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Defining the effects of storage on platelet bioenergetics: The role of increased proton leak

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ABSTRACT

The quality of platelets decreases over storage time, shortening their shelf life and potentially worsening transfusion outcomes. The changes in mitochondrial function associated with platelet storage are poorly defined and to address this we measured platelet bioenergetics in freshly isolated and stored platelets. We demonstrate that the hypotonic stress test stimulates both glycolysis and oxidative phosphorylation and the stored platelets showed a decreased recovery to this stress. We found no change in aggregability between the freshly isolated and stored platelets. Bioenergetic parameters were changed including increased proton leak and decreased basal respiration and this was reflected in a lower bioenergetic health index (BHI). Mitochondrial electron transport, measured in permeabilized platelets, showed only minor changes which are unlikely to have a significant impact on platelet function. There were no changes in basal glycolysis between the fresh and stored platelets, however, glycolytic rate was increased in stored platelets when mitochondrial ATP production was inhibited. The increase in proton leak was attenuated by the addition of albumin, suggesting that free fatty acids could play a role in increasing proton leak and decreasing mitochondrial function. In summary, platelet storage causes a modest decrease in oxidative phosphorylation driven by an increase in mitochondrial proton leak, which contributes to the decreased recovery to hypotonic stress.

1. Introduction

Platelet transfusions are an important clinical treatment for patients undergoing chemotherapy, radiation, after surgery or trauma, and for patients with inherited platelet disorders such as Bernard–Soulier syndrome and idiopathic thrombocytopenic purpura [1,2]. Platelets from healthy human volunteers which are collected and stored in the blood bank undergo a progressive decline in function and quality during storage, which is generally characterized as the platelet storage lesion [3]. Key features of the platelet storage lesion include change in morphology, decreased aggregation, increased glycolytic rate, decreased plasma pH, decreased mitochondrial function, increased expression of activation markers, and a decreased hypotonic stress response [4–8]. Successful platelet transfusion requires an increment in platelet count of 30,000–50,000/μl per unit transfused, accompanied by normal function in primary hemostasis [9]. Due to the platelet storage lesion and the potential for bacterial contamination, hospitals discard platelets past day 5 of collection, leading to shortages and economic loss [10]. In this manuscript, we will define the bioenergetic changes of stored platelets, and the mechanisms underlying these changes.

The processes of platelet activation and aggregation require both glycolysis and oxidative phosphorylation, raising the question whether the metabolic impairments of the storage lesion impact on platelet function. It has been reported that ATP, ADP and AMP levels decrease during storage suggesting a deficiency in glycolysis and/or oxidative phosphorylation [11]. A number of studies have reported decrease in glucose levels, and an increase in lactate, in the platelet storage bags, suggesting an active glycolytic pathway and possible inhibition of mitochondrial function [12–14]. Since this increase in glycolytic rate occurs despite an increase in fatty acids, which we would expect could support mitochondrial function, it also suggests a possible mitochondrial defect. Indeed, a decrease in mitochondrial membrane potential has been reported over storage time, but studies regarding mitochondrial respiratory activities are conflicting. Some reports have shown that the capacity to consume oxygen over 7 days of storage was not altered, as

Abbreviations: AA, antimycin A; ACD, acid citrate dextrose; Asc, ascorbate; BHI, Bioenergetic Health Index; ECAR, extracellular acidification rate; HSA, human serum albumin; HSR, hypotonic stress response; TMPD, Tetramethyl-p-Phenylenediamine; XF, Extracellular Flux.

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measured by blood gas analyzer or Clark oxygen electrodes [12,15]. Other studies have demonstrated an almost complete loss of mitochondrial function. For example, one study reported a decrease of basal respiration of approximately 60% at day 8 whereas others report an almost complete loss of function at the second day which contrasts with other reports that show essentially little or no change [8,16,17]. Typically, these studies use very small sample sizes and varying protocols for platelet storage which are different from those used in transfusion medicine [8,16,18]. In addition, isolation of mitochondria from the platelets to analyze complex activities may lead to damage to the organelle which can be avoided by using cellular bioenergetic analysis [19]. These factors could partially explain the inconsistency of these data. Since both glycolysis and oxidative phosphorylation play an important role in platelet function [20,21] it is important to resolve these issues so effective strategies can be developed to improve the performance of stored platelets following transfusion.

In order to better understand the metabolic and functional changes during storage, 38 platelet concentrates, between ages day 6 and 9, were obtained from the blood bank, and their mitochondrial and glycolytic functions were measured using the Seahorse Extracellular flux analyzer, and compared to freshly isolated platelets from healthy donors. We found that aggregation following thrombin stimulation was not significantly different in the stored platelets, but recovery after the hypotonic stress response was impaired in the stored platelets. Additionally, stored platelets showed a decrease in basal, and ATP linked OCR, and an increase in proton leak and reserve capacity. We also observed that the changes in bioenergetics in the stored platelets were primarily due to an increase in proton leak which we ascribed to the uncoupling effect of fatty acids. In support of this hypothesis, addition of human serum albumin (HSA) to stored platelets attenuated the increase in proton leak, suggesting that it could be used as a possible additive to preserve mitochondrial function in stored platelets.

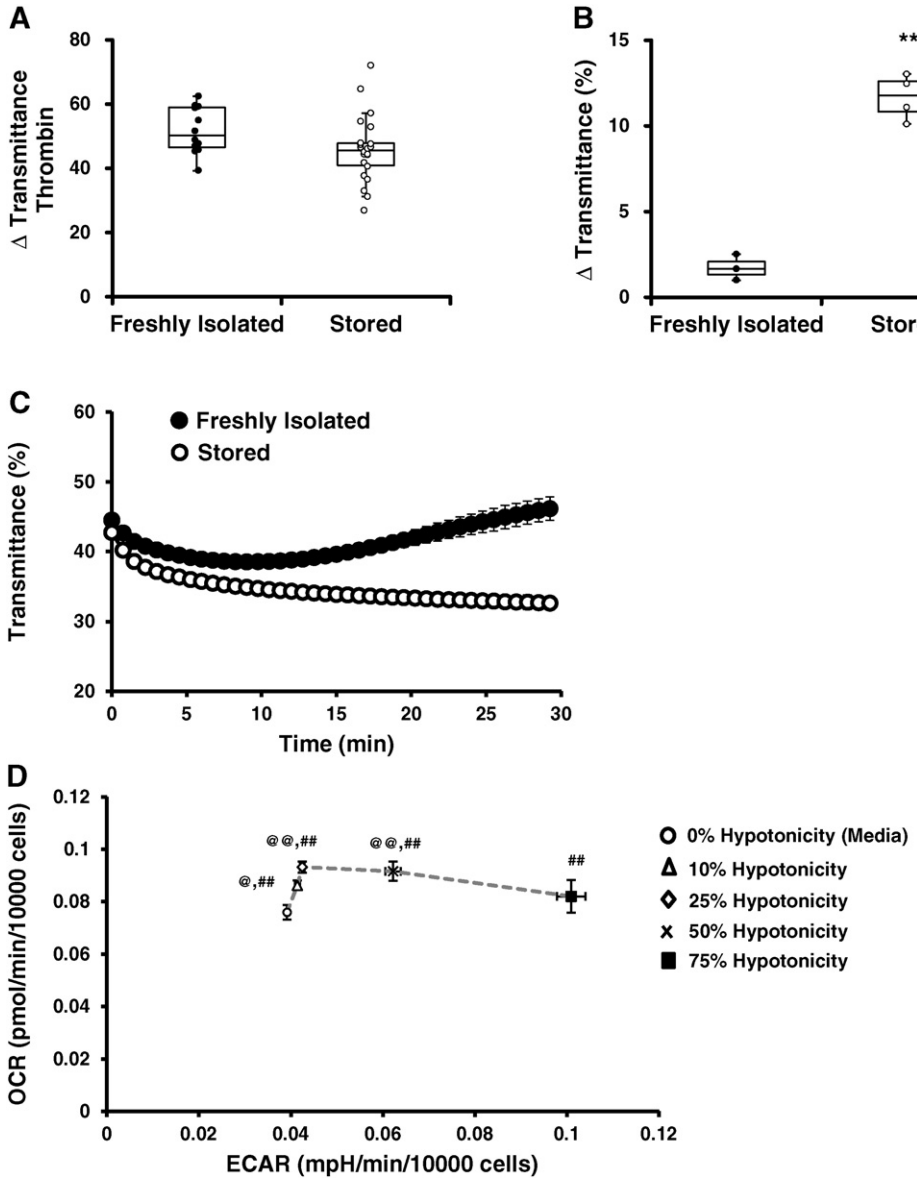

Fig. 1. Aggregation and hypotonic stress response of freshly isolated and stored platelets. Platelets from fresh blood or storage bags were isolated, followed by evaluation of (A) aggregation using thrombin (0.5 U/ml) and (B) hypotonic stress response using equal volume of water. (C) Change in light transmittance after hypotonic stress response assay and (D) OCR vs. ECAR plot of platelets exposed to different hypotonic stress levels (0–75%) from one healthy volunteer donor. Extent of aggregation is expressed as change in light transmittance after thrombin (0.5 U/ml). HSR represented as change in transmittance after addition of water. Aggregation and HSR data graphed as box plots with lower 25th percentile, median, upper 75th percentile, and whiskers drawn at 1.5 × interquartile range. Data expressed as mean ± SEM. Aggregation — 12 freshly isolated platelets and 22 stored platelets; HSR — 3–4 donors. n = 3 replicates per sample. **p < 0.01, different from freshly isolated. %%p < 0.01, %p < 0.05, OCR different from 0% water. ##p < 0.01, ECAR different from 0% water.