Poly(ADP-Ribose) Polymerase Promotes Cardiac Remodeling, Contractile Failure, and Translocation of Apoptosis-Inducing Factor in a Murine Experimental Model of Aortic Banding and Heart Failure

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Received September 3, 2004; accepted October 28, 2004

ABSTRACT

Oxidant stress-induced activation of poly(ADP-ribose) polymerase (PARP) plays a role in the pathogenesis of various cardiovascular diseases. We have now investigated the role of PARP in the process of cardiac remodeling and heart failure in a mouse model of heart failure induced by transverse aortic constriction (banding). The catalytic activity of PARP was inhibited by the potent isoindolinone-based PARP inhibitor INO-1001 or by PARP-1 genetic deficiency. PARP inhibition prevented the pressure overload-induced decrease in cardiac contractile function, despite the pressure gradient between both carotid arteries being comparable in the two experimental groups. The development of hypertrophy, the formation of collagen in the hearts, and the mitochondrial-to-nuclear translocation of the cell death factor apoptosis-inducing factor (AIF) were attenuated by PARP inhibition. The ability of the inhibitor to block the catalytic activity of PARP was confirmed by immunohistochemical detection of poly(ADP-ribose), the product of the enzyme in the heart. Plasma levels of INO-1001, as measured at the end of the experiments, were in the concentration range sufficient to block the oxidant-mediated activation of PARP in murine cardiac myocytes in vitro. Myocardial hypertrophy and AIF translocation was also reduced in PARP-1-deficient mice undergoing aortic banding, compared with their wild-type counterparts. Overall, the current results demonstrate the importance of poly(ADP-ribose)ylation in the pathogenesis of banding-induced heart failure.

Poly(ADP-ribose) polymerase (PARP)-1, a monomeric enzyme present in eukaryotes, is the major isofrom of an expanding family of poly(ADP-ribose)ating enzymes (for review, see Virág and Szabó, 2002). The main isofrom of the family, PARP-1, primarily functions as a DNA damage sensor in the nucleus. Upon binding to damaged DNA mainly through the second zinc finger domain, PARP-1 forms homodimers and catalyzes the cleavage of NAD into nicotinamide and ADP-ribose and uses the latter to synthesize branched nucleic acid-like polymers poly(ADP-ribose) covalently attached to nuclear acceptor proteins. The biological role of PARP-1 is complex and includes the regulation of DNA repair and maintenance of genomic integrity. PARP-1 has been implicated in a variety of pathophysiological processes. PARP is an energy-consuming enzyme, which transfers ADP ribose units to nuclear proteins. As a result of this process, the intracellular nicotinamide dinucleotide (oxidized) (NAD) and ATP levels remarkably decrease, resulting in cell dysfunction and cell death via the necrotic route (for review, see Virág and Szabó, 2002).

PARP becomes activated in response to DNA single-strand breaks, which can develop as a response to free radical and oxidant cell injury. Oxidative and nitrosative stress triggers the activation of the nuclear enzyme PARP, which contributes to the pathogenesis of various cardiovascular diseases, including myocardial infarction and ischemia-reperfusion and heart failure (for review, see Szabó et al., 2004). Recent studies have implicated the importance of mitochondrial dys-
function and mitochondrial cell death factors, including apoptosis-inducing factor (AIF) (Cregan et al., 2002) in the process of oxidant-induced cell death, and the potential role of PARP in regulating these factors in various cell types, including the myocardium (Yu et al., 2002; Xiao et al., 2004).

In the present study, using INO-1001, a potent PARP inhibitor (Khan et al., 2003; Chen et al., 2004; Murthy et al., 2004; Xiao et al., 2004), we have investigated the role of PARP in the development of myocardial failure and hypertrophy in a banding-induced chronic heart failure. We have also investigated the potential translocation of AIF in this model, and the role of PARP in regulating this process. Some of the key findings obtained with the PARP inhibitor were also confirmed by comparing wild-type and PARP-1-deficient mice undergoing aortic banding.

**Materials and Methods**

**Animals.** C57BL/6 mice were purchased from Charles River Laboratories, Inc. (Wilmington, MA). PARP−/− and wild-type PARP+/+ mice originally derived from Dr. Z. Q. Wang’s laboratory (Wang et al., 1995) were maintained and bred in our animal facility as reported previously (Liaudet et al., 2000). Animals were housed in cages in a specific pathogen-free room and exposed to a cycle of 12-h light/12-h dark and had free access to water and food. An acclimation period of at least 1 week was provided before initiating the experiment.

**Preparation of Mitochondrial, Cytosolic, and Nuclear Fractions.** Fresh ventricles were cut into small pieces in ice-cold MSEP buffer [in millimoles per liter: 225 mM mannitol, 75 mM sucrose, 1.0 mM EGTA, and 20 mM HEPES (KOH), pH 7.4]. The samples were then resuspended in 3 volumes of MSEP buffer supplemented with 1 mM dithiothreitol and protease inhibitors (200 μg/ml cofactor cocktail; Sigma-Aldrich). Subcellular fractions were prepared as described previously, followed by Western blotting for AIF (Gottlieb et al., 1995; Chen et al., 2001). The heart homogenates were filtered through a 50-mesh screen to remove unbroken tissue and then centrifuged at 600 × g for 10 min. The supernatant was centrifuged for 10 min at 8000g. The pellet obtained was used as mitochondrial fraction and was washed twice and resuspended in MSEP buffer. The 8000g supernatant was recentrifuged at 15,000g for 20 min, and the resulting supernatant was used as soluble cytosolic fraction.

To obtain nuclear extracts, the 600g nuclear pellet was washed twice with TMS buffer (240 mM NaCl, 10 mM Tris, pH 7.4, and 1.5 mM magnesium chloride) and incubated with 0.1% Triton X-100 and protease inhibitors (20 μg/ml cocktail solution; Sigma-Aldrich). Subcellular fractions were prepared as described previously, followed by Western blotting for AIF (Gottlieb et al., 1995; Chen et al., 2001). The heart was further homogenized for 5 s at power 3 output by a Polytron homogenizer (PT1200 C; Kinematica, Basel, Switzerland). The homogenates were filtered through a 50-mesh screen to remove unbroken tissue and then centrifuged at 600g for 10 min. The supernatant was centrifuged for 10 min at 8000g. The pellet obtained was used as mitochondrial fraction and was washed twice and resuspended in MSEP buffer. The 8000g supernatant was recentrifuged at 15,000g for 20 min, and the resulting supernatant was used as soluble cytosolic fraction.

**Additional Measurements.** At the conclusion of the studies, mice were sacrificed by an overdose of drug. Before the heart was isolated, the chest was opened to determine whether pleural effusion was present. The heart, lung, and liver were removed and their weights were measured. Organ weights were normalized to body weight. In addition, in a separate set of studies C57BL/6 mice treated with INO-1001 and undergoing aortic banding (see methods described above) were sacrificed at 1 and 9 weeks, plasma samples and myocardial tissues were obtained at 1 and 9 weeks of heart failure, and concentration of INO-1001 was measured by high-performance liquid chromatography (n = 5 animals/group).

**Immunohistochemical Analysis for PARP Activation and AIF Translocation.** Immunohistochemical detection of poly(ADP-ribose) and AIF was performed as described previously (Xiao et al., 2004). Primary antibodies used for the stainings were chicken polyclonal anti-poly(ADP-ribose) antibody (Tupil Biolabs, West Point, PA) and rabbit polyclonal anti-AIF antibody (Chemicon International, Temecula, CA).

**Collagen Staining.** For collagen staining, fixed blocks of ventricular tissue were embedded in paraffin, and 5-μm-thick sections were cut from each block. The sections were deparaffinized, rehydrated, and stained with Masson’s trichrome using a staining kit (HT15; Sigma-Aldrich, St. Louis, MO).

**Measurement of Cardiac Function.** To measure arterial pressure, 2 high-fidelity catheter tip transducers (1.4F; Millar Instruments Inc., Houston, TX) were used. One was inserted into left carotid artery. The pressure in both carotid arteries was measured simultaneously and the pressure gradient between the right carotid artery and advanced to the left ventricle for measurement of LV pressure and LV dP/dt. After measurement of LV function, the needle was promptly removed to yield a constriction of 0.4 mm in diameter. The chest was closed in layers. The mice were kept on a heating pad until recovery from anesthesia. For the sham operation, the aorta was not ligated. Mice were studied at 9 weeks (pharmacological inhibitor studies) or 29 weeks (wild-type versus PARP-1 knockout mice) after TAC.

**Administration of INO-1001.** The isoindolinone-based PARP inhibitor INO-1001 (Khan et al., 2003; Murthy et al., 2004) (30 mg/kg/day) or vehicle was administered orally (via drinking water) to aortic banded or sham-operated mice after surgical operation and continued to 9 weeks. INO-1001 was dissolved into 5% dextrine injection USP (Abbott Labs, Pomezia, Italy) and diluted 100 times with water. The drug solution was changed every 2 days.

**In Vitro Myocyte Isolation and PARP Inhibition Studies.** Isolation of ventricular myocytes from mice was conducted as described previously (Chen et al., 2004). Hearts were excised and mounted in a Langendorff perfusion apparatus and perfused for 5 min with Ca2+-free Krebs-Henseleit bicarbonate (KHB) buffer containing 120 mM NaCl, 5.4 mM KCl, 1.2 mM MgSO4, 1.2 mM NaH2PO4, 5.6 mM glucose, 20 mM NaHCO3, 10 mM 2,3-butanedione monoxime (Sigma-Aldrich), and 5 mM taurine (Sigma-Aldrich).
gassed with 95% O₂, 5% CO₂, followed by a 15 to 20 min perfusion with same KHB buffer containing 25 μM CaCl₂ and 0.1% collagenase (Worthington Biochemicals, Freehold, NJ) and 0.1% BSA. Thereafter, ventricular tissue was chopped and incubated for 10 min in the same medium supplemented with 1% BSA. The ventricular tissue was then dispersed and filtered. The resultant myocytes were washed twice with fresh KRB buffer containing 1% BSA and 25 μM CaCl₂, and then the Ca²⁺ concentration was increased to 1.0 mM gradually. Finally, the myocyte pellets were washed and suspended in MEM medium containing 1.2 mM Ca²⁺ (MI808 Sigma-Aldrich). Myocytes were plated in laminin-precoated plates or glass coverslips at a density of 10⁵ cells/cm². After 1-h plating in MEM medium with 5% fetal calf serum, the medium was changed to fetal bovine serum-washed twice with fresh KRB buffer containing 1% BSA and 25 μM CaCl₂, and then the Ca²⁺ concentration was increased to 1.0 mM gradually. Finally, the myocyte pellets were washed and suspended in MEM medium with 5% fetal calf serum, the medium was changed to fetal bovine serum-free MEM. After overnight culture, myocytes were subjected to H₂O₂ treatment at 200 μM in the presence or absence of various concentrations of INO-1001. Activation of PARP was measured by incorporation of radiolabeled NAD⁺ as described previously (Soriano et al., 2001).

**Statistical Analysis.** Results are expressed as mean ± S.E.M. Differences between means were evaluated by unpaired two-tailed Student’s t test.

### Results

**Effect of INO-1001 on Hemodynamics.** To examine the effect of INO-1001 on hemodynamic loading conditions of aortic banded mice, we measured the mechanical function. Table 1 summarizes the hemodynamic results. At 9 weeks after banding, left ventricular systolic pressure increased significantly in banded mice compared with sham-operated mice. Hearts from vehicle-treated banded mice showed a 22 and 14% reduction in maximal dp/dt (+dp/dt) and minimal dp/dt (−dp/dt) compared with those from sham-operated mice, indicating pressure overload-impaired cardiac function. Treatment with INO-1001 prevented the pressure overload-induced decrease in contractile function despite that the pressure gradient between both carotid arteries was comparable in the vehicle-treated banded group (51.2 ± 5.1 mm Hg) versus the INO-1001-treated banded group (50.2 ± 9.5 mm Hg).

**Effect of INO-1001 on the Load-Induced Increase in Heart Weight and Mortality.** To examine whether INO-1001 attenuates load-induced cardiac hypertrophy, we treated aortic banded mice with INO-1001 or vehicle for 9 weeks after surgery. Aortic banded mice had a significant increase in heart weight at end of the experiment compared with sham-operated mice. Heart weight or LV weight to tibiaal length (HW/TL, LV/TL) ratio increased by 100.5 or 117.9% in the vehicle-treated banded mice. Importantly, however, in INO-1001-treated banded mice, HW/TL or LV/TL ratio rose by 39.0 or 60.9%, which was significantly lower than in the vehicle-treated banded mice (Fig. 1). The body weights and tibial lengths were not different among these groups. Furthermore, 9 weeks after TAC, aortic banded mice exhibited increased lung weight (Table 1), indicating the development of pulmonary congestion. In contrast, INO-1001-treated banded mice showed a significant decrease in lung weight.

The all-cause mortality rate of TAC was not different for vehicle-treated banded mice and INO-1001-treated banded mice (Fig. 2). All sham-operated mice survived to the time of experiment. Forty-five mice of 72 in the vehicle-treated banded group and 32 mice of 61 in the INO-1001-treated mice died prematurely. Twenty-one mice in vehicle-treated banded mice (29.2%), and 11 mice in the INO-1001-treated banded mice (18.1%) died of heart failure, judged by postmortem findings. These mice had massive pleural effusion and severe lung congestion (lung wet weight 400.1 ± 29.6 mg, range 189–782 mg). All mice developed severe hypertrophy (heart weight 216 ± 12.7 mg, range 135–451.8 mg).

There was a massive staining of collagen in the myocardium of banded mice, which was markedly reduced in the INO-1001-treated group of banded animals (Fig. 3).

The concentration of INO-1001 in plasma at 9 weeks after treatment in aortic banded mice was 1.24 ± 0.96 μM (n = 4). However, at 1 week after the start of the experiment, plasma PARP inhibitor levels amounted to only 0.27 ± 0.12 μM, indicating a gradual buildup of plasma INO-1001 concentrations in the animals subjected to its administration in the drinking water. Similarly, INO-1001 levels in the heart only amounted to 0.18 ± 0.09 μM at 1 week, whereas they increased to 0.41 ± 0.12 μM at 9 weeks. In vitro pretreatment with 1 μM INO-1001 fully prevented PARP activation in cardiac myocytes challenged with hydrogen peroxide (n = 4). At a 10 times lower concentration (0.1 μM INO-1001), PARP activation in hydrogen peroxide-challenged myocytes was reduced by 56 ± 7% (p < 0.05, n = 4).

**Effect of INO-1001 on the Load-Induced PARP Activation and AIF Translocation.** To examine whether PARP was activated in the myocardium in response to chronic pressure overload, mice were killed 1, 4, and 9 weeks after the operation. Pressure overload caused increase in PARP activity, which reached its peak at 1 week, in both vehicle-treated and INO-1001-treated banded mice hearts compared with

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>INO-1001</th>
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<tr>
<td></td>
<td>Sham</td>
<td>Banded</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>31.2 ± 0.9</td>
<td>29.8 ± 0.8</td>
</tr>
<tr>
<td>Lung weight, mg</td>
<td>184.1 ± 7.8</td>
<td>289.8 ± 41.3*</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>1.29 ± 0.05</td>
<td>1.30 ± 0.04</td>
</tr>
<tr>
<td>Lung weight/body weight, mg/g</td>
<td>5.9 ± 0.3</td>
<td>10.3 ± 1.8*</td>
</tr>
<tr>
<td>Aortic pressure gradient, mm Hg</td>
<td>3.1 ± 1.4</td>
<td>51.2 ± 5.0*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>352 ± 36</td>
<td>323 ± 21</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>126.5 ± 3.0</td>
<td>152.4 ± 6.9*</td>
</tr>
<tr>
<td>dp/dt, mm Hg/ms</td>
<td>5.78 ± 0.24</td>
<td>4.51 ± 0.21*</td>
</tr>
<tr>
<td>−dp/dt, mm Hg/mS</td>
<td>5.47 ± 0.20</td>
<td>4.71 ± 0.32*</td>
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*p < 0.05 versus respective sham-operated group; †p < 0.05 versus vehicle-treated banded group.
sham-operated mice heart. PARP activation in INO-1001-treated banded mice hearts was reduced and was similar to baseline level from 4 weeks after treatment (Fig. 4).

PARP activation signals AIF release from mitochondria, resulting in a caspase-independent pathway of cell death (Yu et al., 2002; Xiao et al., 2004). We used immunohistochemical method to confirm AIF translocation in myocardial tissue. As shown in Fig. 5, pressure overload induced AIF translocation. This phenomenon was also confirmed by subcellular fractionation, followed by AIF Western blotting (Fig. 6). Similar to the results of PARP activation, the peak of AIF translocation occurred at 1 week after aortic banding and then decreased in both groups. INO-1001 prevented AIF translocation from 4 weeks after operation (Fig. 5).

**Studies in Wild-Type and PARP-1-Deficient Animals.**
To further characterize the involvement of PARP in heart failure, PARP<sup>−/−</sup> and wild-type littermate mice (PARP<sup>+/+</sup>) were subjected to long-term aortic banding. After 29 weeks, both PARP<sup>−/−</sup> and PARP<sup>+/+</sup> mice displayed increased contractile function in response to pressure overload (data not shown). Aortic banding caused a significant myocardial hypertrophy in PARP<sup>+/+</sup> mice compared with sham-operated
mice. The percentage of increase HW/TL and LVW/TL was 49.4 and 59.9%, respectively. The increased percentage of HW/TL and LVW/TL in PARP/H11002/H11002 mice was 23.2 and 29.5%, which was significantly (p < 0.05) lower than in the PARP/H11001/H11001 mice. The pressure gradient was comparable in both PARP/H11002/H11002 and PARP/H11001/H11001 banded mice (96.0 ± 28.8 versus 99.4 ± 20.5 mm Hg, n = 4 in each group). These results demonstrate that PARP−/− mice develop less hypertrophy compared with PARP+/+ mice. AIF translocation from the mitochondrial to nuclear fraction was noted in the wild-type mice subjected to aortic banding, but not in the PARP−/− banded mice (Fig. 8). However, the extent of these changes at 29 weeks was less pronounced at 29 weeks than at 9 weeks, perhaps indicating that by 29 weeks of banding the peak of the AIF translocation has passed.

Discussion

The current study shows that aortic banding in mice induces chronic heart failure (CHF), evidenced by depression of left ventricular function and hypertrophy and collagenization of the heart. These results are consistent with earlier reports using similar experimental models. The impaired cardiac function is associated with a diffuse activation of PARP in the myocardium. The ability of INO-1001 to inhibit PARP in the cardiac myocytes was confirmed via immunohistochemical detection of poly(ADP-ribose) activation. In addition, plasma and myocardial concentrations of the PARP inhibitor were found sufficient to block oxidant-induced PARP activation in cardiac myocytes in vitro. Direct functional measurements demonstrate the maintenance of cardiac output in the PARP inhibitor-treated animals undergoing aortic banding.

Reactive oxygen species (superoxide, hydrogen peroxide, and hydroxyl radical) are overproduced in the failing myocardium (Ekelund et al., 1999; Mihm et al., 2001). There is also an overproduction of nitric oxide, due to the expression of the inducible isoform of NO synthase (Fukuchi et al., 1998; Vejstrup et al., 1998). Importantly, a recent study has suggested correlation between chronic overexpression of NO synthase and peroxynitrite generation with cardiac enlargement, conduction defects, sudden cardiac death, and, less commonly, heart failure in mice (Mungrue et al., 2002). In the present, banding-induced CHF model, evidence was also
found for up-regulation of the endothelial isoform of NOS (Dai et al., 2004). The combination of NO and superoxide yields peroxynitrite, which is able to trigger DNA single-strand breakage and activation of PARP (for review, see Vírag and Szabó, 2002). Peroxynitrite generation has been demonstrated in various models of CHF, including the current model of banding (Bouloumie et al., 1997), and this species has been shown to impair cardiac function via multiple mechanisms (Mihm et al., 2001; Pacher et al., 2003). The increased sensitivity of the myocytes to the toxic effects of NO has been directly demonstrated in a banding-induced cardiac hypertrophy model (Brookes et al., 2001).

A recently characterized mechanism of myocardial dysfunction involves the activation of the nuclear enzyme PARP by DNA single-strand breaks generated in response to increased oxidative and nitrosative stress. Evidence for the importance of this pathway has been demonstrated in hearts subjected to regional or global ischemia and reperfusion (for review, see Szabó et al., 2004). The mode of PARP inhibitors’ cardioprotective action involves a conservation of myocardial energetics, as well as a prevention of the up-regulation of various proinflammatory pathways (cytokines, adhesion receptors, and mononuclear cell infiltration) triggered by ischemia and reperfusion (for review, see Vírag and Szabó, 2002). It is conceivable that PARP inhibition exerts beneficial effects in the current model by affecting both above-referenced pathways of injury and also by suppressing positive feedback cycles initiated by them. It is more likely that the reduction in myocardial hypertrophy by INO-1001 (which is thought to be mediated by the up-regulation of growth factors) is mediated by the PARP-mediated modulation of signal transduction and gene expression pathways. Indeed, recent studies implicate the ability of PARP to regulate the expression of growth factors in vitro (Obrosova et al., 2004).

Multiple reports indicate the importance of PARP activation in the development of mitochondrial dysfunction under conditions of oxidative stress (Yu et al., 2002; Du et al., 2003a,b). Even though the major isoform of the PARP family, PARP-1, is widely considered as a nuclear enzyme, there may...
The error bars represent the SEM. *p < 0.05 versus respective sham-operative mice. †p < 0.05 versus PARP−/− mice.

Fig. 7. Effects of long term pressure overload in PARP−/− mouse hearts. Heart weight to tibia length ratio in PARP−/− and PARP+/− mice were determined at 29 weeks after TAC. The error bars represent the SEM. *p < 0.05 versus respective sham-operative mice. †p < 0.05 versus PARP−/− mice.

Fig. 8. AIF translocation in hearts of wild-type and PARP-1-deficient mice at 29 weeks of CHF. Aortic banded wild-type and PARP-1 deficient mice were killed at 29 weeks after operation. AIF levels in mitochondrial and nuclear fractions were detected by Western blotting. In wild-type mice there was evidence for loss of AIF in mitochondrial fractions and an increase in nuclear fractions. Similar changes were not seen in the PARP-1-deficient hearts undergoing CHF.

also be a mitochondrial isoform (Du et al., 2003a), and there is a nuclear-to mitochondrial signaling process, which initiates early mitochondrial alterations, as demonstrated in thymocytes (Virág et al., 1998), in neurons (Yu et al., 2002; Komjáti et al., 2004), in cardiac myocytes in vitro (Chen et al., 2004), and in myocardial infarction in vivo (Xiao et al., 2004). The current findings, demonstrating that PARP inhibition with INO-1001 or genetic PARP-1 deficiency reverse mitochondrial-to-nuclear translocation of AIF in CHF, are consistent with prior studies demonstrating that PARP-1 regulates the translocation of this cell death factor.

It is interesting to note that PARP inhibitor prevented PARP accumulation at 4 or 9 weeks after the banding, but this inhibitory effect is not obvious at 1 week. The same pattern was seen in Fig. 5, where the inhibition of AIF translocation by INO-1001 was less effective at 1 week than at 4 or 9 weeks. Considering the gradual increase in plasma and myocardial INO-1001 levels seen in the present study, it is conceivable that myocardial INO-1001 levels at 1 week did not yet reach sufficient concentrations at the beginning of the study to provide full inhibition of PARP. Additional studies, with delayed administration of the PARP inhibitor are needed to determine the therapeutic window of opportunity and to evaluate whether delayed treatment with PARP inhibitors is able to arrest cardiac hypertrophy or restore myocardial function in CHF.

Prior studies have demonstrated the importance of apoptosis, caspases (Hayakawa et al., 2003; Chandrashekhar et al., 2004), and a variety of cellular pathways, including protein kinase C (PKC) activation (Takeiishi et al., 1998; Koide et al., 2003) in the pathogenesis of CHF. There are also many studies demonstrating TUNEL positivity in a small (approximately 0.3%) population of cells in murine CHF models (Jiang et al., 2003). In human samples, the percentage of TUNEL positivity can be higher (up to 2%) (Di Napoli et al., 2003; Hughes, 2003). What, then, is the relationship between PARP activation, AIF translocation, and the previously described pathways of apoptosis and CHF? First of all, we would like to emphasize that although AIF is termed “apoptosis-inducing” factor, its role goes beyond apoptosis, and it plays a role in a variety of cell death processes (necrotic, oxidant stress induced, and mixed type) (Cregan et al., 2004; Hong et al., 2004). Thus, AIF translocation per se should not be viewed as a hallmark or marker of apoptosis. Indeed, the very small percentage of TUNEL-positive myocytes seen in CHF myocardium (which has also been observed in the current model; our unpublished data), is consistent with this view. AIF translocation can lead to DNA fragmentation, which, however, is caspase-independent. Therefore, its pattern is different from the typical, caspase-mediated DNA cleavage pattern and may not be detectable by conventional TUNEL assays. Furthermore, it is not yet clear whether AIF translocation in CHF is a sufficient or merely a necessary factor for its ability to induce its hallmark large-scale chromatin condensation.

We also need to distinguish between PARP cleavage (a marker, but not necessarily an executor of apoptosis) and PARP activation (induced by DNA strand breaks). The latter process can only occur when PARP is uncleaved, because cleaved PARP is catalytically inactive (for review, see Duriez and Shah, 1997; Virág and Szabó, 2002). The fact that we see widespread poly(ADP-ribose) accumulation in the CHF myocardium indicates that some (possibly most of the) PARP remains in the uncleaved and functionally active state. In fact, a recent report finds no evidence for PARP cleavage in murine or human hearts with CHF (Pillai et al., 2004). The protection against CHF reported by caspase inhibitors therefore is most likely related to a pathway that is parallel and independent from the pathway governed by PARP activation.

Regarding potential connections between PKC and PARP, recent work, conducted using an in vitro model of diabetic complications (endothelial cells placed in high glucose) demonstrates that protein kinase C activation is governed by GAPDH, and in that experimental system PARP inhibitors indirectly inhibit PKC activation, by preventing GAPDH poly(ADP-ribosyl)ation (Du et al., 2003b). At this point, it is
unknown whether PARP/PKC interactions are also present in CHF. In a prior model of CHF induced by chronic coronary ligation, the phosphanithidone-based PARP inhibitor PJ34 was found to attenuate myocardial hypertrophy, and improved the endothelial function of blood vessels (Pacher et al., 2002). The current study extends these findings. The novel aspects of the current work include direct demonstration that PARP inhibition improves cardiac output, attenuates cardiac fibrosis, and tends to reduce CHF-related mortality in a model of banding-induced CHF. The current report is also the first one to demonstrate the phenomenon of mitochondrial-to-nuclear translocation of AIF in CHF and implicates the regulatory role of PARP in this process. While the present work was in review, a study (Pillai et al., 2004) demonstrated an overexpression of PARP-1 enzyme in murine and human CHF models. Thus, the increased poly(ADP-ribose) staining seen in our studies may be a combined consequence of PARP activation and PARP upregulation. The Pillai et al. (2004) report also shows that (similar to our results), PARP-1-deficient mice are protected against the hypertrophy response induced by aortic banding. Overall, our studies (Pacher et al., 2002; current study) as well as other recent studies (Pillai et al., 2004) support the view that PARP activation importantly contributes to the pathogenesis of cardiovascular dysfunction in experimental models of CHF and strengthens the notion that PARP inhibition may represent a novel approach for the experimental therapy of CHF.

References


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