

protein). Specifically, our *in vivo* and *in vitro* data indicate that I/R and Dox markedly increase myocardial CaMKII activation in wild type but not RIP3-deficient mice, and that inhibition of CaMKII protects the heart from I/R- and Dox-induced cardiomyocyte necroptosis, cardiac remodeling and heart failure. Mechanistically, RIP3 activates CaMKII via both direct phosphorylation and indirect reactive oxidative species-dependent oxidation, and subsequently triggers opening of the mitochondrial permeability transition pore (mPTP) and myocardial necroptosis.

Conclusion: These findings identify CaMKII as a new RIP3 substrate and delineate a RIP3-CaMKII-mPTP myocardial necroptosis pathway, a promising target for the treatment of ischemia- and oxidative stress-induced myocardial damage and heart failure.

WE-028

Cardiac-protection of acetylcholine on ischemia/reperfusion injury via regulation of TNF- α /TNFR signal pathway

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Background:

Recent studies reported ischemic heart disease is accompanied by substantial withdrawal of vagal activity, and over-production of tumor necrosis factor- α (TNF- α) worsen cardiac injury. However, it is not fully clear that the replacement of ACh for myocardial ischemia/reperfusion (I/R) modulated the production of TNF- α and TNF- α receptor1/2 (TNFR1/2) signal pathway.

Methods:

Langendorff- perfused rat hearts and H9c2 cells were subjected to global ischemia and reperfusion, or hypoxia/reoxygenation, respectively. Real-time PCR, western blot, TUNEL and Si RNA were used.

Results:

1) ACh abolished hypoxia-induced up-regulation of TNF- α mRNA and protein, caspase-3 activation, and reactive oxygen species (ROS) in cardiomyocytes. ACh treatment prevented the hypoxia-induced increase in p38 MAPK and JNK phosphorylation, and increased ERK phosphorylation in H9c2 cells. Co-treatment

with atropine, a non-selective muscarinic acetylcholine receptor antagonist, or methoctramine, a selective type-2 muscarinic acetylcholine (M₂) receptor antagonist, abrogated the above effects of ACh.

2) Following Langendorff- perfused rat myocardial I/R injury, the cardiac dysfunction and myocardial infarction significantly increased and the expression of TNFR1, apoptosis signal regulating kinase 1 (ASK1) and activated caspase-8 were increased in left ventricle. Instead of TNFR1, TNFR2, Akt and ERK were not affect by I/R. Treated with ACh not only improved the cardiac function, decreased infarction area and apoptosis by TUNEL and Bcl-2/Bax, but also down-regulated the expression of TNF- α and TNFR1, and reduced the activity of ASK1 and caspase-8, finally inhibiting the cardiomyocyte apoptosis. Meantime, ACh up-regulated TNFR2 expression, Akt and ERK phosphorylation, which involved in survival pathway to protect myocardium against I/R injury.

3) Si RNA TNFR1 in H9c2 cells reduced HR-induced phosphorylation of ASK1 and caspase-3 activation. In addition, Si RNA TNFR2 eliminated ACh-increased phosphorylation of Akt and ERK after HR in H9c2 cells.

Conclusion:

ACh protected myocardium against I/R injury via inhibition TNF- α production and regulation of TNFR1/2 pathway.

WE-029

Acute hyperglycemia abolishes cardioprotection by remote ischemic preconditioning

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Background: Remote ischemic preconditioning (RIPerC) has a promising therapeutic insight to improve the prognosis of acute myocardial infarction. Chronic comorbidities such as diabetes are known to interfere with conditioning interventions by modulating cardioprotective signaling pathways, such as e.g., mTOR pathway and autophagy. However, the effect of acute hyperglycemia on RIPerC has not

been studied so far. Therefore, here we investigated the effect of acute hyperglycemia on cardioprotection by RPerC.

Methods: Wistar rats were divided into normoglycemic (NG) and acute hyperglycemic (AHG) groups. Acute hyperglycemia was induced by glucose infusion to maintain a serum glucose concentration of 15–20 mM throughout the experimental protocol. NG rats received mannitol infusion of an equal osmolality. Both groups were subdivided into an ischemic (Isch) and a RPerC group. Each group underwent reversible occlusion of the left anterior descending coronary artery (LAD) for 40 min in the presence or absence of acute hyperglycemia. After the 10-min LAD occlusion, RPerC was induced by 3 cycles of 5-min unilateral femoral artery and vein occlusion and 5-min reperfusion. After 120 min of reperfusion, infarct size was measured by triphenyltetrazolium chloride staining. To study underlying signaling mechanisms, hearts were harvested for immunoblotting after 35 min in both the NG and AHG groups.

Results: Infarct size was significantly reduced by RPerC in NG, but not in the AHG group (NG + Isch: 46.27 ± 5.31 % vs. NG + RPerC: 24.65 ± 7.45 %, $p < 0.05$; AHG + Isch: 54.19 ± 4.07 % vs. 52.76 ± 3.80 %). Acute hyperglycemia per se did not influence infarct size, but significantly increased the incidence and duration of arrhythmias. Acute hyperglycemia activated mechanistic target of rapamycin (mTOR) pathway, as it significantly increased the phosphorylation of mTOR and S6 proteins and the phosphorylation of AKT. In spite of a decreased LC3II/LC3I ratio, other markers of autophagy, such as ATG7, ULK1 phosphorylation, Beclin 1 and SQSTM1/p62, were not modulated by acute hyperglycemia. Furthermore, acute hyperglycemia significantly elevated nitrate stress in the heart (0.87 ± 0.01 vs. 0.50 ± 0.04 μg 3-nitrotyrosine/mg protein, $p < 0.05$).

Conclusions:

This is the first demonstration that acute hyperglycemia deteriorates cardioprotection by RPerC. The mechanism of this phenomenon may involve an acute hyperglycemia-induced increase in nitrate stress and activation of the mTOR pathway.

WE-030

LAPTM4b protects hearts from ischemia/reperfusion injury by promoting autophagy flux

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Myocardial injury following ischemia/reperfusion (I/R) is a common clinical scenario in patients suffering from ischemic heart disease. During myocardial I/R over-activated autophagy and accumulated autophagosome contribute to cardiomyocytes death. Thus, understanding how to accelerate autophagosome clearance and promote autophagy flux is important for the development of new cardioprotective approaches to alleviate I/R injury. Lysosome is responsible for the eventual degradation of autophagosome, however, how to promote lysosome flux via the regulation of lysosome function remains poorly understood. In the present study, we found a lysosomal membrane protein lysosomal-associated transmembrane protein 4b (LAPTM4b) was down regulated during myocardial I/R. Overexpression of LAPTM4b in neonatal rat cardiomyocytes preserved cell viability from hypoxia/reoxygenation (H/R) injury. Moreover, overexpression of LAPTM4b activated lysosomal function and promoted autophagy flux characterized by a decrease in the autophagosome of H/R myocytes, while knockdown of LAPTM4b blocked autophagy flux and aggravated cell death. We then constructed *LAPTM4b* knockout (*LAPTM4b^{-/-}*) mice by using Crispr/Cas9 system. The size of myocardial infarction/area at risk after I/R was significantly larger in *LAPTM4b^{-/-}* mice than that in wide-type mice. Our results firstly report the involvement of a lysosomal protein LAPTM4b in myocardial I/R injury through the regulation of autophagy flux and acceleration of autophagosome clearance.

Key words: LAPTM4b; autophagy flux; ischemia/reperfusion injury;

WE-031

The Role of Calcium-sensing Receptors and Spermine in Hypoxia-induced Pulmonary Vascular Remodeling and the Mechanism