Apoptosis Inhibitors for Heart Disease

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Apoptosis may play a driving role in the transition from compensated hypertrophy to failure in the work-overloaded myocardium. In this issue of Circulation, Hayakawa et al demonstrate that chronic treatment with the broad-range caspase inhibitor IDN 1965 improved cardiac function and prevented or delayed the progression to heart failure in pregnant Goq-overexpressing transgenic mice, a model of peripartum cardiomyopathy. This timely article addresses 2 important issues in cardiovascular disease: the contribution of apoptosis to development and progression of heart failure and the potential use of caspase inhibitors as therapeutic agents for this condition.

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Goq, Hypertrophy, and Heart Failure

One pathway for translating work overload and hormonal stimulation into a myocardial growth response is through activation of receptors coupled to the Gq/11 family of heterotrimeric guanine nucleotide–binding (G) proteins. Compelling evidence has been presented for a functional relationship between increased Goq activity and pathological hypertrophy, here defined as hypertrophy that progresses to decompensation. Transgenic mouse hearts expressing moderate levels of activated Goq undergo dose-dependent hypertrophy, along with activation of hypertrophy-associated marker genes. All known upstream activators of Gq/11, including angiotensin II, norepinephrine, endothelin-1, and prostaglandin F2α, have been shown to mediate hypertrophy of cardiac myocytes in vitro and in a number of in vivo studies. Activation of Gq by these hypertrophic stimuli triggers dissociation of Goq and Gβγ subunits, followed by activation of phosphatidylinositol-specific phospholipase C-β (PLC) by GTP-bound Goq. The pleiotropic response to PLC includes activation of protein kinase C, Ras, mitogen-activated protein kinases, calcineurin/nuclear factor of activated T cells, and calmodulin kinase pathways, all of which probably contribute to hypertrophic growth.

Myocardial hypertrophy is a physiological and compensatory response to increased hemodynamic load but is frequently associated with poor clinical outcomes, including the development of cardiac systolic and diastolic dysfunction and ultimately heart failure. The conditions that lead to decompensation of hypertrophy are not understood but presumably involve factors modulating the hypertrophic process or a compensatory response to hypertrophy itself. Goq-mediated hypertrophy is associated with impaired intrinsic contractility and blunted β-adrenergic responses; higher transgene dosages of Goq lead rapidly to cardiac decompensation, biventricular failure, pulmonary congestion, and death. Mice with Goq-mediated hypertrophy are prone to develop dilated cardiomyopathy (DCM) and heart failure in response to hemodynamic loads that are typically tolerated by their wild-type littermates. In particular, the volume-overload stress of pregnancy promotes DCM and a rapid transition to heart failure. These findings have led to the hypothesis that hypertrophy and heart failure lie along a continuum of responses to Goq activation, in which moderate stimulation of Goq mediates hypertrophy by inducing growth-related genes, whereas higher levels of activation cause cardiomyopathy and failure, possibly by promoting apoptosis. Corresponding loss-of-function experiments in other laboratories have demonstrated a blunted hypertrophic response to pressure overload in transgenic mice expressing a dominant inhibitor peptide of Goq and in mice with cardiac-targeted conditional deletion of Goq. A strong case can therefore be argued for Goq as a mediator not only of the early phases of pressure-overload hypertrophy but also of the subsequent transition to DCM and heart failure.

Increased Apoptosis, DCM, and Heart Failure

Low rates of cardiac myocyte apoptosis have been detected in various forms of heart disease in humans, but the relevance of apoptosis to the development and progression of heart failure is still under debate (see Haider et al and Nadal-Ginard et al for reviews). In the Goq mouse model, the transition from hypertrophy to dilation and failure is associated with and may be causally linked to markedly increased rates of cardiac myocyte apoptosis. Overstimulation of angiotensin II receptors or expression of constitutively active Goq can cause apoptosis of cardiac myocytes in culture. Adams et al demonstrated that moderate overexpression of wild-type Goq caused hypertrophy of cardiac myocytes in vitro but that higher expression of wt-Goq or expression of a constitutively active Goq mutant caused apoptosis. Similarly, in Goq-overexpressing transgenic mice, heart failure resulting from pregnancy or aortic constriction is accompanied by high rates of cardiac myocyte apoptosis. These results suggest that the multiple stresses that lead to pathological hypertrophy, including pressure overload and neurohormonal activation, may do so by stimulating Goq-mediated apoptosis.

In support of this view, Goq-dependent DCM has been linked to the activation of an intrinsic (mitochondrial) pathway of apoptosis. Expression of constitutively active Goq in cultured cardiac myocytes causes opening of the mitochon-
drial permeability transition pore (MPTP), release of cytochrome c, loss of the mitochondrial membrane potential, and activation of effector caspases. In these studies, death by Gaq activation could be blocked by caspase or MPTP inhibitors. More recently, the proapoptotic Bcl-2 family member Nix/BNIP3L (Nix) was identified as a potential downstream effector of Gaq-initiated apoptosis. Expression of Nix is induced in the hearts of Gaq-transgenic mice as well as in hearts under acute pressure overload from aortic constriction and in hypertrophied human hearts from hypertensive patients. Mice with cardiac-targeted overexpression of Nix directed by the α-MHC promoter died within 2 weeks of birth with classic manifestations of heart failure. Conversely, double transgenic mice overexpressing both Gaq and a dominant negative (truncated) Nix cDNA (sNix) were protected from pressure-overload cardiomyopathy. The identification of Nix as part of the Gaq pathway represents an important step in the delineation of this pathway of heart failure. How overstimulation of Gaq induces Nix and precisely how pressure-overload activates Gaq remain unknown. Previous work has implicated mitogen-activated protein kinases, including c-Jun NH2-terminal kinase and p38, as possible downstream effectors of Gaq, and these are possible intermediaries for activation of Nix.

Caspase Inhibition Blocks Apoptosis and Heart Failure in Pregnant Gaq Mice

The demonstration that apoptosis plays a central role in Gaq-mediated heart failure and the possibility that the same or a similar pathway may operate in the broader context of pressure-overload DCM makes this an attractive system to analyze the effects of pharmaceutical intervention. Caspase inhibitors may provide 2 levels of protection for cardiac myocytes that are undergoing apoptosis. Caspase inhibitors may block and possibly reverse the death program. Caspase inhibitors may also inhibit the cleavage of multiple intramyocyte substrates, including sarcomeric components, degradation of which may cause contractile dysfunction. In the present study, Hayakawa et al. treated Gaq transgenic mice for 28 days by continuous infusion with the broad-range caspase inhibitor IDN-1965 and measured the levels of apoptosis, left ventricular chamber dimension, contractile function, and hemodynamics at the end of treatment. Caspase inhibition was initiated early in pregnancy and continued up to postpartum day 14, a stage in this model at which perinatal mortality peaks and significant ventricular dilatation has occurred. Treatment with IDN-1965 effectively reduced caspase 3–like activity and terminal dUTP nick end-labeling–positive myocytes, each by ≈90%. The most striking result was that treatment appeared to eliminate the 30% mortality seen in vehicle-treated mice. Cardiac function and hemodynamics were also improved. At the end of treatment, mean left ventricular end-diastolic dimension, percent fractional shortening, and several other functional parameters were significantly better in the treated than in the untreated pregnant Gaq mice. Overall, ≈40% of the pregnancy-associated myocardial dysfunction in Gaq mice was prevented by caspase inhibition; however, hearts in the 14-day postpartum treatment group were still severely dilated, and the mice were presumably still at significant risk of heart failure and death. Consequently, it is not clear whether the improved survival in treated mice represents deaths prevented or postponed. Nonetheless, this important study supports a causal role for apoptosis in Gaq-mediated heart failure and provides the first direct evidence of a therapeutic role for chronic anticaspase treatment in a model of DCM.

At the same time, the data suggest that there may be quantitative differences in the effects of caspase inhibitors on the separate end points of apoptotic index, mortality and contractile function. Several possibilities may account for this. One possibility is that the low residual caspase activity and apoptosis in the treated mice were sufficient to promote contractile dysfunction and allow progression of DCM. Another interpretation is that cell loss is only partially responsible for promoting heart failure or that cell loss proceeds by both caspase-dependent and -independent mechanisms (see Chen et al. and Kubasiak et al.).

Gaq and Mitochondrial Damage

Still another explanation, perhaps more likely, is that caspase inhibition blocks terminal apoptosis but does not prevent Gaq-mediated mitochondrial damage. In this case, the cells that are salvaged may be functionally impaired and bioenergetically compromised, contributing to further deterioration of myocardial function. Apoptosis in Gaq-transgenic postpartum mice has been shown to involve the translocation of activated Nix to the mitochondria and stimulation of the release of caspase activators, including cytochrome c. Progression of apoptosis through the stage of cytochrome c release may cause sustained mitochondrial dysfunction because the reuptake or resynthesis of cytochrome c may not be sufficient to replace what is lost when the MPTP is open. In fact, the contractile function of cultured cardiac myocytes overexpressing Gaq was preserved when apoptosis was blocked by an MPTP inhibitor but not by a caspase inhibitor. Consequently, it would be interesting to compare, in this model, the effects of caspase inhibitors with those of MPTP inhibitors that would block cytochrome c release, or those of the dominant negative sNix, which would inhibit Nix function upstream of the mitochondrion. If Nix is the sole Bcl-2 family intermediate in the Gaq pathway, which seems to be the case, neutralization of Nix should alleviate mitochondrial dysfunction as well as inhibiting caspase activity and apoptosis.

Caspase Inhibitors as Therapeutic Agents for Heart Disease

It will be important to determine whether the efficacy of caspase inhibitors is reproducible in other models of heart disease in which apoptosis is suspected to play a role. Caspases are potential targets for the treatment of both ischemic and nonischemic cardiomyopathy. High rates of apoptosis in the infarct zone correlate with unfavorable left ventricular remodeling in patients with acute myocardial infarction. One setting in which caspase inhibition may prove to exert significant therapeutic benefit is in the prevention of cardiac dysfunction caused by sepsis. Rats receiving intravenous injections of the broad-range caspase inhibitor z-VAD.fmk experienced less apoptosis and were significantly protected against endotoxin-induced myocardial dysfunction. Conversely, in some animal models, pannecaspase inhibitors can effectively block apoptosis associated with reperfusion damage, but most, although not all, reports have shown minimal impact.
Inhibitors of caspase, calpain, or the MPT may improve the subsequent remodeling phase, and may involve a higher number of aspects of chronic caspase inhibition that remain unresolved. Key issues include minimization of the risk of using specific inhibitors of proapoptotic Bcl-2 proteins (1), MPTP (2), or caspases (3). The most effective therapy may be that which acts higher up in the hierarchy and prevents secondary damage, including mitochondrial dysfunction, and involve apoptosis but also to prevent cleavage of sarcomeric contractile proteins and maintain contractile function.

Conclusions
The studies by Hayakawa et al. (1) set an important precedent for chronic administration of caspase inhibitors for the treatment of DCM and possibly for other forms of heart disease that are associated with elevated levels of apoptosis. Obviously, a number of aspects of chronic caspase inhibition remain unresolved. Key issues include minimization of the risk of tumor induction and overcoming technical problems related to delivery. The optimal timing, duration, and approach to caspase inhibition will probably need to be established for each condition (see Figure). It is possible that a combination of agents that can directly target specific, disease-appropriate components of the apoptosis cascade, such as Nix, Bad, or BNIP3, as well as broadly acting pan-caspase or MPTP inhibitors, will provide a more complete block of apoptosis while preserving mitochondrial and myofilament integrity.

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References


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