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Review Article

PARP and PARG as novel therapeutic targets

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CONTENTS

Summary

Poly(ADP-ribose) is synthesized by poly(ADP-ribose) polymerase (PARP) from β-nicotinamide adenine dinucleotide (NAD^+). It is mainly degraded by poly(ADP-ribose) glycohydrolase (PARG). The expanding family of PARP currently consists of $PARP_{1\text{-}3}$, vPARP, $Tankyrase_{1\text{-}2}$, and more members are being characterized. Similarly, the PARG family awaits more homologs to be identified. $PARP_1$, which is activated by DNA damage, accounts for > 95% poly(ADP-ribose) synthesis. Poly(ADP-ribose) has a half-life of < 1 min *in vivo*, due to its immediate degradation by PARG. The $PARP_1$/PARG cycle results in depletion of NAD^+ and ATP, which can be prevented by inhibiting $PARP_1$ or PARG. After $PARP_1$ was implicated in facilitating DNA repair, pharmaceutical companies began developing PARP inhibitors as potentiators to enhance chemotherapy and radiation therapy in cancers. Recent studies using $PARP_1$ knockout mice and PARP inhibitors validated targeting the poly(ADP-ribose) pathway for ameliorating ischemia injury and abating inflammation. Multiple families of PARP and PARG inhibitors have been identified. A number of these inhibitors have demonstrated efficacy in animal models of cerebral ischemia, traumatic brain injury, Parkinson's disease, myocardial ischemia, retinal ischemia, kidney ischemia, type 1 diabetes, septic shock, hemorrhagic shock, arthritis, inflammatory bowel disease, multiple sclerosis and potentiation of chemotherapy. The therapeutic utility of PARP inhibitors is expected to be studied soon in clinical trials.

Introduction

The earliest encounter with poly(ADP-ribose) can be traced back to experiments by Chambon in Mandel's lab in 1963 (1). While studying eukaryotic RNA polymerase, they stumbled across the incorporation of ATP into an acid-insoluble fraction, which was initially thought to be polyadenylic acid, "polyA". The polyA synthesis was DNA-dependent and enhanced by nicotinamide mononucleotide (NMN). The discovery was followed by a series of painstaking biochemical analysis by Sugimura *et al.* and Hayaishi *et al.*, who characterized the polyA as poly(ADP-ribose) and delineated its complex structure (2-6) (Fig. 1). The NMN enhanced polyA synthesis was not directly from ATP, as initially presumed. Rather, the NAD^+, formed from NMN and ATP by NMN adenylyltransferase (EC 2.7.7.1) (7), served as a substrate for poly(ADP-ribose) synthesis (8).

In ADP-ribosylation reactions, NAD^+ is consumed as a substrate to generate ADP-ribose as a covalently modifying unit and nicotinamide as a by-product, in contrast to the role of NAD^+ as an electron transporter in the cyclic reaction between NAD^+ and NADH. Poly(ADP-ribose) consists of a homo-polymer with repetitive ADP-ribose units linked by α(1"-2')ribosyl-ribose glycosidic bonds and of branching residues with α(1"'-2")ribosyl-glycosidic bond (Fig. 1). It is made up of a few to hundreds of ADP-ribose units, with an average of one branch every 30-40 units (9). Poly(ADP-ribosyl)ation is one of the most conserved pathways present in most cells and tissues examined in eukaryotes, except for yeast. The first enzyme identified to catalyze the process is now called $PARP_1$ (EC 2.4.2.30., also known as PARS). $PARP_1$ has been cloned from lower eukaryotes like *Dictyostelium discoideum* (10) to *Homo sapiens* (11, 12). Equally conserved over evolution is PARG, which degrades poly(ADP-ribose). PARG has been cloned from lower species such as *Caenorhabditis elegans* (13) and *Arabidopsis thaliana* (14) and to higher ones like *Homo sapiens* (15). Although the physiologic roles of poly(ADP-ribosyl)ation are yet to be fully established, recent discovery of the involvement of poly(ADP-ribose) turnaround in multiple animal models of diseases has prompted pharmaceutical research to harness potential therapeutic benefits of the pathway (16). As the key enzymes for poly(ADP-ribosyl)ation, $PARP_1$ and PARG are prominent targets for modulating the poly(ADP-ribose) cycle.

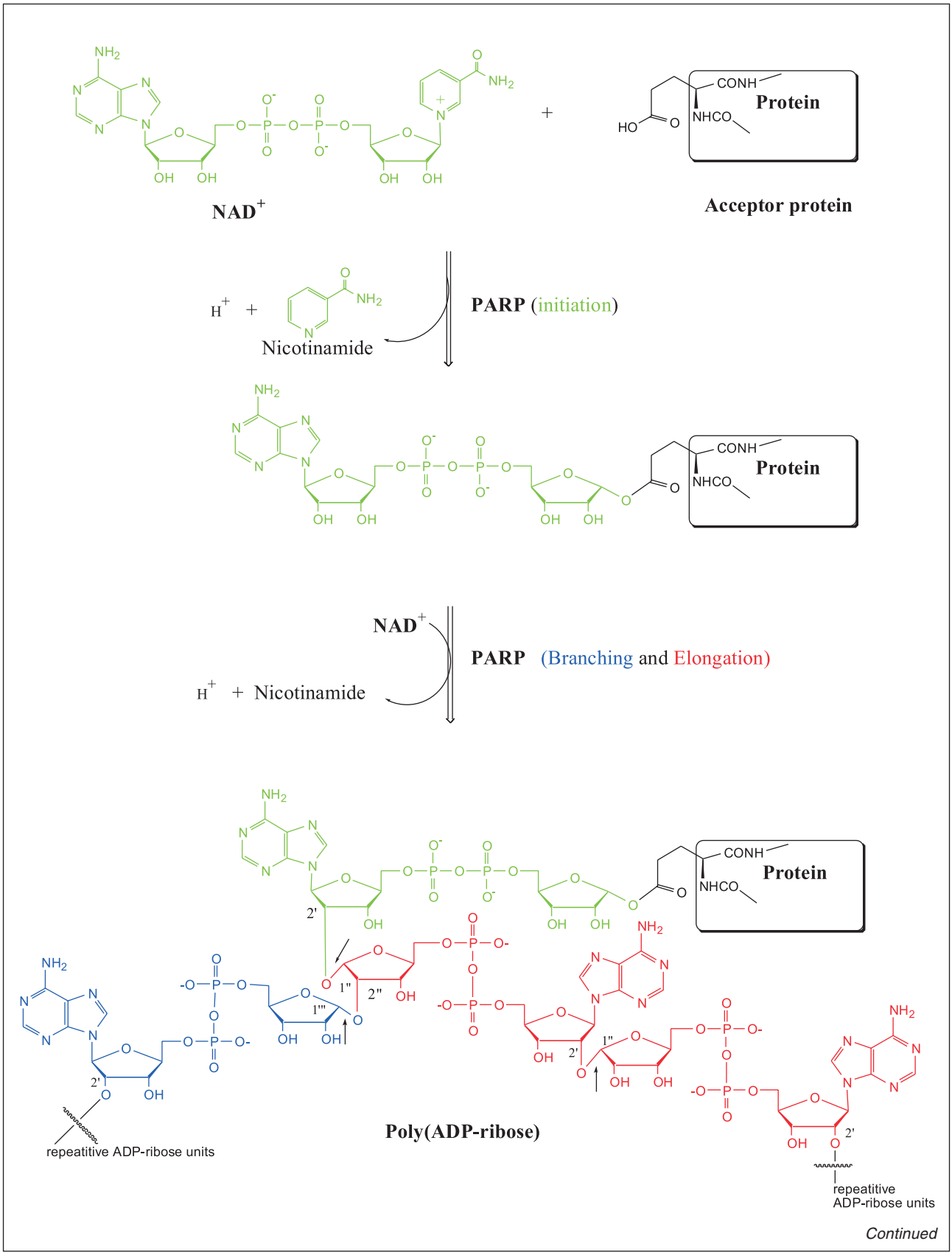

Fig. 1. Structure of poly(ADP-ribose).

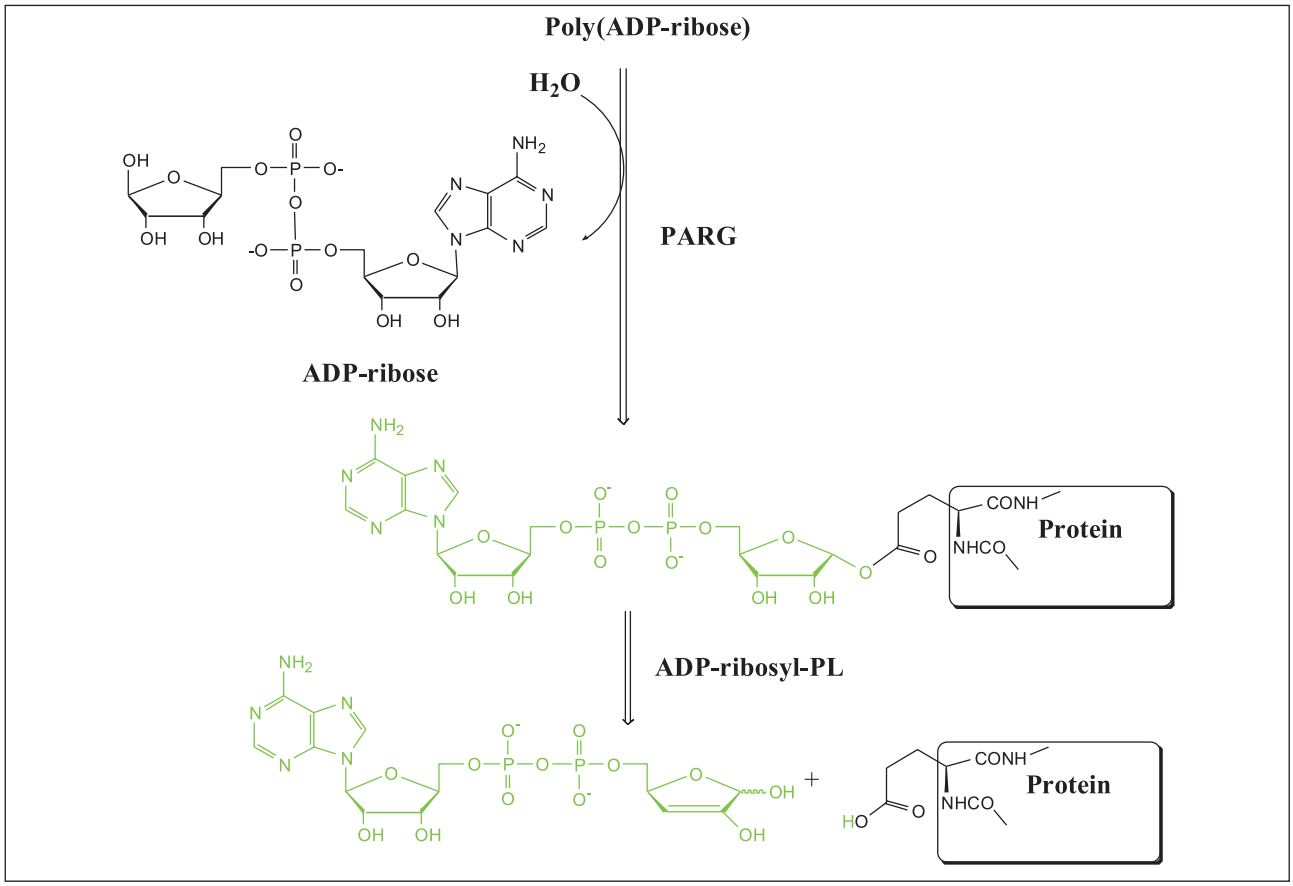

Fig. 1. Structure of poly(ADP-ribose) (Cont.). PARP catalyzes the transfer of ADP-ribose from NAD^+ to the carboxylic group of specific glutamate in acceptor proteins in initiation, and then to ADP-ribose in elongation and branching. PARG cleaves the ribose-ribose bonds of the linear and branched portion of the polymer, namely the glycosidic (1"-2') and glycosidic (1"'-2") linkages of poly(ADP-ribose). Cleavage sites are indicated by arrows. The putative ADP-ribosyl protein lyase (PL) cleaves the protein proximal ADP-ribose glutamic ester bond. The elimination reaction catalyzed by the PL yields an unsaturated sugar, 5"-ADP-3"-deoxypent-2"-enofuranose to give a dehydrated form of ADP-ribose (123).

Enzymes for poly(ADP-ribose) metabolism

PARP family

The prototypical enzyme, responsible for the majority of poly(ADP-ribose) synthesis, is $PARP_1$ in the expanding PARP family that now includes newly discovered members of $PARP_{2-3}$, vPARP, $Tankyrase_{1-2}$ (17-23). Identified, characterized and purified three decades ago, $PARP_1$, a 110 kDa nuclear protein, is abundantly present in most of the eukaryotic tissues. It is the key enzyme for poly(ADP-ribose) anabolism (Figs. 1 and 2). This evolutionarily conserved enzyme appears to be involved in major cellular functions, such as maintaining genomic integrity by limiting sister chromatid exchange, and facilitating chromatin structural changes during DNA repair (24, 25). It has also been suggested to participate, either directly or indirectly, in gene expression, DNA replication, DNA rearrangement, differentiation and mutagenesis (26).

NAD^+ is the sole natural substrate that $PARP_1$ uses to covalently modify proteins through poly(ADP-ribosyl)ation. Partial proteolysis analysis has defined three major domains for $PARP_1$, the DNA-binding domain at the N-terminus, auto-ADP-ribosylation domain in the middle and the catalytic domain at the C-terminus (27). Many protein acceptors of poly(ADP-ribose), such as histones, topoisomerases, DNA and RNA polymerases, DNA ligases, and Ca^{2+}- and Mg^{2+}-dependent endonuclease, are involved in maintaining DNA integrity (26). $PARP_1$ itself serves as a major acceptor through auto-ADP-ribosylation (26). The activity of p53, the tumor suppressor protein, can also be regulated by poly(ADP-ribosyl)ation (28).

For a long time, $PARP_1$ was the only known PARP, until the discovery of two PARP isoforms in plant (29) and the residual poly(ADP-ribose) synthesis activity in mice whose $PARP_1$ gene was deleted (30). Molecular cloning and sequence analysis has now added more members to the PARP family, which includes $PARP_2$ (17), $PARP_3$ (18), $Tankyrase_1$ (20), $Tankyrase_2$ (21-23), vPARP (19) and more are being characterized. The functions of newly identified PARP are under study and their potential as drug targets are yet to be defined. The remainder of this review deals primarily with $PARP_1$ as a therapeutic target.

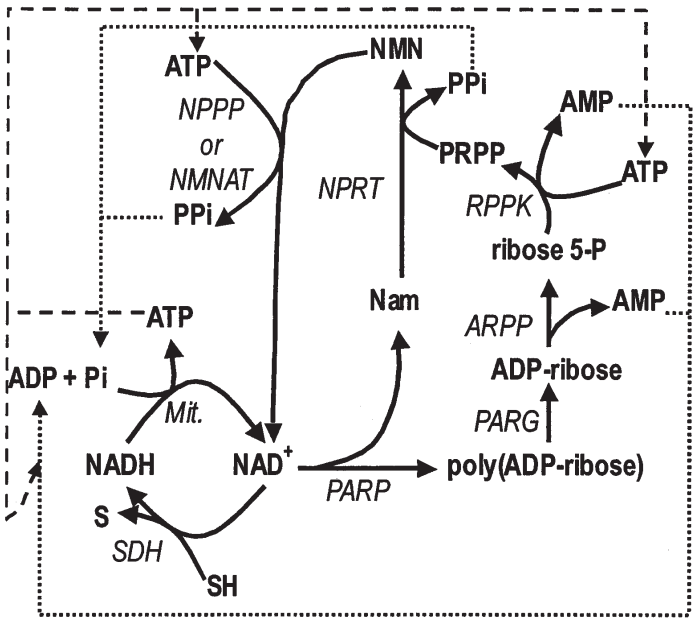

Fig. 2. Poly(ADP-ribose) metabolism consumes NAD^+. The net result of poly(ADP-ribose) synthesis and degradation is converting NAD^+ to ADP-ribose. It takes 4 ATP to regenerate NAD^+ from the NAD^+ salvage synthesis pathway. Overactivation of PARP results in depletion of NAD^+/ATP and necrosis due to the rapid turnover rate. PARP/PARG inhibitors can effectively prevent the energy drop and cell death. ARPP: ADP-ribose pyrophosphotase; Mit: mitochondria; NAD^+: nicotinamide adenine dinucleotide; NADH: reduced NAD^+; Nam: nicotinamide; NMN: nicotinamide mononucleotide; NMNAT: NMN adenylyl transferase (also referred to as NPPP: NAD^+ pyrophosphorylase); NPRT: nicotinamide phosphoribosyl transferase; PARG: poly(ADP-ribose) glycohydrolase; PARP: poly(ADP-ribose) polymerase; PRPP: 5-phosphoribosyl-1-pyrophosphate; PPi: pyrophosphate; RPPK: ribose phosphate pyrophosphokinase; S: oxidized substrate; SDH: substrate dehydrogenase; SH: reduced substrate.

PARG and others

PARG is a key enzyme for poly(ADP-ribose) catabolism, essential for completing the pathway (Figs. 1 and 2). The growth of yeast, which lacks endogenous PARP, was significantly retarded when recombinant $PARP_1$ was transformed and expressed in yeast (31). The growth arrest could be reversed by growing the yeast strain in the presence of PARP inhibitors, *e.g.*, 3-methoxybenzamide (32). This suggested that accumulation of poly(ADP-ribose) had a detrimental effect on yeast cell cycle. Coexpression of PARG with $PARP_1$ in yeast restored the normal growth pattern (32). Thus, it appears that PARG is absolutely necessary for normal growth of all poly(ADP-ribose) producing cells.

PARG catalyzes hydrolysis of poly(ADP-ribose) (Fig. 1). The mechanism of PARG action has been proposed to include three steps: i) endoglycosidic cleavage; ii) endoglycosidic cleavage plus exoglycosidic, progressive degradation; iii) exoglycosidic, distributive degradation. PARG cleaves the ribose-ribose bonds of linear and branched portion of polymer, specifically the glycosidic (1"-2') and glycosidic (1"'-2") linkages of poly(ADP-ribose). The final products of the reaction are mono-ADP-ribosyl protein and ADP-ribose. ADP-ribose is known to be a weak PARG inhibitor with an IC_{50} of 0.1 mM (33). Molecular cloning of PARG from rat predicts a full-length protein of 111 kDa (34). Expression of the C-terminal fragment of 59 kDa is enough for the catalytic activity. In N-terminus, PARG contains a bipartite nuclear localization sequence, which explains its localization in nucleus, as $PARP_1$ does. Immunohistochemical analysis, however, reveals perinuclear and cytoplasmic localization of PARG, perhaps as a result of partial proteolysis as a means of PARG translocation (35). Genomic sequence of *Arabdoposis thaliana* and *Caenorhabditis elegans* identifies PARG gene homologs (13, 14). It is possible that mammalians also possess PARG isoforms.

Another putative enzyme involved in poly(ADP-ribose) catabolism is ADP-ribosyl protein lyase (Fig. 1). It presumably cleaves the protein proximal ADP-ribose-glutamic ester bond. Very little is known about protein lyase.

Roles of poly(ADP-ribose) metabolism

Poly(ADP-ribose) has a very short half-life ($t_{1/2}$ < 1 min) *in vivo* (36). The transient nature of the polymer is attributable to its fast degradation by PARG. Once poly(ADP-ribose) is formed, it is almost immediately hydrolyzed by the constitutively active PARG. $PARP_1$ and PARG constitute a circle for poly(ADP-ribose) production and degradation. Rapid poly(ADP-ribose) turnover results in a drop of cellular NAD^+ level, since each ADP-ribose

unit in the polymer is directly derived from NAD^+. Extensive $PARP_1$ and PARG activation leads to severe depletion of NAD^+ in cells suffering from massive DNA damage, which triggers the pathway in the first place. The cyclic reactions by $PARP_1$ and PARG convert a large amount of NAD^+ to ADP-ribose. From the "salvage resynthesis pathway", it requires an input of four ATP to regenerate NAD^+ from nicotinamide and ADP-ribose (Fig. 2). Depletion of NAD^+ is typically associated with the lowering of the ATP level as well. In less than 1 hour, overstimulation of $PARP_1$ can cause a drop of NAD^+ and ATP to less than 20% of the normal level (37). The phenomenon is described as "the PARP suicidal pathway" (37). During ischemia, anaerobic respiration already results in significantly low ATP production. Subsequent free radical production during reperfusion damages DNA and activates $PARP_1$. NAD^+/ATP depletion due to poly(ADP-ribose) turnover leads to necrotic cell death. Based on the poly(ADP-ribose) mechanism, $PARP_1$ or PARG lend themselves as new targets for inhibition to preserve the cellular energy and to increase the survival of ischemic tissues.

Poly(ADP-ribose) synthesis is also involved in the induced expression of a number of genes essential for inflammatory response (38). Although details for this mechanism are still far from clear, it appears to involve interactions of $PARP_1$ in the NF-κB translocation pathway (39, 40). The interaction seems to involve neither the catalytic nor DNA binding activity of PARP (41). However, auto-ADP-ribosylation of PARP may affect its association with NF-κB (42). PARP inhibitors suppress production of inducible nitric oxide synthase in macrophages (43), P-type selectin and intercellular adhesion molecule-1 (ICAM-1) in endothelial cells (44). The indirect activity offers an explanation for the antiinflammatory effects exhibited by PARP inhibitors. PARP inhibition is able to reduce necrosis by preventing translocation and infiltration of neutrophils to the injured tissues, a process dependent on P-selectin and ICAM-1 expression. Both macrophage and neutrophil activation lead to a further outburst of free radical production that may also result in tissue damage from the PARP suicidal pathway. PARP inhibitors possess dual effects of preserving cellular energy levels and abating the inflammatory response.

$PARP_1$ has long been believed to facilitate DNA repair, since $PARP_1$ inhibition delayed the process (45). Most antineoplastic agents eliminate fast-growing tumor cells by disrupting DNA replication and halting the cell cycle. PARP inhibitors were first developed as candidates to potentiate the effect of chemotherapeutic agents and radiation therapy in cancer treatment (46, 47). Since the 1980s, many PARP inhibitors have been developed and found to effectively lower the doses of chemotherapeutic compounds to kill cancer cells *in vitro* and *in vivo* (48, 49). The putative role of $PARP_1$ in DNA repair also led to speculation that PARP inhibitors may increase tumors, which has not been substantiated, as three strains of independently generated $PARP_1$ knockout mice have survived and reproduced normally for several generations now (25, 50, 51). The role of $PARP_1$ in DNA repair appears to be more complicated than initially anticipated. It seems that PARP's role in gene expression may influence the expression of growth-related cell cycle genes essential for tumor growth (52). Recent experimental results indicate $PARP_1$ may play a different role in tumorigenesis and cancer metastasis. For example, in the double knockout mice of $PARP_1^{-/-}$ and $p53^{-/-}$, the incidence of tumors was actually delayed significantly in comparison to $PARP_1^{+/+}$ and $p53^{-/-}$ mice (53).

Potential clinical indications for PARP/PARG inhibitors

$PARP_1$/PARG activation has been found in a variety of animal models of disease (Table I). In general, there are corroborative results from experiments either using $PARP_1$ knockout mice or through pharmacological inhibition of the enzymes. Both approaches have achieved significant reduction of tissue damage during ischemia and inflammation, validating the targeting of the poly(ADP-ribose) pathway for a broad spectrum of diseases. The following section deals with only a few major clinical indications.

Cerebral ischemia and other CNS injuries

The discovery that $PARP_1$ activation-mediated neuronal death resulted from glutamate neurotoxicity, which could be prevented by PARP inhibitors (54), immediately implied that PARP could be a therapeutic target for treating cerebral ischemia, neuronal degeneration and other neurological disorders where excessive excitotoxicity is a known culprit. Definitive supporting evidence first came from subsequent studies using $PARP_1^{-/-}$ mice and testing PARP inhibitors *in vivo* in animal models of stroke. Profound neuroprotection was observed when $PARP_1^{-/-}$ mice were subjected to middle cerebral artery occlusion (MCAO) (55, 56). $PARP_1^{-/-}$ mice can tolerate brain ischemia and reperfusion with minimum infarction while their wild-type litter mates suffer from typical neural damage. In wild-type mice, there was a transient increase of poly(ADP-ribose) together with a drop of NAD^+ in contralateral but not ipsilateral regions during stroke. This is consistent with $PARP_1$/PARG activation causing transient poly(ADP-ribose) accumulation and drastic depletion of NAD^+. In contrast, in $PARP_1^{-/-}$ mice, no poly(ADP-ribose) synthesis was detected and the NAD^+ level remained unchanged during stroke (56). Around the same time, various PARP inhibitors, including nicotinamide; 3-aminobenzamide; 3,4-dihydro 5-[4-(piperidinyl)butoxy]-1(2*H*)-isoquinolinone (DPQ); GPI 6150 (1,11b-dihydro-[2*H*]benzopyrano[4,3,2-*de*]isoquinolin-3-one); 5-iodo-6-amino-1,2-benzopyrone and PJ-34 (*N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N,N*-dimethylacetamide HCl (Fig. 3), were tested in different models of transient and permanent cerebral ischemia and found to be

Table I: Poly(ADP-ribose) pathway in animal models of disease.

Model	Efficacy in PARP inhibitors	Efficacy in PARP1$^{-/-}$ mice	Evidence of PARP activation in humans	Ref.
Cerebral ischemia	Protection in MCAO	Reduced infarct	Positive poly(ADP-ribose) staining	57-63, 110
Traumatic brain injuries	Protection	Reduced injuries		71-73
Spinal cord injury	Protection			111, 112
Parkinson's disease	Protection in MTPT	Survival of dopamine neurons		66-68
Alzheimer's disease			Increased poly(ADP-ribose) in postmortem staining	113
Multiple sclerosis	Reduced damage			114
Heart ischemia	Protection	Reduced infarct		44, 80-82, 115
Type 1 diabetes	Protection in STZ	Reduced damage		51, 69, 90-92
Hemorrhagic shock	Promoted survival	Reduced mortality		94, 116-118
Septic shock	Promoted survival	Reduced mortality		39,69, 93, 119, 120
Arthritis	Reduced edema	Diminished inflammation		95-98
Gout	Reduced neutrophil infiltration			69
Radiosensitizer	Enhanced effect			47
Chemosensitizer	Enhanced effect			46, 48, 49
Inflammatory bowel disease	Alleviated symptoms	Diminished inflammation		121, 122
Retinal ischemia	Reduced cell death			84
Kidney ischemia	Protection			88, 89
Intestine ischemia	Protection	Protection		85-87

neuroprotective in both situations (56-63). Immunohistochemical staining also revealed diminished poly(ADP-ribose) accumulation in the ischemic region in rats treated with 3-aminobenzamide, DPQ and GPI 6150 (60, 64, 65). The results suggested that the neuroprotective effects exhibited by these compounds resulted from PARP inhibition.

Stroke damage is not the only neural insult linked to PARP. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) destruction of dopamine neurons is virtually abolished in $PARP_1^{-/-}$ mice (66), substantiating earlier observations that PARP inhibitors protect against MPTP damage (67, 68). In the MPTP model, twice-daily i.p. injection of 25 mg/kg GPI 6150 for 5 days resulted in a 42% reduction in the loss of tyrosine hydroxylase innervation in the striatum of CD-1 mice (69). In an animal model of traumatic brain injury, neural lesion in the cortex was associated with acute PARP activation (70). PARP inhibition by GPI 6150 treatment in the rats significantly attenuated the extent of the trauma-induced lesion area (71). Another form of neural damage involving $PARP_1$/PARG cycle is head trauma. In a controlled cortical impact model of traumatic brain injury, $PARP_1^{-/-}$ mice performed better motor and memory function after the impact than their littermates, although no difference in the contusion volumes were observed between $PARP_1^{-/-}$ and wild-type mice (72, 73).

In vitro *and* in vivo *effects of PARG inhibition*

The lack of potent and bioactive inhibitors has precluded *in vivo* investigation of PARG's role for a long time. There are only a couple of reports showing *in vitro* effects of PARG inhibition. For example, PARG inhibition by macrocircular ellaitannin oenothein B interfered with DNA repair (74), suggesting that PARG inhibition may achieve similar effects as PARP inhibitors. Ellaitannin oenothein B belongs to the tannin carbohydrate family of a natural product extracted from tealeaf (Fig. 4) (75). The basic functional unit of the tannin family is 1,2,3,4,6-*O*-pentagalloyl-β-D-glucose (PGG) (Fig. 4). It inhibited PARG with an IC_{50} of 5.5 μM. We used PGG to test whether PARG inhibition would reduce oxidative cytotoxicity as PARP inhibition does (76). We found PGG could protect P388D1 macrophage cells against H_2O_2 cytotoxicity with an EC_{50} of 50 μM. The result supports the notion of PARG as an alternative target to develop inhibitors for blocking oxidative cell death. Independently, Ying and Swanson obtained gallotannin, another PARG inhibitor from the tannin family, and found that it led to a significant reduction of H_2O_2-induced cell death in astrocytes and neurons (77, 78). Evidence for *in vivo* efficacy of PARG inhibition came more recently when we tested a small molecule GPI 16552 (MW = 503) in a rat model of transient cerebral focal ischemia. Both pretreatment and posttreatment with 40 mg/kg significantly reduced the infarct volume after a 2 hours of MCAO (79). Thus, PARG inhibitors may possess similar therapeutic potential as PARP inhibitors.

Other types of ischemia-reperfusion injury

The widespread tissue distribution of $PARP_1$ suggests the $PARP_1$/PARG pathway may contribute to multiple organ injuries during ischemia and reperfusion or during

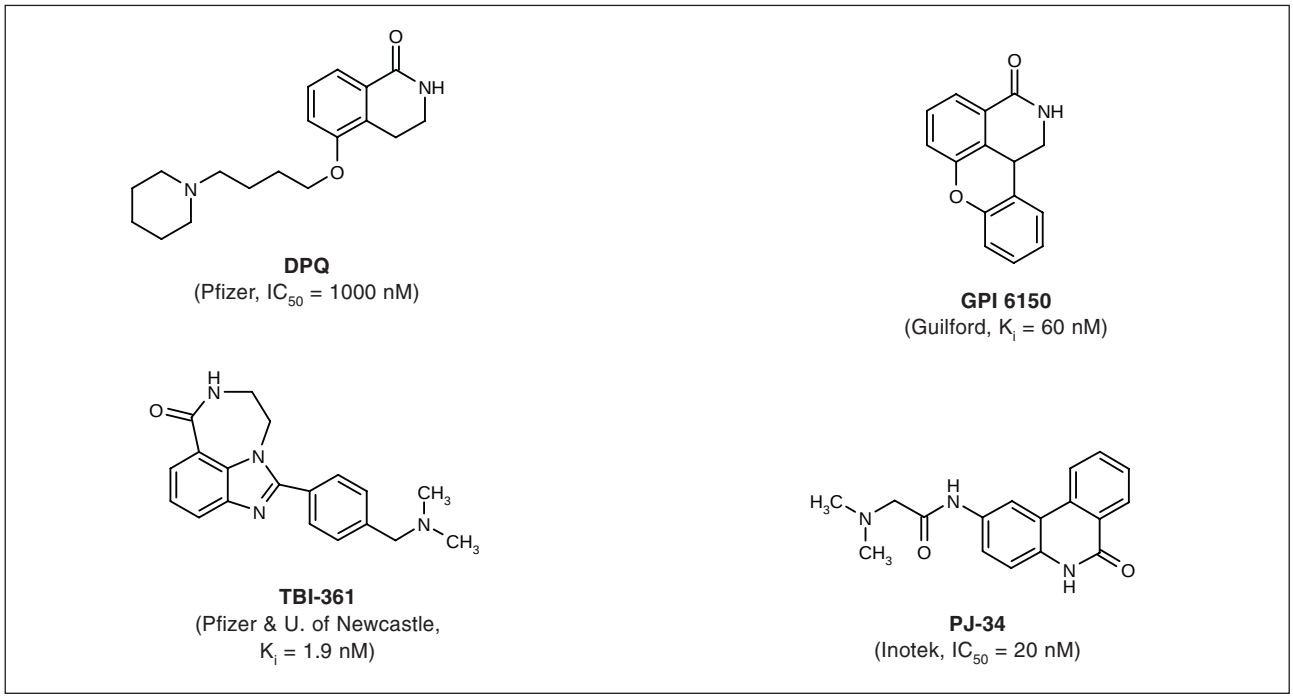

Fig. 3. Chemical structures of some prototypical PARP inhibitors.

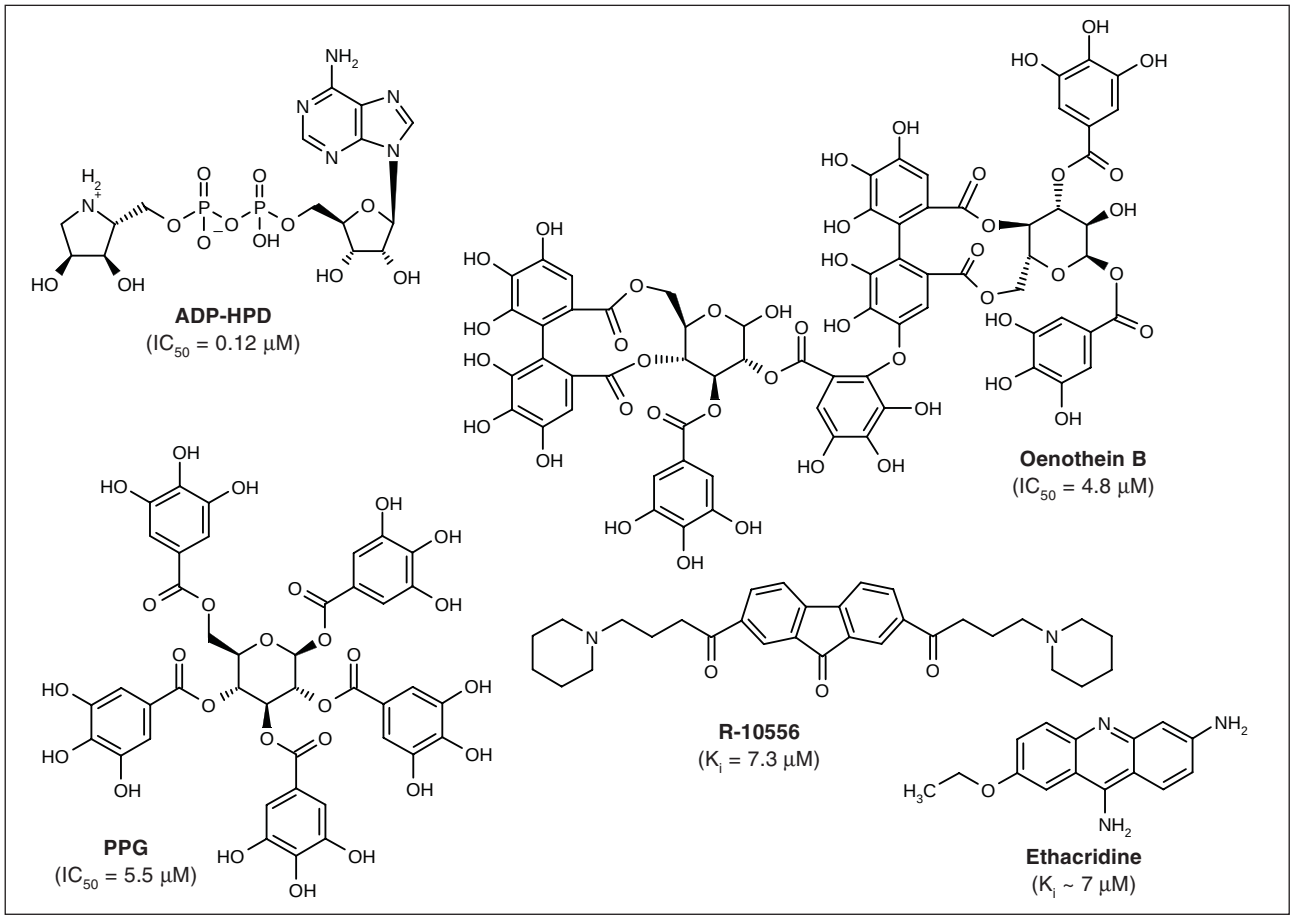

Fig. 4. Chemical structures of representative PARG inhibitors.

surgical procedures such as transplantation and coronary artery bypass. PARP inhibitors have been tested in various animal models of ischemia. In a rabbit model of regional heart ischemia, PARP inhibitors reduced the infarct caused by reperfusion (80). PARP inhibition attenuated the myocardial dysfunction caused by global ischemia and reperfusion in the isolated, perfused rabbit heart, suggesting that myocardiac contractility was maintained by treatment with PARP inhibitors. Similar results from a rat model of regional heart ischemia were reported (81). When $PARP_1^{-/-}$ mice were subjected to regional heart ischemia, coronary artery occlusion produced 40% less myocardial injury in PARP deficient mice than in wild-type mice (44). The myocardiocytes that survived the ischemia/reperfusion by PARP inhibition or deficiency also preserved its cellular function (82). In a rat model of regional heart ischemia, GPI 6150 decreased infarct size by 48% (83). Immunohistochemical staining detected a significant increase in poly(ADP-ribose) production immediately after reperfusion and GPI 6150 effectively inhibited PARP *in vivo* (83). Such a correlation suggested that the cadioprotective effect of GPI 6150 could be attributed to its ability to inhibit PARP.

Protection against ischemia and reperfusion injuries by PARP inhibitors is not limited to brain and heart. There are reports that PARP inhibitors offer protection during ischemia and reperfusion in skeletal muscle (80), retina (84) intestine (85-87) and kidney (88, 89) (Table I).

Inflammation-related injuries

PARP activation has been implicated in a variety of diseases in which inflammation is involved. PARP inhibition is found to be effective in preventing pathogenesis in animal models of type 1 diabetes, hemorrhagic shock, septic shock, rheumatoid arthritis, gout, inflammatory bowel disease and multiple sclerosis (Table I).

One animal model to study type 1 insulin-dependent diabetes uses streptozotocin (STZ) to destroy β-islet cells and illicit hypoglycemia. $PARP_1^{-/-}$ mice were completely protected from STZ-induced diabetes and pancreatic islet cell loss (51, 90, 91). In the STZ model, GPI 6150, given at 25 min before and 2 h and 45 min after STZ, significantly prevented the surge of plasma glucose level by 60% in ICR mice 48 h later (69). 5-Iodo-6-amino-1,2-benzopyrone was also found to have a similar protective effect in STZ-induced diabetic mice (92).

Sepsis leads to an uncontrolled systemic inflammation that progresses to shock and multiple organ failure, a leading cause of mortality in intensive care units. The complex vascular dysfunction is initiated by an endotoxin such as lipopolysaccharide (LPS). $PARP_1^{-/-}$ mice are resistant to death induced by septic shock (39, 93). Similarly, in a hemorrhagic shock model, $PARP_1^{-/-}$ mice were protected from the rapid decrease in blood pressure after resuscitation and showed an increased survival time (94). In a mouse model of shock, a daily single i.p. dose of 60 mg/kg GPI 6150 for 3 days reduced the LPS-induced mortality by 40% in C57/B1 mice (69). In another porcine model of septic shock, PJ-34 treatment significantly increased the survival rate (93).

Free radicals have been known to be responsible for inducing pathological tissue destruction in arthritis. Recent studies established a link between arthritic tissue damage and PARP activation as a result of DNA damage by free radicals. In rodent models of arthritis, carrageenan or zymosan induced paw edema was significantly reduced by treatment with PARP inhibitors (95-97). $PARP_1^{-/-}$ mice were resistant to zymosan-induced multiple organ failure and neutrophil infiltration (98). Inhibition of cytokine-induced gene expression by PARP inhibitors could be one of the mechanisms to suppress the inflammatory response. Histological analysis of collagen-injected paws revealed less neutrophil infiltration in joints of $PARP_1^{-/-}$ mice. GPI 6150 was tested in rat models of acute and chronic inflammation, carrageenan-induced paw edema, adjuvant-induced arthritis and zymosan-induced multiple organ failure (97). The compound reduced paw swelling, suppressed neutrophil infiltration and ameliorated organ damage.

Blocking the poly(ADP-ribose) cycle

Because of the involvement of poly(ADP-ribose) metabolism in various pathologic conditions, there have been tremendous efforts to synthesize potent, small-molecule PARP and PARG inhibitors for treating diseases underlined by the poly(ADP-ribose) pathway.

PARP inhibitors

More than two decades of research by both academic and pharmaceutical laboratories has yielded multiple classes of PARP inhibitors with hundreds of small molecules (99, 100). PARP inhibitors discovered in the early 1980s belong to a class of monoaryl amides, *e.g.*, 3-aminobenzamide. This type of compound is usually a weak $PARP_1$ inhibitor. In the early 1990s, synthesis of PARP inhibitors as potentiators for chemotherapy and radiation therapy led to benzamide analogues whose potencies were greatly enhanced by a lactam functionality. Recent efforts in the search for PARP inhibitors to treat ischemia and inflammation resulted in more potent multiring compounds.

A common structural feature for most of the $PARP_1$ inhibitors is a carboxamide attached to an aromatic ring or the carbamoyl group built within a polyaromatic heterocyclic skeleton to form an aromatically fused lactam. Structural studies of ligand-enzyme interactions indicate that the carboxamide or lactam functionality is essential for effective PARP inhibition (101).

Benzamide derivatives were first recognized as PARP inhibitiors. They mimic the structure of the natural product nicotinamide, a moiety of PARP substrate NAD^+. This group of PARP inhibitors has been widely used as a tool to study the function and malfunction of PARP because of

commercial availability. In general, bezamide-related compounds suffer from low potency (IC_{50} > 1 μM for PARP inhibition), poor enzyme selectivity and side effects at high concentrations (102). Such attributes hinder their further development as drug candidates.

Later on, PARP drug discovery was accelerated by studies of extensive structure-activity relationship and computer-aided drug design. This activity identified several classes of polycyclic lactams whose potency was improved 1000-fold. Representative compounds for bi-, tri- and tetra-cyclic lactams include DPQ (103), TBI-361 (104, 105) and PJ-34 (106) and GPI 6150 (107), respectively (Fig. 3). As discussed in the previous sections, some of the new generation PARP inhibitors have demonstrated *in vivo* efficacy in animal models, providing a framework to further improve physical, chemical, pharmacokinetic and toxicological properties desirable for clinical application.

PARG inhibitors

The development of PARG inhibitors lags behind that of PARP inhibitors. Only two types of PARG inhibitors have sufficient documentation. They are the ADP-ribose analogues, such as ADP-(hydroxymethyl)pyrrolidinediol (ADP-HPD) and the tannin-carbohydrate complexes, such as oenothein B (Fig. 4). The former is based on the nonhydrolyzable ADP-ribose structure (33). ADP-HPD was designed to mimic the structural oxocarbonium ions of ADP-ribose, presumably a transition-state intermediate in poly(ADP-ribose) hydrolysis. ADP-HPD is the most potent known PARG inhibitor (IC_{50} = 0.12 μM, MW = 542). However, this class of nucleotide analogue inhibitors is not favored for drug development because of their poor membrane permeability. The second class of PARG inhibitors, tannin-carbohydrate complexes, are weak PARG inhibitors (IC_{50} > 1.0 μM, MW = 1,000-10,000) (75). Hydrolyzable tannins are commonly known for nonspecific protein interaction due to high tendency of conjugation by the poly-phenol groups. The high molecular weight of these compounds may also be a disadvantage for using them as effective therapeutic agents since they diverge from the typical small-molecule drug profile (108). Compound 1,2,3,4,6-*O*-penta-β-D-galloylglucose (PPG) (IC_{50} = 5.5 μM, MW = 940) represents the small unit in the tannin-carbohydrate group.

In addition to the above two families, various other compounds have been claimed as PARG inhibitors. They include acridine derivatives, *e.g.*, 6,9-diamino-2-ethoxyacridine (ethacridine), tilorone analogues, *e.g.* R-10556 (Fig. 4), daunomycin or daunorubicin hydrochloride, ellipticine, proflavine (109). It appeared that some of these molecules inhibit PARG indirectly through interactions with DNA or poly(ADP-ribose). In general, none of these compounds are potent enough for testing as PARG inhibitors in biological system. Further development of small-molecule PARG inhibitors will depend on high-throughput screening to generate new leads and solving the crystal structure of PARG to facilitate computer-assisted drug design.

Perspectives

Recent progress in PARP preclinical research has accumulated a large body of evidence supporting eventual clinical trials based on its novel mechanism. It is anticipated that the potential benefit of PARP inhibitors will soon be tested clinically, once pharmacologic and toxicologic studies favor further clinical development of the leading PARP inhibitors. The efficacy of PARP inhibitors in various animal models of disease indicates that poly(ADP-ribose) metabolism may affect a broad spectrum of pathologic conditions, especially since the pathway regulates the level of NAD^+, a vital molecule mediating multiple essential cellular functions. Maintaining NAD^+ levels may also have an indirect effect on other NAD^+-utilizing enzymes, such as mono-ADP-ribosyltransferase, cyclic-ADP-ribose synthase/*CD38* and NAD^+-dependent histone deacetylase/*SIR2*. PARG offers an alternative target for the poly(ADP-ribose) pathway. While $PARP_1$ and PARG inhibition share similar effects, especially at maintaining NAD^+ levels, they vary in other aspects. For example, PARG inhibitors would not interfere with the initial activation and auto-ADP-ribosylation of $PARP_1$, and would thus be less likely to hinder DNA repair that is presumably facilitated by poly(ADP-ribosyl)ation. Rather, PARG inhibitors would prolong the half-life of the polymer and enhance the effect of poly(ADP-ribosyl)ation. Creating PARG knockout mice may offer insights to the further understanding of the roles of PARG.

References

1. Chambon, P., Weil, J.D., Mandel, P. *Nicotinamide mononucleotide activation of a new DNA-dependent polyadenylic acid synthesizing nuclear enzyme.* Biochem Biophys Res Commun 1963, 11: 39-43.

2. Reeder, R.H., Ueda, K., Honjo, T. et al. *Studies on the polymer of adenosine phosphate ribose. II. Characterization of the polymer.* J Biol Chem 1967, 242: 3172-9.

3. Fujimura, S., Hasegawa, S., Shimizu, Y. et al. *Polymerization of the adenosine 5'-diphosphate-ribose moiety of nicotinamide-adenine dinucleotide by nuclear enzyme.* Biochim Biophys Acta 1967, 145: 247-59.

4. Fujimura, S., Hasegawa, S., Shimizu, Y. et al. *The polymerization of adenosine 5'-diphosphate-ribose moiety of NAD by nuclear enzyme.* Biochim Biophys Acta 1967, 149: 369-76.

5. Miwa, M., Saito, H., Sakura, H. et al. *A ^{13}C NMR study of poly(adenosine diphosphate ribose) and its monomer: Evidence of α-(1" leads to 2') ribofuranosyl ribofuranoside residue.* Nucleic Acids Res 1977, 4: 3997-4005.

6. Miwa, M., Saikawa, N., Yamaizumi, Z. et al. *Structure of poly(adenosine diphosphate ribose): Identification of 2'-[1"-ribo-*

syl-2"-(or 3"-)(1"'-ribosyl)]adenosine-5',5",5"'-tris(phosphate) as a branch linkage. Proc Natl Acad Sci USA 1979, 76: 595-9.

7. Emanuelli, M., Carnevali, F., Saccucci, F. et al. *Molecular cloning, chromosomal localization, tissue mRNA levels, bacterial expression, and enzymatic properties of human NMN adenylyltransferase.* J Biol Chem 2001, 276: 406-12.

8. Chambon, P., Weill, J.D., Doly, J. et al. *On the formation of a novel adenylic compound by enzymatic extracts of liver nuclei.* Biochem Biophys Res Comm 1966, 25: 638-43.

9. Alvarez-Gonzalez, R., Jacobson, M.K. *Characterization of polymers of adenosine diphosphate ribose generated in vitro and in vivo*. Biochemistry 1987, 26: 3218-24.

10. Kofler, B., Wallraff, E., Herzog, H. et al. *Purification and characterization of NAD^+:ADP-ribosyltransferase (polymerizing) from Dictyostelium discoideum.* Biochem J 1993, 293: 275-81.

11. Suzuki, H., Uchida, K., Shima, H. et al. *Molecular cloning of cDNA for human poly(ADP-ribose) polymerase and expression of its gene during HL-60 cell differentiation.* Biochem Biophys Res Commun 1987, 146: 403-9.

12. Alkhatib, H.M., Chen, D.F., Cherney, B. et al. *Cloning and expression of cDNA for human poly(ADP-ribose) polymerase.* Proc Natl Acad Sci USA 1987, 84: 1224-8.

13. *Genome sequence of the nematode C. elegans: A platform for investigating biology. The C. elegans Sequencing Consortium*. Science 1998, 282: 2012-8.

14. *Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. The Arabidopsis Genome Initiative.* Nature 2000, 408: 796-815.

15. Ame, J.C., Apiou, F., Jacobson, E.L. et al. *Assignment of the poly(ADP-ribose) glycohydrolase gene (PARG) to human chromosome 10q11.23 and mouse chromosome 14B by in situ hybridization.* Cytogenet Cell Genet 1999, 85: 269-70.

16. Zhang, J. *PARP inhibition: A novel approach to treat ischemia/reperfusion and inflammation related injuries*. Emerging Drugs: The Prospect for Improved Medicines. 1999, 4: 209-21.

17. Christenson, E., Demaggio, A.J., Goldman, P.S. et al. *Human poly(ADP-ribose) polymerase 2 materials and methods.* WO 0077179.

18. Kock, M., Hoger, T., Kroger, B. et al. *Poly(ADP-ribose) polymerase gene.* WO 09964572.

19. Rome, L.H., Kickhoefer, V.A. *Human minor vault protein p193.* US 6156879.

20. De Lange, T., Smith, S. *Tankyrase, a TRFI binding protein*. WO 9964606.

21. Berthelsen, J., Toma, S., Isacchi, A. *Tankyrase homolog protein (THP), nucleic acids, and methods related to the same.* WO 0104326.

22. Morin, G.B., Funk, W.D., Pliatyszek, M.A. *A second mammalian tankyrase.* WO 0061813.

23. Christenson, E., Demaggio, A.J., Goldman, P.S. et al. *$Tankyrase_2$ materials and methods.* WO 0100849.

24. Oikawa, A., Tohda, H., Kanai, M. et al. *Inhibitors of poly(adenosine diphosphate ribose) polymerase induce sister chromatid exchanges*. Biochem Biophys Res Commun 1980, 97: 1311-6.

25. de Murcia, J.M., Niedergang, C., Trucco, C. et al. *Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells.* Proc Natl Acad Sci USA 1997, 94: 7303-7.

26. Ueda, K., Hayaishi, O. *ADP-ribosylation.* Ann Rev Biochem 1985, 54: 73-100.

27. Kameshita, I., Matsuda, Z., Taniguchi, T. et al. *Poly(ADP-Ribose) synthetase. Separation and identification of three proteolytic fragments as the substrate-binding domain, the DNA-binding domain, and the automodification domain.* J Biol Chem 1984, 259: 4770-6.

28. Smulson, M.E., Simbulan-Rosenthal, C.M., Boulares, A.H. et al. *Roles of poly(ADP-ribosyl)ation and PARP in apoptosis, DNA repair, genomic stability and functions of p53 and E2F-1.* Adv Enzyme Regul 2000, 40: 183-215.

29. Babiychuk, E., Cottrill, P.B., Storozhenko, S. et al. *Higher plants possess two structurally different poly(ADP-ribose) polymerases*. Plant J 1998, 15: 635-45.

30. Shieh, W.M., Ame, J.C., Wilson, M.V. et al. *Poly(ADP-ribose) polymerase null mouse cells synthesize ADP-ribose polymers.* J Biol Chem 1998, 273: 30069-72.

31. Kaiser, P., Auer, B., Schweiger, M. *Inhibition of cell proliferation in Saccharomyces cerevisiae by expression of human NAD^+ ADP-ribosyltransferase requires the DNA binding domain ("zinc fingers").* Mol Gen Genet 1992, 232: 231-9.

32. Perkins, E., Sun, D., Nguyen, A., et al. *Novel inhibitors of poly(ADP-ribose) polymerase/$PARP_1$ and $PARP_2$ identified using a cell-based screen in yeast.* Cancer Res 2001, 61: 4175-83.

33. Slama, J.T., Aboul-Ela, N., Goli, D.M. et al. *Specific inhibition of poly(ADP-ribose) glycohydrolase by adenosine diphosphate (hydroxymethyl)pyrrolidinediol.* J Med Chem 1995, 38: 389-93.

34. Lin, W., Ame, J.C., Aboul-Ela, N. et al. *Isolation and characterization of the cDNA encoding bovine poly(ADP-ribose) glycohydrolase.* J Biol Chem 1997, 272: 11895-901.

35. Winstall, E., Affar, E.B., Shah, R. et al. *Preferential perinuclear localization of poly(ADP-ribose) glycohydrolase.* Exp Cell Res 1999, 251: 372-8.

36. Wielckens, K., Schmidt, A., George, E. et al. *DNA fragmentation and NAD depletion. Their relation to the turnover of endogenous mono(ADP-ribosyl) and poly(ADP-ribosyl) proteins.* J Biol Chem 1983, 257: 12872-7.

37. Berger, N.A. *Poly(ADP-ribose) in the cellular response to DNA damage*. Radiat Res 1985, 101: 4-15.

38. Szabo, C. *Role of poly(ADP-ribose)synthetase in inflammation.* Eur J Pharmacol 1998, 350: 1-19.

39. Oliver, F.J., Menissier-de Murcia, J., Nacci, C. et al. *Resistance to endotoxic shock as a consequence of defective NF-κB activation in poly (ADP-ribose) polymerase-1 deficient mice*. EMBO J 1999, 18: 4446-54.

40. Hassa, P.O., Hottiger, M.O. *A role of poly(ADP-ribose) polymerase in NF-κB transcriptional activation.* Biol Chem 1999, 380: 953-9.

41. Hassa, P.O., Covic, M., Hasan, S. et al. *The enzymatic and DNA binding activity of PARP-1 are not required for NF-κB coactivator function*. J Biol Chem 2001, 276: 45588-97.

42. Chang, W.J., Alvarez-Gonzalez, R. *The sequence-specific DNA binding of NF-κB is reversibly regulated by the automodification reaction of PARP-1.* J Biol Chem 2001, 276: 47664-70.

43. Hauschildt, S., Scheipers, P., Bessler, W.G. et al. *Induction of nitric oxide synthase in L929 cells by tumour-necrosis factor α is prevented by inhibitors of poly(ADP-ribose) polymerase.* Biochem J 1992, 288: 255-60.

44. Zingarelli, B., Salzman, A.L., Szabo, C. *Genetic disruption of poly(ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury.* Circ Res 1998, 83: 85-94.

45. Durkacz, B.W., Omidiji, O., Gray, D.A. et al. *(ADP-ribose)n participates in DNA excision repair.* Nature 1980, 283: 593-6.

46. Nduka, N., Skidmore, C.J., Shall, S. *The enhancement of cytotoxicity of N-methyl-N-nitrosourea and of γ-radiation by inhibitors of poly(ADP-ribose) polymerase*. Eur J Biochem 1980, 105: 525-30.

47. Arundel-Suto, C.M., Scavone, S.V., Turner, W.R. et al. *Effect of PD 128763, a new potent inhibitor of poly(ADP-ribose) polymerase, on X-ray-induced cellular recovery processes in Chinese hamster V79 cells.* Radiat Res 1991, 126: 367-71.

48. Bowman, K.J., White, A., Golding, B.T. et al. *Potentiation of anti-cancer agent cytotoxicity by the potent poly(ADP-ribose) polymerase inhibitors NU1025 and NU1064.* Br J Cancer 1998, 78: 1269-77.

49. White, A.W., Almassy, R., Calvert, A.H. et al. *Resistance-modifying agents. 9. Synthesis and biological properties of benzimidazole inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase.* J Med Chem 2000, 43: 4084-97.

50. Wang, Z.Q., Auer, B., Stingl, L. et al. *Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease.* Genes Dev 1995, 9: 509-20.

51. Masutani, M., Suzuki, H., Kamada, N. et al. *Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin-induced diabetes*. Proc Natl Acad Sci USA 1999, 96: 2301-4.

52. Simbulan-Rosenthal, C.M., Ly, D.H., Rosenthal, D.S. et al. *Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase.* Proc Natl Acad Sci USA 2000, 97: 11274-9.

53. Conde, C., Mark, M., Oliver, F.J. et al. *Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice*. EMBO J 2001, 20: 3535-43.

54. Zhang, J. Dawson, V.L., Dawson, T.M., Snyder, S.H. *Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity.* Science 1994, 263: 687-9.

55. Eliasson, M.J.L., Sampei, K., Mandir, A.S. et al. *Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia.* Nat Med 1997, 10: 1089-95.

56. Endres, M., Wang, Z.-Q., Namura, S. et al. *Ischemic brain injury is mediated by the activation of poly(ADP-ribose) polymerase.* J Cereb Blood Flow and Metab 1997, 17: 1143-51.

57. Takahashi, K., Greenberg, J.H., Jackson, P. et al. *Neuroprotective effects of inhibiting poly(ADP-ribose) synthetase on focal cerebral ischemia in rats.* J Cereb Blood Flow Metab 1997, 17: 1137-42.

58. Lo, E.H., Bosque-Hamilton, P., Meng, W. *Inhibition of poly(ADP-ribose) polymerase reduction of ischemic injury and attenuation of N-methyl-D-aspartate-induced neurotransmitter dysregulation.* Stroke 1997, 29: 830-6.

59. Endres, M., Scott, G.S., Salzmann, A.L. et al. *Protective effects of 5-iodo-6-amino-1,2-benzopyrone, an inhibitor of poly(ADP-ribose) synthetase against peroxynitrite-induced glial damage and stroke development.* Eur J Pharmacol 1998, 351: 377-82.

60. Sun, A.-Y., Cheng, J.-S. *Neuroprotective effects of poly(ADP-ribose) polymerase inhibitors in transient focal cerebral ischemia of rats.* Acta Pharmacol Sinica 1998, 19: 104-8.

61. Zhang, J., Li, J.-H., Lautar, S. *Post-ischemia protection by a potent PARP inhibitor in transient cerebral focal ischemia.* Soc Neurosci Abstr 1998, 24: 1226.

62. Tokime, T., Nozaki, K., Sugino, T. et al. *Enhanced poly(ADP-ribosyl)ation after focal ischemia in rat brain.* J Cereb Blood Flow Metab 1998, 18: 991-7.

63. Ayoub, I.A., Lee, E.J., Ogilvy, C.S. et al. *Nicotinamide reduces infarction up to two hours after the onset of permanent focal cerebral ischemia in Wistar rats*. Neurosci Lett 1999, 259: 21-4.

64. Takahashi, K., Greenberg, J.H., Pieper, A.A. et al. *Post-treatment with an inhibitor of poly(ADP-ribose) polymerase attenuates cerebral damage in focal ischemia.* Brain Res 1999, 829: 46-54.

65. Lautar, S., Pieper, A.A., Verma, A. et al. *Post-ischemia treatment with GPI 6150 diminishes poly(ADP-ribose) accumulation during ischemia-reperfusion injury in rat brain*. Soc Neurosci Abstr 1998, 24: 1226.

66. Mandir, A.S., Przedborski, S., Jackson-Lewis, V. et al. *Poly(ADP-ribose) polymerase activation mediates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism.* Proc Natl Acad Sci USA 1999, 96: 5774-9.

67. Zhang, J., Pieper, A., Snyder, S.H. *Poly(ADP-ribose) synthetase activation: An early indicator of neurotoxic DNA damage.* J Neurochem 1995, 65: 1411-4.

68. Cosi, C., Colpaert, F., Koek, W. et al. *Poly(ADP-ribose) polymerase inhibitors protect against MPTP-induced depletions of striatal dopamine and cortical noradrenaline in C57B1/6 mice*. Brain Res 1996, 729: 264-9.

69. Zhang, J. *Beneficial effect of GPI 6150 treatment in multiple animal models of diseases.* In: PARP as a Therapeutic Target. J. Zhang (Ed.). CRC Press 2002: 239-56.

70. LaPlaca, M.C., Raghupathi, R., Verma, A. et al. *Temporal patterns of poly(ADP-ribose) polymerase activation in the cortex following experimental brain injury in the rat*. J Neurochem 1999, 73: 205-13.

71. LaPlaca, M.C., Zhang, J., Raghupathi, R. et al. *Pharmacologic inhibition of poly(ADP-ribose) polymerase is neuroprotective following traumatic brain injury in rats.* J Neurotrauma 2001, 18: 369-76.

72. Whalen, M.J., Clark, R.S., Dixon, C.E. et al. *Reduction of cognitive and motor deficits after traumatic brain injury in mice deficient in poly(ADP-ribose) polymerase.* J Cereb Blood Flow Metab 1999, 19: 835-42.

73. Whalen, M.J., Clark, R.S., Dixon, C.E., et al. *Traumatic brain injury in mice deficient in poly-ADP(ribose) polymerase: A preliminary report.* Acta Neurochir Suppl 2000, 76: 61-4.

74. Maruta, H., Matsumura, N., Tanuma, S. *Role of (ADP-ribose)n catabolism in DNA repair*. Biochem Biophys Res Commun 1997, 236: 265-9.

75. Aoki, K., Nishimura, K., Abe, H. et al. *Novel inhibitors of poly(ADP-rribose)glycohydrolase*. Biochem Biophys Acta 1993, 1158: 251-6.

76. Li, J.-H., Zhang, J. *Pharmaceutical compositions containing poly(ADP-ribose) glycohydrolase inhibitors for inhibiting or decreasing free radical-induced cellular energy depletion, cell damage, or cell death.* WO 00025787.

77. Ying, W., Swanson, R.A. *The poly(ADP-ribose) glycohydrolase inhibitor gallotannin blocks oxidative astrocyte death.* Neuroreport 2000, 11: 1385-8.

78. Ying, W., Sevigny, M.B., Chen, Y. et al. *Poly(ADP-ribose) glycohydrolase mediates oxidative and excitotoxic neuronal death.* Proc Natl Acad Sci USA 2001, 98: 12227-32.

79. Lu, X.-C.M., Lin, Q., Massuda, E., Li, W., Li, J.-H., Zhang, J. *Post-ischemia treatment of poly(ADP-ribose) glycohydrolase inhibitor provides neuroprotection against transient focal cerebral ischemia in rat.* Soc Neurosci Abst 2001, 763.18.

80. Thiemermann, C., Bowes, J., Myint, F.P. et al. *Inhibition of the activity of poly(ADP-ribose) synthetase reduces ischemia-reperfusion injury in the heart and skeletal muscle.* Proc Natl Acad Sci USA 1997, 94: 679-83.

81. Zingarelli, B., Cuzzocrea, S., Zsengeller, Z. et al. *Protection against myocardial ischemia and reperfusion injury by 3-aminobenzamide, an inhibitor of poly(ADP-ribose) synthetase.* Cardiovascular Res 1997, 36: 205-15.

82. Grupp, I.L., Jackson, T.M., Hake, P. et al. *Protection against hypoxia-reoxygenation in the absence of poly(ADP-ribose) synthetase in isolated working hearts.* J Mol Cell Cardiol 1999, 31: 297-303.

83. Walles, T., Pieper, A.A., Li, J.-H. et al. *Demonstration that poly(ADP-ribose) accumulation occurs in the postischemic heart and is associated with myocardial necrosis.* Circulation Suppl. 1998, 98: I-260.

84. Lam, T.T. *The effect of 3-aminobenzamide, an inhibitor of poly-ADP-ribose polymerase, on ischemia/reperfusion damage in rat retina.* Res Commun Mol Pathol Pharmacol 1997, 95: 241-52.

85. Liaudet, L., Szabo, A., Soriano, F.G. et al. *Poly(ADP-ribose) synthetase mediates intestinal mucosal barrier dysfunction after mesenteric ischemia.* Shock 2000, 14: 134-41.

86. Cuzzocrea, S., Zingarelli, B., Costantino, G. et al. *Beneficial effects of 3-aminobenzamide, an inhibitor of poly(ADP-ribose) synthetase in a rat model of splanchnic artery occlusion and reperfusion*. Br J Pharmacol 1997, 121: 1065-74.

87. Mazzon, E., Dugo, L., De Sarro, A. et al. *Beneficial effects of GPI 6150, an inhibitor of poly(ADP-ribose) polymerase in a rat model of splanchnic artery occlusion and reperfusion.* Shock 2002, 17: 222-7.

88. Chatterjee, P.K., Zacharowski, K., Cuzzocrea, S. et al. *Inhibitors of poly(ADP-ribose) synthetase reduce renal ischemia-reperfusion injury in the anesthetized rat in vivo.* FASEB J 2000, 14: 641-51.

89. Martin, D.R., Lewington, A.J., Hammerman, M.R. et al. *Inhibition of poly(ADP-ribose) polymerase attenuates ischemic renal injury in rats.* Am J Physiol Regul Integr Comp Physiol 2000, 279: R1834-40.

90. Burkart, V., Wang, Z.Q., Radons, J. et al. *Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic β-cell destruction and diabetes development induced by streptozocin.* Nat Med 1999, 5: 314-9.

91. Pieper, A.A., Brat, D.J., Krug, D.K. et al. *Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes.* Proc Natl Acad Sci USA 1999, 96: 3059-64.

92. Mabley, J.G., Suarez-Pinzon, W.L., Hasko, G. et al. *Inhibition of poly(ADP-ribose) synthetase by gene disruption or inhibition with 5-iodo-6-amino-1,2-benzopyrone protects mice from multiple-low-dose-streptozotocin-induced diabetes.* Br J Pharmacol 2001, 133: 909-19.

93. Marton, A., Szabo, E., Virag, L. et al. *Protective effect of a novel inhibitor of poly(ADP-ribose) synthetase in a porcine model of peritonitis.* FASEB J 2001, Abst 1123.

94. Liaudet, L., Soriano, F.G., Szabo, E. et al. *Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose)polymerase.* Proc Natl Acad Sci USA 2000, 97: 10203-8.

95. Miesel, R., Kurpisz, M., Kroger, H. *Modulation of inflammatory arthritis by inhibition of poly(ADP-ribose) polymerase.* Inflammation 1995, 19: 379-87.

96. Szabo, C., Virag, L., Cuzzocrea, S. et al. *Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly(ADP-ribose) synthase*. Proc Natl Acad Sci USA 1998, 95: 3867-72.

97. Mazzon, E., Serraino, I., Li, J.H. et al. *GPI 6150, a poly(ADP-ribose) polymerase inhibitor, exhibits an anti-inflammatory effect in rat models of inflammation*. Eur J Pharmacol 2001, 415: 85-94.

98. Szabo, C., Lim, L.H., Cuzzocrea, S. et al. *Inhibition of poly (ADP-ribose) synthetase attenuates neutrophil recruitment and exerts antiinflammatory effects.* J Exp Med 1997, 186: 1041-9.

99. Li, J.-H., Zhang, J. *PARP inhibitors*. Drugs 2001, 4: 804-12.

100. Curtin, N.J., Golding, B.T. et al. *New poly(ADP-ribose) polymerase inhibitors for chemo-and radiotherapy of cancer.* In: From DNA Damage and Stress Signaling to Cell Death. G.D. Murcia, S. Shall (Eds.). Oxford University Press 2000: 177–206.

101. Ruf, A., de Murcia, G., Schulz, G.E. *Inhibitor and NAD^+ binding to poly(ADP-ribose) polymerase as derived from crystal structures and homology modeling.* Biochemistry 1998, 37: 3893–900.

102. Milam, K.M., Cleaver, J.E. *Inhibitors of poly(adenosine dephosphate-ribose) synthesis effect on other metabolic processes.* Science 1984, 223: 589-91.

103. Suto, M.J., Turner, W.R., Werbel, L.M. *Substituted dihydroisoquinolinones and related compounds as potentiators of the lethal effects of radiation and certain chemotherapeutic agents; selected compounds, analogs and process.* US 5177075.

104. Webber, S.E., Skalitzky, D.J. et al. *Tricyclic inhibitors of poly(ADP-ribose) polymerases.* WO 0116136.

105. Curtin, N.J., Almassy R. et al. *Anti-cancer chemo- and radio-sensitization in vitro and in vivo by novel potent $PARP_1$ inhibitor, TBI-361.* 13th Int Symp ADP-Ribosylation (June, New York) 2001, Abst 122.

106. Abdelkarim, G.E., Gertz, K., Harms, C. et al. *Protective effects of PJ34, a novel, potent inhibitor of poly(ADP-ribose) polymerase (PARP) in vitro and in vivo models of stroke.* Int J Mol Med 2001 7: 255–60.

107. Li, J.-H., Kalish, V.J., Lautar, S., Zhang, J. *GPI 6150, a novel PARP inhibitor, is cardioprotective and neuroprotective in rat models of ischemia.* 220th Natl Meet ACS (Aug 20-24, Washington, DC) 2000, MEDI 154.

108. Lipinski, C.A., Lombardo, F. et al. *Experimental and computational approaches to estimate solubility and permeability in*

drug discovery and development settings. Adv Drug Deliv Rev 1997, 23: 3-25.

109. Tavassoli, M., Tavassoli, M.H., Shall, S. *Effect of DNA intercalators on poly(ADP-ribose) glycohydrolase activity.* Biochim Biophy Acta 1985, 827: 228-34.

110. Love, S., Barber, R., Wilcock, G.K. *Neuronal accumulation of poly(ADP-ribose) after brain ischaemia.* Neuropathol Appl Neurobiol 1999, 25: 98-103.

111. Mao, J., Price, D.D., Zhu, J. et al. *The inhibition of nitric oxide-activated poly(ADP-ribose) synthetase attenuates transsynaptic alteration of spinal cord dorsal horn neurons and neuropathic pain in the rat.* Pain 1997, 72: 355-66.

112. Scott, G.S., Jakeman, L.B., Stokes, B.T. et al. *Peroxynitrite production and activation of poly(adenosine diphosphate-ribose) synthetase in spinal cord injury.* Ann Neurol 1999, 45: 120-4.

113. Love, S., Barber, R., Wilcock, G.K. *Increased poly(ADP-ribosyl)ation of nuclear proteins in Alzheimer's disease.* Brain 1999, 122: 247-53.

114. Scott, G.S., Hake, P., Kean, R.B. et al. *Role of poly(ADP-ribose) synthetase activation in the development of experimental allergic encephalomyelitis.* J Neuroimmunol 2001, 117: 78-6.

115. Pieper, A.A., Walles, T., Wei, G. et al. *Myocardial postischemic injury is reduced by polyADPribose polymerase-1 gene disruption.* Mol Med 2000, 6: 271-82.

116. Szabo, A., Hake, P., Salzman, A.L., Szabo, C. *3-Aminobenzamide, an inhibitor of poly(ADP-ribose) synthetase, improves hemodynamics and prolongs survival in a porcine model of hemorrhagic shock.* Shock 1998, 10: 347-53.

117. McDonald, M.C., Filipe, H.M., Thiemermann, C. *Effects of inhibitors of the activity of poly(ADP-ribose) synthetase on the organ injury and dysfunction caused by haemorrhagic shock*. Br J Pharmacol 1999, 128: 1339-45.

118. McDonald, M.C., Mota-Filipe, H., Wright, J.A. et al. *Effects of 5-aminoisoquinolinone, a water-soluble, potent inhibitor of the activity of poly(ADP-ribose) polymerase on the organ injury and dysfunction caused by haemorrhagic shock.* Br J Pharmacol 2000, 130: 843-50.

119. Zingarelli, B., Salzman, A.L., Szabo, C. *Protective effects of nicotinamide against nitric oxide-mediated delayed vascular failure in endotoxic shock: potential involvement of polyADP ribosyl synthetase*. Shock 1996, 5: 258-64.

120. Kuhnle, S., Nicotera, P., Wendel, A., Leist, M. *Prevention of endotoxin-induced lethality, but not of liver apoptosis in poly(ADP-ribose) polymerase-deficient mice*. Biochem Biophys Res Commun 1999, 263: 433-8.

121. Zingarelli, B., Szabo, C., Salzman, A.L. *Blockade of poly(ADP-ribose) synthetase inhibits neutrophil recruitment, oxidant generation, and mucosal injury in murine colitis.* Gastroenterology 1999, 116: 335-45.

122. Cuzzocrea, S., Mazzon, E., Mazzon, S. et al. *GPI 6150, a PARP inhibitor, reduces the colon injury caused by dinitrobenzene sulfonic acid in rats*. Biochem Pharmacol 2002, in press.

123. Oka, J., Ueda, K. et al. *ADP-ribosyl protein lyase. Purification, properties and identification of the product*. J Biol Chem 1984, 259, 986-95.