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Platelet Storage Temperature Determines Recovery of GPVI-Function *In Vivo*

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Background: Platelets (PLTs) are currently stored at 22°C (RT, room temperature) for clinical purposes. This approach ensures long circulation time but has numerous downsides, including limited storage time due to the risk of bacterial growth and increased costs due to bacterial testing or pathogen reduction processing. PLTs stored at 4°C were the standard of care in the 1960s and 1970s. In our previous study with healthy volunteers, we showed that humans who received cold-stored PLTs have a significantly weaker response to collagen (an agonist that acts predominantly via GPVI) compared to RT-stored PLTs. If and how cold-stored PLTs recover their function in vivo is poorly understood.

Methods: We obtained human PLTs by an apheresis collection and sampled either at baseline (fresh) or after five days at RT or 4°C. To test the response to GPVI-dependent agonists, we stimulated platelet-rich plasma or washed PLTs with collagen and the GPVI-specific agonist convulxin (CVX) and tested for activated integrin and α-degranulation by flow cytometry. Platelet aggregation, in response to GPVI-dependent agonists, was tested by aggregometry. We checked for GPVI expression levels by flow cytometry and for signaling events downstream of GPVI by immunoblotting. To allow for recovery of function in vitro, we incubated either 4°C-stored, or RT-stored PLTs with fresh, platelet-depleted

blood for 15min, and perfused the reconstituted whole blood through a microfluidic block and post device to quantify the contractile forces of platelet aggregates. Additionally, we performed platelet force measurements at the single cell level using a traction force microscopy approach.

To validate a murine model of platelet storage and transfusion, we replicated functional studies in vitro by testing mouse PLTs for integrin activation and α-degranulation by flow cytometry. Platelet aggregation in response to collagen, CVX, and the GPVI-specific antibody JAQ-1 with crosslinking anti-IgG was also tested. To evaluate the platelet function after transfusion, we obtained whole blood from UbiC-GFP mice and isolated platelet-rich plasma followed by storage for 24 hours at either 4°C or RT. To allow tracking of stored PLTs in vivo, we transfused the UbiC-GFP PLTs into wild-type C57BL/6J mice and tested for integrin activation of endogenous and transfused PLTs.

Results: In human PLTs, we found a significantly increased integrin response in 4°C-stored PLTs stimulated with collagen in flow cytometry studies in vitro. Similarly, the aggregation response of 4°C-stored PLTs to collagen was significantly increased compared to RT-stored PLTs in vitro. In line with these findings, we observed more PLCγ2 phosphorylation and Syk phosphorylation at baseline in 4°C-stored PLTs compared to RT-stored PLTs, suggesting more pre-activation downstream of GPVI. However, no differences in PLCγ2 phosphorylation or Syk-phosphorylation were found between RT and 4°C-stored PLTs after stimulation with CVX, and no significant differences in surface expression levels of GPVI were detected between RT and 4°C. Stored platelets in plasma showed superior function after 4°C-storage in aggregation and flow cytometry assays. In contrast, we found similar contractile forces of platelet aggregates when RT-stored or 4°C-stored PLTs were added to platelet-depleted fresh blood. Additionally, at the single cell level, we found a similar magnitude of platelet forces in RT-stored and 4°C-stored PLTs.

Similar to human PLTs, mouse PLTs showed significantly more integrin activation, P-selectin exposure, and aggregation in 4°C-stored PLTs compared to RT. To test the recovery of function of stored mouse platelets in vivo, we transfused GFP-positive PLTs into GFP-negative wild-type mice. Contrary to our pre-transfusion results, we found a significantly lower integrin activation response to CVX in 4°C-stored platelets after transfusion, consistent with our previous results in healthy human volunteers.

Summary: The in vivo recovery of function of stored PLTs is an underappreciated phenomenon in platelet storage biology, and most studies are solely based on functional in vitro data. Based on our post-transfusion results, storage temperature affects the ability to recover function in vivo significantly in human and mouse platelets. Whether these differences lead to differences in clinical outcomes needs to be investigated in clinical trials.

Disclosures

Sniadecki: *Stasys Medical Corporation:* Current equity holder in private company, Other: Co-founder; *Curi Bio:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees.

Author notes

* Asterisk with author names denotes non-ASH members.