POSTER SESSION

1024 Myocardial Ischemia: Basic Insights

Sunday, March 30, 2003, Noon-2:00 p.m.
McCormick Place, Hall A
Presentation Hour: Noon-1:00 p.m.

1024-106 A Functional Erythropoietin Receptor in a Rat Heart Is Linked to Anti-Apoptotic Effects

Peter van der Meer, Erik Lipsic, Rudolf A. de Boer, Rob H. Henning, Dirk J. van Veldhuisen, Wiek H. van Gilst, University of Groningen, Groningen, The Netherlands, University Hospital Groningen, Groningen, The Netherlands

Background Recent experiments show that erythropoietin (EPO) plays a protective role in brain ischemia. In this condition, the EPO receptor (EPO-R) are upregulated and administration of EPO provides protection against apoptosis. It is unknown whether the EPO-R is expressed in the heart and if EPO exerts similar beneficial effects during hypoxic stress in cardiac cells. We report the first evidence that a functional EPO-R is expressed in rat cardiac tissue and EPO plays an anti-apoptotic role. **Methods** First we studied the functionality of the EPO-R, by incubating rat cardiac tissue (n=4) for one hour with increasing doses EPO (control, 10^{-2} U/ml, 10^{-1} U/ml, and 1 U/ml) and measuring STAT-5 phosphorylation by Western Blotting. To further evaluate the effects of EPO, we induced low-flow ischemia (30 min at 1.7 ml/min) followed by 45 min of reperfusion in Langendorff perfused rat hearts. Four groups of hearts were used: control hearts (n =6), control hearts that were perfused with EPO (0.3U/ml) (n =6), hearts subjected to low flow ischemia with EPO (n=6) and without EPO administration (n=6). After the experiments, cardiac tissue was harvested for RT-PCR, immunohistochemistry and apoptosis detection, assessed by determining the Bcl-2/Bax ratio. **Results** Incubation of rat cardiac tissue with 10^{-2} U/ml, 10^{-1} U/ml and 1 U/ml EPO showed a 2.4-, 3.7-, and 4.1-fold increase in phosphorylated STAT-5 expression, respectively. Furthermore, EPO-R mRNA was detected in both normal and ischemic hearts, assessed by RT-PCR. With immunohistochemistry we found that cardiomyocytes, smooth muscle cells and endothelial cells expressed the EPO-R. Administration of EPO during ischemia and reperfusion exerted an increase in the Bcl-2/Bax ratio, compared with the non-treated group. **Conclusion** A functional EPO-R is present in rat heart as evidenced by STAT-5 phosphorylation and tissue expression. Furthermore, administration of EPO may protect cardiac cells from apoptosis following ischemia and reperfusion.

1024-107 Early Activation of Matrix Metalloproteinase After Myocardial Infarction in Rats

Jason Wollmuth, Nicholle Johnson, Steven Goldman, Mohamed A. Gaballa, University of Arizona Sarver Heart Center, Tucson, AZ, Southern Arizona VA Health Care System, Tucson, AZ

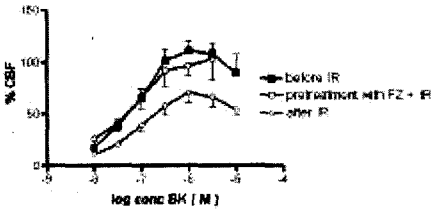
We examined MMP activation early after MI in rats. MMP zymographic activity was measured in LV myocardial extracts. Immediately after MI, there is an increased (P<0.05) in LV end-diastolic pressure (LVEDP), decrease (P<0.05) in both LV dP/dt and LV systolic pressure. Maximum increase in LVEDP and decrease in LVdP/dt, and LV systolic pressure occured at 5-minute after MI,. Myocardial MMP-2 was detected in normal control tissue (17.84±6.7 II/µg protein), and at each time point examined between immediately and 21 days after MI. The increase in MMP-2 activity reached significance at 4 min after MI (34.98±7.64 vs. 22.96 ± 4.22 II/µg protein, P<0.05). MMP-2 activity decreased at times >5 minutes after MI (24.4±4.3 vs 34.98±7.6 II/µg protein, P<0.05) and increased at 24 and 48 hrs after MI (49.7±12.9, 55.6±13.7, respectively vs.17.84±6.7, II/µg protein, P<0.05). There was another rise in MMP-2 activities at 7 days (65.8±12.7 vs. 17.8±6.7, II/µg protein, P<0.05). Finally, MMP-2 activity was decreased at 21 days after MI (65.8±12.7 vs. 55.6±13.7 II/µg protein, P<0.05) compared to MMP-2 activity at 7days after MI. MMP-9 activity was not observed in normal hearts or early after MI (<60 minutes). However, MMP-9 activity increased at 3 hours (16.98 ± 4.69 II/µg protein, P<0.05), at 24 (38.52 ± 11.0 II/µg protein, P<0.05), and at 48 hours (53.84 ± 20.59 II/µg protein, P<0.05. MMP-9 activity was absent at 7 and 21 days post MI. MMP-13 activity was not observed in normal hearts but showed a increase immediately after MI (34.15 ± 14.61 II/µg protein, P<0.05). At 2 minutes (41.79 ± 16.25 II/µg protein, P<0.05), at 4 minutes (60.46 ± 20.12 II/µg protein), and at 5 minutes (113.43 ± 45.54 II/µg protein, P<0.05) after MI. This activity, however, was not seen again at any time points after 5 minutes, except at 24 hr after MI (89.4±34.9 II/µg protein). Active MMP-3 was seen in normal heart (control, 32.5±6.4) and at each time point measured except at 7 days after MI, where it decreased (12.5±3.7 vs 32.5±6.4 II/µg protein, P<0.05). However, proMMP-3 was only seen at 7 days after MI. In conclusion, LV myocardial MMP activity increases within minutes of MI and early changes in hemodynamic may be the precipitating factor.

1024-108 Pretreatment With Folic Acid Prevents Ischemia/Reperfusion Induced Endothelial Dysfunction

An L. Moens, Marc Claeys, Johan M. Bosmans, Jean-Pierre Timmermans, Christiaan J. Vrints, University of Antwerp, Antwerp, Belgium

Background: Endothelial dysfunction is one of the components of ischemia/reperfusion(IR) injury. This study was designed to determine whether pre-treatment with folate can prevent endothelium-dependent coronary vasomotion after IR. Folate regenerates tetrahydrobiopterin, an essential cofactor of NO-synthase, lowers the homocysteine level and improves the antioxidant defence system. In patients with cardiovascular risks or with chronic coronary artery disease, folate improves endothelial function.

Methods and Results: Vasodilator responses to bradykinin , an endothelium dependent vasodilator (Bk, 50 µl , $10^{-8,5}$ to 10^{-6} M) were recorded before and after global zero-flow normotherme I/R (40/40 min.) on 14 isolated perfused rat hearts. Coronary bloodflow (CBF) changes were expressed as the percentage of baseline flow. Folate (4,5 .10^{-6}M) was added on the affluent of 7 hearts during 30 min as a pre-treatment before IR. 7 control hearts received only Krebs-solution as pre-treatment. IR caused a significant reduction of the vasodilator responses to Bk ($p<0.01$). However, after pretreatment with folate, the vasodilator respons to Bk remained unchanged after IR ($p>0.05$).



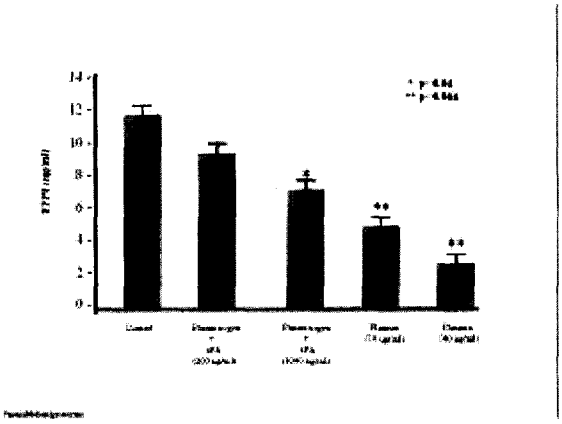
Conclusion: Pretreatment with folic acid can prevent IR-induced endothelial dysfunction. This pre-treatment mechanism represents possibly the basis for a new strategy in the therapy of patients with acute coronary syndromes.

1024-109 Plasmin-Mediated Proteolysis of Tissue Factor Pathway Inhibitor: Potential Contributing Mechanism to Rethrombosis Despite Heparin Administration Following Fibrinolytic Therapy

Richard C. Becker, YouFu Li, Frederick A. Spencer, University of Massachusetts Medical School, Worcester, MA

Background: Coronary arterial reocclusion following successful fibrinolysis occurs in 10 to 15% of patients despite heparin-based anticoagulant therapy. We have shown previously that unfractionated heparin (UFH) depletes endothelial cell tissue factor pathway inhibitor (TFPI), an important component of vascular thromboresistance. Because plasmin, the active enzyme generated from plasminogen by fibrinolytics is a non-specific protease, we determined its effect on endothelial cell TFPI.

Methods/Results: Human vascular endothelial cells (third passage) were grown to confluence on 24-well polysterene culture dishes precoated with gelatin at a density of 1.0 $X10^5$ cells per dish. The cells were incubated for two hours with varying concentrations of plasminogen, tPA and plasmin. TFPI (antigen) was measured by ELISA. All experiments were performed in triplicate. The results are summarized in the figure shown below.



Conclusions: Plasmin, a non-specific protease generated in high concentrations following fibrinolytic therapy, decreases endothelial cell surface TFPI. The combined effects of UFH and plasmin may substantially impair antiprotease activity and vascular thromboresistance, contributing to coronary arterial rethrombosis.

1024-110 Aldosterone Antagonism Improves Endothelial Dependent Vasorelaxation in Heart Failure via a Nitric Oxide Mediated Mechanism

Trunk Tran, Kent G. Meredith, Jason Wollmuth, Steven Goldman, Hoang Thai, Mohamed A. Gaballa, University of Arizona Sarver Heart Center, Tucson, AZ, Southern Arizona VA Health Care System, Tucson, AZ

Impaired nitric oxide (NO) mediated endothelial dependent vasorelaxation is significant in heart failure (HF). Clinical data demonstrate that therapies which improve NO mediated vasorelaxation by modulating the renin-angiotensin-aldosterone system also decrease mortality in subjects with HF. In the Randomized Aldactone Evaluation Study, the aldosterone antagonist, spironolactone, reduced mortality of HF patients. Our study was designed to determine if spironolactone improves NO mediated endothelial dependent vasorelaxation. Myocardial infarcted (MI) adult (8-10 weeks old) Sprague Dawley rats (n=10) in HF were treated with spironolactone (7 mg/kg/day) for 4 weeks. At the end of the treatment period, all animals underwent hemodynamic studies. The heart and tho-