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FIRST TRIANNUAL REPORT (YEAR 1)

for period February 1, 1992, to May 31, 1992

Report date: June 4, 1992

ONR Grant No. N00014-92-J-1244

Evaluation of Dried Storage of Platelets for Transfusion: Physiologic Integrity and Hemostatic Functionality.

Principal Investigator: Arthur P. Bode, Ph.D.
East Carolina University School of Medicine
Greenville, NC

Attachment: Report from subcontract principal investigator, Marjorie S. Read, Ph.D., The University of North Carolina at Chapel Hill.

Administration of New Grant

The award document was received on 2/05/92 and spending authority at ECU was set up on 2/11/92. A new subcontract agreement was enacted with UNC-Chapel Hill on 2/25/92 to grant spending authority at both performance sites. The alacrity with which this grant processing was accomplished highlights the importance attached to the project by the involved administrations. The investigators have met several times now since the award to implement the new study plan.

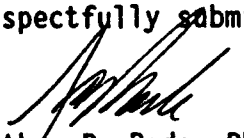
No new collaborations were required in the proposal versus the prior project. Purchases of equipment have been processed through the state-mandated bid system. At ECU, the proposed computer workstation has been obtained, installed, and is fully functional, replacing the aged PC-XT formerly engaged in plot generation, data record keeping, and word processing. Bids have been opened on the requested lyophilizer to be placed at ECU but delivery and installation of the equipment is still pending. The original budgeted amount of $9800 has been augmented by $11,000 from the continued previous grant (N00014-89-J-1712) to accept a much more capable instrument than was previously planned. The 600 series Virtus freeze-dryer compressor and shelf drying unit will be outfitted with a side manifold for handling large volume samples such as platelets from a whole unit of blood in a single container. It is expected that installation of the lyophilizer will be completed by the end of June.

Scientific Progress

The first set of goals in the new project overlap with continuation of activities described in the prior (extended) grant project. Animal model studies with para-platelets are proceeding to test hemostatic efficacy and circulatory lifespan (see attached report from the performance site at Univ. North Carolina - Chapel Hill). At ECU, we have prepared a very large batch of nearly 300 aliquots of lyophilized para-platelets to begin a controlled trial of storage conditions. Four batches of 50-70 aliquots have been placed in dessicators out on the bench at room temperature, or in a refrigerator at 4-6°C, or in a freezer at -20°C, or in an ultra-low freezer at -70°C. The initial workup included flow cytometry analysis, hypotonic shock response, aggregometry, and morphology studies; also, these platelets represent the adaptation to human platelets of the standard para-platelet protocol being characterized for functionality in the animal model experiments.

When the new lyophilizer is installed at ECU, work will begin right away on developing large, sterile platelet preparations from human blood collected in the standard blood bag sets used by the Red Cross and other blood suppliers in current blood banking protocols. At the same time, experiments will continue in dogs and pigs to assess the thrombotic and hemostatic potential of rehydrated para-platelets in the fresh or stored state. Work will also continue on fully developing and testing a permanganate-based platelet preparation as an adjunct procedure to compare to the results with para-platelets. We hope to have significant new data on all three parts of this effort in the next report.

Respectfully submitted,



Arthur P. Bode, Ph.D.
Principal Investigator

Quarterly Report

First Quarter: Januarv 1, 1992-March 31, 1992.

Office of Naval Research: Grant No.N00014-92-J-1244

Project Title: Dehydration of Platelets for Transfusion.

Performance Site: University of North Carolina at Chapel Hill.

Principal Investigator: Marjorie S. Read, Ph. D.

Co-Principal Investigator: Robert Reddick, M.D.

Progress Report- June 5, 1992.

Statement A per telecon Capt Steven Lewis
NMRDC/Code 404
Bethesda, MD 20889-5044

NWW 8/14/92

A. Studies conducted during the past three months.

We have limited the studies conducted on the UNC campus to platelets stabilized with paraformaldehyde. Two sets of experiments were begun during this quarter and are still being studied.

1. Studies were begun on a pig made thrombocytopenic by whole body irradiation. The purpose of this study is to evaluate the performance of rehydrated platelets in the absence of normal fresh platelets. The evaluation of tissues and blood samples taken from this animal are still underway. An 81 pound female pig was transported to North Carolina State College of Veterinary Medicine where it received a whole body irradiation dose of 8 Grays. The pig was returned to The Francis Owen Blood Research Laboratory where a CBC was done daily to follow the hematologic changes. Hematocrit, platelet count, hemoglobin and white cell population remained within normal range for five days. On day 5, the platelet count dropped to 86,000/cmm and on day 6, it was 11,000/cmm. The pig developed an elevated temperature. By day 7, the ear bleeding time (BT) was greater than 15 min. The pig suffered a wound to the hoof which required cauterization to stop bleeding. Rehydrated platelets were infused to a level of 55,000/cmm and a bleeding time was taken. Additional rehydrated platelets were transfused and the circulating platelet count was raised to 490,000/cmm. The BT was corrected to 7 min 45 sec. The animal was allowed to walk around freely and again sustained an injury to the hind toe which stopped bleeding without additional interference. We gave multiple transfusions during the day for a total of three times the original platelet count. We were unable to get a sustained platelet count above 25-30,000/cmm and the saline ear bleeding time by the end of the day was again >15 min. By day 8, the pig's temperature was 105.4. Rehydrated platelets were infused but the bleeding time was not corrected. The stenosis and injury protocol was followed. There was no cyclic flow and no thrombosis. We were unable to raise the platelet count above 32,000/cmm. The Baumgartner experiments performed at the time using vessels and blood collected from the pig are being studied and evaluated. At necropsy, we observed no abnormality of the spleen and no gross evidence of splenic uptake of infused platelets. Plasma samples are being studied for presence of endotoxin which could explain the rapid loss of platelets from the circulation and our failure to raise the circulating platelet count to a normal level. Circulating endotoxin, as result of infection, might have caused platelet lysis. Tissue from the spleen, kidney, lung, heart, blood vessels,etc. were taken for study. Blood and blood clots were fixed for EM. Data from this animal is still being collected.

2. Studies of canine platelets for presence of platelet von Willebrand Factor are nearly complete. Dogs are being used as a model for transfusion of rehydrated platelets as studied in the standard arterial injury and stenosis protocol. This system is being used to evaluate the ability of rehydrated platelets to form normal thrombi. The protocol is also being used in the vWD dog as a control for abnormal thrombosis. It is necessary to understand the role of canine vWf in fresh and rehydrated platelets. We have studied platelets from 6 normal dogs, 11 hemophilia A dogs, 3 vWD dogs, and 1 dog doubly deficient for hemophilia A and vWF. Canine platelets were compared to normal human and pig platelets for platelet vWF antigen and platelet vWF multimer distribution. The platelet lysates and releasates were prepared by five different procedures. The platelets were disrupted by freeze-thaw, sonication, and triton X-100. Platelets were stimulated to undergo release with A23187 and MgCl2. Electron microscopy of samples before and after treatment confirmed either disruption or release as evidenced by platelet degranulation. The platelet vWF antigen level was measured by Laurell rocket and cross immunoelectrophoresis. The platelet vWF activity level was measured by the standard botrocetin macroscopic assay. No vWF was detected, antigenically or functionally from fresh, normal canine platelets.

The role of platelet vWF in the canine, therefore, is different from human and pig. Canine plasma vWF, like porcine plasma vWF, is required for arterial thrombosis. The role of platelet GP1b, shown to be retained on the rehydrated platelet surface is still being studied.

The presence of fibrinogen in rehydrated platelets and the ability of the rehydrated platelet to undergo release is being studied.