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Review on Activator and Inhibitors of Programmed Cell Death: Vital Role in Heart Diseases

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Abstract

Apoptosis of cardiomyocytes is seen in acute myocardial infarction where it may contribute to infarct size, and also in chronic heart failure, where it may be responsible for the gradual decline in cardiac function. Apoptosis of vascular smooth muscle cells is both physiological, in vessel remodelling, and pathological, in disease states such as atherosclerosis and arterial aneurysm formation. Apoptosis is regulated by both pro- and anti-apoptotic stimuli, and both activators and inhibitors of apoptosis signalling within the cell. Treatment to inhibit apoptosis in the heart can be targeted to inhibit ischaemia or reperfusion injury, to enhance endogenous protective mechanisms within cardiomyocytes, or to disrupt apoptosis signalling. The benefits of conventional heart failure treatment may be due in part to the inhibition of cardiomyocyte apoptosis.

Keywords: Apoptosis, Ischemia, Atherosclerosis, Cardiac Heart Failure.

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1. Introduction

Much has been discovered about the molecular control of apoptosis since its initial description as a series of morphological events. Apoptosis, or programmed cell death, is a process through which multicellular organisms dispose of cells efficiently. Apoptosis defines a type of cell death distinct from the more conventional necrotic death, seen classically in myocardial infarction, on the basis of characteristic morphological features. Apoptosis is frequently followed by secondary necrosis of cells, especially if there is failure of clearance or ingestion of apoptotic bodies [1]. There is a great deal of overlap between apoptosis and necrosis in morphological features and biochemical events.

Apoptosis in Heart Disease: Heart disease is the leading cause of morbidity and mortality in the developed world. Apoptosis, a highly regulated cell death process, plays an important role in numerous pathologic conditions involving the heart, and the inhibition of apoptosis is emerging as a potential therapeutic strategy.

Detection of Apoptosis

Apoptotic cells undergo a characteristic cascade of biochemical events, many of which are useful in detecting apoptotic cells. In particular, apoptotic cells expose specific membrane phospholipids that can be detected with labelled marker proteins (for example, phosphatidylserine detected with fluorescently labelled annexin V) and

cleave their DNA into specific fragments that are the basis for the enzyme linked assays to detect fragmented DNA (for example, terminal UTP nick end labelling, or TUNEL) [2]. Biochemical signalling during apoptosis, such as activation or cleavage of specific caspase enzymes can also be used on both cells and tissue samples. While helpful, the gold standard for detecting apoptosis is still based on morphology at both the light and particularly the electron microscopic level, where the features are easily distinguished.

The adult cardiomyocyte has limited (if any) ability to proliferate. Correspondingly, apoptosis is observed infrequently in adult hearts. In contrast, cardiomyocyte apoptosis plays a critical role in heart formation, such as formation of septa between cardiac chambers and valves. This evidence suggests that defects in apoptosis can result in congenital heart disease. Major foci of apoptosis include zones of fusion of the atrioventricular or bulbar cushions, and both aortic and pulmonary valves in non-myocytes. Myocyte apoptosis also occurs in the interventricular septum and right ventricular wall after birth, during the transition from fetal to adult circulations. The conducting tissue also undergoes apoptosis, and aberrant apoptosis is implicated in congenital heart block and long QT syndrome or the persistence of accessory pathways.

Apoptosis in Heart

Although the prevalence of apoptosis is very low in most forms of chronic heart failure, Apoptosis has been shown to be involved in both acute and chronic loss of cardiomyocytes in myocardial infarction, ischemic heart disease, reperfusion injury, various forms of cardiomyopathy, and the development of both acute and chronic heart failure³. Animal and human studies have demonstrated that apoptosis is present in the border zone of the infarcted myocardium in the early phase, confirming the important role of apoptosis in acute myocardial loss after myocardial infarction⁴. Apoptosis is also present months later suggesting that apoptosis may also play a role in remodeling and in the subsequent development of heart failure. Cardiomyocyte loss is the most important determinant of morbidity and mortality after myocardial infarction, preventing cardiomyocyte loss becomes a critical issue in the management of myocardial infarction. The prevalence of apoptosis is very low in most forms of CHF (usually <0.1% terminal deoxynucleotidyl transferase dUTP nick end labeling-positive cells), whether or not apoptosis significantly contributes to the pathogenesis of HF or is an epiphenomenon associated with end-stage HF is still debated [5]

2. Mechanism of Apoptosis

Over the last two decades, much work has been done to identify and elucidate the molecular mechanisms that regulate and execute apoptosis. These studies have shown that apoptosis is a tightly regulated, cell death process that involves close interactions among various pro- and anti-apoptotic molecules. It is generally agreed that apoptosis cannot be strictly identified by only one or two characteristics. In fact, a number of different types of mechanisms have been identified and characterized such as intrinsic vs. extrinsic pathway or caspase-dependent vs. caspase-independent apoptosis.

- Caspase-mediated apoptosis
- Family of cysteine proteases that cleave target proteins at specific aspartate residues
- Initiator caspases: Caspase-9 and -8
- Effector caspases: caspase-3
- Either by extrinsic (involving death receptors) or intrinsic (mitochondria mediated) pathways – Depicted through below picture- (Fig.-2)^{6,7}

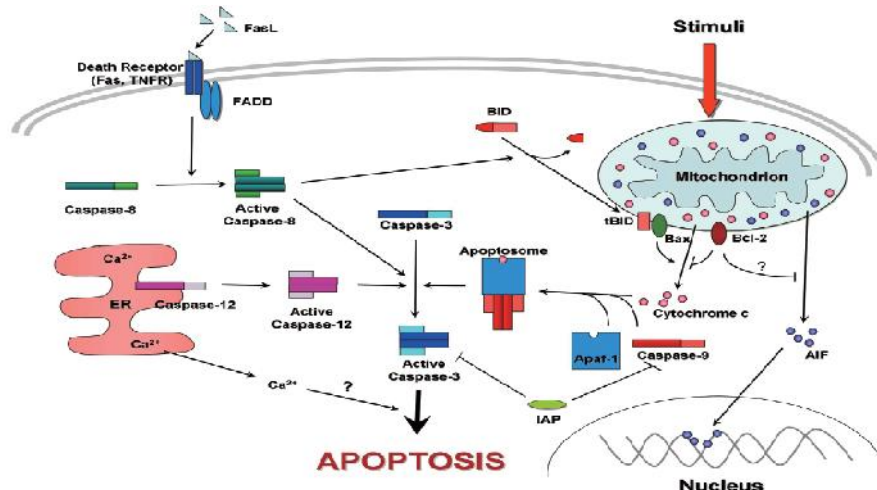


Figure 1. Mechanism of Apoptosis

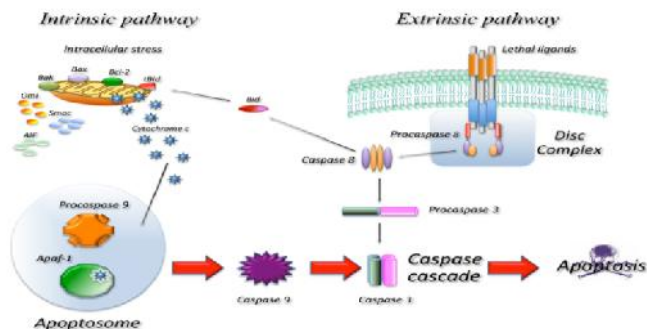


Figure 2. Intrinsic and Extrinsic Pathway [8]

Caspase Inhibitor: Caspase Inhibitor of apoptosis proteins (IAPs) are prototypical inhibitors of caspases that block caspase function, usually by directly binding to the caspases. Cardiac-specific over expression of cIAP2 reduces infarct size after I/R in isolated perfused hearts. Another important caspase inhibitor is ARC, which is found in high levels in skeletal muscle and heart. ARC interacts with upstream caspases and has been shown to block caspase-2 and -8, as well as cytochrome c release the over expression of ARC decreases infarct size after I/R [9].

Apoptosis-Inducing Factor:

AIF is a flavoprotein localized in the mitochondrial intermembrane space and is required for oxidative phosphorylation. Upon apoptotic stimulation, AIF is released into the cytosol and translocates into the nucleus to induce DNA fragmentation without caspase activation. This notion is supported by the fact that microinjection of AIF into cells induces apoptotic changes, such as chromatin condensation, that are not blocked by a caspase inhibitor (zVAD.fmk). In the heart, AIF has been implicated in apoptosis induced by oxidative stress, ischemia reperfusion, and heart failure *in vitro* and *in vivo*. AIF also accumulates in the cytosolic and nuclear fractions of the heart following I/R. Significant activation of caspase-independent apoptosis in the Dahl salt-sensitive rat model of heart failure. AIF-induced apoptosis is activated in cardiomyocytes, especially in hypertrophic cardiomyocytes AIF has also been shown to possess an essential pro-survival function. During apoptotic stimulation, the pro-apoptotic function of AIF is recognized when AIF is released from mitochondria and translocates to the nucleus, where it promotes DNA damage [10].

Non-Apoptotic Cell Death

Non-apoptotic mechanisms, such as necrosis and autophagy, are also important cell death processes in heart. Necrosis, which has often been viewed as an accidental and uncontrolled cell death process, might also be a highly orchestrated type of programmed cell death, such as apoptosis, and this subset of regulated necrosis is termed necroptosis. Unlike apoptosis or necrosis, autophagy is primarily involved in survival. Autophagy enables cells to dispose of cytoplasm and organelles by fusing vesicles containing cellular components and lysosomes¹¹. Autophagy has features resembling apoptosis, including a possible association with the caspases and Bcl-2. That there is considerable controversy at present around differentiating the various types of cell death. There may be a spectrum of different mechanisms, and which mode of cell death predominates depends on the specific type of apoptotic stimuli, the degree of insult, and the intracellular ATP concentration. These are important and controversial issues at this time, and further studies are needed to clarify these modes of cell death [12].

Table.1

Apoptosis	Necrosis
Condensation /Clumping of nuclear chromatin	Nuclear Chromatin non-specific degraded
Loss of cell-cell contact, cell shrinkage, and fragmentation, with formation of membrane bound process and vesicles containing fragments of nuclear material or organelles	Cell volume increases
Adjacent cells phagocytes the end product, the apoptotic body	
Minimal disruption of cell membranes or release of lysosomal enzymes, with consequently little inflammatory reaction	Cell membrane integrity lost early, release of lysosomal enzymes and subsequent inflammation.
Organelle structure and function maintained until late into the process	Organelle structure and function lost early



Diseases in which Apoptosis has Been Implicated

Cardiac (myocyte)

- a. Idiopathic dilated cardiomyopathy
- b. Ischaemic cardiomyopathy
- c. Acute myocardial infarction
- d. Arrhythmogenic right ventricular dysplasia
- e. Myocarditis

Cardiac (conducting tissues)

- a. pre-excitation syndromes
- b. heart block,
- c. congenital complete atrioventricular heart block,
- d. long QT syndromes

Vascular

- a. Atherosclerosis
- b. Restenosis after angioplasty/stenting
- c. Vascular graft rejection
- d. Arterial aneurysm formation

Apoptosis in Ischaemia/Infarction

Ischaemia is a potent inducer of both myocyte necrosis and apoptosis in vitro. Thus, deprivation of oxygen alone, or in addition to serum withdrawal and deprivation of glucose, induces neonatal myocyte apoptosis. Ischaemia alone can induce apoptosis in the ischaemic territory in vivo, and this may be reduced by reperfusion. However, although reperfusion may limit ischaemia induced apoptosis, reperfusion itself may accelerate the appearance of apoptosis in the reperfused regions.

Myocardial infarction has been considered to be a prime example of necrotic cell death, because of the breakdown of cellular energy metabolism. However, apoptosis of cardiomyocytes also occurs in a temporally and spatially specific manner. Thus, acute myocardial infarction manifests both forms of cell death with apoptosis particularly occurring at the hypoperfused “border” zones, between a central area of necrosis and viable myocardium. The central, unperfused region also manifests apoptosis, particularly within the first six hours, although between 6–24 hours necrosis is more common. Apoptosis in the remote non-infarcted myocardium may be partly responsible for myocardial remodelling and dilatation after myocardial infarction, and may be amenable to treatment [13].

- a. Ischaemia is a potent inducer of both myocyte necrosis and apoptosis
- b. Reperfusion itself may accelerate the appearance of apoptosis in the reperfused regions
- c. Acute myocardial infarction manifests both forms of cell death, with apoptosis particularly occurring at the hypoperfused “border” zones, between a central area of necrosis and viable myocardium
- d. The central, unperfused region also manifests apoptosis, particularly within the first six hours
- e. Responsible for myocardial remodelling and dilatation after myocardial infarction, and may be amenable to treatment¹³

Apoptosis in Heart Failure

The finding that cardiomyocyte apoptosis occurs in the end stage human heart indicates that apoptosis may contribute to heart failure in a variety of situations. Aging is associated with myocardial cell loss, and cardiomyocyte apoptosis may be the mechanism of the gradual deterioration in cardiac function. In humans undergoing transplantation, apoptosis can be observed, with some studies suggesting higher levels in ischaemic versus idiopathic dilated cardiomyopathy [14].

- a. Cardiomyocyte apoptosis occurs in the end stage human heart indicates that apoptosis may contribute to heart failure in a variety of situations
- b. Aging leads to myocardial cell loss, and
- c. Cardiomyocyte apoptosis : mechanism of the gradual deterioration in cardiac function.
- d. In humans undergoing transplantation, apoptosis can be observed, with some studies suggesting higher levels in ischaemic versus idiopathic dilated cardiomyopathy [14].
- e. The transition from compensated to decompensated hypertrophy is also associated with myocyte apoptosis in animals, and
- f. High levels of apoptosis are seen in arrhythmogenic right ventricular dysplasia, a condition characterised by myocardial replacement with fibrofatty material [15].
- g. There is increasing evidence that toxic cardiomyopathies, such as that induced by doxorubicin (Adriamycin), are associated with cardiomyocyte apoptosis.
- h. Although the evidence that apoptosis promotes heart failure is persuasive, the present problem is defining by what extent. Toxic cardiomyopathies, (Adriamycin): cardiomyocyte apoptosis.



- i. Vastly different rates of apoptosis have been reported in both human and animal heart failure, with rates of up to 35.5% [15].
- j. Death rates may be seen only in very localised areas, given that apoptosis takes less than 24 hours to complete, such rates would result in rapid involution of the heart.
- k. Recently, rates of < 0.5% have been consistently reported in end stage HF, which make far more physiological sense.
- l. In addition, in end stage HF necrosis is still (up to seven times) more frequent than apoptosis [15].

Apoptosis in the Vessel Wall

- a. Vascular smooth muscle cells (VSMCs) within the vessel wall can both divide and undergo apoptosis throughout life.
- b. However, the normal adult artery shows very low apoptotic and mitotic indices.
- c. In diseased tissue additional factors are present both locally, such as inflammatory cytokines, inflammatory cells, and the presence of modified cholesterol, and systemically, such as blood pressure and flow.
- d. These factors substantially alter the normal balance of proliferation and apoptosis, and apoptosis in particular may predominate in many disease states [16].

Remodelling

- a. Remodelling defines a condition in which alterations in vessel size can occur through processes that do not necessarily require large changes in overall cell number or tissue mass.
- b. Physiological remodelling by cell proliferation apoptosis results in closure of the ductus arteriosus and reduction in lumen size of infra-umbilical arteries after birth, and remodelling occurs in primary atherosclerosis, after angioplasty and in restenosis.
- c. Although surgical reduction in flow results in compensatory VSMC apoptosis, the role of VSMC apoptosis per se in determining the outcome of remodelling is unclear [17].

Arterial Injury and Aneurysm Formation

Acute arterial injury at angioplasty is followed by rapid induction of medial cell apoptosis. In animal models injury results in medial cell apoptosis 30 minutes to six hours after injury with adventitial and neointimal apoptosis occurring later. In humans, restenosis after angioplasty has been reported to be associated with either an increase or decrease in VSMC apoptosis, and again the role of VSMC apoptosis in either the initial injury or the remodelling process in restenosis in humans requires further study. The most common form of arterial aneurysm in humans is characterised by a loss of VSMCs from the vessel media, with fragmentation of elastin and matrix degradation, leading to progressive dilatation and eventually rupture. Apoptosis of VSMCs is increased in aortic aneurysms compared with normal aorta, associated with an increase in expression of a number of pro-apoptotic molecules. In particular, the presence of macrophages and T lymphocytes in aneurysms suggests that inflammatory mediators released by these cells may promote VSMC apoptosis. Moreover, the production of tissue metalloproteinases by macrophages may accelerate apoptosis by degrading the extracellular matrix from which VSMCs derive survival signals [17].

Restenosis after angioplasty has been reported to be associated with either an increase or decrease in VSMC apoptosis, and again the role of VSMC apoptosis in either the initial injury. Common form of arterial aneurysm- loss of VSMCs from the vessel media, with fragmentation of elastin and matrix degradation, progressive dilatation and eventually rupture. Apoptosis of VSMCs is increased in aortic aneurysms compared with normal aorta, associated with an increase in expression of a number of pro-apoptotic molecules. Presence of macrophages and T lymphocytes in aneurysms suggests that inflammatory mediators released by these cells may promote VSMC apoptosis. Production of tissue metalloproteinases by macrophages may accelerate apoptosis by degrading the extracellular matrix from which VSMCs derive survival signals [17].

3. Atherosclerosis

Rupture of atherosclerotic plaques is associated with a thinning of the VSMC-rich fibrous cap overlying the core. Rupture occurs particularly at the plaque shoulders, which exhibit lack of VSMCs and the presence of inflammatory cells. Apoptotic VSMCs are evident in advanced human plaques including the shoulder regions, prompting the suggestion that VSMC apoptosis may hasten plaque rupture. Increased VSMC apoptosis occurs in unstable versus stable angina lesions. Loss of VSMCs would be expected to promote plaque rupture, there is no direct evidence of the effect of apoptosis per se in advanced human atherosclerosis. Apoptotic cells in advanced lesions - macrophages next to the lipid core. Loss of macrophages from atherosclerotic lesions would be predicted to promote plaque stability rather than rupture, since macrophages can promote VSMC apoptosis by both direct interactions and by release of cytokines. Macrophage apoptosis - plaque rupture. Macrophages are the most common cell types found at rupture sites [18].



Effect of VSMC Apoptosis

The effect of VSMC apoptosis is clearly context dependent. Intimal VSMC apoptosis in advanced atherosclerotic plaques - plaque rupture, or medial apoptosis may promote aneurysm formation. In neointima formation post injury, both intima and media can limit neointimal formation at a defined time point.

Apoptosis also has number of deleterious effects.

- a. Exposure of phosphatidylserine on the surface of apoptotic cells provides a potent substrate for the generation of thrombin and activation of the coagulation cascade
- b. Apoptotic cells release membrane bound microparticles that are systemically procoagulant.
- c. VSMC apoptosis may be directly pro-inflammatory, with release of chemoattractants and cytokines from inflammatory cells [19].

Therapeutic Options for Apoptosis Treatment

The prevention of cardiomyocyte apoptosis is now a very important therapeutic aim. Critical to determining therapeutic benefit is not just inhibiting apoptosis markers at a single defined time point, but actually improving cardiac function. Many agents prevent the development of the morphological appearance of apoptosis or a biochemical marker (for example, DNA fragmentation) without inhibiting cell death. The ability to delay death may serve no useful purpose and may even be deleterious if that cell undergoes subsequent necrosis, with concomitant inflammation. In contrast, some studies have indicated that inhibition of apoptosis improves ventricular remodelling and contractility after infarction [20].

Although the long term effects of this inhibition are unknown, clinically meaningful improvements in cardiac function have been achieved

Apoptosis can be interrupted at many points in the signalling pathway

Prevention of apoptotic myocyte death may be directed at

- a. Inhibiting/preventing the stimulus,
- b. Inhibiting the regulatory mechanisms determining the decision to die, or
- c. inhibiting the pathways executing apoptosis

The cascade of events leading to cardiomyocyte apoptosis, and also the point at which a cell is irreversibly committed to die, crucially determine the approach to inhibiting apoptosis

Table 2. Potential inhibitors and signalling pathways of cardiomyocyte apoptosis²⁰

Stimulus	Signalling pathway	Potential inhibitor
Ischemia reperfusion	ERK / SAPK	Activation of ERK, inhibition of SAPK signalling
Pressure overload	ERK/SAPK	
Neurohormonal factor (e.g catecholamines)	G protein coupling	Beta Blockers
Ischemia	Lack of growth factor signalling	Activation of Akt/ERK pathways (for example, by IGF-1)
Death receptor ligands	Adaptor molecule caspases	Decoy receptors/receptors antagonists IAPs/Caspase inhibitors
ERK- Extracellular signal related Kinase; IAP- Inhibitor of Apoptosis Protein; SAPK- Stress Activated Protein Kinase.		

Inhibiting/Preventing the Pro-Apoptotic Stimulus [21]

- a. Ischemia/reperfusion, hypertrophy caused by increased afterload, and myocardial remodelling following infarction all are associated with myocyte apoptosis.
- b. This suggests that current treatment of proven benefit in these diseases may already act by inhibition of apoptosis.
- c. The beneficial effects of b blockers in chronic heart failure and ischaemic heart disease may counteract the pro-apoptotic effect of excess catecholamines.
- d. Indeed, carvedilol can inhibit ischaemia/reperfusion induced myocyte apoptosis, and angiotensin converting enzyme inhibitors may protect against angiotensin II induced apoptosis.
- e. Approaches aimed at reducing myocardial stretch, or oxidative stress, or improving myocardial perfusion may have the same effect.
- f. Finally, many pathways leading to apoptosis are triggered by specific death ligands, with either apoptosis or the disease itself manifesting upregulation of death receptors.



- g. Inhibition of delivery of death ligands—for example, by scavenging ligands through soluble receptors or receptor antagonists—may reduce apoptosis mediated through these pathways.
- h. However, it should be noted that other signals emanate from death receptors. For example, Fas activation reduces the membrane potential and induces afterdepolarisations in cardiac myocytes; inhibiting Fas induced apoptosis may allow escape of other Fas signalling, promoting arrhythmias

4. Protection against Apoptosis

- a. Many molecules protect cells from apoptosis, including anti-apoptotic Bcl-2 family members, IAPs, and decoys for death receptors.
- b. Although these agents inhibit apoptosis mediated by many stimuli, and may therefore be clinically useful, at present they cannot be selectively expressed without gene transfer into the heart, with all its inherent problems.
- c. The potential administration of soluble survival factors following the apoptotic stimulus.
- d. Many growth factors, including IGF-1, cardiotrophin-1, and the neuregulins, inhibit apoptosis following ischaemia, serum withdrawal, myocyte stretch, and cytotoxic drugs
- e. Overexpression of IGF-1 reduces apoptosis in non-infarcted remote zones & promotes favourable remodelling postmyocardial infarction.
- f. Activation of the cardiotrophin-1 receptor also inhibits cardiac dilatation following aortic banding, suggesting that reduced cardiomyocyte apoptosis can be translated into improved function.
- g. These agents signal through the AKT and ERK pathways, respectively, that are known to be anti-apoptotic in many cell types²¹.
- h. Some agents are potential therapeutics for long term administration.
- i. Heart failure is characterised by increased plasma concentrations of catecholamines and TNF α .
- j. The beneficial effects of β blockers in heart failure may therefore be achieved by prevention of myocyte apoptosis²¹.
- k. Licensed inhibitors of TNF α are now available, although recent randomised controlled trials (RENAISSANCE and RECOVER) suggest that a soluble TNF receptor antagonist (etanercept) does not benefit patients with heart failure.
- l. Evidence identifying the type 2 angiotensin II receptor as inducing apoptosis in models of heart failure has suggested that its inhibition may be beneficial
- m. Execution of apoptosis and cellular disintegration and packaging requires the activation of downstream signalling pathways, including mitochondrial amplification and activation of caspases.
- n. Augmentation of endogenous inhibitors of caspases, such as the IAPs, could therefore inhibit apoptosis induced by many stimuli.
- o. Pharmacological inhibition of caspases using cell permeable analogues of cleavage sites can inhibit myocyte apoptosis over the short term.
- p. However, their long term benefits are unknown, as cells that are destined to die may do so anyway, and delaying apoptosis may not provide long term benefit [21].

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