M25 INSULIN VASODILATATION IS ABOLISHED BY BOTH L-NMMA AND ANGIOTENSIN II

SJ CLELAND, JR PETRIE, S UEDA, HL ELLIOTT and JMC CONNELL

Department of Medicine and Therapeutics, University of Glasgow, Glasgow, United Kingdom

Objective: There is evidence that insulin produces vasodilatation by promoting endothelial nitric oxide release. We investigated the effect of a local insulin infusion on forearm blood flow with co-infusion of either L-NMMA (the substrate inhibitor of nitric oxide synthase), angiotensin II (ANG II) or placebo.

Design and Methods: Ten healthy male volunteers were studied on three occasions in a double-blind, random-order, placebo-controlled design. Changes in forearm blood flow ratio were assessed at ten-minute intervals using bilateral venous-occlusion strain-gauge plethysmography. After baseline readings, D-glucose (75μmol/min) was infused for 30 minutes via the brachial artery along with either L-NMMA (4pmol/min), ANG II (20pmol/min) or placebo. Insulin (5mU/min) was then co-infused for a further 90 minutes. Blood was sampled from a deep forearm vein for estimation of insulin, glucose and potassium concentrations. The Wilcoxon Signed Rank Test was used for statistical analysis.

Results: Significant forearm vasodilation occurred with L-NMMA [-40.6% (-50.8,-19.1)] and ANG II [-27.9% (-41.7, -20.9)], [median (interquartile range)]. Insulin and D-glucose infusion caused significant vasodilatation on the placebo day [30.9% (5.7, 49.4)] (p<0.05). However, insulin had no significant effect on either L-NMMA induced vasodilatation [-32.6% (-49.4,-18.5)] or ANG II induced vasodilatation [-26.2% (-35.2, -9.2)].

Conclusions: Local infusion of insulin and D-glucose causes significant forearm vasodilatation. Co-infusion of either L-NMMA or ANG II abolishes this effect. We conclude that insulin may exert its vascular effects by promoting endothelial nitric oxide release, but other mechanisms may also be involved.

M26 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ALTERS ENDOTHELIAL CELL FUNCTION

J C BROCKELSBY, R P WELLPINGS and P N BAKER

Department of Obstetrics and Gynaecology, City Hospital, Nottingham University, Nottingham NG5 1PB

Pre-eclampsia is primarily a disorder of the maternal endothelium, an as yet unidentified circulating factor, probably originating in the placenta, causes widespread endothelial activation. Plasma from patients with pre-eclampsia alters endothelial cell function (notably prostacyclin and nitric oxide production). We have recently reported that vascular endothelial growth factor (VEGF) is significantly elevated in pregnancies complicated by pre-eclampsia. VEGF is produced by the placenta and has been shown to have specific effects on endothelial cells. Our hypothesis is that VEGF may be the circulating factor, and we have investigated whether it mimics the endothelial activation induced by plasma from patients with pre-eclampsia.

The majority of studies that have addressed the question of a circulating factor have only examined activation in macrovascular human umbilical vein cells. However, pre-eclampsia is a disease of the microvasculature. We have recently reported that plasma from patients with pre-eclampsia has differential effects on microvascular compared to macrovascular endothelial cells. We then studied the effects of VEGF on a variety of endothelial cells: 1) human umbilical vein (HUVEC) 2) human dermal microvascular (HDMV) iii) human decidual microvascular (decidual) and iv) bovine microvascular (B-96). All cells were grown to confluence, quiescent for 72 hrs and then stimulated with increasing concentrations of VEGF (0-1.0 nM) for 24 hrs. Media was taken for measurement of 6-keto prostaglandin F1 alpha, stable metabolite of prostacyclin), nitrite, lactate dehydrogenase and protein was harvested from the cells.

There was a significant concentration-dependent increase in prostacyclin production (P<0.05) in HUVEC, HDMV and B-96 but not HDMV. A significant increase in the production of nitrite (P<0.05) was observed in the HDMV cell, however there was no significant increase in the other three cell types. LDH levels (a marker of cell membrane damage) were increased in all four cell types by a VEGF concentration of 1.0 nM, suggesting that the altered prostacyclin and nitric oxide production did not merely reflect membrane damage.

We wish to correct our previous claim that VEGF does not alter endothelial cell function. The cell specific nature of the result emphasizes the need to study an appropriate cell type. This data is consistent with our hypothesis that VEGF may be the circulating factor which is central to the pathogenesis of pre-eclampsia.

ACKNOWLEDGEMENTS: JCB is funded by a grant from ACTION RESEARCH. Grant No: S/P/27922

M27 ANTI-PHOSPHOLIPID AUTOANTIBODY-POSITIVE SERA INHIBIT VEIN ENDOTHELIAL SERINE-PI3K-DIDYLYSERINE DS-PHOSPHATASE RECOGNITION OF APOPTOTIC CELLS BY MACROPHAGES (Mo)

H CHURCH, S BROWN, Y BEN, S LOZIOU*, M WALPORT* and J SAVILL

Department of Medicine, University Hospital, Nottingham and *Division of Rheumatology, Royal Postgraduate Medical School

Cells preferentially die by apoptosis leading to swelling, injury-limiting and non-phlogistic clearance of intact dying cells by phagocytes. If dying cells evade phagocytosis then uncontrolled release of their contents may exacerbate tissue injury and fuel autoimmune responses in disorders such as SLE. Anti-phospholipid autoantibodies (aPL) occur in 20-40% of SLE patients and may recognize PS. Since PS is preferentially exposed by apoptotic cells and may mark them for recognition by phagocytes such as the thioglycollate-elicited murine peritoneal inflammatory Mo (MoPI), we sought evidence that aPL interacted with exposure of PS by apoptotic cells and their recognition by MoPIs.

Sera were obtained from 30 patients with SLE or primary antiphospholipid antibody syndrome (PAPS) and the presence of IgM and IgG aPL determined by the standard anticardiolipin (aCL) assay. Initially serum from a SLE patient strongly positive for IgM and IgG aCL (+ve control) was compared with serum from a SLE patient without detectable aCL (-ve control). Neat aCL -ve serum weakly inhibited MoPIs phagocytosis of human apoptotic neutrophils (APMs) to 80.2±6.7% of medium-only control (mean±SD, n=6) whereas neat aCL +ve serum strongly inhibited uptake to 5.4±3.8% of control. This effect was concentration-related; at 1 in 2 dilution aCL -ve serum had no effect but aCL +ve serum inhibited MoPIs uptake of APMs to 59±10% of control. Sera from normal individuals had no effect at any dilution. Since the aCL +ve serum (but not the aCL -ve or normal control sera) consistently exhibited concentration-dependent inhibition of the binding by Fc receptor-negative apoptotic rat mesangial cells (RMCs) of FITC-labelled annexin V assessed by flow cytometry, we studied (in observer-blind experiments) the effect of each serum from the panel upon apoptotic RMC binding of this marker of PS exposure. Sera from PAPS and aCL +ve SLE patients markedly reduced peak mean channel fluorescence of FITC-annexin V binding by 148.6±32.5 (mean±SD, n=20) at 1:20 and 98.2±48 at 1:10. However, sera from 10 aCL -ve SLE patients also inhibited FITC-annexin V binding but to a lesser extent - 101±49.3 at 1:20 and 98.2±48 at 1:10.

These preliminary data indicate that aPL detected in the aCL assay interfere with Fc-dependent recognition of apoptotic cells by phagocytes and may contribute to the pathogenesis of SLE. Moreover, sera from SLE patients negative in the aCL assay may also interfere with Fc exposure by apoptotic cells.

M28 QT DISPERSION IS RELATED TO AUTONOMIC TONE IN PATIENTS WITH CHRONIC HEART FAILURE

CE BONNAR, JR MAC FADVEN, J ROBSON, A DUNCAN and AD STRUTHERS

Department of Clinical Pharmacology, Ninewells Hospital & Medical School, University of Dundee, DD1-9SY, Scotland

BACKGROUND: A wealth of experimental evidence links the autonomic nervous system with ventricular arrhythmias and sudden death. Increased QT dispersion increases susceptibility to ventricular arrhythmias and predicts sudden death in patients with chronic heart failure (CHF). This suggests that autonomic tone may be related to QT dispersion in CHF patients.

PATIENTS: We examined this possibility in 23 patients with stable CHF: mean age (67 ± 7 yrs), 20 M, LVEF (30 ± 8 %). Patients were receiving conventional therapy for CHF and were in sinus rhythm, without bundle branch block.

METHODS: QT dispersion was measured from resting 12-lead ECG's by a single blinded observer. Native QT dispersion (QT disp), lead adjusted QT dispersion (A.QT disp) and the SD of QT intervals (SD QT) were...