Ischemic preconditioning attenuates ischemia/reperfusion-induced activation of caspases and subsequent cleavage of poly(ADP-ribose) polymerase in rat hearts in vivo

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Abstract

Recently, we have demonstrated that ischemic preconditioning (IP) both limits infarct size and decreases internucleosomal DNA fragmentation in rat hearts in vivo, and that there was a direct correlation between myocardial infarct size and DNA fragmentation even after IP. In this study, we examined the ability of IP to attenuate processing and activation of caspase-1 and caspase-3, and cleavage of poly(ADP-ribose) polymerase (PARP), after prolonged ischemia and reperfusion using the same in vivo animal model. Rats that underwent IP and controls (Ctrl) were subjected to 30 min of left coronary artery occlusion followed by 180 min of reperfusion. IP was accomplished by five 5-min cycles of ischemia, each followed by 5 min of reperfusion. The amount of soluble nucleosomes was measured by enzyme-linked immunosorbent assay. Cleavage of caspases-1 and -3, and of one of their substrates PARP, was analyzed by Western blotting. Nucleosomal DNA fragmentation was significantly reduced in ischemic left ventricular (LV) tissue obtained from IP compared with Ctrl animals. The proforms of caspases-1 and -3, and the active form of PARP were not cleaved in the nonischemic LV region of both IP and Ctrl hearts. In contrast, the proform of caspase-3 and the active form of PARP were cleaved in the ischemic LV region of Ctrl hearts, while processing of caspase-1 was increased. Cleavages of caspases-1 and -3, and inactivation of PARP were prevented by IP. The results of this study indicate that IP attenuates both internucleosomal DNA fragmentation and caspases processing, and suggest that the prevention of caspases activation by IP may be important steps in protecting the heart against ischemia/reperfusion injury in vivo.

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Keywords: Apoptosis; Ischemia; Reperfusion; Preconditioning

1. Introduction

Ischemic preconditioning (IP) is one of the most potent mechanisms to protect against myocardial ischemic injury \cite{1-8}. Gottlieb et al. found that apoptosis was reduced by preconditioning in rabbit cardiomyocytes in culture during metabolic inhibition/recovery \cite{9}. Consistent with this finding, we previously demonstrated that: (i) both internucleosomal DNA fragmentation and infarct size were significantly decreased after prolonged ischemia and reperfusion in IP rat hearts in vivo; (ii) the TdT-mediated dUTP nick-end labeling (TUNEL) assay localized fewer and sparsely stained nuclei within the infarct zone of IP hearts compared with nonIP hearts; (iii) there was a direct correlation between myocardial infarct size and internucleosomal DNA fragmentation \cite{10}. Similar results were recently reported by Maulik et al. in isolated perfused rat hearts \cite{11}. Taken together, these data suggest that IP reduces irreversible ischemic injury in part by decreasing apoptosis after prolonged ischemia and reperfusion.

Apoptosis is a regulated and energy-requiring mechanism that involves a cascade of biochemical events resulting in cell shrinkage, chromatin condensation, DNA loss, and DNA fragmentation. This process is thought to play a role in the pathogenesis of myocardial ischemic injury, as well as in other ischemic and nonischemic disorders. In this study, we examined the ability of IP to attenuate processing and activation of caspases and cleavage of PARP after prolonged ischemia and reperfusion using the same in vivo animal model. Rats that underwent IP and controls (Ctrl) were subjected to 30 min of left coronary artery occlusion followed by 180 min of reperfusion. IP was accomplished by five 5-min cycles of ischemia, each followed by 5 min of reperfusion. The amount of soluble nucleosomes was measured by enzyme-linked immunosorbent assay. Cleavage of caspases-1 and -3, and of one of their substrates PARP, was analyzed by Western blotting. Nucleosomal DNA fragmentation was significantly reduced in ischemic left ventricular (LV) tissue obtained from IP compared with Ctrl animals. The proforms of caspases-1 and -3, and the active form of PARP were not cleaved in the nonischemic LV region of both IP and Ctrl hearts. In contrast, the proform of caspase-3 and the active form of PARP were cleaved in the ischemic LV region of Ctrl hearts, while processing of caspase-1 was increased. Cleavages of caspases-1 and -3, and inactivation of PARP were prevented by IP. The results of this study indicate that IP attenuates both internucleosomal DNA fragmentation and caspases processing, and suggest that the prevention of caspases activation by IP may be important steps in protecting the heart against ischemia/reperfusion injury in vivo. © 1999 Elsevier Science B.V. All rights reserved.

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fragmentation, and ultimately cell death [12]. Cysteine proteases related to the Caenorhabditis elegans cell death gene ced-3, also called caspases, are considered to be key mediators of the apoptotic machinery [13,14]. The first mammalian homologue of ced-3 identified was interleukin-1β-converting enzyme (ICE) or caspase-1 according to the new nomenclature [15]. Caspase-1 is expressed in many tissues as a proenzyme of 45 kDa, which is processed by proteolytic cleavage to its active form [16]. To date, several homologues of caspase-1 have been characterized. Among these, caspase-3 (also called CPP32, Apopain or YAMA) is synthesized as an 32 kDa inactive precursor requiring cleavage at specific aspartate residues in order to be transformed into active protease [17,18]. One of the substrates for caspase-3 during apoptosis is the 116 kDa nuclear DNA repair enzyme, poly(ADP-ribose) polymerase (PARP). Cleavage of PARP by either caspase-3 or other effector caspases yields an 85 kDa inactive fragment which is considered to be a useful marker for activation of caspases during apoptosis [18–20].

Recently, Yaoita et al. demonstrated that z-VAD-fmk, a caspase inhibitor known to inhibit caspase-1, -3 and -4, attenuated ischemia/reperfusion injuries in rat hearts in vivo [21]. Since IP appears to reduce both infarct size (i.e., myocyte cell death) and internucleosomal DNA fragmentation in rat hearts in vivo [10], we hypothesized that IP may decrease myocyte cell death by attenuating caspases activation. To test our hypothesis, we examined the ability of IP to attenuate processing and activation of caspase-1 and caspase-3, and subsequent cleavage of PARP, after prolonged ischemia and reperfusion in our in vivo animal model.

2. Materials and methods

2.1. Animal model of acute myocardial ischemia and reperfusion

IP and control (Ctrl) animals were subjected to a protocol of ischemia and reperfusion described previously [5,6,10]. Briefly, female Sprague-Dawley rats weighing 225–250 g were anesthetized with intraperitoneal pentobarbital (40 mg/kg) and ventilated via a tracheostomy on a Harvard rodent respirator (tidal volume 0.5 to 1.5 ml; respiratory rate 95–105 breaths per min). A midline sternotomy was performed, and a reversible coronary artery snare occluder was placed around the proximal left coronary artery (LCA). A 1 s test occlusion, too short to induce preconditioning, was performed to visually test the occluder and its positioning. After a subsequent 20-min stabilization period, all animals were then randomly subjected to one of the two protocols described below. All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health (NIH publication 85-23, revised 1985), and were approved by the Committee on Animal Research at the University of California, San Francisco, CA, USA.

2.2. Experimental protocol

Animals (five per group) were randomly assigned to one of two groups: Ctrl animals with no preconditioning, and IP animals. The IP protocol consisted of five consecutive 5-min episodes of LCA occlusion, each followed by a 5-min period of reperfusion, as previously described [10]. NonIP animals had a comparable 50-min nonischemic period with the snare occluder open. IP and Ctrl animals were then subjected to 30-min period of LCA occlusion followed by 180 min of reperfusion. Throughout the experiments, core body temperature of the animals was monitored by a rectal thermometer and was maintained constant (between 36.8 and 37°C) by a heating pad and heating lamps. To differentiate between ischemic and nonischemic tissue, the LCA was reoccluded after the 180-min perfusion period, and 1 ml of phthalocyanine blue dye was injected into the left ventricular (LV) cavity in vivo and allowed to perfuse the nonischemic portions of the heart. The entire heart was excised, rinsed of excess blue dye, trimmed of right ventricular and atrial tissue, and sliced transversely into 5 sections about 2 mm thick. Samples from both ischemic (unstained) and nonischemic (blue-stained) cardiac tissue were collected to assess DNA fragmentation by enzyme-linked immunosorbent assay (ELISA) and caspase-1, caspase-3 and PARP cleavage by Western blotting as described below.

2.3. ELISA

The amount of soluble mono- and oligonucleosomes was measured using an ELISA kit (Boehringer Mannheim) as previously described [10]. Transmural samples of fresh tissue (±25 mg) from completely normal areas (central portion of the septum) and ischemic areas (central portion of the LV free wall, halfway from the edge of the septum) were isolated from one slide with the phthalocyanine blue dye perfusion as a guide, washed of blood, homogenized in tissue grinder and incubated for 30 min at room temperature in 400 μl lysis buffer supplied with the kit. The homogenate was centrifuged at 13 000 g for 20 min. The supernatant (i.e., cytosolic fraction) was further diluted 50-fold in phosphate-buffered saline buffer (137 mmol/l NaCl; 2.7 mmol/l KCl; 4.3 mmol/l Na₂HPO₄·7H₂O; 1.4 mmol/l KH₂PO₄ pH 7.4) and directly used as antigen source in the sandwich ELISA. Incubation buffer instead of sample solution and DNA–histone complex included in the kit were used as background control and positive control, respectively. Three separate spectrophotometric measurements (A405/A490) were averaged, and the background value of the immunoassay was subtracted from
each of these averages. The positive control was used as internal control for daily variability of the assay.

2.4. Western blotting

To detect processing of caspase-1 and -3 (ICE and CPP32, respectively) and cleavage of PARP, transmural samples of fresh tissue (±25 mg) obtained from the same animals were isolated as described above, and homogenized (Polytron, Brinkmann) in 0.75 ml lysis buffer [1% Triton X-100 lysis buffer containing 20 mmol/l Tris–HCl (pH 8.0), 137 mmol/l NaCl, 10% (v/v) glycerol, 2 mmol/l EDTA, 6 mol/l urea, 1 mmol/l pefabloc, 0.14 unit of aprotinin per ml, 20 μmol/l leupeptin, and 1 mmol/l sodium orthovanadate] at 4°C. Insoluble material was removed by centrifugation at 4°C for 15 min at 14 000 g, and the protein concentration of the soluble fraction was determined with the bicinchoninic acid (BCA) protein assay kit (Pierce). Equal quantities of lysates were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) (7.5%, 15%) and transferred to Immobilon-P membranes (Millipore). Separate Western blot experiments were performed using two different antibodies: (1) an antihuman caspase-1 rabbit polyclonal antibody (1:1000; Upstate Biotechnology) that recognizes the proenzyme of 45 kDa and the p20 subunit of active caspase-1; (2) an antihuman caspase-3 rabbit polyclonal antibody (1:300 dilution; Upstate Biotechnology) that recognizes caspase-3 precursor (32 kDa) but failed to recognize the p12 subunit of caspase-3 (manufacturer’s information); and (3) an antihuman PARP rabbit polyclonal antibody (1:750 dilution; Upstate Biotechnology) that recognizes both the 116 kDa of PARP and its 85 kDa fragment. The species cross-reactivity of anticaspase-3 and antiPARP have been tested in rat PC-12 cells, while the immunizing sequence used to raise the anticaspase-1 has 100% homology to the rat sequence [22]. Western blots were incubated with the appropriate antibody and then washed in Tris-buffered saline containing 0.1% Tween 20. Antigen–antibody complexes were detected with horseradish peroxidase-coupled secondary antibodies and the enhanced chemiluminescence system (Renaissance; NEN). Finally, the blots were exposed to reflection NEF films (NEN). Autoradiographs of antiPARP Western blot were analyzed by densitometry and quantified using Intelligent Quantifier (BioImage) software.

2.5. Statistical analysis

All values are expressed as mean±SEM. Comparisons between groups were assessed by one-way analysis of variance with post hoc analysis with the Student–Newman–Keuls test. Statistical significance was defined as a value of \( p<0.05 \).

3. Results

3.1. Quantification of DNA fragmentation

Quantitative analysis of soluble mono- and oligonucleosomes was performed by ELISA both in Ctrl and IP animals. As illustrated in Fig. 1, DNA fragmentation was low in nonischemic LV tissue obtained from both IP and Ctrl animals (0.06±0.01U vs. 0.07±0.01U; \( n=5; \ p=\) nonsignificant [NS]). In contrast, DNA fragmentation was markedly increased in the ischemic LV tissue obtained from Ctrl hearts after 30 min of LCA occlusion and 180 min of reperfusion. However, DNA fragmentation was significantly reduced in ischemic LV tissue obtained from IP animals compared with Ctrl animals following prolonged LCA occlusion and reperfusion (0.36±0.07U vs. 1.14±0.13U; \( n=5; \ p<0.001 \)).

3.2. Processing of caspase-1 and caspase-3

To characterize the effect of IP on some of the components of the apoptotic pathway, we performed Western blot analysis for caspases-1 and -3. Both caspases are constitutively expressed in cells as inactive proenzymes of 45 kDa and 32 kDa, respectively. These proenzymes need to be cleaved at specific aspartate residues to generate two smaller active subunits which oligomerize to form an active protease complex. As illustrated in Fig. 2A, all tissue samples (\( n=5 \)) showed an immunoreactive band of 45 kDa corresponding to the proform of caspase-1. Non-
ischemic and ischemic LV tissue obtained from both Ctrl and IP animals expressed similar amount of this proform. However, the active subunit of 20 kDa was only highly detectable in the ischemic LV tissue obtained from Ctrl animals (lane 2) while in the nonischemic LV tissue obtained from both Ctrl and IP animals, this band was faint (lanes 1) or nearly absent (lane 3). This generation of the active p20 subunit of caspase-1 following ischemia was prevented by IP as shown in lane 4.

All nonischemic LV tissue samples (n=5) obtained from Ctrl and IP animals showed a strong signal for a band of 32 kDa corresponding to the inactive precursor of caspase-3 (Fig. 2B, lanes 1 and 3). In contrast, the inactive proform of caspase-3 was markedly reduced in the ischemic LV tissue obtained from Ctrl animals (lane 2), most likely due to proteolytic cleavage of this proform to generate the active subunits. This cleavage of the inactive proform of caspase-3 was prevented by IP (lane 4).

3.3. Cleavage of PARP

PARP, one of the known substrates of caspase-3, is a nuclear enzyme involved in DNA repair. During the apoptotic process, PARP is inactivated by a single proteolytic cleavage of the 116 kDa active enzyme that generates a 85 kDa fragment. As illustrated on Fig. 3A, the 116 kDa form was uncleaved in all nonischemic LV tissue samples (lanes 1, 3, 5 and 7). In contrast, this active form was almost completely cleaved and, in turn, the 85 kDa fragment was more abundant in the ischemic LV tissue obtained from Ctrl animals (lanes 2 and 8). This cleavage and inactivation of PARP was reduced by IP (lanes 4 and 6).

Autoradiographs of Western blots were also analyzed by densitometry to determine the relative intensity of the 116 and 85 kDa bands. The relative cleavage and inactivation of PARP is shown in Fig. 3B. The cleavage of PARP was limited and not significantly different in nonischemic LV tissue obtained from IP and Ctrl animals (8.3±4.8% vs. 10.8±3.7%; n=5; p=NS). In contrast, in ischemic LV tissue obtained from Ctrl animals, the active form of p116 was markedly cleaved compared to the nonischemic LV tissue obtained from the same group (95.5±1.2% vs. 10.8±3.7%; n=5; p<0.001). In ischemic LV tissue obtained from IP animals, this cleavage was markedly attenuated compared to Ctrl animals (10.9±3.0% vs. 95.5±1.2%; n=5; p<0.001). Actually, PARP cleavage was almost completely prevented by IP since a nonsignificant difference was observed between ischemic and nonischemic LV tissue obtained from IP (8.3±4.8% vs. 10.8±3.7%; n=5; p=NS). These results suggest that caspase activity plays a role in myocyte cell death after ischemia and reperfusion.

4. Discussion

In this present study, we found that caspase-1 and caspase-3 activation, and PARP cleavage were early events that accompany internucleosomal DNA fragmentation induced by prolonged ischemia and reperfusion in rat
mentation, a hallmark of apoptosis, was restricted in the infarcted area after prolonged ischemia and reperfusion in rat hearts in vivo [25], which was confirmed by the TUNEL assay [10]. Moreover, we found a close correlation between infarct size and degree of internucleosomal DNA fragmentation in this in vivo model [10]. However, our results did not allow a conclusion regarding the relationship between apoptotic cell death and necrosis.

In the present study, we demonstrate that prolonged ischemia followed by reperfusion is accompanied by processing of the apoptotic proteases, caspases-1 and -3, and inactivation of one of their substrates, PARP, a nuclear DNA repair enzyme, in the ischemic portion of nonIP rat hearts in vivo. The generation of the active form of caspase-1 was always associated with the disappearance of the proform of caspase-3 and both the inactivation of its substrate PARP and DNA fragmentation in the ischemic LV tissue obtained from Ctrl animals. Taken together with our previous data, these results suggest that activation of caspases-1 and -3 following ischemia/reperfusion may lead to inactivation of PARP, increased DNA fragmentation, and finally cell death. Since caspases are known to interact each other and act as complexes, it is highly possible that ischemia/reperfusion regulates not only caspases-1 and -3 activation but also other cysteine proteases of the effector phase of apoptosis. Furthermore, Black et al. recently colocalized caspase-3 with apoptotic myocytes after in vivo myocardial ischemia/reperfusion in rats [26]. All these findings support the hypothesis that ischemia followed by reperfusion is a noxious stimulus able to activate the physiological cell death mechanism in vivo.

Fig. 3. Cleavage of PARP. (A) Equal amount of protein (100 μg) from control (Ctrl) (lanes 1, 2, 7 and 8) and ischemic preconditioned (IP) (lanes 3, 4, 5 and 6) rat hearts were subjected to SDS-PAGE and immunoblotting with a rabbit polyclonal antibody against human PARP. Arrows denote full-length PARP (p116) and its 85 kDa inactive fragment (p85). Positions of proteins of known molecular mass (kDa) are indicated. (B) The relative intensity of the p85 and p116 bands was determined with ImageQuant software (Molecular Dynamics) and the percentage of cleavage product (p85 band) was plotted. Each bar represents the mean±SE for the data from one group of animals. * p<0.001 vs. nonischemic area (NI). ** p<0.001 vs. Ctrl. Note that cleavage of PARP p116 active form observed in ischemic LV regions of Ctrl animals was almost completely prevented by IP.

hearts in vivo. Furthermore, we demonstrated for the first time that these events were attenuated by IP.

4.1. Apoptosis in the ischemic and reperfused myocardium

Although it has been accepted for almost a century that necrosis (i.e., a catastrophic metabolic failure resulting directly from severe damage) was the only mechanism of cardiomyocyte cell death during ischemia, recent observations support the evidence that apoptosis may be the prominent form of ischemia-related cell death in the heart [23,24]. Consistent with this new and attractive concept, we reported previously that internucleosomal DNA frag-
myocardial ischemia (i.e., IP) protect the heart against subsequent episodes of prolonged ischemia by delaying lethal cell injury, thereby limiting infarct size [1–8]. The mechanism by which transient ischemia prevent ischemic myocyte cell death remains not well understood. Recently, we demonstrated that both DNA fragmentation (i.e., nucleosome-sized DNA ladders, soluble nucleosomes and DNA-nick end labeling) and infarct size were significantly decreased after prolonged ischemia and reperfusion in IP rat hearts in vivo using the same experimental protocol [10]. We observed a close correlation between infarct size and the amount of internucleosomal DNA fragmentation in this animal model. Moreover, the correlation was maintained after IP [10]. These results were consistent with a previous report by Gottlieb et al. showing that IP was able to prevent programmed cell death in vitro [9]. On the basis of these findings, we suggested that IP reduces irreversible ischemic injury (i.e., myocyte cell death) in part by preventing apoptosis after prolonged ischemia and reperfusion in vivo. Since then, Maulik et al. demonstrated that apoptotic cell death and DNA fragmentation were completely abolished by IP in isolated perfused rat hearts [11]. Similar findings were also recently demonstrated by Wang et al. in dog hearts [33].

Continuing the same experimental protocol, we report here that IP attenuates processing of both caspases-1 and -3, and cleavage of PARP in rat hearts in vivo. Since apoptotic proteases are considered to be key components in the effector phase of apoptosis, these results support our previous working hypothesis that inhibition of programmed cell death may play a major role in the mechanism of adaptive protection of the heart. By preventing activation of both caspases-1 and -3, IP may act upstream from the “executioner” and, as a consequence, decrease DNA fragmentation as observed in this study and previously in our in vivo model [10]. The observations that z-VAD-fmk, a potent inhibitor of caspases-1, -3 and -4, reduces ischemia/reperfusion-induced cell death in cardiomyocytes in culture [9] and in vivo [21] strongly support this theory.

Intracellular signaling events by which IP prevents activation of caspases in the heart remain unknown. Consistent observations indicated that protein kinase C activation [34,35] and reduction in intracellular acidosis are important steps in preconditioning the cells [5,36,37]. Recently Gottlieb and coworkers proposed that activation of the vacuolar proton ATPase by protein kinase C during IP may attenuate intracellular acidification during metabolic inhibition and, thereby, protect myocytes from apoptosis [9,38]. Interestingly, another recent report from Maulik et al. suggested that IP may activate the tyrosine kinase–phospholipase D signaling pathway in rat hearts, resulting in the activation of the mitogen-activated protein kinase (MAPK) pathway [39]. Although programmed cell death pathways resulting in apoptosis are not well understood, several observations highlight the important role that integration of multiple intracellular signaling pathways plays in the regulation of cell death. For example, it has been suggested that apoptosis may be regulated in part by an imbalance between different MAPK pathways in different cell systems [40,41]. Previous studies have also examined the contribution of neutrophils to apoptosis in reperfused myocardium in vivo [42–44]. According to this hypothesis, Wang et al. have recently suggested that IP may attenuate myocardial apoptosis in part by inhibiting polymorphonuclear neutrophil activation [33]. Implication of such mechanisms in the regulation of caspases activity in the IP heart needs to be further studied.

5. Conclusion

This study indicates that both caspases-1 and caspase-3 activation, and PARP cleavage are early events that accompany internucleosomal DNA fragmentation induced by prolonged ischemia and reperfusion in rat hearts in vivo. Furthermore, we demonstrate for the first time that these events are highly prevented by IP. These results indicate that IP attenuates both internucleosomal DNA fragmentation and caspases processing, and suggest that the prevention of caspases activation by IP may be important steps in protecting the heart against ischemia/reperfusion injury in vivo.

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References

297.


[18] Tewari M, Quan LT, O’Rourke K et al. Yama / CPP32
[19] Tewari M, Quan LT, O’Rourke K et al. Yama / CPP32


