250 mM group demonstrated less hemolysis compared to control (21.06% v. 38.50%, $p < 0.0001$) but had similar lysis levels at hypoosmotic saline concentrations. There were no significant differences between the groups in RBC granularity, rates of eryptosis, and band 3 expression.

Conclusion: Whole blood stored with 250 mM amitriptyline demonstrated reduced severity of the RBC storage lesion as evidenced by greater RBC size, lower microparticle and free hemoglobin concentrations, and decreased osmotic fragility. These data suggest that acid sphingomyelinase inhibition by amitriptyline is effective in blunting the RBC storage lesion in stored whole blood.

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Introduction

- The red blood cell (RBC) storage lesion describes the biochemical, functional, and morphological changes that occurs to RBCs in stored blood.
- Increased duration of storage is associated with increased morbidity and mortality after transfusion.
- Acid sphingomyelinase contributes to this storage lesion by catalyzing the hydrolysis of sphingomyelin into ceramides, leading to increased microparticle production and hemolysis of RBCs.
- Amitriptyline is a known inhibitor of acid sphingomyelinase.

Hypothesis: Treatment of murine whole blood with amitriptyline reduces the severity of the RBC storage lesion.

Methods

- Whole blood from C57BL/6 male mice was collected via cardiac puncture and stored in citrate phosphate double dextrose.
- Amitriptyline was added at increasing concentrations (2.5, 25, and 250 μ M; n=5 for each group).
- On day 10, RBC size, living RBCs, eryptosis, band 3 expression, and microparticle concentrations were measured utilizing flow cytometry. Osmotic fragility was assessed by suspending stored RBCs in increasingly hypoosmotic saline solutions. Cell free hemoglobin was also measured.

Results

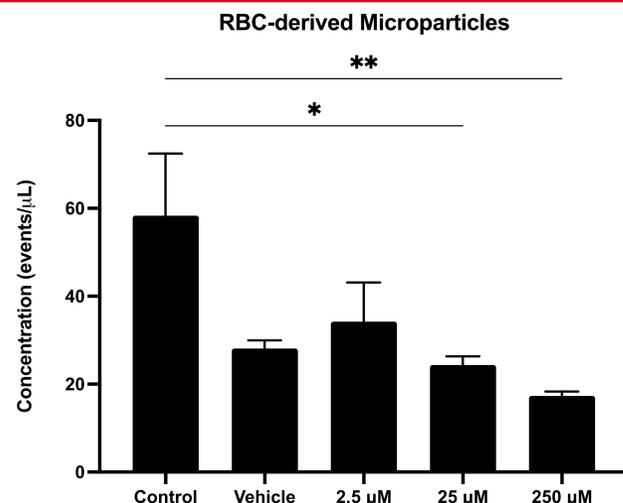


FIGURE 1: Microparticles were isolated via from day 10 stored whole blood and tagged with TER-119 to identify those of RBC origin. *p<0.05, **p<0.01.

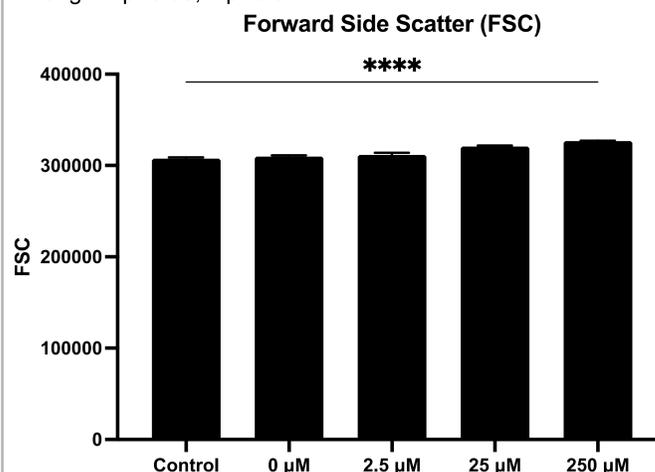


FIGURE 3: Forward side scatter as a representation of RBC size. ****p<0.0001

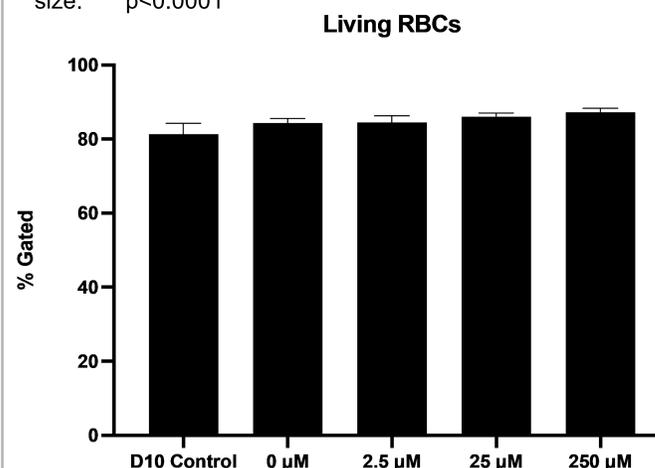


FIGURE 5: Living RBCs were measured on flow cytometry using Calcein/Annexin V stains.

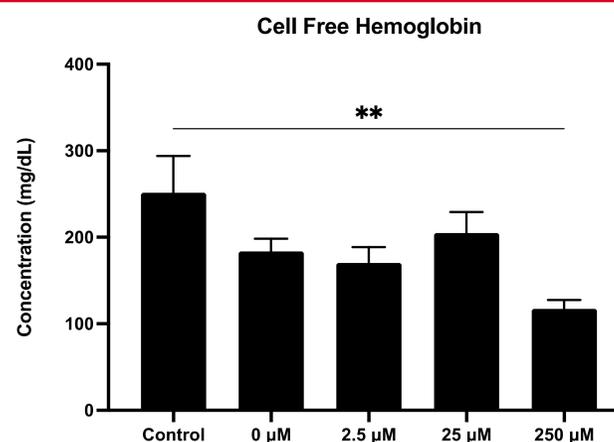


FIGURE 2: Free hemoglobin concentrations were measured using a multiplate analysis **p<0.01.

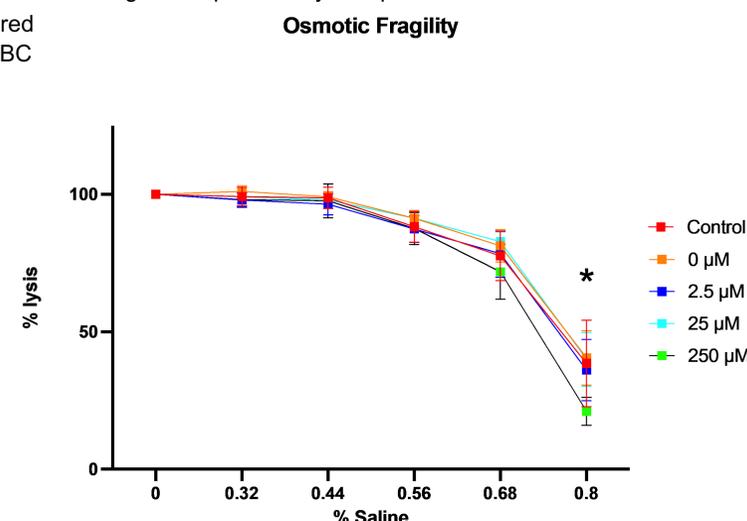


FIGURE 4: Osmotic fragility was observed by suspending D10 whole blood in increasingly hypoosmotic saline solutions. *250 μ M v. all D10 groups.

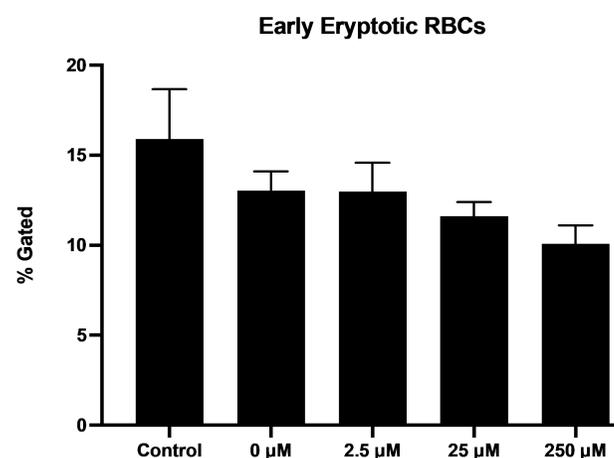


FIGURE 6: Eryptosis was measured on flow cytometry.

Results cont.

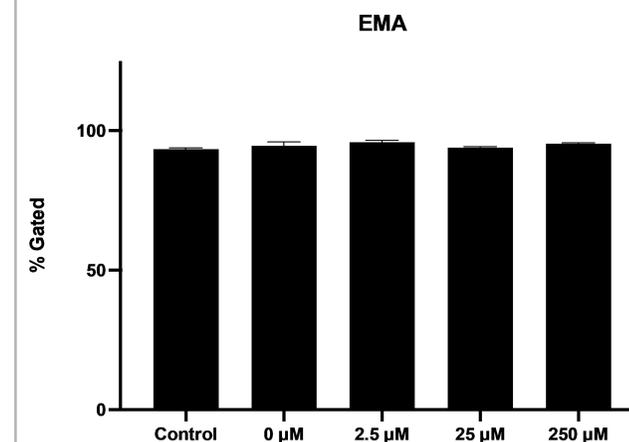


FIGURE 7: Band 3 protein integrity was measured on flow cytometry using eosin-5-maleimide (EMA).

Conclusions

- Whole blood stored with 250 μ M amitriptyline demonstrated greater RBC size, lower microparticle and free hemoglobin concentrations, and decreased osmotic fragility.
- There were no significant differences for living RBCs, eryptosis, or band 3 expression.

These data suggest that acid sphingomyelinase inhibition by amitriptyline is effective in blunting the RBC storage lesion in stored whole blood.

Next Steps:

- Resuscitate murine hemorrhagic shock models with whole blood stored in amitriptyline.
- Observe the effects of amitriptyline on the storage of human whole blood.

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