Signaling Pathways That Protect the Heart Against Apoptosis Induced by Ischemia and Reperfusion

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Summary

Ischemia and reperfusion injury commonly occurs in ischemic heart disease, resulting in apoptotic or necrotic cell death. Apoptotic cell death is highly regulated. Two mechanisms of apoptosis involve the extrinsic death receptor pathway and the intrinsic mitochondrial pathway. Both pathways lead to the activation of effector caspases, resulting in cell death. The mitochondrial pathway plays a key role in initiating apoptosis after ischemia and reperfusion. The phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC), and extracellular signal-regulated kinase (ERK) signaling pathways protect the heart against ischemia and reperfusion injury. They inhibit mitochondrial cytochrome c release into the cytosol by regulating the Bcl-2 family proteins and activating the mitoKATP channel, thereby blocking the process of apoptosis.

Key words: Apoptosis; signal transduction; cardioprotection; ischemia and reperfusion injury; mitochondria; caspases; Bcl-2; PI3K; PKC; ERK.

1. Introduction

Ischemic heart disease is a leading cause of death in the United States. Each year millions of people suffer a heart attack, and hundreds of thousands die of acute myocardial infarction. The survivors from heart attack suffer ischemia and reperfusion injury. Cell death can occur in two different ways, necrosis and apoptosis. Necrosis is an irreversible process, leading to membrane disruption, cell swelling, and cellular debris that stimulate an inflammatory response. In contrast, apoptosis is a highly regulated form of cell death, characterized by cell shrinkage, chromatin condensation, DNA fragmentation, and organelle dismantling without an inflammatory response (1). To maintain normal heart function, cardiomyocytes need be protected against apoptosis. The loss of cardiomyocytes results in greater demands on the remaining myocytes, which have to work harder to compensate. The added stress induces further cardiomyocyte apoptosis resulting in a vicious cycle leading to congestive heart failure. Therefore, controlling the process of apoptosis is a logical strategy to prevent heart failure in patients with ischemic heart disease. This chapter provides a review of the mechanism of apoptosis during ischemia and reperfusion, then discuss signaling pathways that regulate the process of apoptosis to prevent cell death in the heart.

2. Ischemia and Reperfusion Injury

It is well known that ischemia followed by reperfusion induces damage to the heart; however, it is still controversial whether ischemia alone can cause apoptotic
cell death. Fliss and Gattinger (2) demonstrated apoptosis in ischemic rat myocardium in the absence of reperfusion. Kajstura et al. (3) reported both apoptosis and necrosis contribute to infarct size after a prolonged period of ischemia in the rat heart. Several studies (2,4,5) demonstrated that reperfusion accelerated the apoptotic cell death initiated by ischemia, but Ohno et al. (6) reported that necrotic but not apoptotic cell death was detected in the rabbit heart after coronary artery occlusion by using immunogold electron microscopy and *in situ* nick end labeling. In the rabbit myocardium, Gottlieb et al. (7) reported that apoptosis was detectable only after reperfusion. Anversa et al. (8) showed the transition from apoptosis to necrosis in the ischemic myocardium. Although apoptosis and necrosis are different mechanisms of cell death, they may share the same early events. Differences in detection methods, time points, and animal species may contribute to these different findings. Further studies are needed to determine the mechanisms of cell death induced by ischemia and ischemia-reperfusion.

3. Mechanism of Apoptosis

Apoptosis can be induced by either the intrinsic mitochondrial pathway or the extrinsic cell death receptor pathway (Fig. 1). Both pathways are activated in the heart subjected to ischemia and reperfusion. Ischemia and reperfusion increase intracellular calcium and free radicals, stimulating the intrinsic mitochondrial pathway, and also increase levels of death receptor ligands such as Fas ligand (FasL) (9,10) and tumor necrosis factor-1α (TNF-1α) in the heart (11), leading to activation of the extrinsic pathway. The mitochondria play a key role in initiating the process of apoptosis, which is regulated by Bcl-2 family proteins. Cell death is mediated by caspases (12).

4. Death Receptor Pathway

The death receptors belong to the tumor necrosis factor receptor (TNFR) gene superfamily. They are composed of an N-terminal extracellular region required for ligand binding, a single transmembrane spanning region, and an intracellular C-terminal region containing the death domain. After binding of ligands, death receptors such as Fas and the TNF-1α receptor form a homotrimeric complex, which recruits adaptor proteins that interact with the death domains, leading to the recruitment and subsequent activation of caspase-8 (13,14). The activation of caspase-8 then activates downstream effectors caspase-3 and caspase-7, executing cells by apoptosis (15,16). The death receptors are regulated by ischemia and reperfusion. Increased expression of Fas has been reported in the heart subjected to ischemia and reperfusion (4). The coronary effluent from post-ischemic isolated and perfused hearts contained significantly increased levels of FasL. Isolated hearts from Fas deficient mice had a marked reduction in cell death and infarct size following ischemia and reperfusion (17). Signaling through the TNF-1α receptor may also play a role in ischemia and reperfusion injury. Over-expression of TNF-1α in transgenic mice increased cardiac apoptosis. Increased levels of TNF-1α were found in the hearts after ischemia and reperfusion (11). The circulating levels of TNF-1α are directly related to disease severity (18), and inhibition of TNF-1α production improved heart function (19). However, several studies reported that TNF-1α may have a beneficial effect in the heart. TNF-1α pretreatment decreased lactate dehydrogenase (LDH)