Methods: Human umbilical endothelial cells were cultured to confluence and stimulated with either thrombin or histamine with or without inhibitors of protein kinases possibly involved in the regulation of eNOS activity. Phosphorylation of eNOS was analyzed with a phosphospecific eNOS(Ser1179) antibody by Western blotting.
Results: Stimulation (using short term 12-O-tetradecanoylphorbol-13-acetate (TPA) treatment) or inhibition (by GF 109203X) of protein kinase C had no effect on eNOS phosphorylation and neither did the Rho-dependent kinase inhibitorY27632, the calmodulin kinase inhibitor KN-62, the ERK-inhibitor PD98059 or the p38 inhibitor SB203580. Conversly, H89 which is an inhibitor of both protein kinase A (PKA) and the AMP-activated kinase (AMPK) strongly inhibited eNOS phosphorylation. Neither thrombin nor histamine caused a rise in endothelial cyclic-AMP and neither forskolin nor dibutyryl-cyclic-AMP affected eNOS phosphorylation contradicting the possible role of PKA in the activation pathway. On the other hand, thrombin and histamine caused phosphorylation of the AMPK downstream substances acetyl-CoA carboxylase and elongation factor-2.
Conclusion: The G-protein activators thrombin and histamine stimulate eNOS phosphorylation on Ser1179 via an Akt-independent, AMPK-dependent pathway.

1122-175 Ox-Low-Density Lipoprotein Are Potent Antigens for Dendritic Cell in Plaque Instability: Role of Lipoxygenase-1

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Experimental data identify ox-LDL has the most important antigens in atherosclerosis and plaque rupture. Recently, we identified lectin-like oxidized low-density lipoprotein receptor (LOX-1), as a susceptibility gene for myocardial infarction. Experimental data had shown that ox-LDL are a potent stimulus for maturation of monocyte derived dendritic cells (MDDC). MDDC stimulated by ox-LDL are able to activate T-lymphocyte toward a Th1 response similar to that observed during the acute phases of coronary instability.
We hypothesized that LOX-1 could be involved in ox-LDL induced maturation of MDDC and could drive the activation of immune system toward a Th1 response.
Methods: peripheral blood monocyte were collected from healthy donors and CD14+ cells were cultured in the presence of GM-CSF and IL-4 to differentiate in immature dendritic cells and stimulated at day 5 of differentiation by ox-LDL (80 micg/ml) or lipopolysaccharide (LPS) (1 ng/ml) in the presence and absence of a blocking monoclonal antibody of LOX-1 (10 micg/ml). Analysis of CD-80, HLA-DR, CD-86, CD-14, CD1a were performed by flow-cytometry at day 6. Capture of DiI labelled ox-LDL by mature dendritic cells were studied by flow-cytometry after maturation induced by LPS or ox-LDL in the presence or absence of LOX-1 antibody.
Results: Pre-treatment with LOX-1 antibody negatively modulates the differentiation of MDDC induced by ox-LDL but not by LPS. Induction of maturation induced by LPS or ox-LDL reduced the amount (mean fluorescence intensities, MFI) of DiI-labelled ox-LDL captured by dendritic cells. Pre-treatment at day 6 of maturation with LOX-1 antibody have no effect in cells stimulated with LPS, conversely in cells stimulated with ox-LDL, LOX-1 antibody reduced by 75% the binding of DiI labelled ox-LDL.
Conclusions: we identified LOX-1 as one of the major receptor involved in up-take of ox-LDL and maturation of MDDC. Moreover activation of MDDC lead to a reorganization of membrane receptor with a dominant role of LOX-1. Inhibition of dendritic cells activation induced by ox-LDL by the use of LOX-1 antibody could have important role in clinical stabilization of patients presenting with ACS.

1122-176 NAD(P)H Dependent Superoxide Production in Human Platelets: The Role of Angiotensin II and Protein Kinase C

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Background: In health Endothelial Cells (ECs) have tightly controlled pathways for the production and degradation of Superoxide ($0_2^{\cdot-}$). In hypertension there seems to be loss of this control in animal/ in vtiro studies, with an increase in $0_2^{\cdot-}$ production mainly by the NAD(P)H oxidase system. This increase in $0_2^{\cdot-}$ appears to be stimulated by a Protein Kinase C (PKC) mediated effect on Angiotensin II (AT II) acting via the AT II type 1 receptor. Platelets are known to release $0_2^{\cdot-}$.In this study we postulate that the systems responsible for $0_2^{\cdot-}$ production in ECs are also responsible in platelets making the latter an excellent moel for the study of ECs.
Method:We studied 20 patients with known essential hypertension who were off medication for 4 weeks. Platelet suspensions were prepared by sequential centrifugation and standardised assays were stimulated with AT II or Phorbol Myristate Acetate (PMA). Valsartan (an AT II type 1 receptor inhibitor), DPI/ Quinacrine (a flavoprotein oxidoreductase inhibitor) or Chelerythrine (a PKC inhibitor) were added to the platelet samples from each individual.$0_2^{\cdot-}$ was measured using the lucigenin-enhanced chemiluminescent technique.
Results: AT II and PMA stimulated platelet $0_2^{\cdot-}$ production. This was markedly reduced by Valsartan, Chelerythrine and DPI/ Quinacrine. Conclusions: AT II stimulates an increase in platelet production of $0_2^{\cdot-}$ from NAD(P)H oxidase predominantly via the AT II type 1 receptor. This is at least in part mediated by PKC activation. Platelets are thus an excellent model of ECs for the study of $0_2^{\cdot-}$ production, in terms of both ease of accessibility and similarity of systems.

POSTER SESSION

1123

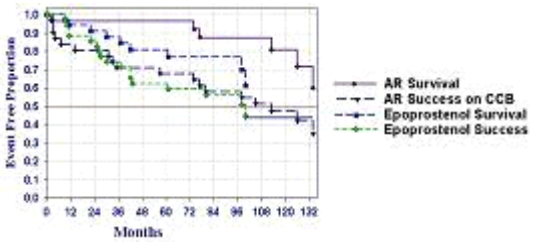
Insights Into Venous Throembolism and Pulmonary Hypertension

Tuesday, March 09, 2004, 9:00 a.m.-11:00 a.m.
Morial Convention Center, Hall G
Presentation Hour: 9:00 a.m.-10:00 a.m.

1123-177 Treatment for Children With Idiopathic Pulmonary Arterial Hypertension: Long-Term Survival and Outcomes

Delphine Yung, Greg Maislin, Allison Widlitz, Erika S. Berman Rosenzweig, Kelly Schmitt, Daniela Brady, Ellen O'Brien, Diane Kerstein, Evelyn M. Horn, Robyn J. Barst, Columbia University College of Physicians and Surgeons, New York, NY

Background: Treatment for idiopathic pulmonary arterial hypertension (IPAH) in children has been calcium channel blockers (CCB) for acute responders (AR) with vasodilator testing and chronic epoprostenol for nonresponders (NR). We sought to determine factors associated with long-term survival and treatment success, i.e. without death or transplant, and without transition to epoprostenol for patients on CCB.
Methods: A previously identified cohort of 79 patients under age 16 diagnosed with IPAH between 1982 and 1995 at Columbia University was followed through 2002.
Results: For AR, survival rates at 1, 5 and 10 years were 97%, 97%, and 81%, respectively. For AR, success rates on CCB at 1, 5 and 10 years were 84%, 68%, and 47%. Survival rates on epoprostenol at 1, 5 and 10 years were 94%, 81%, and 61%, respectively. Success rates on epoprostenol at 1, 5 and 10 years were 89%, 63%, and 45%. With multivariate analysis, baseline variables associated with success on CCB were AR status, NYHA class I, II, or III, and right atrial pressure. When AR status changed to NR status, success on CCB decreased significantly. Both survival and success on epoprostenol were only associated with age at the start of epoprostenol.
Conclusion: These findings suggest that children with IPAH who are AR should be treated with CCB therapy with transition to epoprostenol if they change to NR status. Epoprostenol therapy should be considered in patients with higher NYHA class, and listing for transplantation should be considered in older patients.



1123-178 The Lonflit Study: Evaluation of Venous Thrombosis in 5,000 Long-Haul Passengers

Belcaro Giovanni, Cesarone Maria Rosaria, Chieti University, San Valentino (Pe), Italy

The LONFLIT studies have evaluated the frequence of deep venous thrombosis (DVT) and edema in subjects flying 7-12 hours. By ultrasound femoral, popliteal, soleal veins were scanned. A global analysis was performed to evaluate the frequence of DVT in a population sample including 5000 passengers; 4922 (age range 25-69; M:F=43% vs 57%) completed the study. Subjects were divided in high, moderate and low risk for DVT (Am College of Cardiology Guidelines). Global analysis indicates the incidence of clots detected after flights: 3.25% of subjects had a clot; most subjects (93.66%) had a variable range of leg edema. An epidemiological evaluation (questionnaires) on 6448 passengers, indicates that 54% of subjects flying between 7-12 hours (age range 25-75) can be considered at low-risk, 35% at moderate and 11% at high risk. The possible incidence of DVT could be extrapolated to a number between 2 and 3% for the general flying population. Our analysis excluded younger subjects (<25) and subjects older than 75 who constitute a significant number of passengers. As 20 000 000 travellers fly every year for more than 10 hours the problem should be considered with attention. The finding small (<1 cm) vein clots, which may be spontaneously lysed in hours is an interesting observation of doubtful clinical value. Prevention with specific stockings, low-molecular weight heparin (Lovenox) and antithrombotic drugs, as shown in randomized trials reduce DVT incidence particularly in high-risk subjects.

FREQUENCE OF DVT AND EDEMA

RISK LEVEL	NUMBER	LOST	% WITH DVT	NUMBER OF DVT	% WITH EDEMA
LOW	1476	58	1.35	20	88
MODERATE	1787	61	2.5	45	93
HIGH	1659	54	5.9	98	100
TOTAL	4922	173	3.25	163	93.66