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Forum Review

Growth Factor Signaling for Cardioprotection Against Oxidative Stress-Induced Apoptosis

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ABSTRACT

The heart is subjected to oxidative stress during various clinical situations, such as ischemia–reperfusion injury and anthracycline chemotherapy. The loss of cardiac myocytes is the major problem in heart failure; thus, it is important to protect cardiac myocytes against cell death. Various growth factors, including insulin-like growth factor, hepatocyte growth factor, endothelin-1, fibroblast growth factor, and transforming growth factor, have been shown to protect the heart against oxidative stress. The mechanism of growth factor-mediated cardioprotection may involve the attenuation of cardiac myocyte apoptosis. The present article summarizes the current knowledge on the molecular mechanisms of growth factor-mediated antiapoptotic signaling in cardiac myocytes. Insulin-like growth factor-1 activates phosphatidylinositol 3′-kinase and extracellular signal-regulated kinase pathways. Recent data showed that GATA-4 might be an important mediator of cardiac myocyte survival by endothelin-1 and hepatocyte growth factor. These growth factors, as well as mediators of growth factor-signaling, may be useful in therapeutic strategies against oxidative stress-induced cardiac injury. *Antioxid. Redox Signal.* 5, 741–749.

INTRODUCTION

THE HEART is subjected to oxidative stress during various pathological conditions, such as myocardial ischemia–reperfusion injury and anthracycline-induced cardiomyopathy. The uses of antioxidants have been explored for reducing cardiac damage induced by oxidative stress. More recently, growth factors, such as insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), endothelin-1 (ET-1), transforming growth factor-β1 (TGF-β1), and basic fibroblast growth factor (bFGF), have been shown to protect the heart against oxidative stress. Therefore, these growth factors appear to serve as "functional antioxidants." This article summarizes current knowledge of the mechanisms of growth factor-mediated protection against oxidative stress-induced cardiac myocyte apoptosis.

OXIDATIVE STRESS AND CARDIAC DAMAGE

Ischemic injury to myocardium in response to coronary occlusion remains to be the leading cause of death in the United States and other Western countries. Postischemic dysfunction persists after reperfusion despite restoration of normal coronary flow, and this may be mediated via the generation of reactive oxygen species (ROS). Evidence of the role of ROS in ischemia–reperfusion injury was obtained in experiments in which the administration of antioxidant enzymes, such as superoxide dismutase and catalase, were shown to enhance the recovery of cardiac functions after reperfusion (10). Production of free radicals was detected immediately after reperfusion as monitored by the electron paramagnetic resonance spectroscopy (11).

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The anthracycline antibiotics, including daunorubicin (DNR) and doxorubicin (Dox), have been used as effective cancer chemotherapeutic agents for >20 years. DNR is the first anthracycline developed and has been found to be useful against acute leukemia, whereas Dox was found to have more diverse use being effective also against solid tumors. Despite the usefulness of these agents in eliminating cancer cells, severe cardiotoxicity may occur in patients treated with anthracyclines. The mechanism by which anthracyclines cause irreversible myocardial damage may include the generation of ROS (57) and lipid peroxidation (1). The quinone moiety of the anthracyclines acts as a catalyst for the formation of ROS (16). The role of ROS in anthracycline cardiotoxicity was confirmed by a study of transgenic mice overexpressing catalase (35).

CARDIAC MYOCYTE APOPTOSIS

Myocyte loss during acute myocardial infarction may involve apoptotic cell death. In human infarcted myocardium, apoptotic myocytes were found in the hypoperfused border zone between the central infarct area and noncompromised myocardial tissue (65). Myocytes in the infarct regions have up-regulated levels of apoptotic regulatory proteins (49). Animal models of myocardial infarction suggest that 5–33% of myocytes are apoptotic within the central infarct area (7, 18). Caspase inhibitors reduce infarct size and improve acute functional parameters of the heart in rats, suggesting the role of apoptosis in myocardial infarction. Hypoxia and reoxygenation also induce apoptosis of cultured cardiac myocytes (23, 71).

Recent data demonstrated that anthracyclines induce cardiac myocyte apoptosis. In Dox-treated mice, Kang *et al.* (36) detected myocardial apoptosis. Dox also induced cardiac myocyte apoptosis in rats (5). Further, apoptosis has been demonstrated in response to treatment of cultured cardiac muscle cells with Dox (36) or DNR (40).

INSULIN-LIKE GROWTH FACTOR

IGF-1 is a 70-amino acid polypeptide of ~7.5 kDa that regulates cell differentiation and proliferation in a variety of cell types (20). In the heart, IGF-1 exerts multiple actions, such as regulating cardiac muscle cell growth, calcium signaling, and differentiation (62). IGF-1 has also been shown to protect the heart against oxidative stress. Buerke *et al.* (13) showed that an injection of IGF-1 attenuated myocardial ischemia–reperfusion injury in rats. Further, IGF-1 was found to attenuate the incidence of cardiac myocyte apoptosis. Similarly, overexpression of IGF-1 in transgenic mice protected cardiac myocytes from apoptosis (44). In cultured cardiac myocytes, IGF-1 attenuated apoptosis induced by serum withdrawal, Dox (75), or ethanol (15) by inhibiting the induction of Bax and the activation of caspase 3.

The mechanism of IGF-1-mediated cardiac myocyte survival appears to involve phosphatidylinositol 3′-kinase (PI 3-kinase) and extracellular signal-regulated kinase (ERK) (29, 60). Expression of constitutively active PI 3-kinase or Akt attenuates apoptosis of cardiac myocytes (47, 77). Experiments using neonatal rat cardiac myocytes by Wu *et al.* (77) demonstrated that IGF-1-mediated attenuation of Dox-induced apoptosis (Fig. 1A), as well as the activation of caspase 3, was inhibited by LY294002, an inhibitor of PI 3-kinase. Furthermore, the expression of a constitutively active mutant of PI 3-kinase (Ad5-p110*) attenuated the Dox-induced apoptosis (Fig. 1B). Fujio *et al.* (21) also reported that Akt activation protected cardiac myocytes against ischemia–reperfusion injury in mice. Known downstream targets of Akt include Bad. However, neither

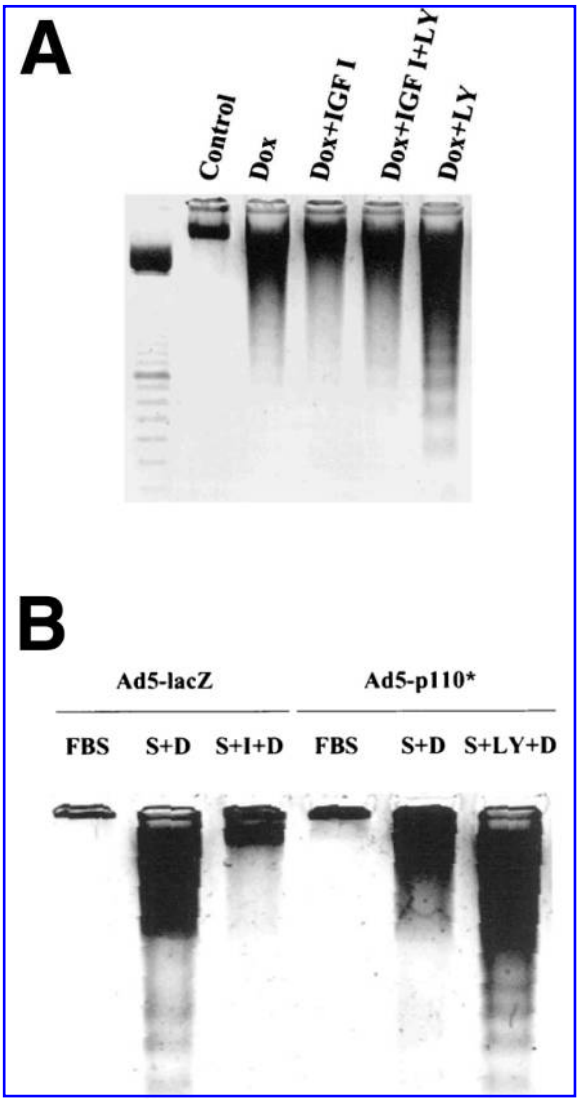

FIG. 1. Role of PI 3-kinase in IGF-1-mediated attenuation of apoptosis. (**A**) Cardiac myocytes were serum-deprived overnight, and apoptosis was induced by 0.4 μ*M* Dox for 6 h. IGF-1 and LY294002 were added 1 h before Dox treatment. (**B**) Cells were infected with adenovirus expressing β-galactosidase (Ad5-lacZ) or adenovirus expressing constitutively active PI 3-kinase (Ad5-p110*), serum-deprived overnight (S), and incubated with Dox (D), IGF-1 (I), and/or LY294002 (LY). Equal amounts of DNA were resolved with 1.5% agarose gel. Work of Wu *et al.* (77) is being republished with permission. FBS, fetal bovine serum.

IGF-1 nor constitutively active PI 3-kinase induced a phosphorylation of Bad (77), indicating that the downstream mechanism does not involve Bad. In transgenic mouse hearts, Yamashita *et al.* (78) demonstrated that disrupting the activation of Akt abolished the antiapoptotic effects of IGF-1 during ischemia–reperfusion injury.

The mitogen-activated protein kinase kinase (MEK)/ERK pathway has been implicated in the mechanism of cardiac myocyte survival. In cultured rat neonatal cardiac myocytes, Aikawa *et al.* (3) demonstrated that the inhibition of MEK by PD98059 induced apoptosis and potentiated hydrogen peroxide (H_2O_2)-induced apoptosis. PD98059 and a dominant negative mutant of MEK abolished the inhibition of serum deprivation-induced apoptosis by cardiotrophin 1 (67). Ping *et al.* (61) demonstrated that the prevention of cellular injury by ischemic preconditioning was abolished by PD98059 in adult rabbit cardiac myocytes subjected to simulated ischemia. ERK may regulate the phosphorylation state of an antiapoptotic protein Bcl-2 (30). Foncea *et al.* (19) reported that ERK, but not protein kinase C, is involved in protection of cardiac myocytes against apoptosis by IGF-1.

HEPATOCYTE GROWTH FACTOR

HGF was first found to be a potent mitogen of hepatocytes (53), and has been purified, cloned, and sequenced (54). The HGF receptor was identified as the c-*met* proto-oncogene product (12, 56). cMet is expressed in hepatocytes, epithelial cells, melanocytes, endothelial cells, microglial cells, neurons, and hemopoietic cells. Adult cardiac myocytes also express cMet (40, 74).

HGF appears to be an oxidative stress-inducible factor, as myocardial ischemia–reperfusion enhanced the expression of HGF in rats (58). Clinical studies showed that plasma HGF levels were elevated in patients with acute myocardial infarction (48, 66). Studies using animal models indicated that HGF is cardioprotective as HGF was found to attenuate myocardial ischemia–reperfusion injury in rats (55, 73) and rapid pacing-induced heart failure in a canine model (2). In adult rat ventricular myocytes, HGF was found to attenuate cell death induced by DNR (40) or H_2O_2 (74).

HGF has been shown to inhibit apoptosis in a number of noncardiac tissues. Revoltella *et al.* (63) reported that C2.8 mouse embryonic hepatocytic cells require exogenous HGF to survive and proliferate in serum-free medium. Apoptosis of hepatocytes induced by interferon-γ was inhibited by HGF (52). In human endometrial epithelial cells, HGF suppressed apoptosis induced by Fas antigen (72). A protective role of HGF in acute renal failure was suggested because HGF attenuated renal epithelial cell apoptosis (46). Liu (45) reported that HGF triggered a phosphorylation and the resultant activation of proapoptotic Bad via the PI 3-kinase/Akt pathway and simultaneously up-regulated antiapoptotic Bcl-x_L. HGF was shown to block the induction of apoptosis by various DNA-damaging agents, including Dox, x-rays, and ultraviolet radiation in breast cancer cells (17). In this study, HGF was shown to prevent the down-regulation of Bcl-x_L that was associated with the induction of apoptosis. These results suggest that PI 3-kinase-dependent inactivation of proapoptotic protein Bad and up-regulation of Bcl-x_L may be responsible for antiapoptotic action of HGF.

In cardiac myocytes, HGF was found to up-regulate Bcl-x_L (55), whereas this growth factor failed to activate Akt (74). Nakamura *et al.* (55) demonstrated that the administration of HGF in rats induced the expression of Bcl-x_L in cardiac myocytes as monitored by immunohistochemical staining (Fig. 2A) and by western blot analysis (Fig. 2B). To understand the mechanism of HGF-mediated Bcl-x_L up-regulation, our laboratory used HL-1 adult mouse rat cardiac muscle cells as a model. In these cells, HGF enhanced the Bcl-x_L expression, and this was inhibited by the adenovirus-mediated expression of a dominant negative mutant of MEK (Fig. 3A), suggesting that HGF signaling for Bcl-x_L expression may involve the MEK/ERK pathway (41). As the promoter/enhancer region of the *bcl*-x gene contains nuclear factor-κB (NF-κB) and GATA elements, we examined the effects of HGF on NF-κB and GATA factors. Results showed that HGF failed to activate the NF-κB DNA binding activity. However, as shown in Fig. 3B, HGF increased the DNA binding activity of GATA-4 via MEK

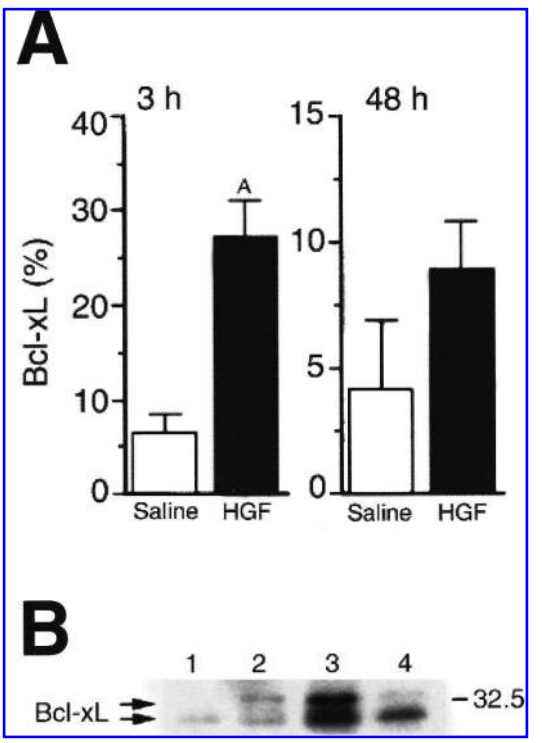

FIG. 2. HGF enhances Bcl-x_L expression. Rats were injected with HGF and subjected to left coronary artery ligation to elicit ischemia–reperfusion injury. Hearts were excised 3 and 48 h after reperfusion. (**A**) Immunohistochemical staining for Bcl-x_L. Bar graphs represent percentage of Bcl-x_L-positive myoyctes. $^Ap < 0.05$. (**B**) Changes in Bcl-x_L expression in myocardial extracts were detected by western blot. Lane 1, sham-operated left ventricle; lane 2, ischemia–reperfused left ventricle with saline treatment; lane 3, ischemia–reperfused left ventricle with HGF treatment; lane 4, normal rat spleen for positive control. Work of Nakamura *et al.* (55) is being republished with permission.

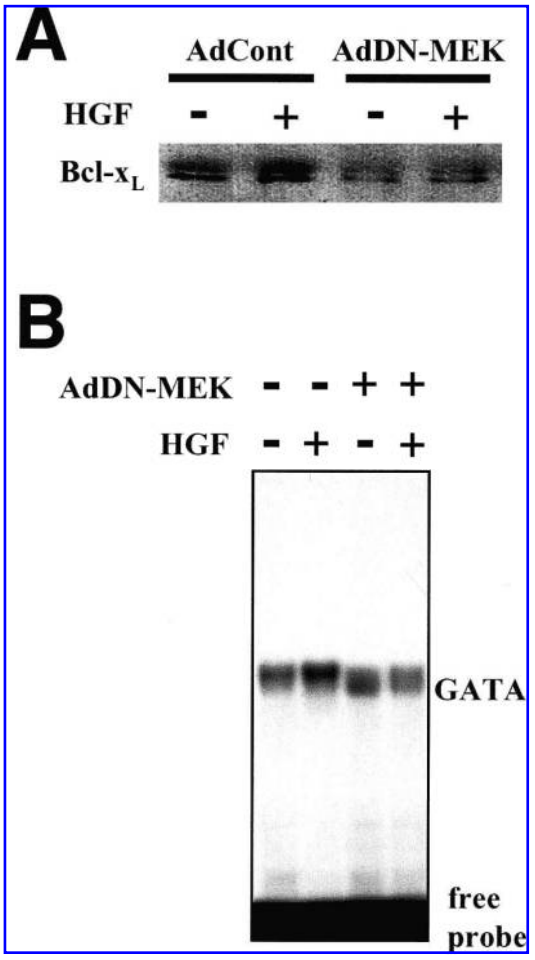

FIG. 3. Role of MEK in HGF-induced enhancement of Bcl-x_L expression and GATA-4 activation. (**A**) HL-1 cardiac muscle cells were infected with adenovirus expressing dominant negative MEK (AdDN-MEK), then treated with HGF for 24 h. Cell lysates were prepared and subjected to western blot analysis using a Bcl-x_L antibody. AdCont, adenovirus control. (**B**) HL-1 cells were infected with adenovirus expressing dominant negative MEK, then treated with HGF for 10 min. Nuclear extracts were prepared and subjected to electrophoretic mobility shift assays with oligonucleotide containing GATA binding sequence. Work of Kitta *et al.* (41) is being republished with permission.

(41). We also found that HGF phosphorylated a serine residue at position 105 in the GATA-4 molecule. Furthermore, adenovirus-mediated overexpression of GATA-4 promoted cardiac myocyte survival (38). Thus, HGF may elicit cell survival signaling via the activation of the MEK/ERK/GATA-4 pathway.

ENDOTHELIN-1

ET-1, a peptide composed of 21 amino acids, is a potent vasoconstrictor originally identified in vascular endothelial cells. ET-1 has also been shown to induce growth of vascular smooth muscle cells, as well as cardiac myocytes. In addition, ET-1 serves as a preconditioning agent against atrial and ventricular damage during ischemia–reperfusion injury (14). Levels of ET-1 in plasma and myocardium are increased in human and animal models of heart failure (33, 64). Recently, ET-1 has been shown to attenuate anthracycline-inducedcardiac myocyte injury (68). ET-1 exerts antiapoptotic activity in various cell types, including cardiac myocytes (4).

The mechanism of ET-1-mediated cardiac muscle cell survival was studied by Kakita *et al.* (34). In neonatal rat ventricular myocytes, ET-1 attenuated the apoptosis induced by H_2O_2. ET-1 also activated the calcineurin–NFAT (nuclear factor of activated T-cells) pathway, and calcineurin inhibitors (cyclosporin A and FK506) attenuated the ET-1 protection against H_2O_2-induced apoptosis. Further, H_2O_2 failed to induce apoptosis in cells expressing an activated calcineurin mutant. ET-1 activated Bcl-2 expression in a cyclosporin A-inhibitable fashion. These results suggest that ET-1 activates the calcineurin–NFAT pathway and enhances the expression of Bcl-2. NFAT has been shown to activate the GATA-4-dependent gene transcription (51). In HL-1 adult mouse cardiac muscle cells, ET-1-mediated activation of GATA-4 (39) is associated with its ability to partially protect against apoptosis induced by DNR as monitored by the neutral comet assay (Fig. 4). Thus, GATA-4 may be involved in ET-1-mediated cardiac muscle cell survival.

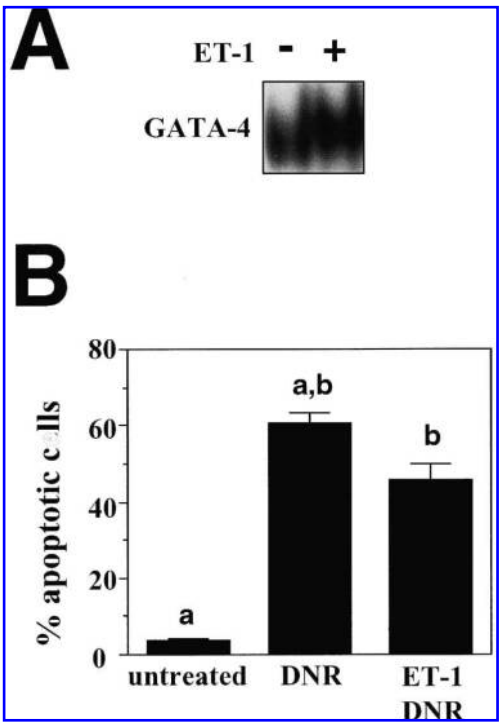

FIG. 4. Effects of ET-1 on GATA-4 activity and apoptosis. (**A**) HL-1 cells were treated with ET-1 (30 n*M*) for 10 min. Nuclear extracts from untreated and ET-1-treated cells were prepared and subjected to electrophoretic mobility shift assays with ^{32}P-labeled oligonucleotide containing the consensus GATA sequence. (**B**) HL-1 cells were pretreated with ET-1, then treated with DNR for 24 h. Apoptotic cells were identified using the neutral comet assay. Values represent means ± SE (n = 4). (a) and (b) denote the values that are significantly different from each other at $p < 0.05$.

FIBROBLAST GROWTH FACTOR

bFGF is a single-chain 18-kDa protein. The level of bFGF is increased in pericardial fluid of patients with ischemic heart disease (22). In porcine (27) and canine (31) models, bFGF was found to protect the heart against ischemic injury. In neonatal rat ventricular myocytes, Iwai-Kanai *et al.* (32) demonstrated that bFGF attenuated apoptosis induced by H_2O_2 or lipopolysaccharide, which activates inducible nitric oxide synthase. The bFGF-induced antiapoptotic effect was blocked by PD98059, a MEK inhibitor.

TRANSFORMING GROWTH FACTOR

TGF-β can also induce cardioprotection (43). Recently, TGF-β1 was also found to exert cardioprotective effects through its antiapoptotic activity. In cultured rat ventricular myocytes, Baxter *et al.* (6) found that TGF-β1 attenuated apoptosis induced by hypoxia–reoxygenation. Reduction of apoptosis was abrogated by PD98059, suggesting the role of the MEK/ERK pathway. These results were also obtained in intact rat hearts subjected to regional ischemia and reperfusion (6). The mechanism of antiapoptotic actions of TGF-β1 may involve its ability to up-regulate Bcl-2 (26).

GATA-4 AND CELL SURVIVAL

Studies of ET-1 (34) and HGF (41) suggest that GATA-4 may play an important role in cardiac muscle cell survival. GATA-4 is a member of the GATA family of zinc finger transcription factors, which plays important roles in transducing nuclear events that modulate cell lineage differentiation during development (50). Six GATA family members have been identified and shown to alter transcription of target genes via binding to the consensus 5′-WGATAR-3′ sequence. Three members of this family, GATA-4/5/6, are expressed in the heart. Functionally relevant GATA-binding sites have been identified in numerous cardiac transcriptional regulatory regions. A number of genes that contain GATA regulatory sites in their promoters have been shown to be up-regulated during cardiac hypertrophy, including those encoding α- and β-myosin heavy chains, myosin light chains, troponin C, Na^+–Ca^{2+} exchanger, angiotensin type 1a receptor, atrial natriuretic factor, and brain natriuretic factor. GATA-4 plays a crucial role in the development of cardiac hypertrophy and failure (50). Molkentin *et al.* (51) reported that calcineurin-induced cardiac hypertrophy may be mediated through an interaction between NFAT-3 and GATA-4. Studies using adenovirus expressing GATA-4 mutant have shown that signal transduction pathways for GATA-4 activation also involve a phosphorylation of the GATA-4 molecule (41).

In addition to its established role in cardiac hypertrophy, our studies indicated that GATA-4 may also play a role in cell survival of cardiac myocytes (39, 41). Thus, this transcription factor may also regulate pathophysiological conditions, such as myocardial ischemia–reperfusion injury, ischemic preconditioning, and environment- and drug-induced cardiomyopathy where apoptosis and survival of cardiac myocytes play an important role.

It has been reported that another member of the GATA family of transcription factors, GATA-1, exerts antiapoptotic activities. Weiss and Orkin (76) demonstrated that GATA-1-deficient erythroid precursors undergo apoptosis. In erythroleukemia cells, induction of apoptosis by estrogen was associated with estrogen receptor-dependent inhibition of GATA-1 (8, 9). Gregory *et al.* (24) reported that GATA-1 induces the expression of antiapoptotic protein Bcl-x_L. Consistently, the *bcl-x* gene has two GATA consensus motifs that reside in the 5′ promoter region (25). GATA-1 has also been shown to regulate the expression of Bcl-2 (70). GATA elements are found in promoters of other genes that may be involved in antiapoptotic activities, such as nitric oxide synthases (37) and antioxidant enzymes (59). These results suggest the role of GATA-1 in prevention of apoptosis and cell survival.

GATA-4 may play a similar role as apoptosis was found to be associated with a decrease in the expression of GATA-4 in ovarian cells (28). In isolated adult rat ventricular myocytes and in HL-1 cardiac muscle cells, we also found that apoptotic agents such as anthracyclines down-regulate the mRNA and protein expression of GATA-4 (38). Furthermore, overexpression of GATA-4 via adenovirus-mediated gene transfer attenuated cardiac myocyte apoptosis, and the expression of a dominant negative mutant of GATA-4 induced apoptosis (38). Adenovirus-mediated expression of wild-type and mutant GATA-4 suggested that Bcl-x_L is regulated by GATA-4 in cardiac myocytes (41). In HL-1 adult mouse cardiac muscle cells, GATA-4 is activated by cell survival factors such as HGF and ET-1 via MEK/ERK-dependent phosphorylation (41), whereas its expression is down-regulated by apoptotic agents like DNR, Dox (38), and nitric oxide (unpublished observations). More recently, work from my laboratory demonstrated that GATA-4 is an oxidative stress responsive transcription factor as the exposure of cells to mercury rapidly phosphorylated and activated GATA-4, which elicited cell survival signaling; this was followed by down-regulation of GATA-4 expression and the induction of apoptosis (69). Thus, GATA-4 can induce cell survival and the GATA-4 down-regulation may be involved in the mechanism of the induction of cardiac myocyte apoptosis.

SUMMARY AND CONCLUSIONS

Oxidative stress plays an important role in cardiac pathology during ischemia-reperfusion injury, hypoxia, sepsis, anthracycline chemotherapy, and exposure to heavy metals. Accumulating evidence suggests that various growth factors are able to protect cardiac myocytes against apoptosis induced by oxidative stress. In addition, many growth factors are released and produced in response to stress and may serve as endogenous cardioprotective factors during such situations as myocardial ischemic preconditioning, the most potent mechanism for cardioprotection. Exogenous administrations of these growth factors and mediators of growth factor signaling via pharmacological as well as genetic means may be useful for preventing cardiac muscle damage, which could occur during ischemia–

reperfusion injury, anthracycline-induced cardiomyopathy (Fig. 5), and other diseases, and environmental insult.

Mechanisms of growth factor-mediated cardioprotection are under intense investigation, and many growth factors appear to use PI-3 kinase/Akt and MEK/ERK pathways. Our laboratory identified GATA-4 as a target of the MEK/ERK pathway, which mediates cardiac myocyte survival at least in part via the regulation of antiapoptotic protein Bcl-x_L (38, 41). Although not discussed in this article, other signal transduction pathways, such as p38 motogen-activated protein kinase, Stat, and NF-κB, may also play roles in cardiac myocyte survival. Interactions of these growth factors with other endogenous mediators of cardioprotection, such as tumor necrosis factor (42), may also be important.

Further studies on understanding molecular mechanisms of growth factor signaling for cardioprotection should provide invaluable information to help therapeutic strategies against oxidative stress to myocardium.

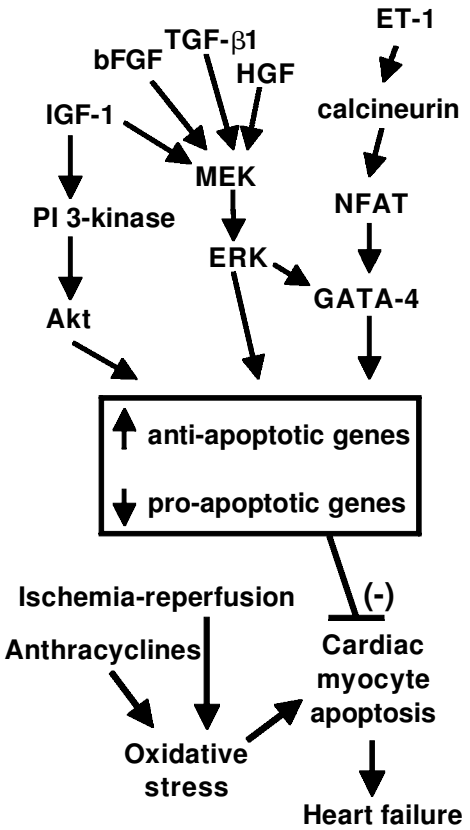

FIG. 5. Schematic representation of proposed growth factor signaling for protection of cardiac myocytes against oxidative stress-induced apoptosis. Pathological conditions such as ischemia–reperfusion injury and cardiac damage exerted by anthracycline cancer chemotherapeutic agents (DNR and Dox) cause oxidative stress, which in turn induces apoptosis of cardiac myocytes, resulting in heart failure. Growth factors are released in response to oxidative stress and induce signal transduction pathways exerting antiapoptotic actions to protect cardiac myocytes from being lost.

ACKNOWLEDGMENTS

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ABBREVIATIONS

bFGF, basic fibroblast growth factor; DNR, daunorubicin; Dox, doxorubicin; ERK, extracellular signal-regulated kinase; ET-1, endothelin-1; HGF, hepatocyte growth factor; H_2O_2, hydrogen peroxide; IGF-1, insulin-like growth factor-1; MEK, mitogen-activated protein kinase kinase; NF-κB, nuclear factor-κB; NFAT, nuclear factor of activated T-cells; PI 3-kinase, phosphatidylinositol 3′-kinase; ROS, reactive oxygen species; TGF-β1, transforming growth factor-β1.

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