Dominant-negative p38α mitogen-activated protein kinase prevents cardiac apoptosis and remodeling after streptozocin-induced diabetes mellitus

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Thandavarayan RA, Watanabe K, Ma M, Gurusamy N, Veeraveedu PT, Konishi T, Zhang S, Muslin AJ, Kodama M, Aizawa Y. Dominant-negative p38α mitogen-activated protein kinase prevents cardiac apoptosis and remodeling after streptozocin-induced diabetes mellitus. Am J Physiol Heart Circ Physiol 297: H911–H919, 2009. First published July 17, 2009; doi:10.1152/ajpheart.00124.2009.—The p38 mitogen-activated protein kinase (MAPK) is activated during heart diseases that might be associated with myocardial damage and cardiac remodeling process. Diabetic cardiomyopathy is associated with increased oxidative stress and inflammation. The purpose of this study was to investigate the role of p38α MAPK after experimental diabetes by using transgenic (TG) mice with cardiac-specific expression of a dominant-negative mutant form of p38α MAPK. The elevation of blood glucose was comparable between the nontransgenic (NTG) and TG mice. The expression of phospho-p38 MAPK and phospho-MAPK-activated protein kinase 2 levels were significantly suppressed in TG mice heart than in NTG mice after diabetes induction. Left ventricular (LV) dimension in systole was smaller, and the percent fractional shortening was higher in diabetic TG mice compared with diabetic NTG mice. In addition, diabetic TG mice had reduced cardiac myocyte diameter, content of cardiac fibrosis, LV tissue expressions of atrial natriuretic peptide, transforming growth factor β1, and collagen III compared with diabetic NTG mice. Moreover, LV expression of NADPH oxidase subunits, p22, p67, gp91, and Nox4, reactive oxygen species and lipid peroxidation levels were significantly increased in diabetic NTG mice, but not in diabetic TG mice. Furthermore, myocardial apoptosis, the number of caspase-3-positive cells, and the downregulation of antiapoptotic protein Bcl-XL were less in diabetic TG mice compared with diabetic NTG mice. In conclusion, our data establish that p38α MAPK activity is required for cardiac remodeling after diabetes induction and suggest that p38α MAPK may promote cardiomyocyte apoptosis by downregulation of Bcl-XL.

p38 mitogen-activated protein kinase; oxidative stress

Diabetes mellitus causes various cardiovascular complications, which have become the major cause of morbidity and mortality in the diabetic population (10). Diabetic cardiomyopathy is now well documented and is characterized by left ventricular (LV) remodeling, which involves both diastolic and systolic dysfunction (7, 47). Experimental models of diabetes mellitus, such as streptozocin (STZ)-induced type 1 diabetes mellitus, imitate the structural and cellular abnormalities of diabetic cardiomyopathy (39). These abnormalities include among others, cardiac apoptosis, hypertrophy, fibrosis, and cardiac inflammation, which lead to LV dysfunction (13–15, 37). However, the signaling pathways leading to the above cardiac changes are not well understood.

p38 Mitogen-activated protein kinase (p38 MAPK), a member of the MAPK family, is activated by physical and chemical stress factors, resulting in growth promotion, apoptosis, oxidative stress, and vasoconstriction (9, 27, 38, 41). The p38 MAPK contains different isoforms, such as α, β, δ, and ε (33), of which p38α MAPK is found to be the major isoform in human heart (21). The p38 MAPK cascade includes a MAPK kinase kinase, such as apoptosis signal regulating kinase-1, a MAPK kinase (MAPKK), such as MAPKK-3 (MKK3) or MAPKK-6 (MKK6), and a MAPK, such as p38α (33). Each protein in the cascade activates the subsequent kinase by phosphorylation of specific amino acid residues. Once activated, p38α MAPK phosphorylates a variety of intracellular targets, including transcriptions factors and protein kinases, and some of these targets may promote apoptosis (33). Chronic exposure of human mesangial cells to high glucose concentration activates the p38 MAPK pathway (46). High concentration of glucose in human endothelial cells induced apoptosis via activation of p38 MAPK-mediated bax-caspase pathway (28). p38 MAPK is activated by various stresses and is shown to play a pivotal role in the development of interstitial fibrosis in human and experimental diabetic nephropathy (2). Hyperglycemia aggravates pancreatic fibrosis in pancreatic stellate cells through activation of p38 MAPK pathway (30). We and others (13, 35) have revealed that p38 MAPK is activated in the diabetic myocardium, and pharmacological inhibition of p38 MAPK attenuates diabetes-induced LV dysfunction (45). Although previous studies (42, 45) have indicated the role of p38
MAPK in the diabetic myocardium, the specific role of p38 MAPK is not known.

In the present study, to clarify the role of p38α MAPK in experimental diabetes, we attempted to use p38α MAPK knockout mice in this study. The p38α MAPK knockout mice died during the embryonic development (1, 4, 36). Thus we used transgenic (TG) mice with cardiac-specific overexpression of dominant-negative (DN) mutant of p38α MAPK to examine the role of p38α MAPK in the diabetic myocardium. We speculated that DN p38α MAPK mice would be resistant to ventricular remodeling after experimental diabetes.

MATERIALS AND METHODS

Generation of TG mice. TG DN p38α MAPK mice in Swiss Black genetic background were generated, as described previously (49), at the Neuroscience Transgenic Facility of Washington University School of Medicine. Progeny were analyzed by polymerase chain reaction to detect transgene integration using mouse-tail DNA as template. TG mice were compared with nontransgenic (NTG) littermates in every experiment. Mice were maintained with free access to water and chow throughout the period of study, and animals were treated in accordance with the Guidelines for Animal Experimentation of our institute. All animals were handled according to the approved protocols and animal welfare regulations of the Institutional Review Board at Niigata University of Pharmacy and Applied Life Sciences.

Diabetes induction. Diabetes was induced by a single intraperitoneal injection of STZ (150 mg/kg body wt; Sigma-Aldrich, St. Louis, MO) dissolved in vehicle (20 mM sodium citrate buffer, pH 4.5) to 10- to 12-wk-old male TG and NTG mice. As a control, vehicle (100 μl of citrate buffer) was injected in age-matched TG and NTG mice. Random blood glucose measurements were performed using Medisafe chips (Terumo, Tokyo, Japan) at a 7-day interval. Animals were considered to be diabetic if they had a random blood glucose levels >300 mg/dl. All other physiological, anatomic, and biochemical studies were performed on animals at 28 days after STZ injection.

Transcardiac echocardiography. Two-dimensional echocardiography studies were performed in anesthetized mice (pentobarbital, 50 mg/kg ip) to evaluate cardiac function using a commercial system (Sonos 5500; Aloka, Tokyo, Japan). The short-axis view of the LV was recorded to measure the LV dimension in systole and diastole, as well as the percent fractional shortening (%FS). Hearts were harvested for analysis from control and diabetic mice. The LV was quickly dissected and cut into two parts. One part was immediately transferred into liquid nitrogen and then stored at −80°C for Western blotting analysis, all primary and secondary antibodies were used at a dilution of 1:1,000 and 1:5,000, respectively. Films were scanned, and band densities were quantified by densitometric analysis using Scion image software (Epson GT-X700, Tokyo, Japan).

Cytochrome c reduction assay. NADPH-dependent superoxide production was examined using superoxide dismutase (SOD)-inhibitable cytochrome c reduction (22). Total protein from myocardial tissue (final concentration 1 mg/ml) was distributed in 96-well plates (final volume 200 μl/well). Cytochrome c (500 μmol/l) and NADPH (100 μmol/l) were added in the presence or absence of SOD (200 U/ml) and incubated at room temperature for 30 min. Cytochrome c reduction was measured by reading absorbance at 550-nm wavelength on a microplate reader. Superoxide production was calculated from the difference between absorbance with and without SOD and extinction coefficient for change of ferricytochrome c to ferrocyanochrome c, i.e., 21.0 mmol−1·cm−1.

Thiobarbituric acid reactive substances assay. Levels of thiobarbituric acid reactive substances (TBARS) were measured in homogenates of heart tissues using a commercial kit (Oxitek, ZeptoMetrix, Buffalo, NY). Hearts that had been used in the functional experiments were homogenized in normal saline, and 100 μl of protein suspension was combined with 2.5 ml of thiobarbituric acid from the assay kit, incubated for 1 h at 85°C, cooled to room temperature, and centrifuged at 3,000 rpm for 15 min. The supernatant was analyzed by spectrophotometer (532 nm) for the reaction product between thiobarbituric acid and malondialdehyde (MDA), which latter results from lipid peroxidation. TBARS levels were expressed as nanomoles of MDA per milligram protein. The standard curve comprised 0–100 nmol/ml MDA.

Myocardial cell size measurement. Paraffin-embedded LV tissue sections stained with hematoxylin and eosin, were used for measuring cell size. Short-axis diameter of cardiac myocyte was measured for 10 myocytes selected per field (~50 fields were selected per section) at 400-fold magnification by light microscopy (CIA-102, Olympus, Tokyo, Japan). Each average value was obtained based on the data from 10 myocytes and was used as an independent sampling data (13, 15).

Measurement of myocardial fibrosis. The area of myocardial fibrosis in LV tissue sections stained with Azan-Mallory was quantified using a color image analyzer (CIA-102, Olympus, Tokyo, Japan) and measuring the blue fibrotic areas as opposed to the red mycardium at ×200 magnification. The results were presented as the ratio of the fibrotic area to the whole area of the myocardium (15). Digital photographs were taken using the color image analyzer (CIA-102; Olympus, Tokyo, Japan).

Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling assay. Frozen LV tissues embedded in OCT compound were cut into 4-μm-thick sections and fixed in 4% paraformaldehyde (pH 7.4) at room temperature. Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) apoptosis analysis was performed as specified in the in situ apoptosis detection kit (Takara Bio, Shiga, Japan), and sections were examined under fluorescence with horseradish peroxidase-coupled secondary antibodies (Santa Cruz Biotechnology) and chemiluminescence developing agents (Amersham Biosciences, Buckinghamshire, UK). The level of expression of each protein in control NTG mice was taken as one arbitrary unit. For Western blotting analysis, all primary and secondary antibodies were used at a dilution of 1:1,000 and 1:5,000, respectively. Films were scanned, and band densities were quantified by densitometric analysis using Scion image software (Epson GT-X700, Tokyo, Japan).
Table 1. Changes in blood glucose level, body and heart weights, HW/BW, and echocardiographic measurements in NTG and TG mice

<table>
<thead>
<tr>
<th></th>
<th>NTG</th>
<th>Diabetic</th>
<th>TG</th>
<th>Diabetic</th>
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<tr>
<td>Blood glucose level, mg/dl</td>
<td>137.6±3.1</td>
<td>561.6±26.3b,d</td>
<td>132.5±4.6</td>
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<td>Body weight, g</td>
<td>27.91±0.36</td>
<td>21.27±0.16d</td>
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<td>Heart weight, mg</td>
<td>109.0±0.002</td>
<td>90.5±0.002c</td>
<td>116.7±0.002</td>
<td>110.5±0.008</td>
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<tr>
<td>HW/BW, mg/g</td>
<td>3.90±0.04</td>
<td>4.26±0.10b</td>
<td>3.97±0.05</td>
<td>4.10±0.20</td>
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<tr>
<td>LVDd, mm</td>
<td>2.51±0.17</td>
<td>2.73±0.30</td>
<td>2.56±0.26</td>
<td>2.68±0.08</td>
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<tr>
<td>LVDs, mm</td>
<td>1.22±0.06</td>
<td>1.77±0.07b</td>
<td>1.1±0.06</td>
<td>1.23±0.06b</td>
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<tr>
<td>FS, %</td>
<td>52.07±3.56</td>
<td>36.22±3.44b,d</td>
<td>58.0±3.64</td>
<td>47.25±1.86c,c</td>
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Values are means ± SE (n = 10 animals). TG, transgenic; NTG, nontransgenic; control, nonstreptozotocin-induced age-matched mice; diabetic, mice 28 days after streptozotocin injection; HW/BW, the ratio of heart weight to body weight; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; FS, fractional shortening. *P < 0.05 vs. control NTG mice; †P < 0.01 vs. control NTG mice; ‡P < 0.05 vs. control TG mice; §P < 0.01 vs. control TG mice; #P < 0.05 vs. diabetic NTG mice; §§P < 0.01 vs. diabetic NTG mice.

RESULTS

Changes in body weight, heart weight, and ratio of heart weight to body weight. Changes in body weight (BW), heart weight (HW), and ratio of HW to BW (HW/BW) are shown in Table 1. BWs of TG and NTG mice were significantly decreased at 28 days after STZ injection, compared with control animals (Table 1). However, the decrease in BW was significantly less in TG mice compared with NTG mice. HW was reduced, but HW/BW was significantly increased in NTG mice at 28 days after STZ injection relative to its control animals, but the changes were not significant in TG mice (Table 1).

Microscopy at 200-fold magnification (CIA-102, Olympus, Tokyo, Japan). For each animal, five sections were scored for apoptotic nuclei.

Immunofluorescence. For immunofluorescence, tissues were fixed in 10% buffered formaldehyde solution and embedded in paraffin. Sections underwent microwave antigen retrieval, blocked with 10% goat serum in PBS, and incubated with polyclonal rabbit anti-caspase-3 antibody (Cell Signaling Technology). Binding sites of the primary antibody were revealed with fluorescein isothiocyanate conjugated secondary antibody (Sigma Aldrich). Samples were visualized with a fluorescence microscope at ×400 magnification (CIA-102, Olympus, Tokyo, Japan).

Statistical analysis. Data are represented as means ± SE. Statistical analysis among groups was determined by t-test or by two-way analysis of variance, followed by Tukey’s method. Differences were considered as statistically significant at P < 0.05.
analysis revealed that LV expression of phospho-p38 MAPK and p-MAPKAPK-2, a well described substrate of p38 MAPK, was significantly increased in NTG and TG mice at 28 days after STZ injection compared with respective control mice (Fig. 1). However, TG mice had significantly ($P < 0.01$) less p38 MAPK and MAPKAPK-2 activation compared with NTG mice (Fig. 1).

LV cardiomyocyte hypertrophy, interstitial fibrosis, and related protein expression. Increased average cross-sectional diameter of cardiac myocytes, indicating the LV cardiomyocyte hypertrophy, was evident in NTG mice at 28 days after STZ injection compared with control TG and NTG mice, and this increase was significantly ($P < 0.05$) attenuated in TG mice compared with NTG mice (Figs. 2, A–D and 3A). Furthermore, a marked increase in HW/BW was observed in NTG mice at 28 days after STZ injection (Table 1). Myocardial fibrosis was significantly elevated in NTG mice at 28 days after STZ injection compared with control TG and NTG mice (Figs. 2, E–H and 3B). These fibrotic changes in the heart were significantly reduced in TG mice ($P < 0.01$) compared with NTG mice at 28 days after STZ injection (Figs. 2, E and H, and 3B). The expression of molecular markers of cardiomyocyte hypertrophy, such as ANP and profibrotic protein collagen III and TGF-$\beta_1$, were also significantly elevated in NTG mice at 28 days after STZ injection relative to control TG and NTG mice, but significantly ($P < 0.05$) attenuated in TG mice relative to NTG mice (Fig. 4).

**TUNEL analysis, caspase-3 activation, and Bcl-xL expression.** The number of TUNEL-positive cells in LV sections was not differing between control TG and NTG mice (Figs. 2, I and K, and 3C), and, expectedly, the number of TUNEL-positive cells was markedly increased in LV sections of NTG mice after diabetes induction relative to control mice (Figs. 2, I–K, and 3C). Interestingly, the number of TUNEL-positive cells after diabetes induction was much less in TG mice ($P < 0.01$) than in NTG mice (Figs. 2, J and L, and 3C). As shown in Figs. 2, M–P, and 3D, NTG mice displayed significantly higher caspase-3-positive cells compared with control TG and NTG mice at 28 days after STZ injection. In contrast, the number of caspase-3-positive cells did not differ significantly between diabetic TG and control mice. Furthermore, the level of Bcl-xL expression was significantly downregulated in NTG mice, but...
not in TG mice, at 28 days after STZ injection compared with its respective genetic controls (Fig. 5).

**Electron spin resonance spectrometric analyses.** Electron spin resonance (ESR) spectroscopic analyses showed that the formation of *OH signals in heart homogenates of diabetic TG mice was lower compared with diabetic NTG mice heart homogenates (Fig. 6, A and B). The *OH signals relative to the internal standard of manganese ion in diabetic TG mice were lower compared with diabetic NTG mice (Fig. 6, A and B). No signals were detected in respective genetic control animals.

**STZ injection induces ROS and oxidative stress.** Diabetic cardiomyopathy has been reported to be associated with enhanced ROS generation and oxidative stress (5). NADPH-dependent O$_2^\cdot$ production by LV homogenates assessed by the cytochrome c reduction was significantly increased in the hearts of diabetic NTG mice compared with its respective genetic controls (Fig. 6C). Interestingly, the ROS production was significantly lower in the hearts of diabetic TG mice compared with diabetic NTG mice (Fig. 6C). Cardiac lipid peroxidation was determined by TBARS assay. TBARS was significantly increased in the NTG diabetic heart, but not in the TG diabetic heart compared with the control animals (Fig. 6D). Because NADPH oxidase is the main source of ROS in the cardiovascular tissues (20, 22, 25, 26, 40), we next measured

**Fig. 3.** Bar graph shows quantitative analysis of cardiomyocyte cell diameter (A), interstitial fibrosis (B), cardiomyocyte apoptosis (C), and caspase-3 positive cells (D) in control and diabetic myocardium, respectively. Open and solid bars represent NTG and TG mice, respectively. Each bar represents means ± SE (n = 4–5). *P < 0.05 vs. control NTG mice; **P < 0.01 vs. control NTG mice; #P < 0.05 vs. control TG mice; ###P < 0.01 vs. control TG mice; $P < 0.05$ vs. diabetic NTG mice; $$P < 0.01$ vs. diabetic NTG mice.

**Fig. 4.** LV atrial natriuretic peptide (ANP), transforming growth factor (TGF)-β1, and collagen III expression in control and diabetic mice. Representative Western immunoblots (A) and densitometry analysis (B–D) using Scion image software are shown for ANP (A and B), TGF-β1 (A and C), and collagen III (A and D) normalized against GAPDH. Open and solid bars represent NTG and TG mice, respectively. Each bar represents means ± SE (n = 4–5). **P < 0.01 vs. control NTG mice; ###P < 0.01 vs. control TG mice; $P < 0.05$ vs. diabetic NTG mice; $$P < 0.01$ vs. diabetic NTG mice.
the protein expression of $\text{p22}_{\text{phox}}$, $\text{p47}_{\text{phox}}$, $\text{p67}_{\text{phox}}$, $\text{gp91}_{\text{phox}}$, and $\text{Nox4}$ in the hearts of control and diabetic animals. Myocardial expression of $\text{p22}_{\text{phox}}$, $\text{p67}_{\text{phox}}$, $\text{gp91}_{\text{phox}}$, and $\text{Nox4}$ were significantly elevated in NTG mice, but not in TG mice, at 28 days after STZ injection compared with its respective genetic controls (Fig. 7). There was no significant difference in $\text{p47}_{\text{phox}}$ protein expression in diabetic and control groups (data not shown). These data suggest that the increased cardiac superoxide production, lipid peroxidation level, and NADPH oxidase subunits may contribute to the increased ROS, which caused the cardiac damage in diabetic mice.

**DISCUSSION**

Cardiac remodeling occurs in response to diverse pathophysiological stimuli, such as oxidative stress, cardiomyopathy, hypertension, and ischemic heart disease. Experimental models of diabetes mellitus, such as STZ-induced type 1 diabetes mellitus, imitate the structural and cellular abnormalities of diabetic cardiomyopathy (39). These abnormalities include, among others, cardiac apoptosis, hypertrophy, fibrosis, and cardiac inflammation, which lead to LV dysfunction (13–15, 37). The p38 MAPK family is activated by physical and chemical stress factors, resulting in growth promotion, apoptosis, oxidative stress, and vasoconstriction (9, 27, 38, 41). The p38 MAPK present as four different isoforms, such as $\alpha$, $\beta$, $\delta$, and $\varepsilon$ (33). p38$\alpha$ MAPK is found to be the major isoform and highly detectible in human heart (21). Although studies have implicated the role of p38 MAPK (42, 45), the specific role of p38$\alpha$ MAPK in the diabetic myocardium is not known. Our study results indicate that cardiac-specific overexpression of DN p38$\alpha$ MAPK significantly attenuated LV dysfunction, cardiac apoptosis, hypertrophy, and fibrosis associated with diabetic cardiomyopathy. These results indicate that p38$\alpha$ MAPK isoform plays an important role in the mediation of cardiac remodeling processes associated with diabetic cardiomyopathy.

The role of p38 MAPKs in cardiac hypertrophy has been suggested by studies using overexpressed, active forms of their upstream activators MKK3 and MMK6 in cardiomyocytes. In these studies, it was found that the active mutants elicited characteristic hypertrophic responses (43, 48). Recently, we have reported that diabetic cardiomyopathy has been associated with LV cardiomyocyte hypertrophy and fibrosis (13, 15, 37). The cardioprotective effects of insulin against ischemia-

Fig. 5. Representative Western immunoblots (A) and densitometry analysis (B) using Scion image software for Bcl-X$_{L}$ in control and diabetic mice. Blots were normalized against GAPDH. Open and solid bars represent NTG and TG mice, respectively. Each bar represents means $\pm$ SE ($n = 4–5$). **$P < 0.01$ vs. control NTG mice; $\#P < 0.01$ vs. control TG mice; $\#\#P < 0.01$ vs. diabetic NTG mice.

Fig. 6. STZ injection elevates the myocardial ROS and oxidative stress. A and B: representative electron spin resonance spectra and analysis of the hydroxyl radical signal relative to the internal standard of manganese ion. Hydroxyl radical signals were not detected (ND) in hearts of control animals. Mn (3) and Mn (4) indicate the internal standard signals of manganese ion (Mn$^{2+}$). C: superoxide production by LV homogenates of control and diabetic animals. D: bar graph shows malondialdehyde (MDA) levels in control and diabetic mice. Open and solid bars represent NTG and TG mice, respectively. Each bar represents means $\pm$ SE ($n = 4–5$). **$P < 0.01$ vs. control NTG mice; $\#P < 0.01$ vs. control TG mice; $\#P < 0.05$ vs. diabetic NTG mice. $\#\#P < 0.01$ vs. diabetic NTG mice.
reperfusion injury are abolished by the activation of p38 MAPK (6). Cardiac phosphorylation of p38 MAPK was significantly reduced by atorvastatin in STZ-induced diabetic animals and improves cardiac function (42). Reorganization of actin cytoskeleton induced during cellular stresses is mediated via the p38 MAPKAPK-2-HSP27 pathway (12, 16). In this study, we found enhanced activation of p38 MAPK and its downstream effector, p38 MAPKAPK-2 in NTG group mice relative to TG mice, after diabetes induction. p38 MAPK activation contributes for the negative inotropic and restrictive diastolic effect and in the development of ventricular end-systolic remodeling (24). High glucose can induce the activation of p22phox, a component of the NADPH oxidase, via de nova synthesis of diacyl glycerol (17). We also found that the myocardial expression of p22phox, p67phox, gp91phox, and Nox 4 were significantly increased in NTG mice at 28 days after STZ injection. Moreover, NTG mice had higher level of ROS content and lipid peroxidation after diabetes induction relative to TG mice. These results show that oxidative stress was elevated in diabetic myocardium. The development of LV hypertrophy in diabetic NTG mice were confirmed with distinct LV chamber dilation, decreased %FS, increased HW/BW, and increased LV expression of ANP. However, the above cardiac dysfunctions were significantly attenuated in TG mice. These results clearly indicate that p38α MAPK plays a significant role in the oxidative stress, cardiac dysfunction, and development of LV cardiomyocyte hypertrophy after experimental diabetes.

Pharmacological inhibition of p38 MAPK significantly attenuated the expression of cardiac inflammatory markers, such as TNF-α, IL-1β, and IL-6, and collagen content associated with diabetic cardiomyopathy (45). Inhibition of p38α MAPK has inhibited the collagen production, and in vitro studies have demonstrated that TGF-β1-induced collagen expression in myoblasts is mediated via p38 MAPK activation (32). TGF-β1-induced extracellular matrix production by neonatal rat primary cardiac fibroblasts is inhibited by SB-203055, an inhibitor of the p38 MAPK pathway (3), and activated mutants of MKK3 and MKK6, both upstream kinases of the p38 MAPK pathway, have been associated with an increase in cardiac fibrosis (24). In this study, the percentages of cardiac myocyte fibrosis, as well as the expression of cytokine TGF-β1, and extracellular fiber collagen III were significantly elevated in NTG mice at 28 days after STZ induction. Moreover, NTG mice had higher level of ROS content and lipid peroxidation after diabetes induction relative to TG mice. These results show that oxidative stress was elevated in diabetic myocardium. The development of LV hypertrophy in diabetic NTG mice were confirmed with distinct LV chamber dilation, decreased %FS, increased HW/BW, and increased LV expression of ANP. However, the above cardiac dysfunctions were significantly attenuated in TG mice. These results clearly indicate that p38α MAPK plays a significant role in the oxidative stress, cardiac dysfunction, and development of LV cardiomyocyte hypertrophy after experimental diabetes.

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During ischemia in perfused heart, inhibition of p38 MAPKs activity protects against hypoxic-induced apoptosis and necrosis. Hypoxia was also found to lead to intracellular acidosis, which augments p38α activation and leads to apoptotic cell death (50). ROS generated from the mitochondria during ischemia and reperfusion activates p38 MAPK, and inhibition of p38α significantly prevented cell death arising from ischemia-reperfusion (19). High concentration of glucose in human endothelial cells induced apoptosis via activation of p38 MAPK-mediated bax-caspase pathway (28), and it has been suggested that TNF-induced p38 MAPK-mediated phosphorylation of Bcl-XL, in endothelial cells leads to degradation of Bcl-XL by proteasomes and subsequent induction of apoptosis (11). We observed a marked reduction in cardiomyocyte apoptosis and caspase-3-positive cells in TG mice after diabetes induction. The reduction in apoptosis observed in TG cardiac tissue may be a consequence of reduced downregulation of Bcl-XL. The precise mechanism by which p38α MAPK mod-
ulates Bcl-XL downregulation remains to be determined. Moreover, Fiordaliso et al. (8) reported that myocyte death in STZ-induced diabetes is angiotensin (ANG) II dependent, and we have also observed marked elevation in ANG II levels with significant cardiac apoptosis after diabetes induction (13) and p38 MAPK phosphorylation (activation). It is, therefore, possible that cardiac apoptosis associated with diabetes may also be mediated by the ANG II-p38 MAPK-Bcl-XL axis.

There are several potential limitations to be acknowledged in this study. First, our high-dose STZ model resembled more like type 1 diabetes, and, therefore, our results may not be applied to other forms of diabetes. Additionally, the relationship between ventricular performance and ongoing myocyte death and accumulated cell loss was analyzed only at 4 wk after the induction of diabetes. Longer intervals must be examined to establish whether chronic myocyte loss leads to cardiac dysfunction in this model. Furthermore, the ROS production assay was performed using tissue homogenates, which is another limitation. However, ROS production in the cellular levels was not performed in the present study, and previous studies using this method have repeatedly documented that diabetes heart is indeed associated with increased ROS production (44). Finally, the lack of pharmacological intervention was a major limitation of the present study that could have more strongly demonstrated the role of p38α MAPK in diabetic cardiomyopathy. Although these limitations must be considered when the accumulated results are interpreted, the current observations point to the p38α MAPK as a determinant of the development of diabetic cardiomyopathy.

Taken together, our results suggest that inhibition of p38α MAPK may represent a useful therapeutic target to ameliorate cardiac dysfunction, apoptotic cell death, and remodeling, such as hypertrophy and fibrosis associated with diabetic cardiomyopathy. Our data support the pharmacological inhibition of p38 MAPK as a new promising therapeutic strategy regarding the prevention of diabetic complications. However, further studies have to prove whether these findings can be translated to human conditions.

The results of this study provide preliminary evidence that p38α MAPK inhibitors may be useful for the treatment of diabetic cardiomyopathy. Our laboratory’s previous work and the work of other groups also support the use of p38α MAPK inhibitors to limit pathological cardiac remodeling after myocardial infarction (23, 31, 34). Although numerous p38α MAPK inhibitors have been developed, none is currently approved for use in human patients, and clinical trials evaluating the efficacy of these agents in cardiovascular disease have not been published (18).

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