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Review

Apoptosis in cardiac diseases: stress- and mitogen-activated signaling pathways

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Abstract

Apoptosis is a form of cell death that involves discrete genetic and molecular programs, de novo protein expression and a unique cellular phenotype. Evidence for the existence of apoptosis in the human heart has been reported in various cardiac diseases, including ischemic and non-ischemic heart failure, myocardial infarction and arrhythmias. Among the most potent stimuli that elicit cardiomyocyte apoptosis are: oxygen radicals (including NO), cytokines (FAS/TNFα-receptor signaling), stress conditions (chemical or physical, e.g., radiation), sphingolipid metabolites (ceramide) and autocoids, e.g., angiotensin II. Apoptosis of cardiac myocytes may contribute to progressive pump-failure, arrhythmias and cardiac remodeling. The recognition of numerous molecular targets associated with cardiomyocyte apoptosis may provide novel therapeutic strategies for diverse cardiac ailments, as recently suggested by pharmacologic studies in experimental animals. This review paper is aimed to highlight the role of protein kinase signaling pathways in apoptosis with special attention to the stress-activated protein kinases (SAPK) and mitogen-activated protein kinases (MAPK) systems.

Keywords: Heart failure; Protein kinases; Signal transduction

1. Introduction

Apoptosis was first reported in a seminal paper by a group of pathologists studying cell population regulation [1]. In this paper, the authors described a form of cell death marked by its singularity, unique morphology and resolution without apparent 'traces' (e.g., inflammation) in the tissue of origin. These features of cell death were contrasted to necrosis, a form of cell death, due to noxious stimuli that leads to cell membrane disruption, swelling, disintegration, cell-content leakage and local inflammation. Featuring prominently in the apoptotic process are the 'apoptotic bodies' (fragments of dense DNA surrounded by apparently intact plasma membrane) and DNA condensation and fragmentation (the latter noted as a 'ladder' when separated on DNA-gel electrophoresis). The apoptosis phenotype has been associated later on with 'programmed cell death' (PCD) described first in the nematode, *C. elegans*, where genetically specified deletions of cells during development followed a timed-activation of specific genes (*ced*-3/4) [2]. It is now quite common to use apoptosis and PCD interchangeably. In this review, apoptosis represents the cellular phenotype resulting from activation of genomic programs that lead to DNA damage and cell death. Reports on cardiomyocyte apoptosis in human cardiac disease were only recently published [3,4]. Using two key markers of apoptosis that monitor the breakup of nuclear DNA: DNA 'ladder' and TUNEL (terminal deoxy-uridine-nick-end-labeling) histochemistry, apoptosis of cardiomyocytes and non-myocytes were identified in the following cardiac diseases: (1) ischemic and idiopathic dilated cardiomyopathy, associated with clinical heart failure; (2) acute myocardial infarction; (3) congenital arrhythmogenic dysplasias; (4) myocarditis; (5) arrhythmias. The reported incidence of cardiac myocyte apoptosis in these conditions varied considerably with estimates of 0.1% to 30%, depending on

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the disease specimen, methodology, and area of sampling. The rate of cardiac cell deletion of both myocytes and non-myocytes by apoptosis is difficult to assess in vivo especially in the human situation; in vitro, the resolution of the apoptotic process from initiation to complete engulfment is quite rapid (hours or a few days) and therefore even a low prevalence, e.g., 0.1%, recycled over years may lead to substantial depletion of cardiac cells. At the present time, the contribution of cardiac myocyte apoptosis to initiation and progression of the above-cited heart diseases cannot be accurately estimated.

2. Stimuli that elicit cardiomyocyte apoptosis

Significant research has been launched over the past 5 years to identify stimuli that elicit cardiomyocyte apoptosis and to decipher their signal transduction pathways [5]. It is important to note that much of the information is derived from (1) in vitro studies; (2) non-human (and often non-adult) cardiomyocytes, and (3) highly controlled (artificial) conditions. Nevertheless, circumstantial evidence supports the existence of many of the same stimuli in human cardiac disease as enumerated below. (1) Stress conditions such as ischemia (especially when followed by reperfusion) and oxygen radicals can elicit cardiomyocyte apoptosis, as has been demonstrated in both cell cultures and isolated cardiac perfusion studies [6,7]. In the former condition, deprivation of growth factors, energy sources (glucose) occurs, and endogenous antioxidants are usually present. (2) Cytokines, such as TNFα, have been shown to produce cardiomyocyte apoptosis in culture. Cytokines may figure prominently especially in advanced heart failure where very high levels of circulating TNFα (and other cytokines) are present [8–10]. Endogenous synthesis of TNFα in the heart (where its receptors are present) may be equally important. (3) Nitric oxide (NO) produced primarily by the Type II NOS (inducible nitric oxide synthase) elicits cardiomyocyte apoptosis possibly in association with peroxynitrite ($ONOO^-$) production. Activation of iNOS in heart failure has been established. (4) Neurohormonal factors such as angiotensin II (ATII) acting via the AT-receptors, have been shown to produce cardiomyocyte apoptosis [11]. Elevated circulating levels of ATII, correlating to disease stage, and in situ cardiac ATII production, possibly by a non-ACE pathway, may play an important role in this respect. (5) Mechanical stress has been shown to elicit apoptosis in cardiac muscle preparations in vitro. This physical form of stress is likely to exist in situations of cardiac remodeling leading to dilated myopathy and sphericity where increase in wall tension/stress is fundamental to the heart failure condition. Taken together, diverse stimuli are capable of producing apoptosis in cardiac myocytes, many of which co-exist in advanced heart failure. It is difficult at this time to dissect out the most important contributing factors in chronic human cardiac diseases where multiple humoral and local pro-apoptotic stimuli exist.

3. Signaling pathways of apoptosis in cardiac myocytes

Five possible pro-apoptotic signaling pathways in cardiac myocytes have emerged which may provide opportunities for specific pharmacological interventions. The data on these signal transduction pathways are largely derived from in vitro and mostly cultured neonatal cardiomyocytes. Discrete stimuli may activate multiple signal transduction pathways and 'cross talk' between various pathways is likely to be the common situation. Activation of apoptotic pathways may be 'intercepted' and aborted by anti-apoptotic regulatory mechanisms; thus, checkpoints that provide 'rescue' opportunities may be important in determining execution of the apoptotic programs.

The five major signaling pathways that have been suggested to convey apoptotic stimuli in cardiac myocytes are:

1. Redox-regulated systems (activated by oxygen radicals and NO/ONOO). [12,13]
2. The Fas/TNFα family of cytokine receptors operating via unique 'death domains' that are linked to several intracellular signaling pathways. [14,15]
3. Caspases, a family of cysteine-proteases operating in a cascade that is activated either by receptor originating signals or mitochondrial-associated cytochrome C. [5,10,16,17].
4. G-protein-coupled receptor (GPCR)-dependent stimulation induced by ligands/agonists. One such system is ATII and its receptor signaling system Gαi/Gαq, but other, novel GPCR pathways associated with Gαq have also been recently described [18,19].
5. Phospholipase-C type biochemical reactions that lead to sphingomyelinase activation and generation of sphingolipids like ceramide [20].

The scope of this brief review may not accommodate detailed deliberations on each of the discrete signaling events enumerated above, for which the reader is encouraged to resort to recent reviews [5]. More important though, are emerging principles that can be summarized as follows: (a) a single pro-apoptotic stimulus may lead to activation of single or multiple pathways of apoptosis; (b) the final common pathway of apoptotic signaling pathways involves breakdown of numerous nuclear proteins of cytoarchitectural function, transcription modulation and cell cycle regulation; (c) 'checkpoints' that regulate the apoptotic process are present in cardiomyocytes as is also the case for other cells; in this respect, the Bcl-2 family of proteins and the Bax-associated proteins may modulate

both cell membrane or mitochondrial-activated apoptosis [21].

4. Phospholipids as secondary messengers in apoptosis

Ceramides have been implicated as key mediators in numerous signaling pathways leading to diverse cellular phenotypes including cell proliferation, differentiation and apoptosis. Ceramides, along with phosphocholine, are products of sphingomyelin hydrolysis by sphingomyelinases (which are sphingomyelin-specific type C phospholipases). Accumulation of ceramides in response to various stimuli such as interferon-γ, tumor necrosis factor α (TNFα), IL-1β, FAS/APO-1 (CD95) and other cytokines is well established (for review see [22]). Furthermore, sphingomyelin hydrolysis is also induced by cellular stresses such as nutritional withdrawal or irradiation, two known pro-apoptotic conditions. Ceramides were shown to activate ceramide-activated protein kinases (CAPK) that phosphorylate *raf-1* which in turn activates extracellular-signal regulated kinase-2 (ERK2) type of MAPK via phosphorylation of MAPK/ERK kinase (MEK) [23] (see Fig. 1). Ceramide may also serve as upstream activator of *ras* possibly through vav exchange factor [24]. Ceramide may also activate the stress–response kinase cascade via MEKK, resulting in activation of JNK-1 via SEK (See Fig. 1) [25], thereby activating nuclear transcriptional mechanisms. Finally, it has been suggested that ceramides induce NF-κB translocation and activation possibly by indirect pathways that result in phosphorylation (by PKC) of the cytosolic IκBα inhibitor [26]. The subsequent activation of many transcriptional events including genes known to be associated with cardiac apoptosis (iNOS, TNFα) may provide a key link of ceramides to apoptosis.

However, the stimulation of numerous signaling pathways by ceramide may result in opposing biological outcomes depending on cell type, stimuli and conditions such as pH, ions and nutrition. It is believed that key stimuli and receptor signaling pathways leading to apoptosis are CD95 and TNF-R1. Both receptors have 'death domains' which are critical for the rapid, transient sphingomyelin hydrolysis by the acidic sphingomyelinase [27] which results in apoptosis.

In conclusion, stimuli that are know to impact on failing cardiac myocytes (e.g.TNFα) activate specific sphingomyelinases that result in ceramide production and activation of apoptotic programs within the cells. However, the precise role of these phospholipids in cardiac apoptosis awaits further investigation with selective chemical tools that allow highly specific inhibition of selected elements of the ceramide synthesis and action.

5. MAP kinase signaling pathways

Cells respond to various stimuli by activating one or more intracellular signaling pathways that in turn can result in alterations in gene expression, cytoskeleton and motility and can lead to apoptosis, proliferation or differentiation. One of the pathways that has been extensively

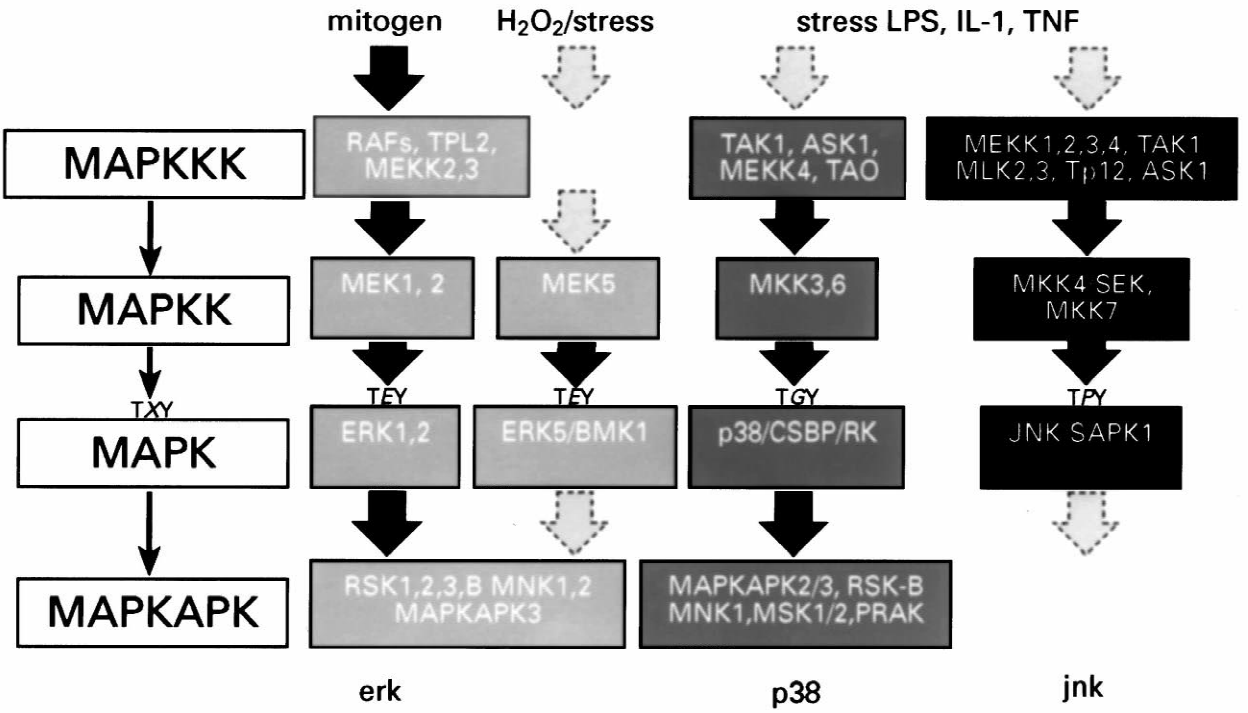

Fig. 1. Cascades of protein kinase signaling. Abbreviations are detailed in text and in reviews [29,66].

studied for its role in apoptosis is the MAP kinase pathway. The MAP kinases, or mitogen-activated protein kinases, phosphorylate target proteins on serine or threonine residues adjacent to a proline. The name of the family comes from the observation that the first discovered member responded to mitogens, but it has since been established that the MAP kinases can respond to a range of stimuli [18].

All of the MAPK members are catalytically inactive in unstimulated cells, and are activated in response to the appropriate stimulus by phosphorylation on both a threonine and tyrosine that appear in a threonine-X-tyrosine motif close to the active site. This phosphorylation is carried out by a dual specificity MAPKK (MAP kinase kinase), which in turn is activated through phosphorylation by a MAPKKK (MAP kinase kinase kinase). As illustrated in Fig. 1, the MAP kinases fall into three main families, the ERKs (extracellular regulated kinases), the JNK/SAPKs (*c-jun* amino-terminal kinase/stress-activated protein kinases) and the p38 MAPKs. Each family contains multiple isoforms encoded by different genes and splice variants, and differs from other family members in the amino acid X in the threonine-X-tyrosine activation motif (ERK has Thr-Glu-Tyr, JNK has Thr-Pro-Tyr, p38 has Thr-Gly-Tyr) and the size of the loop that contains it. This reflects the finding that each MAPK family is activated by a different MAPKK. In contrast, each MAPKK can be activated by several different MAPKKKs.

The stimuli that trigger each MAPK pathway differ. While ERKs respond primarily to mitogenic stimuli such as growth factors and PMA, the JNKs and p38 MAPKs respond to physiological stresses such as heat, chemical, oxidative, osmotic, pH, hypoxia, growth factor withdrawal and UV. This has led to the latter two being often referred to as stress-activated protein kinases (SAPKs) [29]. However, there are many stimuli that can activate more than one pathway at a time. Thus LPS and the two proinflammatory cytokines IL-1 and TNFα can stimulate all three pathways, albeit to differing extents [30,31], and similar results are seen with several cytokines and with G-protein-coupled receptor ligands. The particular MAPK pathways stimulated may be determined by the choice of MAPKKK, since some of these appear to be more promiscuous than the MAPKK and MAPKs.

Our understanding of the role of the different MAPKs in the physiological responses of cells to various stimuli has been aided by the use of dominant negative fragments of several components of the MAPK pathways and through the use of low molecular weight, cell permeable inhibitors of two of the MAPK pathways (Fig. 2). MEK 1 and 2 are two MAPKK that phosphorylate and activate ERK in response to mitogenic stimuli (Fig. 1). Two inhibitors of MEK were discovered. PD98059 was discovered by screening for inhibitors of a mutant, constitutively active form of the enzyme, but the inhibitor actually inhibits the activation of MEK1 by binding to the inactive form and is ten-fold less effective on MEK2 [32]. In contrast, U0126 was discovered in a screen for compounds that inhibited

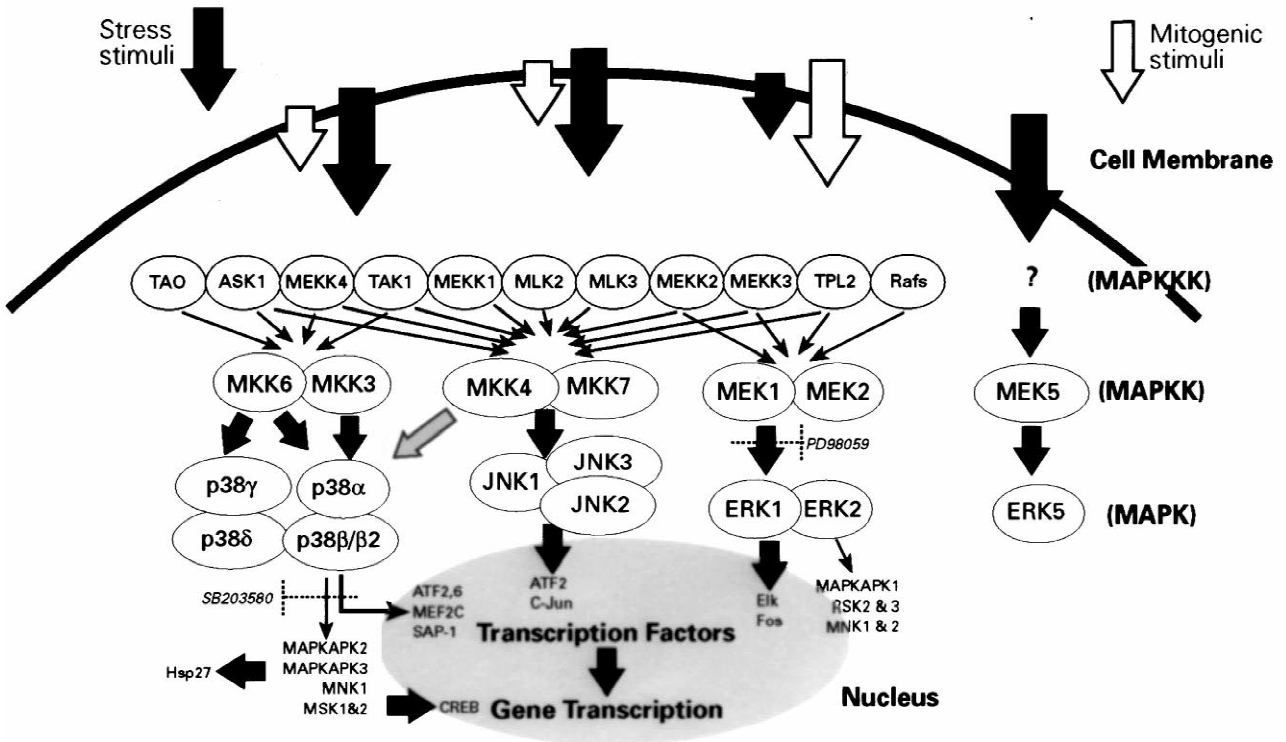

Fig. 2. Interaction of the various protein-kinase signaling pathways and site of inhibitor action. Inhibitors and kinase abbreviations are provided in text and in reviews [29,66]. CREB=cAMP response element binding protein. Hsp27=Heat shock protein 27.

AP-1 dependent transcription, and was subsequently characterized as an inhibitor of both MEK1 and MEK2 [33]. Both are non-ATP competitive inhibitors but compete with each other for the same binding site on MEK. When added to cells, both MEK inhibitors block the activation of ERKs in response to various mitogenic stimuli.

The p38 inhibitors, exemplified by the pyridinyl imidazole SB203580, were discovered by their ability to inhibit LPS-stimulated production of IL-1 and TNFα from human monocytes, and were subsequently used to identify and clone the molecular target, which turned out to be p38 MAP kinase [34]. SB203580 inhibits the kinase activity of p38 and its nearest homologue, p38β/β2, but does not inhibit the more distantly related homologues p38γ and p38δ [35,36]. When added to cells, SB203580 blocks the activation of MAPKAP kinase-2, a direct substrate of p38, in response to various stress and cytokine stimuli [37].

The physiological pathways regulated by the ERK, p38 MAP kinase and JNK pathways as determined through inhibitor and dominant negative studies have been recently reviewed [29,38,39]. From many studies, it is clear that MAP kinase pathways can regulate gene expression at the transcription, mRNA stabilization and translation levels [40,41]. In the case of transcription regulation, this can be directly correlated with the ability of the MAPKs or their substrate MAPKAPK/RSKs to phosphorylate specific transcription factors [42,43]. MAPKs can modulate the enzyme activity of targets such as PLA_2, tyrosine hydroxylase and the RSK/MAPKAP kinases through direct phosphorylation [44]. P38 MAPK activation can also lead to structural changes in the cell through alterations in hsp27 phosphorylation [45]. Finally, there is evidence that the MAPK pathways can influence proliferation, apoptosis, differentiation, aggregation and migration in different cell types [46–49].

The specific contribution of each MAPK pathway to a physiological response varies from cell to cell, and also depends on stimulus. In some cases MAPK pathways can cooperate, and in other cases they antagonize [28,46]. However, in several of these cases, inhibition of just one of the pathways has a physiological effect. Further obscuring this picture has been the reliability of extending studies using transformed cell lines to primary cultures and in vivo physiology. An example of this confusion emerges from studies of apoptosis. In neural cells, p38 is proapoptotic [46] whereas it is anti-apoptotic in TNF treated fibroblast cell lines [50].

6. SAPK and MAPK in cardiac cell hypertrophy and apoptosis

Evidence in support for linkage between SAPK/MAPK activation and cardiac cell hypertrophy and apoptosis has been derived from several lines of investigations in in vitro and in vivo systems. Wang et al. [51,52] used in vitro gene transfer technology to over-express wild type and constitutively active forms of MKK7 (or JNKK2) in neonatal rat cardiomyocytes. This procedure resulted in activation of JNK without affecting other mitogen-activated protein kinases, including extracellular signal-regulated protein kinase and p38 (see Fig. 2 for orientation). Specific activation of the JNK pathway in cardiac myocytes induced characteristic features of cardiomyocyte hypertrophy along with increased expression of atrial natriuretic factor and changes in sarcomeric organization. Similarly, activation of JNKs through transfection of MEKK1 and MKK4 also led to hypertrophy [53]. In contrast, co-activation of both JNK (via MKK7) and p38 (by dual expression of MKK3 or MKK6) induced phenotypic alterations compatible with apoptosis without hypertrophy. While gene overexpression studies harbor the caveat of possibly non-physiological levels of products, these data provide strong direct evidence that activation of JNK alone is sufficient to induce cardiac cell hypertrophy. Because co-activation pathways (JNK+p38) induced apoptosis with a lack of hypertrophy, this suggests that interaction or convergence of SAPK/MAPK pathways may ultimately lead to apoptosis possibly following a hypertrophic phase. These in vitro derived data support a role for SAPK/MAPK in the pathophysiology of cardiac injury in response to various conditions, including ischemia and reperfusion injury and hypertrophy in vivo [53–55].

Wang et al. 1998 [52] have shown that the p38 MAPK pathway is activated in cardiac tissue of murine hearts subjected to chronic transverse aortic constriction (Fig. 3). In in vitro cultures of neonatal rat cardiomyocytes, the p38 pathway was shown to have complex role in cardiac myocytes. Infection of cardiac myocytes with recombinant adenoviruses encoding up-stream activators of p38 kinases (constitutively active mutants of MKK3b and MKK6b)

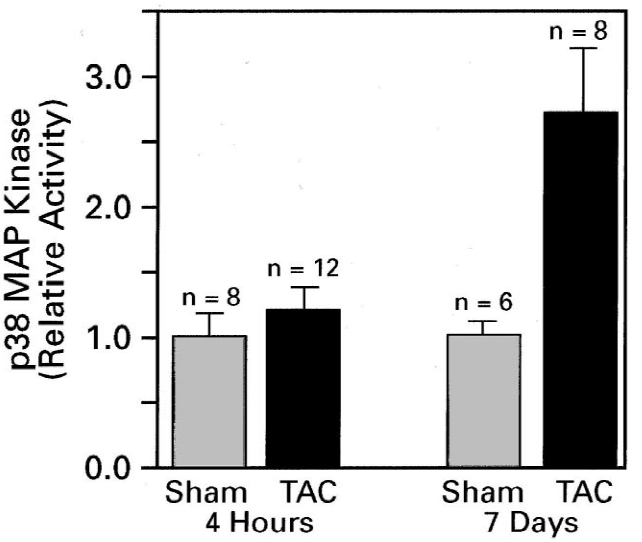

Fig. 3. p38 MAPK activation in a pressure overload murine model. TAC: transortic activation. Reproduced with permission from Ref [52].

(see Figs. 1 and 2 for orientation) elicited typical hypertrophic responses including ANF expression [52]. The hypertrophic response was enhanced by co-transfection of p38β (wild type) and suppressed by a dominant-negative p38β mutant. In contrast, MKK3bE-induced cell death was augmented by co-transfection of the p38α isoform and suppressed by a p38α dominant negative mutant. These data suggest a highly differentiated p38 pathway that includes divergent functions for different molecules of the p38 MAPK family.

7. p38 MAPK inhibitors in cardiac ischemia and apoptosis

Myocardial ischemia and reperfusion was shown to activate p38 MAPK in vivo (52–54, Fig. 3). In order to establish whether activation of p38 MAPK plays a role in myocardial cell apoptosis and infarction, we have used the potent and selective inhibitor of p38 MAPK, SB203580 [37] in a rabbit heart (Langendorff preparation) model of ischemia and reperfusion [56]. In this model, ischemia alone caused a moderate increase in p38 MAPK (3.5 fold over baseline) while reperfusion after ischemia further increased p38 MAPK by 6.3 fold. Activation of p38 MAPK is a rapid event occuring over minutes that precedes cellular and organ lesions. Administration of SB203580 before the ischemic insult resulted in dose-dependent inhibition of p38 MAPK and markedly diminished the consequences of the ischemia/reperfusion injury, including apoptosis (>50%), creatinine kinase loss (34%) and infarct size (>50%). Confirmation of inhibition of apoptosis was done by both TUNEL and DNA-'ladder' criteria. Most importantly, the p38 MAPK inhibitor accelerated the recovery of coronary flow, cardiac contractility and left ventricular pressure. It is also of interest to note that SB203580 did not inhibit JNK, another kinase activated by stress/ischemia, indicating the specific role of p38 MAPK in myocardial injury associated with ischemia and reperfusion. These data, while preliminary, should encourage more detailed work in many other models of cardiac injury, including heart failure. If such studies are consistently positive, clinical investigations will be warranted pending on the safety and tolerability of the p38 MAPK inhibitors.

8. SAPK inhibition in cardiac ischemia and apoptosis — potential role in adrenergic blockers and antioxidants

Beta-adrenergic receptor blockers (β-blockers) are proven drugs for cardioprotection in a variety of heart diseases including myocardial infarction and heart failure [57,58]. While the primary mechanism of cardioprotection by β-blockers is believed to be reduction in cardiac work, new information derived from studies with the multiple-action β-blocker, carvedilol and the non-selective β-blocker propranolol, indicate that these agents may prevent apoptosis of cardiac myocytes subjected to ischemia and reperfusion injury. In a rabbit model of in vivo ischemia (30 min) and reperfusion (I/RP, 4 h), robust apoptosis has been demonstrated by TUNEL and 'DNA ladder' markers [56]. Administration of carvedilol (a multiple action, β-blocker, α1-blocker and antioxidant) immediately before reperfusion, reduced the number of apoptotic myocytes by 77% (Fig. 4). Most notably, carvedilol significantly reduced infarct size [59]. Propranolol, the non-selective β-blocker, administered at equipotent β-blocking doses as carvedilol also provided significant protection against I/RP induced apoptosis, although to a lesser extent (39%). In this model, I/RP resulted in robust activation of JNK/SAPK in the ischemic myocardium only; this increase of JNK was significantly diminished by carvedilol (53%) yet no consistent effect on JNK activation was found in propranolol-treated rabbits (Fig. 4). Furthermore, expression of *Fas* in the ischemic myocardium was also significantly reduced by carvedilol [56]. The antioxidant properties of carvedilol might have contributed to its anti-apoptotic and cardiac protection since propranolol, an equipotent β-blocker that has much lesser anti-oxidant actions [60], displayed less anti-apoptotic capacity. The agents that have been used in this study are not specific JNK inhibitors, since no direct inhibition of the JNKs have been demonstrated with carvedilol or propranolol. However, the association of JNK suppression to apoptosis inhibition and improved function suggests a role of these kinases in ischemia induced cardiac apoptosis and injury. Further investigation with potent and specific JNK inhibitors will determine if inhibition of JNK contributes to the effect of carvedilol in cardiac apoptosis.

9. The significance of cardiac cell apoptosis in the evolution of heart diseases

The emerging evidence in recent literature on cardiac apoptosis strongly suggests that this form of cell death indeed exists in the human heart during various disease conditions. Although the evidence derived from human specimens is largely based on histological and phenotypic observations, cell based systems provide strong support for the existence of pro-apoptotic pathways in cardiomyocytes. However, a key question that remains unanswered is whether cardiac cell apoptosis has a significant role in any of the cardiac diseases where it is found. While this question cannot be decisively answered at this time, some suggestions as for possible mechanisms whereby apoptosis contributes to heart disease can be offered. Loss of cardiomyocytes could lead to loss of 'cardiac mass' and hence 'diminished pump power'. This possibility although

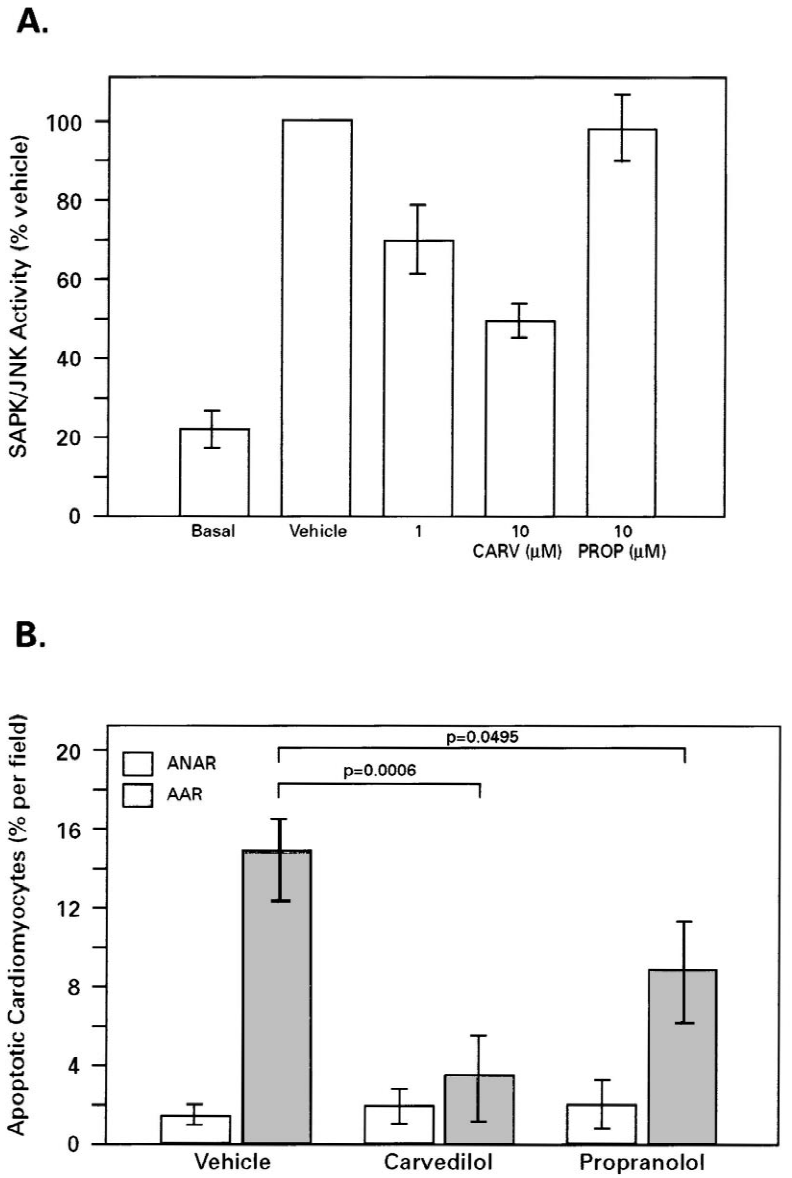

Fig. 4. Effect of carvedilol or propranolol on cardiac cell apoptosis and injury following ischemia and reperfusion (see Ref. [59]). Panel A: Effects of carvedilol and propanolol on ischemia/reperfusion-induced activation of JNK/SAPK. CARV=carvedilol; PROP=propranolol; concentration of agents are expressed in μm (micromolar). Panel B: Percentage of nuclei staining positive for TUNEL in heart tissues exposed to 30 min of ischemia and 4 h or reperfusion compared to vehicle treated rabbits. Carvedilol and propanolol were administered intravenously at 1 mg/kg, 5 min before reperfusion. AAR=area at risk; ANAR: area not at risk.

plausible, is difficult to assess as the rate and incidence of the apoptotic cycle in the heart has not been assessed. However, Adams et al. [18] have shown that robust apoptosis induced by *Gαq* overexpression in transgenic mice results in severe heart failure with markedly dilated chambers and perinatal death, indicating that robust apoptosis exercised over a brief period may on its own, without ischemia or growth factor deprivation, result in fulminant heart failure. Furthermore, in cardiac selective 'knock out' mice where the *gpl30* gene was deleted, heart failure develops following pressure overload by apoptosis without an ischemic or inflammatory component [61]. While 'quantity' cannot be dismissed as a possible mechanism, other possible factors such as aberrant electrical conduction at apoptotic *sites* may lead to arrhythmias. Apoptosis may also result in 'cardiac remodeling' due to 're-alignment' of neighboring cardiomyocytes [62]. This latter mechanism is unique to the heart, where function is extremely dependent on the optimal geometrical and structural alignment. Thus, apoptosis, even if limited in its local scope, may result in confounding mechanical and electrical disturbances.

10. Novel therapeutic opportunities for heart diseases based on anti-apoptotic agents

The potential for development of cardioprotective agents that are mechanistically based on modulation of apoptosis is rapidly emerging. Diverse opportunities may be exploitable, emanating both from enhancing anti-apoptotic capacities within the cardiac myocytes such as the *bcl-2* system, or inhibition of key pro-apoptotic stimuli and their signal transduction pathways. The former option, enhancing anti-apoptotic pathways may turn out to be a difficult task as the discrete regulatory pathways of the anti-apoptotic genes have not been clarified as yet. However, some interesting 'proof of concept' studies have been recently reported. Experiments conducted in *bcl-2* transgenic (TG) mice provided proof of concept that enhanced expression of anti-apoptotic pathways may provide protection from ischemic injury [63]. In the latter study, *bcl-2* TG mice were exposed to transient cerebral ischemia and neuronal death followed over extended periods. The *bcl-2* TG mice demonstrated significant protection against ischemia induced neuronal loss. While such studies have not as yet been reported in experimental models of cardiac ischemia or heart failure, in vitro studies of cardiomyocyte apoptosis evoked by p53 overexpression have been performed with co-transfection with *bcl-2* [21]. In this model of cardiac myocyte apoptosis, *bcl-2* provided strong anti-apoptotic action and prevented cell death. These data suggest that there is a potential for developing pharmacological strategies aimed at enhancing the expression and/or action of *bcl-2* as a means of arresting cardiac cell apoptosis. However, specific strategies that enable this objective have not as yet been reported. Alternatively, it may be more plausible to expect that agents acting at critical 'checkpoints' downstream of the key 'final common pathway(s)' elements leading to apoptosis may prove a superior strategy to prevent apoptosis. Several anti-apoptotic agents that may provide cardioprotection due to an anti-apoptotic mechanism have been reported: (a) p38 MAPK inhibitors; (b) caspase inhibitors; (c) β-adrenergic receptor blockers; (d) antioxidants and growth factors that inhibit SAPK [64,65].

11. Conclusion

Apoptosis is now recognized as a fundamental process in cell biology that is critical for tissue and organ development, physiologic adaptation and disease. The heart is like other organs — apoptosis plays a role in cardiac development, maturation and diverse disease conditions. The discrete stimuli and the molecular mechanisms that initiate and propagate apoptosis in human heart disease are largely unknown. In vitro studies in experimental models suggest multiple stimuli that activate highly diverse signaling pathways. The pathways of apoptosis display redundancies, regulatory 'checkpoints' and possibly convergence into a final common pathway where a 'point of no return' completes the process. Three possible consequences of cardiac myocytes apoptosis are postulated: (1) compromise in cardiac contractility due to loss of myocytes; (2) conduction disturbances leading to arrhythmias; (3) cardiac remodeling due to disruption of the geometrical alignment of cardiac myocytes. If indeed cardiomyocyte apoptosis plays an important role in initiation and progression of cardiac diseases, drugs that effectively and specifically inhibit apoptosis might be useful therapeutic agents for diverse cardiac diseases. Opportunities may emerge from either enhancing anti-apoptotic mechanisms (e.g., up-regulation of *bcl-2*) or inhibition of key targets in the pro-apoptotic pathways such as SAPKs and MAPKs. In conclusion, the recognition of apoptosis as a discrete, genomically mediated cell death in the myocardium, has opened new conceptual paradigms in heart disease research. Most importantly, the understanding on a molecular basis of the key executioners of apoptosis in cardiac myocytes may provide new opportunities for development of novel cardioprotective agents.

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