GSK-3β, a Therapeutic Target for Cardiomyocyte Protection

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Glycogen synthase kinase-3β (GSK-3β) is a multifunctional Ser/Thr kinase that plays important roles in necrosis and apoptosis of cardiomyocytes. A major mechanism of cell necrosis is the opening of the mitochondrial permeability transition pore (mPTP), which consists of multiple protein subunits, including adenine nucleotide translocase (ANT). The threshold for mPTP opening is elevated by phosphorylation of GSK-3β at Ser9, which reduces activity of this kinase. How inactivation of GSK-3β suppresses mPTP opening has not been fully understood, but evidence to date suggests that preservation of hexokinase-II in the mPTP complex, inhibition of cyclophilin-D-ANT binding, inhibition of p53 and inhibition of ANT into the mitochondria are contributory. GSK-3β phosphorylation is a step to which multiple protective signaling pathways converge, and thus GSK-3β phosphorylation is crucial in cardioprotection of a variety of interventions against ischemia/reperfusion injury. Apoptosis of cardiomyocytes by pressure overload or ischemia/reperfusion is also suppressed by inactivation of GSK-3β-δ, in which reduced phosphorylation of p53, heat shock factor-1 and myeloid cell leukemia sequence-1 and inhibition of Bax translocation might be involved. Considering predominant roles of GSK-3β in cardiomyocyte death, manipulation of this protein kinase is a promising strategy for myocardial protection in coronary artery disease and heart failure. (Circ J 2009; 73: 1184–1192)

Key Words: Apoptosis; Heart failure; Myocardial infarction; Preconditioning; Signal transduction

Mechanisms of Cardiomyocyte Necrosis During Ischemia/Reperfusion

When myocardial blood perfusion is interrupted, for example by occluding the coronary artery, ischemic cardiomyocytes suffer from several critical intracellular events that can lead to cell necrosis and/or prime the cells to reperfusion-induced necrosis. First, intracellular level of high-energy phosphate is reduced due to oxygen deficiency. Although ischemic cardiomyocytes cease contraction within a few minutes after the onset of ischemia, adenosine triphosphate (ATP) consumption continues during ischemia mainly by mitochondrial ATPase for maintenance of mitochondrial membrane potential.6,7 Anaerobic glycolysis supplies ATP during the early period of ischemia, but it is ultimately halted by inhibition of glyceraldehyde phosphate dehydrogenase due to accumulated H+ and NADH.8 Second, intracellular Na+ accumulates due to Na+-H+ exchangers and Na+ channels and due to reduced Na+ efflux via the Na+-K+ pump.9–11 This Na+ overload predominates ischemic cardiomyocytes to Ca2+ influx by the Na+-Ca2+ exchanger. Third, Ca2+ influx via the Na+-Ca2+ exchanger, reduced Ca2+ uptake into the sarcoplasmic reticulum and reduced Ca2+ efflux via the sarcolemmal Ca2+ pump induce Ca2+ overload in ischemic cardiomyocytes.9–12 However, elevation of intracellular Ca2+ is modest during ischemia because acidosis inhibits the Na+-Ca2+ exchanger and cytosolic Ca2+ is taken up by the mitochondria as long as its membrane potential is maintained by use of ATP.9–11,13

Severely ischemic cardiomyocytes ultimately ‘starve to death’, although sarcomemal damage by detergent actions of accumulated long-chain Acyl-CoA and by Ca2+-activated proteases and phospholipases might also be involved in ischemia-induced necrosis.9 Nevertheless, reperfusion is necessary to salvage ischemic cardiomyocytes from necrosis. However, reperfusion per se can trigger lethal mechanisms. Reperfusion washes out H+ in the extracellular space, which
retracts inhibition of the reverse mode operation of the Na\(^+\)-Ca\(^{2+}\) exchanger, resulting in massive Ca\(^{2+}\) influx into cardiomyocytes.\(^9\)–\(^11\) The abrupt increase in intracellular Ca\(^{2+}\) level induces activation of calpain, a Ca\(^{2+}\)-activated protease, which compromises Na\(^+\)-K\(^+\) pump function\(^10\) and Ca\(^{2+}\) overload of the mitochondria.\(^6\),\(^7\),\(^8\),\(^9\),\(^11\) At the same time as Ca\(^{2+}\) overload, cardiomyocytes abruptly develop cell edema after reperfusion as a result of osmotic loading during ischemia. ATP production restituted by re-supply of oxygen in Ca\(^{2+}\)-overloaded and swelling myocytes with fragile sarcolemma induces hypercontraction and sarcolemmal rupture. Involvement of this mechanical factor in cardiomyocyte necrosis is supported by findings that suppression of myocardial contraction during the early period of reperfusion by the use of butanedione monoxime significantly reduced infarct size.\(^14\),\(^15\) However, a major mechanism of lethal reperfusion injury is likely to be opening of the mitochondrial permeability transition pore (mPTP) at the time of reperfusion, which will be discussed in detail below.

**Apoptosis of Cardiomyocytes After Ischemia/Reperfusion**

Most, if not all, of dead cardiomyocytes after ischemia/reperfusion show morphology of necrosis under examinations with macro- and micro-histochemistry and electron microscopy. However, contribution of apoptosis to myocyte loss after ischemia/reperfusion is rather inconclusive.\(^16\) A major problem in this issue is limitations of methods used for detecting apoptosis. Terminal deoxynucleotidyl tranferase-mediated dUTP in-situ nick end labeling (TUNEL) and detection of non-random fragmentation of DNA (DNA laddering) are somewhat non-specific, and TUNEL-positive cardiomyocytes after ischemia/reperfusion in situ actually showed necrotic morphology under electron microscopy.\(^17\)

However, there are some observations suggesting that apoptosis is involved in cardiomyocyte death after ischemia/reperfusion. Ischemia/reperfusion induces activation of caspase-3, -8, -9 and translocation of Bax and Bcl-2 in the myocardium.\(^18\),\(^19\) Myocardial infarct size (ie, size of necrotic tissue mass) was reduced to 37–78% of controls by caspase inhibitors\(^20\),\(^21\) or genetic deletion of Fas,\(^22\) and this protection was associated with reduction in TUNEL-positive cells in reperfused regions. Furthermore, recent studies have shown that apoptotic process is converted to necrotic process when essential co-factors for apoptosis are lacking.\(^23\)–\(^25\) ATP is one of such co-factors, and switching from apoptosis to necrosis by inhibition of mitochondrial ATP generation has been shown in non-cardiac cells.\(^23\),\(^24\) Taken together, these findings are consistent with the notion that apoptotic cell death triggered by intrinsic mechanisms and/or Fas-Fas ligand system is ultimately converted to necrotic cell death in the myocardium during ischemia/reperfusion. However, this possibility remains to be critically tested.

**mPTP and Reperfusion Injury**

The mPTP is a non-selective large conductance channel in the mitochondrial inner membrane, which is physiologically closed. Opening of mPTPs is involved in cell death induced by a variety of causes (for example, ischemia/reperfusion, alcohol, endotoxin, anti-cancer agents).\(^26\) Although the molecular structure of the mPTP has not yet been clarified, several proteins have been suggested to be subunits of this multi-protein complex.\(^26\)–\(^29\) In a classic model of mPTP, the pore is formed by adenine nucleotide translocase (ANT) in the inner membrane and voltage-dependent anion channel (VDAC) (Figure 1A). In this model, binding of cyclophilin-D (CypD), a matrix protein, to ANT increases sensitivity of ANT to Ca\(^{2+}\), a trigger of mPTP opening. A crucial role of CypD in mPTP opening has been indicated by independent studies demonstrating that deletion of a gene coding...
mitochondrial CypD (Ppif) markedly increased resistance of cells to mPTP opening and necrosis.\textsuperscript{30–32} However, recent studies using ANT- and VADAC-deficient mice indicate that ANT and VDAC are not indispensable for mPTP opening.\textsuperscript{33,34} Based on these findings, a new model of mPTP was recently proposed by Sollott’s group (Figure 1B)\textsuperscript{35} In this new model, ANT and VDAC are regulatory factors of an unknown channel protein.

In addition to Ca\textsuperscript{2+}, reactive oxygen species (ROS), accumulated inorganic phosphate and depletion of ATP promote opening of mPTPs.\textsuperscript{35,36} Although the relative importance of each factor is not clear, all of these mPTP opening stimuli are induced in cardiomyocytes subjected to long-sustained ischemia and reperfusion. Studies using radio-labeled deoxyglucose as a tracer of opened mPTPs indicated that mPTPs open within 5–10 min after reperfusion but not during ischemia.\textsuperscript{37} This finding is consistent with the notion that ROS production and Ca\textsuperscript{2+} influx upon reperfusion are triggers of mPTP opening in the reperfused myocardium.

Because molecules smaller than 1.5 kDa pass through mPTPs, irreversible mPTP opening abolishes mitochondrial membrane potential, disabling the mitochondria to produce ATP. Without ATP generation, reperfused cardiomyocytes cannot recover Na\textsuperscript{+} and Ca\textsuperscript{2+} homeostasis and thus cannot maintain cell viability. Results of experiments using pharmacological inhibitors of mPTP and CypD knockout mice generally support the notion that opening of mPTPs is responsible for cardiomyocyte necrosis after ischemia/reperfusion.\textsuperscript{32,34,38–40}

In contrast to its role in cell necrosis, the role of mPTP in apoptosis is still controversial. Earlier studies using non-cardiac cells have shown that mPTP opening induces Bax translocation to the mitochondria, leading to apoptosis.\textsuperscript{41,42} Furthermore, inhibition of mPTP by pharmacological inhibitors (cyclosporine A, bongkrekic acid, NIM811) suppressed apoptosis induced by H\textsubscript{2}O\textsubscript{2} in vitro\textsuperscript{34–45} and by ischemia/reperfusion in vivo.\textsuperscript{46} However, susceptibility of CypD deleted cells to apoptosis induced by etoposide, saturosporin, TNF-\textalpha plus cyclohexamide was not different from that of normal control cells.\textsuperscript{32} There is no clear explanation for the contradictory observations, but it may reflect complex cross-talks of redundant signaling pathways of apoptosis and necrosis.\textsuperscript{47–49}

### GSK-3\beta and mPTP

Johaszoa et al first reported that GSK-3\beta activity is a determinant of the threshold for mPTP opening in cardiomyocytes.\textsuperscript{27} They showed that the threshold for ROS-induced opening of mPTPs is elevated by inhibitory phosphorylation of GSK-3\beta, pharmacological inhibitors of GSK-3\beta and knockdown of GSK-3\beta protein expression using siRNA. Recently, Gomez et al examined the role of GSK-3\beta in mPTP regulation by using the amount of loading Ca\textsuperscript{2+} required to induce irreversible Ca\textsuperscript{2+} release in isolated mitochondria as an index of the threshold for mPTP opening.\textsuperscript{48} Postconditioning significantly elevated the threshold of mPTP opening in cardiac mitochondria from wild-type mice, but such a protective effect was not detected in mitochondria from transgenic mice expressing GSK-S9A. These findings indicate that GSK-3\beta activity promotes mPTP opening in response to ROS and/or Ca\textsuperscript{2+} overloading.

How inactivation of GSK-3\beta (or its phosphorylation at Ser9) increases the threshold for mPTP opening remains unclear. However, several mechanisms have been suggested to date. First, hexokinase II (HK-II) might be preserved in the mPTP complex by inactivation of GSK-3\beta.\textsuperscript{49,50} Although experiments were conducted by using non-cardiac cells, Pastorino et al showed that activation of GSK-3\beta induces release of HK-II from the mitochondria via phosphorylation of VDAC, which enhanced susceptibility of the cells to necrosis.\textsuperscript{49} A role of HK-II as a stabilizer of mPTP opening was also indicated by finding that the inhibitory effect of leukemia inhibitory factor on mPTP opening was significantly attenuated by dissociation of HK-II from the mitochondria by the use of glucose-6-phosphate or HK-II.
dissociating peptide.\(^{50}\)

Second, our recent study suggests that binding of phospho-GSK-3\(\beta\) to ANT suppresses interaction of ANT with CypD, a trigger of mPTP opening.\(^{61}\) In isolated rat hearts, reperfusion after sustained ischemia induced translocation of cytosolic GSK-3\(\beta\) to the mitochondria, where it formed a complex with ANT and VDAC. Phosphorylation of GSK-3\(\beta\) by ischemic preconditioning and erythropoietin (EPO) receptor activation was dependent on protein kinase C (PKC) and phosphatidylinositol 3-phosphate kinase (PI3K), and phospho-GSK-3\(\beta\) was bound to ANT but not to VDAC at reperfusion (Figure 2A). Interestingly, this phospho-GSK-3\(\beta\)-ANT interaction was associated with reduction of CypD-ANT binding by 60% compared with the untreated control group (Figure 2B). Because neither ANT nor CypD is a putative substrate of GSK-3\(\beta\), modification of CypD-ANT interaction by phospho-GSK-3\(\beta\) is presumably an indirect one.

Third, p53-mediated regulation of mPTP might be suppressed by inhibition of GSK-3\(\beta\) activity. Phosphorylation of p53 by GSK-3\(\beta\) enhances its functional activity and translocation to the nucleus and mitochondria.\(^{52}\) Venkatapuram et al showed that an inhibitor of p53, piifithrin-\(\alpha\), sensitized the myocardium to ischemia-induced protection, which is presumably phospho-GSK-3\(\beta\)-mediated.\(^{53}\) This beneficial effect of piifithrin-\(\alpha\) was abolished by an activator of mPTP, atracyloside, indicating that a target of the p53 inhibitor was mPTP.

As another possible mechanism of protection by GSK-3\(\beta\) inactivation, Das et al recently showed that inhibition of GSK-3\(\beta\) suppressed ATP hydrolysis by reducing ATP transport from the cytosol to the mitochondria.\(^{54}\) This effect was associated with reduced phosphorylation of VDAC, suggesting modification of ATP transport through VDAC. Suppression of ATP hydrolysis during ischemia would prevent both ATP depletion and accumulation of inorganic phosphate, 2 factors promoting mPTP opening. All of the mechanisms discussed here are not mutually exclusive and might contribute in concert to mPTP inhibition (Figure 3).

### GSK-3\(\beta\) and Tolerance of Cardiomyocytes to Necrosis

Involvement of GSK-3\(\beta\) phosphorylation in myocardial protection has been suggested in a wide variety of interventions against infarction. Elevation (or preservation) of phospho-Ser9-GSK-3\(\beta\) level on reperfusion and anti-infarct tolerance have been observed in rat, rabbit or mouse hearts treated with ischemic preconditioning, ischemic post-conditioning, bradykinin, opioids, bradykinin, or resveratrolo.\(^{55,57,60,61}\) GSK-3\(\beta\) phosphorylation by these apparently unrelated interventions is explained by the fact that GSK-3\(\beta\) is a substrate of multiple pro-survival protein kinases, including Akt, PKC-\(\varepsilon\), extracellular signal-regulated kinase (ERK) and protein kinase G, and GSK-3\(\beta\) phosphorylation is therefore a step to which multiple protective signaling pathways converge. Direct inhibition of GSK-3\(\beta\) by the use of structurally different pharmacological inhibitors (SB-216763, lithium chloride) administered before either ischemia or reperfusion limits infarct size.\(^{55,57,60,72,73}\) It is interesting to note that inhibitors of GSK-3\(\beta\) increase the level of phospho-GSK-3\(\beta\), which is explained by GSK-3\(\beta\)-mediated positive regulation of protein phosphatase-1 (Figure 3).\(^ {74}\) Nevertheless, these findings support the notion that inactivation of GSK-3\(\beta\) by phosphorylation at Ser9 is a common mechanism of protection of cardiomyocytes against necrosis in many cardioprotective interventions.

We hypothesized that phospho-Ser9-GSK-3\(\beta\) at the time of reperfusion determines the extent of reperfusion-induced myocardial necrosis, because it suppresses mPTP opening. To test this possibility, different levels of GSK-3\(\beta\) phosphorylation were induced in rat hearts in situ by IPC, administration of EPO, the combination of these two, or inhibitors of PKC or PI3K together with IPC-EPO combination before 20min ischemia/2h reperfusion. Levels of phospho-GSK-3\(\beta\) at 5 min after reperfusion were tightly correlated with infarct sizes (% of ischemic area) 2h after reperfusion (r=0.809, P<0.05), indicating that approximately 60% of infarct size variation is explained by variation in the level of GSK-3\(\beta\) phosphorylation.\(^ {57}\) This relationship between phospho-GSK-3\(\beta\) and infarct size is unlikely to be nonspecific, because there was no correlation between infarct size with level of phospho-Ser473-Akt or phospho-Tyr705-STAT3 on reperfusion.

However, that role of phospho-GSK-3\(\beta\) in cardiomyocyte...
protection might not be equivalent across animal species. To our surprise, protective effects of ischemic preconditioning and postconditioning were not lost in GSK-3αβ knock-in mice in which Ser21 of GSK-3α and Ser9 of GSK-3β were changed to Ala. Furthermore, pharmacological inhibitors of GSK-3β failed to limit infarct size in these knock-in mice and also in wild-type mice. In contrast, another study using transgenic mice showed apparently opposite results. Gomez et al. reported that the infarct size-limiting effect of postconditioning was lost in mice cardiосpecifically expressing GSK-3β-S9A and that SB216763 could mimic postconditioning in wild-type mice. The reasons for this apparent discrepancy in results of the 2 mouse studies remains unclear, although differences in intracellular distribution of mutant GSK-3β, function of extra-cardiac GSK-3β and function of GSK-3α are possibilities. In addition, Skysschally et al. recently reported that the infarct size-limiting effect of ischemic postconditioning was maintained in pigs in which phosphorylation of Akt, ERK and GSK-3β was inhibited by inhibitors of PI3K and ERK. Collectively, the negative results in mouse and pig studies suggest that role of phosphorylated GSK-3β as a determinant of myocyte tolerance to reperfusion-induced necrosis may be species dependent.

Role of GSK-3β in Apoptosis of Cardiomyocytes

It is known that GSK-3β has 2 opposite roles in apoptotic death of non-cardiac cells depending on the trigger of apoptosis. GSK-3β activity restrains pro-apoptotic signaling from death receptors (ie, TNF-R1, Fas, DR4, DR5). Inhibition of GSK-3β in the cells with activated death receptors is facilitated by active GSK-3β. Activation of GSK-3β results in mouse and pig studies suggest that role of GSK-3β as a determinant of myocyte tolerance to reperfusion-induced necrosis may be species dependent.

In contrast, apoptosis induced by intrinsic mechanisms is facilitated by active GSK-3β. Pro-apoptotic functions of GSK-3β have been shown in apoptosis by withdrawal of growth factors[18-83] DNA damage[52-54] mitochondrial toxins[85] ischemia[86,87] oxidant stress[88] and other conditions that trigger apoptosis via the mitochondrial pathway. Phosphorylation of 4 GSK-3β substrates (p53, heat shock factor-1 [HSF-1], myeloid cell leukemia sequence-1 [MCL-1], Bax) is involved in the pro-apoptotic functions. Phosphorylation of p53 in the nucleus leads to p53-mediated transcription of pro-apoptotic genes[82,89] phosphorylation of HSF-1 inhibits its function as a survival-promoting transcription factor[90,91] phosphorylation of MCL-1 induces ubiquitinylation and subsequent degradation of this anti-apoptotic Bcl-2 protein[92,93] and phosphorylation of Bax promotes its localization in the mitochondria and apoptosis[81] Contribution of the 4 GSK-3β substrates to apoptosis appears different depending on cell types, experimental conditions and triggers of apoptosis.

Although the role of GSK-3β in apoptosis of cardiomyocytes has not been fully clarified, evidence to date suggests its significant contribution to apoptosis induced by ischemia/reperfusion[95,94-96] hypoxia/re-oxygenation[97] adrenoceptor activation[98] and pressure overload[99] Apoptosis by these insults was suppressed by overexpression of the adrenomedullin gene[95] or kallikrein gene[96] or treatment with statins[97] all of which induced phosphorylation of GSK-3β. Furthermore, this protection from apoptosis was inhibited by dominant-negative Akt or active GSK-3β mutant and mimicked by pharmacological inhibitors of GSK-3β[94-98] Transgenic mice that cardio-specifically express dominant-negative GSK-3β showed significantly fewer apoptotic cardiomyocytes after pressure overloading by aortic constriction[99] These findings indicate that GSK-3β activity determines the fate of cardiomyocytes exposed to apoptosis inducers. However, the intracellular localization of phospho-GSK-3β responsible for the anti-apoptotic function and the mechanism downstream of GSK-3β phosphorylation in cardiomyocytes remain unclear.

Recently, we tested the hypothesis that translocation of phosphorylated GSK-3β to the mitochondria is important for inhibition of oxidant stress-induced apoptosis of cardiomyocytes[100] Apoptosis of H9c2 cells was induced by hydrogen peroxide or hypoxia/reoxygenation. EPO and insulin-like growth factor-1 significantly suppressed apoptosis of H9c2 cells, which was associated with activation of Akt and phosphorylation of GSK-3β at Ser9. Furthermore, H9c2 cells transfected with S9A were insensitive to EPO-induced protection, and siRNA knockdown of GSK-3β could mimic the effect of the EPO receptor activation on H2O2-induced apoptosis. However, translocation of GSK-3β to the mitochondria after EPO receptor activation was not detected by tracing EGFβ-tagged GSK-3β or by co-immunostaining of GSK-3β and mitochondria. Instead, these methods showed that GSK-3β was phosphorylated within the mitochondria after EPO receptor activation in a PI3K-dependent manner.

No significant changes were detected for p53, MCL-1 or Bcl-2 after H2O2 challenge or EPO treatments in our protocols, but translocation of Bax to mitochondria in response to oxidant stress was suppressed. These results suggest that phosphorylation of GSK-3β pre-existing in the mitochondria by Akt affords protection from oxidant stress-induced apoptosis, possibly by suppressing Bax translocation in cardiomyocytes. Phosphorylation of GSK-3β in the mitochondria[100] is possibly achieved by translocation of Akt to the mitochondria[80,82].

Do We Need a Novel Therapy for Cardiomyocyte Protection in the PCI Era?

Introduction of reperfusion therapy and its continuous refinement have improved myocardial salvage in patients with acute myocardial infarction (AMI) and their prognosis. Our recent analysis of data on infarct size and ischemic zone size in the literature indicates that current reperfusion therapy salvages more than 50% of the ischemic myocardium in approximately half of the patients with AMI. However, there is wide variation of the effect of reperfusion therapy, and the infarct size is larger than 75% of the ischemic zone despite successful coronary reperfusion in 25% of AMI patients. Patients with infarct size larger than 20% of the left ventricle at the acute phase of AMI were also approximately 25% of the total patients. Interestingly, the variation of infarct size cannot be explained by symptom-to-treatment time alone. Collectively, it is clear that a novel and potent therapy is necessary for at least approximately 25% of AMI patients in order to improve their clinical outcomes.

Recently, 2 randomized clinical studies, AMISTAD II[102] and J-WIND[103] showed that administration of adenosine and that of human atrial natriuretic peptide, respectively, significantly reduced infarct size in AMI patients. However, the protection afforded by these pharmacological agents was not associated with improvement of prognosis or suppres-
sion of ventricular remodeling after AMI. Possible reasons for the apparent discrepancy between infarct size limitation and lack of clinical benefit were extensively discussed in our recent review. Nevertheless, it is notable that ‘significant’ limitation of infarct size might not always reduce mortality after AMI or its surrogate markers such as ventricular dimension.

Myocardial necrosis is a major problem not only in coronary artery disease but also in heart failure. Necrosis of cardiomyocytes, in addition to their apoptosis, has been observed in animal models of heart failure and in end-stage heart failure of patients with dilated cardiomyopathy and aortic stenosis. Recent studies have shown that plasma troponin concentration increases during acute severe heart failure and during exacerbation of chronic heart failure, and their prognosis correlates with the concentration of troponin.

However, no current therapy directly targets myocardial necrosis in failing hearts.

GSK-3β Inhibitors for Clinical Application
Lithium chloride is the only GSK-3β inhibitor in clinical use for bipolar mood disorders at the present time. Findings in experiments discussed in the present article indicate that GSK-3β inhibitors are promising agents for use in adjunctive therapy with PCI for AMI patients. Side effects of the agent for this use could be minimal, because a single dose administration of a GSK-3β inhibitor would be sufficient to inhibit mPTP opening at the time of reperfusion. However, chronic use of a GSK-3β inhibitor for heart failure, for example, could be problematic because its effects on ventricular hypertrophy and tumor growth might result in adverse outcomes. Actually such serious side effects have not been reported for chronic use of lithium chloride. However, more specific and potent inhibitors of GSK-3β afford significant protection in human hearts as well. Nevertheless, no current therapy directly targets myocardial protection by inhibition of GSK-3β in vivo. However, protection of the human heart was shown in animal models of heart failure, and in end-stage heart failure of patients with dilated cardiomyopathy and aortic stenosis. Recent studies have shown that plasma troponin concentration increases during acute severe heart failure and during exacerbation of chronic heart failure, and their prognosis correlates with the concentration of troponin.

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