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Role of Poly(ADP-ribose) polymerase 1 (PARP-1) in Cardiovascular Diseases: The Therapeutic Potential of PARP Inhibitors

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Abstract

Accumulating evidence suggests that the reactive oxygen and nitrogen species are generated in cardiomyocytes and endothelial cells during myocardial ischemia/reperfusion injury, various forms of heart failure or cardiomyopathies, circulatory shock, cardiovascular aging, diabetic complications, myocardial hypertrophy, atherosclerosis, and vascular remodeling following injury. These reactive species induce oxidative DNA damage and consequent activation of the nuclear enzyme poly(ADP-ribose) polymerase 1 (PARP-1), the most abundant isoform of the PARP enzyme family. PARP overactivation, on the one hand, depletes its substrate, NAD^+, slowing the rate of glycolysis, electron transport, and ATP formation, eventually leading to the functional impairment or death of the endothelial cells and cardiomyocytes. On the other hand, PARP activation modulates important inflammatory pathways, and PARP-1 activity can also be modulated by several endogenous factors such as various kinases, purines, vitamin D, thyroid hormones, polyamines, and estrogens, just to mention a few. Recent studies have demonstrated that pharmacological inhibition of PARP provides significant benefits in animal models of cardiovascular disorders, and novel PARP inhibitors have entered clinical development for various cardiovascular indications. Because PARP inhibitors can enhance the effect of anticancer drugs and decrease angiogenesis, their therapeutic potential is also being explored for cancer treatment. This review discusses the therapeutic effects of PARP inhibitors in myocardial ischemia/reperfusion injury, various forms of heart failure, cardiomyopathies, circulatory shock, cardiovascular aging, diabetic cardiovascular complications, myocardial hypertrophy, atherosclerosis, vascular remodeling following injury, angiogenesis, and also summarizes our knowledge obtained from the use of PARP-1 knockout mice in the various preclinical models of cardiovascular diseases.

Keywords

Angiogenesis; Apoptosis; Cardiomyopathy; Diabetes; DNA repair; Heart failure; Inflammation; Necrosis; Nitric oxide; Peroxynitrite; Poly(ADP-ribose) polymerase; Vascular remodeling

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Conflict of interest: CS is one of the founders and has been from 1998–2005 Chief Scientific Officer of Inotek, Inc., the developer of PARP inhibitors; PP owns stock in the company. The trust, established for the members of CS family, owns stock in Inotek, Inc. [Correction added after online publication Oct 3, 2007: PP replaced by CS]

INTRODUCTION

The nuclear enzyme poly(ADP-ribose) polymerase 1 (PARP-1, EC 2.4.2.30) is the most abundant isoform of the PARP enzyme family, which is continuously undergoing expansion (Jagtap and Szabo 2005; Schreiber et al. 2006; Szabo et al. 2006a; Virag 2005; Virag and Szabo 2002). PARP-1, is a 116-kDa protein consisting of three main domains: the N-terminal DNA-binding domain containing two zinc fingers, the automodification domain, and the C-terminal catalytic domain. The primary structure of the enzyme is highly conserved in eukaryotes, with the catalytic domain showing the highest degree of homology between different species. PARP-1 functions as a DNA damage sensor and signaling molecule binding to both single- and double-stranded DNA breaks. Upon binding to damaged DNA, PARP-1 forms homodimers and catalyzes the cleavage of NAD^+ into nicotinamide and ADP-ribose to form long branches of ADP-ribose polymers on glutamic acid residues of a number of target proteins including histones and PARP-1 (automodification domain) itself. Poly(ADP-ribosylation) deliberates negative charge to histones leading to electrostatic repulsion among histones and DNA, a process implicated in chromatin remodeling, DNA repair, and transcriptional regulation. Numerous transcription factors, DNA replication factors, and signaling molecules have also been shown to become poly(ADP-ribosylated) by PARP-1. The effects of PARP-1 on the function of these proteins is achieved by noncovalent protein–protein interactions or by covalent poly(ADP-ribosyl)ation. Poly(ADP-ribosyl)ation) is a fast dynamic process, which is also indicated by the short (<1 min) *in vivo* half-life of the polymer, and determined by two catabolic enzymes poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosyl protein lyase (Virag and Szabo 2002).

Until recently, it was thought that the regulation of PARP happens primarily at the level of DNA breakage: Recognition of DNA breaks was considered to be the primary regulator (activator) or the catalytic activity of PARP (Jagtap and Szabo 2005; Schreiber et al. 2006; Szabo et al. 2006a; Virag 2005; Virag and Szabo 2002). Excitedly, recent studies have provided evidence that PARP-1 activity can also be modulated by several endogenous and exogenous factors, including various kinases, estrogen, thyroid hormones, active forms of vitamin D, polyamines, purines, and caffeine metabolites (Szabo et al. 2006a). Poly(ADP-ribosyl)ation is involved in the regulation of multiple physiological and pathophysiological cellular functions such as DNA repair, gene transcription, cell cycle progression, cell death, chromatin function, and genomic stability, the discussion of which is a subject of several recent detailed overviews (Jagtap and Szabo 2005; Schreiber et al. 2006; Szabo et al. 2006a; Virag 2005; Virag and Szabo 2002), and is beyond the scope of this review article. In this review we will highlight the main mechanisms and pathways that underlie the rationale for the development of various PARP inhibitors for diverse cardiovascular indications.

PARP-1 AND ITS INHIBITORS

Triggers, Consequences, and Inhibitors of PARP-1 Activation

According to the unifying concept (Virag and Szabo 2002) cells exposed to DNA damaging agents can enter three major pathways based on the intensity of the trigger. Moderate genotoxic stimuli facilitate PARP-1 activation leading to DNA repair by signaling cell cycle arrest and by interacting with DNA repair enzymes such as XRCC1 and DNA-PK. Consequently, DNA damage is restored and cells survive without the risk of passing on mutated genes in this pathway. This scenario may occur in cells that are exposed to certain genotoxic agents (e.g., antitumor drugs), when the damage caused by these agents is largely dependent on the activation of PARP; under these circumstances the inhibition of PARP augments cytotoxicity, which can be exploited for anticancer therapy. More severe DNA damage triggers the second apoptotic cell death pathway during which caspases (the main executor enzymes of the apoptotic machinery) inactivate PARP-1 by cleaving it into two fragments by destroying its

ability to respond to DNA strand breaks, thereby preventing the loss of cellular ATP associated with PARP activation and allowing the maintenance of the cellular energy essential for the execution of apoptosis. This route is intended to prevent cells from the pathological consequence of the third pathway mentioned later in which cells die by necrosis, a less controlled mechanism also posing a risk for neighboring cells. As such, PARP cleavage has been proposed to function as a molecular switch between apoptotic and necrotic modes of cell death (Boulares et al. 1999; Levrand et al. 2006; Virag and Szabo 2002). Extensive oxidative and/or nitrosative stress triggers the third pathway by inducing extensive DNA breakage, overactivation of PARP, and consequent depletion of the cellular stores of its substrate NAD^+, impairing glycolysis, Krebs cycle, and mitochondrial electron transport, and eventually resulting in ATP depletion and consequent cell dysfunction and death by necrosis. In this case, pharmacological inhibition of PARP or genetic deletion of the PARP-1 preserves cellular NAD^+ and ATP pools in oxidatively and/or nitrosatively stressed cardiomyocytes, endothelial or other cell types, thereby allowing them to function normally, or, if the apoptotic process has initiated, to utilize the apoptotic machinery and die by apoptosis instead of necrosis (Bhatnagar 1997; Bowes et al. 1998a, 1999; Fiorillo et al. 2006; Gilad et al. 1997; Levrand et al. 2006). The inhibition of this third pathway by PARP inhibitors may offer tremendous therapeutic benefit; for instance, in severe cardiovascular conditions (e.g., during the myocardial ischemia reperfusion following myocardial infarction, bypass surgery, cardiac transplantation, cardiac arrest, aortic reconstructive surgery, just to mention a few) by preventing acute cell death. In addition to its previously mentioned functions in cell death, two recently discovered roles of PARP have been described, which are crucial from the therapeutic perspective of most cardiovascular disorders to be described.

The first additional role of PARP-1 is its involvement of regulating the mitochondria-to-nucleus translocation of apoptosis-inducing-factor (AIF), a 67-kDa mitochondrial death-promoting protein, which induces DNA fragmentation by initiating the activation of a yet unidentified nuclease (Susin et al. 1999). PARP-1 activity appears to be essential for AIF to translocate to the nucleus in cells exposed to oxidative stress, a process most likely mediated by small PAR fragments signaling into the mitochondria (Andrabi et al. 2006; Yu et al. 2002, 2006). As such, AIF is currently believed to play an important role in PARP-1-dependent cell death (Andrabi et al. 2006; Dawson and Dawson 2004; Yu et al. 2002, 2006), supporting the hypothesis that a nuclear mitochondrial crosstalk dependent on poly(ADP-ribosylation) is critical in determining the fate of oxidatively injured cells. Interestingly, this crosstalk may also involve a PARP-1-dependent activation of the MAP kinase JNK1 via a pathway using members of the TNF signaling cascade (RIP1 and TRAF2) (Xu et al. 2006). Further studies are required to clarify this intriguing aspect of PARP-1 biology.

The second additional role of PARP-1 is its involvement in the regulation of the expression of various proteins implicated in the inflammation at the transcriptional level [(e.g., inducible nitric oxide synthase (iNOS), intercellular adhesion molecule-1 (ICAM-1), [COX-2, and major histocompatibility complex class II (MHC Class II)], which is of particular importance. The absence of functional PARP-1 (either genetic or pharmacological) decreased the expression of a host of proinflammatory mediators, including cytokines, chemokines, adhesion molecules, and enzymes (e.g., iNOS), and it also reduced tissue infiltration with activated phagocytes in experimental models of inflammation, circulatory shock, and ischemia reperfusion (Szabo 2006). NF-κB is a key transcription factor in the regulation of these proteins and PARP has been shown to act as a co-activator in the NF-κB-mediated transcription (Oliver et al. 1999). Poly(ADP-ribosylation) can loosen up chromatin structure and thereby make genes more accessible for the transcriptional machinery. These seminal observations have been extended to show that PARP-1 further participates in the activation of other essential proinflammatory signaling cascades, including JNK (Zingarelli et al. 2004a, 2004b) and p38 MAP-kinases (Ha et al. 2002), as well as the transcription factors activator-protein-1 (AP-1), stimulating factor-1

(Sp-1), octamer-binding transcription factor-1 (Oct-1), Yin Yang-1 (YY-1), and signal transducer and activator of transcription-1 (STAT-1) (Ha et al. 2002). The ability of PARP inhibitors to suppress the expression of proinflammatory genes may be further exploited in various cardiovascular disorders associated with acute (e.g., myocardial infarction, coronary bypass and aortic reconstructive surgeries and septic shock) and/or chronic inflammation (e.g., atherosclerosis, cardiovascular aging), inflammatory diseases, and various forms of cancer. Some of the key pathophysiological roles of PARP are shown in a simplified scheme (Fig. 1).

Many pharmacological inhibitors of PARP have been developed over the last two decades. Some of the prototypical PARP inhibitor structures are shown in Figure 2. The medicinal chemistry and structure–activity relationships of these compounds are beyond the scope of this paper and have been overviewed in a number of recent expert reviews (Donawho et al. 2007;Jagtap et al. 2002,2004,2005;Ratnam and Low 2007;Southan and Szabo 2003;Thomas et al. 2007;Virag and Szabo 2002;Woon and Threadgill 2005). Please note that the inhibitors of lower potency tend to be less specific, as they also exert nonspecific antioxidant effects, as well as effects on many (if not all) of the various isoforms of the PARP family (Jagtap and Szabo 2005;Virag and Szabo 2002).

An area worthy of separate discussion is the relationship of PARP inhibition with sirtuin activation. The latter enzyme(s) are NAD^+-dependent deacetylases and it is likely that changes in NAD^+ metabolism due to PARP activation or inhibition could impact sirtuin function (Chong et al. 2005; Frye 1999). Activation of sirtuins has been shown to be protective in a number of aging related disorders and these pathways likely interact (Hassa et al. 2006). Furthermore, the PARP-1-dependent cardiac myocyte cell death during heart failure may also be mediated by NAD^+ depletion and reduced Sir2alpha deacetylase activity (Pillai et al. 2005a).

Due to confidentiality reasons, the structures and the potencies of many of the PARP inhibitors that have entered clinical trials (see separate section below) have not been disclosed in the scientific literature, but they all appear to be highly potent (presumably in the nanomolar range, when tested in the isolated PARP-1 enzyme).

Role of PARP-1 Activation in Various forms of Myocardial Ischemia Reperfusion Injury: Effects of PARP Inhibitors

Reperfusion injury (triggered by the transient disruption of the normal blood supply to target organs followed by reperfusion) is the principal cause of tissue damage occurring in conditions such as myocardial infarction, cardiopulmonary bypass, and aortic reconstructive surgeries, stroke, organ transplantation, and as well as a major mechanism of end-organ damage complicating the course of circulatory shock of various etiologies. The definitive treatment to reduce myocardial damage of ischemic myocardium is reperfusion; however, reperfusion itself leads to additional tissue injury mediated by a multitude of factors including reactive oxygen (superoxide anion, hydrogen peroxide, and hydroxyl radical) and reactive nitrogen species (e.g., peroxynitrite and nitrogen dioxide) upon reperfusion, as well as to the rapid transcriptional activation of an array of proinflammatory genes (Ferdinandy and Schulz 2003; Pacher et al. 2005b, 2006b, 2007; Turko and Murad 2002; Ungvari et al. 2005). Immediate consequences are the local sequestration and activation of polymorphonuclear leukocytes, leading to a rapid amplification of the initial inflammatory response and ROS generation, so-called “respiratory burst” (Lucchesi 1990). The sources of reactive oxygen species in reperfusion injury can be multiple such as mitochondria, xanthine oxidase, NAD(P)H oxidases, cyclooxygenase, and NOS (Griendling et al. 2000; Pacher et al. 2005b, 2006b, 2007; Ungvari et al. 2005). The burst of reactive oxygen and nitrogen species immediately upon reperfusion initiates a chain of deleterious cellular responses eventually leading to coronary endothelial dysfunction; adherence of neutrophils to endothelium, transendothelial

migration, and the release of inflammatory mediators; transient impairment of left ventricular systolic contractile function or “myocardial stunning”; acute diastolic dysfunction; cellular calcium overload; re-energization-induced myocyte hypercontracture; arrhythmia; and cell death (Pacher et al. 2007; Ungvari et al. 2005).

There is marked overactivation of PARP in the reperfused myocardium, which parallels with the decline of the contractile function and myocardial NAD^+ and ATP contents in preclinical models of myocardial infarction and cardiopulmonary bypass. Consequently, the pharmacological inhibition of PARP with various inhibitors (3-AB, nicotinamide, ISQ, 5-AIQ, IQD, BGP-15, GPI 6150, PJ-34, and INO-1001) or genetic deletion of PARP-1 in mice (Grupp et al. 1999; Pieper et al. 2000; Yang et al. 2000; Zhou et al. 2006; Zingarelli et al. 1998, 2003, 2004b), rats (Bowes et al. 1999; Docherty et al. 1999; Farivar et al. 2005; Fiorillo et al. 2002, 2003; Halmosi et al. 2001; Liaudet et al. 2001a; Pieper et al. 2000; Szabados et al. 2000; Szabo et al. 2002b, 2005b, 2006b, 2006c; Thiemermann et al. 1997; Wayman et al. 2001; Xiao et al. 2004; Zingarelli et al. 1997), rabbits (Thiemermann et al. 1997), dogs (Szabo et al. 2004b, 2004c), and pigs (Bowes et al. 1998b; Faro et al. 2002; Hauser et al. 2006) markedly improves the outcome of myocardial ischemia-reperfusion damage (in all *in vitro* or *ex vivo* models) associated with hypoxia/reoxygenation, coronary artery occlusion/reocclusion, cardiopulmonary bypass, and cardiac transplantation, which is also a subject of numerous recent overviews (Pacher et al. 2005b, 2007; Szabo 2005a; Szabo and Bahrle 2005; Ungvari et al. 2005). The favorable effects of PARP inhibitors in these preclinical models involve improvement in myocardial intracellular energy status and myocardial contractility; attenuation of the proinflammatory gene/mediator expression and neutrophil infiltration into the reperfused myocardium; and decrease of cardiomyocyte and endothelial cell necrosis (Tables 1 and 2) (Bowes et al. 1998b, 1999; Docherty et al. 1999; Farivar et al. 2005; Faro et al. 2002; Fiorillo et al. 2002, 2003; Grupp et al. 1999; Halmosi et al. 2001; Hauser et al. 2006; Liaudet et al. 2001a; Pacher et al. 2005b, 2007; Pieper et al. 2000; Szabados et al. 2000; Szabo 2005a; Szabo and Bahrle 2005; Szabo et al. 2002b, 2004b, 2004c, 2005c, 2006b, 2006c; Thiemermann et al. 1997; Ungvari et al. 2005; Wayman et al. 2001; Xiao et al. 2004; Yang et al. 2000; Zhou et al. 2006; Zingarelli et al. 1997).

Several recent human studies have investigated the role of peroxynitrite (a reactive nitrogen species formed from the diffusion-limited reaction of nitric oxide and superoxide anion) also known to be an obligatory trigger of oxidative DNA damage and consequent PARP activation, in myocardial ischemia/reperfusion (I/R) in patients undergoing open heart surgery (Hayashi et al. 2001, 2003; Mehlhorn et al. 2003). These studies analyzed myocardial nitrotyrosine immunoreactivity (footprint of peroxynitrite formation and nitrative stress) from left ventricular biopsy specimen (Mehlhorn et al. 2003) or plasma nitrotyrosine levels from coronary sinus effluent and/or arterial blood (Hayashi et al. 2001, 2003) before and at the end of cardiopulmonary bypass. They found that the difference between plasma nitrotyrosine level from coronary sinus effluent and arterial blood (index of myocardium-derived peroxynitrite generation) peaked at 5 minutes following reperfusion, and was significantly correlated with the peak coronary sinus effluent and arterial blood difference in plasma malondialdehyde concentrations (index of myocardial oxidative stress and lipid peroxidation), and with postoperative maximum creatinine kinase level (index of myocardial injury) (Hayashi et al. 2001). The cardioplegia-induced myocardial I/R was also accompanied by increased iNOS expression, nitrotyrosine and 9-isoprostane formation (Mehlhorn et al. 2003), further supporting the role of both peroxynitrite and reactive oxygen species (ROS) in mediating the myocardial injury. Increased immunostaining for nitrotyrosine and iNOS were also demonstrated from the left ventricular biopsy specimens of patients with hibernating myocardium, a state of chronic contractile dysfunction present at rest in a territory subtended by a stenosed coronary artery that recovers following revascularization, most likely originated from repetitive episodes of transient ischemia (Baker et al. 2002), and in human coronary

arteries of patients with human transplant coronary artery disease, a major cause of late mortality after cardiac transplantation (Ravalli et al. 1998), and during cardiac allograft rejection (Szabolcs et al. 1998). At present, human studies have not yet been conducted to investigate if PARP is activated in human cardiomyocytes and endothelial cells during the myocardial reperfusion injury. Nevertheless, a recent study (Toth-Zsamboki et al. 2006) investigated multiple aspects of human myocardial ischemia/reperfusion-related pathology by analyzing serum, plasma, and isolated peripheral leukocyte samples from cardiovascular patients with acute ST-segment elevation myocardial infarction and successful primary angioplastic intervention and provided evidence for: (a) oxidative/peroxidative imbalance (increased total plasma peroxide concentration and nitrotyrosine production), (b) angioplasty-triggered DNA damage (substantiated by increased levels of serum 8-hydroxy-2′ deoxyguanosine [8OHdG]), (c) rapid activation of PARP-1 in circulating human peripheral leukocytes (demonstrated by immunohistochemistry and Western blotting) following revascularization of the occluded coronary artery, and (d) translocation of the AIF from mitochondria to nuclei (which may well be a consequence of PARP activation). These observations further support the theory that recanalization of an occluded blood vessel triggers oxidative/nitrosative stress in humans, and demonstrate that local myocardial I/R triggered by percutaneous interventions in acute myocardial infarction is capable of generating systemic oxidative responses (Toth-Zsamboki et al. 2006).

Role of PARP-1 Activation in Various forms of Heart Failure, Cardiomyopathies, and Myocardial Hypertrophy

Multiple lines of evidence support the increased superoxide generation by various enzymatic other sources (e.g., xanthine oxidase, NADPH oxidases, cyclooxygenases, and the mitochondria) coupled with increased NO abundance (presumably from iNOS and/or nNOS overexpression) in various preclinical models of heart failure, favoring the generation of reactive oxidant peroxynitrite, which coupled with ROS may impair the cardiovascular function by various mechanisms (Pacher et al. 2005b, 2007; Schulz 2007; Turko and Murad 2002), one of which is ultimately oxidative DNA damage and PARP activation. Augmented myocardial nitrotyrosine formation, iNOS expression, and matrix metalloproteinase (MMP-2) and/or PARP activation were reported in acute and chronic mouse models of doxorubicin-induced heart failure (Bai et al. 2004; Mihm et al. 2002; Pacher et al. 2003; Pacher et al. 2002b; Pacher et al. 2006a; Szenczi et al. 2005; Weinstein et al. 2000), in heart failure induced by permanent left anterior coronary artery ligation in mice (Feng et al. 2001) and rats (Mihm et al. 2001; Pacher et al. 2002c, 2006a) or by pacing in dogs (Cesselli et al. 2001). Moreover, a novel peroxynitrite decomposition catalyst FP15 (Pacher et al. 2003) and PARP inhibitors PJ-34 (Pacher et al. 2002b) or INO-1001 (Pacher et al. 2006a) attenuated the development of cardiac dysfunction, myocardial nitrotyrosine formation, and increased the survival in doxorubicin-induced mouse cardiomyopathy models (Bai et al. 2004; Pacher et al. 2002b, 2003b, 2006a; Szenczi et al. 2005), and also in a rat model of chronic heart failure induced by permanent left anterior coronary artery ligation in rats (Pacher et al. 2002c, 2006a). In the latter model PARP inhibition with PJ-34 or INO-1001 was also associated with improved heart failure-associated decreased endothelial function and decreased myocardial hypertrophy and adverse remodeling (Pacher et al. 2002c, 2006a). Importantly, recent studies have also demonstrated overexpression of PARP-1 or increased activity in biopsies from human subjects with heart failure (Molnar et al. 2006; Pillai et al. 2005b).

In chronic heart failure associated with advanced aging or hypertension in rats and/or mice, increased ROS formation and nitrosative stress and increased poly(ADP-ribosyl)ation were also reported both in cardiomyocytes and endothelial cells (Booz 2007; Csiszar et al. 2005; Escobales and Crespo 2005; Pacher et al. 2005b, 2007; Turko and Murad 2002; Ungvari et al. 2005). Pharmacological inhibition of PARP attenuated the myocardial hypertrophy and

improved endothelial function in these animal models of diseases (Csiszar et al. 2005; Pacher et al. 2002e, 2002f, 2004a, 2004b; Radovits et al. 2007) (Tables 1 and 2).

Recently, overexpression of the PARP-1 and increased poly(ADP-ribosyl)ation in myocardium of mice with aortic banding-induced CHF were also demonstrated (Pillai et al. 2005b; Xiao et al. 2005). In addition, genetic deletion of PARP-1 or pharmacological inhibition protected against hypertrophy response, heart failure, and cardiovascular dysfunction induced by aortic banding or angiotensin II infusion, and prevented the mitochondrial-to-nuclear translocation of the cell death and apoptosis-inducing factors (Balakumar and Singh 2006; Pillai et al. 2005b, 2006; Szabo et al. 2004a; Xiao et al. 2005) (Tables 1 and 2).

In mouse and rat models of diabetic cardiomyopathies the depression of myocardial systolic and diastolic function is also associated with a significant increase in protein nitration and poly (ADP-ribosyl)ation in the cardiac myocytes and endothelial cells and impaired vascular endothelial relaxation, which is remarkably improved by PARP inhibitors (Garcia Soriano et al. 2001; Mabley and Soriano 2005; Pacher et al. 2002d; Pacher and Szabo 2005, 2006; Soriano et al. 2001; Szabo 2005c). Importantly, the vascular nitrotyrosine and PAR content is increased in type-2 prediabetic and diabetic patients and is the predictor of impaired endothelial function (Szabo et al. 2002a).

PARP-1 knockout mice are resistant to cardiovascular collapse associated with endotoxic, septic, or hemorrhagic shock, and PARP inhibitors exert beneficial effects in these conditions by numerous complex interrelated mechanisms discussed in several recent overviews (Evgenov and Liaudet 2005; Szabo, 2006, 2007). Furthermore, both nitrotyrosine and PARP activity were found to be increased in myocardial biopsy specimens of human subjects with sepsis (Kooy et al. 1997; Soriano et al. 2006).

Role of PARP-1 Activation in Endothelial Dysfunction, Atherosclerosis, Vascular Remodeling, and Angiogenesis

Increasing evidence supports the view that the endothelial dysfunction associated with diabetes, hypertension, heart failure, and atherosclerosis is related to the local formation of reactive oxygen and nitrogen species in the vicinity of the vascular endothelium (Csiszar et al. 2005; Griendling et al. 2000; Pacher et al. 2007; Ungvari et al. 2005). Peroxynitrite may contribute to the vascular dysfunction by various complex interrelated mechanisms overviewed recently (Pacher et al. 2007), which may involve upregulation of adhesion molecules in endothelial cells, endothelial glycocalyx disruption, enhancement of neutrophil adhesion, inhibition of voltage-gated K^+ (K_v) and Ca^{2+}-activated K^+ channels in coronary arterioles and vascular prostacyclin synthase, and apoptosis and/or PARP-dependent cell death in endothelial and vascular smooth muscle cells (Pacher et al. 2007), among many others. Indeed, PARP activation appears to be involved in the vascular dysfunction associated with circulatory shock (Evgenov and Liaudet 2005; Szabo 2006, 2007), myocardial ischemia reperfusion injury (Szabo 2005a; Szabo and Bahrle 2005), heart failure (Pacher et al. 2005b; Ungvari et al. 2005), hypertension (Escobales and Crespo 2005), diabetes (Mabley and Soriano 2005; Pacher et al. 2005a; Pacher and Szabo 2005, 2006; Szabo 2005c), and cardiovascular aging (Csiszar et al. 2005), both in experimental models and in patients, and the pharmacological inhibition of PARP improves endothelium-dependent relaxation in these pathological conditions (Tables 1 and 2, and see also above).

Experimental, clinical, and epidemiological studies have unraveled the significance of the cross-talk between inflammation, generation of reactive oxygen and nitrogen species, and lipid metabolism in the pathogenesis of atherosclerosis and vascular remodeling following injury (reviewed in Harrison et al. 2003; Pacher et al. 2007; Rubbo and O'Donnell 2005). Additional evidence suggests that atherosclerosis is not only associated with decreased NO bioavailability,

but also with alterations in signal-transduction components downstream of NO, including among others, the NO receptor sGC, particularly in neointima (Evgenov et al. 2006). The classic hypothesis envisions reactive oxygen and nitrogen species oxidatively damage LDL trapped in the arterial intima forming oxidized LDL, which in turn initiates numerous events (e.g., foam cell formation, monocyte recruitment, and adhesion to the endothelium, inhibition of macrophage motility, smooth muscle cell proliferation, promotion of cytotoxicity, and attenuation of vascular reactivity) facilitating the development of atherosclerotic lesions.

Numerous studies have demonstrated increased 3-nitrotyrosine and iNOS expression in human atherosclerotic tissue (Pacher et al. 2007), which correlated with plaque instability in patients, supporting the pathogenetic role of peroxynitrite in atherosclerosis (Pacher et al. 2007). Consistently, elevated levels of oxidative DNA damage and DNA repair enzymes (e.g., PARP-1) were found in human atherosclerotic plaques (Martinet et al. 2002). In an ApoE mouse model of atherosclerosis fed on a high-fat diet PARP inhibition improved endothelial function (Benko et al. 2004), reduced atherosclerotic plaque size, and promoted factors of plaque stability (Oumouna-Benachour et al. 2007), presumably by reduction of inflammatory factors and cellular changes related to plaque dynamics.

Accumulating evidence suggests that reactive oxygen and nitrogen species and downstream effector pathways (e.g., PARP-1) play an important role in the pathogenesis of restenosis following vascular injury (Azevedo et al. 2000; Beller et al. 2006; Jacobson et al. 2003; Muscoli et al. 2004). Various studies demonstrated increased 3-nitrotyrosine immunoreactivity and/or iNOS overexpression in media and neointima following ballon injury (a model of restenosis), and increased 3-nitrotyrosine/tyrosine ratio in the serum of patients following stent implantation (Azevedo et al. 2000; Beller et al. 2006; Inoue et al. 2006; Jacobson et al. 2003; Muscoli et al. 2004). The serum 3-nitrotyrosine/tyrosine ratio appears to be an independent predictor of angiographic late lumen loss in patients (Inoue et al. 2006).

PARP inhibitors are being developed for the treatment for cancer, both in monotherapy as well as in combination with radiation and chemotherapeutic agents in humans, but the discussion of this subject, which is covered by several excellent recent overviews, is beyond the scope of this synopsis. Until recently it was thought that PARP inhibitors enhance the death of the cancer cells primarily by the interference with DNA repair at various levels (reviewed in Graziani and Szabo 2005; Jagtap and Szabo 2005; Ratnam and Low 2007; Tentori and Graziani 2005). Recent studies have established a novel concept that PARP inhibitors may decrease angiogenesis, either by inhibiting growth factor expression or by inhibiting growth factor-induced cellular proliferative responses (Obrosova et al. 2004; Rajesh et al. 2006a, 2006b). Several structurally distinct PARP inhibitors (3-aminobenzamide, PJ-34, 5-aminoisoquinolinone-hydrochloride, and 1,5-isoquinolinediol) showed antiangiogenic effects by decreasing VEGF- and FGF-induced proliferation, migration, and tube formation of human umbilical vein endothelial cells, and also in an *ex vivo* rat aortic ring assay of angiogenesis. These findings might also have implications to the mode of PARP inhibitors' anticancer effects *in vivo*.

PARP Inhibitors in Clinical Trials

A number of PARP inhibitors have entered the stage of clinical testing, and many of these clinical candidates focus on cancer therapy, which is overviewed in more detail by Graziani and Szabo (2005), Haince et al. (2005), Plummer (2006), Ratnam and Low (2007), and Tentori and Graziani (2005). Based on murine data (Thomas et al. 2007) generated in cancer models using Agouron/Pfizer's AG-014699, a phase I study was conducted to evaluate the safety of i.v. AG014699, when administered with temozolomide in solid tumors. The compound exhibited no dose-limiting toxicities (Ratnam and Low 2007). A subsequent phase II trial was conducted, which involved 40 evaluable patients with metastatic malignant melanoma. In this

study, 18% of the study subjects demonstrated partial responses, with notable side effects (temozolomide-related myelosuppression and one toxic death) (Plummer et al. 2006; Ratnam and Low 2007).

KuDOS'/AstraZeneca's oral PARP inhibitor KU-0059436 is currently in a phase I trial in patients with advanced tumors in the UK and the Netherlands (Fong et al. 2006; Ratnam and Low 2007). To date, the available data are only of a pharmacokinetic nature. However, anecdotal reports indicate a partial response in a patient with ovarian cancer, and stabilization of the disease for 24 weeks in a patient with metastatic soft tissue sarcoma.

Inotek—INO-1001. Inotek (Beverly, MA) in partnership with Genentech (South San Francisco, CA) is developing INO-1001, both for cardiovascular indications, as well as for cancer. For cardiovascular indications, it has been granted orphan drug status by the U.S. Food and Drug Administration for the prevention of postoperative aortic aneurysm repair complications and according to a 2005 review (Jagtap and Szabo 2005) is considered for several phase II trials for various cardiovascular indications. The first human clinical study with a PARP inhibitor in a cardiovascular indication has been conducted by Inotek. In this phase II prospective, single-blind, multi-center, dose escalation study of a single dose of intravenous INO-1001 (200 mg, 400 mg, or 800 mg) administered to 30 patients between the ages of 48 and 63 years presenting with acute ST-segment elevation myocardial infarction (STEMI), who were to be treated with primary percutaneous coronary intervention (PCI), the primary endpoint was to evaluate the safety, tolerability, and pharmacokinetic profile of INO-1001. The secondary objectives of the study were to characterize the pharmacodynamic profile of INO-1001 and to evaluate various biomarkers of necrosis and inflammation. The PARP inhibitor INO-1001 was found to induce a tendency to reduce the plasma levels of C-reactive protein and the inflammatory marker IL-6, without reducing plasma markers of myocardial injury. No drug-related serious adverse events were observed in the patients receiving the drug during the study period (Morrow et al. 2007).

INO-1001 is also being studied in combination therapy in metastatic melanoma and glioma and as a single agent in cancer for BRCA1- and BRCA2-deficient tumors. A preliminary analysis of a phase I trial, which evaluated INO-1001 in combination with temozolomide in unresectable stage III/IV melanoma, reported one patient with objective tumor regression (Wang et al. 2006). Recent preclinical data also indicate that INO-1001 is effective in enhancing the antitumor effects of chemotherapy agents such as doxorubicin against p53-deficient breast cancer (Mason et al. 2007).

Additional, early-stage human cancer trials include Abbott's ABT-888 (Donawho et al. 2007), BiPAR Sciences' (Brisbane, CA) BSI-201, and MGI Pharma's (Bloomington, MN) GLP-21016. The latter compound is likely to derive from Guilford Pharmaceutical's program (Lapidus et al. 2006; Tentori et al. 2003), a Baltimore-based pharmaceutical company that has merged with MGI. ABT-888 is being studied in a phase 0 clinical trial by the National Cancer Institute under an exploratory Investigational New Drug application (Kummar et al. 2007; Ratnam and Low 2007). BSI-201 is being tested as intravenous monotherapy in solid tumors with the objective to determine a maximum tolerated dose and a pharmacokinetic profile (Ratnam and Low 2007).

CONCLUSIONS

Taken together, the evidence summarized above strongly supports the crucial role of the ROS/RNS-PARP pathway in mediating cardiac and endothelial dysfunction associated with various forms of cardiovascular injury and heart failure. The information available to date supports the view that PARP activation is a pivotal feature of myocardial infarction and I/R of the heart,

and that the pharmacological inhibition of PARP may provide significant benefits in these conditions by salvaging cardiomyocytes and endothelial cells, and by reducing the plasma markers of myocyte necrosis, as well as by downregulating the inflammatory responses. The first clinical data appear to be suggestive of a therapeutic benefit; additional human data are expected to become available in 2007–2008 on the latter subject. A multitude of novel pharmacological inhibitors of PARP have entered clinical testing as cytoprotective agents and as adjunct antitumor therapeutics, and several tetracycline antibiotics unexpectedly turned out to be potent PARP-1 inhibitors. Consequently, there is considerable expectation that these new drugs may become efficient therapies in the near future to combat cardiovascular diseases and cancer. While the clinical benefit of PARP inhibitors is being tested, additional new areas of research are also opening up in the preclinical front, which will certainly warrant additional studies. Some of these areas include the role of free poly(ADP-ribose) polymer in cell injury (Andrabi et al. 2006), the interactions between PARP and poly(ADP-glycohydrolase) (Meyer et al. 2007; Poitras et al. 2007), the role of PARP in the development of immunocompetence (Aldinucci et al. 2007), the regulatory role of PARP on the release of the proinflammatory nuclear protein HMGB-1 (Ditsworth et al. 2007) as well as the ever-increasing array of endogenous modulators of PARP activity, which now include, among others, sex hormones, the active form of vitamin D (Mabley et al. 2007), caffeine derivatives, as well as many other molecules (Szabo et al. 2006a).

ADDENDUM

The full chemical names of some of the compounds mentioned in the text are:

PJ-34: [11C]2-(dimethylamino)-N-(5,6-dihydro-6-oxophenanthridin-2-yl)acetamide

L-2286: 2-[(2-piperidin-1-yletil)thio]quinazolin-4(3H)-one

BGP 15: O-(3-piperidino-2-hydroxy-1-propyl) nicotinic acid-amidoxime

GPI 6150: 1,11b-dihydro-[2H]benzopyrano [4,3,2-de]isoquinolin-3-one

The chemical names of INO-1001, AG-014699, KU 0059436, ABT 888, BSI-201 or GLO-21016 were not found in the published literature or available databases.

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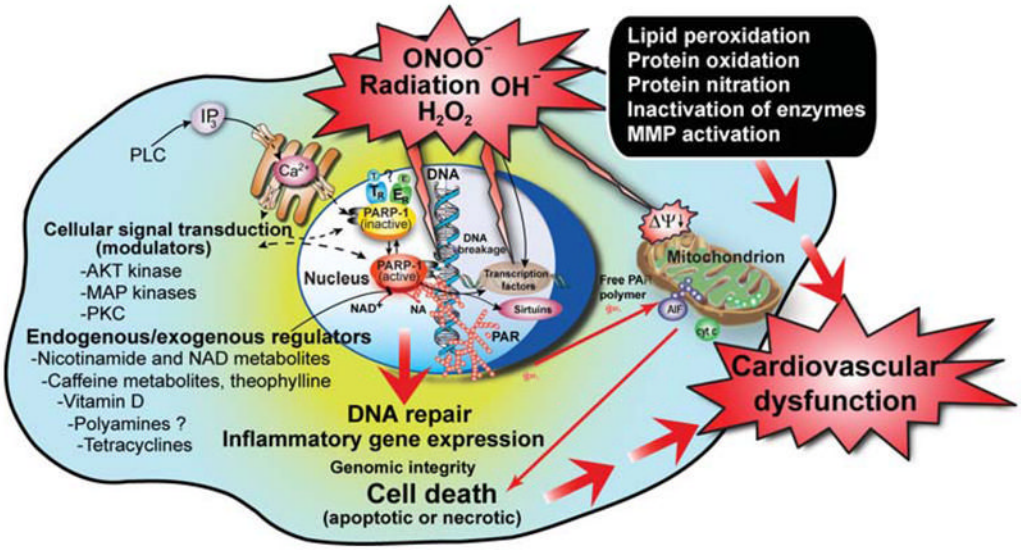

FIG. 1.

Triggers, mechanisms of PARP-mediated cell death, and exogenous/endogenous regulators/modulators of PARP activity. Reactive oxygen and nitrogen species (e.g., peroxynitrite)-dependent cytotoxicity in various cardiovascular diseases is mediated by a multitude of effects including lipid peroxidation, protein nitration and oxidation, DNA oxidative damage, activation of matrix metalloproteinases (MMPs), and inactivation of a series of enzymes. Mitochondrial enzymes are particularly vulnerable to attacks by peroxynitrite, leading to reduced ATP formation and induction of mitochondrial permeability transition by opening of the permeability transition pore, which dissipates the mitochondrial membrane potential ($\Delta\psi_m$). These events lead to cessation of electron transport and ATP formation, mitochondrial swelling, and permeabilization of the outer mitochondrial membrane, allowing the efflux of several proapoptotic molecules, including cytochrome or C and apoptosis-inducing factor (AIF). In turn, cytochrome or C and AIF activate a series of downstream effectors, which eventually result in the fragmentation of nuclear DNA. In addition to its damaging effects on mitochondria, peroxynitrite inflicts more or less severe oxidative injury to DNA, resulting in DNA strand breakage, which in turn activates the nuclear enzyme poly(ADP-ribose) polymerase (PARP). Activated PARP consumes NAD to build up poly(ADP-ribose) polymers (PAR), which are themselves rapidly metabolized by the activity of poly(ADP-ribose) glycohydrolase (PARG). Some free PAR may exit the nucleus and travel to the mitochondria, where they amplify the mitochondrial efflux of AIF (nuclear to mitochondria crosstalk). Depending on the severity of the initial insult by peroxynitrite or other oxidants, the injured cell may either recover or die. In the latter case, the cell may be executed by apoptosis in the case of moderate PTP opening and PARP activation with preservation of cellular ATP, or by necrosis in case of widespread PTP opening and PARP overactivation, leading to massive NAD consumption and collapse of cellular ATP. Various endogenous factors can influence PARP activity either by inhibiting the binding of its substrate NAD^+ to the active site of the enzyme or by forming a complex with PARP. An example for the latter may include estrogen (E) and thyroid hormones (T) and for the former nicotinamide (NA), NAD^+ metabolites, caffeine metabolites, and vitamin D. PARP activity can also be modulated by various kinases by phosphorylation (e.g., MAP kinases and PKC), and PARP can modulate kinase (e.g., AKT) activity. Various exogenous factors such as caffeine and its endogenously formed metabolites, theophylline, and tetracycline antibiotics may also modulate PARP activity. Overall, PARP appears to be a subject of multiple lines of endogenous regulators, and it is conceivable that

the processes regulated by PARP (e.g., DNA repair and cellular NAD homeostasis) are under a similarly dynamic control by a multitude of factors and influences.

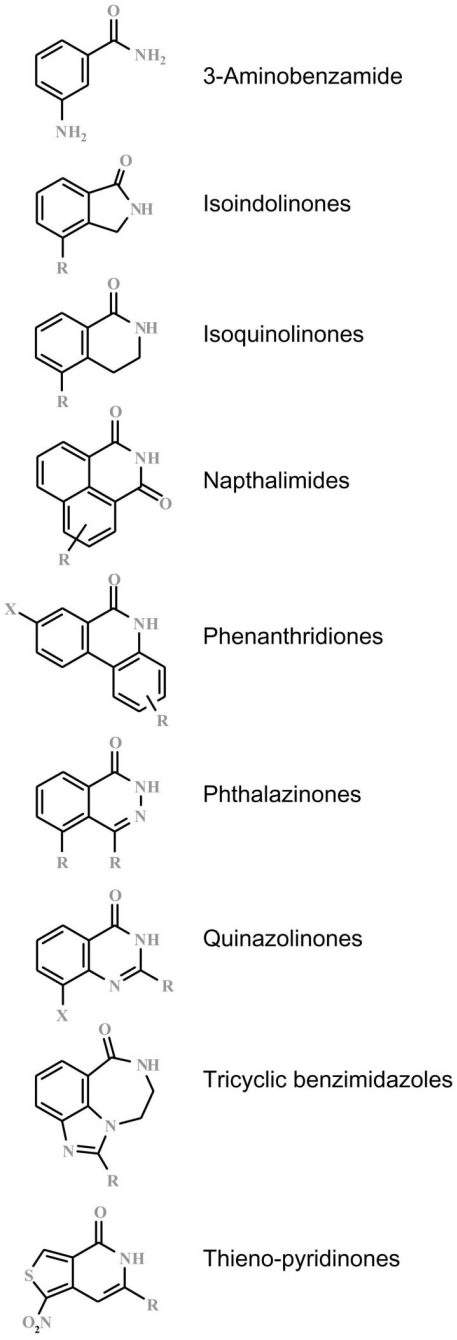

FIG. 2.
Some prototypical PARP inhibitor structures.

TABLE 1

Therapeutic effects of genetic deletion of PARP-1 in cardiovascular diseases

Disease/trigger	Experimental model	PARP-1 neutralization	Key findings	References
Myocardial ischemia reperfusion (I/R) or ischemic preconditioning (IPC)				
Global I/R	Mouse heart	PARP-1−/− phenotype	Improved LV function, reduction of NAD+ consumption, improved mitochondrial function	Grupp et al. 1999; Pieper et al. 2000; Zhou et al. 2006
Regional I/R	Mouse	PARP-1−/− phenotype	Decreased infarct size, neutrophil infiltration, and circulating TNFalpha, IL-10 and nitrate; reduced of ICAM-1/P-selectin expression	Yang et al. 2000; Zingarelli et al. 1998
Regional I/R	Mouse	PARP-1−/− phenotype	Reduced myocardial damage and apoptosis, reduced NF-kappa B activation	Zingarelli et al. 2003
Regional I/R	Mouse	PARP-1−/− phenotype	Decreased infarct size, reduction of the phosphorylative activity of JNK, reduced AP-1 activation, increased HSF-1 and HSP70 activation	Zingarelli et al. 2004b
Ischemic preconditioning	Mouse	PARP-1−/− phenotype	Suppression of the benefit of IPC	Liaudet et al. 2001b
Heart failure and myocardial hypertrophy				
Doxorubicin	Mouse	PARP-1−/− phenotype	Improved contractile function, reduced myocyte death, decreased mortality	Pacher et al. 2002b
Aortic banding	Mouse	PARP-1−/− phenotype	Decreased hypertrophy, myocardial collagen formation, and the mitochondrial-to-nuclear translocation of the cell death factor apoptosis-inducing factor (AIF)	Pillai et al. 2005b; Xiao et al. 2005
Angiotensin II infusion	Mouse	PARP-1−/− phenotype	Protected from angiotensin II-induced cardiac hypertrophy	Pillai et al. 2006
Circulatory shock				
Endotoxic and septic shock	Mouse	PARP-1−/− phenotype	Improved contractile function, decreased mortality and tissue injury	Oliver et al. 1999; Pacher et al. 2002a; Soriano et al. 2002
Hemorrhagic shock	Mouse	PARP-1−/− phenotype	Protection from the rapid decrease in blood pressure after resuscitation, increased survival time, and decreased tissue injury	Liaudet et al. 2000

TABLE 2

Therapeutic effects of PARP inhibitors in cardiovascular disease

Disease/trigger	Experimental model	PARP inhibitor	Key findings	References
Oxidant-or hypoxia reoxygenation-induced cell death in vitro				
H_2O_2, peroxynitrite, hypoxia reoxygenation	Rat and human cardiomyoblasts	3-AB, nicotinamide, ISQ, PJ-34	Improved mitochondrial respiration, reduced cell death, protection against the reduction of action potential duration, improved cellular ATP levels	Bhatnagar 1997; Bowes et al. 1998a 1999; Fiorillo et al. 2006; Gilad et al. 1997
Angiogenesis				
VEGF and bFGF	Human umbilical vein endothelial cells(HUVECs), aortic ring explants	3-AB, PJ-34, 5-AIQ, IQD	Dose-dependent reduction of VEGF- or bFGF-induced proliferation, migration, and tube formation of human umbilical vein endothelial cells *in vitro*, and prevention of the sprouting of rat aortic ring explants	Rajesh et al. 2006a, 2006b
Myocardial ischemia reperfusion, ischemic preconditioning				
Global I/R	Rat heart	3-AB, BGP-15, ISQ	Improvement of LV dysfunction, reduction of ATP and NAD+ catabolism, improved ATP and CK recovery	Docherty et al. 1999; Halmosi et al. 2001; Szabados et al. 2000
Regional I/R	Rat heart	3-AB	Reduction of infarct size	Bowes et al. 1999
Regional I/R	Rat	3-AB, GPI 6150	Reduction of infarct size and LV dysfunction, preservation of myocardial ATP stores, reduction of neutrophil infiltration	Liaudet et al. 2001a; Pieper et al. 2000; Zingarelli et al. 1997 Wayman et al. 2001
Regional I/R	Rat	5-AIQ	Reduction of infarct size	
Regional I/R in diabetic animals	Rat	INO-1001	Improved left ventricular function, reduced infarct size and mitochondrial-to-nuclear translocation of the cell death effector apoptosis-inducing factor (AIF) in myocardial infarction	Xiao et al. 2004
Global and regional I/R	Rabbit, rabbit heart	3-AB, nicotinamide, ISQ	Reduction of infarct size and improvement of left ventricular function	Thiemermann et al. 1997
Cardiopulmonary bypass, crystalloid cardioplegia and extracorporal circulation	Dog	PJ-34, INO-1001	Better recovery of left and right ventricular systolic function, increased coronary blood flow, improved vascular and pulmonary function	Szabo et al. 2004b; Szabo et al. 2004c
Regional I/R	Pig	3-AB, PJ-34, INO-1001	Reduction of infarct size and improvement of left ventricular function	Bowes et al. 1998b; Faro et al. 2002; Hauser et al. 2006
Cardiac transplantation	Rat	3-AB, 5-AIQ, PJ-34, INO-1001	Decreased myocardial damage, reduced oxidative stress, improved cellular NAD^+ and ATP levels, improved contractile and endothelial function, reduced ICAM-1 and P-selectin expression; decreased allograft rejection	Farivar et al. 2005; Fiorillo et al. 2002, 2003; Szabo et al. 2006b; Szabo et al. 2002b; Szabo et al. 2006c; Szabo et al. 2005
Ischemic preconditioning	Rat	3-AB	Suppression of the benefit of IPC	Liaudet et al. 2001b
Segment-elevated myocardial infarction followed by revascularization	Human	INO-1001	Reduction in plasma levels of C-reactive protein; tendency for reduced plasma levels of IL6	Morrow et al. 2007
Circulatory shock				
Endotoxic shock	Mouse, rat	3-AB, PJ-34, INO-1001	Improved myocardial contractility, vascular function and survival, decreased inflammation and tissue injury	Jagtap et al. 2002; Pacher et al. 2002a, 2006a; Szabo et al. 1997; Tasatargil et al. 2005; Veres et al. 2003
Hemorrhagic shock	Rat, pig	3-AB, 5-iodo-6-amino-1,2-benzopyrone	Improved hemodynamics, prolonged survival	Szabo et al. 1998; Szabo 1998; Szabo and Billiar 1999
Heart failure, myocardial hypertrophy, and hypertension				
Doxorubicin	Mouse	PJ-34, INO-1001	Improved contractile function, reduced myocyte death, decreased mortality	Pacher et al. 2002b, 2006a
Chronic myocardial ischemia	Rat	PJ-34, INO-1001	Improved left ventricular and vascular function, reduced hypertrophy and remodeling	Pacher et al. 2002c, 2006a

Disease/trigger	Experimental model	PARP inhibitor	Key findings	References
Chronic heart failure following isoproterenol-induced myocardial infarction	Rat	L-2286	Reduced the progression of postinfarction heart failure, attenuated cardiac hypertrophy and interstitial fibrosis, and preserved the integrity of mitochondrial respiratory complexes. L-2286 also repressed the hypertrophy-associated increased phosphorylation of panPKC, PKC alpha/betaII, PKC delta, and PKC epsilon, which could be responsible for the activation of the antihypertrophic GSK-3beta.	Palfi et al. 2006
Aortic banding	Mouse	INO-1001	Decreased hypertrophy, myocardial collagen formation, and the mitochondrial-to-nuclear translocation of the cell death factor apoptosis-inducing factor (AIF)	Xiao et al. 2005
Aortic banding, exercise	Mouse	5-AIQ	Decreased pathological hypertrophy, myocardial collagen formation, improved cardiac function, without affecting physiological hypertrophy	Balakumar and Singh 2006
Hypertension				
Genetic hypertension	Spontaneously hypertensive rats	PJ-34	Improved endothelial function	Pacher et al. 2002f
Angiotensin II	Mouse, rat	PJ-34, INO-1001	Improved endothelial function	Szabo et al. 2004a
Cardiovascular aging				
Advanced aging	Rat	PJ-34, INO-1001	Improved cardiac and vascular function	Pacher et al. 2002e, 2002f, 2004b
Atherosclerosis, restenosis				
High fat diet	Apolipoprotein E (ApoE)-deficient mice	INO-1001	Chronic treatment with the PARP inhibitor INO-100 reduced the degree of the endothelial dysfunction	Benko et al. 2004
High fat diet	ApoE-deficient mice	Thieno[2,3-c] isoquinolin-5-one	Reduced plaque number and size and altered structural composition of plaques promoting its stability	Oumouna-Benachour et al. 2007
Endarterectomy	Rat	INO-1001	Reduced neointima hyperplasia following vascular injury	Beller et al. 2006
Diabetic cardiovascular complications				
NOD diabetes	Mouse	PJ-34	Improved contractile and vascular function	Pacher et al. 2002d
STZ diabetes	Mouse, rat	PJ-34	Improved contractile function	Garcia Soriano et al. 2001; Pacher et al. 2002d; Soriano et al. 2001

Abbreviations: 3-aminobenzamide (3-AB); 1,5-dehydroxyisoquinoline (ISQ); 5-aminoisoquinolinone (5-AIQ); 1,5-isoquinolinediol (IQD); N-(6-oxo-5,6-dihydro-phenanthridin-2-yl) -N,N-dimethylacetamide (designated as PJ-34), vascular endothelial growth factor (VEGF); basic fibroblast growth factor (bFGF).

There are numerous excellent recent overviews on the oxidative-nitrosative stress-PARP pathway (Pacher et al. 2007; Szabo 2003; Szabo et al. 2006a; Virag and Szabo, 2002; Virag et al. 2003) and its role in various cardiovascular pathologies such as circulatory shock (Evgenov and Liaudet 2005; Szabo 2006, 2007), myocardial ischemia reperfusion (Szabo 2005a; Szabo and Bahrle 2005), heart failure (Pacher et al. 2005b; Ungvari et al. 2005), hypertension (Escobales and Crespo 2005), diabetic endothelial dysfunction and cardiomyopathy (Mabley and Soriano 2005; Pacher et al. 2005a; Pacher and Szabo 2005, 2006; Szabo 2005c), cardiovascular aging (Csiszar et al. 2005), and on the development of various PARP inhibitors for therapeutic indications (Donawho et al. 2007; Graziani and Szabo 2005; Jagtap and Szabo 2005; Ratnam and Low 2007; Southan and Szabo 2003; Szabo 2005b; Tentori and Graziani 2005; Thomas et al. 2007; Virag and Szabo 2002).