1145 Basic Concepts in Myocardial Ischemia

Tuesday, April 01, 2003, 9:00 a.m.-11:00 a.m.
McCormick Place, Hall A
Presentation Hour: 9:00 a.m.-10:00 a.m.

Trimezidine (TMZ) was reported to protect myocardium from ischemia by mechanisms not fully understood. To better understand them, we used an ex-vivo model of global myocardial ischemia, perfused in a Langendorff apparatus. Thirty min of coronary ischemia were divided in 3 groups: A - 180 minutes of perfusion with a modified Krebs solution, B - 60 minutes of perfusion with the same solution, followed by 120 minutes of ischemia, C - as in B, but in the presence of TMZ 25 μM. Mitochondria were then isolated and used to determine the activity of complex I, II, and IV of the mitochondrial respiratory chain (MRC). Parameters evaluated were: Respiratory Control Ratio (RCR), Mitochondrial Membrane's Electrical Potential (Δ Y2), Phosphorylation Lag Phase (PLP) and Enzymatic Activities (EA) of MRC's complexes.

TMZ significantly improved the EA of MRC's complex I, resulting in higher RCR and Δ Y2 values, with no changes in PLP. In conclusion, TMZ improved the efficiency of the oxidative system, with a better preservation of the mitochondrial respiratory chain. One can assume that the activity of complex I is independent of the changes in enzyme activities of complexes II-III and IV of the mitochondrial respiratory chain (MRC). Parameters evaluated were: Respiratory Control Ratio (RCR), Mitochondrial Membrane's Electrical Potential (Δ Y2), Phosphorylation Lag Phase (PLP) and Enzymatic Activities (EA) of MRC's complexes.

Table 1: Results

<table>
<thead>
<tr>
<th>Groups/Parameters</th>
<th>Ischemic</th>
<th>TMZ</th>
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<tbody>
<tr>
<td>RCR</td>
<td>56–5%</td>
<td>101±16%*</td>
</tr>
<tr>
<td>Δ Y (Glutamate/Malate)</td>
<td>99±1%</td>
<td></td>
</tr>
<tr>
<td>EA - Complex I</td>
<td>63±5%</td>
<td>154±11%*</td>
</tr>
<tr>
<td>EA Complexes II-III</td>
<td>66±5%</td>
<td>66±4%</td>
</tr>
<tr>
<td>EA Complex IV</td>
<td>7±7%</td>
<td>7±5%</td>
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1145-190
Trimezidine-Mediated Cardioprotection during Ischemia is Mediated by Mitochondrial Respiratory Chain's Complex I Activity
Pedro Monteiro, Paulo J. Oliveira, Lino M. Goncalves, Luis A. Providencia, Basic Research Unit Coimbra University Hospital, Coimbra, Portugal

Background: The mechanisms that result in myocardium being reversibly damaged (myocardial stunning) as a consequence of brief ischemia followed by reperfusion remain unresolved. Previous studies indicate a role for oxygen free radicals (OFR) formation and intracellular calcium overload in the pathogenesis of stunning. The relatively short time course of the onset of injury suggests that modifications to key proteins may be critical to the cellular manifestation. We have utilized proteomics to investigate the molecular basis of stunning and show alterations to multiple proteins may be reversed by scavenging OFR.

Methods: Left ventricular (LV) samples were taken from rabbit hearts either after 75 min of control perfusion or 15 min low flow (1 min/mg) ischemia followed by 60 min reperfusion (15/60R). A final group underwent 15/60R, with OFR scavenger, N-(2-mercapto propionyl)-glycine (MPG, 3mM) added (n=6/group). Isovolumetric LV pressure was measured throughout 2-DE and mass spectrometry to identify differentially expressed proteins.

Results: Rate-pressure product (RPP, % baseline) at the end of the protocol was impaired in 15/60R (65±6%, p<0.01) compared to control, consistent with stunning. In contrast, RPP was preserved in the MPG protected hearts (106±9%, NS). Comparative analysis revealed 37 differences (p<0.05) in expression from 5 functional groups: mitochondrial matrix, actin-myosin, calcium signaling, cytoskeleton, and protein pool. These differences may be the result of post-translational modifications, for instance fragmentation. The modifications to myosin light chain-2, alpha actinin, and Na/K ATPase were reversed by the addition of MPG.

Conclusion: The molecular mechanism of stunning is most likely a multifactorial process, involving damage to and/or alteration of several key protein systems. This study shows that scavenging OFR results in the amelioration of some of these modifications.

1145-102
Powerful Microvascular and Myocardial Protection by Ne4+1-Exchange Inhibition and Ischemia Preconditioning: Evidence for a Causal Link in the Rabbit
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Objectives and Backgrounds: Two independent cardioprotective interventions, ischemic preconditioning (PC) and Na+/H+-exchange inhibition (isopropylamine (IPA) 1000 μg/kg), were studied with respect to their differential effects on microvascular and myocardial salvage.

Methods: Isovolumetric left ventricular (LV) samples were taken from rabbit hearts in a Langendorff model of ischemic injury (20 min) followed by reperfusion (30 min). Isolated rabbit hearts were subjected to 30 min of global ischemia and 30 min of reperfusion in the absence of glucose and oxygen, and compare coronary vascular resistance (CVR) (9.6±0.8 vs. 12.8±0.5, p<0.05). During the final 1 min of ischemia preserved coronary flow measured (0.00±0.03 vs. 1.51±0.12 at 30 min, 2.70±0.7 vs. 1.00±0.30 at 4 hr, 2.34±0.24 vs. 1.80±0.19 at 5 days, p<0.05), reduced infarct size (3±8 vs. 31±4.3 vs. 0.00±0.00) compared with control (n=11). Myocardial Contrast Echocardiography at 4 hr showed contrast defect in risk area in control but not in dV1-1. TUNEL and CD31 or a-actinin co-stain showed the evidence of dV1-1 reduced apoptosis in vascular endothelial cells and myocytes and dV1-1 reduced 50% of apoptosis.

Conclusion: Microvascular function preservation and reduced infarcted size can be