

Role of the thiol redox control in myocardial ischemia-reperfusion injury

Norbert Nagy

Department of Pharmacology

In our experiments, we have been using isolated working hearts to study the role of redox-signaling mechanisms in cardioprotection.

In the first part of our research, we studied the potential role of the peroxiredoxin 6 (Prdx6) in I-R induced injury. Prdx6 is a novel peroxidase enzyme belonging to the Prdx family, which in mammals contains five more peroxiredoxins (Prdx1–Prdx5). Like glutathione peroxidase (GSHPx) and catalase, Prdx6 possesses H_2O_2 -scavenging activities, and, like the former, it also removes hydroperoxides. Since significant amounts of catalase and GSHPx are present in the heart contributing toward the attenuation of H_2O_2 and hydroperoxides formed during I-R injury and thereby providing cardioprotection, we investigated whether Prdx6 also has any role in this process. In this study we used Prdx6^{-/-} mice to assess the role of Prdx6 in ischemic injury. Western blot analysis revealed the absence of any Prdx activity in the Prdx6^{-/-} mouse heart, while the GSHPx-1 and catalase levels remained unchanged. Randomly selected hearts from Prdx6^{-/-} mice and wild-type mice were subjected to 30 min of global ischemia followed by 120 min of reperfusion at normothermia. The hearts from the Prdx6^{-/-} mice were more susceptible to ischemic reperfusion injury as evidenced by reduced recovery of left ventricular function, increased myocardial infarct size, and higher amount of apoptotic cardiomyocytes compared with wild-type mouse hearts. These Prdx6^{-/-} hearts were also subjected to a higher amount of oxidative stress as evidenced by the presence of higher amount of malondialdehyde. Our finding thus indicates a non-redundant role of Prdx6 in myocardial ischemic reperfusion injury as catalase, and GSHPx could not make up for the deficiency of Prdx6 activities.

In the second part of our experiments, we intend to determine the potential role of the glutaredoxin2 (Glxr2) in cardiac disorders. Mitochondrial Glxr2 has been recognized as an important redox regulator in mammalian organs including heart. This study examined if myocardial overexpression of Glxr2 in the heart could rescue the cardiac cells from apoptosis and necrosis induced by ischemia and reperfusion. The isolated hearts from Glxr2 transgenic mice and non-transgenic (wild type) littermates were subjected to 30 min of global ischemia followed by 2 h of reperfusion in working mode. The hearts from Glxr2 transgenic mice displayed significantly improved contractile performance and reduced myocardial infarct size and cardiomyocyte apoptosis. There was a reduction in cytochrome-*c* release and activation of caspase 3 and caspase 9. Glxr2 overexpression also reduced the ischemia/reperfusion-mediated loss of mitochondrial cardiolipin, decreased the activities of ROS and preserved GSH/GSSG ratio. Glxr2 mediated survival signal appeared to be stemmed from PI-3-kinase-Akt survival signaling pathway and involved the activation of redox sensitive transcription factor NFκB and anti-apoptotic protein Bcl-2. Our results indicate a crucial role of mitochondrial Glxr2 in cardioprotection.

In the third part of our work, we have been examining the cardioprotective abilities of PR39 gene therapy. PR-39, a proline-arginine-rich angiogenic response peptide, has been implicated in myocardial ischemic-reperfusion injury. In this study, male C57Bl/6 mice were randomized to intramyocardial injection of 10⁹ plaque forming units (p.f.u.) adenovirus encoding PR39 (PR39), FGFR1 dominant negative signaling construct (FGFR1-dn), empty vector (EV), or PR39 adenovirus plus 4 μg of plasmid encoding a HIF-1α dominant negative construct (PR39 + HIF-1α-dn). Seven days later, hearts were subjected to 20 min of ischemia and 2 h reperfusion *ex vivo* and aortic and coronary flow, left ventricular developed pressure (LVDP), and LVdp/dt were measured. Myocardial infarct (MI) size and cardiomyocyte apoptosis were measured by TTC staining and TUNEL, respectively. PR39 expression was robust up to 14 days after gene transfer and was absent after EV and FGFR1-dn. Hemodynamics showed no differences at baseline, and heart rate remained unchanged in all groups throughout the experiment. After I–R, hemodynamics remained unchanged in PR39 hearts, but deteriorated significantly in the other groups, except for aortic flow, which remained significantly higher in FGFR1-dn than in EV and PR39 + HIF-1α-dn ($p < 0.05$), although it was lower than in PR39 ($p < 0.05$). MI was $8.7 \pm 0.9\%$ in PR39, $23.8 \pm 1.1\%$ in FGFR1-dn, $29.9 \pm 2.2\%$ in EV, and $30.8 \pm 2.7\%$ in PR39 + HIF-1α-dn. In PR39, HIF-1α protein was higher than in FGFR1-dn and EV. Importantly, co-transfection of HIF1α-dn with PR39 completely abolished cardioprotection by PR39. Cardioprotection by PR39 is likely conveyed by protective metabolic and survival responses through HIF-

1 α stabilization and not by angiogenesis, because baseline coronary flow was the same in all groups. Abrogation of FGFR1 signaling conveyed an intermediate degree of cardioprotection.

In the last parts of our research, we investigated the contribution of dexamethasone treatment on the recovery of postischemic cardiac function and the development of reperfusion-induced arrhythmias in ischemic/reperfused isolated rat hearts. Electrocardiograms were monitored to determine the incidence of reperfusion-induced ventricular fibrillation. Dexamethasone pretreatment significantly reduces the occurrence of ventricular fibrillation. Cytochrome-*c* release was also observed in the cytoplasm and it was interfered with dexamethasone pretreatment. The results suggest that the inhibition of cytochrome-*c* release is involved in the dexamethasone-induced cardiac protection and actinomycin D prevented the dexamethasone-induced cardiac protection.

Keywords: Peroxiredoxin; Glutaredoxin; PR39 gene therapy; Dexamethasone; Ischemia/Reperfusion; Isolated heart