

PROPOSAL NARRATIVE

Of Squirrels and Men: A Story of Platelet Storage

A. Significance of the Project

Many people throughout the country not only need blood but donate blood as well. One of the uses of this donated blood is for a platelet transfusion. Platelets are cells in the blood that help to form life saving clots if a person is injured. Currently, platelets are stored at room temperature because if stored in the cold they will develop platelet storage lesions, lose function, and are rapidly cleared from the circulation. However the fact that platelets are stored at room temperature increases their risk of bacterial contamination. As a result, platelets are currently limited to a five day shelf life, with expiration of up to half of the platelet supply before they can be used. One thing that would allow us to store platelets for a longer period of time would be if we were able to store platelets at lower temperatures. However, human platelets that undergo cooling for even a brief period of time at 4°C are rapidly cleared from the circulation after transfusion. This has been shown both *in vivo*, and using cultured human liver Hep G2 cells (2-4). Chilled platelets also experience other structural and functional changes as a result of being exposed to colder temperatures that can also lead to increased aggregation and degranulation upon warming.

Hibernating mammals such as ground squirrels have developed several physical adaptations that allow them to survive in extreme environments. One of these adaptations is that their platelets seem to be resistant to the cold-induced storage lesions that human platelets would undergo if exposed to the same conditions (1). This leads us to believe that the behavior of ground squirrel platelets is different than that of other mammals including humans. One of the major differences

is that the ground squirrel platelet levels return to normal once the animals are aroused from up to six months at 4°C, whereas human or other non-hibernating mammal's platelets under these conditions would rapidly be cleared from circulation by the liver. If ground squirrel platelets are resistant to cold-induced clearance *in vivo*, and the mechanism for how this is done can be determined, this could have direct clinical applications allowing human platelets to be stored at 4°C.

B. Objectives

The objectives of performing the following experiments are to perfect an assay using cultured human Hep G2 cells to see how human and ground squirrel platelet uptake is affected by different storage temperatures. This will be one of the first steps in our ultimate goal of improving platelet storage in a clinical setting.

C. Research Methods

As stated above, the overall goal of this experiment is to see if ground squirrel platelets are taken up by human liver cells and the effect that temperature has on this uptake. Hep G2 cells are liver hepatocellular carcinoma cells that we culture in the lab in tissue culture flasks. Human platelets will be obtained by drawing blood from a donor, and centrifuging the red and white blood cells away from the platelets. The platelets will then be mixed with plasma and stored at either 4°C or 37°C for 48 hours. These human platelets will then be labeled with CM-Orange, which is a type of fluorescent dye. The labeling process allows the location of the platelets to be followed with a flow cytometer which detects fluorescence. These labeled platelets will then be fed to

Hep G2 cells in a tissue culture flask for 30 minutes to allow platelet uptake. Any remaining platelets will be washed off, and the Hep G2 cells knocked off the flask with the enzyme trypsin. The Hep G2 cells will then be run through a flow cytometer which allows us to measure the uptake of the fluorescent platelets by the Hep G2 cells. Hep G2 cells that have not taken up a platelet will not be fluorescent. This portion of the experiment allows us to determine a baseline measurement of platelet uptake on the flow cytometer. We can also compare our results with published experiments that stored human platelets at different temperatures.

Once this assay has been perfected, we can repeat the experiment with the ground squirrel platelets using the same protocol stated above. We will collect platelets from ground squirrels that are housed on the UW-L campus in the Health Science Center and store the platelets at 4°C and 37°C for 48 hours. These platelets will then be labeled with CM-Orange and fed to the Hep G2 cells. We will then be able to run these assays on the flow cytometer to view the difference in uptake of these platelets compared to that of humans. We expect to see cold storage increase the rate of human platelet uptake by Hep G2 cells, but cold storage should have no effect on ground squirrel platelet uptake. Once the assay is working we can vary the duration of platelet storage and times of incubation to help determine the mechanism of any differences observed between human and ground squirrel platelet storage.

These Hep G2 assays were developed by a collaborator of ours at Harvard, Dr. Karin Hoffmeister. If we cannot get the assays to work here, she has offered to show me how to do the assays in her lab in Boston. Part of the funds requested are to travel to Boston to learn how to perform these assays.

References

1. **Cooper ST, Richters KE, Melin TE, Liu Z, Hordyk PJ, Benrud RR, Geiser LR, Cash SE, Shelley CS, Howard DR, Ereth MR, and Sola-Visner MC.** The hibernating 13-lined ground squirrel as a model organism for potential cold storage of platelets. In: *AM J PPhysiol*, 2012.
2. **Grozovsky R, Hoffmeister KM, and Falet H.** Novel clearance mechanisms of platelets. *Current opinion in hematology* 17: 585-589, 2010.
3. **Rumjantseva V, Grewal PK, Wandall HH, Josefsson EC, Sorensen AL, Larson G, Marth JD, Hartwig JH, and Hoffmeister KM.** Dual roles for hepatic lectin receptors in the clearance of chilled platelets. *Nature medicine* 15: 1273-1280, 2009.
4. **Sorensen AL, Rumjantseva V, Nayeb-Hashemi S, Clausen H, Hartwig JH, Wandall HH, and Hoffmeister KM.** Role of sialic acid for platelet life span: exposure of beta-galactose results in the rapid clearance of platelets from the circulation by asialoglycoprotein receptor-expressing liver macrophages and hepatocytes. *Blood* 114: 1645-1654, 2009.

D. Final Products and Dissemination

A poster will be presented on my results at the 2013 NCUR held at UW-L. The results may also be part of a peer reviewed publication submitted by Dr. Cooper.

E. Budget justification

Over the course of this experiment, I will be spending a minimum of 10-15 hours per week working in the lab possibly more during times when more extensive portions of the experiment are being performed. These duties will include the culture of the Hep G2 cells 2-3 times per week along with running samples on the flow cytometer once or twice per week. Platelet samples will also have to be collected at minimum once per each time the experiment is run.

If I cannot get the assays to work in our lab, I would like to travel to a colleague's lab at Harvard to learn the assay there. This would require flying to Boston for a few days. For two nights lodging in Boston, the University travel maximum is listed as \$230. Dr. Cooper said he could cover the difference.

2. Letter of Support

Dear review committee,

I am writing on behalf of Cinderella Slipper's application for an undergraduate research grant. Cinderella is currently a dual major in Biochemistry and Cellular and Molecular Biology at the University of Wisconsin - La Crosse. I have known Cinderella for a year as her research and academic advisor. Cinderella has expressed an interest in pursuing a research career and I think she is well suited for the rigors of the field.

In my research lab Cinderella is working on a clinically relevant project exploring the effects of hibernation on platelets in ground squirrels. When ground squirrels hibernate, their blood flow drops dramatically, yet they do not form stasis induced blood clots. We have found that their platelets are sequestered in the spleen during hibernation and are released within two hours of

arousal from hibernation in the spring. These released platelets are not cleared rapidly and are still functional. Cold storage of platelets from other mammals results in surface changes that cause the platelets to be rapidly cleared from circulation, negating their value in transfusions. Cinderella's work is focused on looking at clearance of ground squirrel platelets that have been stored in the cold by cultured liver cells (HepG2 cells). In her experiments she cultures HepG2 cells, fluorescently labels platelets that had been stored at 4°C or 37°C, feeds the platelets to the HepG2 cells, and then looks for platelet uptake by running the HepG2 cells on a flow cytometer. If Cinderella can determine if the surface changes that lead to rapid clearance of chilled human platelets are blocked in ground squirrel platelets during cold storage, we may be able to create methods to store human platelets in the cold for transfusions.

These are difficult and time consuming assays, and if Cinderella has the time to focus on them this summer, I think she can make good progress and hopefully prepare a figure for a future publication. My colleague Karin Hoffmeister at Harvard developed these assays, and would be willing to help train Cinderella if we can fly her to Boston, a letter of support she wrote for my current NIH grant is also attached. The travel funding is requested for Cinderella to go to Karin's lab in Boston to learn these assays. If Cinderella does not need to travel to Boston, these funds would obviously be returned. I can cover the supplies and equipment needed for the rest of the experiments from my laboratory budget.

I have not had Cinderella in class yet, as she is a junior. She has a good overall GPA given the difficult science courses she has taken in her dual major. Cinderella is also very independent, and once I taught her how to do tissue culture she has been maintaining the cell line with no supervision. She has also become quite proficient on the flow cytometer. I think that receiving

an undergraduate research grant would allow Cinderella to continue her research, and also broaden her background and experiences by interacting with researchers at other universities.

Sincerely,

Scott Cooper, Professor of Biology