PLENARY IV

Thrombosis

PLATELET PROSTAGLANDIN ENDOPEROXIDES FORMATION IN HYPERLIPIDEMIAS. R. Bizios, L.K. Wong, R. Vaillancourt, R.S. Lees, and A.C. Carvalho. Massachusetts Institute of Technology Arteriosclerosis Center, Cambridge, Massachusetts, U.S.A. and Cardiac and Hematology Units, Massachusetts General Hospital, Boston, Massachusetts, U.S.A.

To evaluate the role of prostaglandin endoperoxide formation in the mechanism(s) of platelet hypersensitivity in hyperlipoproteinemias, a study was undertaken in 8 type II patients, 3 type IV, and 8 normal volunteers. Platelet aggregation and release were studied in all subjects. Simultaneously, EDTA-platelets were washed by centrifugation and suspended in modified Ringer's solution. One ml suspensions ($0.8-1 \times 10^6/\mu\text{l}$) were added to $4.5 \mu\text{g}$ of ^{14}C -Arachidonic Acid (AA; sp. act. 50 mCi/mmol) and incubated for 30 sec and 5 min at 37°C . The methylated reaction products were separated by thin layer chromatography. AA, PGE_2 , $\text{PGF}_2\alpha$, and thromboxane B_2 (PHD) (Upjohn) were used to identify and locate the respective bands. The products were analyzed by mass spectrometry. TLC-plates were both scanned and scraped for radioactive counting. The chromatograms displayed a pattern similar to that reported by Samuelsson et al. Compared with normal, hypersensitive platelets of type II patients generated 25% more thromboxane A_2 (TXA_2) after 30 sec incubation with AA, while at 5 min PHD was increased by 30% and TXA_2 was still elevated by a factor of 2. The behavior of type IV platelets was similar to normal.

Aspirin-treated platelets produced one product (HETE). Among the AA derivatives studied, only eluted TXA_2 induced platelet release.

Our data suggest that platelet hyperreactivity in type II patients may be mediated by the increased production and long survival of thromboxane A_2 .

HUMAN ENDOTHELIAL CELL INJURY MECHANISMS IN VITRO. R.T. Wall, L.A. Harker, G. Striker and L. Quadracci. University of Washington School of Medicine, Seattle, Washington, U.S.A.

Since endothelial cell desquamation constitutes the initial event leading to acute and chronic vascular diseases, we have developed an in vitro system for studying injury mechanisms using cultured cells. The system involves the incubation of test material with confluent, ^{51}Cr -labeled, cultured human umbilical vein endothelial cells in 100% pooled human serum; injury was measured as % endothelial cell ^{51}Cr release (ECR) into the supernatant media. Baseline spontaneous ECR was $6.0\% \pm 1.5$ while specific rabbit antihuman endothelial cell antibody (1:64 dilution) in the presence of fresh complement released $90\% - 3$ of the total releasable ^{51}Cr activity. Consistent with in vivo predictions, homocysteine in concentrations of $0.5-40 \text{ mM}$ induced dose response ECR up to 20%. Neither methionine nor homocysteine increased ECR over controls. Also as expected from experimental work endotoxin (*S. enteritidis*) caused dose response ECR, i.e., $24\% \pm 2$ at 10 ug/ml . Control studies with cultured human smooth muscle cells (SMC) demonstrated no ECR with homocysteine but significant release occurred with endotoxin.

Specific complement dependent cytotoxic anti-endothelial cell antibody was demonstrated in the serum of two thrombotic thrombocytopenic purpura (TTP) patients at a 1:1 dilution, inducing ECR of $23.1\% - 1.4$ and $21.0\% \pm 0.25$. The antibody was also demonstrated by immunofluorescent techniques and was absorbed from the serum using human endothelial cells. One disease-free, six month survivor showed no cytotoxic activity. Serum from a patient with adult hemolytic-uremic syndrome demonstrated antibody dependent cell mediated cytotoxicity with release of $22\% \pm 1.1$ when normal non-immune lymphocytes were added to heat inactivated serum. Control studies with SMC showed no ECR with TTP sera. We conclude that assays of endothelial cell ^{51}Cr release are stable, reproducible and useful in the characterization of injury mechanisms.