Review

Poly(ADP-ribose) polymerase activation in the reperfused myocardium

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Abstract

The activation of poly(ADP-ribose) polymerase (PARP) is now considered a final common effector in various types of tissue injury including systemic inflammation, circulatory shock and ischemia/reperfusion. Free radical and oxidant production and related cytotoxicity during ischemia/reperfusion leads to DNA strand breakage which activates the nuclear enzyme PARP and initiates an energy-consuming, inefficient cellular metabolic cycle with transfer of the ADP-ribosyl moiety of NAD+ to protein acceptors. During the last 5 years, a growing number of experimental studies demonstrated the beneficial effects of PARP inhibition in cell cultures through rodent models and more recently in pre-clinical large animal models of regional and global ischemia/reperfusion injury. The objective of the current review is to provide an overview of the experimental evidence implicating PARP as a pathophysiological modulator of myocardial injury in vitro and in vivo.

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1. Introduction

Recent experimental evidence shows that pathophysiological states such as inflammation, circulatory shock and ischemia/reperfusion generate free radical and oxidant species, which, in turn, produce DNA injury and activate a cellular suicidal cascade triggered by the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP also termed as poly(ADP-ribose) synthetase PARS) and poly(ADP-ribose) transferase (pPADPRT). PARP is an abundant nuclear enzyme present throughout the phylogenetic spectrum. The precise physiologic role of PARP is complex and involves several main functions. First, PARP has been implicated in DNA repair and maintenance of genomic integrity [1–4]. Second, PARP regulates the expression of various proteins at the transcriptional level including pro-inflammatory mediators [5–8] such as the inducible nitric-oxide synthetase [5–7], intracellular adhesion molecule [9,10], and major histocompatibility complex class II [11].

Third, PARP activation has been proposed to represent a cell elimination pathway [4,12–14] through which severely damaged cells are removed from tissues. Fourth, PARP was suggested to play a role in the regulation of replication and differentiation [15–18].

Besides its physiological functions, PARP activation was identified as a key pathway in different pathophysiological conditions and disease states. PARP has a complex role in DNA-damage-induced cell death. 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 [19,20]. The pathway overviewed in the current article 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: PARP cleavage has been considered as an endogenous mechanism that serves to prevent PARP-dependent metabolic suppression and necrosis [21,22].

Depending on the severity of DNA damage, genotoxic stimuli can trigger three different pathways [23]. In the case of mild DNA damage, PARP facilitates DNA repair and thus survival. More severe DNA damage induces apoptotic cell...
death during which caspases, the main executor enzymes of apoptotic process, inactivate PARP cleaving into two fragments (p89 and p24). This pathway allows cells with irreparable DNA damage to become eliminated in a safe way. The most severe DNA damage may cause excessive PARP activation depleting NAD$^+$ and ATP stores. NAD$^+$/ATP depletion blocks apoptosis and results in necrosis.

The mechanisms leading to tissue injury and organ dysfunction after ischemia/reperfusion or hypoxia/reoxygenation are multiple. However, there is good evidence that reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide, as well as the reactive nitrogen species peroxynitrite contribute to reperfusion injury in the previously ischemic myocardium [24–27], which, in turn, leads to PARP activation with subsequent myocardial and vascular injury. The current review provides an overview of the experimental evidence implicating PARP as a pathophysiological modulator of myocardial reperfusion injury in vitro and in vivo.

2. Pharmacologic inhibition of PARP

The endogenous inhibitor of PARP, nicotinamide, and the compound 3-aminobenzamide have long served as “benchmark” inhibitors of PARP, i.e., experimental agents suitable for laboratory investigations. These compounds inhibit the enzyme with a low potency, have limited cell uptake and cellular residence time, and exert nonspecific effects, act as antioxidants [23]. More recently, several other classes of more potent and selective PARP inhibitors have been synthesized. Most PARP inhibitor compounds fall into the categories of monoaryl amides and bi-, tri-, or tetracyclic lactams. A common structural feature for these inhibitors is a carboxamide attached to an aromatic ring or the carbamoyl group built in a polyaromatic heterocyclic skeleton to form a fused aromatic lactam or imide. The best-known classes of PARP inhibitors with established structure–activity relationships are quinazolines, isoquinolones, and the closely related tricyclic 5[H]-phenanthridin-6-ones. Most PARP inhibitors act as competitive inhibitors of the enzyme, i.e., the inhibitors block NAD$^+$ binding to the catalytic domain of the enzyme, although some benzamides have also been shown to exert additional effects, such as inhibition of PARP binding to DNA [23].

The most potent compounds from the recent bi- and tricyclic structures can be characterized by low-micromolar to mid-nanomolar inhibitory potencies in whole-cell-based assays and by effective inhibition of PARP and effective biological effects in the low milligram-per-kilogram dosing range [28,29]. For example, PJ34, a novel phenanthridin compound and related substances inhibit PARP activation in whole-cell-based assays in the concentration range of 10 nM to 1 μM, with an EC$_{50}$ in the 100- to 300-nM range, and they exert in vivo anti-inflammatory and anti-reperfusion actions in the dose range of 3–30 mg/kg [28]. Because PARP inhibition is an active and highly competitive area of investigation, it is likely that the most potent and effective compounds (i.e., the likely candidates for drug development) are not yet available in the scientific literature but rather may ultimately emerge in the various databases of published patents and pending patent applications. The published scientific and patent literature has recently been overviewed by Cosi [30].

The earliest studies with PARP inhibitors in the setting of ischemia/reperfusion injury used mainly 3-aminobenzamide and/or nicotinamide. Meanwhile recent data are also available using novel isoquinolones such as 5-aminooquino- lone [10,31] or tricyclic 5[H]-phenanthridin-6-ones such as PJ34 [10,32,33].

So far, no study systemically investigated the effects of timing of PARP inhibition during ischemia/reperfusion. In most studies, PARP inhibitor was applied directly prior to and/or during reperfusion period.

3. Evidence for PARP activation 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 [10,33–35]. The time course of PARP activation is rather prolonged: depending on the experimental model used, it is present at 1 h after the start of reperfusion, and continues to be present as late as 24 h after reperfusion [34,36]. 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 massive, early DNA single-strand breakage, which remains unrepaired for prolonged time periods, is responsible for the prolonged pattern of PARP activation. After regional ischemia/reperfusion, the site of the most pronounced PARP activation is the peri-infarct zone (i.e. area at risk) and the area of necrosis. Most of the poly(ADP-ribose) staining was seen in cardiac myocytes [34,35], indicating that the heart tissue itself, rather than the infiltrating mononuclear cells, is the main site of PARP activation. In the area of necrosis, more diffuse staining pattern can be seen, which is likely to reflect the fact that the cellular content (and thus the poly-ADP-riboseylated 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 direct inhibition of myocyte dysfunction culminating in myocyte necrosis. The likely sites of the PARP inhibitors’ beneficial effects are the ischemic core and the peri-infarct zone, which contains viable cells, in which PARP is markedly activated.

Activation of PARP has also been demonstrated after global ischemia/reperfusion in vitro in an isolated perfused
heart system [37] and in vivo after heart transplantation [10] and after cardiopulmonary bypass with cardioplegic arrest [33] showing a diffuse distribution pattern of PARP in both myocardial and endothelial cells. In this setting, PARP activation could be shown to occur primarily during reperfusion but not during ischemia (Fig. 1), which underlines the role of PARP activation in reperfusion injury.

4. In vitro studies

4.1. Studies in cell culture

Though numerous in vitro studies describe the role of PARP activation in cell injury (overviewed in Ref. [23]), only few studies investigated the effects of PARP activation in cell types relevant to cardiac reperfusion injury—namely myocytes and endothelial cells. Gilad et al. [38] demonstrated for the first time in H9c2 cardiac myoblasts that myocardial oxidant injury after application of peroxynitrite and hydrogen peroxide caused PARP activation with subsequent reduction of mitochondrial respiration. In this study, the suppression of mitochondrial respiration was ameliorated by pharmacological inhibition of PARP. Hypoxia (1 h) and reoxygenation (1–24 h) also resulted in significant activation of PARP, and caused suppression of mitochondrial respiration, which was prevented by the inhibition of PARP. These results were confirmed in rat [39] and human cardiac myoblasts [24]. Recently, Uchiyama et al. [40] showed PARP activation in neonatal rat cardiac myocytes in nitric oxide induced cell necrosis, which was abolished by pharmacologic PARP inhibition.

PARP activation in response to oxidative stress in endothelial cells was described for the first time by Junod et al. [41]. Exposure to hydrogen peroxide led to an increase in PARP activity and a profound depletion of ATP and NAD⁺. In cultured bovine pulmonary artery, endothelial cells exposed to hydrogen peroxide increased PARP activity and
LDH release as an indicator of cell necrosis, which was prevented by the pretreatment of the PARP inhibitor 3-aminobenzamide [42]. In cultured human endothelial cells, Zingarelli et al. [43] demonstrated that exogenous peroxynitrite caused a significant suppression of mitochondrial respiration and simultaneous activation of PARP. Pretreatment with 3-aminobenzamide resulted in the prevention of PARP activation and protection against the cytotoxic effect of peroxynitrite. Sharp et al. [44] showed that TNF-α and interleukine-1β induced expression of adhesion molecules is blocked by PARP inhibition. Recently, it could also be shown that PARP inhibition prevents oncosis [45] and tight junction derangement [46] in endothelial cells.

4.2. Isolated heart studies

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 functional PARP gene resulted in a significant prevention of reperfusion injury. We reported that at the end of the reoxygenation, in hearts from wild-type animals, the rate of intraventricular pressure development and in the rate of relaxation significantly decreased [47]. In contrast, in the hearts from the PARP knockout animals, no significant suppression of the rate of intraventricular pressure development and relaxation was observed [47].

Our findings in the isolated perfused heart and the in vivo models (see below) have recently been confirmed by Pieper et al. [35] using PARP-deficient mice. Cardiac contractility, nitric oxide (NO) and reactive oxygen species production, NAD⁺ and ATP levels were measured. Ischemia/reperfusion augmented formation of NO, oxygen free radicals and PARP activity. Ischemia/reperfusion decreased cardiac contractility and NAD⁺ levels, effects that were attenuated in PARP-deficient animals [35].

A series of experiments in isolated hearts show that pharmacologic inhibition of PARP effectively reduce or even prevent reperfusion injury. In 1997, Thiemermann et al. [48] showed that pharmacological PARP inhibition attenuates myocardial dysfunction caused by global myocardial ischemia and reperfusion in the isolated heart. Subsequently, several isolated heart studies confirmed the effectiveness of PARP inhibition in the reduction of global [37,49,50] and regional [39] myocardial ischemia/reperfusion injury.

5. In vivo studies

5.1. Studies on regional ischemia/reperfusion

Based on in vitro studies, the potential utility of pharmacological inhibition of PARP has been proposed as a protective strategy for myocardial reperfusion injury. The role of PARP was first evaluated using pharmacological inhibitors of the enzyme in acute models of regional myocardial reperfusion injury in the rat [43] and in the rabbit [48]. Peroxynitrite formation was evidenced by plasma oxidation of dihydrorhodamine123 and formation of nitrotyrosine in the reperfused heart [43]. Myocardial reperfusion resulted in a marked cellular injury, as measured by an increase in 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, diminished infarct size, and preserved the ATP pools [48]. Subsequent studies have confirmed our results in similar experimental models of myocardial reperfusion [32,34,35,51]. In rabbit [48] and pig [32,51] models of myocardial infarction, pharmacological inhibitors of PARP, such as nicotinamide and 3-aminobenzamide and the novel ultrapotent PARP inhibitor PJ34 all dramatically reduced infarct size. The cardioprotection afforded by the PARP inhibitors was due to a selective inhibition of PARP, since the structurally related but inactive agents, such as 3-aminobenzoic acid and nicotinic acid, did not cause a reduction in infarct size [48].

In vivo regional ischemia/reperfusion studies in PARP-deficient mouse also showed reduced reperfusion injury in PARP-deficient animals in comparison to wild-type controls [9,22]. In this model, genetic disruption of PARP inhibited the expression the expression P-selectin and intracellular adhesion molecule-1 (ICAM-1) in myocardial ischemia reperfusion. Using a model of ischemia followed by delayed reperfusion, Henry and Wang [22] demonstrated genetic disruption of PARP reduces myocardial necrosis, mortality, and the delayed production of inflammatory mediators during the late stages of reperfusion.

5.2. Studies on global ischemia/reperfusion

The effects of PARP activation on global ischemia reperfusion injury in vivo have also been investigated. We described for the first time in a rat heart transplantation model in vivo that different classes of PARP inhibitors effectively prevent PARP activation and myocardial dysfunction after 1 h of cardiac preservation and reperfusion [10] which was recently confirmed by others [52,53] in a nearly identical model. We could also show that the beneficial effects of PARP inhibition are mediated by both the preservation of high-energy phosphate pool and by attenuation of leukocyte–endothelium interaction. Immunohistological staining demonstrated a significantly attenuated expression of the adhesion molecules P-selectin and intracellular adhesion molecule-1 after pharmacologic inhibition of PARP. The studies of Fiorillo et al. [52,53] clearly indicate that during heart transplantation, the activation of PARP, causing energy depletion, results in myocardial cell injury whose dominant
feature, at least in this experimental model, is necrosis rather than apoptosis.

While most previous in vitro and in vivo studies focused on myocardial function and tissue injury markers, in our abovementioned study we provided a detailed description of endothelial function. We showed that endothelial dysfunction during reperfusion is even more pronounced and more prolonged, which was partially reversed by PARP inhibition. This indicates that mechanism different from PARP activation may also play a significant role in endothelial dysfunction during reperfusion.

Most recently, in dog [33] and pig [54] models of cardiopulmonary bypass, myocardial and endothelial function were significantly improved and myocardial injury reduced after cardioplegic cardiac arrest and reperfusion.

5.3. Role of PARP activation in preconditioning

The above data provide a multitude of evidence that the PARP pathway plays a crucial role in myocardial reperfusion injury. 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), it was shown that the protective effect of preconditioning disappears in PARP-deficient mice or in response to the PARP inhibitor 3-amino-benzamide [55]. The protection against reperfusion injury by preconditioning is also associated with partially preserved myocardial NAD$^+$ levels, indicating that preconditioning attenuates PARP activation. This conclusion was further strengthened by poly(ADP-ribose) immunohistochemical measurements, demonstrating that ischemic preconditioning markedly inhibits PARP activation during reperfusion [55]. Because ischemic preconditioning itself induces low levels of oxidative stress and low degree of PARP activation, we proposed that the low level of PARP activation during preconditioning may lead to auto-riboseylation (i.e. 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 naïve (non-preconditioned wild-type) animals during reperfusion [55].

6. Cellular pathways of PARP inhibition in the reperfused myocardium

6.1. Effects of PARP activation on myocardial energy metabolism

The best characterized pathway of PARP activation is related to the myocardial energy metabolism. 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 in specific, leads to elevated intracellular Na$^+$ and Ca$^{2+}$ 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. Several studies proved (overviewed in Ref. [23]) that PARP activation initiates an energy-consuming, inefficient cellular metabolic cycle with transfer of the ADP-ribose moiety of NAD$^+$ to protein acceptors and subsequent depletion of NAD$^+$ and ATP pools. Multiple direct measurements demonstrate that NAD$^+$ and ATP levels are depleted in cells exposed to various forms of oxidative stress, and also in ischemic/reperfused hearts, and 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 reactive oxygen intermediates production and a loss of cardiolipin, an indicator of mitochondrial membrane damage [56]. Inhibition or inactivation of PARP attenuates peroxynitrite-induced $\Delta \Psi_m$ reduction, secondary reactive oxygen intermediate generation, cardiolipin degradation, and intracellular calcium mobilization [57]. Recent emerging evidence also indicates the intramitochondrial presence of PARP, with roles in cell death under conditions of oxidative stress [57]. Compartmentalization of active PARP, NAD$^+$, and DNA damage within mitochondria explains the rapid beneficial effects after exposure to extramitochondrial peroxynitrite [23,56,57]. In addition, compartmentalization of nitric oxide synthase and oxygen radicals within mitochondria is a potential source of intracellularly generated peroxynitrite, which, in turn, may further facilitate the vicious cycle leading to energy depletion and necrotic cell death. Until now, all experiences are restricted on cell cultures, whereas some differences could be found depending on the cell types (i.e. dividing vs. nondividing, immortalized vs. primary cell cultures, mitochondrial density or other factors), and no studies exist which investigated the role of mitochondrial PARP activation under ischemia/reperfusion. Nevertheless, it might be possible that PARP inhibitors provide cardioprotection also by preserving myocardial mitochondrial function.

6.2. Effects of PARP inhibition on the inflammatory pathways

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 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 [58],...
AP-1 complex [59], and NF-κB [8]. Consequently, PARP inhibition interferes with the expression of pro-inflammatory genes, such as the inducible NO synthase and ICAM-1 [5–7,60]. 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 [36]. The regulation by PARP of gene expression may involve the poly-ADP ribosylation of transcription factors or the repair of DNA strand breaks which 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 c-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 [59]. 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 and PARP deficiency have also been shown to suppress TNF-α and IL-10 production in myocardial reperfusion injury [36]. Since 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-κB activation has a pleiotropic effect on the expression of pro-inflammatory genes, given the broad role that NF-κB 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 [61]. Similarly, under conditions of immunostimulation, PARP regulates the expression of a multitude of gene products [62].

6.3. Effects of PARP inhibition on leukocyte–endothelium interaction

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 pro-inflammatory 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 [6,63,64]. The mechanism of regulation of neutrophil trafficking by PARP may involve the regulation of the expression of adhesion molecules as described above and the maintenance of endothelial integrity. It is well known that NO, derived from the vascular endothelium, is a key inhibitor of neutrophil activation, adhesion, and transmigration. There is also accumulating evidence demonstrating that pharmacological inhibition or genetic inactivation of PARP maintains endothelial integrity under conditions of oxidant stress [28,65]. The regulation of endothelium-dependent relaxant ability by PARP is directly related to modulation of intracellular NADPH levels [66]—NADPH being an essential co-factor for NO 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 both reduce neutrophil infiltration and oxidant and free radical generation.

It is unlikely that PARP directly regulates neutrophil function because neutrophil granulocytes do not contain the PARP enzyme [67]. 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 which lack neutrophils (such as cultured cells or isolated buffer-perfused heart systems).

7. Conclusions and future directions

The present review demonstrates that PARP activation plays a central role in the pathophysiological changes during reperfusion and that genetic disruption or pharmacologic inhibition of PARP attenuates or even prevents reperfusion injury. 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, 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). Some limited studies suggest that PARP inhibition is also beneficial in other type of myocardial injury. In a recent study, 2′,3′-dideoxycytidine and 3′-azido-3′-deoxythymidine were found to induce PARP activation in the heart, and the PARP pathway has been proposed to play a role in the cardiomyopathy induced by these compounds [67,68]. Furthermore, PARP inhibition improved myocardial function during the development of heart failure induced by chronic coronary ligation [69], in the myocardial dys-
function associated with systemic inflammation/endotoxic shock [70] and in the later stages of diabetes mellitus [28,71]. With respect to diabetes mellitus, it is noteworthy that recent studies have provided evidence for increased PARP activation in the blood vessels of diabetic and pre-diabetic humans [72].

An important question is whether these effects can be achieved also in the clinical setting. A variety of PARP
inhibitors are in various stages of pre-clinical development, many with potency that greatly exceeds the prototypic agents used in experimental proof-of-concept studies of reperfusion injury. The latest results in large animal cardiopulmonary bypass models, which are nearly identical to the clinical situation, may be encouraging to move forward and to test the efficacy of PARP inhibition in the clinical arena. However, a world of caution should be considered before starting large clinical therapeutic studies. 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 evolutionarily conserved protein. PARP has been implicated in many physiologic housekeeping functions, such as gene repair, transcription, and cell cycling. PARP inhibition and PARP deficiency has also been associated with an increase in sister chromatid exchange [73–75], 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 [76]. Indeed, PARP deficiency has been shown to facilitate the rapid ligation of DNA excision-repair patches [77,78]. Whether PARP inhibition predisposes to malignant transformation is an open question. PARP-deficient mice have not been reported to show 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, vs. 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. These issues must be adequately addressed prior to considering the development of PARP inhibitors for therapeutic purposes. Until such time as its true physiologic functions are more precisely defined, long-term administration of PARP inhibitors to man should be considered with great caution in. However, PARP inhibitors may be particularly useful in the treatment of acute disorders such myocardial ischemia/reperfusion, where the inhibitor is administered only for a short period of time, and thereby reducing the issues of potential toxicity.

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