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Apoptosis Review Series

Don't lose heart - therapeutic value of apoptosis prevention in the treatment of cardiovascular disease

Janice L.V. Reeve [a], Angela M. Duffy [b], Timothy O'Brien [b,c], Afshin Samali [a, b, d] *

[a] *Department of Biochemistry, National University of Ireland, Galway, Ireland*
[b] *Regenerative Medicine Institute, National University of Ireland, Galway, Ireland*
[c] *Department of Medicine, National University of Ireland, Galway, Ireland*
[d] *National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland*

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Abstract

Cardiovascular disease is a leading cause of death worldwide. Loss of function or death of cardiomyocytes is a major contributing factor to these diseases. Cell death in conditions such as heart failure and myocardial infarction is associated with apoptosis. Apoptotic pathways have been well studied in non-myocytes and it is thought that similar pathways exist in cardiomyocytes. These pathways include death initiated by ligation of membrane-bound death receptors, release of pro-apoptotic factors from mitochondria or stress at the endoplasmic reticulum. The key regulators of apoptosis include inhibitors of caspases (IAPs), the Bcl-2 family of proteins, growth factors, stress proteins, calcium and oxidants. The highly organized and predictive nature of apoptotic signaling means it is amenable to manipulation. A thorough understanding of the apoptotic process would facilitate intervention at the most suitable points, alleviating myocardium decline and dysfunction. This review summarizes the mechanisms underlying apoptosis and the mediators/regulators involved in these signaling pathways. We also discuss how the potential therapeutic value of these molecules could be harnessed.

Keywords: cardiovascular disease • apoptosis • ischemia/reperfusion (I/R) • mitochondria • anti-apoptosis • therapy

* Correspondence to: Dr. Afshin SAMALI,
NCBES, National University of Ireland, Galway,
University Road, Galway city, Ireland.

Tel.: +353-91-492440
Fax: +353-91-494596
E-mail: afshin.samali@nuigalway.ie

Introduction

Cardiovascular disease (CVD), a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects. These diseases account for 17,000,000 deaths per annum worldwide. CVD remains the number one cause of death in the Western World, claiming more lives than any of the other major illnesses. It is well accepted that CVD leading to heart failure involves not only contractile dysfunction, but also cardiomyocyte death. Cell death is the ultimate result of convergence of multiple signaling pathways during CVD, triggered by events such as nutrient & oxygen deprivation, ion imbalance and excessive reactive oxygen species (ROS) production. Due to the logistical improbability of successfully hindering all of these diverse stimuli efficiently, delineation of downstream cell death pathways could provide more concisely controlled therapeutic intervention points in the prevention of CVD manifestations.

Cell death

Within tissues, cell death can occur by necrosis, apoptosis or autophagy. Necrosis is a passive, unregulated and irreversible form of cell death, occurring mainly under pathological conditions. A rapid loss of ion-flux control leads to the swelling and rupture of the cell. In contrast, apoptosis is a highly regulated, energy-dependent process involving an elaborate network of signal transduction pathways. It is characterized by caspase activation, cell shrinkage, DNA fragmentation and membrane blebbing. Autophagic cell death, as with apoptosis, is an important 'programmed cell death' associated with DNA fragmentation. Unlike apoptosis it is caspase-independent with morphology resembling necrotic death [1, 2].

Of all forms of cell death apoptosis is the best characterized and its highly regulated nature makes it an attractive target for therapeutic intervention. In general, apoptosis is induced in response to a large variety of stimuli including cytokines, cytotoxic drugs, oxidative stress and ionizing radiation.

Apoptosis in the heart

Apoptotic cell death is a fundamental process in the morphogenesis of the developing heart [3, 4]. Until recently the classical view was that necrosis was the major mode of cardiomyocyte death during CVD. However, accumulating *in vitro* and *in vivo* studies provide compelling evidence that terminally differentiated cardiomyocytes, can and do undergo apoptosis [5]. Apoptosis has important pathophysiological consequences, contributing to the loss and functional abnormalities of the myocardium. Cardiomyocyte apoptosis has been reported in a variety of cardiovascular diseases, including myocardial infarction (MI), end-stage heart failure, arrhythmogenic right ventricular dysplasia and adriamycin-induced cardiomyopathy [6]. Multiple biochemical events contribute to the development of CVD pathologies. In MI, blood flow interruptions starve the myocardium of valuable nutrients and oxygen, increasing lactate dehydrogenase production and ATP hydrolysis to create a more acidic environment. Reoxygenation of the tissue generates an oxidized environment promoting further injury. Though seemingly opposing insults, ischemia and reperfusion both result in apoptosis, with the explosion of reactive oxygen species produced during reperfusion, enhancing the rate of apoptosis and the injury initiated by ischemia [7]. Numerous patient and animal studies have shown biochemical and histological evidence of apoptosis in CVD. Animal models have been instrumental in establishing the existence of cardiomyocyte apoptosis and in the elucidation of the apoptotic mechanisms. Features of myocyte apoptosis were first reported in rabbit and rat heart models of ischemia/reperfusion (I/R) injury by *in situ* end labeling of apoptotic cardiomyocyte nuclei and characteristic DNA fragments observed on agarose gels [8, 9]. Since these pioneering studies, apoptosis has been repeatedly observed in the injured human heart through the immunohistochemical assessment of apoptotic proteins (*e.g.* Bcl-2, Bax) alongside the biochemical examination of DNA fragmentation and nuclear condensation [10–14]. Due to its sporadic occurrence and the prompt clearance of apoptotic cells by phagocytosis, apoptosis in diseased tissue is grossly underestimated.

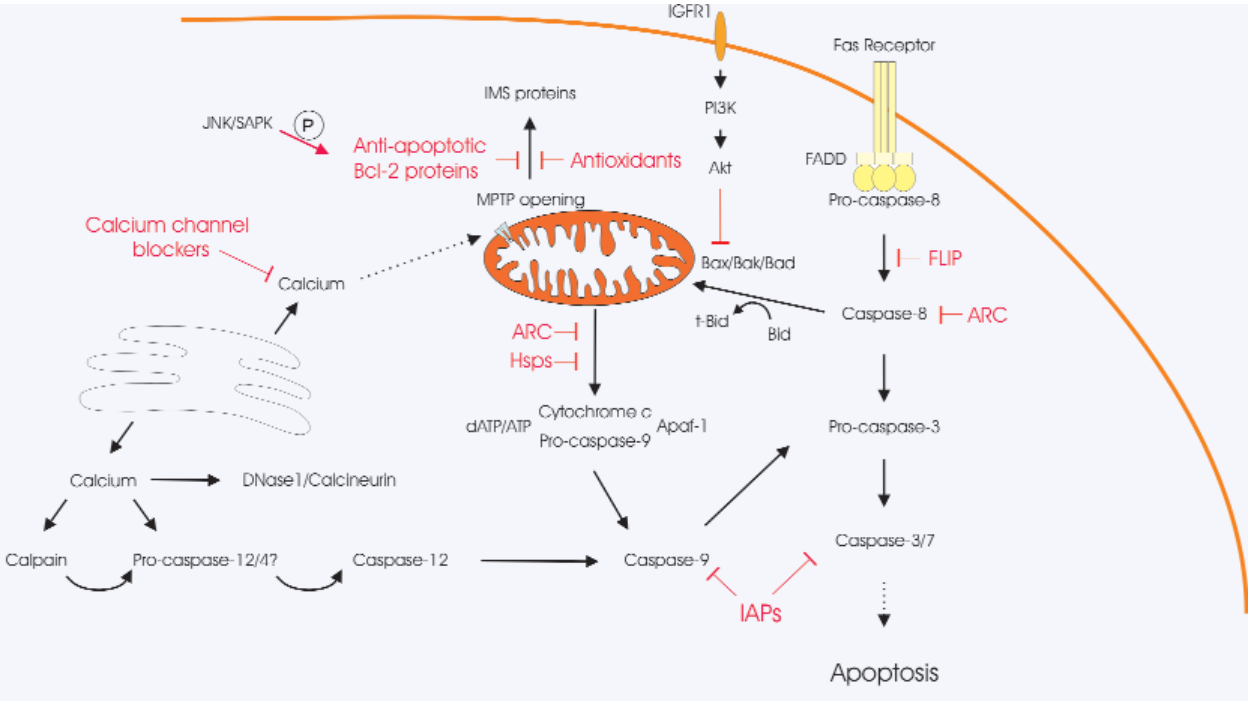

Fig. 1 Mechanisms of caspase inhibition. Caspases are processed and activated via a number of different processes. Membrane-bound death-receptor ligation, release of pro-apoptogenic factors from the mitochondria or following stress at the endoplasmic reticulum. The Bcl-2 family of proteins, stress-inducible chaperones, caspase inhibitory molecules, calcium, oxidants and growth factors modulate apoptosis. Receptor-mediated caspase activation can be inhibited upstream of pro-caspase-8 activation through FLIP recruitment to the DISC. Interaction of ARC with caspase-8 and -2 sequesters their activity. ARC can also maintain mitochondrial membrane integrity, preventing IMS protein release. IAPs exert their inhibitory action downstream through direct interaction with caspases-3, -7 and -9. Anti-apoptotic Bcl-2 proteins, which localize to the mitochondria, ER and nuclear envelope have multiple modes of action. Their protective affects may be due to their mitochondrial pore forming abilities and ion flow regulation, through their ability to heterodimerize and neutralize affects elicited by their pro-apoptotic counterparts or through MPTP protein interaction. Heat Shock Protein (Hsp) apoptosis inhibition is centered at the level of mitochondria. Hsps directly hinder apoptosome formation and effector caspase activation. The cellular antioxidant systems regulate ROS and in so doing, attenuate oxidative stress and apoptosis. Similarly, regulation of cytosolic Ca^{2+} concentration prevents mitochondrial and ER stress-induced apoptosis as well as attenuating apoptotic processes mediated by Ca^{2+} dependent enzymes.

The diverse stimuli that trigger cardiomyocyte apoptosis during CVD, do so by activating one or more signal transduction pathways, which converge to activate a conserved family of aspartic acid-specific cysteine proteases, referred to as caspases [15]. Caspases are constitutively expressed within cells as inactive zymogens and are activated in response to apoptotic stimuli by dimerization or specific proteolytic cleavage. Once activated, they orchestrate the demise of the cell through the cleavage of specific cellular substrates, resulting in the characteristic biochemical and morphological changes associated with apoptosis [2].

Caspase activation pathways

Caspase activation during apoptosis proceeds *via* a number of different pathways (Fig. 1). The extrinsic pathway involves the binding of death ligands to cell surface receptors (*e.g.* Fas/CD95/Apo-1 or TNF receptor), recruitment of adaptor molecules Fas Associated Death Domain (FADD) or TNF Receptor Associated Death Domain (TRADD) to the cytosolic end of the receptor, formation of the Death Inducing Signaling Complex (DISC) at the plasma membrane and pro-caspase-8 dimerization and activation. Once activated, caspase-8 cleaves

and activates downstream pro-caspase-3 (in Type I cells) or Bid (BH3 interacting domain death agonist), a pro-apoptotic Bcl-2 protein, which links the extrinsic and intrinsic pathways (in Type II cells).

Receptor-mediated activation of caspases is reported to occur in failing hearts [16–23]. A correlation exists between death receptor (*e.g.* Fas) expression levels and DNA fragmentation in compromised human cardiomyocytes [22, 23]. Moreover, Fas receptor-mediated apoptosis has been observed in animal models of MI and chronic heart failure (CHF) [17, 18]. The hearts of lymphoproliferative (lpr) mice, which lack a functional Fas receptor, display reduced apoptosis following I/R strengthening the role of the Fas pathway in I/R induced apoptosis [18]. Components of the death receptor-mediated apoptotic pathway are upregulated in cardiomyocytes during myocarditis, particularly in immune-mediated cardiomyopathy [24]. The cytokine, TNF-α, is also associated with the induction of apoptosis during CVD. Greater circulating concentrations of TNF-α correlate with an increasingly compromised heart [19, 21] implying that TNF-α may induce apoptosis in stressed cardiomyocytes [16, 20]. Despite an obvious role of receptor-mediated apoptosis in the heart, cardiomyocytes are typical type II cells, where apoptosis predominantly proceeds *via* the mitochondria [25].

The intrinsic pathway is initiated through the release of cytochrome *c* from the intermembrane space (IMS) of mitochondria. Cytochrome *c* translocation to the cytosol may follow a number of possible mechanisms. However, once in the cytosol, cytochrome *c* binds to Apoptosis Protease Activating Factor 1 (Apaf-1) and in the presence of dATP (or ATP) facilitates Apaf-1 oligomerization and the recruitment of pro-caspase-9. The formation of this caspase-activating complex, termed the apoptosome, results in the activation of procaspase-9 and the effector caspases including procaspase-3 [26].

There is evidence that the mitochondrial pathway is involved in ischemia-induced myocyte apoptosis in the heart [27]. Studies of myocardial I/R injury have consistently shown that mitochondrial-mediated apoptosis contributes to cardiomyocyte loss through IMS proteins released, Bcl-2 protein involvement and pro-caspase-9 activation [28–30]. Due to the magnitude of energy required by heart muscle, mitochondria are particularly abundant in myocytes. Under physiological conditions many pro-survival mechanisms exist to protect the myocardium from inappropriately triggered apoptosis. A recent report claims that neonatal cardiomyocytes are resistant to mitochondrial mediated apoptosis, due to a lack of Apaf-1 [31]. However, it is premature to consider this possibility without further experimentation.

The endoplasmic reticulum (ER) stress-induced apoptotic pathway is a more recent and controversial path to caspase activation. Originally, the pathway was thought to engage the mitochondria and induce caspase activation *via* Apaf-1. However, caspase-12 has been identified as an ER associated caspase, processed in response to chemical inducers of ER stress. Although the origin of the particular ER disturbance may differ, it activates a common signaling pathway termed the unfolded protein response (UPR). Controversy surrounding ER stress-induced apoptosis has centered on the lack of expression of caspase-12 in human cells [32]. However, the search for the functional homologues of human caspase-12 has indicated a possible role for caspase-4 in mediating apoptosis triggered by the ER [33].

The role of the ER and its more specialized form, the sarcoplasmic reticulum (SR), in cardiomyocyte apoptosis is poorly understood. Expression of protein folding chaperones, Grp78, Grp94 and calreticulin, is upregulated in cardiac samples from dilated cardiomyopathy patients [34]. Administration of doxorubicin to rat hearts induced an ER/SR-dependent mechanism of caspase activation rather than receptor- or mitochondria-mediated apoptosis [35]. Considering the immense contribution of SR-stored Ca^{2+} to heart function it seems conceivable that SR injury plays a major role in cardiomyocyte death. Following ER stress, leakage of Ca^{2+} from ER stores into the cytosol may have serious deleterious consequences. Irregular Ca^{2+} concentrations activate the Ca^{2+}- dependent protease, calpain which is thought to process caspase-12 [36].

The contribution of each caspase activation mechanism to cardiomyocyte death and the extent of cross-talk between these different apoptotic cascades has yet to be fully delineated. As cardiomyocytes have extensive ER/SR networks and excessive cytosolic Ca^{2+} is the principle trigger of Mitochondrial Permeability Transition Pore (MPTP) opening, the mitochondria and ER are

inextricably linked [37, 38]. Stress-sensors at the ER or mitochondria respond to cellular stresses and relay vital information to each other *via* Ca^{2+} signals [39]. Anti-apoptotic Bcl-2 family members (discussed in the next section) localize to the intracellular membranes of the mitochondria, ER and the nuclear envelope and are important players in this cross-talk.

The MPTP regulates mitochondrial membrane permeabilization and IMS protein release. Mitochondrial function and viability is dependent on the preservation of mitochondrial membrane potential ($\Delta\Psi m$). The MPTP is composed of the voltage gated anion channel (VDAC) on the outer mitochondrial membrane, the adenine nucleotide translocase (ANT) on the inner mitochondrial membrane and cyclophilin D in the matrix [40]. Cytosolic Ca^{2+} concentrations raised above normal physiological levels results in MPTP opening. Unlike small solutes, larger proteins cannot move freely across the inner mitochondrial membrane. An osmotic imbalance is thereby generated, causing mitochondrial swelling, outer mitochondrial membrane rupture and release of IMS factors [41]. MPTP inhibitors, cyclosporin A and sanglifehrin A, are cardioprotective [41–43] emphasizing the importance of intact mitochondria to heart function.

The mitochondrion has been identified as a central control point for the integration of diverse death signals during apoptosis. Many of the key molecules involved in apoptosis are associated with the mitochondrion. These molecules include cytochrome *c*, Smac/Diablo, Omi/HtrA2 (high temperature requirement protein A2), AIF (apoptosis-inducing factor), EndoG (Endonuclease G) and Puma. When released into the cytosol, these molecules function both in the initiation and execution of the apoptotic program. It is therefore unsurprising to find that the mitochondrion serves as a control point for cross-talk between the intrinsic, extrinsic and ER stress pathways of apoptosis.

Several studies have demonstrated the presence of active caspases in diseased cardiac tissue and apoptotic cardiomyocytes. Caspase activation has been observed in the myocardium of end-stage heart failure patients [11], cultured cardiac myocytes in models of staurosporine- [44], hypoxia [45], and serum- and glucose-deprivation-induced apoptosis [28]. In addition, caspase activation is implicated in I/R injury in the heart [7].

It appears that caspase-9 may be activated during ischemia whereas caspase-8 is activated after reperfusion in cultured cardiomyocytes and in isolated perfused hearts [46]. In addition, sustained activation of caspase-9 during reperfusion was dependent on caspase-8 mediated cleavage of Bid. Pretreatment of hearts with a caspase-8 inhibitor reduced both caspase-8 and caspase-9 activity following I/R [47]. Activation of the apoptotic pathways in the heart may lead to contractile dysfunction prior to cell death. Overexpression of caspase-3 in the heart resulted in depressed cardiac function and myofibril disruption, without triggering a full apoptotic response, whereas another report demonstrated activated caspase-3 resulted in a reduction of cardiac function by causing sarcomere disorganization. In addition, myosin light chain was identified as a substrate for caspase-3 and its cleavage in failing myocardium was associated with a reduction in myocyte contractile performance [48]. Caspase-3 cleaves cardiac myofibrillar proteins, such as α-actin, α-actinin, and troponin T [49].

Endogenous regulators of apoptosis

FLIP

Cardiomyocytes are relatively resistant to FasL despite the expression of Fas receptors. Indeed, cardiac-specific overexpression of TNF-α did not cause increased myocyte apoptosis [50], suggesting that although the death-receptor-mediated pathway could be important in certain situations (*e.g.* autoimmune-mediated heart failure), this pathway might typically be more difficult to activate in cardiomyocytes. One inhibitor of the Fas signaling pathway is the FLICE-Inhibitory Protein (FLIP), which is highly expressed in the heart under normal physiological conditions [51]. FLIP is a cytoplasmic protein, which inhibits caspase-8 recruitment and activation by Fas. It has been proposed that FLIP plays a role in the resistance to FasL in cardiomyocytes and that the enhanced sensitivity to Fas-induced apoptosis may be due to the downregulation of FLIP [52]. For instance, FLIP protein levels were decreased in the heart after I/R, as well as in neonatal myocytes after doxorubicin treatment [53]. Both conditions have been reported to make

cardiomyocytes susceptible to FasL-induced apoptosis [25, 54]. In addition, hypoxia and oxidative stress caused a downregulation of FLIP, which correlated with an increase in FasL-induced apoptosis in cardiomyocytes [52]. The presence of, cytoplasmic caspase inhibitory molecules, IAPs, also obstruct the function of active caspases -3, -7 and -9 through direct interaction [55–58]. However, SMAC/DIABLO on its release from mitochondrial IMS, binds to and inactivates IAPs, thus facilitating caspase re-activation.

Bcl-2 family of proteins

The mitochondria-mediated pathway is modulated by the Bcl-2 family of proteins. At least 18 members of the Bcl-2 family have been identified and structurally they contain at least one of four conserved Bcl-2 homology domains (BH1–BH4). The members of the family can be either anti-apoptotic or pro-apoptotic. Anti-apoptotic proteins (*e.g.* Bcl-2 and Bcl-x_L) contain BH1 and BH2 domains, with most comprised of all four motifs [59]. Pro-apoptotic Bid, Bim, Noxa and Puma contain the BH3 domain alone whereas, Bax and Bak have multiple domains [60]. Bcl-2 proteins can homo and heterodimerize. Truncated Bid triggers the release of cytochrome *c*, through interaction with the pro-apoptotic proteins Bak and Bax.

Following a death signal BH3-only-proteins are transcriptionally-induced or posttranslationally altered. Cells deficient in either Bax or Bak can initiate apoptosis following BH3-only-protein simulation; but, cells deficient in both proteins cannot induce the intrinsic pathway [61, 62]. Pro-apoptotic members interact with MPTP proteins to further enhance MPTP opening [63–65].

Apoptosis repressor with CARD (ARC)

Protective mediators such as ARC are expressed at high levels in heart and muscle [66]. ARC inhibits extrinsic death by interacting with the CARD of caspases -2 and -8 [67]. In addition, Yaniv *et al.* [52] reported that ARC expression decreases in hypoxic-cultured cardiomyocytes. Other studies have demonstrated that protection mediated by ARC may not necessarily be through the inhibition of caspases. Ekhterae *et al.* [68] demonstrated that ARC suppressed hypoxia-induced apoptosis by blocking cytochrome *c* release from the mitochondria in a caspase-independent manner. Similarly, Neuss *et al.* [66] reported that ARC inhibited oxidant stress-mediated cell death by preserving mitochondrial function independently of caspase inhibition. Furthermore, ARC has been reported to be cardioprotective where ARC delivered by TAT protein transduction into isolated rat hearts resulted in reduced infarct size after I/R [69] .

Apoptosis suppressive strategies

A diverse range of stimuli contribute to the development of CVD, culminating in cardiac cell death. Their complete eradication through therapeutic intervention is currently infeasible. However, the merits of targeting the apoptosis-associated response are clear. *In vitro* and *in vivo* experiments have demonstrated that the converging mechanistic network is amenable to control. An ideal intervention approach should target the earlier events of apoptosis, preventing the occurrence of downstream morphological changes and their associated affects on cellular and cardiac function. Current understanding of cardiac apoptosis indicates that all caspase activation mechanisms converge and act on mitochondria. Anti-apoptotic strategies developed will therefore need to protect mitochondrial membrane integrity thereby reducing apoptogenic protein release, curbing ATP depletion and regulating ROS produced. Anti-apoptosis therapies should exploit the cells endogenous defense systems or mimic their beneficial functions. Efforts in developing novel anti-apoptotic therapies have concentrated on acute conditions over the more variable and complex chronic diseases. Medications used in emergency procedures, such as MI, are much more clear-cut than the long-term medication regimes outlined for chronic CVD patients. In these instances a combination of treatment strategies will be required to improve heart condition.

Calcium modulation

Ca^{2+} channel manipulation is routinely used in CVD treatment. Ca^{2+} homeostasis is vital for heart contractility. Deregulated Ca^{2+} occurs in CVD and is prevalent in the failing heart due to altered Ca^{2+} pump expression [70]. Regulation of these channels maintains cellular Ca^{2+} concentrations, prevents prolonged MPTP opening and reduces apoptosis. The modulation of Ca^{2+} also benefits non-mitochondrial-mediated apoptotic processes. Ca^{2+} activates calpain, endonucleases and the phosphatase calcineurin, the latter of which leads to Bad dephosphorylation and intrinsically-activated apoptosis. Ultimately cytosolic Ca^{2+} concentration determines compromised cardiomyocyte fate [5, 71–73].

β adrenergic receptor 1 (β_1AR) and 2 (β_2AR) are expressed in the heart with opposing functions [74]. Ligand binding to β_1AR initiates apoptosis with β_2AR attenuating this activity. β_1AR is predominant in the injured heart and ensures that apoptosis prevails [74]. β-blockers prevent stimulation of β_1AR, and their use in patients with heart failure lengthens diastole, prevents apoptosis and improves contractility [70]. L-type Ca^{2+} channel blockers, on the other hand, prevent Ca^{2+} influx, promote blood vessel dilation, reduce myocardium contraction and decrease apoptosis [75] by attenuating cytochrome *c* release and prolonging pro-survival ERK1/2 activation [76].

Bcl-2 family

Depletion of Ca^{2+} from mitochondrial and ER stores results in apoptosis [77]. Bcl-2 enhances mitochondrial Ca^{2+} load and maintains $\Delta\Psi m$ counteracting pro-apoptotic Bcl-2 family members which decrease $\Delta\Psi m$ and deplete mitochondrial Ca^{2+}. Potentiating the effects of anti-apoptotic Bcl-2 proteins minimizes the ill-effects of pro-apoptotic members. Overexpression of Bcl-2 in non-cardiac cell lines is protective, preventing apoptosis by diverse stimuli [78]. This protection translates to the myocardium where apoptosis resistance is conferred and heart function maintained after I/R, ischemia and TNF-α treatments [79–82].

Antioxidants

ROS are important cellular signaling mediators. Oxidative stress occurs when the endogenous antioxidant defense mechanisms cannot cope with ROS clearance. Disproportionate free radicals are associated with cardiomyocyte apoptosis in aging, I/R injury and heart failure [83, 84]. Oxidant damage induces apoptosis but at higher levels, ROS, initiates necrosis through caspase inactivation and ATP depletion. Excessive ROS production causes mitochondrial damage and dysfunction [85–87]. While ischemia causes some cell death on its own, reperfusion is associated with accelerated apoptosis, resulting, in part, from a burst of ROS production within the first few minutes of reperfusion [8]. Antioxidants, which reduce oxidative stress by removing free radicals from the cell, confer protection against I/R injury. Addition of antioxidants significantly decreased apoptosis of cultured cardiomyocytes in a model of I/R [88], whereas long-term treatment with antioxidants attenuated myocyte apoptosis following MI [89]. Moreover, hearts from glutathione peroxidase null $(GSHPx)^{-/-}$ mice displayed increased levels of I/R induced apoptosis, whereas, hearts from transgenic mice overexpressing GSHPx were more resistant to I/R injury [90]. Similarly, overexpression of MnSOD (Manganese Superoxide Dismutase) reduced myocardial I/R injury in transgenic mice [91], whereas hearts from $Cu/Zn\text{-}SOD^{-/-}$ mice were more susceptible to I/R injury [92]. Vitamin E, tocopherol, is cardioprotective, an effect mediated by its antioxidant capacity [93]. The association between vitamin supplementation and an improved antioxidant defense is weak [94]; rather the benefits of vitamin consumption appear secondary to endogenous defense [95]. Improvement of these endogenous antioxidant systems or addition of exogenous compounds would improve the efficacy of ROS scavenging.

Caspase Inhibition

Though caspase activation occurs relatively late during cellular injury many studies support the beneficial effects of protease inhibition in the injured heart. Besides endogenous caspase inhibitors, IAPs, ARC and cFLIP, pharmacologically derived

inhibitors may prove more compliant. Studies to date have shown a very positive outcome for their *in vivo* use. For example, prolonged treatment with various broad range caspase inhibitors reduced cardiomyocyte apoptosis, preserved rat heart function following MI [96], reduced detrimental left ventricular (LV) remodeling [97], reduced infarct size and LV pressure in rat [98] and rabbit I/R models [99], and reduced TUNEL positivity in rat I/R hearts [100]. However, a limitation of many of these studies is a lack of follow-up to determine functional recovery of cardiomyocytes. Terminating a prolonged administration or indeed the reduced effects of a single dose of drug could have detrimental affects on the function of rescued cardiomyocytes. Also, inhibition of such a downstream event as an activated caspase cascade, may be short-lived, overshadowed by an accumulation of dysfunctional or mutant cells.

Heat Shock Proteins (Hsps)

Heat shock proteins are molecular chaperones that co-ordinate the proper folding and assembly of proteins [101]. Inducible Hsps exist, allowing for increased activity at particularly stressful times, *e.g.*, the compromised heart [102–106]. Through protein-protein interactions Hsps intercept apoptotic pathways. Non-cardiac cell lineages have shown Hsp70 prevents outer mitochondrial membrane permeabilization, Hsp27 binds cytochrome *c* [107, 108] and αB-crystallin inhibits caspase-3 processing [104]. Mitochondrial Hsp60 binds Bax and Bak in cardiomyocytes [109], reduced Hsp60 instigates apoptosis [110, 111] through Bax released [109].

There are many ways in which Hsp70 can inhibit the execution of caspase-dependent apoptosis and caspase-independent cell death. Most are centered on mitochondria-mediated events. For example, direct binding of the Apaf-1 by Hsp70 was shown to prevent apoptosome formation and caspase-9 activation in cell-free systems [112, 113]. However, recent findings suggest that inhibition of caspase activation by Hsp70 is due to interference with mitochondrial release of cytochrome *c* [108]. Although Hsp70 has been shown to be protective in a TNF-α-induced cell death model, recent studies indicate that whether or not Hsp70 is protective in a given pathway, depends on whether or not activation of the mitochondrial pathway is required [114]. Alternatively, Hsp70 may interfere with post-mitochondrial signaling, through direct binding of pro-caspases-3 and -7 [115]. In many ways, the protective effects of Hsp27 supplement those described for Hsp70. For example, while Hsp70 may prevent release of cytochrome *c* from mitochondria, phosphorylated Hsp27, through direct cytochrome *c* binding, inhibits apoptosome formation and subsequent activation of pro-caspase-9. In addition, Hsp27 has been shown to inhibit caspase-3 activity, by interacting with pro-caspase-3 and by preventing its cleavage by caspase-9 [116, 117]. At the level of mitochondria, Hsp27 may also exert its protective effect by inhibiting the release of SMAC/DIABLO [117].

Conditioning

Overexpression of these chaperones or improvement in their efficacy would enhance endogenous cardioprotective mechanisms. The beneficial effects of Hsps are reduced in compromised myocardium [118]. Hsp potency needs to be augmented in this setting by non-aggressive stimuli so as to prevent further aggravation and elicit only beneficial effects [119]. Thermotolerance experiments have shown that pre-empting of a lethal heat stress with a short sub-lethal heat challenge results in the transient expression of protective chaperones and consequently affords protection. Similarly, by exposing cells to short sub-lethal bursts of ischemia, proteins expressed give a transient protection against subsequent lethal ischemic injury [120, 121]. Unfortunately, therapeutic ischemic preconditioning is not a feasible option for human hearts. The difficulties associated with predicting ischemic susceptibility limits its utility *in vivo*. However, understanding the protective mechanisms activated would facilitate their exploitation and pharmacological augmentation.

More accessible to the surgeon, postconditioning may prove a more viable option for patients presenting with MI. Conditioning, of blood-deprived hearts, with repetitive short bursts of reperfusion, reduced infarct size following lethal reperfusion injury to levels comparable with ischemic preconditioned hearts [122–124]. Like preconditioning [125–127], postconditioning may exert its effects at

the level of the mitochondria, through an increased antioxidant capacity [123, 128, 129]. Postconditioning protects the heart through the activation of pro-survival kinases of the reperfusion injury salvage kinase (RISK) pathway [124].

Chemical or pharmacological preconditioning may at some point prove viable. Preliminary studies have shown promise. Sub-lethal doses of tunicamycin, an inhibitor of N-linked glycosylation and an ER stress inducer, increased Grp78 expression in cardiomyocytes. These pharmacologically primed cells showed protection against later insults of I/R and oxidative stress by maintaining Ca^{2+} homeostasis and reducing lactate dehydrogenase released [130]. Whether these chemical preconditioning reagents confer toxicity will ultimately decide if pre-treatment of patient hearts, genetically or environmentally predisposed to heart conditions, is feasible.

Conclusion

Accumulating evidence from *in vivo* and *in vitro* studies suggest that apoptosis plays a pivotal role in the developing heart and the progression of the pathogenesis of several cardiovascular diseases. Thus, inhibition of cardiomyocyte apoptosis may represent a novel approach for treatment of cardiac disease. Apoptosis is not the only mode of cell death in the heart and it is not clear how much apoptosis contributes to the progression of heart disease. It will also be important to determine whether inhibition of apoptosis will convert cardiomyocyte death to an alternative non-apoptotic mode. Clearly, further investigations are required to determine the importance of apoptosis and the mechanisms that control activation of the apoptotic machinery in cardiac diseases. With a thorough understanding of all of the heart's apoptotic pathways, their initiation, execution and cross-talk, it should be possible to intercept and block these cascades. Anti-apoptotic strategies, to date, have shown great potential. Effective therapies must prevent a conversion from apoptosis to necrosis and must be cardiomyocyte specific. As type II cells, maintenance of cardiomyocyte mitochondrial membrane integrity is vital. With the importance of Ca^{2+} to heart contractility, the implications of ER stress and the evidence of inter-organelle cross-talk, anti-apoptotic strategies will need to look closely at the ER and its involvement in myocyte apoptosis. Cardiac cell rescue will only be constructive if, thereafter, the cell remains functional. The sporadic execution of apoptosis may require multiple therapeutic strategies be deployed to numerous apoptotic targets. Exploitation of the cells' own anti-apoptotic systems may prove most beneficial. By improving the potency and efficacy of these mechanisms we would harness the benefits of years of evolutionary enhancement.

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