

VEIN CONTRACTION AND SMOOTH MUSCLE CELL EXTENSIONS AS CAUSES OF ENDOTHELIAL SLOUGHING DURING GRAFT PREPARATION. F.G. Baumann, F.P. Catinella, J.N. Cunningham, Jr. and F.C. Spencer. Dept. of Surgery, New York Univ. Med. Ctr., N.Y. USA.

In this study light, transmission and scanning electron microscopy were used to investigate the effects of various methods of vein graft preparation on endothelial and smooth muscle cells (SMC) of the dog cephalic vein. After removal, veins were stored in one of three heparinized solutions at 10°C for 5 minutes or 1 hour: autologous blood, Plasma-Lyte or Plasma-Lyte with 0.6 mg/ml Papaverine added. The vein wall proved very sensitive to dissection, manipulation and introduction of fixative and reacted to such stimuli with a severe contraction which not only diminished the luminal diameter, but also resulted in formation of medial SMC cytoplasmic extensions and protrusion of the endothelial cells into the lumen. The SMC cytoplasmic extensions were particularly frequent in the immediate subendothelial area and seem to play a role in lifting up, separating or desquamating the endothelial cells. Such findings also are relevant to the chronic effects of arterial spasm. Among the stored veins, those soaked in blood showed the greatest vessel wall contraction and endothelial cell loss. Veins soaked in Plasma-Lyte-Papaverine had the most relaxed and normal appearance and the least endothelial cell loss. If the Papaverine treated veins were subsequently subjected to brief distension at 100 mmHg, however, large gaps appeared between the endothelial lining cells. The results suggest that Papaverine treatment greatly reduces vein graft endothelial cell loss due to contraction, but Papaverine-induced relaxation should be reversed before the graft is subjected to arterial pressure.

DOES ADP-INDUCED PLATELET AGGREGATION MEDIATE LUNG VASCULAR INJURY? A.B. Malik, F.L. Minnear, M.V. Tahamont, D.G. Moon and J.E. Kaplan. Department of Physiology, Albany Medical College, Albany, NY 12208.

We determined the effects of ADP-induced platelet aggregation on lung fluid and protein exchange to examine whether platelet aggregation mediates lung vascular injury. The studies were made in intact sheep in which pulmonary lymph was obtained, and the protein concentration of lymph was compared to that of plasma. Two groups were studied: Control sheep receiving i.v. infusion of 10 mg/kg of ADP and experimental sheep in which platelets were depleted with anti-platelet serum prior to ADP infusion. In the control group, ADP decreased the platelet count from $178,554 \pm 62,750$ to $103,500 \pm 47,828$ cells/mm³, suggesting the entrapment of platelet in the pulmonary circulation. The pulmonary arterial pressure (P_{pa}) increased from 13.1 ± 1.8 to 15.9 ± 1.2 mmHg. Lung lymph flow (\dot{Q}_{lym}) increased from 8.4 ± 1.8 to 11.4 ± 2.3 ml/hr ($p < 0.05$) and transvascular protein clearance ($\dot{Q}_{lym} \times \text{lymph/plasma protein concentration}$), a measure of protein exchange, increased from 6.7 ± 1.3 to 9.4 ± 3.0 ml/hr ($p < 0.05$). These increases could be explained by an increase in microvascular pressure (P_{mv}) and ultrafiltration since mechanically elevation of P_{mv} produced the same changes in \dot{Q}_{lym} and clearance. Platelet depletion prevented the ADP-induced increases in \dot{Q}_{lym} , clearance and P_{pa} . Thus, ADP-induced platelet aggregation does not mediate lung vascular injury, but increases fluid filtration by increasing the microvascular pressure. This effect may be mediated by release of pulmonary vasoconstrictor substances such as thromboxane A₂ and serotonin after platelet aggregation.

EFFECTS OF ASPIRIN AND SULFINPYRAZONE ON PLATELETS, VASCULAR CELLS AND THEIR INTERACTIONS. M.R. Buchanan, M.J. Vazquez and M.A. Gimbrone, Jr. Department of Pathology, Brigham and Women's Hospital, Boston, MA

Sulfinpyrazone (SUL) and aspirin (ASA) are potentially useful antithrombotic drugs. Both drugs are thought to exert this effect by inhibiting the platelet enzyme, cyclooxygenase (C-O), thus preventing thromboxane A₂ synthesis. Recent data, however, suggest that these drugs also may affect vessel wall cells. To study this further, we examined the effects of SUL and ASA on i) the adhesion of ³H-adenine-labelled washed human platelets to cultured bovine endothelial (EC) and smooth muscle cells (SMC), ii) EC and SMC DNA synthesis (³H-thymidine incorporation) and iii) cell growth. Pretreatment of platelets with 100μM ASA or 250μM SUL (concentrations sufficient to inhibit C-O), did not affect platelet adhesion to untreated EC or SMC. However, adhesion of untreated, ASA- and SUL-platelets was increased 25, 28 and 44% resp. when EC were pretreated with 650μM SUL for 24 hr. In contrast, adhesion of ASA-platelets to EC pretreated with 100μM ASA (sufficient to inhibit prostacyclin), was unaffected. Platelet adhesion to SMC pretreated with 650μM SUL for 24 hr was decreased when platelets also were pretreated with ASA (20%, $p < 0.05$) or SUL (27%, $p < 0.02$). Pretreatment of SMC with SUL for only 2 hr had no effect. DNA synthesis in EC and SMC treated with 62.5 and 250μM SUL for 24 hr, was inhibited >35% and >95% resp. Preliminary data suggest that this inhibitory effect may last longer in SMC. To study the effect of SUL on cell growth, EC and SMC were plated at 2×10^4 cells/cm² and fed with culture medium containing 0, 62.5 or 625μM SUL on day 0, 1, 3 and 4.5. EC growth rate and final density were unaffected over 7 days. SMC growth rate also was unaffected, but the final density of SMC treated with 650μM SUL was $31 \pm 2\%$ less than untreated SMC at 7 days ($p < 0.01$). These data indicate that SUL has direct effects on EC and SMC that may influence i) platelet-vessel wall interactions and ii) vascular cell proliferation.

EFFECT OF REMOVING SIALIC ACID FROM VASCULAR ENDOTHELIUM IN LIVING ANIMALS ON ITS INTERACTION WITH CIRCULATING PLATELETS. G.V.R. Born, P.Görög and Ingrid Schraufstatter. Department of Pharmacology, King's College, Strand, London WC2R 2LS, UK

The non-adherence of platelets in the circulation has been attributed mainly to two mechanisms: electrostatic repulsion due to net excess negative surface charges on platelets and endothelial cells; and release of endothelial prostaglandin I₂ (prostacyclin) which inhibits platelets. Our experiments were intended to provide evidence on the relative contribution of these mechanisms.

Rabbits were injected with ³H-5-hydroxytryptamine to label the circulating platelets. After about 2h, the rabbits were anaesthetised with nembutal. Both common carotid arteries were constricted in two places about 3 cm apart and perfused free of blood. One common carotid was perfused with saline as control and the other with neuraminidase (from Sigma or Behring Werke: 0.5 u/ml saline) for 30 min. These conditions apparently brought about maximal release of sialic acid from the endothelial surface. The constrictions were removed from the arteries to restore the blood flow. After 10 min both carotids were removed. Separate segments were prepared for determinations of radioactivity and prostaglandin I₂ and for microscopy.

In seven experiments, the effects of neuraminidase was to increase the deposition of platelets, as measured by their radioactivity, about 4-fold (mean: range 2.0 - 7.3) above controls. Neuraminidase had no significant effect on prostaglandin I₂ production, determined by bioassay and radioimmunoassay.

It is concluded that diminution in electrostatic repulsion between circulating platelets and vascular endothelium by removal of sialic acids from endothelial surface glycoproteins permits adherence of platelets irrespective of prostaglandin I₂.