TOPIC 11 – Myocardial hypoxia, reperfusion, stroke - A

April 12th, Thursday 2012

0081
TIMP-1 produced by cardiac fibroblasts improves cardiomyocytes viability during ischemia-reperfusion

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Background: Cardiac fibroblasts (CF) are the largest cell population in the heart. They are involved in physiological and pathological conditions like heart failure and remodelling. Preliminary data obtained in our laboratory showed that conditioned medium (CM) of CF increases neonatal rat cardiomyocytes (NRC) viability after a sequence of ischemia reperfusion (I/R). In this CM several factors were identified and TIMP-1 (Tissue Inhibitor of Metalloproteinase-1) was the most highly expressed.

Hypothesis: We hypothesized that TIMP-1 was implicated in the modulation of cardioprotection against ischemia reperfusion injury.

Method: NRC were isolated from 2 days old Wistar rats and submitted to 3 hours of simulated ischemia, followed by 21 hours of simulated reperfusion. Recombinant protein TIMP-1 (0.5nM) was added into NRC medium at reperfusion. After I/R, cell viability was evaluated using MTT assay. Western blot was performed in order to detect Akt and ERK1/2 proteins and their phosphorylated forms. LY24002 and PD98059 were used as inhibitors to investigate PI3K/Akt and ERK1/2 signalling pathway respectively. For in vivo experiment, myocardial ischemia reperfusion was induced on C57Bl6 mice by coronary artery ligation during 60 minutes through a left thoracotomy. Animals (n=8/group) received 5 minutes before reperfusion an IV bolus of the drug vehicle or TIMP-1 mouse recombinant protein at the concentration of 0.75μg/kg. Area at risk and area of necrosis were evaluated after 24 hours of reperfusion.

Results: We showed that TIMP-1 increases NRC viability after I/R and induces an activation of Akt and ERK 1/2 signalling pathway. Akt and ERK 1/2 inhibitors reverse this protecting effect. In vivo experiments showed that TIMP-1 reduces infarct size by 50% vs control.

Conclusion: Our data show that TIMP-1 protects NRC against ischemia reperfusion injury by increasing viability. This cardioprotection could be mediated by an activation of Akt and ERK1/2 signalling pathways.

0418
In vivo rat acute myocardial Ischemia-Reperfusion (I/R) Injury

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Myocardial ischemia is a disease that remains highly lethal despite recent advanced medical treatment. It is caused by the sudden interruption of coronary flow by occlusion of the coronary artery, which sequentially causes irreversible cardiomyopathy, tissue loss, and scar formation.

Nitric oxide (NO) is vital for the protection against ischaemic heart disease. Arginine is up-regulated during I/R and this enzyme compete with NO synthase (NOS) for arginine, the substrate of NO.

We investigated the protective effect of N-omega-hydroxy-nor-L-arginine (nor-N0HA), the arginine blockade, whereby inhibition of arginase activity increases the bioavailability of NO by shifting utilization of the substrate arginine from arginase to NOS.

Rats were subjected to 30min of coronary artery ligation, followed by 2h of reperfusion. The validity of this model was attested by the stability of PaO₂, PaCO₂ and pH recorded before, during and after ligation.

An I.V. administration of nor-N0HA was performed before or after ligation. The efficacy of nor-N0HA was assessed by troponin levels, marker of cardiomyocyte damage, measured before and after the ligation and by infarct size.

The plasma levels of troponin were generally undetectable before the induction of ischemia, but during reperfusion, the levels of troponin was equivalent in all the ischaemic group with a tendency to decrease for nor-N0HA treatment.

Concerning infarct size, nor-N0HA, injected before ischemia, induced a significant decrease in the infarct size (-24%), expressed in function of the risk area.

In conclusion, nor-N0HA afforded a cardioprotection effect. This finding represents an important and novel mechanism in cardiac I/R injury, and could serve as a new therapeutic target in ischaemic heart disease.

0175
Cardioprotective effects of statins administered during reoxygenation is associated with anti apoptotic effects in human myocardium, in vitro

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Introduction: The importance of apoptosis in reperfusion injury is well established. We have showed that pravastatin, a statin, administered during reoxygenation induced cardioprotection (Anesth Analg 2010;110(suppl.; S423). This study investigated the effect of pravastatin during reoxygenation on the expression of markers of apoptosis in human myocardium, in vitro.

Methods: After the approval of CPP, right atrial appendages were obtained during cannulation for CPB from patients scheduled for cardiac surgery. Right atria were pinned in a chamber containing Tyrode’s solution (34°C, 1 Hz) exposed to 30min hypoxia and 60min reoxygenation (Control), pravastatin was administered throughout the reoxygenation (Prav). The protein expression of BAD, phospho-BAD, caspase 3, Pim-1 kinase and Bcl-2 were measured 15min after the start of reoxygenation and at the end of reoxygenation period (15min and 60min reox) using Western immunoblotting. Statistical comparison was made by analysis of variance.

Results: At 15min reox and 60min reox, pravastatin increase the ratio phospho-BAD (Ser112)/BAD total and the level of caspase 3 as compared to the respective Control (P<0.01). At 60min reox, pravastatin abolished the decrease of caspase 3 expression observed in Control, suggesting that pravastatin preserve the myocardium against the caspase 3 activation. At 60min reox, in Control Bcl-2 expression was decreased as compared to that observed at 15min of reoxygenation (P<0.01). At 60min reox, the level of Bcl-2 was enhanced in presence of pravastatin as compared to respective Control (P<0.01), suggesting that pravastatin abolished the decrease of Bcl-2 observed in Control. At 15min reox and 60min reox, the Pim-1 expression was enhanced in presence of pravastatin as compared to the correspondent Control (P<0.01).

Conclusions: Pravastatin induced cardioprotection is associated to the phosphorylation of BAD, the activation of Pim-1 and Bcl-2, and maintain of the caspase 3 level.
Reperfusion induced ventricular fibrillation increases cellular autophagy in mouse heart: implication of Beclin 1

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Introduction: Autophagy is a highly orchestrated cellular process by which proteins and organelles are degraded via an elaborate lysosomal pathway. In the heart, autophagy occurs at low levels under normal conditions, and during stress, dysregulation of this process is responsible for heart dysfunction and failure. Although autophagy is enhanced in various pathophysiological conditions, such as during ischemia and reperfusion (IR), whether post-ischemic ventricular fibrillation (VF) could be involved in its activation has never been challenged. Consequently the aim of this work was to study, in hearts subject to IR, alterations of activation pathways of autophagy following VF.

Methods: First a myocardial global IR on isolated, Langendorff buffer perfused heart was performed on 22 C57BL/6 mice. Autophagy activation pathways were then studies in hearts developing VF (VF, n=9) or not VF (Ctrl, n=13). In order to evaluate autophagy, LC3B, ATG-5, ATG-7, ATG-12, Bcl-2 and Beclin-1 expressions in LV tissues were evaluated using western immunoblotting.

Results and Discussion: In our experiment it has been observed that the ratio between LC3B-II and LC3B-I was drastically increased following VF pointing out an increase in autophagy by VF. This increase appears to be due to an increase in LC3B-II content without change in LC3B-I. No effect of VF was observed on the ATG pathway. Indeed, no difference was observed in the expression of ATG-7 and ATG-5 as well as in the conjugation of ATG-5 and ATG12. A main result of this study was to point out that the activation of autophagy may be due to the increase of Beclin-1 expression without any change in Bcl-2 content. Consequently the main result of this experiment is to point out that occurrence of VF during IR is responsible for an increase in cellular autophagy which is the consequence of an increase in Beclin-1 expression.