Regulation of cardiomyocyte apoptosis by redox-sensitive transcription factors

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Abstract Reperfusion of ischemic myocardium results in apoptotic cell death and DNA fragmentation. Several transcription factors are known to regulate the apoptotic cell death. This study sought to examine the regulation of cardiomyocyte apoptosis by these transcription factors. Isolated working rat hearts were divided into six groups: control, 15 min ischemia, 60 min ischemia, 15 min ischemia followed by 2 h reperfusion, ischemic stress adaptation by subjecting the hearts to four cyclic episodes to 5 min ischemia, each followed by 10 min of reperfusion, and adaptation followed by 15 min ischemia and 2 h reperfusion. Redox-regulated transcription factors, NFκB and AP-1 and the expression of two anti- and pro-apoptotic genes, Bcl-2 and p53 were determined. The results demonstrated NFκB and AP-1 progressively and steadily increased as a function of the duration of ischemia. In the adapted heart, NFκB binding remained high while AP-1 binding was lowered to almost baseline value. The anti-oxidant gene, Bcl-2 was downregulated in the ischemic/reperfused heart, but upregulated in the preconditioned myocardium. Significant induction of the expression of p53 occurred after ischemia and reperfusion. Apoptotic cells were barely detected in the adapted myocardium which was subjected to the same ischemia/reperfusion protocol. The results demonstrate for the first time differential regulation of cardiomyocyte apoptosis by pro- and anti-apoptotic transcription factors and genes as a function of different durations of ischemia and reperfusion.

Key words: Ischemia/reperfusion; NFκB; AP-1; Bcl-2; p53; Myocardium

1. Introduction

Programmed cell death or apoptosis is recognized as a physiological counter part of cell replication, and is the contributing cause of cardiomyocyte cell death during ischemia/reperfusion, myocardial infarction, and heart failure [1,2]. It is an energy requiring process; needs de novo pro apoptotic gene expression (p53, bax etc.), and directed by an in born genetic program. The terminal result of this program is the fragmentation of nuclear DNA which is associated with ultrastructural changes in cellular morphology, while the functional integrity of the cell membrane still remains intact [3]. However, the exact mechanisms by which apoptosis is initiated in vivo still remains unclear. Our initial studies have demonstrated increased apoptosis in concert with nuclear fragmentation in the ischemic reperfused myocardium [4]. Myocardial adaptation to ischemic stress by repeated ischemia and reperfusion inhibited apoptotic cell death and DNA laddering apparently by reducing oxidative stress [5].

There are two well-known redox-regulated proteins, NFκB and AP-1, which participate in the regulation of apoptosis in some cell types [6]. The transcription factor NFκB, a nuclear protein of the Rel oncogene family, is involved in the regulation of numerous genes [7]. It exists in the cytoplasm as an inactive form, and stabilized by an inhibitory subunit IκB, which inhibits its DNA binding activity. The transcriptional factors c-Jun and c-Fos form heterodimers or homodimers which binds to DNA and the complex formed by this proteins is AP-1 [8]. Recent studies have revealed that like AP-1, NFkB also stimulates or inhibits oxidative stress induced apoptosis in a trigger-dependent or a cell type specific manner [9]. In various cell lines wild-type-P53 (transcription factor, pro-apoptotic gene), induced by DNA damage has been shown to induce programmed cell death or apoptosis. It is reported that p53 protein functions as an active transcription factor in lesioned brain [10]. How activation of p53 promotes apoptosis is unclear, but it might involve Bax [11], a series of p53-inducible genes [12] or signaling through Fas-related pathways [13]. There are other p53 effectors which include caspases that execute apoptotic cell death [14]. Several studies on the master controller gene of apoptosis, p53, reveal conflicting results [15]. The proteins bcl-2 (anti-apoptotic or anti-death) and bax (pro-apoptotic) have important regulatory influences.

No study has been performed to show the behavior of these redox-sensitive transcription factors during different durations of ischemia and reperfusion. To explore molecular mechanisms involved in apoptosis, redox sensitive transacting molecules, AP-1, NFκB as well as p53 and bcl-2 were determined in the heart as a function of ischemic and reperfusion time. In order to determine how these redox-regulated molecules govern apoptosis, cardiomyocyte apoptosis was also evaluated during different periods of ischemia, reperfusion and ischemic preconditioning.

2. Materials and methods

2.1. Chemicals

p65 antibody was obtained from Santa Cruz Biotechnological Co., CA, USA. Gel-Shift Assay kits for NFκB and AP-1 were purchased from Promega, Madison, WI, USA. cDNA probe for bcl-2 was obtained from Oncor, Gaithersburg, MD, USA. P53 anti-sense and sense primers were purchased from Stratagene, La Jolla, CA, USA.

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DNA labelling kit was purchased from Boehringer Mannheim, Indianapolis, IN, USA. [γ-^{32}p]ATP and [α-^{32}P]dCTP were obtained from Amersham, Arlington Heights, IL, USA. All other chemicals were of high purity and obtained from Sigma Chemical Co., St. Louis, MO, USA.

2.2. Isolated perfused heart preparation

Isolated perfused heart preparation was performed [16]. The hearts were divided into six groups, six in each group. After stabilization the hearts were perfused with KHB buffer for 195 min (Group I), Group II hearts were subjected to 15 min of global ischemia, Group III hearts were subjected to 60 min of ischemia only, Group IV hearts were perfused with KHB for 1 h and then subjected to 15 min of global ischemia followed by 120 min of reperfusion. In Group V, the hearts were subjected to ischemic preconditioning by repeated ischemia and reperfusion. For this, global ischemia was induced for 5 min followed by 10 min of reperfusion, repeating the process four times (4×PC) as described previously [17]. Group VI hearts were subjected to 4×PC followed by 15 min ischemia and 120 min of reperfusion. The first 10 min of reperfusion was in the retrograde mode to allow for post-ischemic stabilization and thereafter in the antegrade working mode to allow for assessment of functional parameters. For NFκB and AP-1 binding activity as well as for Bcl-2 and p53 gene expression, left ventricles from the control and experimental hearts were kept frozen at liquid nitrogen temperature. The extent of myocardial apoptosis was also evaluated in the heart after each experiment.

2.3. Electrophoretic mobility shift assay (EMSA) for NFκB and AP-1

Gel shift assay was performed according to the manufacturer's (Promega) protocol with slight modification. Nuclear proteins were isolated from the heart and gel-shift assays for NFκB and AP-1 were performed [18].

2.4. Northern blot analysis of Bcl-2

Total RNA was extracted from the heart tissues by the acid-guanidinium thiocyanate-phenol-chloroform method as described previously [19]. The results of densitometric scanning were normalized relative to the signal obtained by using GAPDH cDNA.

2.5. RT-PCR for P53

The cDNA was synthesized by reverse transcription reaction (255 μl) of 1 μg of total RNA isolated from control, ischemic, ischemic/reperfused and adapted rat myocardium. The resulting cDNAs were amplified by PCR using 1.5 μl of the RT reaction product in PCR buffer containing 2.5 units *Taq* DNA polymerase (Boehringer Mannheim, Indianapolis, IN, USA), 200 mM dNTPs and 0.6 mM of sense (5′-CCTGCCCTCAACAAGATGTTTTG-3′) and antisense (5′-TCAAAGCTGTTCCGTCCCAG-3′) primer of P53 and sense 5′-TGGAATCCTGTGGCATCCATGAAAC-3′ and antisense 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′ primers of β-Actin (as internal control) for 25 cycles in a thermal cycler. When the sequence for β-actin was used in the initial cycle, denaturation was extended to 4 min. Subsequent cycles were: denaturation 94°C 30 s; annealing 58°C 30 s; elongation 72°C 30 s; and final extension 72°C 10 min. After amplification, 10 μl of each PCR product, along with a ladder of known molecular weights were electrophoresed in TBE buffer in a 2% agarose gel containing 1 mg/ml ethidium bromide, and photographed with a Kodak DC120 Digital Camera Dedicated system which provided photographs and semiquantitative measures of the fluorescent intensity of the bands. Quantitation of the data was done by densitometric scanning using β-actin as a control.

2.6. Evaluation of apoptosis

Immunohistochemical detection of apoptotic cells was carried out using TUNEL [20] assay method in conjunction with an antibody against myosin heavy chain to specifically identify apoptotic cardiomyocytes.

2.7. Statistical analysis

For statistical analysis, a two-way analysis of variance (ANOVA) followed by Scheffe's test was first carried out using Primer Computer Program (McGraw-Hill, 1988) to test for any differences between groups. If differences were established, the values were compared using Student's *t*-test. The values were expressed as mean ± S.E.M. The results were considered significant if P was less than 0.05.

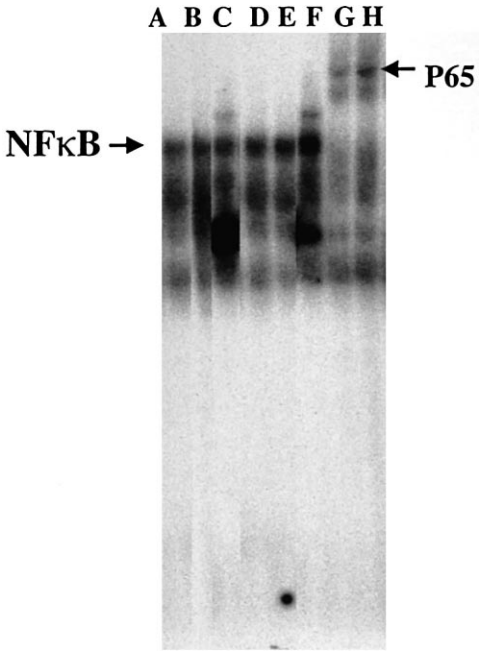

Fig. 1. Effect of different durations of ischemia, ischemia/reperfusion and ischemic preconditioning on the DNA binding activity of NFκB. Lane A = perfused control heart, lane B = 15 min ischemia, lane C = 60 min ischemia, lane D = 15 min ischemia and 120 min reperfusion, lane E = PC, lane F = PC+120 min reperfusion, lanes G and H = Band shift (super) by using P65 antibody. Results are representative of at least six different experiments per group.

3. Results

3.1. The NFκB binding activity during different duration of ischemia, ischemia reperfusion and ischemic preconditioning

NFκB binding activity was found to be very low in non-ischemic control hearts (Fig. 1A). Global ischemia for 15 and 60 min significantly increased the translocation of NFκB from the cytosol to the nucleus (Fig. 1B,C). NFκB binding activity was also increased significantly (5-fold, Fig. 1F) for the ischemically preconditioned hearts when compared to the ischemic reperfused myocardium (Fig. 1D). To confirm the specificity of NFκB binding activity, we performed supershift assays with polyclonal antibody recognizing NFκB p65 subunit (as shown in lanes 1G and 1H).

3.2. The consensus AP-1 binding activity during different duration of ischemia, ischemia/reperfusion and ischemic preconditioning

EMSA indicated increased AP-1 binding activity in the ischemic heart (15 min as well as 60 min) rat heart (Fig. 2, lane B and lane C) compared the to control perfused Group (lane A). Reperfusion of 15 min ischemic heart for 120 min also demonstrated significantly higher AP1 binding activity compared to the control. In the ischemically adapted groups (4×PC), the binding activity of AP-1 was significantly affected. The densitometry scanning by image analyzer showed significant decrease in its binding activity in the ischemic preconditioned group compared to the ischemic as well as the ischemic/reperfused group (the value is similar to the control group).

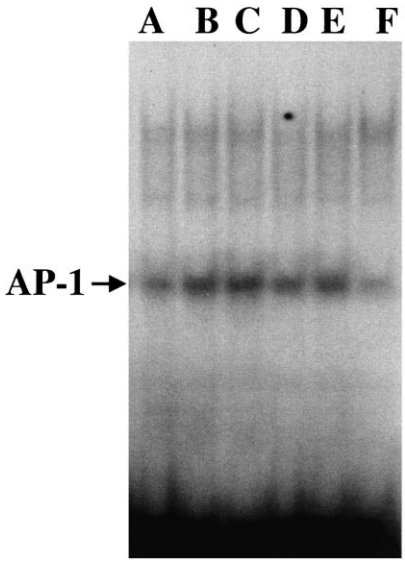

Fig. 2. Effect different durations of ischemia, ischemia/reperfusion and ischemic preconditioning on AP1 activity. Where lane A = perfused control heart, lane B = 15 min ischemia, lane C = 60 min ischemia, lane D = 15 min ischemia and 120 min reperfusion, lane E = PC, lane F = PC+120 min reperfusion. Results are representative of at least six different experiments per group.

3.3. The expression of p53 during ischemia, ischemia/reperfusion and ischemic preconditioning

The level of of p53 mRNA was assessed by performing RT-PCR on samples prepared from control perfused, 15 min and 60 min of ischemia, ischemic-reperfused, and ischemic preconditioned rat myocardium. The results of densitometric scanning (means ± S.E.M.) for six different experiments for each time points are shown with the blot. Transcript of p53 was barely detectable in the control perfused (Fig. 3A), ischemic (Fig. 3B,C) and preconditioned (Fig. 3E,F) hearts. Maximal induction was achieved in the ischemic-reperfused myocardium (Fig. 3D). β-Actin RT-PCR products, analyzed as internal control, were unchanged during the course of the experiments.

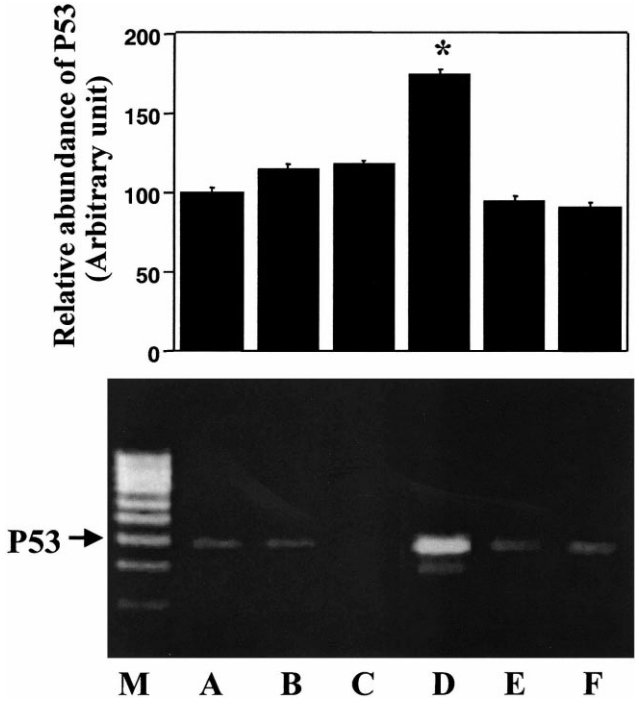

Fig. 3. Effect of different durations of ischemia, ischemia/reperfusion and ischemic preconditioning on the relative abundance of P53 transcripts in heart tissue. Lane M = DNA molecular weight marker. lane A = perfused control heart, lane B = 15 min ischemia, lane C = 60 min ischemia, Lane D = 15 min ischemia and 120 min reperfusion, lane E = PC, lane F = PC+120 min reperfusion. $*P < 0.05$ as compared to the baseline control.

3.4. The expression of Bcl2 during ischemia, ischemia/reperfusion and ischemic preconditioning

Northern blot analysis revealed Bcl-2 gene upregulation in ischemically adapted hearts (4×PC, 4×PC+ischemia/reperfusion, Fig. 4E,F) compared to the control hearts. To the contrary, prolonged reperfusion (2 h) after acute ischemia (15 min) downregulated Bcl-2 gene significantly as shown in Fig. 4D. Ischemia alone had no influence on Bcl-2 gene expression as shown in Fig. 4B,C. The results of densitometric scanning (means ± S.E.M.) for six different experiments for each time point are now shown with the blot.

3.5. Evaluation of apoptotic cells during ischemia, ischemia/reperfusion and preconditioning

We were unable to detect apoptotic cardiomyocytes in the control and in the ischemic hearts which were not subjected to reperfusion (Fig. 5A). Even 60 min of global ischemia could not induce apoptosis, there was no sign of fragmented nuclear DNA in these biopsies. Apoptotic cells were identified only in the reperfused hearts. The extent of apoptosis increased with the progression of reperfusion time. Apoptotic cardiomyocytes were first evidenced after 90 min of reperfusion. The number of apoptotic cells increased (24%) after 2 h reperfusion as evidenced from the immunohistochemical staining of

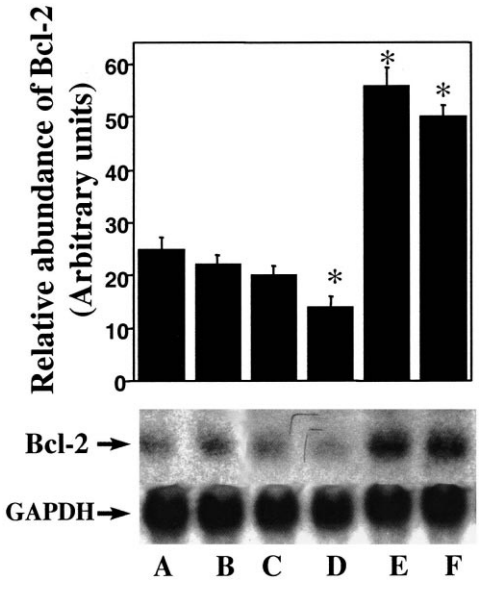

Fig. 4. Effect of different durations of ischemia, ischemia/reperfusion and ischemic preconditioning on the relative abundance of Bcl-2 mRNA in heart tissue by Northern blot. Analysis as described in Section 2. The results of densitometric scanning (mean ± S.E.M.) for six different experiments for each time point are shown by bar graph. Where A = perfused control heart, B = 15 min ischemia, C = 60 min ischemia, D = 15 min ischemia and 120 min reperfusion, E = PC, F = PC+120 min reperfusion. $*P < 0.05$ as compared to the baseline control.

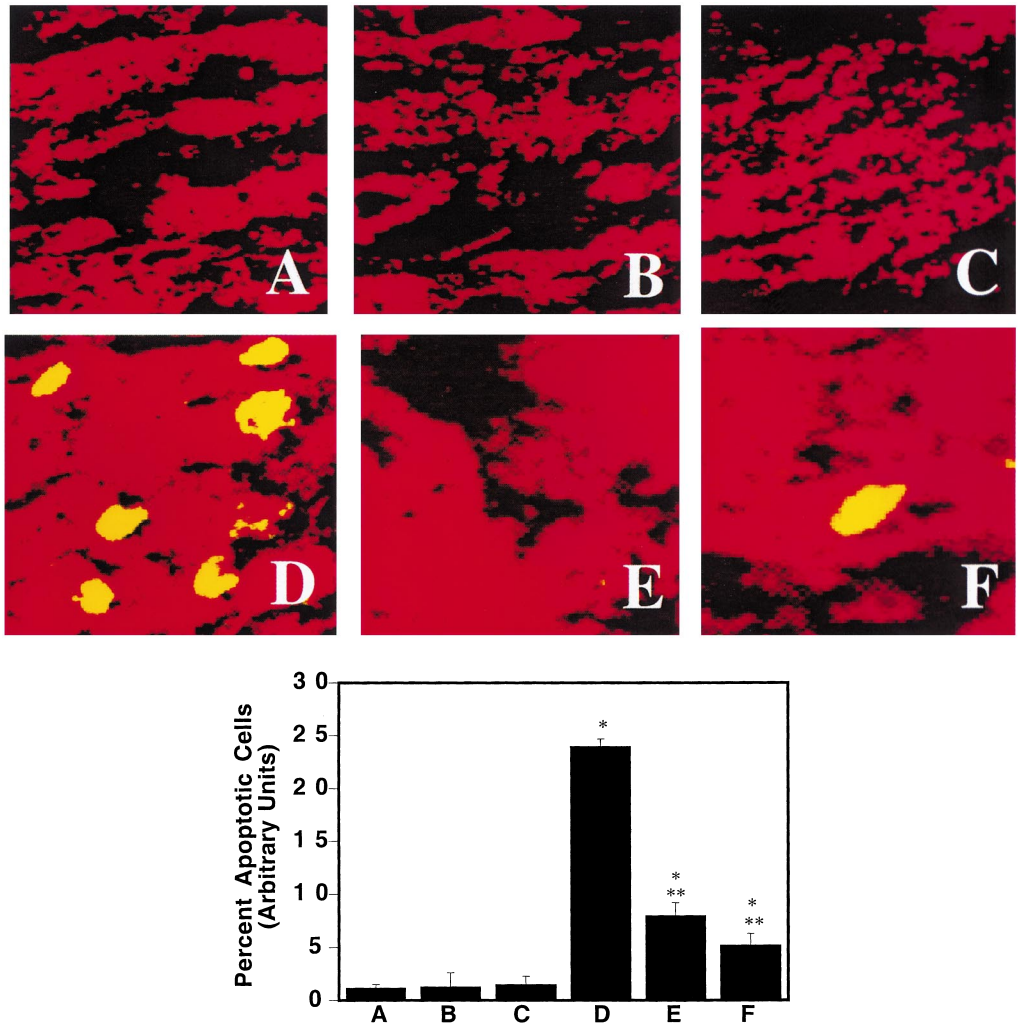

Fig. 5. Evaluation of cardiomyocyte apoptosis using antibody against myosin heavy chain in conjunction with Tunnel staining. Representative photomicrographs showing apoptotic cardiomyocytes. Bar graphs showing average number of cardiomyocytes undergoing apoptosis. Results are expressed as means ± S.E.M. of six different rats per group. *$P < 0.05$ compared to control, **$P < 0.05$ compared to the ischemic/reperfused group. A = perfused control heart, B = 15 min ischemia, C = 60 min ischemia, D = 15 min ischemia and 120 min reperfusion, E = adapted, F = adapted followed by 120 min reperfusion.

the extended DNA in these hearts (Fig. 5B). Ischemic preconditioning reduced the number of apoptotic myocytes in the reperfused myocardium significantly (to 5%).

4. Discussion

Recurrent episodes of myocardial ischemia are commonly observed in patients with coronary artery disease who suffer from frequent angina pectoris or angioplasty of the left anterior descending coronary artery. Reversibly injured myocardium (by a short episode of ischemia followed by another short period of reperfusion) renders the heart more resistant to a longer ischemic-reperfusion period. Such adaptation is mediated through the upregulation of the heart's own cellular defense via the accumulation of intracellular mediators and reprogramming of gene expression. Recent studies suggest that the cytoprotection resulted from ischemic adaptation could be applied to human heart and may constitute anti-ischemic therapy to cure ischemic heart disease. Altered gene expression in the cardiovascular tissues of diseased heart has been reported [21]. Cardiomyocytes exposed to hypoxia revealed apoptotic cell death as evidenced by DNA fragmentation in conjunction with the expression of Fas mRNA [22]. Cardiomyocyte apoptosis has been demonstrated after ischemia and reperfusion [4], failing human heart [23] myocardial infarction [24] and in senescent hearts [25]. The possible cause of the altered gene regulation is the abnormal binding activities of some of the transcription factors like NFκB and AP-1. Reactive oxygen species are involved in signal transduction pathways leading to the modulation of both AP-1 and NFκB which have been implicated in the regulation of gene transcription. Recently, a novel signal transduction pathway responsible for activating NFκB has been described [26]. Cytoplasmic IκB complexes are major targets in signal transduction pathways [27]. A novel therapeutic strategy has been adopted to reduce the extent of myocardial infarction, using in vivo transfer of a 'decoy' *cis* element to bind the critical transcription factor NFκB and thereby block the transactivation of cytokines and adhesion molecule genes necessary for the myocardial reperfusion injury [28].

In this report, we demonstrate for the first time the binding activities of NFκB and AP-1 during different durations of

ischemia, reperfusion and ischemic adaptation. Myocardial adaptation to ischemia was achieved by four cyclic episodes of 5 min of ischemia each followed by 10 min of reperfusion. Such adaptation, was previously shown to exert powerful cardioprotective effects for ischemic hearts [29]. The results of the present study demonstrated that the binding activities of NFκB and AP-1 progressively and steadily increased as a function of the duration of ischemia. During subsequent reperfusion, NFκB binding activity goes down while AP-l activity continues to increase with reperfusion time. In adapted myocardium, NFκB activity remains high while AP-1 binding activity comes down to the baseline level. In the majority of cells, NFκB exists as a cytoplasmic complex by binding with its inhibitory protein IκBα. Phosphorylation of IκBα by oxidative stress resulting from ischemia/reperfusion can cause dissociation of IκB from NFκB. We have shown in Fig. 1, an early activation of NFκB by ischemia in the myocardium which may be a signaling mechanism for the induction of immediate-early gene expression during subsequent stress adaptation. The binding of NFκB remained high during subsequent ischemia and reperfusion. Preconditioning of the heart by repeated ischemia and reperfusion also resulted in increased NFκB binding activities. When the preconditioned myocardium was subjected to ischemia/reperfusion protocol, significantly higher binding activity of NFκB prevailed while AP-1 binding became downregulated. We have also investigated the loss of IκBα protein after dissociation in the cytoplasm caused by ischemia/reperfusion and preconditioning. The kinetics of NFκB binding activity in the nuclear extracts correlated with the kinetics for IκBα protein disappearance in the cytoplasm. The data (not shown) suggest that the disappearance of IκBα protein from cytoplasm resulted in the translocation of NFκB complex to the nucleus as an active form.

The results of our study indicate a direct correlation of cardiomyocyte apoptosis with AP-l and indirect or minimal correlation with NFκB. For example, ischemia as well as reperfusion of ischemic myocardium resulted in the activation of AP-l simultaneously causing apoptotic cell death (Figs. 2 and 5). Conversely, ischemic stress adaptation downregulated AP-1 (close to baseline control) and attenuated apoptosis. These results are consistent with previous observations that increased expression of components of AP-1 is linked to apoptosis [30]. A recent study demonstrated that c-Jun/AP-1, but not NFκB, is a mediator for oxidant-initiated apoptosis in glomerular mesangial cells [31]. In this study, using Northern blot analysis and transient transfection assays with reporter plasmids, the authors showed that H_2O_2 activated both AP-1 and NFκB. Downregulation of c-Jun/AP-1 using a transdominant negative mutant of c-Jun-inhibited apoptotic cell death while use of a transdominant negative mutant of p50 NFκB subunits did not affect H_2O_2-mediated apoptosis. NFκB on the other hand was activated during ischemia ischemia/reperfusion, but significantly activated during ischemic stress adaptation. A previous study demonstrated that activation of NFκB is a necessary step for myocardial adaptation to ischemic stress [5,32]. The results, thus, suggests that NFκB may play a dual role as pro- and anti-apoptotic factor.

Recent analysis of the bcl-2 gene family reveals a complex network regulating apoptosis. Within this bcl-2 gene family, some of the candidates can suppress apoptosis while the others can induce apoptosis [33]. Apoptosis initiated by various different stimuli can be blocked by overexpressing bcl-2. Therefore, investigation of the expression and regulation of the bcl-2 family may provide some light on the mechanisms to the susceptibility by ischemia-reperfusion-induced apoptosis and reduction of apoptosis by stress adaptation. Furthermore, apoptosis induced by c-Myc over expression has been found to be p53-dependent and these cells can be rescued by bcl-2 gene expression [34]. One of the report suggests that growth arrest and apoptosis are controlled by the p53 gene [35]. It is already reported that the increased expression of wild-type p53 can induce apoptosis in myeloid leukemia and colon cancers [36]. Both Apo-1/Fas and Bax have been reported to be positively regulated by p53 on a transcriptional level [37]. Therefore, the regulation and expression of the p53 gene may be of central importance to induction of apoptosis in normal and tumor cells. These findings prompted us to investigate the regulation/status of these two genes p53 (pro-apoptotic) and bcl-2 (anti-apoptotic) in ischemic, ischemic-reperfused and preconditioned heart. The level of p53 mRNA as assessed by performing RT-PCR revealed important findings in our system for the first time. Transcript of p53 was barely detectable in control (perfused with buffer only) and 15 min as well as 60 min ischemic rat myocardium as shown Fig. 3. Whereas reperfusion of ischemic myocardium induced p53 significantly which established the role of p53 in ischemic reperfused myocardium to induce apoptosis. Ischemic preconditioning prevented p53 activation and inhibited apoptosis. Bcl-2 had just an opposite trend of p53. The activation of bcl-2 was associated with the inhibition of apoptosis (Figs. 4 and 5) in the preconditioned myocardium and downregulation of this anti-death gene occurred in concert with the significant amount of apoptosis in the ischemic/reperfused myocardium. We previously demonstrated that prolonged reperfusion after ischemia caused downregulation of bcl-2 in concert with enhanced DNA fragmentation [38]. The results of the present study confirmed the previous findings and further demonstrated differential induction of the expression of bcl-2 as a function of various durations of ischemia, reperfusion and ischemic stress adaptation.

In summary, our results indicate that ischemia/reperfusion-mediated cardiomyocyte apoptosis is regulated by several redox-sensitive transcription factors and genes. Apoptosis occurs in concert with an increase in AP-land p53 and decrease in bcl-2. Consistent with this finding, a reduction of AP-1 and p53 and an increase in bcl-2 inhibits apoptosis. NFκB appears to function both as a pro- and an anti-apoptotic transcription factor.

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References

[1] Hamet, P., Moreau, P., Dam, T.V., Orlov, S.N., Tea, B.S., de Blois, D. and Tremblay, J. (1996) J. Hypertens. 14, S65–70.
[2] Maclellan, W.R. and Schneider, M.D. (1997) Circ. Res. 81, 137–144.
[3] Bromme, H.J. and Holz, J. (1996) Mol. Cell. Biochem. 163, 261–275.
[4] Maulik, N., Kagan, V.E., Tyrin, V.A. and Das, D.K. (1998) Am. J. Physiol. 274, H242–H248.
[5] Maulik, N., Yoshida, T., Engelman, R.M., Deaton, D., Flack,

J.E., Rousou, J.A. and Das, D.K. (1998) Mol. Cell. Biochem. 186, 139–145.
[6] Sun, Y. and Oberley, L.W. (1998) Free Radic. Biol. Med. 21, 335–348.
[7] Ghosh, S., Gifford, A.M., Riviere, L.R., Tempst, P., Nolan, G.P. and Baltimore, D. (1990) Cell 62, 1019–1029.
[8] Angel, P. and Karin, M. (1991) Biochim. Biophys. Acta 1072, 129–157.
[9] Beg, A.A. and Baltimore, D. (1996) Science 274, 787–789.
[10] Hughes, P.E., Alexi, T., Yoshida, T., Schreiber, S.S. and Knusel, B. (1996) Neuroscience 74, 1143–1160.
[11] Miyashita, T. and Reed, J.C. (1995) Cell 80, 293.
[12] Polyak, K., Xia, Y., Zweier, J.L., Kinzler, K.W. and Vogelstein, B. (1997) Nature 389, 300.
[13] Owen-Schaub, L.B., Zhang, W., Cusack, J.C., Angelo, L.S., Santee, S.M., Fujiwara, T., Roth, J.A., Deisseroth, A.B., Zhang, W. and Kruzel, E. (1995) Mol. Cell. Biol. 15, 3032–3040.
[14] Thornberry, N.A. and Lazebnik, Y. (1998) Science 281, 1312.
[15] Clark, A.R., Purdie, C.A., Harrison, D.J., Morris, R.G., Bird, C.C., Hooper, M.L. and Wyllie, A.H. (1993) Nature 362, 849–852.
[16] Engelman, D.T., Watanabe, M., Engelman, R.M., Rousou, J.A., Kisin, E., Kagan, V.E., Maulik, N. and Das, D.K. (1995) Cardiovasc. Res. 29, 133–140.
[17] Maulik, N., Watanabe, M., Zu, Y.-L., Huang, C.-K., Cordis, G.A., Schley, J.A., Das, D.K. (1996) 396, 233–237.
[18] Maulik, N., Sato, M., Price, B.D. and Das, D.K. (1998) FEBS Lett. 429, 365–369.
[19] Das, D.K., Engelman, R.M. and Kimura, Y. (1993) Cardiovasc. Res. 27, 578–584.
[20] Maulik, N., Yoshida, T. and Das, D.K. (1998) Free Radic. Biol. Med. 24, 869–875.
[21] Nishio, Y., Kashiwagi, A., Taki, H., Shinozaki, K., Maeno, Y., Kojima, H., Maegawa, H., Haneda, M., Hidaka, H., Yasuda, H., Horiike, K. and Kikkawa, R. (1998) Diabetes 47, 1318–1325.
[22] Tanaka, M., Ito, H., Adachi, S., Akimoto, H., Nishikawa, T., Kasajima, T., Marumo, F. and Hiroe, H. (1994) Circ. Res. 75, 426–433.
[23] Olivetti, G., Abbi, R., Quaini, F., Kajstura, J., Cheng, W., Nitahara, J.A., Quaini, E., Loreto, C.Di., Beltrami, C.A., Krajewski, S., Reed, J.C. and Anversa, P. (1997) N. Engl. J. Med. 336, 1131–1141.
[24] Kajstura, J., Cheng, W., Reiss, K., Clark, W.A., Sonnenblick, E.H., Krajewski, S. and Reed, J.C. (1996) Lab. Invest. 74, 86–107.
[25] Kajstura, J., Cheng, W., Sarangarajan, R., Li, P., Li, B., Nitahara, J.A., Chapnick, S., Reiss, K., Olivetti, G. and Anversa, P. (1996) Am. J. Physiol. 271, H1215–H1228.
[26] Baeuerle, P.A. (1991) Biochim. Biophys. Acta 63, 63–80.
[27] Geng, Z., Rong, Y. and Lau, B.H.S. (1997) Free Radic. Biol. Med. 23, 345–350.
[28] Morishita, R., Sugimoto, T., Aoki, M., Kida, I., Tomita, N., Moriguchi, A., Maeda, K., Sawa, Y., Keneda, Y., Higaki, J. and Ogihara, T. (1997) Nature Med. 3, 894–899.
[29] Das, D.K., Maulik, N. and Moraru, II. (1995) J. Mol. Cell Cardiol. 27, 181–193.
[30] Marti, A., Jehn, B., Costello, E., Keon, N., Ke, G., Martia, F. and Jaggi, R. (1994) Oncogene 9, 1213–1223.
[31] Ishikawa, Y., Yokoo, T. and Kitamura, M. (1997) Biochem. Biophys. Res. Commun. 240, 496–501.
[32] Xuan, Y.T., Tang, X.L., Banerjee, S., Takano, H., Li, R.C., Han, H., Qiu, Y., Li, J.J. and Bolli, R. (1999) Circ. Res. 84, 1095–1099.
[33] Williams, G.T. and Smith, C.A. (1993) Cell 74, 777–779.
[34] Lotem, J. and Sachs, L. (1993) Cell Growth Differ. 4, 41–47.
[35] Jing, M.C., Yang-Yen, H.F., Lin, J.-K. and Young Yen, J.J. (1996) Oncogene 13, 609–616.
[36] Yonish-Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A. and Oren, M. (1991) Nature 352, 345–347.
[37] Miyashita, T., Krajewski, S., Krajewska, M., Wang, H., Lin, K., Liebermann, D., Hoffmann, B. and Reed, J.C. (1994) 0ncogene 9, 1799–1805.
[38] Maulik, N., Yoshida, T., Engelman, R.M., Rousou, J.A., Flack, J.E., Deaton, D. and Das, D.K. (1997) Surg. Forum 48, 245–249.