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Preclinical Investigations of Lyophilized Platelet Preparations

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Grant

6. AUTHOR(S)
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Pathology, Chapel Hill, NC 27514

8. PERFORMING ORGANIZATION REPORT NUMBER:

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
The U.S. Office of Naval Research NMRDC; National Naval Medical Center
8901 Wisconsin Ave.
Bethesda, MD 20889

10. SPONSORING/MONITORING AGENCY REPORT NUMBER:

11. SUPPLEMENTARY NOTES:
This project has been extended to Dec. 31, 1996.

12a. DISTRIBUTION AVAILABILITY STATEMENT
Unlimited

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13. ABSTRACT (Maximum 200 words)
This project is a collaborative effort between UNC-Chapel Hill and East Carolina University to test the efficacy and safety of preparations of lyophilized blood platelets for transfusion. The development and testing of these preparations is an extension of previous work done under grants N00014-92-J-1244 and N00014-89-J-1712 from the U.S. Office of Naval Research. The present emphasis has been on infusions of rehydrated platelet preparations into animal models of hemostasis for the arrest of hemorrhage and/or correction of bleeding time test results. Functionality comparable to liquid stored platelet concentrates was obtained and no adverse effects were noted. Experiments in vitro demostrated the adhesion of rehydrated platelets to exposed blood vessel subendothelium, and evidence of activation response (Thromboxane formation, neo-antigen expression) as a result. These findings are encouraging for the eventual pharmaceutical production and clinical trial of our lyophilized platelet preparations under an FDA IND. Armour Pharmaceutical Corp. (now Centeon Corp.) has been licensed to carry out this goal.

14. SUBJECT TERMS
Blood platelets, lyophilization, transfusion, platelet adhesion, animal models, hemostasis

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January 31, 1996

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Dear Sir,

Please find enclosed two copies of the Annual Progress Report for the period October 1, 1994 - Sept 30, 1995. If you have any questions I may be contacted at 919-816-5020. Thank you.

Arthur P. Bode, Ph.D.
Principal Investigator

APB/tsb

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ANNUAL PROGRESS REPORT

Work Unit No. (including P.E.): Grant No. N00014-93-I-1034

PRINCIPAL INVESTIGATOR: Arthur P. Bode

INSTITUTION: East Carolina University School of Medicine

SUBCONTRACT PRINCIPAL INVESTIGATOR: Marjorie S. Read

INSTITUTION: The University of North Carolina at Chapel Hill

GRANT TITLE: Preclinical Investigations of Lyophilized Platelet Preparations

REPORTING PERIOD: 1 Oct 94 - 30 Sept. 95 (12 months)

AWARD PERIOD: 1 Sept 93 - 31 Aug 96 (with no-cost extension)

OBJECTIVE: To test the hemostatic properties and side effects of infusions in animal models of reconstituted lyophilized platelet preparations developed in our prior ONR project activity; also to study the responsiveness of the rehydrated platelets to in vitro stimulation for release of granule contents, generation of thromboxanes, and adhesion properties.

APPROACH: The main emphasis of experimentation at ECU in this reporting period has been on: (1) the adhesion and activation chamber, and (2) transfusion of rehydrated canine platelets in order to convert the bleeding time in dogs with a platelet function defect and thrombocytopenia due to prolonged extracorporeal recirculation of blood during cardiopulmonary bypass. The Baumgartner experiments were analyzed for the ability of the platelet preparations to adhere to exposed thrombogenic sites on the subendothelium of denuded canine blood vessel strips, and for evidence of activation in the non-adherent platelets (CD62, CD63 neo-antigens expression, thromboxane formation). In the dog bypass model, a single large bolus of homologous lyophilized platelets were infused at the end of 2 hours of CPB while the subject animal was still on the extracorporeal circulation pump or immediately after weaning from the pump. Bleeding time tests were done during the pump time and immediately prior to platelet infusion, and then followed for up to 4 hours post-infusion.

At UNC-CH, rehydrated platelets were examined by SDS PAGE and 2D- gels for biochemical changes induced by processing, and the effects on the releasate after stimulation with

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thrombin or other agonists. Also, the microbicidal properties of the paraformaldehyde fixation and washing protocol used in preparing our lyophilized platelets were examined by collaborators in the UNC Transfusion Service by inoculation of platelet units prior to processing with several strains of bacteria obtained from the CDC.

ACCOMPLISHMENTS (less 12 months): We have continued to demonstrate that the rehydrated platelets adhere to thrombogenic denuded vessel strips to an extent nearly equivalent to fresh platelets, and do not adhere to intact vessels in the perfusion chamber at high shear. We have compiled more evidence of Thromboxane production by the lyophilized platelet preparations in this system as evidence of an activation response, but the neo-antigen expression has proved to be highly variable. Still, evidence of an increase in procoagulant activity and release of granule contents after in vitro stimulation with strong agonists supports the notion that these platelets are capable of an activation response to physiologic stimuli although somewhat slower or reduced compared to fresh platelets. Correction of in vivo bleeding time in the canine CPB model has proved difficult to demonstrate when the infusion of rehydrated platelets is given after coming off the pump, probably due to problems in managing circulatory pressure, but the last three experiments have shown a persistence of at least 3 hours in lowering of the bleeding time by the platelet infusion. Corroborative data have been obtained from peripheral blood samples taken during these runs and tested on the Clot Signature Analyzer in vitro bleeding time device.

The gel analysis of rehydrated platelets and releasates has shown very few qualitation differences versus fresh platelets, although there is overall less protein released from the rehydrated platelets in response to in vitro stimulation. There was no evidence of neo-antigens created by the preparation protocol, suggesting that these preparations may have low immunogenicity in vivo. Moreover, the bacteriocidal studies showed that the platelet processing with paraformaldehyde and washing steps results in complete oblation of microbial survival or growth in inoculated units.

SIGNIFICANCE: The rehydrated platelets have now proven to be hemostatic in vivo under a variety of conditions. There is no evidence of inappropriate thrombosis or adhesion to non-thrombogenic sites. The residual degree of responsiveness that appears to remain in the rehydrated platelets may explain their hemostatic effectiveness in vivo and the persistence of this effect. We will need to determine how important the responsiveness properties demonstrated in vitro relate to the hemostatic functionality of the platelet preparations in vivo.

WORK PLAN (next 12 months): The project has entered a no-cost extension period (up to 31 Aug 96) to finish up experimentation in progress. This will include more biochemical studies of the relationship between in vitro responsiveness and in vivo hemostatic effect, and more preliminary studies or the circulatory lifespan of rehydrated platelets.

RELATED WORK: We have tested several lots of lyophilized platelets produced by Armour Pharmaceutical Corp. under the licensing agreement initiated a year ago. The recent results show that their product is much like ours especially in terms of hemostatic effectiveness and responsiveness. We are enthused that a commercial product with these essential properties may go forward in to clinical trials in the near future.

PUBLICATION, REPORTS AND ABSTRACTS (last 12 months):

(1) MS Read, RL Reddick, AP Bode et al. "Preservation of hemostatic and structural properties of rehydrated lyophilized platelets: Potential for long-term storage of dried platelets for transfusion." Proc. Natl. Acad. Sci. 92 (Jan):397-401, 1995.

(2) AP Bode. "Preclinical testing of lyophilized platelets s a product for transfusion medicine." Transfusion Science 16(2):183-185, 1995.

(3) MS Read and AP Bode. "Platelet storage: Efforts to extend the shelf-life of platelet concentrates." Molecular Medicine Today 1 (7):322-328, 1995.

(4) Presentation of one abstract to the American Association of Blood Banks (Nov. '94), and three abstracts to the American Society of Hematology (Dec. '94).