330A ABSTRACTS - Myocardial Ischemia and Infarction

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

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Background Recent experiments show that erythropoietin (EPO) plays a protective role in brain ischemia. In this context, the EPO receptor (EPO-R) is upregulated and the presence of EPO is protective 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 (w/1) 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 (0.3 U/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 Bolz-Boax 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 endocardial cells expressed the EPO-R. Administration of EPO during ischemia and reperfusion exerted an increase in the Bolz-Boax 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.

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

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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 LV dp/dt and LV systolic pressures. Maximum increase in LVdP/dt and LV systolic pressure occurred at 5 minutes after MI. Myocardial MMP-2 was detected in normal control tissue (1.84±0.7 U/g protein), and at each time point examined between immediately and 21 days post MI. The increase in MMP-9 activity was significant at 7 days after MI (34.98±1.7 U/g protein, P<0.05). MMP-2 activity decreased at times >5 minutes after MI (24.4±3.3 vs. 19.7±3.1 U/g protein, P<0.05) and increased at 24 and 48 hrs after MI (49.7±12.9, 55.6±13.7, respectively vs. 17.8±6.7, 11.9±4.5 U/g protein, P<0.05). There was another rise in MMP-2 activities at 7 days (65.8±12.7 vs. 17.8±6.7, 11.9±4.5 U/g protein, P<0.05). Finally, MMP-2 activity was decreased at 21 days after MI (56.8±12.7 vs. 55.6±13.7 U/g protein, P<0.05) compared to MMP-2 activity at 7 days after MI. MMP-9 activity was not observed in normal hearts or early after MI (40±6 U/g protein, P<0.05), at 24 (38.5±6.1 U/g protein, P<0.05), at 48 hours (53.8±4.2 U/g 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 (34.1±0.1 U/g protein, P<0.05). At 2 minutes (41.9±6.25 U/g protein, P<0.05), at 4 minutes (90.4±6.12 U/g protein), and at 8 minutes (115.9±4.5 U/g protein, P<0.05) after MI. This activity, however, was not seen again at any time points after 5 minutes, except at 21 hr after MI (89.4±3.9 U/g protein). Active MMP-3 was seen in normal heart (controls: 5±5.4) and at each time point measured except at 7 days after MI, where it decreased (12.5±3.7 vs. 32.5±6.4 U/g 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 hemodynamics may be the precipitating factor.

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

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Background: Endothelial dysfunction is one of the components of ischemia/reperfusion injury. This study was designed to determine whether pretreatment with folic acid can prevent endothelium-dependent coronary vasomotion after IR. Folate receptors of P-selectin, an essential cosignaller of NO synthase, lowers the homocysteine levels 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 were measured in 14 isolated perfused rat hearts. Coronary bloodflow (CBF) changes were expressed as the percentage of baseline flow. Folic acid (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 (n=0.01). However, after pretreatment with folic acid the vasodilator responses 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 Retrombosis Despite Heparin Administration Following Fibrinolytic Therapy

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Background: Coronary arterial reclosure following successful fibrinolysis occurs in 10 to 15% of patients despite heparin-based anticoagulant therapy. We have shown previously that unfunctionalized urokinase (UHF) depletes endothelial cell tissue factor pathway inhibitor (TFPI), an important component of vascular antithrombosis. Because plasmin, the active enzyme generated from plasminogen by fibrinolysis in 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 polystyrene culture dishes precoated with gelatin at a density of 1.0 X 10^5 cells per dish. The cells were incubated for two hours with varying concentrations of plasminogen. UPA 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 UHF and plasmin may substantially impair antiprotease activity and vascular thrombore sistence, contributing to coronary artery rethrombosis.

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

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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 natriuretic-angiotensin-aldosterone system also decrease mortality in subjects with HF. In the Randomized Aldosterone 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/d) for 4 weeks. At the end of the treatment period, all animals underwent hemodynamic studies. The heart and th-