



# Oxidative stress and regional ischemia-reperfusion injury: the peroxy-nitrite–poly(ADP-ribose) polymerase connection

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**Keywords:** poly(ADP-ribose) polymerase, nitric oxide, peroxy-nitrite, cytokines, oxidative stress, myocardial infarction, heart, preconditioning

Coronary Artery Disease 2003, 14:000–000

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Sponsorship: This study was supported by a grant from the National Institutes of Health (R01HL59266) to Csaba Szabó. Lucas Liaudet was supported by a grant from the ADUMED Foundation (Switzerland).

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## Introduction

Poly(ADP-ribose) polymerase (PARP), the enzyme also referred to as poly(ADP-ribose) synthetase (PARS) and poly(ADP-ribose) transferase (pPADPRT), is an abundant nuclear enzyme present throughout the phylogenetic spectrum. The precise physiologic roles of PARP remain undefined: its traditional role as a DNA-repair enzyme has been questioned by recent studies. PARP plays diverse roles, participating in DNA repair, chromatin relaxation, cell differentiation, DNA replication, transcriptional regulation, the control of the cell cycle, p53 expression and apoptosis and transformation [1].

Recent work identifies PARP activation as a key pathway of oxidant- and free radical-mediated myocardial reperfusion injury. *In-vitro* studies in cardiac myocytes and other cell types demonstrated that when activated in response to DNA single strand breaks, PARP initiates an energy-consuming, inefficient cellular metabolic cycle by transferring ADP-ribose units to nuclear proteins. This process rapidly depletes the intracellular NAD<sup>+</sup> and ATP pools leading to cellular dysfunction and cell necrosis. This pathway has been well characterized *in vitro* and is overviewed elsewhere [1–3]. The current chapter provides an overview of the experimental evidence implicating PARP as a pathophysiological modulator of myocardial injury *in vivo*.

## Evidence for activation of poly(ADP-ribose) polymerase in the reperfused heart

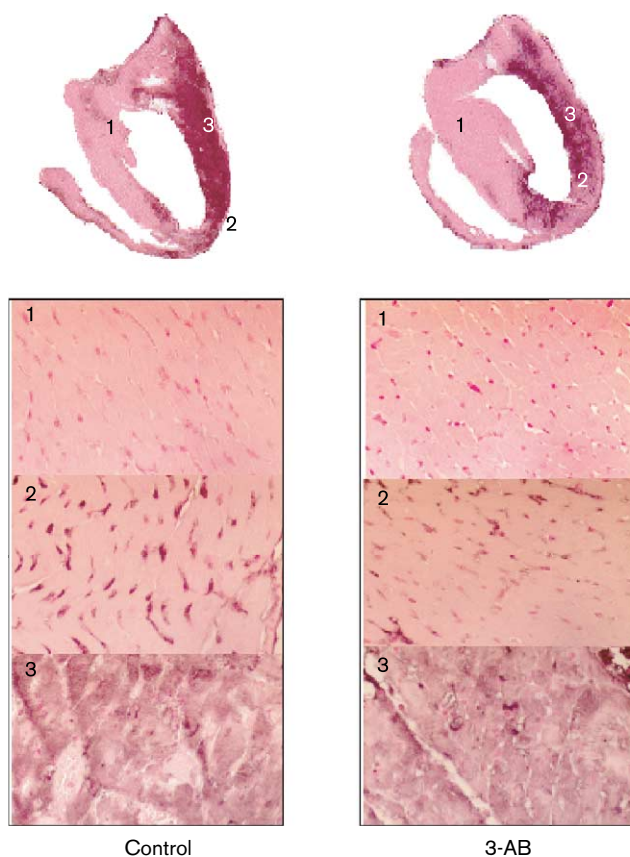
Recent work, utilizing immunohistochemical detection of poly(ADP-ribose) formation in the myocardium, demonstrates that PARP is activated in the reperfused myocardium [4,5]. The time course of PARP activation is rather prolonged: it is present at 2 h after the start of reperfusion and continues to be present as late as 24 h after reperfusion (Fig. 1). This delayed pattern of PARP

activation is likely related to the continuing presence of free radical and oxidant production in the reperfused myocardium. It is also conceivable that a massive, early DNA single strand breakage that remains unrepaired for a prolonged period of time is responsible for the prolonged pattern of PARP activation. The site of the most pronounced PARP activation is the area of necrosis and peri-infarct zone (that is, the area at risk). Most of the poly(ADP-ribose) staining was seen in cardiac myocytes [4,5], indicating that the heart tissue itself, rather than the infiltrating mononuclear cells, is the main site of PARP activation. A more diffuse staining pattern can be seen in the area of necrosis: this pattern is likely to reflect the fact that the cellular contents (and thus the poly-ADP-ribosylated proteins) are now more-or-less uniformly distributed in the necrotic area, due to myocardial necrosis and the associated breakdown of the cell membrane permeability. Because PARP activation triggers cellular necrosis due to cellular energetic collapse (see below), the primary mode of PARP inhibitors' cardioprotective effects is related to a direct inhibition of myocyte necrosis. The peri-infarct zone, which contains viable cells and in which PARP is markedly activated, is the likely site of the PARP inhibitors' beneficial effects.

Activation of PARP has also been demonstrated *in vitro*, in an isolated perfused heart system [6]. One of the enzymes that undergoes poly(ADP-ribose)ylation is PARP itself (auto-riboseylation) [6]. Ischemia-reperfusion-induced self-ADP-riboseylation of PARP can be attenuated by pharmacological inhibitors of PARP [6].

Limited information is available on the activation of PARP in the heart in conditions other than myocardial ischemia-reperfusion. In a recent study, 2',3'-dideoxycytidine and 3'-azido-3'-deoxythymidine were found to induce PARP activation in the heart and the PARP

Fig. 1



Immunohistochemical localization of poly(ADP-ribose) polymerase (PARP) activation in the reperfused myocardium. Top: poly(ADP-ribose) formation, an indicator of PARP activation, as determined in whole heart sections from rats exposed to 1 h ischemia and 23 h of reperfusion. A massive staining is evident in the left ventricular free wall of control animals, which is clearly reduced in rats treated with 3-aminobenzamide (3-AB). Bottom: microscopically, the normal, non-ischemic myocardium from the interventricular septum (zone 1) shows no sign of PARP activation, whereas in the ischemic myocardium, PARP activation is mainly located in the nuclei of myocytes in the peri-infarction zone (zone 2), a pattern which is reduced by treatment with 3-AB. In the infarcted myocardium, severe architectural alterations are coexisting with a more diffuse pattern of PAR staining (zone 3). Magnification, x400.

pathway has been proposed to play a role in the cardiomyopathy induced by these compounds [7,8].

Recent work identified several minor isoforms of PARP [1–3]. The original PARP enzyme is now sometimes called PARP-1. In most pathophysiological conditions, it appears that activation of PARP-1 plays the crucial role in modulating the intracellular metabolic events. Nevertheless, a significant degree of residual PARP activation was found in the hearts of PARP-1-deficient mice, emphasizing the potential, additional role of the minor PARP isoforms in poly(ADP-ribose) formation [5,9].

### Protection against myocardial injury by poly(ADP-ribose) polymerase inhibitors

Based on *in-vitro* studies demonstrating that various cultured cells, including cultured rat cardiac myoblasts, are protected against hydrogen peroxide- and peroxy-nitrite-mediated cell necrosis by PARP inhibitors [10], at the Third International Shock Congress (Hamamatsu, Japan, 1995) we proposed the potential utility of pharmacological inhibitors of PARP as protective agents in myocardial reperfusion injury [11]. We have subsequently evaluated the role of PARP in an acute model of myocardial reperfusion injury in the rat [12]. Peroxynitrite formation was evidenced by plasma oxidation of dihydrorhodamine 123 and formation of nitrotyrosine in the reperfused heart [12]. Myocardial reperfusion resulted in marked cellular injury, as measured by an increase of plasma creatine phosphokinase activity and development of a large infarcted area. Pharmacological inhibition of PARP with 3-aminobenzamide significantly improved the outcome of myocardial dysfunction, as evidenced by a reduction in creatine phosphokinase levels and diminished infarct size, and preserved the ATP pools [12]. Other investigators have confirmed our results in similar experimental models of myocardial reperfusion. In rabbit and pig models of myocardial infarction, pharmacological inhibitors of PARP, such as nicotinamide and 3-aminobenzamide, dramatically reduced the infarct size [13,14]. The cardioprotection afforded by the PARP inhibitors was due to a selective inhibition of PARP, because the structurally related but inactive agents, such as 3-aminobenzoic acid and nicotinic acid, did not cause a reduction in infarct size [13]. Over the last several years, a multitude of studies have demonstrated the cardioprotective effects of various pharmacological PARP inhibitors in cultured myocytes, in perfused heart systems and in various *in-vivo* models of myocardial reperfusion injury [4–6,10,12–24]. Inhibition of PARP activity was found to facilitate energy recovery during reperfusion and reversed some of the functional deterioration caused by reperfusion in isolated hearts. Table 1 overviews the published studies demonstrating myocardial protection in various models of reperfusion injury [4–6,10,12–27] and other models of myocardial failure [7,8,28–31].

### Protection against myocardial injury in poly(ADP-ribose) polymerase-deficient animals

The development of transgenic mice lacking the functional gene for PARP provided the unique opportunity to unequivocally define the role of PARP in myocardial injury and also to investigate some of the cellular mechanisms underlying this disease. Using a murine model of myocardial injury after early reperfusion, we found that absence of a functional PARP gene resulted in a significant prevention of reperfusion injury. One-hour ligation and 1 h reperfusion of the left anterior descend-

**Table 1** Protection against myocardial injury by pharmacological inhibition or genetic inactivation of poly(ADP-ribose) polymerase *in vitro* and *in vivo*

Experimental model	Inducer of injury	Mode of PARP inhibition	Effect of PARP inhibition	References
<i>Studies in vitro</i>				
Rat cardiomyoblasts	H <sub>2</sub> O <sub>2</sub> , peroxyxynitrite H/R	3-AB, Nicam, ISO	Reduction of cell necrosis, improvement of mitochondrial respiration	[10,16]
Human cardiomyoblasts	H <sub>2</sub> O <sub>2</sub>	3-AB, Nicam, ISO	Reduction of cell death	[14,15]
<i>Studies in isolated hearts</i>				
Mouse heart	Global I/R	PARP <sup>-/-</sup> phenotype	Reduction of NAD <sup>+</sup> consump- tion	[5,23]
Rat heart	Global I/R	BGP-15, ISO	Suppression of LV dysfunction Reduction of NAD <sup>+</sup> and ATP catabolism	[6,17]
Rat heart	Global I/R	Lipoamide (antioxidant)	Reduction of LV dysfunction Reduction of myocardial da- mage	[19]
Rat heart	Regional I/R	3-AB	Decrease of infarct size	[16]
Rabbit heart	Global I/R	3-AB	Decrease of infarct size Reduction of LV dysfunction	[13]
<i>Studies in vivo (rodents)</i>				
Mouse	Regional I/R	PARP <sup>-/-</sup> phenotype	Decrease of infarct size, neutro- phil infiltration and circulating IL- 10, TNF- $\alpha$ and nitrate Reduction of P-selectin/ICAM-1 expression	[21,22]
Mouse	IPC	PARP <sup>-/-</sup> phenotype	Suppression of the benefit of IPC	[24]
Rat	Regional I/R	3-AB GPI6150	Preservation of myocardial ATP stores decrease of infarct size, reduc- tion of LV dysfunction, reduction of neutro- phil infiltration	[4,5,12]
Rat	Cardiac transplantation	PJ34	Improved myocardial contracti- lity, protection against endothelial dysfunction	[25]
Rat, mouse	IPC	3-AB, PJ34	Suppression of the benefit of IPC	[24]
Rat, mouse	AZT, ddC, DOX		Induction of cardiomyopathy through the formation of ROS and PARP activation	[7,8,28]
Rat, mouse	Hyperglycemia	PJ34	Protection against myocardial failure	[29]
Rat	Endotoxin	PJ34	Improved myocardial contracti- lity	[30]
<i>Studies in vivo (large animals)</i>				
Rabbit	Regional I/R	3-AB, Nicam, ISO	Decrease of infarct size	[13]
Pig	Regional I/R	3-AB PJ-34	Decrease of infarct size, improvement of LV function	[14] [20]
Pig, Dog	Bypass and I/R	PJ34	Improved myocardial contracti- lity	[26,27]
Pig	Endotoxin	PJ34	Improved myocardial contracti- lity	[31]

H/R, hypoxia/reoxygenation; 3-AB, 3-aminobenzamide; Nicam, nicotinamide; ISO, 1,5 dihydroxyisoquinoline; I/R, ischemia/reperfusion; PARP, poly(ADP-ribose) polymerase; NAD<sup>+</sup>, nicotinamide adenine dinucleotide; LV, left ventricular; IL-10, interleukin 10; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; ICAM-1, intercellular of adhesion molecule-1; IPC, ischemic preconditioning; AZT, 3'-azido-3'-deoxythymidine; ddC, 2',3'-dideoxycytidine; DOX, doxorubicin; ROS, reactive oxygen species.

ing branch of the coronary artery induced massive myocardial necrosis and triggered neutrophil infiltration in wild-type mice [21]. When the reperfusion after 1 h ischemia was prolonged to 24 h, wild-type mice also developed high mortality [22]. In PARP<sup>-/-</sup> mice plasma levels of creatine phosphokinase activity were significantly reduced, the histological features of myocardium were improved, neutrophil infiltration was reduced and survival was improved [21,22].

Significant protective effects of PARP deficiency can also be demonstrated in isolated perfused hearts. We reported that at the end of the reoxygenation in hearts from wild-type animals, there is a significant suppression in the rate of intraventricular pressure development and in the rate of relaxation [23]. In contrast, in the hearts from the PARP knockout animals, no significant suppression of the rate of intraventricular pressure development and relaxation was observed [23].

Our findings, both in isolated perfused hearts and in the *in-vivo* models, have recently been confirmed by Pieper and colleagues, using PARP-deficient mice [5]. *In-vivo* PARP activation in heart tissue slices was assayed through conversion of [<sup>33</sup>P]NAD<sup>+</sup> into poly(ADP-ribose) and also monitored by immunohistochemical staining for poly(-ADP-ribose). Cardiac contractility, nitric oxide and reactive oxygen species production and NAD<sup>+</sup> and ATP levels were measured [5]. Ischemia-reperfusion augmented formation of nitric oxide and oxygen free radicals and PARP activity. Ischemia-reperfusion decreased cardiac contractility and NAD<sup>+</sup> levels, effects that were attenuated in PARP-deficient animals [5]. PARP deficient mice were recently found to be resistant against the effect of the cardiotoxic drug doxorubicin [28].

### Potential role of poly(ADP-ribose) polymerase in myocardial preconditioning

The above data provide a multitude of evidence that the PARP pathway plays a crucial role in myocardial reperfusion injury. Our recent work also demonstrates that PARP is necessary for the phenomenon of ischemic myocardial preconditioning. Using a combined approach (pharmacological inhibition of PARP and PARP-deficient mice), we recently observed that the protective effect of preconditioning disappears in PARP<sup>-/-</sup> mice or in response to the PARP inhibitor 3-aminobenzamide [24]. The protection against reperfusion injury by preconditioning is also associated with partially preserved myocardial NAD<sup>+</sup> levels, indicating that PARP activation is attenuated by preconditioning. This conclusion is further strengthened by poly(ADP-ribose) immunohistochemical measurements, demonstrating that ischemic preconditioning markedly inhibits PARP activation during reperfusion [24]. Because ischemic preconditioning itself induces low levels of oxidative stress and a low degree of PARP activation, we proposed that the low level of PARP activation during preconditioning may lead to auto-ribosylation (that is, auto-inhibition) of PARP. This process could, in turn, protect against the deleterious effects of ischemia and reperfusion, via inhibition of the subsequent, massive activation of PARP, which occurs in naive (non-preconditioned wild-type) animals during reperfusion [24].

### The cellular modes of the cardioprotective action of poly(ADP-ribose) polymerase inhibition

#### The energetic pathway

The best-characterized pathway is related to the energetic hypothesis. The myocardial contraction process is tightly regulated by an efficient conversion of chemical into mechanical energy. Disruption of cellular energetics in general, or of the mitochondrial function specifically, leads to elevated intracellular Na<sup>+</sup> and Ca<sup>2+</sup> levels and

progressive intracellular acidosis, which will affect myocardial contraction and excitability. Disturbances in the energy-generation process and in the mitochondrial function severely compromise the myocardial contractile apparatus. Multiple direct measurements demonstrate that NAD<sup>+</sup> and ATP levels are depleted in cells exposed to various forms of oxidative stress and also in ischemic/reperfused hearts and that these alterations are reversed by PARP deficiency or PARP inhibition (see above). In addition, PARP activation promotes mitochondrial damage and dysfunction. *In-vitro* studies demonstrated that exposure of cultured cells to oxidants induces a time- and dose-dependent decrease in mitochondrial transmembrane potential ( $\Delta\Psi_m$ ), which is associated with an increase in secondary reactive oxygen intermediates production and a loss of cardiolipin, an indicator of mitochondrial membrane damage [32]. Inhibition or inactivation of PARP attenuates peroxynitrite-induced  $\Delta\Psi_m$  reduction, secondary reactive oxygen intermediate generation, cardiolipin degradation and intracellular calcium mobilization [32]. Thus, PARP inhibitors may provide cardioprotection by preserving myocardial mitochondrial function.

#### The inflammatory mediator pathway

The role of PARP in experimental models of disease is not confined to its effects on intracellular energetics and resultant cellular dysfunction. *In-vitro* and *in-vivo* investigations have recently revealed that inhibition of PARP activation has unexpected actions in regulating the expression, activation and nuclear translocation of key pro-inflammatory genes and proteins. The absence of PARP or its pharmacological inhibition has been shown to suppress the activation of MAP kinase [33], AP-1 complex [34] and NF- $\kappa$ B [35]. Consequently, PARP inhibition interferes with the expression of pro-inflammatory genes, such as the inducible nitric oxide synthase and intercellular adhesion molecule-1 (ICAM-1) [21,22]. PARP inhibition blocks ICAM-1 expression in cultured endothelial cells stimulated *in vitro* by a combination of pro-inflammatory cytokines and in the vascular tissues of hearts subjected to reperfusion [22]. The regulation by PARP of gene expression may involve the poly-ADP ribosylation of transcription factors or the repair of DNA strand breaks that interfere with transcription. PARP may also alter the activation of pro-inflammatory pathways via its influence on the expression of AP-1, a heterodimer composed of *c-fos* and *jun* factors. High levels of transcriptional activation of human ICAM-1 and *c-fos* require AP-1 binding to 5' flanking regulatory regions. In cultured cells PARP inhibition blocks oxidant-induced *c-fos* mRNA expression and AP-1 activation [34]. Since the *c-fos* promoter contains an AP-1 consensus site, *c-fos* activation could trigger a positive-feedback cycle of gene expression.

PARP inhibition or PARP deficiency has also been shown to suppress tumour necrosis factor- $\alpha$  and IL-10 production in myocardial reperfusion injury [22]. Because MAP kinase plays a major role in the pleiotropic transduction of intracellular inflammatory cascades, the anti-inflammatory effects of PARP inhibition may be accounted for at this level of gene regulation. One may also expect that PARP-dependent regulation of NF- $\kappa$ B activation has a pleiotropic effect on the expression of pro-inflammatory genes, given the broad role that NF- $\kappa$ B plays in the transcriptional activation of cytokine and chemokine genes. A microchip analysis study recently completed has investigated the changes in the expression of 15 000 genes in wild-type and PARP-deficient fibroblasts. The study has demonstrated that under baseline conditions there is a significant alteration in the expression of a whole host of genes [36]. Even more pronounced differences were found in immunostimulated cells [37].

#### **The endothelial injury and neutrophil activation pathways**

Infiltration of neutrophils is a crucial event for ischemia and reperfusion injury. In the early stages of reperfusion after ischemia, neutrophils move out of the circulation into inflamed tissue. Neutrophils augment the reperfusion damage to vascular and parenchymal cellular elements by the release of proteolytic enzymes, free radicals and proinflammatory mediators. A growing body of experimental data suggests that activation of PARP is an important modulator of leukocyte–endothelial cell interactions. Inhibition of PARP is frequently associated with a reduction of neutrophil infiltration in the site of injury in various experimental models of inflammation including arthritis and colitis [38–40]. The mechanism of regulation of neutrophil trafficking by PARP may involve (1) the regulation of the expression of adhesion molecules (see above) and (2) the maintenance of endothelial integrity. With respect to (2), it is well known that nitric oxide, derived from the vascular endothelium, is a key inhibitor of neutrophil activation, adhesion and trans-migration. There is also accumulating evidence demonstrating that pharmacological inhibition or genetic inactivation of PARP maintains endothelial integrity under conditions of oxidant stress [41,42]. We have recently demonstrated that the regulation of endothelium-dependent relaxant ability by PARP is directly related to modulation of intracellular NADPH levels [42], NADPH being an essential co-factor for nitric oxide synthase. Through the above mechanism, one can hypothesize that during myocardial injury, free radicals and oxidants injure the vascular endothelium, which reduces NO production, which then leads to neutrophil infiltration and further injury (positive feedback cycle). PARP inhibition, by interrupting this cycle, may reduce both neutrophil infiltration and oxidant and free radical generation.

That PARP directly regulates neutrophil function is unlikely, for neutrophil granulocytes do not contain the PARP enzyme [1]. Also, the regulation of neutrophil infiltration by PARP cannot be the sole or exclusive mechanism of cardioprotection, because PARP inhibition continues to be effective in experimental systems that lack neutrophils (such as studies utilizing cultured cells and studies using isolated buffer-perfused heart systems, see above).

#### **Additional mechanisms**

Several additional mechanisms may contribute the reduction of myocardial injury in response to PARP inhibition. PARP-related changes in cellular energetics and related processes involving calcium sequestration, biosynthetic processes and maintenance of the normal cell shape and adherence may be involved. Poly(ADP-ribose) itself may directly play a role in regulating myocardial contractility, as has been shown for ADP-ribose [43], and it may also affect gene expression.

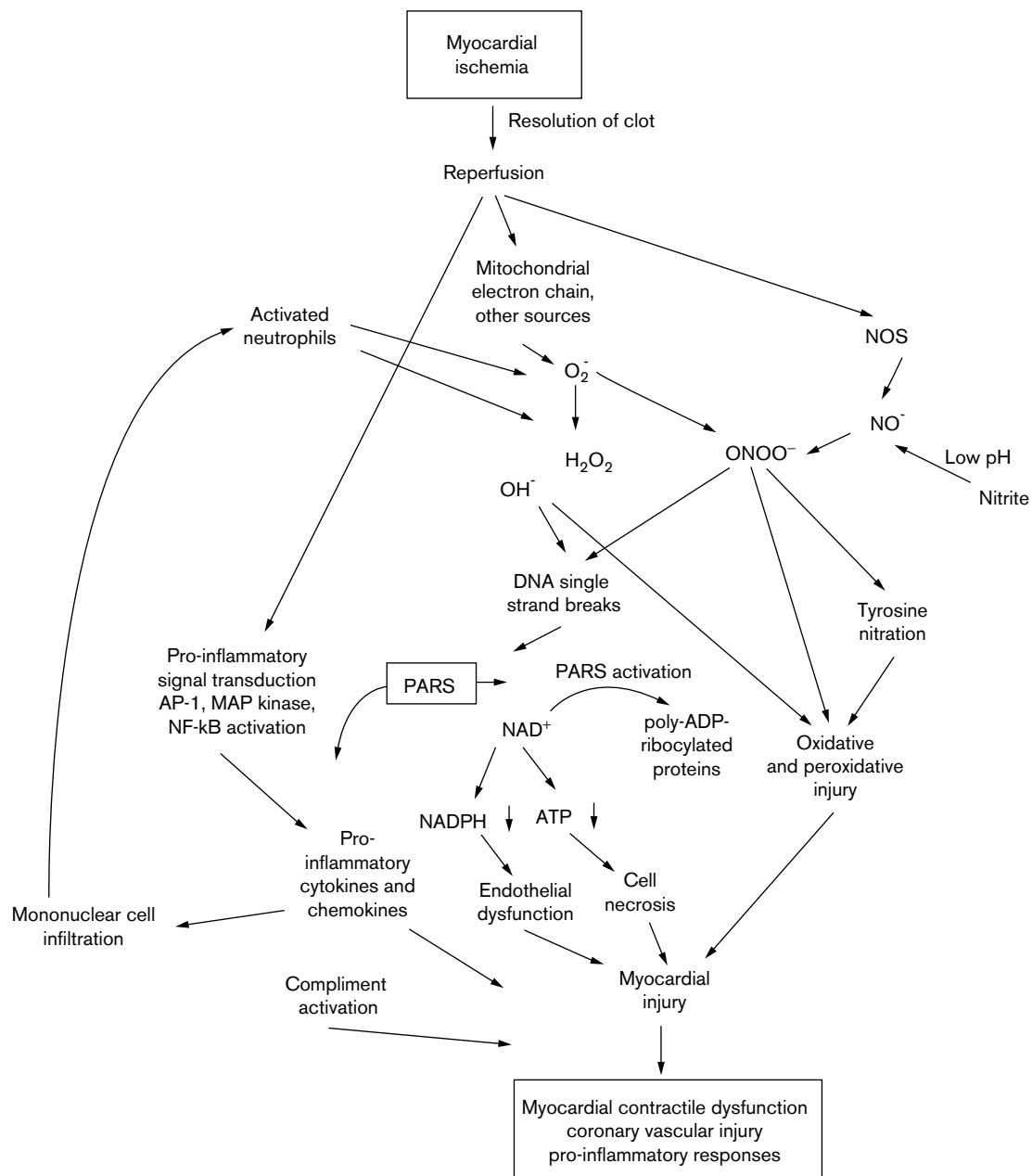
Much of the cell death-related literature focuses on PARP cleavage (as opposed to PARP activation). PARP cleavage by caspases is a marker of apoptotic cell death and has been shown to occur in various models of myocardial ischemia-reperfusion injury [44,45]. The pathway proposed herein has no relationship to the PARP cleavage pathway: pharmacological inhibition of PARP inhibits the process of cell necrosis (rather than apoptosis). In fact, the cleaved form of PARP is catalytically inactive and PARP cleavage has been considered an endogenous mechanism that serves to prevent PARP-dependent metabolic suppression and necrosis [46,47].

#### **Conclusions**

Taken together, a self-amplifying vicious cycle, regulated by PARP, exists in myocardial ischemia and reperfusion (Fig. 2). Early production of oxidants by dysfunctional mitochondria after reperfusion leads to DNA damage and activation of PARP, which in turn causes further derangement of cellular energetic status and induces endothelial injury and production of inflammatory mediators and expression adhesion molecules. The loss of the endothelial barrier function is then responsible for the infiltration of neutrophils, which in turn produces additional oxidants. Pharmacological inhibition of PARP ameliorates the endothelial and myocardial dysfunction by interrupting the vicious cycle at various interacting levels (energetic failure, mediator production, neutrophil infiltration and oxidative damage).

It is appropriate to ask whether PARP inhibition is a candidate for clinical treatment of myocardial reperfusion injury. A variety of PARP inhibitors are in various stages of preclinical development, many with potency that greatly

Fig. 2



Representative scheme of the poly(ADP-ribose) polymerase (PARP) pathway in myocardial ischemia and reperfusion injury. The reduction of oxygen supply during ischemia alters the mitochondrial function, leading to the production of reactive oxidant species. Superoxide and nitric oxide react to yield peroxynitrite. Peroxynitrite and hydroxyl radicals induce the single strand breakage in DNA, which, in turn, activates PARP. The activation of PARP rapidly depletes the cellular  $\text{NAD}^+$  and ATP pools. The cellular energy exhaustion maintains the mitochondria in a reduced state, therefore allowing further production of reactive oxidants at reperfusion. Depletion of  $\text{NAD}^+$  and ATP leads to cellular dysfunction. Depletion of NADPH leads to reduced endothelial nitric oxide formation. The cellular dysfunction is further enhanced by promotion of pro-inflammatory gene expression by PARP, via promotion of NF- $\kappa$ B, AP-1 and MAP kinase activation. The oxidant-induced pro-inflammatory molecule and adhesion molecule expression and the endothelial dysfunction induce neutrophil recruitment and neutrophil activation, which initiates a positive feedback cycle of oxidant generation, PARP activation and cellular injury. PARP, poly(ADP-ribose) synthetase.

exceeds the prototypic agents used in experimental proof-of-concept studies of reperfusion injury. PARP inhibitors may be particularly useful in the treatment of

acute disorders, where the inhibitor is administered only for a short period of time, and therefore the issues of potential toxicity are reduced. Although the exact

physiologic role of PARP remains a matter of dispute, it is logical to suppose it plays an important role since it is an abundant and evolutionally conserved protein. PARP has been implicated in many physiologic housekeeping functions, such as gene repair, transcription and cell cycling. Until such time as its true physiologic functions are more precisely defined, there should exist a considerable caution in the long-term administration of PARP inhibitors to humans. PARP inhibition and PARP deficiency have also been associated with an increase in sister chromatid exchange [48–50], which may raise the risk of malignant transformation. PARP activation leads to cell death and some have argued that its physiologic role is to eliminate genetically damaged cells, thereby reducing oncogenic potential [51]. Indeed, PARP deficiency has been shown to facilitate the rapid ligation of DNA excision-repair patches [52,53]. Whether PARP inhibition predisposes to malignant transformation is an open question. PARP-deficient mice have not been reported to have an increased frequency of malignancies, although this issue has not yet been systematically investigated. A clear distinction must also be made between pharmacological PARP inhibition and genetic PARP deficiency: the latter condition will also affect cellular processes due to the absence of protein–protein interactions that PARP is known to participate in. An additional complicating factor is the multitude of PARP-like enzymes; these isoenzymes are likely to serve different physiological functions. It will be necessary to systematically investigate the function of each of the PARP isoforms, in order to evaluate the benefits against the risks deriving from their selective inhibition. These issues must be adequately addressed prior to considering the development of PARP inhibitors for therapeutic purposes.

## Annotated references

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In this study, activation of poly(ADP-ribose) polymerase (PARP) in the reperfused myocardium of rats was demonstrated using immunohistochemical techniques to detect the formation of poly(ADP-ribose). The activation of PARP, which was located mainly in the cardiomyocytes, was found both at the early (2h) and late (24h) stages of reperfusion and was reduced by treatment with 3-aminobenzamide, resulting in a decreased infarct size and an improved myocardial contractility.

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In this study, activation of poly(ADP-ribose) polymerase (PARP) in reperfused rat hearts *in vivo* was demonstrated by assaying the conversion of radiolabelled NAD<sup>+</sup> into poly(ADP-ribose) as well as by immunohistochemical techniques. This work also showed in an isolated mouse heart preparation that hearts from

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