CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
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Project Number
S0421

Project Title
The Inhibition of Lipid Raft-Mediated Platelet Plug Formation by Methyl-beta-cyclodextrin in an in vitro System

Abstract

Objectives/Goals
The purpose of the study is to prolong coagulation through the introduction of cholesterol-binding methyl-beta-cyclodextrin (MBCD). It is thought that MBCD, through its known prevention of the formation of lipid rafts, will inhibit platelet aggregation and plug formation.

Methods/Materials
Healthy, medication free human donors supplied the blood, which was drawn into a one-ninth volume of acid-citrate-dextrose (ACD) at a pH of 8.8. After centrifugation to separate the platelet-rich plasma (PRP), platelets were isolated from the PRP through sepharose gel filtration. A desired concentration of 3.0*10^8 platelets per ml was achieved through dilution of the platelet suspension, and 2.5 mM Thrombin Receptor-Activating peptide 1 (TRAP1) was added in a 1:1000 volume dilution to activate the platelets. To determine a standard curve for the clotting times, against which the data could be compared, 20 trials of a clotting assay were run. Likewise, to determine the curve for the clotting times, in which MBCD was introduced, 20 trials of a different chemical (drug) assay were run.

Results
The standard curve assay yielded platelet plug formation times that spanned from .533 minutes for the PNP-rich group to 6.017 minutes for the PNP-deficient group. The factor XI-deficient plasma increased the clotting times at regular intervals. At the same platelet concentration as the standard curve assay, the chemical assay yielded expected results. While the average times for the control were initially relatively disparate, the trends became similar as the concentration of MBCD increased. The greatest increase measured was from 1.983 minutes for the MBCD-free group to 9.915 minutes for the group with the highest concentration of MBCD.

Conclusions/Discussion
Results show that when MBCD is introduced into an in vitro model of platelet plug formation, coagulation times increase by as much as five-fold; on average, however, the time change for all groups was a 126% increase. The data suggests that lipid rafts are related to platelet activity, specifically aggregation, because it has been shown that inhibition of the rafts prolongs coagulation times. Further experimentation will probably determine (a) at what concentration MBCD is most effective in prolonging coagulation times, (b) how MBCD affects binding of proteins to lipid rafts, and (c) other roles lipid rafts may play in protein reception and hemostasis as a whole.

Summary Statement
The effects of the decrease in concentration of lipid rafts on the period of platelet plug formation are studied by decreasing the rafts' assembly through the introduction of the cholesterol-binding chemical methyl-beta-cyclodextrin.

Help Received
Grant funding and equipment used at the Temple University School of Medicine; advisement and consultation from Drs. Frank Baglia and Fredda London