Oxygen Free Radical Scavengers Prevent Damage to Proteins of the Sarcomere Initiated by Reversible Ischemia/Reperfusion Injury

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Background: Prolonged contractile dysfunction is a characteristic feature of brief periods of ischemia with subsequent reperfusion, or myocardial stunning. It has been shown that a loss of calcium response of the myofilament may influence this loss of contractile function. In the past, myocardial stunning has also been attributed to the proteolysis of sarcromeric proteins. Such changes in contractile response have been reversed by the addition of oxygen free radical (OFR) scavengers.

Methods: Left ventricular (LV) samples were taken from rabbit hearts after either 75 min normal perfusion (control) or 15 min low flow (1 ml/min) ischemia followed by 60 min reperfusion (15I/60R) (n=6/group). A further group (n=6) underwent the 15I/60R protocol, but with the hydroxyl radical scavenger, N-(2-mercaptopropionyl) glycine (MPG, 3 mM) added to the perfusate (15I/60R+MPG). Isovolmetric LV pressure was measured throughout. Whole cell and myofilament-associated protein profiles were generated by two-dimensional gel electrophoresis and mass spectrometry utilised to identify differentially abundant sarcomeric proteins as determined by image analysis.

Results: Rate pressure product at the end of the protocol was impaired in 15I/60R (61±6% baseline) in comparison to control (90±6%, mean ± SEM; p<0.01) but was preserved (106±5% baseline) in MPG treated hearts. Comparative analysis of control and stunned myocardium revealed modifications to multiple proteins of the sarcomere (>1.5-fold difference in visible abundance). Cleavage of α-actin, a skeletal LIM domain protein as a consequence of 15I/60R was prevented by addition of MPG. The addition of MPG also abolished the removal of αMHC from the myofilament-associated fraction following 15I/60R.

Discussion: The cleavage of essential protein constituents may contribute to impaired contractile function, which is characteristic of stunning. The removal of αMHC from the myofilament may partially explain the reduced calcium response. With the addition of the OFR scavenger MPG we observed the prevention of these modifications, indicating that damage to sarcromeric proteins may be related to the presence of OH radicals during reperfusion.

Calmodulin Kinase Inhibition Improves Cardiac Function After Myocardial Infarction

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Background: Calmodulin kinase (CaMK) signaling is linked to cardiomyopathy and CaMK activity is increased in patients and animal models with cardiomyopathy from myocardial infarction (MI). Methods: We created surgical MI by ligation of the left coronary artery in transgenic (TG) mice with genetic CaMK inhibition by cardiac expression of a specific CaMK inhibitory peptide, IP. TG mice expressing an inactive scrambled peptide, CP, and wild type (WT) littermates were used as controls. There was no difference in baseline heart weight/body weight ratios or left ventricular (LV) fractional shortening (FS) in age- and gender-matched mice.

Results: 3 weeks after MI, IP TG mice had relatively preserved LV function (FS = 37.2 ± 3.1%, n = 12) compared to CP TG mice (FS = 20.6 ± 1.6%, n = 10) and WT mice (FS = 26.4 ± 2.9%, n = 11, P < 0.001) (Figure). We measured ANP expression, a marker for cardiomyopathy, from whole hearts 3 weeks post-MI using real time PCR normalized to β-actin. ANP expression was significantly increased in WT mice (26.4 ± 2.9%, n = 11, P < 0.001) compared to CP TG mice (20.6 ± 1.6%, n = 10) and WT mice (FS = 26.4 ± 2.9%, n = 11, P < 0.001). This response is significantly less in IP TG mice (10.5 ± 2.2%, n = 12) compared to CP TG mice (20.6 ± 1.6%, n = 10) and WT mice (FS = 26.4 ± 2.9%, n = 11, P < 0.001) (Figure).

Conclusions: Our results show that increased CaMK activity following MI is mechanistically linked to worsening cardiac function and that CaMK inhibition can preserve LV function after MI.
Background: We recently demonstrated that sildenafil citrate, a potent phosphodiesterase-5 inhibitor, induces a preconditioning-like effect through synthesis of nitric oxide and opening of mitochondrial KATP channels in adult rabbits. The purpose of this study was to demonstrate the effects of sildenafil on myocardial functional improvement and infarct size reduction during ischemia/reperfusion injury in infant rabbits.

Methods: 8-week-old rabbits were treated with sildenafil (0.7mg/kg IV) or saline 30 minutes prior to ischemia for 30 minutes and reperfusion for 3 hours. Transesophageal echocardiography was utilized to assess left ventricular cardiac output (LVCO) and aortic Velocity Time Integral (VTI). Sections of left ventricular myocardium were analyzed for infarct size using triphenyltetrazolium staining and computer morphometry.

Results: The sildenafil group had significant reduction in infarct size (15.5±1.1 vs 33.1±1.5 in controls, % area risk, mean±SE, n=10-15/group, p<0.05). Sildenafil treated rabbits had a 34% decline in mean arterial pressure (MAP) and a 8% increase in heart rate (HR) compared to controls after drug administration (p<0.05) but were comparable to controls prior to ischemia. The controls had a decline in LVCO and aortic VTI after ischemia (18% and 16% lower than baseline values, respectively, p<0.05), while the LVCO and aortic VTI increased in the sildenafil group (20% and 15% higher than baseline values, respectively, p<0.05). This is a statistically significant increase in LVCO and aortic VTI in the sildenafil group compared to controls (n=6/group, p<0.05).

Conclusion: For the first time, we have shown that sildenafil citrate promotes myocardial protection in infant rabbits as evidenced by preservation and even elevation in post-ischemic LVCO and aortic VTI and reduction in infarct size. This may prove to be a viable model for myocardial ischemia in pediatric cardiac surgery involving cardiopulmonary bypass, circulatory arrest, or low flow states. As such, the mechanism of cardioprotection, either through preconditioning, enhanced isotropy, or hemodynamic effects, needs further investigation.

Acute Myocardial Infarction Size Determines N-Terminal Pro-Brain Natriuretic Peptide Release as Measured by Contrast Enhanced Magnetic Resonance Imaging

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Background: Plasma N-terminal pro Brain Natriuretic Peptide (NTBNP) concentrations are elevated post Acute Coronary Syndromes (ACS) and predict left ventricular (LV) remodeling. It is unclear whether NTBNP release is due to myocardial necrosis or wall stress. We assessed the relationship between NTBNP and infarct size using rest contrast enhanced Magnetic Resonance imaging (ceMR).

Methods: 36 male and 18 female (median range) age 61-34(83) years index hospital admissions with ACS clinical treatment: 24 primary thrombolysis, 30 conservative) were consecutively recruited. Exclusions: serum creatine >200µmol/l or history of chronic renal fail, hypertension, cardiomyopathy, significant valve disease or cor pulmonale. Blood sampling for troponin I (TnI) took place at 8-12 hours and for serum creatinine and renal function: creatinine 98 (22) µmol/l. Log NTBNP was unrelated to LV ejection fraction (mean (SD) 56 (10) %, LV mass (130 (36) g), LV end diastolic (137 (35) ml) or systolic (60 (23) ml) volumes. Log NTBNP was unrelated to LV ejection fraction (mean (SD) 56 (10) %), LV mass (130 (36) g), LV end diastolic (137 (35) ml) or systolic (60 (23) ml) volumes. Log NTBNP was unrelated to LV ejection fraction (mean (SD) 56 (10) %), LV mass (130 (36) g), LV end diastolic (137 (35) ml) or systolic (60 (23) ml) volumes.

Conclusion: Haemodynamic measures: mean (SD) heart rate 54 (11) bpm, systolic BP 122 (19) mmHg, diastolic BP 76 (12) mmHg. Renal function: creatinine 98 (22) µmol/l. Log NTBNP was unrelated to LV ejection fraction (mean (SD) 56 (10) %), LV mass (130 (36) g), LV end diastolic (137 (35) ml) or systolic (60 (23) ml) volumes.

Conclusion: In patients with ACS the amount of myocardium damaged, assessed using ceMR, correlates strongly with an early increase in both plasma NTBNP and TnI. The correlation between NTBNP and TnI is not strong suggesting that there is an additional mechanism underlying its release. There is no relationship between NTBNP and LV function and dimensions.