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Review

# Targeting cell death in the reperfused heart: Pharmacological approaches for cardioprotection

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ABSTRACT

During acute myocardial infarction and in the reperfused heart, loss of cardiomyocytes is mostly caused by apoptosis and necrosis. As apoptosis was considered as the only form of regulated cell death for many years, initial studies investigating cardiomyocyte cell death mainly focused on direct inhibition of apoptosis. However, it has become clear that ischemic conditioning protocols – the application of alternating periods of non-lethal ischemia and reperfusion – can reduce necrotic cell death in the reperfused heart. Research on the signal-transduction pathways responsible for this phenomenon resulted in the discovery of many pharmacological targets to limit cell death after reperfusion, in which the activation of survival kinases and inhibition of mitochondrial permeability transition pore (MPTP) play an important role. Very recently, a regulated form of necrotic cell death (called 'necroptosis') was identified together with potential pharmacological inhibitors, which may also protect the myocardium from lethal reperfusion injury. This review highlights the role of apoptosis and necrosis in the reperfused hearts, including its execution and regulation and the emerging role of programmed necrosis (necroptosis). Furthermore, we will focus on the results of pharmacological interventions in experimental studies as well as relevant proof-of-concept clinical trials trying to limit apoptosis, necrosis and necroptosis in the reperfused heart. Although the list of cardioprotective compounds is promising, large multi-centre clinical trials, with enough statistical power, will be necessary to determine whether they can improve clinical outcome and can be applied in patients as adjuvant therapy next to reperfusion.



## 1. Introduction

Acute myocardial infarction (MI) remains one of the leading causes of morbidity and mortality in the western world as this leads to irreversible loss of cardiomyocytes [1]. Myocyte cell death during ischemia–reperfusion (I/R) is mainly caused by apoptosis and necrosis. Currently, the most effective therapy is early reperfusion, as infarct size is a major determinant of cardiac remodelling and prognosis after MI [2,3]. However, within minutes after the restoration of blood flow, reperfusion itself results in additional damage also known as myocardial ischemia–reperfusion (I/R) injury [4]. Based on experimental studies it has become clear that I/R injury contributes to a significant amount of cell death taking place after the onset of reperfusion, also referred to as lethal reperfusion injury [5,6]. Although numerous experimental studies have shown that both pharmacological postconditioning and ischemic postconditioning can lead to infarct size reduction, translation of these cardioprotective approaches has largely failed in the clinical setting [7–9]. Pharmacological approaches that target programmed cell death and especially the role of programmed necrosis, a relatively new level of cell death regulation, may offer novel therapeutic opportunities to limit cell death in the ischemic heart [10,11].

## 2. Cell death in the reperfused heart

Cell death can be classified based on various criteria, but the major types are apoptosis, autophagy and necrosis [12]. Where apoptosis was considered as a regulated form of cell death (*i.e.* the cell starts its suicide program), necrosis was generally seen as a passive and unregulated process resulting from externally-induced cellular injury. Autophagy on the other hand is mainly considered to be a survival mechanism by which cells recycle their proteins, lipids and organelles under energy and nutrient-deprived conditions, which may become detrimental [13]. This process is induced in the ischemic heart and seems to be cardioprotective rather than being responsible for myocyte death and therefore beyond the scope of this review. The reader is referred to a comprehensive review on the role of autophagy in cardiac disease elsewhere [14].

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Apoptosis was reported to be detectable from approximately 4 h after coronary artery occlusion and involves cardiomyocytes as well as non-cardiomyocytes [15–18]. Real-time imaging of apoptotic cell death in mice even showed that apoptosis occurs within minutes after ischemia when using Annexin-V labelling [19,20]. Ischemia–reperfusion was suggested to be a stronger stimulus for acute apoptosis than permanent occlusion [21,22], which could even be visualized in patients suffering from myocardial infarction [23]. Necrosis, in contrast, appeared at 2 h and continued to increase until 24 h after MI [15]. Therefore, the process of necrosis-induced myocardial cell death mainly takes place within the first 24 h, after which the inflammatory phase begins [24,25].

During the last decades, our view and understanding on cell death in the ischemic heart have changed markedly. As apoptosis was seen as the only regulated form of cell death for many years, genetic and pharmacological approaches targeting necrosis are relatively rare [26]. During the last two decades, a growing body of evidence clearly demonstrated that oxidative stress and its effect on mitochondria are very important in lethal reperfusion injury and ischemic cell death [27,28]. It has become clear that at least some part of this necrotic cell death can be regulated, in which opening of the mitochondrial permeability transition pore (MPTP) plays an important role [29,30]. Moreover, emerging evidence has demonstrated that the serine/threonine kinase activity of receptor-interacting protein 1 (RIP1) and its interaction with RIP3 after death receptor stimulation are necessary for programmed necrosis [31,32]. Necrostatin-1 (Nec-1), a small molecule capable of inhibiting the kinase activity of receptor interacting protein-1 (RIP1), was shown to inhibit programmed necrosis without affecting other RIP1-mediated processes and efficiently prevented necrotic cell death [33,34].

Next to irreversible loss of cardiomyocytes, another important aspect of post-MI recovery is inflammation [25,35]. Apoptosis is characterized by cell shrinkage and the formation of apoptotic bodies in order to avoid inflammation (as plasma integrity is generally maintained). In contrast, necrosis is accompanied by a gain in cell volume, rupture of the plasma membrane and loss of intracellular contents leading to a profound immune response. The possibility to target (programmed) necrosis in the ischemic heart can therefore exert beneficial effects in terms of cardioprotection and may indirectly influence the inflammatory response [36,37]. This is particularly important as inflammation-induced ROS can lead to necrotic cell death as well [38], which could explain why modulation of pro-inflammatory signals that are re-expressed post-MI can attenuate cardiac remodelling accordingly [39–42].

## 3. Apoptosis

Apoptosis is a well-defined process by which the cell undergoes cell death following a variety of different stimuli, finally resulting in the activation of a special family of death proteases known as caspases [43,44]. Over 14 different caspases have been identified, of which many are involved in regulating apoptosis. One type of caspases (*i.e.* initiator caspases) act upstream thereby initiating the apoptotic cascade, including caspase-2, -8, -9 and -10. On the other hand, caspase-3 and -7 were found to be involved in downstream signalling in the apoptosis pathway and are therefore called effector caspases.

Like in non-cardiac cells and tissues, caspases are the central players in myocardial apoptosis during pathological conditions such as myocardial infarction and heart failure [45,46]. Caspases are synthesized in an inactive form and remain present in the cytosol as pro-caspases. Once activated, initiator (upstream) caspases cleave and activate the effector caspases, caspase-3 and caspase-7. These downstream caspases then inactivate the enzyme poly ADP-ribose polymerase (PARP), cleave structural nuclear proteins and induce DNA fragmentation via endonucleases including caspase-activated DNAse (CAD) [47]. Apoptosis can be separated into two distinct pathways – the extrinsic pathway and the intrinsic pathway – both leading to the activation of downstream effector caspase-3 and -7 (Fig. 1).

### 3.1. The extrinsic pathway

The extrinsic pathway is initiated by binding of extracellular death ligands, tumour necrosis factor-α (TNF-α) and Fas-ligand, to their transmembrane death receptors present on the cell surface [48]. After binding to the death receptor, several death adaptor molecules are recruited originating from the cytoplasmic side, including TNF-receptor-associated death domain (TRADD), TNF-receptor-associated factor (TRAF) [49]. This multiprotein complex, known as the death inducing signalling complex (DISC) or Complex I, then starts binding initiator (pro)caspase-8. As more procaspase-8 will be recruited, autoactivation leads to formation of active caspase-8, thereby causing activation of effector caspases such as caspase-3 more downstream.

### 3.2. The intrinsic pathway

The intrinsic (mitochondrial) pathway is induced by a variety of extracellular and intracellular stimuli such as physical stress or oxidative stress and DNA damage. The balance between pro-apoptotic (Bid, Bax, Bak) and anti-apoptotic (Bcl-2, Bcl-xL) members of the Bcl-2 protein family is crucial for the initiation of this pathway [50]. Upon stimulation, Bax translocates to the mitochondria to form a complex with Bak on the outer mitochondrial membrane (OMM). The OMM then becomes permeabilized, leading to the release of cytochrome *c* and other pro-apoptotic proteins (apoptogens) [51]. Once in the cytosol, cytochrome *c* interacts with apoptotic protease activating factor-1 (APAF-1), forming a complex known as the apoptosome. This complex then activates caspase-9, enabling further activation of effector caspases-3/7 and thus the execution of apoptotic cell death [52]. Other apoptogens that are released upon OMM permeabilization include SMAC (second mitochondria-derived activator of caspase, also

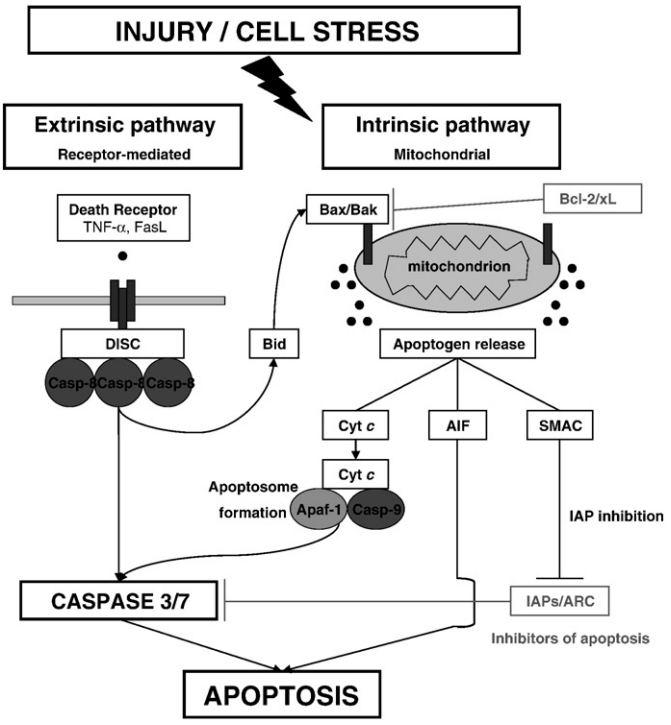


**Fig. 1.** Schematic overview of both the extrinsic (death receptor-mediated) and intrinsic (mitochondrial) pathways leading to apoptosis, as well as the cross-talk between extrinsic apoptosis via Bid cleavage by caspase-8 resulting in Bax/Bak translocation.