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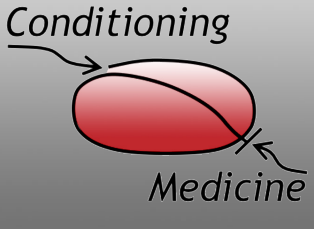

REVIEW ARTICLE | OPEN ACCESS

RISK, SAFE, and eNOS pathways in conditioning cardiac protection

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Ischemic and reperfusion injury can be reduced by cardiac preconditioning and postconditioning. The mechanisms underlying preconditioning and postconditioning protection may involve the activation of several cardioprotective survival pathways. This article reviews (1) the role of the reperfusion injury salvage kinase (RISK) pathway, survivor activating factor enhancement (SAFE) pathway, and endothelial nitric oxide synthase (eNOS) pathway in conditioning cardiac protection, (2) mechanisms by which these signaling pathways interact, and (3) the physiological consequences of these interactions. A clear understanding of the mechanisms underlying preconditioning and postconditioning protection is necessary for the development of molecular-targeted therapy with cardioprotective potential.

Introduction

The clinical treatment of acute myocardial infarction focuses on restoring coronary artery blood flow, either using pharmacologic agents (thrombolysis, anti-platelet agents) or by mechanical means (percutaneous coronary intervention or coronary artery bypass graft surgery) (Pong et al., 2014). However, reperfusion following ischemia can be associated with significant tissue damage, due to rapid changes in pH, Ca^{2+} overload, generation of reactive oxygen species, and opening of the mitochondrial permeability transition pores (Sanada et al., 2011). Ischemic and reperfusion injury can be reduced by cardiac preconditioning and postconditioning.

Paradigms for cardioprotection

It has been shown that several short cycles of non-injurious ischemia and reperfusion significantly protect from a subsequent sustained ischemic insult (Murry et al., 1986). This phenomenon was defined as "ischemic preconditioning" (IPC). The concept of IPC has further evolved into "ischemic conditioning", a broader term that encompasses a number of related endogenous cardioprotective strategies. Ischemic preconditioning is considered the most powerful intervention available to protect the heart against myocardial ischemia reperfusion injury (IRI) and has become the paradigm of choice for cardioprotection (Rossello et al., 2017).

The mechanisms underlying the effects of preconditioning and postconditioning involve the activation of cardioprotective survival pathways, including extracellular signal regulated kinases 1/2 (ERK1/2) and Akt kinase (together termed the reperfusion injury salvage kinases or RISK pathway) and the tumor necrosis factor alpha (TNFα) and signal transducer and activator 3 (STAT3) pathways (together termed the survival activating factor enhancement or SAFE pathways) (Pong et al., 2014). Both the RISK and SAFE cardioprotective pathways may activate endothelial nitric oxide synthase (eNOS), which may serve an additional signaling role in mediating postconditioning protection (Kitakaze and Hori; 1998, Liu et al., 1991; Otani, 2009).

The RISK pathway

Activation of the RISK pathway occurs during two phases, which are crucial in mediating protection – the preconditioning phase (prior to the ischemic episode, this phase is also known as the "trigger phase") (Yellon and Downey, 2003) and at the reperfusion phase (Hausenloy et al., 2017). The RISK pathway is a combination of two parallel cascades, the phosphatidylinositol-4,5-biphosphate 3-kinase (PI3K)/Akt) and MAP kinase kinase 1 (MEK1)/ERK1/2 pathways. The PI3K/Akt signaling cascade activates a series of events: the activation of PI3K results in the phosphorylation of pyruvate dehydrogenase kinase 1 (PDK1), which in turn activates Akt to subsequently recruit a wide range of pro-survival downstream targets such a glycogen synthase kinase-3 beta (GSK-3β), ribosomal protein s6 kinase (p70s6k) and eNOS (Oudit, Penninger, 2009).

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Phosphorylation of GSK-3β(Ser9), a down-stream target of Akt and ERK1/2, inhibits GSK-3β and enhances myocardial survival against ischemia-reperfusion injury (IRI) (Rossello et al., 2017). The activation of the PI3K/Akt pathway inhibits the mitochondrial permeability transition pore (MPTP) opening and leads to activation of the mitochondrial ATP-dependent potassium channel, resulting in decreased cell death (Lacerda et al., 2009). It is believed that MPTP, which opens within the first 15 min of reperfusion, is the underlying target for protection against reperfusion injury (Rossello and Yellon, 2018).

Phosphorylation of PI3K/Akt kinases is controlled by the activation of phosphatase and tensin homolog (PTEN), which is a dual lipid and protein phosphatase that antagonizes the PI3K/Akt signaling pathway (Rossello et al., 2017). Whereas PI3K activity results in an increased production of the second messenger phosphatidylinositol (3,4,5)-triphosphate (PIP3) to activate the pro-survival downstream cascade, PTEN dephosphorylates PIP3 to phosphatidylinositol 4,5-biphosphare (PIP2) to down-regulate Akt activation (Rossello et al., 2017). It has been shown that there is a reduction in the activity of PTEN following IRI in an isolated perfused rat heart model (Cai and Semenza, 2005). Other data have shown that ischemic preconditioning (IPC) induces inhibition of PTEN. It has been suggested that IPC not only activates pro-survival kinases but also inhibits their major counter regulators (Rossello et al., 2017).

It has been shown that short-term activation of these RISK kinases is protective, triggering downstream pro-survival pathways (Rossello et al., 2017), whereas long-term activation is considered to be harmful due to their growth-inducing effects and induction of cardiac hypertrophy (Nagoshi et al., 2005). The RISK pathway is activated by both IPC and postconditioning strategies (Hausenloy et al., 2017). Pharmacological agents such as growth factors, natriuretic peptides, estrogen, volatile anesthetics, and statins (Hausenloy and Yellon, 2007) show a cardioprotective potential which is linked to the involvement of the RISK pathway.

The SAFE pathway

The SAFE pathway, which involves TNFα, janus kinase (JAK), and STAT3, has been described as an alternative RISK-independent cascade mediating cardioprotective effects, which is related to STAT3 translocation to the mitochondria by modulating electron transport chain activity and inhibiting the MPTP opening (Boengler et al., 2010). Through inhibition of the MPTP opening, the SAFE pathway promotes cardiomyocyte survival (Hadebe et al., 2018). Activated STAT3, which is present in both the nucleus and the mitochondria during ischemia–reperfusion (Wegrzyn et al., 2009, Szczepanek et al., 2015), interacts with nuclear factor kappa B signaling to upregulate mitochondrial fusion by activation of optic atrophy 1 expression (Nan et al., 2017). Several publications have shown that STAT5, but not STAT3 may play a critical role for cardioprotection in humans (Davidson et al., 2012; Heusch et al., 2017). Interestingly, in children undergoing tetralogy of Fallot repair, remote IPC was associated with the activation of both STAT3 and STAT5 (Wu et al., 2017). Inhibition of STAT3 (byAG490) inhibits STAT3 phosphorylation and abolishes IPC-induced cardioprotection (Gent et al., 2017). Also, administration of a JAK/STAT3 pathway inhibitor suppresses the phosphorylation of STAT3, and results in an increased number of apoptotic cells (Xuan et al., 2001), therefore suggesting a cardioprotective role of STAT3.

eNOS

The signaling cascade based on protein kinase G and involving nitric oxide has also been proposed to mediate cardioprotection (Cohen and Downey, 2007; Rossello et al., 2018). eNOS is known to be activated by the RISK and SAFE cardioprotective pathways, and is thought to play signaling roles in mediating postconditioning protection (Kitakaze and Hori, 1998; Liu et al., 1991; Otani, 2009). Despite its well-known role as an endogenous vasodilator, a direct role for eNOS-derived NO in improving microvascular regional blood flow after postconditioning has not been demonstrated. In a recent study it was shown that eNOS knockout mice do not show any improvement from postconditioning, and myocardial contrast echocardiography replenishment curves showed more pronounced defects in reperfusion compared with wild-type mice (Pong et al., 2014). Furthermore, the finding that Akt phosphorylation is still increased in eNOS knockout mice, while postconditioning protection is not observed, suggests that eNOS activity is required for the protective effects of Akt pathway activation (Pong et al., 2014). These results suggest that effects on regional microvascular blood flow and reperfusion, mediated by eNOS, may interact with known cardioprotective mechanisms to modulate tissue outcome, and that cardioprotection may require a minimum degree of reperfusion to protect tissues.

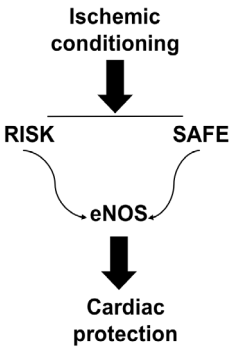

Figure 1. Schematic diagram of conditioning-induced cardiac protection. Ischemic conditioning may involve the RISK and SAFE pathways activating eNOS in promoting cardiac protection.

Conclusion

A clear understanding of the mechanisms underlying preconditioning and postconditioning is necessary for the development of molecular-targeted therapy with cardioprotective potential, which is linked to the involvement of the RISK, SAFE, and eNOS pathways. A crucial role of these pathways in cardioprotection may help uncover important therapeutic targets for the treatment of acute myocardial infarction.

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