POLY(ADP-RIBOSE) POLYMERASE AND THE THERAPEUTIC EFFECTS OF ITS INHIBITORS

Prakash Jagtap and Csaba Szabó*‡*

Abstract | Poly(ADP-ribose) polymerases (PARPs) are involved in the regulation of many cellular functions. Three consequences of the activation of PARP1, which is the main isoform of the PARP family, are particularly important for drug development: first, its role in DNA repair; second, its capacity to deplete cellular energetic pools, which culminates in cell dysfunction and necrosis; and third, its capacity to promote the transcription of pro-inflammatory genes. Consequently, pharmacological inhibitors of PARP have the potential to enhance the cytotoxicity of certain DNA-damaging anticancer drugs, reduce parenchymal cell necrosis (for example, in stroke or myocardial infarction) and downregulate multiple simultaneous pathways of inflammation and tissue injury (for example, in circulatory shock, colitis or diabetic complications). The first ultrapotent novel PARP inhibitors have now entered human clinical trials. This article presents an overview of the principal pathophysiological pathways and mechanisms that are governed by PARP, followed by the main structures and therapeutic actions of various classes of novel PARP inhibitors.

ZINC FINGER
Protein module in which conserved cysteine or histidine residues coordinate a zinc atom. Some zinc-finger regions bind specific DNA sequences.

**Inotek Pharmaceuticals Corp., Suite 419E, 100 Cummings Center, Beverly, Massachusetts 01915, USA.*
‡Department of Human Physiology, Semmelweis University Medical School, Budapest, Hungary.
Correspondence to C.S.
e-mail: szabocsaba@aol.com
doi:10.1038/nrd1718

Approximately 40 years ago, Chambon and colleagues reported that the addition of NAD^+ to rat liver nuclear extracts stimulated the synthesis of a polyadenylic acid, which was later identified as poly(ADP-ribose) or PAR[1]. This observation initiated intensive research in the area of poly(ADP-ribosyl)ation and led to the discovery of the first PAR polymerase (PARP) isoform (now termed PARP1), which was followed by the identification of several other PARP family members[2–4]. Although research on the roles of the various PARP isoforms is expanding, most of our knowledge relates to PARP1, which has the principal role in regulating the cellular processes that are most relevant in terms of pathophysiology and experimental therapy[4]. We therefore focus on the roles of PARP1 in this review.

PARP1 is one of the most abundant proteins in the nucleus. This 116-kDa protein consists of three main domains: an amino (N)-terminal DNA-binding domain (DBD) of 46 kDa, an automodification domain of 22 kDa and a carboxy (C)-terminal catalytic domain of 54 kDa (FIG. 1). The catalytic function of PARP1 relates to its role as a DNA-damage sensor and signalling molecule. The ZINC FINGERS of PARP recognize single- and double-stranded DNA breaks. PARP1 subsequently forms homodimers and catalyses the cleavage of NAD^+ into nicotinamide and ADP-ribose; the latter is used to synthesize branched nucleic acid-like polymers that are covalently attached to acceptor proteins (FIG. 1). Most of the biological effects of PARP relate to the various aspects of this process: covalent poly(ADP-ribosyl)ation that influences the function of target proteins; PAR oligomers that, when cleaved from poly(ADP-ribosyl)ated proteins, confer distinct cellular effects; the physical association of PARP with nuclear proteins to form functional complexes; and the lowering of the cellular level of its substrate, NAD^+.

Poly(ADP-ribosyl)ation is a dynamic process, as indicated by the short half-life of the polymer. PAR glycohydrolase (PARG) and ADP-ribosyl protein lyase catabolize PAR; the former cleaves the ribose–ribose

bonds of both the linear and branched portions of PAR, whereas the latter removes the protein-proximal ADP-ribose monomer[5].

Although the regulation of PARP1 can occur at the level of expression, it is principally regulated at the level of its catalytic activity. The best-characterized mechanism for the downregulation of enzyme activity is through auto-poly(ADP-ribosyl)ation[3,4]. Nicotinamide exerts a weak negative-feedback inhibitory effect on PARP1. PARP1 is a highly abundant nuclear protein, which can be activated (up to 100-fold) by DNA strand breaks, and so changes in the expression or abundance of PARP1 are not of primary regulatory relevance. Nevertheless, several recent reports have shown up- or downregulation of PARP1 under pathophysiological conditions, including its upregulation in chronic heart failure[6].

Cellular signalling mechanisms modulate the activation of PARP1. An endogenous inhibitory pathway relates to protein kinase C (PKC)[7], whereas a DNA strand break-independent calcium-mediated pathway of PARP activation is linked to the activation of the phospholipase C–inositol 1,4,5-trisphosphate pathway[8].

It is not clear how much basal activity is shown by PARP1 and how this activity is regulated. PARP1 binds with high affinity and in a cooperative manner to various DNA structures (for example, cruciform, curved or supercoiled structures), which influence basal PARP activity[9]. When considering the basal activity of PARP, it should be noted that oxidants and free radicals are produced as byproducts of oxidative phosphorylation and other pathways[10]. A basal low-level DNA-strand breakage might have a role in maintaining basal PARP1 activity.

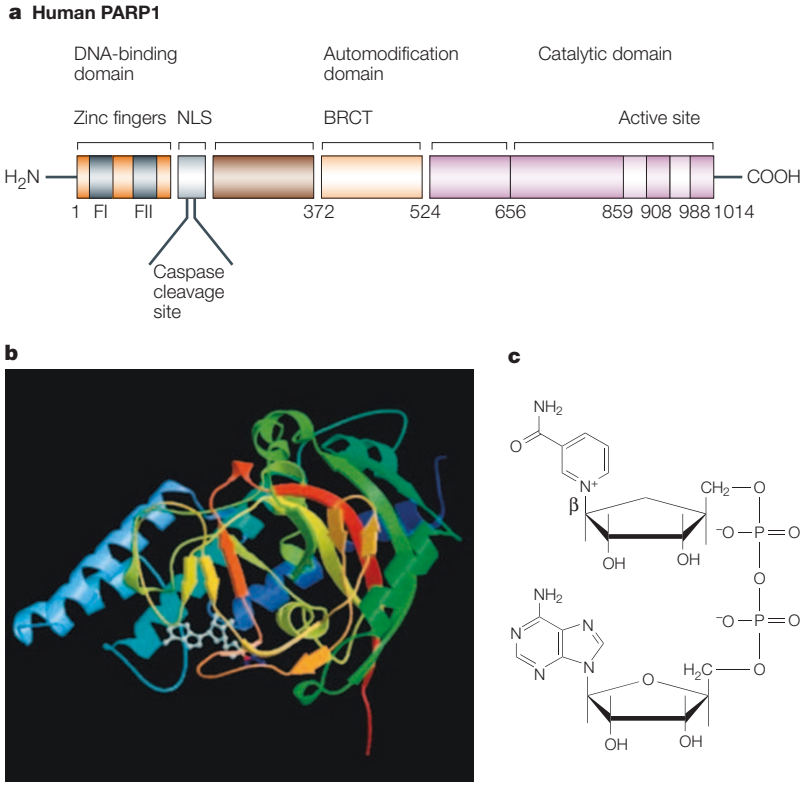


Figure 1 | **Structure of poly(ADP-ribose) polymerase-1 (PARP1). a** | A schematic representation of the modular organization of human PARP1 (hPARP1); the location of some modules is indicated by amino-acid numbering. The amino (N)-terminal DNA-binding domain contains two zinc fingers, which are responsible for DNA binding and some protein–protein interactions. A DNA-nick sensor, which is a nuclear-localization signal (NLS) in the caspase-cleavage site (DEVD), can be found in this DNA-binding domain. The automodification domain is a central regulating segment with a breast cancer-susceptibility protein-carboxy (C) terminus motif (BRCT), which is common in many DNA-repair and cell-cycle proteins, and serves protein–protein interactions. The C-terminal region accommodates the catalytic centre of PARP. There is high amino-acid sequence similarity between different species in the primary structure of the PARP1 enzyme, with the catalytic domain showing the greatest amount of similarity. The PARP catalytic fragment (CF), which contains the active site, comprises residues 655–1,014 (human numbering) and is composed of two parts: a purely α-helical N-terminal domain (NTD) from residues 662 to 784 is formed by an up-up-down-up-down-down motif of helices, in which the connections are 9–14 residues long; and a C-terminal domain is found between residues 785 and 1,010, which includes the NAD^+ binding site. The core of this region consists of a five-stranded antiparallel β-sheet and a four-stranded mixed β-sheet. These two consecutive sheets are connected by a single pair of hydrogen bonds. The central β-sheets are surrounded by five α-helices, three 310-residue helices, and a three- and a two-stranded β-sheet in a 37-residue excursion between two central β-strands. **b** | A ribbon representation of the chicken PARP1-CF (terminal end amino-acids 662–1,014), which was co-crystallized with the NAD analogue carba-NAD. The diagram shows the interaction of carba-NAD (an inhibitor substrate analogue) with the NAD^+-binding site of PARP-CF. The observed bound ADP moiety of carba-NAD is shown and denotes the acceptor site. **c** | The structure of carba-NAD. The ring oxygen of the nicotinamide ribose is replaced by a methylene group, which prevents ADP-ribosyl transfer and hydrolysis of the nicotinamide moiety by cleavage of the β-glycosidic bond. Adapted with permission from REF. 114 © (2003) American Society of Experimental Pharmacology and Therapeutics.

PARP and DNA repair

PARP1 has been implicated in DNA repair and the maintenance of genomic integrity[2–4,11,12]. This 'guardian angel' function of PARP is evidenced by delayed DNA base-excision repair (BER) and the high frequency of sister chromatid exchange in the absence of functional PARP1 after exposure to ionizing radiation or alkylating agents[13].

Multiple mechanisms are involved in the regulation by PARP of the DNA BER pathway. Dantzer reported that lysates from PARP1-deficient fibroblasts had no long-patch-repair activity and showed reduced short-patch-repair activity[14]. PARP1 interacts with multiple nuclear components of the single-strand break repair (SSBR) and BER complexes, including the nick-sensor DNA ligase III, the adaptor factor XRCC1 and DNA-repair effectors, such as DNA polymerase-β and DNA ligase III. PARP1 cooperatively interacts with PARP2 in some of these functions[3,12]. The following steps are involved in the process: first, the sensing of the DNA break; second, the translation and amplification of the damage signal, the poly(ADP-ribosyl)ation of PARP1 itself (automodification) and of histones H1 and H2B (heteromodification), the triggering of chromatin-structure relaxation and increasing the access of DNA-repair enzymes to the break; third, the recruitment of XRCC1 to the damaged site (mediated through its poly(ADP-ribosyl)ation), followed by the assembly of the SSBR repair complex; fourth, end processing, whereby polynucleotide kinase that is stimulated by XRCC1 converts the DNA ends to 5′-phosphate and 3′-hydroxyl moieties; fifth, gap filling, during which DNA polymerase that is stimulated by PARP1 fills the gap; and sixth, the ligation step that is catalysed by DNA ligase III.

A further DNA-repair pathway that is implicated in conjunction with PARP involves the Cockayne syndrome-B protein (CSB)[15], which accelerates the repair by increasing the accessibility of packaged DNA. This process is triggered by the direct poly(ADP-ribosyl)ation of histones and chromatin decondensation. PARP and CSB cooperate in the opening of the chromatin, which is triggered by stalled transcription machinery, thereby permitting the more rapid repair of DNA modifications. PARP also interacts with the Werner syndrome nuclear protein (WRN), which possesses 3′–5′ exonuclease and ATPase-dependent 3′–5′ helicase activities. PARP1, but not PARP2, interacts with WRN, thereby inhibiting both of its activities[16].

Another role of PARP in DNA repair relates to the regulation of the topological state of DNA and, therefore, the activity of DNA topoisomerase I. This enzyme has essential roles in controlling the level of DNA supercoiling and relieving the torsional stress that is generated during replication, transcription, recombination and chromatin remodelling. Topoisomerase I sometimes gets trapped in a covalent complex with nicked DNA through the 3′-phosphate terminus, which inhibits its DNA-ligase activity. If unrepaired, this can lead to genomic instability or apoptosis. Camptothecins, which are a prominent class of anticancer drugs that are also known as topoisomerase I poisons, act through the stabilization of topoisomerase I-cleavage complexes. ADP-ribose polymers target specific domains of topoisomerase I, and induce the enzyme to remove itself from cleaved DNA and close the resulting gap[17,18].

PAR also serves as a supply of energy in the DNA-ligation step[19]. Extensive poly(ADP-ribosyl)ation (and cytosolic NAD^+ depletion) might therefore be viewed as pathological overactivation of a physiological mechanism that normally serves to transfer cytoplasmic energetic pools to nuclear energetic pools to support DNA repair.

How important is the DNA-repair function of PARP1 in normal cellular functions? This question is hard to address for two main reasons. First, the literature is conflicting, even in the context of BER, and several studies have failed to show consistent differences in DNA repair between wild-type and PARP1-deficient systems, or after treatment with PARP inhibitors[20–22]. The importance of PARP1 might be dependent on the experimental model that is used, the cell type, and the type and degree of DNA damage. Second, PARP1-deficient mice seem to be healthy, have a normal lifespan and do not develop spontaneous tumours. This is consistent with the hypothesis that PARP1 has a relatively minor role in DNA-repair pathways under normal conditions — after all, it is not a DNA-repair enzyme *per se*. Considering the cooperative actions of PARP1 and PARP2 in DNA-repair processes, double-deficient models might be deemed appropriate for the study of DNA repair or tumorigenesis. However, this approach is not possible, as PARP1/PARP2 double-knockout embryos die[12]. Another way of addressing this question is to investigate radiation-induced DNA alterations and/or induced or spontaneous tumorigenesis in PARP1-deficient mice. Studies in this direction have yielded conflicting results. PARP1-deficient mice that were placed on a p53-knockout background or treated with oral carcinogens yielded variable findings, which ranged from an enhancement of the frequency of induced tumours to a reduction in carcinogenesis[23–28].

Importantly, the results that are obtained from studies using PARP-deficient experimental systems usually do not distinguish between findings that are related to the physical absence of the enzyme (its 'scaffolding' function) and those owing to the lack of catalytic activity (its 'enzymatic' function). Therefore, the results obtained with PARP-deficient cell lines do not necessarily mirror those seen in cells that are treated with PARP inhibitors. For example, although PARP-deficient cells have disturbed cell-cycle progression and establish a tetraploid population, similar findings are not seen in wild-type cells that are treated with the PARP inhibitor GPI-6150 (REFS 29,30).

Further studies are required to clarify the physiological importance of PARP1 in the maintenance of genomic integrity and DNA repair. Overall, it seems to have more important roles in DNA repair under stress conditions (such as radiation, carcinogen exposure or exposure to certain antitumour drugs) than in normal (non-stressed) cells.

PARP, signal transduction and gene expression

A physiological role of PARP, which is also relevant from pathophysiological and therapeutic viewpoints, is its ability to regulate transcription. Three principal mechanisms are involved: first, the effects of PARP on histones, which are followed by changes in the chromatin structure; second, the regulation of DNA methylation by PARP; and third, the participation of PARP in enhancer/promoter-binding complexes.

The first two processes represent more generic regulatory mechanisms. In the first, poly(ADP-ribosyl)ation confers negative charge to histones, which leads to electrostatic repulsion from the DNA. Poly(ADP-ribosyl)ation also loosens the chromatin and makes various genes accessible to the transcriptional machinery. Additionally, polyanionic PAR — either attached to protein substrates or as free polymer — serves as a local matrix for core histones that are released from destabilized NUCLEOSOMES[31]. In the second mechanism, PARP1 binds to DNA methyltransferase-1, which is a key enzyme in DNA methylation and a global regulator of gene expression, and thereby inhibits its catalytic function[32].

Many functional interactions between PARP1 and various nonhistone proteins have been described. For instance, PARP acts as a co-activator in nuclear factor-κB (NF-κB)-mediated transcription[32–35]. There is no consensus in the literature as to whether the modulation of NF-κB-mediated transcription by PARP is dependent on the catalytic activity of the enzyme or, alternatively, on its physical presence[32–35]. These interactions are dependent on the cell type, the model system and the nature of the stimulus that is used. The PARP inhibitor PJ-34 suppresses NF-κB-mediated

NUCLEOSOME
A packing unit for DNA within the cell nucleus, which gives the chromatin a 'beads-on-a-string' structure, in which the 'beads' consist of complexes of nuclear proteins (histones) and DNA, and the 'string' consists of DNA only. A histone octamer forms a core around which the double-stranded DNA helix is wound twice.

gene expression in immunostimulated macrophages[36], but exerts variable effects in endothelial cells that are placed into elevated extracellular glucose[37,38]. Although NF-κB-dependent transcriptional activation is usually promoted by PARP1, there are also examples of NF-κB-target genes the expression of which is silenced by PARP1 (REF. 39).

Further mechanisms that are involved in the regulation of NF-κB activation by PARP might include direct poly(ADP-ribosyl)ation[4,31] as well as the regulation by PARP1 of the expression levels of histone acetyltransferases, p300 and cAMP response element-binding protein (CREB)-binding protein (CBP). The expression of these factors is reduced in PARP1-deficient cells. As p300 and CBP are coactivators of NF-κB, PARP1 might participate in NF-κB-dependent transcription by maintaining the expression of histone acetyltransferases[40]. The inhibition of NF-κB activation in the absence of functional PARP1 has also been shown in murine models of myocardial reperfusion injury and colitis[41,42].

PARP1 is also required for the activation of other inflammation-related transcription factors, such as activator protein-1 (AP1), AP2, transcription-enhancer factor-1 (TEF1), *trans*-acting transcription factor-1 (SP1), octamer-binding transcription factor-1 (Oct1), yin-yang-1 (YY1) and signal transducer and activator of transcription-1 (STAT1)[4,31]. There might be several mechanisms underlying these effects. One mechanism that could be involved in these regulatory functions relates to the role of PARP1 as a transcriptional coactivator, stimulating (or sometimes inhibiting) the activity of DNA-binding transcription factors. PARP1 might be specifically recruited to target promoters through interactions with DNA-binding factors. In other contexts, PARP1 might contact enhancer or promoter DNA directly (for example, by recognizing certain DNA structures or sequences)[4,31].

Several other signal-transduction pathways are affected by PARP under specific conditions (FIG. 2). For example, the cytoprotective AKT/PKB pathway is regulated by PARP: a pro-inflammatory stimulus and the absence of the catalytic activity of PARP are both required for the phosphorylation (activation) of AKT[43]. The pharmacological inhibition of PARP attenuates the activation of the endotoxin-induced extracellular signal-regulated kinase (ERK)1/2 and p38 mitogen-activated protein (MAP) kinase in a tissue-specific manner[44]. Interaction between PARP and MAP kinase has also been reported: the PARP inhibitor INH2BP suppresses endotoxin-induced MAP kinase activation in cultured macrophages[45]. In PARP-deficient glial cells, the downregulation of the inflammatory response is linked to a reduction in the MAP kinase-mediated phosphorylation of activating transcription factor 2 (ATF2) and CBP[46]. The regulation of PKC activation by PARP in endothelial cells that are placed in elevated extracellular glucose conditions involves an indirect mechanism, through the poly(ADP-ribosyl)ation and inhibition of GAPDH[38].

The identification of PARP-regulated genes has been achieved using DNA-chip technology. In wild-type versus PARP1-deficient fibroblasts, 91 out of 11,000 genes were found to be differentially expressed[47], which confirmed the role of PARP1 as a basal transcriptional regulator. In endotoxin-stimulated glial cells, the expression of interleukin-6 (IL-6), pro-IL-1, intercellular cell-adhesion molecule 1 (ICAM1), tumour necrosis factor-α (TNFα), inducible cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS) was reduced in the absence of PARP1 (REF. 48). In endothelial cells, 18 PARP1-dependent early-response genes to TNFα have been identified[39]. In murine myocardial infarction, the expression of several AP1-dependent genes of proinflammatory mediators and heat-shock proteins is altered by PARP1 deficiency[49].

The PARP-mediated expression of genes might have physiological purposes during development or the physiological inflammatory response. When these processes over-amplify themselves, positive-feedback cycles of inflammation and organ damage are initiated. PARP inhibitors can downregulate the activation of multiple inflammatory pathways and suppress the production of various inflammatory mediators. This contributes to their therapeutic action in acute and chronic inflammatory diseases, as discussed later.

PARP and apoptosis

PARP has been implicated in both apoptosis and necrosis. Initial studies on PARP and cell death focused on the cleavage of PARP and its potential role in apoptosis. PARP is recognized by cell biologists as the 'death substrate', because PARP1 was one of the first identified substrates of caspases. During apoptosis, caspase 7 and caspase 3 cleave PARP1 into two fragments: p89 and p24. The cleavage of PARP1 separates its DBD from its catalytic domain, which inactivates the enzyme. PARP1 cleavage is more appropriately viewed as a marker of apoptosis, rather than an executor of the process[4,50]. The cleavage process is independent from the catalytic activity of PARP and is not influenced by pharmacological inhibitors of the enzyme[4]. In addition to caspases, the nuclear action of matrix metalloproteinase 2 has been shown to cleave PARP in myocytes[51]. $PARP^{+/+}$ and $PARP^{-/-}$ hepatocytes, thymocytes and primary neurons undergo similar amounts of apoptosis in response to Fas, TNFα, etoposide, dexamethasone and ceramide[52,53]. The normal development pattern that is seen in PARP1-deficient mice is indicative that PARP1 does not have an active role in apoptosis. In fact, PARP cleavage prevents the overactivation of PARP (and the PARP-dependent cellular energetic crisis) and thereby maintains cellular energy for certain ATP-sensitive steps of apoptosis[54,55].

PARP overactivation promotes cell necrosis

In addition to numerous biochemical and morphological similarities (for example, the role of the mitochondrial permeability transition) and differences between apoptosis and necrosis (for example, in DNA cleavage and the role of caspases), a distinctive feature

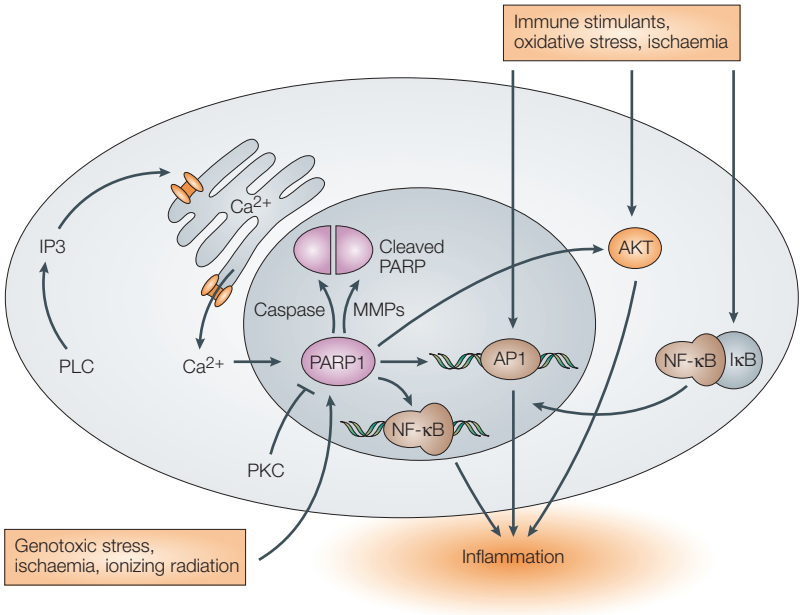


Figure 2 | **Activation and inactivation of poly(ADP-ribose) polymerase (PARP): interactions with specific signal-transduction pathways.** PARP can be catalytically activated by DNA strand breaks or by the inositol (1,4,5)-triphosphate (IP3)/calcium route. PARP can be auto-inactivated by auto-poly(ADP-ribosyl)ation (not shown) and can be cleaved into inactive fragments by caspases or matrix metalloproteinases (MMPs). The phosphorylation of PARP by protein kinase-C (PKC), through the inhibition of its DNA binding, also suppresses PARP activity. PARP, through physical interactions and/or catalytic actions, promotes activator protein-1 (AP1) and nuclear factor-κB (NF-κB)-dependent signal-transduction processes and can suppress the activation of the cytoprotective AKT pathway; therefore, the inhibition of PARP can suppress AP1 and NF-κB activation and induce AKT phosphorylation. The pro-inflammatory effects of PARP1 might be attributed to the facilitation of various signal-transcription pathways. PARP is involved in the general regulation of signal-transduction processes by interactions with histones and with DNA-methylation processes (not shown). IκB, inhibitor of NF-κB; PLC, phospholipase C.

of the latter is the disintegration of the plasma membrane (as opposed to the compaction of apoptotic cells, which is followed by elimination). The leakage of cell content from necrotic cells into the surrounding tissue exacerbates the inflammatory process. In many acute cardiovascular diseases, necrosis of the cells is the primary mode of cell death. Cell necrosis-associated markers are used clinically (for example, the plasma levels of creatine kinase or troponin released from necrotic myocytes are used as diagnostic markers of myocardial infarction). Data showing protection against cell necrosis caused by the inhibition or inactivation of PARP1 (REFS 4,56,57; TABLE 1) prove that not only apoptosis but also necrosis can be amenable to pharmacological interventions — alongside apoptosis, necrosis can be considered as the other form of regulated cell death.

The oxidant- and free-radical-mediated necrosis of pancreatic β-cells, neurons, thymocytes and other cell types can be prevented by PARP inhibitors or deficiency[4,56–61]. Nitric oxide, through the generation of the cytotoxic oxidant peroxynitrite (which is formed when nitric oxide reacts with superoxide), is one of the principal pathophysiological triggers of DNA injury and PARP activation[62,63].

The idea that PARP overactivation can lead to cell death was originally put forward by Berger[64,65]. In stroke, heart attack and other forms of reperfusion injury, the main steps of the process (as summarized in REFS 4,66,67) are as follows. First, the occlusion of a blood vessel prevents blood flow to the organ. Second, the restoration of the blood flow (spontaneously or in conjunction with a medical intervention, such as thrombolysis or angioplasty) triggers the generation of various oxidants and free radicals. Third, these species (such as hydrogen peroxide, hydroxyl radical and peroxynitrite) induce a range of oxidative and nitrosative injuries to the cells, including protein and lipid modifications, mitochondrial dysfunction and DNA damage (base modifications and DNA-strand breakage). Fourth, PARP senses the DNA breaks and becomes activated. Fifth, this process leads to the consumption of NAD, initially mainly from the cytosolic pool[68]. Sixth, the cell attempts to regenerate NAD from nicotinamide, which is converted to nicotinamide mononucleotide by phosphoribosyl transferase using phosphoribosyl pyrophosphate obtained from ATP (the latter is also necessary to metabolize nicotinamide mononucleotide into NAD^+ by mononucleotide adenylyl transferase). Seventh, PARP-activation-induced depletion of the cellular pyridine nucleotide pool impairs important NAD^+-dependent cellular pathways, including glycolysis and mitochondrial respiration. NAD^+ deficiency allows only the ATP-consuming part of anaerobic glycolysis to take place, thereby decreasing the synthesis of pyruvate and the mitochondrial formation of NADH. NADH-deficient mitochondria undergo depolarization, which converts the ATP synthase into an ATPase. Eighth, and finally, the ensuing cellular energetic starvation leads to the shutdown of energy-requiring processes (including those that are involved in the maintenance of membrane integrity, such as the membrane sodium/potassium ATPase), which leads to a breakdown of membrane potential and leakage of the membrane. These events promote futile cycles that, ultimately, lead to cell death through the necrotic route[4,69] (FIG. 3). This type of necrosis, coupled with the overproduction of PAR, can be seen, for example, in the ischaemic core area of the heart during myocardial infarction (FIG. 4).

The depletion of the cytosolic NAD^+ pool renders the cells unable to use glucose as a metabolic substrate. Under conditions in which glucose is the only available metabolic substrate, this is especially deleterious (for example, in the brain, where glucose is normally the only metabolic substrate that is transported rapidly across the blood–brain barrier). The restoration of cellular NAD^+ (with high extracellular concentrations of NAD^+ or tricarboxylic-acid-cycle substrates, such as α-ketoglutarate and pyruvate) can counteract PARP-mediated necrosis in oxidatively injured cells[68,70,71].

Positive-feedback cycles modulate the above processes. First, in diseases, PARP-mediated energetic depletion frequently occurs in oxidatively/nitrosatively injured cells, where many cellular functions are already adversely affected, the cellular antioxidant pool is

Table 1a | **PARP inhibition in animal models of inflammation, reperfusion, degenerative and vascular diseases**

Organ	Disease model	PARP activation confirmed?	PARP inhibitors	PARP1 deficiency protective?	Main results
Brain	Stroke	Yes	Benzamide, 3-AB, ISQ DPQ, PHT, INH2BP, GPI-6150, PJ-34, INO-1001, ONO-1924H, DR2313, G-PH	Yes (effective in male animals only)	Reduction in necrosis of the neurons, improvement in neurological status, protection against white-matter damage and AIF translocation
	Traumatic brain injury	Yes	3-AB, GPI-6150, PJ-34, INO-1001	Yes	Improved neurological status
	Parkinson's disease	Yes	GPI-6150, ISQ, F-Q	Yes	Improved neurological outcome and dopamine loss
	Meningitis	Yes	3-AB	Yes	Improved survival, improved neurological status and reduced inflammatory mediator production
	Hypoglycaemia	Yes	ISQ	Not tested	Improved survival and behavioural status
Heart	Myocardial infarction	Yes	3-AB, NA, ISQ, GPI-6150, 5-AIQ, PJ-34, INO-1001	Yes	Reduced myocardial necrosis, reduced infarct size, improved myocardial contractility, reduced inflammatory mediator production and reduced neutrophil infiltration
	Cardiopulmonary bypass	Yes	PJ-34, INO-1001	Not tested	Improved myocardial contractility and reduced remote injury (pulmonary and mesenteric)
	Transplantation	Yes	3-AB, PJ-34, INO-1001	Not tested	Improved myocardial contractility, reduced inflammatory mediator production, extension of transplant survival and synergy with cyclosporine treatment
	Ischaemic cardiomyopathy	Yes	PJ-34, INO-1001	Not tested	Improved myocardial contractility, improved survival
	Aortic banding-induced heart failure	Yes	INO-1001	Yes	Improved myocardial contractility, reduced hypertrophy and improved survival
	Diabetic cardiomyopathy	Yes	PJ-34, INO-1001	Not tested	Improved myocardial contractility
	Doxorubicin-induced myocardial failure	Yes	PJ-34, INO-1001	Yes	Improved myocardial contractility and no reduction in the antitumour effect of doxorubicin
	Ageing-associated heart failure	Not tested	PJ-34, INO-1001	Not tested	Improved myocardial contractility
Vasculature	Diabetic endothelial dysfunction	Yes	PJ-34, INO-1001	Yes	Prevention or reversal of diabetic endothelial dysfunction
	Hypertension	Yes	PJ-34	Not tested	Protection against the development of endothelial dysfunction
	Ageing	Yes	PJ-34, INO-1001	Not tested	Protection against the development of endothelial dysfunction
	Balloon angioplasty	Yes	PJ-34	Yes	Reduced hypertrophy and improved endothelium-dependent relaxation
	Endothelial injury by homocysteine or AII	Yes	PJ-34	Yes (for AII)	Protection against the loss of endothelium-dependent relaxation
Lung	Interstitial pulmonary fibrosis	Not tested	NA	Not tested	Combined treatment with taurine and niacin almost completely ameliorated bleomycin-induced increases in lung collagen accumulation
	ARDS	Yes	Benzamide, PJ-34	Yes	PARP inhibition suppresses endotoxin-induced pulmonary damage, reduces inflammatory cell accumulation and inflammatory mediator production and NMDA-receptor-mediated lung oedema formation
	Hyperoxic lung injury	Yes	Not tested	Yes	Improved survival in PARP1-deficient mice exposed to hyperoxia
	Ovalbumin-induced asthma	Yes	3-AB, 5-AIQ, PJ-34	Yes	Reduced inflammatory mediator production and improved pulmonary function

Table 1b | **PARP inhibition in animal models of inflammation, reperfusion, degenerative and vascular diseases**

Organ	Disease model	PARP activation confirmed?	PARP inhibitors	PARP1 deficiency protective?	Main results
Endocrine pancreas	Diabetes	Yes	NA, 3-AB, INH2BP	Yes (some conflicting data)	Protection against necrosis of islets and reduction in the degree of hyperglycaemia
GI tract	Colitis	Yes	3-AB, NA, INH2B, PJ-34, 5-AIQ, ISQ, GPI-6150, INO-1001	Yes (also antisense against PARP2 is effective)	Improved survival, protection against gut shortening, reduced neutrophil infiltration, lipid peroxidation, nitrosative damage and ICAM1 expression, and attenuation of inflammatory markers
	Mesenteric I/R injury	Yes	3-AB, NA, GPI-6150, PJ-34, INO-1001	Yes	Protection against histological damage, neutrophil infiltration and mucosal barrier failure
Joint	Arthritis	Not tested	Nicotinamide, 3-AB, PJ-34	Yes (unpublished)	Reduced arthritis severity and incidence, and synergistic inhibition of arthritis by thalidomide and PARP inhibition
Skeletal muscle	Reperfusion injury	Not tested	3-AB	Not tested	Reduction in reperfusion injury necrosis markers
Kidney	Reperfusion injury	Yes	Benzamide, 3-AB, 5-AIQ	Yes	PARP inhibitors accelerate the recovery of normal renal function after I/R injury
	Transplantation	Yes	PJ-34	No	No effect on the function of the transplanted kidney
Liver	Acetaminophen toxicity	Yes	3-AB, PJ-34, 5-AIQ	No	Reduced hepatic necrosis with some agents (I-AR and PJ-34)
	I/R	Yes	PJ-34	Yes	Improved survival, reduced hepatic necrosis and protection against hepatic leukostasis
Eye	Uveitis	Not tested	PJ-34	Not tested	Protection from leukocyte migration
	Retinal I/R	Not tested	3-AB	Not tested	Reduced I/R damage to the retina
	Diabetic retinopathy	Yes	PJ-34	Yes (unpublished)	Inhibition of the development of acellular capillaries and ghosts, prevention of inflammatory response and reduced leukostasis
	Optic nerve transection	Yes	3-AB	Not tested	Inhibition of secondary retinal ganglion cell death
Ear	Cochlear I/R injury	Not tested	3-AB	Not tested	Improvement of cochlear function
	Acoustic trauma	Not tested	3-AB, NA	Not tested	Improvement of cochlear function
Skin	Sulphur mustard-induced vesication	Not tested	3-AB, NA	Not tested	Protection against NAD^+ depletion and microvesicle formation
Peripheral nerve	Diabetic neuropathy	Yes	ISQ, PJ-34	Yes	Protection against loss of motor and sensory nerve conductance
	Cavernous nerve injury	Yes	INO-1001	Not tested	Improved erectile function
Many organs	Haemorrhagic, endotoxic and septic shock	Yes	3-AB, NA, PJ-34, INH2BP, GPI-6150, 4-OHQ	Yes	Protection against haemodynamic decompensation, improved survival, protection against gut hyperpermeability and myocardial, vascular, hepatic and renal failure, and reduced inflammatory mediator production
	Thoracoabdominal I/R	Yes	PJ-34	Yes	Improved survival and neurological status
	HIV	Not tested	Benzamides, benzopyrones and INH2BP	Yes	PARP deficiency reduces viral replication and integration, although the data are conflicting, especially with pharmacological PARP inhibitors

3-AB, 3-aminobenzamide; 4-OHQ, 4-hydroxyquinazoline; 5-AIQ, 5-aminoisoquinolinone; AII, angiotensin II; AIF, apoptosis-inducing factor; AmNAP, 4-amino-1,8-naphthlalimide; ARDS, acute respiratory distress syndrome; DPQ, 3,4-dihydro-5-[4-1(1-piperidinyl)butoxy]1(2H)-isoquinolinone; DR2313, 2-methyl-3,5,7,8-tetrahydrothipyrano[4,3-d]pyrimidine-4-one; F-Q, Fujisawa's quinazolin-4(3H)-one derivative; GI, gastrointestinal; G-PH, Guilford's novel aza-5[H]-phenanthridin-6-one derivative; GPI-6150, 1,11b-dihydro-[2H]benzopyrano[4,3,2-de]isoquinolin-3-one; INH2BP, 5-iodo-6-amino-1,2-benzopyrone; INO-1001, Inotek's isoindolinone-derivative; I/R, ischaemia/reperfusion; ISQ, 1,5 dihydroxyisoquinoline; NA, nicotinamide; NMDA, *N*-methyl-D-aspartate; ONO-1924H, compound 3; PHT, 6(5H)-phenanthridinone; PJ-34, *N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N*,*N*-dimethylacetamide. For further discussion of the results summarized here, see main text and REFS 4,78 and the tables therein.

diminished and energetic processes do not function perfectly. Second, the poly(ADP-ribosyl)ation of various proteins by over-activated PARP can also set the stage for unexpected biochemical processes (for example, the inhibition of GAPDH)[38]. Third, mitochondrial injury promotes the release of mitochondrial cell-death factors (such as the release of apoptosis-inducing factor (AIF))[72–74]. The AIF-related mechanisms have received much attention recently. AIF released from a mitochondrion shuttles to the nucleus and induces peripheral chromatin condensation, large-scale fragmentation of DNA and, ultimately, cytotoxicity. Neutralization of AIF has been shown to exert neuroprotective effects in neuro-injury models *in vitro*[72–74]. The exact mechanisms by which PARP activation triggers AIF release have not yet been delineated.

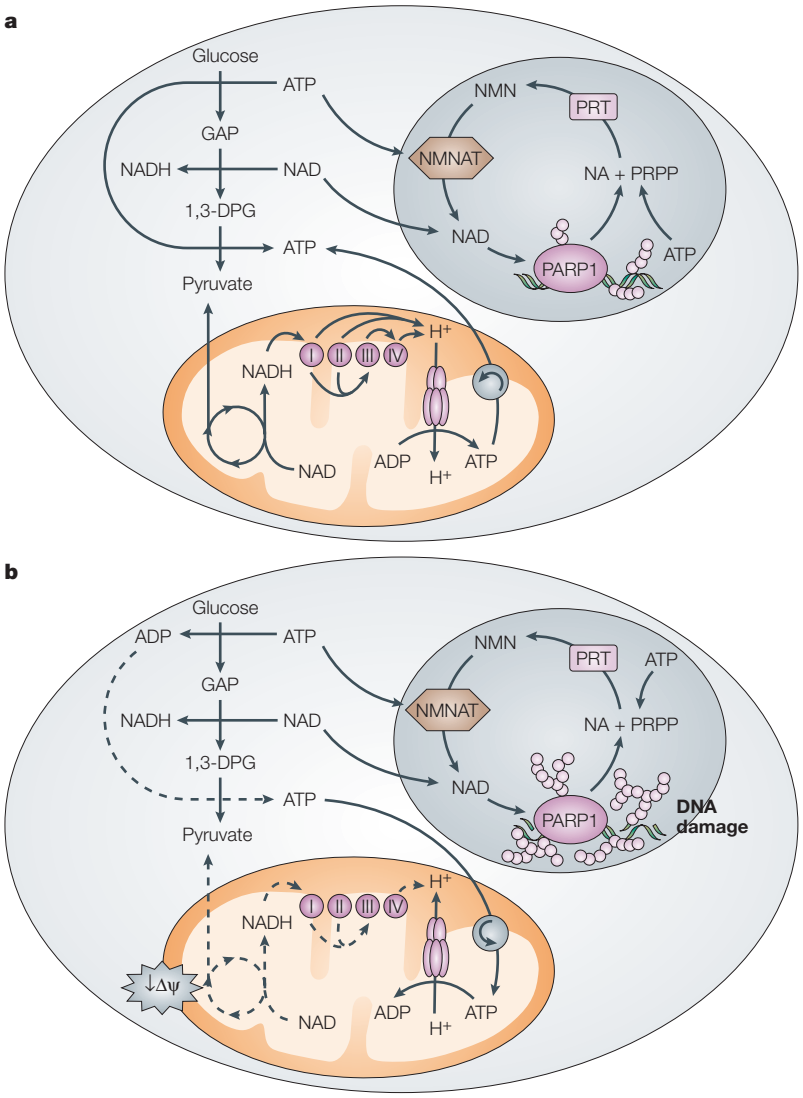


Figure 3 | **Pathways involved in promoting cellular necrosis in response to massive poly(ADP-ribose) polymerase (PARP) activation in oxidatively/nitrosatively injured cells. a** | The metabolic transformation of glyceraldehyde-3-phosphate (GAP) into 1,3-diphosphoglycerate (1,3-DPG) requires NAD^+ and fuels the ATP-producing part of anaerobic glycolysis, which leads to the synthesis of pyruvate. In the presence of NAD^+ and pyruvate, the Krebs cycle supplies NADH to the respiratory chain, which triggers electron flux among respiratory complexes. Mitochondrial respiration generates a proton gradient across the inner membrane that is used by ATP synthase. **b** | When PARP1 is activated on a large scale, it metabolizes NAD^+ into polymers of ADP-ribose and nicotinamide, with the consequent depletion of the pyridine nucleotide pool. Therefore, NAD^+-dependent cellular metabolic pathways, such as anaerobic glycolysis and mitochondrial respiration, are impaired. NAD^+ resynthesis uses additional cellular energy: nicotinamide is converted to nicotinamide mononucleotide (NMN) by phosphoribosyl transferase (PRT), which uses phosphoribosyl pyrophosphate (PRPP) that is obtained from ATP. ATP is also necessary to convert NMN into NAD^+ by mononucleotide adenylyl transferase (NMNAT). Overall, the cellular energetic depletion leads to a loss of cellular functions, dissipation of membrane potential ($\Delta\psi$) and cell death through the necrotic route. Adapted, with permission, from REF. 69 © (2002) Elsevier Science.

Both the release of AIF and the poly(ADP-ribosyl)ation of GAPDH might be triggered by free PAR oligomers that have been split off from poly(ADP-ribosyl)ated proteins through the action of PARG[38,74]. Some of the above processes must also be re-evaluated in the context of recent observations concerning the functional role of an intramitochondrial version of PARP[75–77].

TABLE 1 summarizes some of the diseases in which PARP inhibition or deficiency provides therapeutic benefit *in vivo*. Comprehensive reviews of previous pharmacological studies showing the cytoprotective effects of PARP inhibitors of various structural classes *in vitro* and the therapeutic effects of PARP inhibitors *in vivo* are presented in REFS 4,78 and the tables therein. In addition to various reperfusion diseases of isolated organs, recent preclinical data point to a role of PARP in systemic ischaemia–reperfusion (haemorrhagic shock)[79] in reperfusion injury and systemic inflammation that is induced by thoraco-abdominal aortic aneurysm repair[80], as well as in an experimental model of cavernous nerve injury which models the consequences of radical prostate surgery[81].

Moderate PARP activation decreases the cellular NAD^+ content and impairs cellular functions without killing the cells. At this stage (which, in conjunction with septic shock and systemic inflammatory responses, is referred to as 'pre-necrosis' or 'cytopathic hypoxia'), reversible suppression of cellular oxygen use and reversible cellular dysfunction is observed[82]. By restoring cellular energetics the pharmacological inhibition of PARP facilitates the recovery of cells from this dysfunctional state. Examples of such restorative responses were found in endothelial cells that produced high levels of endogenous oxidants[83] and in intestinal epithelial cells from colitic guts[84].

In vivo, the role of PARP in regulating apoptosis and necrosis in disease is more complex, because certain cell types undergo apoptosis whereas others undergo necrosis. PARP inhibition can differentially affect these processes; for example, in autoimmune diabetes, PARP inhibition prevents oxidant-mediated β-cell necrosis, but promotes the apoptosis of islet-infiltrating lymphocytes[85].

Regulation of cell survival and death by PARP

For a long time, there was controversy over the role of PARP in DNA-damage signalling and, especially, in DNA-damage-induced cell death. The two sides of the argument viewed PARP1 either as an indispensable cellular survival factor or as an active mediator of cell

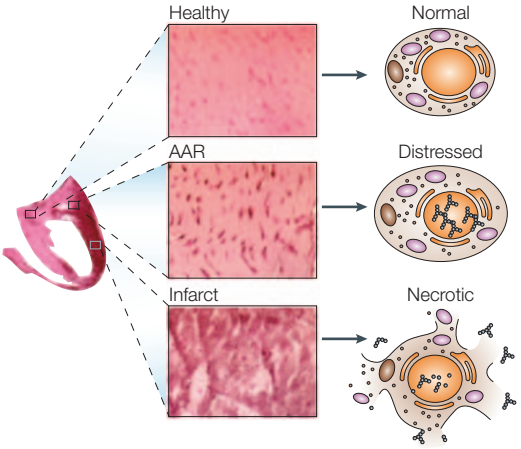


Figure 4 | **Poly (ADP-ribose) polymerase (PARP) and myocardial infarction.** Immunohistochemical localization of PARP activation in the reperfused myocardium and relationship to the status of the cell (normal cells in the healthy myocardium, severely distressed cells in the area at risk (AAR) or necrotic cells in the zone of the infarction). Poly(ADP-ribose) (PAR) formation, an indicator of PARP activation, as determined in whole heart sections (top) from rats exposed to 1 h ischaemia and 23 h reperfusion. A massive staining is evident in the left ventricular free wall of control animals. Microscopically, the normal, non-ischaemic myocardium from the interventricular septum shows no sign of PARP activation, whereas in the ischaemic myocardium, PARP activation is mainly located in the nuclei of myocytes in the peri-infarction zone. In the infarcted myocardium, severe architectural alterations coexist with a more diffuse pattern of PAR staining, due to necrosis of the myocytes and spillage of PAR-containing material into the extracellular space. Adapted from REF. 87.

death. In 2002, Virág and Szabó proposed a unifying concept to integrate previous results[4]. According to this theory, cells that are exposed to DNA-damaging agents can enter three pathways based on the intensity of the stimulus (FIG. 5). In the first pathway, PARP1 activated by mild to moderate genotoxic stimuli facilitates DNA repair, at least partly by interacting with DNA-repair enzymes such as XRCC1 and DNA-dependent protein kinase (DNA-PK). As a result, DNA damage is repaired and cells survive without the risk of passing on mutated genes (a version of this response occurs in cells that are exposed to certain types of anticancer agent as discussed below). In the second pathway, more severe DNA damage induces apoptotic cell death, with caspases inactivating PARP1; this eliminates cells with severe DNA damage. The third pathway can be induced by extensive DNA breakage caused by oxidative or nitrosative stress. The overactivation of PARP depletes the cellular stores of its substrate NAD^+ and, consequently, ATP. The severely compromised cellular energetic state prevents the apoptotic cell-death pathway from functioning. Under these conditions, pharmacological PARP inhibition or the absence of PARP in PARP-deficient mice preserves cellular ATP and NAD^+ pools in oxidatively stressed cells and thereby allows them to either function normally or die by apoptosis (which is preferable to necrosis)[4,69,78]. Therefore, cells with mild repairable DNA damage, as well as those that are severely damaged, are diverted by PARP inhibition to a common pathway (pathway 2), which results in the elimination of cells by apoptosis. The inhibition of PARP in severely injured cells (which would normally undergo necrosis) preserves ATP for the apoptotic system and either prevents cell death or enables it to occur through apoptosis instead of necrosis (FIG. 5).

PARP activation in reperfusion injury

PARP activation contributes to various forms of reperfusion injury in the brain, heart, kidney, gut, liver and other organs. Selected ischaemia–reperfusion injury models in which PARP has a pathogenic role are outlined in TABLE 1 and are reviewed in REFS 4,69,78.

Two organs in which PARP-mediated reperfusion injury has been intensively investigated are the brain[66] and heart[67]. In both of these organs, treatment with pharmacological inhibitors of PARP and PARP1 deficiency reduce the infarct size and improve the functional outcome variables (such as neurological function and myocardial contractility)[4,66,67]. In the acute phase of the disease, the preservation of cellular energetic pools (and thereby the inhibition of parenchymal cell necrosis) is the mode of protection. Additionally, in the delayed stage of the disease, PARP inhibition prevents the activation of various inflammatory pathways.

Direct evidence of PAR accumulation in reperfused organs has been provided by the immunohistochemical detection of PAR polymers[86,87]. In normal parenchyma, low levels of PAR accumulation are seen, whereas in the ischaemic borderzone (the area of risk in the heart or the penumbra in the brain), PARP activation can be seen. These cells might be in a state in which PARP is activated and the cells are dysfunctional but not yet necrotic. In the core of the infarct, necrotic cell death occurs. The diffuse PAR immunostaining is consistent with the necrotic lysis of the cells and the spillage of poly(ADP-ribosyl)ated proteins into the extracellular space (FIG. 4). It has not yet been determined whether PAR or poly(ADP-ribosyl)ated proteins have extracellular signalling roles.

The roles of PARP, and its relationships with various parallel and inter-related pathways of cell injury, are presented in FIG. 6. Conditions that involve the heart and in which PARP activation has a pathogenic role include acute myocardial infarction, cardiopulmonary bypass, cardiac transplantation and chronic heart failure (TABLE 1).

Role of PARP in inflammation

The role of PARP activation and the protective effects of PARP inhibitors have also been demonstrated in various experimental models of inflammation, including acute inflammatory diseases (such as endotoxic shock), as well as chronic inflammation of the gut, joints and various other organs (TABLE 1). For instance, PARP inhibitors and/or PARP deficiency is effective in arthritis, colitis, pulmonary inflammation and systemic inflammatory diseases (circulatory shock). The severity of these inflammatory diseases is suppressed by PARP inhibitors

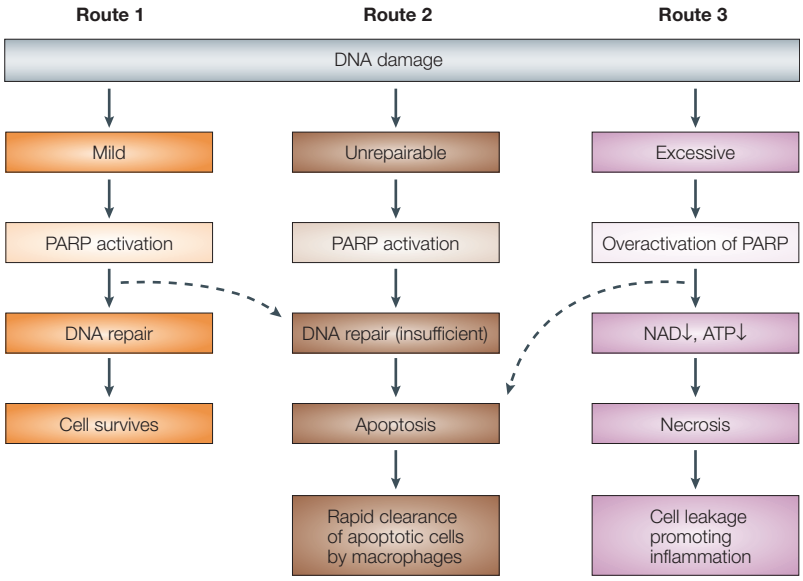


Figure 5 | **Intensity of DNA-damaging stimuli determines the fate of cells: survival, apoptosis or necrosis.** Depending on the intensity of the stimulus, poly(ADP-ribose) polymerase (PARP) regulates three different pathways. In the case of mild DNA damage, poly(ADP-ribosyl)ation facilitates DNA repair and, therefore, survival (route 1). More severe genotoxic stimuli activate an apoptotic pathway that eliminates cells with damaged DNA (route 2). The most severe DNA damage can cause excessive PARP activation, which depletes cellular NAD^+/ATP stores. NAD^+/ATP depletion blocks apoptosis and results in necrosis (route 3). The inhibition of PARP in cells entering route 1 suppresses repair and, therefore, diverts cells to route 2 (dashed arrow on the left). The inhibition of PARP in cells that are entering route 3 preserves cellular energy stores and allows the apoptotic machinery to operate (dashed arrow on the right).

and the production of multiple pro-inflammatory mediators is downregulated (TABLE 1). There is evidence for PARP activation in human samples with inflammatory disease — for instance, in the inflamed guts of colitic patients (Keshavarzian, Zsengeller & Szabo, unpublished observations). The inhibition of PARP also reduces the formation of nitrotyrosine — an indicator of reactive nitrogen species — in inflamed tissues. This finding was, at first, unexpected because PARP activation is perceived to occur downstream from the generation of oxidants and free radicals in various diseases. The mechanism is probably related to the fact that PARP inhibition reduces the infiltration of neutrophils into inflammatory sites[4,88]. This reduces oxygen- and nitrogen-centred free-radical production. The basis for the regulation by PARP of neutrophil infiltration might be related to the reduced expression of adhesion molecules[49,57] and/or the preservation of endothelial integrity[37,89].

Role of PARP in cardiovascular diseases

PARP activation also has a pathogenic role in hypertension, atherosclerosis and diabetic cardiovascular complications. In these diseases, the dysfunction of the vascular endothelium (that is, a reduction in the ability of the endothelial cell to produce nitric oxide and other cytoprotective mediators) is diminished. This sets the stage for many manifestations of cardiovascular disease. The oxidant-mediated endothelial cell injury is dependent on PARP and can be attenuated by pharmacological inhibitors or genetic PARP1 deficiency[37,89]. For instance, elevated extracellular glucose levels (as in diabetes) trigger PARP activation through the mitochondrial release of oxidants, which is followed by DNA-strand breakage[37,38]. In hypertension, angiotensin II acts on its endothelial receptors and upregulates NADPH oxidases. The subsequent generation of superoxide and peroxynitrite triggers DNA-strand breakage and PARP activation[90]. *In vivo* studies show that PARP inhibition improves endothelium-dependent relaxations in hypertensive and diabetic animals[37,89,91]. PARP activation is also involved in the early functional changes that are associated with atherosclerosis and vascular injury[92,93]. The preservation of vascular function might underlie the protective effect of PARP inhibitors against diabetic neuropathy and retinopathy[94,95]. In the context of diabetic complications, the mechanism of endothelial cell injury involves the mitochondrial overproduction of superoxide from mitochondrial complex III, followed by the generation of peroxynitrite; this activity, possibly together with oxygen-derived oxidants and free radicals (for example, hydrogen peroxide and hydroxyl radical), induces DNA-strand breakage that activates PARP. PARP activation leads to poly(ADP-ribosyl)ation and the inactivation of GAPDH[38], which stimulates four important pathways of diabetic complications: the hexosamine pathway, the advanced glycation end-products pathway, the PKC pathway and the aldose reductase pathway.

PARP inhibitors as adjunct anticancer agents

As mentioned previously, PARP has a role in BER, and PARP inhibitors can be used to suppress DNA repair and to promote apoptosis in cells that are treated with certain anticancer agents. The methylating agent temozolomide (TMZ) is used, among others, in the therapy of various central nervous system (CNS) tumours. TMZ resistance develops frequently and diminishes the clinical response. This phenomenon is often the result of the efficient repair of methyl adducts at the O^6 position of guanine mediated by O^6-alkylguanine DNA alkyltransferase (AGT) or functional defects of the mismatch-repair system (MR), which is required for the induction of DNA-strand breaks by O^6-methylguanine. The combination of PARP inhibitors with TMZ interferes with the repair of methylpurines. These do not usually contribute to the cytotoxicity that is induced by TMZ, because they are rapidly removed by the short-patch BER system. When PARP is inhibited, the recruitment-promoting functions of XRCC1 are impaired, which hampers strand rejoining; this leads to the generation of permanent single strand breaks that trigger the apoptotic process. *In vitro* and *in vivo* studies have shown that combining PARP inhibitors with TMZ markedly potentiates the anticancer effect of TMZ and restores the sensitivity of MR deficient tumours to TMZ[96–100].

PARP inhibitors have also been shown to enhance the cytotoxicity of the DNA topoisomerase I poisons irinotecan and topotecan. PARP1 interacts with, and promotes the activity of, topoisomerase

I. Poly(ADP-ribosyl)ated PARP1 and PARP2 counteract the action of camptothecin by facilitating the resealing of DNA-strand breaks[101,102].

The combination of doxorubicin and PARP inhibitors is especially effective in p53-deficient breast cancer lines[103]. In this context, PARP inhibition might be particularly advantageous, as it can also simultaneously prevent the cardiodepressant side effects of doxorubicin[104,105]. Another recently recognized use of PARP inhibitors could be in the

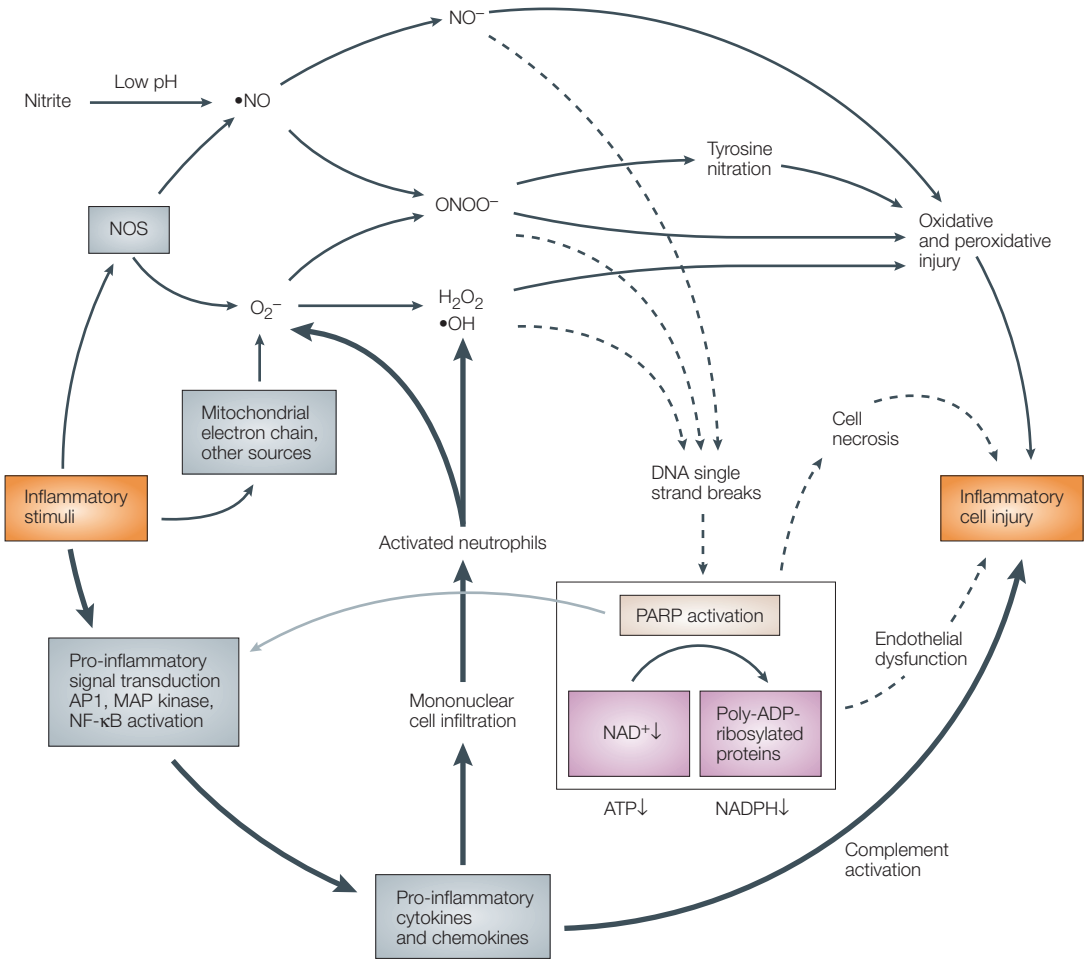


Figure 6 | **Pathophysiological triggers of poly(ADP-ribose) polymerase (PARP) activation and interacting pathways of injury.** The reduction of oxygen supply during ischaemia alters mitochondrial function, which leads to the production of reactive oxidant species. In heart failure, cardiotoxic drugs or inefficient working of the heart leads to the production of oxidants and free radicals from various sources (such as the mitochondria, xanthine oxidase or NADPH oxidase). During stroke, oxidant and free-radical generation is triggered primarily by *N*-methyl-D-aspartate (NMDA)-receptor activation. In inflammatory states, various pro-inflammatory pathways are stimulated in response to autoimmune responses and/or pro-inflammatory microbial components. The corresponding isoforms of nitric oxide synthase (NOS; brain NOS in the central nervous system, endothelial NOS in the cardiovascular system and inducible NOS under inflammatory conditions) produce NO (but under conditions of L-arginine depletion NOS can also produce superoxide). Under low pH conditions (such as tissue hypoxia/acidosis), nitrite can also be converted to NO. Superoxide (which is produced from various cellular sources, including mitochondria) and NO react to yield peroxynitrite. Peroxynitrite and hydroxyl radical induce single-strand breaks in DNA, which, in turn, activate PARP. This rapidly depletes the cellular NAD^+ and ATP pools. Cellular energy exhaustion triggers the further production of reactive oxidants. Depletion of NAD^+ and ATP leads to cellular dysfunction. Depletion of NADPH leads to reduced endothelial NO formation (endothelial dysfunction). The cellular dysfunction is further enhanced by the promotion of pro-inflammatory gene expression by PARP, through the promotion of nuclear factor-κB (NF-κB), activator protein-1 (AP1) and mitogen-activated protein (MAP) kinase activation. PARP can also promote complement activation. The oxidant-induced pro-inflammatory molecule and adhesion-molecule expression, along with the endothelial dysfunction, induce neutrophil recruitment and activation, which initiates positive-feedback cycles of oxidant generation, PARP activation and cellular injury. For instance, tissue-infiltrating mononuclear cells produce additional oxidants and free radicals. PARP is also involved in triggering the release of mitochondrial cell-death factors, such as apoptosis-inducing factor (AIF). There are many oxidative and nitrosative injury pathways that are triggered by oxygen- and nitrogen-centred oxidants and free radicals, which act in parallel or in synergy with PARP-mediated pathways of cell injury. The relative contribution of cell necrosis versus inflammatory cell injury, as well as the relative role of the various pathways shown in the figure, depends on the specific pathophysiological conditions in question.

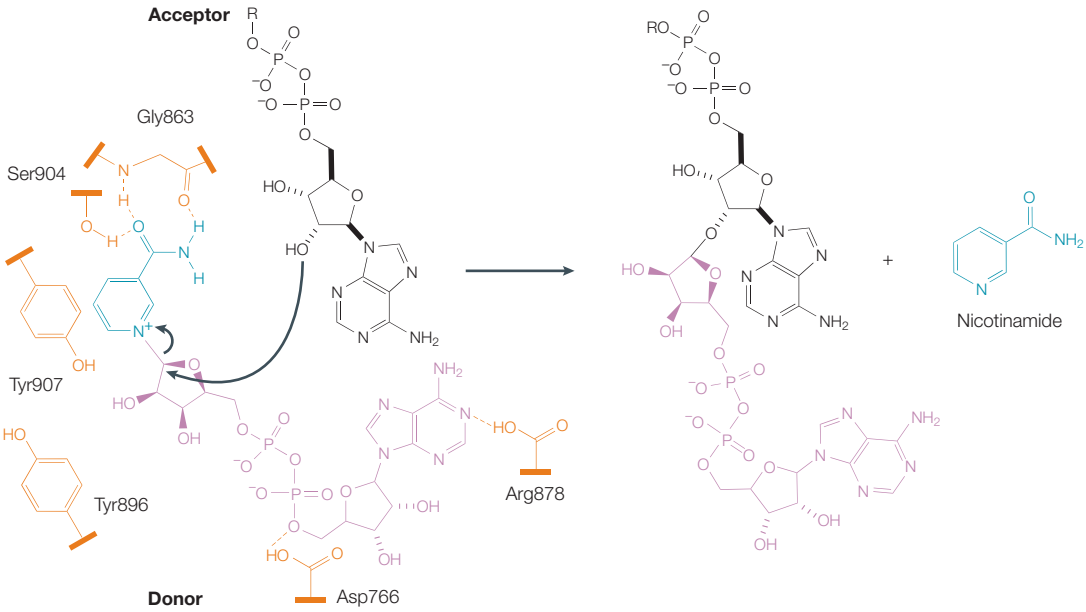


Figure 7 | **Schematic representation of the binding of NAD⁺ to poly(ADP-ribose) polymerase (PARP) protein and the catalytic mechanism of PARP1.** Some of the main amino-acid residues that are involved in the interaction with NAD⁺ are shown in gold. The curved arrows represent the nucleophilic attack of the 2′-hydroxyl of the adenosine ribose of the acceptor site and the release of the nicotinamide of the donor site. The enzyme becomes activated on binding to DNA breaks and uses NAD⁺ as substrate. PARP1 catalyses the cleavage of NAD⁺ into nicotinamide and ADP-ribose.

context of enhancing cancer-cell killing in cells deficient in homologous recombination. Two recent studies demonstrate that cells that are deficient in BRCA1 or BRCA2 are acutely sensitive to killing by PARP inhibitors *in vitro* and *in vivo*[169,170].

A final potential application of PARP inhibitors in cancer treatment might be related to the enhancement of the antitumour effects of radiotherapy[106]. The *trans*-dominant inhibition of PARP[107–109] and various classes of PARP inhibitors exert radiosensitizing effects[110]. The *in vivo* efficacy of PARP inhibition for radiosensitization has been recently shown by a preclinical study, which found that the intraperitoneal administration of the PARP inhibitor AG14361 enhanced the sensitivity of colon carcinoma subcutaneous xenografts to radiation therapy[99].

One concern relating to systemic treatment with PARP inhibitors is the impairment of DNA repair in normal cells, which might render them more susceptible to the toxic effects of chemotherapy or radiotherapy. Although TMZ works primarily against actively proliferating cells, the combination of TMZ and PARP inhibitors can be toxic to resting cells[99]. Systemic toxicity caused by the use of PARP inhibitors as enhancers of cancer therapy might, therefore, involve both self-renewing tissues (such as the gut epithelium and bone marrow) and, perhaps, differentiated resting cells. The question of whether their combination with PARP inhibitors will increase the side effects of the anticancer interventions (in addition to increasing their antitumour efficacy) will be addressed in upcoming clinical trials.

PHARMACOPHORE
The ensemble of steric and electronic features that is necessary to ensure optimal interactions with a specific biological target structure and to trigger (or to block) its biological response.

Pharmacological inhibition of PARP

Ruf and colleagues reported the first crystal structure of the catalytic fragment (CF) of chicken PARP both with and without the nicotinamide-analogue inhibitor 3,4-dihydro-5-methyl-isoquinolinone (PD 128763)[111]. Analysis of the co-crystal structure showed that the inhibitor interacts with PARP-CF through two hydrogen bonds between its lactam group and the peptide backbone of Gly863 and the oxygen atom of the side chain of Ser904, in the same manner as the nicotinamide moiety of NAD⁺ (FIG. 7). These results helped to determine the details of the inhibitor–enzyme interactions of four structurally unrelated PARP inhibitors that all bind in a similar manner to the nicotinamide portion of NAD⁺ (REF. 112). On the basis of structural information available from the crystal structures of PARP1-CF[111–114] and PARP2-CF[115], it is now clear that the majority of PARP inhibitors are based on the benzamide PHARMACOPHORE, which mimics the nicotinamide moiety of NAD⁺ and binds to the donor site of the protein. The amide group of these inhibitors invariably interacts with the backbone atoms of Ser904 and Gly863, whereas the aromatic ring presumably interacts through π–π interactions with Tyr907 and, to some degree, Tyr896, which lines the other face of the pocket and provides further binding energy. The binding interactions that were defined by these crystallography studies are in good agreement with the published structure–activity relationship (SAR) data for most potent PARP inhibitors. Ruf and colleagues[113] reviewed the C terminal of the chicken PARP1-CF, which was co-crystallized with carba-NAD (FIG. 1). The additional

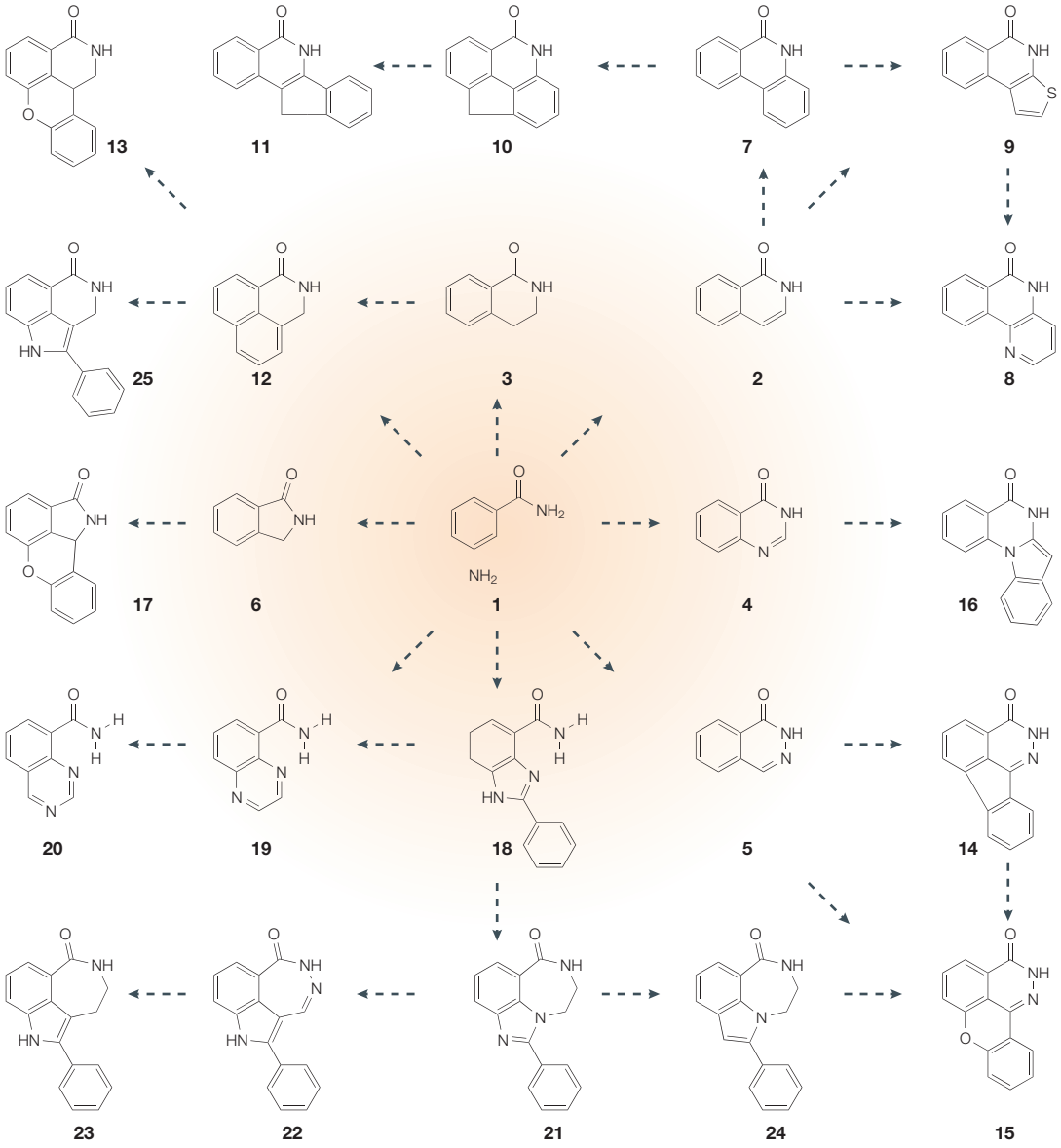


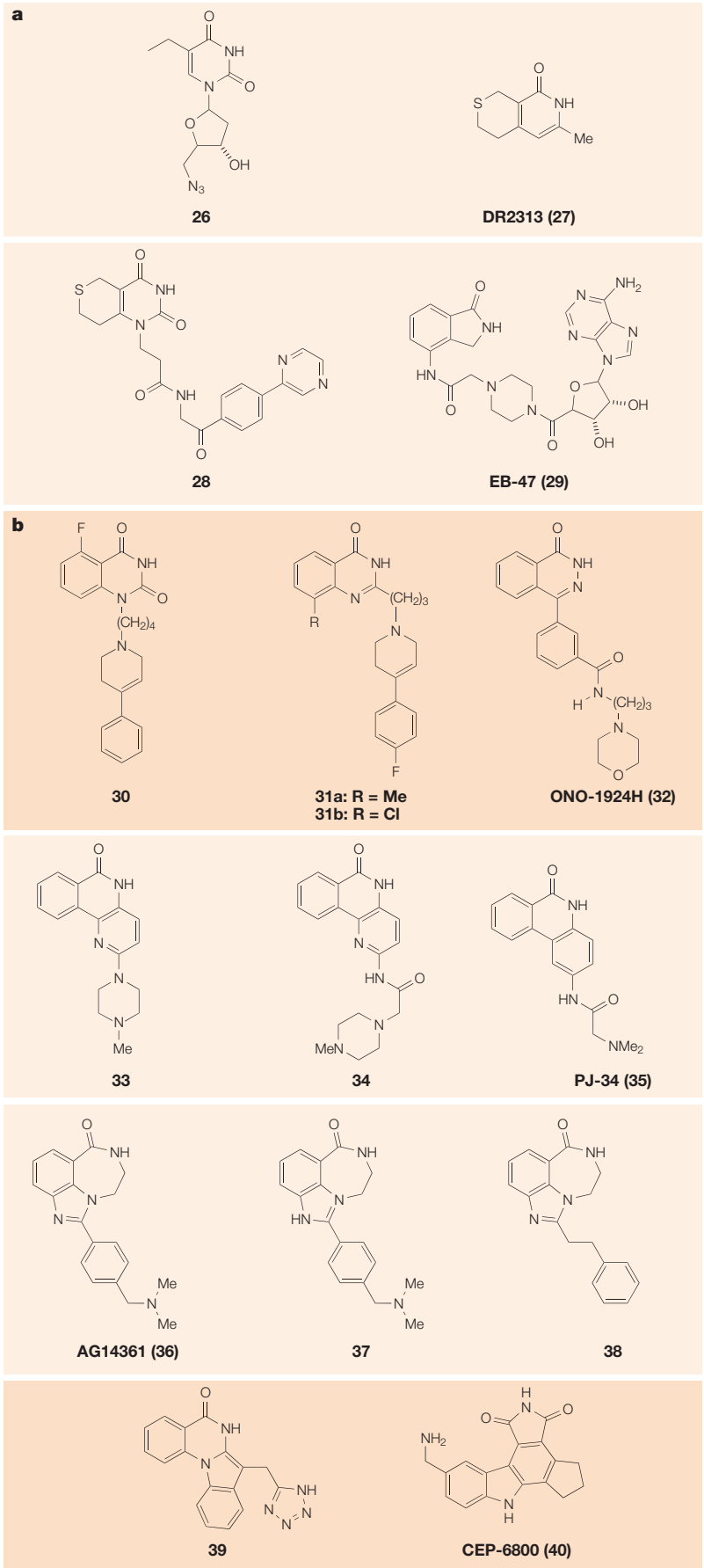
Figure 8 | **Structures of representative classes of poly(ADP-ribose) polymerase (PARP) inhibitors derived from the classical PARP scaffolds (benzamide or cyclic lactams).** Dotted arrows represent the structural similarity between corresponding scaffolds. Note the general presence of a benzamide pharmacophore in all core structures.

methylene group of carba-NAD, which replaces the ring oxygen of the nicotinamide riboside of NAD^+, prevents ADP-ribosyl transfer and the release of nicotinamide by cleavage of the β-glycosidic bond[113,114].

Fujisawa published the crystal structure of the catalytic domain of human recombinant PARP1 complexed with the inhibitor FR257517 (compound 31a)[116]. The quinazolinone part of the compound binds tightly to the nicotinamide–ribose binding site and the 4-fluorophenyl-tetrahydropyridine moiety provides secondary contacts to the adenosine-binding site. The hydrophobic 4-fluorophenyl ring of the inhibitor induces a significant conformational change in the active site of PARP by displacing the side chain of Arg878, which forms the bottom of the active site. Fujisawa also reported X-ray crystallography and molecular modelling results for the quinazolinedione-based PARP inhibitor (compound 30)[117]. The nitrogen atom of the tetrahydropyridine ring directly binds to the COOH of Asp766 and, similarly to FR257517, the tetrahydropyridine moiety of this compound induces a conformational change at the bottom of the adenosine-binding site by displacing Arg878. The terminal phenyl ring lies in a deep pocket and interacts via van der Waals interactions with the protein.

PARP inhibitor discovery

During the past decade, structure-based drug design and an increased understanding of the molecular details of the active site of PARP1 (FIG. 7) have facilitated the discovery of highly potent PARP inhibitors. The wide range of compounds originally published by



Banasik[118,119] has led to the identification of new templates for the design of novel PARP inhibitors[120–122]. Bi- and tricyclic PARP scaffolds (FIG. 8; compounds 2–5 and 7) described by Banasik were later optimized to enhance the potency, pharmacokinetics and water-solubility at desired pH values. More recent medicinal chemistry efforts have been directed at the invention of new cyclic carboxamide scaffolds, and have focused on the synthesis of tri- and tetracyclic PARP scaffolds (FIG. 8; compounds 8–17 and 21–25). We now have a good general understanding of the SARs of these benzamide-pharmacophore-based PARP inhibitors, which has led to the synthesis of highly potent novel inhibitors[121,122].

The strategy of cyclizing an open benzamide structure or creating a further ring system on the existing cyclic amide (that is, lactam) is one of the best approaches to designing new PARP inhibitors. All cyclic carboxamide derivatives that incorporate a carboxamide group in an *anti*- (or *cis*-) configuration[123] into a ring structure are considerably more effective than their open monocyclic amide analogues. Bicyclic PARP scaffolds comprising mainly isoquinolin-1(2H)-one (compound 2), 3,4-dihydro-isoquinolin-1(2H)-one (compound 3), quinazolin-4(3H)-one (compound 4) and phthalazin-1(2H)-one (compound 5) have undergone extensive elaboration to achieve better PARP-inhibitory activity, including highly potent tri- and tetracyclic PARP scaffolds (FIG. 8; compounds 8–17 and 21–25).

Some groups have explored alternative ways in which to inhibit PARP1 activation by using nucleic-acid and nucleoside derivatives[124,125] or have linked them to carboxamide pharmacophores[126] (FIG. 9). Bayer patented several fused uracil derivatives; one β-alanine-glycine linked fused uracil derivative (compound 28) was identified as a potent PARP inhibitor[125]. Inotek produced a series of isoindolinone derivatives that were linked to adenosine through various spacers[126]. The most potent compound, EB-47 (compound 29), which carries a piperazine spacer between the isoindolinone and adenosine structures, has a half-maximal inhibitory concentration (IC_{50}) of 45 nM, which represents a 650-fold increase in the PARP-inhibitory activity compared with the parent isoindolinone compound.

Therapeutic effects and clinical testing

There is continued interest in the discovery and optimization of new PARP inhibitors, which is reflected

Figure 9 | **Further poly(ADP-ribose) polymerase (PARP) inhibitors. a** | Nucleic-acid and nucleoside derivatives (compounds 26–29) are examples of potent PARP inhibitors. An azido uridine analogue (compound 26) showed an inhibition constant (K_i) of 800 nM. The fused-uracil analogues (compounds 27 and 28) showed half-maximal inhibitory concentration (IC_{50}) values of 200 nM and 20 nM, respectively. An adenosine analogue (compound 29) showed an IC_{50} value of 45 nM. **b** | Structures of PARP inhibitors studied in PARP1 catalytic fragment (CF)/inhibitor co-crystal structure or various animal disease models. Compounds 30 and 31a were used for PARP1-CF co-crystal studies. Compounds 31b–40 and INO-1001 (structure yet to be disclosed) have been investigated in various animal disease models.

by recent published patents and articles. Increases in potency of 10^2–10^3-fold have been achieved over the prototypical PARP inhibitors that were originally reviewed by Banasik. Inotek, Pfizer/Newcastle, Fujisawa, Guilford, Glaxo, Ono and Novartis all have early-stage preclinical discovery or clinical development programmes for PARP1 inhibitors. The cytoprotective and therapeutic effects of many earlier-generation PARP inhibitors are reviewed in REFS 4,32,78. Here, we focus on the most recent advances in the field.

GPI-6150 (Guilford; compound 13) and PJ-34 (Inotek; compound 35) have been studied most intensely in disease models. Both compounds show significant therapeutic effects in several animal disease models (TABLE 1). GPI-6150 is cytoprotective *in vitro* and exerts significant protective effects at a dose range of 20–40 mg per kg in experimental models of stroke, traumatic brain injury, neurodegeneration, circulatory shock, diabetes mellitus and various inflammatory conditions (including colitis and gouty arthritis)[127–132] (TABLE 1). The phenanthridinone derivative PJ-34 (compound 35) is cytoprotective *in vitro*, and dose-dependently suppresses pro-inflammatory cytokine and chemokine production in immunostimulated macrophages[133]. In *in vivo* models, 3–30 mg per kg PJ-34 improves the functional outcome in stroke, myocardial infarction and reoxygenation injury, circulatory shock, diabetes and its vascular complications, colitis, arthritis and uveitis. PJ-34 also protects in chronic heart failure and improves the endothelium-dependent vascular relaxant function in various disease models[37,43,80,83,90,91,93,95,104,133–141] (TABLE 1).

Fujisawa synthesized several highly potent quinazolin-4(3H)-one derivatives that are modified at the 2,5- and 2,8-positions[142–144]. The 2,8-disubstituted derivative (compound 31b), has three-methylene unit and 4-(4-fluorophenyl)-tetrahydropyridine substitution at the quinazolinone-2-position. This compound shows strong PARP1 inhibition, with an IC_{50} value of 13 ± 1 nM, and *in vivo* neuroprotective activity. It has 20% bioavailability in the rat (70% in the dog) and a high brain/plasma concentration ratio. In a mouse model of Parkinson disease, this compound prevented the depletion of striatal dopamine, and metabolites of dihydroxyphenylacetic acid and homovanillic acid[117].

Kamanaka and colleagues reported the neuroprotective effects of the phthalazinone-based PARP inhibitor ONO-1924H (compound 32)[145]. In a rat stroke model, ONO-1924H reduced the stroke volume and attenuated the development of neurological deficits.

Scientists from the Universities of Perugia and Florence in Italy, and from GlaxoSmithKline, have reported that thieno-phenanthridin-6-one (compound 9), in which the thieno ring is attached to the isoquinolinone scaffold (compound 2), displays submicromolar activity in the purified bovine PARP1 assay (with an IC_{50} value of 450 nM) and is neuroprotective in models of brain ischaemia[146,147].

Meiji Seika Kaisha reported the neuroprotective effects of DR2313 (compound 27) in *in vivo* models of permanent and transient stroke in rats[148]. DR2313 also showed excellent profiles in terms of water-solubility and CNS penetration.

Guilford reported a new series of compounds that contain aza-5[H]-phenanthridin-6-one (compound 8)[149] and partially saturated aza-5[H]phenanthridin 6-one scaffolds[150]. Two such compounds (compounds 33 and 34) that were evaluated in the transient and permanent stroke models provided a 30–40% reduction in infarct volume.

Researchers at the University of Newcastle-upon-Tyne in the United Kingdom initially investigated the bicyclic carboxamide derivative (compound 18), which forms the cyclic PARP pharmacophore by intramolecular hydrogen bonding. A series of such compounds were further studied by researchers at Agouron (now Pfizer), who produced tricyclic lactam inhibitors (compounds 21–25)[150–155]. Subsequent lead optimization led to the discovery of the potent water-soluble tricyclic PARP inhibitors AG140361 (compound 36), compound 37 and AG140699 (structure not yet disclosed). AG140361 increased the delay of LoVo xenograft growth induced by irinotecan, irradiation or temozolomide by two- to threefold. A combination of AG140361 and temozolomide caused the complete regression of SW620 xenograft tumours[99,154] (FIG. 10). A Phase I dose-escalation trial of AG140699 in combination with temozolomide was initiated in June 2003 by the Cancer Research UK Drug Development Office[156].

Guilford also made a series of compounds using the tricyclic core of a PARP scaffold (compound 21)[157]. The PARP inhibitor compound 38 caused a significant reduction of infarct volume in rat models of transient and permanent brain ischaemia.

Novartis patented a series of substituted derivatives of indoloquinazolinones as PARP inhibitors[158]. The tetrazole-substituted compound 39 has an *in vitro* IC_{50} value of 12 nM and dose-dependently reduced infarct size by up to 60% in a rabbit myocardial infarction model.

Cephalon evaluated the chemo-potentiating capacity of CEP-6800 (compound 40), which was a prototype in the carbazole imide family of PARP inhibitors, in combination with three mechanistically distinct chemotherapeutic agents (temozolomide, irinotecan and cisplatin) against U251MG, glioblastoma, HT29 colon carcinoma, and Calu-6 non-small-cell lung carcinoma xenografts and cell lines[159]. This novel 3-aminomethyl carbazole imide is an inhibitor of both PARP1 and PARP2, and has an inhibition constant (K_i) of 5 nM.

Inotek has developed a series of PARP inhibitors derived from compound 11 (REF. 160). The potent parenteral inhibitor, INO-1001 — which has an *in vitro* IC_{50} value of 1 nM and protective effects in stroke, myocardial infarction and chronic heart failure[73,161–165] — is now in clinical trials for the treatment of reperfusion injury induced by myocardial infarction, cardiopulmonary bypass and thoraco-abdominal aortic aneurysm surgery.

Table 2 | **PARP inhibitors in clinical trials***

PARP	Company	Phase	Disease inhibitor
INO-1001	Inotek	I	TAAA‡
INO-1001	Inotek	I	STEMI§
INO-1001	Inotek	II	Myocardial infarction‖
AG140699	Pfizer/University of Newcastle	I	Malignant melanoma¶

*See Inotek Pharmaceuticals and Northern Institute for Cancer Research in the online links box for information regarding these clinical trials. ‡Thoracoabdominal aortic aneurysm (TAAA; Phase I): prevention of complications in patients undergoing TAAA. §ST-elevated myocardial infarction (STEMI; Phase I): treatment of patients with STEMI undergoing primary percutaneous coronary intervention.‖Myocardial infarction (Phase II): patients undergoing emergent angioplasty after acute myocardial infarction. ¶Malignant melanoma (Phase I): in combination with temozolomide.

Future development directions

As discussed above, PARP inhibitors (or PARP1 genetic deficiency) exert marked protective effects in animal models of a range of acute and chronic diseases. A comparison of the expected benefits of PARP inhibitors with the potential risks posed by these agents for acute life-threatening indications implies that the progression into clinical testing is well justified, as it is unlikely that the short-term administration of PARP inhibitors will pose significant risks to patients who participate in these trials. Indeed, the PARP inhibitor INO-1001 was well tolerated in a single-dose administration regimen in a Phase I trial that was conducted with healthy human volunteers. Prime acute clinical indications include myocardial infarction, cardiopulmonary bypass in high-risk patients, stroke (CNS-uptake permitting) and various malignancies. Scheduled interventions (bypass or surgical interventions such as thoraco-abdominal aortic aneurysm surgery) are attractive because they offer the possibility of pretreatment (relative to the time of reperfusion).

In terms of the risks of acute PARP inhibition, the most frequently raised issue relates to the ultimate benefit that can be derived from the preservation of cells that contain large amounts of damaged (broken) DNA. In the short term, preserving ATP and NAD^+ levels in the affected cells (for example, myocytes and neurons) might create cells with large amounts of DNA damage, which could be unrepairable and might facilitate subsequent mutagenic transformations. Although this is a difficult issue to resolve, it should be noted that PARP inhibitors do not induce these DNA-damage conditions — the damage is caused by the disease itself (that is, by the free-radical and oxidant species that are produced during reperfusion, as shown in FIG. 6). A further point to consider is the role of PARP in enhancing or promoting positive-feedback cycles of tissue injury. By inhibiting these processes, PARP inhibitors might reduce the degree of mitochondrial dysfunction, the release of mitochondrial oxidants and free radicals, and the release of mitochondrial cell-death factors (such as AIF). In addition, by downregulating the inflammatory response and inhibiting the infiltration of activated mononuclear cells (thereby preventing the release of toxic mediators into the tissues), PARP inhibitors might indirectly reduce the net oxidative and nitrosative stress burden that the tissues are exposed to. These issues need to be addressed in future preclinical and clinical investigations.

Because PARP inhibitors represent a new type of therapeutic modality, which has not been tested for efficacy in humans, there is a degree of uncertainty with respect to its ultimate outcome — as is the case with all new therapeutic modalities when they initially enter clinical trials. The human clinical efficacy trials are evaluating parameters that are similar in nature to those studied in preclinical studies (such as indices of tissue necrosis and the functional parameters of the affected organs). As PARP inhibitors show significant cytoprotection in human cells (*in vitro*) and the PARP pathway has been shown to have important pathogenic roles in a wide range of animal species (rodent and non-rodent), it is unlikely that there are marked species differences between animals and humans.

When contemplating the use of PARP inhibitors for chronic disease indications, a range of other issues need to be considered in addition to the usual pharmacokinetic, pharmacodynamic and toxicological considerations. These include the following: the long-term safety of the compound for human use (both mechanism-based and mechanism-independent actions); oral bioavailability, and pharmacokinetic and metabolic issues; the long-term effects (does long-term PARP inhibition affect the expression of PARP and are there any rebound or negative effects on discontinuation?);

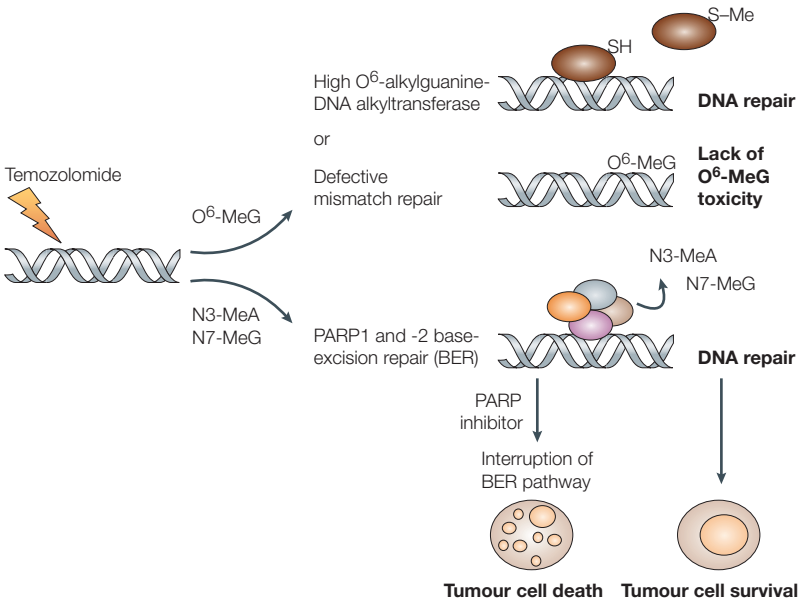


Figure 10 | **PARP inhibition restores tumour-cell sensitivity to temozolomide.** The schematic highlights the most frequent causes of tumour cell resistance to temozolomide: removal of the methyl adduct from O^6-methylguanine (O^6-MeG) by high levels of O^6-alkylguanine-DNA alkyltransferase and tolerance to O^6-methylguanine toxicity due to functional defects of the mismatch repair system. N7-methylguanine (N7-MeG) and N3-methyladenine (N3-MeA) are promptly repaired by the base-excision repair system (BER), co-ordinated by PARP1 and PARP2, and generally do not contribute to the drug toxicity. Inhibition of PARP allows sensitization of resistant tumour cells by interrupting the repair of methylpurines, regardless of the susceptibility of tumour cells to O^6-methylguanine toxicity and, therefore, independently of the functional status of the mismatch repair system or O^6-alkylguanine-DNA alkyltransferase activity.

and the potential interactions of PARP inhibitors with other therapeutics that are already used in the management of the chronic indication in question. It is unlikely that the therapeutic use of PARP inhibitors will inhibit all PARP isoforms, and it is also unlikely that it would lead to the complete and permanent obliteration of the enzymatic activity of PARP1.

On the balance of evidence, the short-term inhibition of PARP seems to be acceptable, in terms of the risk/benefit ratio, for experimental therapy for severe life-threatening diseases (see above). However, the question of whether the risk/benefit ratio is acceptable during chronic PARP-inhibitor treatment for the experimental treatment of chronic diseases remains to be determined (clearly, not all chronic diseases are the same in terms of their severity, risks and therapeutic options, and the selection of an appropriate risk/benefit ratio remains crucial). Patients with chronic cardiovascular diseases are usually treated with a range of therapeutic agents as part of standardized combination therapies. Some of these agents (for example, statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and those used for tight glycaemic control in diabetic patients) might be able to reduce the degree of oxidative and nitrosative stress, and, therefore, the degree of PARP activation in the cardiovascular system[90]. It remains to be seen whether PARP activation persists in humans who have been appropriately treated with state-of-the-art therapies (and, therefore, whether combining a low-dose PARP-inhibitor regimen with the standard battery of therapies[166] might represent a potential approach to enhance therapeutic efficacy, and, at the same time, reduce the potential side effects that are associated with long-term PARP inhibition).

It is also important to briefly consider the issue of the isoform selectivity of PARP inhibitors. Although the catalytic sites of most members of the PARP family are similar, future advances in medicinal chemistry might yield PARP1-selective inhibitors (or, in theory, agents with selectivity for other PARP isoforms). A recent example of such selectivity is certain quinazoline derivatives made by Fujisawa Pharmaceuticals[167]. Such PARP1-selective agents might be superior to isoform-unselective compounds, inasmuch as they might not interfere with the functions of other members of the PARP family (for example, the regulation of DNA repair, the cell cycle, cell division and telomere length). However, it must be noted that there are no truly PARP1-selective inhibitors available at present, so this hypothesis cannot yet be directly tested. Moreover, some data indicate that PARP2 activation (similar to PARP1 activation) might confer cytotoxic and pro-inflammatory actions[168]; therefore, the inhibition of PARP2 might also be of therapeutic benefit in certain disease conditions.

Conclusions

Research in the area of PARP has led to a better understanding of the roles of PARP1 in various normal cellular processes and the pathophysiology of various diseases. In response to oxidative and nitrosative stress, PARP activation initiates an energy-consuming and inefficient cellular metabolic cycle; this leads to cellular and mitochondrial dysfunction, and promotes the functional impairment of the affected cells, which culminates in cell necrosis. PARP also has a role in the regulation of gene transcription. The activation of PARP is involved in the pathogenesis of a range of inflammatory and cardiovascular diseases. PARP inhibitors might have other potential uses as anticancer agents. Many pharmacological classes of compounds have been shown to inhibit the catalytic activity of PARP. Moreover, some of these compounds have now successfully progressed through the preclinical efficacy and safety stages of drug development, and have entered human clinical testing (TABLE 2). These trials will bring us closer to answering the question of whether PARP inhibitors will fulfil their initial promise in the clinical arena.

1. Chambon, P., Weil, J. D. & Mandel, P. NAD activation of a new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. *Biochem. Biophys. Res. Comm.* **11**, 39–45 (1963).
This was the first report on PARP.
2. Ame, J. C., Spenlehauer, C. & de Murcia, G. The PARP superfamily. *Bioessays* **26**, 882–893 (2004).
A recent and comprehensive review on the various PARP isoforms.
3. Nguewa, P. A., Fuertes, M. A., Valladares, B., Alonso, C. & Perez, J. M. Poly(ADP-ribose) polymerases: homology, structural domains and functions. Novel therapeutical applications. *Prog. Biophys. Mol. Biol*. **88**, 143–172 (2005).
4. Virág, L. & Szabó, C. The therapeutic potential of poly (ADP-ribose) polymerase inhibitors. *Pharmacol. Rev*. **54**, 375–429 (2002).
A comprehensive review on the role of PARP in various disease conditions.
5. Davidovic, L., Vodenicharov, M., Affar, E. B. & Poirier, G. G. Importance of poly(ADP-ribose) glycohydrolase in the control of poly(ADP-ribose) metabolism. *Exp. Cell Res*. **268**, 7–13 (2001).
6. Pillai, J. B., Russell, H. M., Raman, J. S., Jeevanandam, V. & Gupta, M. P. Increased expression of poly (ADP) ribose polymerase-1 contributes to caspase-independent myocyte cell death during heart failure. *Am. J. Physiol. Heart Circ. Physiol*. **288**, H486–H496 (2005).
7. Bauer, P. I. *et al*. Inhibition of DNA binding by the phosphorylation of poly ADP-ribose polymerase protein catalysed by protein kinase C. *Biochem. Biophys. Res. Commun.* **187**, 730–736 (1992).
8. Homburg, S. *et al*. A fast signal-induced activation of poly(ADP-ribose) polymerase: a novel downstream target of phospholipase C. *J. Cell Biol.* **150**, 293–307 (2000).
9. Sastry, S. S. & Kun, E. The interaction of adenosine diphosphoribosyl transferase (ADPRT) with a cruciform DNA. *Biochem. Biophys. Res. Commun.* **167**, 842–847 (1990).
10. Wallace, D. C. A mitochondrial paradigm for degenerative diseases and ageing. *Novartis Found Symp*. **235**, 247–263 (2001).
11. Bouchard, V. J., Rouleau, M. & Poirier, G. G. PARP-1, a determinant of cell survival in response to DNA damage. *Exp. Hematol*. **31**, 446–454 (2003).
12. Huber, A., Bai, P., de Murcia, J. M. & de Murcia, G. PARP-1, PARP-2 and ATM in the DNA damage response: functional synergy in mouse development. *DNA Repair (Amst.)* **3**, 1103–1108 (2004).
13. de Murcia, J. M. *et al.* Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. *Proc. Natl Acad. Sci. USA* **94**, 7303–7307 (1997).
A definitive study showing the different radiosensitivity of PARP1-deficient and wild-type mice.
14. Dantzer, F. *et al.* Base excision repair is impaired in mammalian cells lacking poly(ADP-ribose) polymerase-1. *Biochemistry* **39**, 7559–7569 (2000).
This paper discusses the role of PARP in regulating BER.
15. Flohr, C., Burkle, A., Radicella, J. P. & Epe, B. Poly(ADP-ribosyl)ation accelerates DNA repair in a pathway dependent on Cockayne syndrome B protein. *Nucleic Acids Res*. **31**, 5332–5337 (2003).
16. von Kobbe, C. *et al*. Poly(ADP-ribose) polymerase 1 regulates both the exonuclease and helicase activities of the Werner syndrome protein. *Nucleic Acids Res*. **32**, 4003–4014 (2004).
17. Kun, E. & Bauer, P. I. Cell biological functions of PARP I: an overview. *Ital. J. Biochem*. **50**, 15–18 (2001).
18. Yung, T. M., Sato, S. & Satoh, M. S. Poly(ADP-ribosyl)ation as a DNA damage-induced post-translational modification regulating poly(ADP-ribose) polymerase-1-topoisomerase I interaction. *J. Biol. Chem*. **279**, 39686–39696 (2004).
19. Oei, S. L. & Ziegler, M. ATP for the DNA ligation step in base excision repair is generated from poly(ADP-ribose). *J. Biol. Chem*. **275**, 23234–23239 (2000).
20. Vodenicharov, M. D., Sallmann, F. R., Satoh, M. S. & Poirier, G. G. Base excision repair is efficient in cells lacking poly(ADP-ribose) polymerase 1. *Nucleic Acids Res*. **28**, 3887–3896 (2000).

21. Russell, J., Sanderson, R. J. & Lindahl, T. Down-regulation of DNA repair synthesis at DNA single-strand interruptions in poly(ADP-ribose) polymerase-1 deficient murine cell extracts. *DNA Repair* **1**, 547–558 (2002).
22. Allinson, S. L., Dianova, I. I. & Dianov, G. L. Poly(ADP-ribose) polymerase in base excision repair: always engaged, but not essential for DNA damage processing. *Acta Biochim. Pol*. **50**, 169–179 (2003).
23. Tong, W. M. *et al*. Null mutation of DNA strand break-binding molecule poly(ADP-ribose) polymerase causes medulloblastomas in p53(–/–) mice. *Am. J. Pathol.* **162**, 343–352 (2003).
24. Nozaki, T. *et al*. PARP-1 deficiency implicated in colon and liver tumorigenesis induced by azoxymethane. *Cancer Sci.* **94**, 497–500 (2003).
25. Masutani, M., Nakagama, H. & Sugimura, T. Poly(ADP-ribose) and carcinogenesis. *Genes Chromosom. Cancer* **38**, 339–348 (2003).
26. Conde, C. *et al.* Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice. *EMBO J.* **20**, 3535–3543 (2001).
27. Hans, M. A., Muller, M., Meyer-Ficca, M., Burkle, A. & Kupper, J. H. Overexpression of dominant negative PARP interferes with tumor formation of HeLa cells in nude mice: evidence for increased tumor cell apoptosis *in vivo*. *Oncogene* **18**, 7010–7015 (1999).
28. Martin-Oliva, D. *et al*. Crosstalk between PARP-1 and NF-κB modulates the promotion of skin neoplasia. *Oncogene* **23**, 5275–5283 (2004).
29. Simbulan-Rosenthal, C. M. *et al.* Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase. *Proc. Natl Acad. Sci. USA* **97**, 11274–11279 (2000).
30. Simbulan-Rosenthal, C. M. *et al*. Inhibition of poly(ADP-ribose) polymerase activity is insufficient to induce tetraploidy. *Nucleic Acids Res*. **29**, 841–849 (2001).
31. Kraus, W. L. & Lis, J. T. PARP goes transcription. *Cell* **113**, 677–683 (2003).
32. Reale, A., Matteis, G. D., Galleazzi, G., Zampieri, M. & Caiafa, P. Modulation of DNMT1 activity by ADP-ribose polymers. *Oncogene* **24**, 13–19 (2005).
This was the first report on the regulation by PARP of DNA methyl transferase.
33. Hassa, P. O. & Hottiger, M. O. The functional role of poly(ADP-ribose)polymerase 1 as novel coactivator of NF-κB in inflammatory disorders. *Cell. Mol. Life Sci*. **59**, 1534–1553 (2002).
A comprehensive overview on the role of PARP in regulating NF-κB activation.
34. Hassa, P. O., Buerki, C., Lombardi, C., Imhof, R. & Hottiger, M. O. Transcriptional coactivation of nuclear factor-κB-dependent gene expression by p300 is regulated by poly(ADP)-ribose polymerase-1. *J. Biol. Chem.* **278**, 45145–45153 (2003).
35. Nakajima, H. *et al.* Critical role of the automodification of poly(ADP-ribose) polymerase-1 in nuclear factor-κB-dependent gene expression in primary cultured mouse glial cells. *J. Biol. Chem*. **279**, 42774–42786 (2004).
36. Hasko, G. *et al*. Poly(ADP-ribose) polymerase is a regulator of chemokine production: relevance for the pathogenesis of shock and inflammation. *Mol. Med*. **8**, 283–289 (2002).
37. Garcia-Soriano, F. *et al*. Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. *Nature Med*. **7**, 108–113 (2001).
This was the first report implicating the role of PARP in the pathogenesis of diabetic complications.
38. Du, X. *et al*. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J. Clin. Invest.* **112**, 1049–1057 (2003).
39. Carrillo, A. *et al.* Transcription regulation of TNF-α-early response genes by poly(ADP-ribose) polymerase-1 in murine heart endothelial cells. *Nucleic Acids Res.* **32**, 757–766 (2004).
40. Ota, K., Kameoka, M., Tanaka, Y., Itaya, A. & Yoshihara, K. Expression of histone acetyltransferases was down-regulated in poly(ADP-ribose) polymerase-1-deficient murine cells. *Biochem. Biophys. Res. Commun*. **310**, 312–317 (2003).
41. Zingarelli, B. *et al.* Absence of poly(ADP-ribose)polymerase-1 alters nuclear factor-κB activation and gene expression of apoptosis regulators after reperfusion injury. *Mol. Med.* **9**, 143–153 (2003).
42. Zingarelli, B., O'Connor, M. & Hake, P. W. Inhibitors of poly (ADP-ribose) polymerase modulate signal transduction pathways in colitis. *Eur. J. Pharmacol*. **469**, 183–194 (2003).
43. Veres, B. *et al.* Decrease of the inflammatory response and induction of the Akt/protein kinase B pathway by poly-(ADP-ribose) polymerase 1 inhibitor in endotoxin-induced septic shock. *Biochem. Pharmacol.* **65**, 1373–1382 (2003).
44. Veres, B. *et al*. Regulation of kinase cascades and transcription factors by a poly(ADP-ribose) polymerase-1 inhibitor, 4-hydroxyquinazoline, in lipopolysaccharide-induced inflammation in mice. *J. Pharmacol. Exp. Ther*. **310**, 247–255 (2004).
This paper shows that PARP inhibitors affect the activation of many kinase pathways.
45. Szabó, C. *et al*. Regulation of components of the inflammatory response by 5-iodo-6-amino-1,2-benzopyrone, and inhibitor of poly (ADP-ribose) synthetase and pleiotropic modifier of cellular signal pathways. *Int. J. Oncol.* **10**, 1093–1101 (1997).
46. Ha, H. C. Defective transcription factor activation for proinflammatory gene expression in poly(ADP-ribose) polymerase 1-deficient glia. *Proc. Natl Acad. Sci. USA* **101**, 5087–5092 (2004).
47. Simbulan-Rosenthal, C. M. *et al.* Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase. *Proc. Natl Acad. Sci. USA* **97**, 11274–11279 (2000).
48. Ha, H. C., Hester, L. D. & Snyder, S. H. Poly(ADP-ribose) polymerase-1 dependence of stress-induced transcription factors and associated gene expression in glia. *Proc. Natl Acad. Sci. USA* **99**, 3270–3275 (2002).
This report shows that PARP regulates signal transduction and gene expression in immunostimulated cells.
49. Zingarelli, B. *et al.* Differential regulation of activator protein-1 and heat shock factor-1 in myocardial ischemia and reperfusion injury: role of poly(ADP-ribose) polymerase-1. *Am. J. Physiol. Heart Circ. Physiol*. **286**, H1408–H1415 (2004).
50. Ivana-Scovassi, A. & Diederich, M. Modulation of poly(ADP-ribosylation) in apoptotic cells. *Biochem. Pharmacol*. **68**, 1041–1047 (2004).
51. Kwan, J. A. *et al*. Matrix metalloproteinase-2 (MMP-2) is present in the nucleus of cardiac myocytes and is capable of cleaving poly (ADP-ribose) polymerase (PARP) *in vitro*. *FASEB J*. **18**, 690–692 (2004).
52. Leist, M. *et al*. Apoptosis in the absence of poly-(ADP-ribose) polymerase. *Biochem. Biophys. Res. Commun*. **233**, 518–522 (1997).
53. Virág, L. *et al*. Peroxynitrite-induced thymocyte apoptosis: the role of caspases and poly (ADP-ribose) synthetase activation. *Immunology* **94**, 345–355 (1998).
This was the first definitive report showing that PARP activation promotes cell necrosis.
54. Herceg, Z. & Wang, Z. Q. Failure of poly(ADP-ribose) polymerase cleavage by caspases leads to induction of necrosis and enhanced apoptosis. *Mol. Cell. Biol.* **19**, 5124–5133 (1999).
A definitive report showing that, by preventing PARP-mediated cell necrosis, PARP cleavage serves to promote apoptosis.
55. Aikin, R., Rosenberg, L., Paraskevas, S. & Maysinger, D. Inhibition of caspase-mediated PARP-1 cleavage results in increased necrosis in isolated islets of Langerhans. *J. Mol. Med*. **82**, 389–397 (2004).
56. Eliasson, M. J. *et al.* Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. *Nature Med.* **3**, 1089–1095 (1997).
In this study, PARP-knockout mice showed an 80% reduction in cortical necrosis in a murine model of stroke.
57. Zingarelli, B., Salzman, A. L. & Szabó, 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.* **83**, 85–94 (1998).
In this study, PARP-knockout mice showed a 40% reduction in myocardial necrosis in a murine model of myocardial infarction.
58. Zhang, J., Dawson, V. L., Dawson, T. M. & Snyder, S. H. Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science* **263**, 687–689 (1994).
A seminal report linking NO overproduction, PARP activation and neurotoxicity.
59. Heller, B. *et al.* Inactivation of the poly(ADP-ribose) polymerase gene affects oxygen radical and nitric oxide toxicity in islet cells. *J. Biol. Chem*. **270**, 11176–11180 (1995).
A seminal report linking NO overproduction, PARP activation and islet cell toxicity.
60. Virág, L., Salzman, A. L. & Szabó, C. Poly(ADP-ribose) synthetase activation mediates mitochondrial injury during oxidant-induced cell death. *J. Immunol*. **161**, 3753–3759 (1998).
61. Ha, H. C. & Snyder, S. H. Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. *Proc. Natl Acad. Sci. USA* **96**, 13978–13982 (1999).
62. Szabó, C., Zingarelli, B., O'Connor, M. & Salzman, A. L. DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. *Proc. Natl Acad. Sci. USA* **93**, 1753–1758 (1996).
This report describes the identification of peroxynitrite (the reaction product of NO and superoxide) as an endogenous trigger of DNA single-strand breakage and PARP activation.
63. Zingarelli, B., O'Connor, M., Wong, H., Salzman, A. L. & Szabó, C. Peroxynitrite-mediated DNA strand breakage activates poly-adenosine diphosphate ribosyl synthetase and causes cellular energy depletion in macrophages stimulated with bacterial lipopolysaccharide. *J. Immunol*. **156**, 350–358 (1996).
64. Berger, N. A. & Berger, S. J. Metabolic consequences of DNA damage: the role of poly (ADP-ribose) polymerase as mediator of the suicide response. *Basic Life Sci*. **38**, 357–363 (1986).
This paper describes the regulation by PARP activation of NAD depletion, cell suicide and DNA-repair mechanisms in cells with massive DNA damage.
65. Berger, N. A., Whitacre, C. M., Hashimoto, H., Berger, S. J. & Chatterjee, S. NAD and poly(ADP-ribose) regulation of proteins involved in response to cellular stress and DNA damage. *Biochimie* **77**, 364–367 (1995).
66. Skaper, S. D. *et al.* Poly(ADP-ribose) polymerase-1 in acute neuronal death and inflammation: a strategy for neuroprotection. *Ann. NY Acad. Sci*. **993**, 217–228 (2003).
67. Szabó, G., Liaudet, L., Hagl, S. & Szabó, C. Poly(ADP-ribose) polymerase activation in the reperfused myocardium. *Cardiovasc. Res*. **61**, 471–480 (2004).
68. Ying, W., Alano, C. C., Garnier, P. & Swanson, R. A. NAD(+) as a metabolic link between DNA damage and cell death. *J. Neurosci. Res.* **79**, 216–223 (2005).
69. Chiarugi, A. Poly(ADP-ribose) polymerase: killer or conspirator? The 'suicide hypothesis' revisited. *Trends Pharmacol. Sci*. **23**, 122–129 (2002).
70. Ying, W., Garnier, P. & Swanson, R. A. NAD^+ repletion prevents PARP-1-induced glycolytic blockade and cell death in cultured mouse astrocytes. *Biochem. Biophys. Res. Commun*. **308**, 809–813 (2003).
71. Ying, W., Chen, Y., Alano, C. C. & Swanson, R. A. Tricarboxylic acid cycle substrates prevent PARP-mediated death of neurons and astrocytes. *J. Cereb. Blood Flow Metab*. **22**, 774–779 (2002).
72. Wang, H. *et al.* Apoptosis-inducing factor substitutes for caspase executioners in NMDA-triggered excitotoxic neuronal death. *J. Neurosci*. **24**, 10963–10973 (2004).
73. Xiao, C. Y., Chen, M., Zsengeller, Z. & Szabó, C. Poly(ADP-ribose) polymerase contributes to the development of myocardial infarction in diabetic rats and regulates the nuclear translocation of apoptosis-inducing factor. *J. Pharmacol. Exp. Ther.* **310**, 498–504 (2004).
74. Hong, S. J., Dawson, T. M. & Dawson, V. L. Nuclear and mitochondrial conversations in cell death: PARP-1 and AIF signaling. *Trends Pharmacol. Sci*. **25**, 259–264 (2004).
A comprehensive overview of recent progress on the role of PARP in regulating AIF translocation and AIF-mediated cytotoxicity.
75. Du, L. *et al.* Intra-mitochondrial poly(ADP-ribosyl)ation contributes to NAD^+ depletion and cell death induced by oxidative stress. *J. Biol. Chem*. **278**, 18426–18433 (2003).
76. Lai, Y. C. *et al*. Poly-ADP-ribosylation as a post-translational modification of mitochondrial proteins: 82. *Crit. Care Med*. **32**, A21 (2004).
77. Scovassi, A. I. Mitochondrial poly(ADP-ribosylation): from old data to new perspectives. *FASEB J*. **18**, 1487–1488 (2004).
78. Szabó, C. & Dawson, V. L. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia–reperfusion. *Trends Pharmacol. Sci*. **19**, 287–298 (1998).
79. Liaudet, L. *et al*. Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose)polymerase. *Proc. Natl Acad. Sci. USA* **97**, 10203–10208 (2000).
80. Casey, P. J. *et al*. PARP inhibition modulates spinal cord dysfunction after thoracoabdominal aortic ischemia–reperfusion. *J. Vasc. Surg.* **41**, 99–107 (2005).
81. Kendirci, M. *et al.* PARP inhibition restores *in vivo* erectile function in bilateral cavernous nerve injured rats. *J. Sex. Med.* (Suppl. 1), 107 (2004).
82. Khan, A. U. *et al*. Liposomal NAD(+) prevents diminished O(2) consumption by immunostimulated Caco-2 cells. *Am. J. Physiol. Lung Cell Mol. Physiol*. **282**, L1082–L1091 (2002).
83. Soriano, F. G , Pacher, P., Mabley, J., Liaudet, L. & Szabó, C. Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly(ADP-ribose) polymerase. *Circ. Res*. **89**, 684–691 (2001).

84. Jijon, H. B. *et al.* Inhibition of poly(ADP-ribose) polymerase attenuates inflammation in a model of chronic colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **279**, G641–G651 (2000).
85. Suarez-Pinzon, W. L., Mabley, J. G., Power, R., Szabó, C. & Rabinovitch, A. Poly (ADP-ribose) polymerase inhibition prevents spontaneous and recurrent autoimmune diabetes in NOD mice by inducing apoptosis of islet-infiltrating leukocytes. *Diabetes* **52**, 1683–1688 (2003).
86. Endres, M. *et al.* Role of peroxynitrite and neuronal nitric oxide synthase in the activation of poly(ADP-ribose) synthetase in a murine model of cerebral ischemia–reperfusion. *Neurosci. Lett.* **248**, 41–44 (1998).
87. Liaudet, L. *et al.* Suppression of poly (ADP-ribose) polymerase activation by 3-aminobenzamide in a rat model of myocardial infarction: long-term morphological and functional consequences. *Br. J. Pharmacol.* **133**, 1424–1430 (2001).
88. Szabó, C. *et al.* Inhibition of poly (ADP-ribose) synthetase attenuates neutrophil recruitment and exerts antiinflammatory effects. *J. Exp. Med.* **186**, 1041–1049 (1997).
89. Szabó, C., Cuzzocrea, S., Zingarelli, B., O'Connor, M. & Salzman, A. L. Endothelial dysfunction in a rat model of endotoxic shock. Importance of the activation of poly (ADP-ribose) synthetase by peroxynitrite. *J. Clin. Invest.* **100**, 723–735 (1997).
90. Szabó, C. *et al.* Angiotensin II-mediated endothelial dysfunction: role of poly(ADP-ribose) polymerase activation. *Mol. Med.* **10**, 28–35 (2004).
91. Pacher, P., Mabley, J. G., Soriano, F. G., Liaudet, L. & Szabó, C. Activation of poly(ADP-ribose) polymerase contributes to the endothelial dysfunction associated with hypertension and aging. *Int. J. Mol. Med.* **9**, 659–664 (2002).
92. Zhang, C., Yang, J. & Jennings, L. K. Attenuation of neointima formation through the inhibition of DNA repair enzyme PARP-1 in balloon-injured rat carotid artery. *Am. J. Physiol. Heart. Circ. Physiol.* **287**, H659–H666 (2004).
93. Benko, R., Pacher, P., Vaslin, A., Kollai, M. & Szabó, C. Restoration of the endothelial function in the aortic rings of apolipoprotein E deficient mice by pharmacological inhibition of the nuclear enzyme poly(ADP-ribose) polymerase. *Life Sci.* **75**, 1255–1261 (2004).
94. Obrosova, I. G. *et al.* Role of poly(ADP-ribose) polymerase activation in diabetic neuropathy. *Diabetes* **53**, 711–720 (2004).
95. Zheng, L., Szabó, C. & Kern, T. S. Poly(ADP-ribose) polymerase is involved in the development of diabetic retinopathy via regulation of nuclear factor-κB. *Diabetes* **53**, 2960–2967 (2004).
96. Delaney, C. A. *et al.* Potentiation of temozolomide and topotecan growth inhibition and cytotoxicity by novel poly(adenosine diphosphoribose) polymerase inhibitors in a panel of human tumor cell lines. *Clin. Cancer Res.* **6**, 2860–2867 (2000).
This paper describes the synergistic cytotoxic effects of PARP inhibitors and certain antitumour agents, including temozolomide.
97. Tentori, L. *et al.* Effects of single or split exposure of leukemic cells to temozolomide, combined with poly(ADP-ribose) polymerase inhibitors on cell growth, chromosomal aberrations and base excision repair components. *Cancer Chemother. Pharmacol.* **47**, 361–369 (2001).
98. Tentori, L., Portarena, I., Bonmassar, E. & Graziani, G. Combined effects of adenovirus-mediated wild-type p53 transduction, temozolomide and poly (ADP-ribose) polymerase inhibitor in mismatch repair deficient and non-proliferating tumor cells. *Cell Death Differ.* **8**, 457–469 (2001).
99. Calabrese, C. R. *et al.* Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361. *J. Natl. Cancer Inst.* **96**, 56–67 (2004).
100. Curtin, N. J. *et al.* Novel poly(ADP-ribose) polymerase-1 inhibitor, AG14361, restores sensitivity to temozolomide in mismatch repair-deficient cells. *Clin. Cancer Res.* **10**, 881–889 (2004).
101. Yung, T. M., Sato, S. & Satoh, M. S. Poly(ADP-ribosyl)ation as a DNA damage-induced post-translational modification regulating poly(ADP-ribose) polymerase-1-topoisomerase I interaction. *J. Biol. Chem.* **279**, 39686–39696 (2004).
102. Malanga, M. & Althaus, F. R. Poly(ADP-ribose) reactivates stalled DNA topoisomerase I and induces DNA strand break resealing. *J. Biol. Chem.* **279**, 5244–5248 (2004).
103. Munoz-Gamez, J. A. *et al.* PARP inhibition sensitizes p53-deficient breast cancer cells to doxorubicin-induced apoptosis. *Biochem. J.* **386**, 119–125 (2005)
104. Pacher, P. *et al.* Activation of poly(ADP-ribose) polymerase contributes to development of doxorubicin-induced heart failure. *J. Pharmacol. Exp. Ther.* **300**, 862–867 (2002).
105. Szenczi, O. *et al.* Poly(ADP-ribose) polymerase regulates myocardial calcium handling in doxorubicin-induced heart failure. *Biochem. Pharmacol.* **69**, 725–732 (2005).
106. Chalmers, A. J. Poly(ADP-ribose) polymerase-1 and ionizing radiation: sensor, signaller and therapeutic target. *Clin. Oncol. (R. Coll. Radiol.)* **16**, 29–39 (2004).
107. Rudat, V., Kupper, J. H. & Weber, K. J. *Trans*-dominant inhibition of poly(ADP-ribosyl)ation leads to decreased recovery from ionizing radiation-induced cell killing. *Int. J. Radiat. Biol.* **73**, 325–330 (1998).
108. Schlicker, A., Peschke, P., Burkle, A., Hahn, E. W. & Kim, J. H. 4-amino-1,8-naphthalimide: a novel inhibitor of poly(ADP-ribose) polymerase and radiation sensitizer. *Int. J. Radiat. Biol.* **75**, 91–100 (1999).
109. Veuger, S. J., Curtin, N. J., Richardson, C. J., Smith, G. C. & Durkacz, B. W. Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. *Cancer Res.* **63**, 6008–6015 (2003).
110. Brock, W. A. *et al.* Radiosensitization of human and rodent cell lines by INO-1001, a novel inhibitor of poly(ADP-ribose) polymerase. *Cancer Lett.* **205**, 155–160 (2004).
111. Ruf, A., Mennissier, D. M., De Murcia, G. & Schulz, G. E. Structure of the catalytic fragment of poly(AD-ribose) polymerase from chicken. *Proc. Natl Acad. Sci. USA* **93**, 7481–7485 (1996).
This paper describes the characterization of the catalytic site of PARP.
112. Ruf, A., Mennissier, D. M., De Murcia, G. & Schulz, G. E. Inhibitor and NAD^+ binding to poly(ADP-ribose) polymerase as derived from crystal structures and homology modeling. *Biochemistry* **37**, 3893–3900 (1998).
113. Ruf, A., Rolli, V., De Murcia, G. & Schulz, G. E. The mechanism of the elongation and branching reaction of poly(ADP-ribose) polymerase as derived from crystal structures and mutagenesis. *J. Mol. Biol.* **278**, 57–65 (1998).
114. Nguewa, P. A., Fuertes, M. A., Alonso, C. & Perez, J. M. Pharmacological modulation of poly(ADP-ribose) polymerase-mediated cell death: exploitation in cancer chemotherapy. *Mol. Pharmacol.* **64**, 1007–1014 (2003).
115. Oliver, A. W. *et al.* Crystal structure of the catalytic fragment of murine poly(ADP-ribose) polymerase-2. *Nucleic Acids Res.* **32**, 456–464 (2004).
116. Kinoshita, T. *et al.* Inhibitor-induced structural change of the active site of human poly(ADP-ribose) polymerase. *FEBS Lett.* **556**, 43–46 (2004).
117. Hattori, K. *et al.* Rational approaches to discovery of orally active and brain-penetrable quinazolinone inhibitors of poly(ADP-ribose)polymerase. *J. Med. Chem.* **47**, 4151–4154 (2004).
This report describes orally active and CNS-penetrable inhibitors of PARP.
118. Banasik, M. & Ueda, K. Inhibitors and activators of ADP-ribosylation reactions. *Mol. Cell Biochem.* **138**, 185–197 (1994).
119. Banasik, M., Komura, H., Shimoyama, M. & Ueda, K. Specific inhibitors of poly(ADP-ribose) synthetase and mono(ADP-ribosyl)transferase. *J. Biol. Chem.* **267**, 1569–1575 (1992).
A seminal report identifying several PARP-inhibitor structures that later became prototypes for optimization and drug development.
120. Li, J. H. & Zhang, J. PARP inhibitors. *IDrugs* **4**, 804–812 (2001).
121. Southan, G. J. & Szabó, C. Poly(ADP-ribose) polymerase inhibitors. *Curr. Med. Chem.* **10**, 321–340 (2003).
122. Peukert, S. & Schwahn, U. New inhibitors of poly(ADP-ribose)polymerase (PARP). *Expert Opin. Ther. Patents* **14**, 1531–1551 (2004).
123. Suto, M. J., Turner, W. R., Arundel-Suto, C. M., Werbel, L. M. & Seboly-Leopold J. S. Dihydroiso-quinolinones: the design and synthesis of a new series of potent inhibitors of poly(ADP-ribose) polymerase. *Anticancer Drug Design* **6**, 107–117 (1991).
124. Pivaziyan, A. D., Birks, E. M., Wood, T. G., Lin, T. S. and Prusoff, W. H. Inhibition of poly(ADP-ribose) polymerase activity by nucleoside analogs of thymidine. *Biochem. Pharmacol.* **44**, 947 (1992).
125. Steinhagen, H., Gerisch, M., Mittendorf, J., Schlemmer, K. H. & Albrecht, B. Substituted uracil derivatives as potent inhibitors of poly(ADP-ribose)polymerase-1 (PARP-1). *Bioorg. Med. Chem. Lett.* **12**, 3187–3190 (2002).
126. Jagtap, P. G. *et al.* The discovery and synthesis of novel adenosine substituted 2,3-dihydro-1-H-isoindol-1-ones: potent inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1). *Bioorg. Med. Chem. Lett.* **14**, 81–85 (2004).
This paper describes the synthesis and biological evaluation of the adenosine-based PARP inhibitor EB-47.
127. LaPlaca, M. C. *et al.* Pharmacologic inhibition of poly(ADP-ribose) polymerase is neuroprotective following traumatic brain injury in rats. *J. Neurotrauma* **18**, 369–376 (2001).
128. Mazzon, E. *et al.* GPI 6150, a poly(ADP-ribose) polymerase inhibitor, exhibits an anti-inflammatory effect in rat models of inflammation. *Eur. J. Pharmacol.* **415**, 85–94 (2001).
129. Mazzon, E. *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* **17**, 222–227 (2002).
130. Mazzon, E. *et al.* GPI 6150, a PARP inhibitor, reduces the colon injury caused by dinitrobenzene sulfonic acid in the rat. *Biochem. Pharmacol.* **64**, 327–337 (2002).
131. Feng, Y. & LeBlanc M. H. Drug-induced hypothermia begun 5 minutes after injury with a poly(adenosine 5'-diphosphate-ribose) polymerase inhibitor reduces hypoxic brain injury in rat pups. *Crit. Care Med.* **30**, 2420–2424 (2002).
132. Zhang, J. in *PARP as a Therapeutic Target* (ed. Zhang, J. C.) 239–333 (CRC Press, Boca Raton, Florida, 2002).
133. Jagtap, P. *et al.* Novel phenanthridinone inhibitors of poly(adenosine 5'-diphosphate-ribose) synthetase: potent cytoprotective and anti-shock agents. *Crit. Care Med.* **30**, 1071–1082 (2002).
This paper reports on a phenanthridinone-based class of PARP inhibitors with anti-inflammatory effects.
134. Abdelkarim, G. E. *et al.* Protective effects of PJ34, a novel, potent inhibitor of poly(ADP-ribose) polymerase (PARP) in *in vitro* and *in vivo* models of stroke. *Int. J. Mol. Med.* **7**, 255–260 (2001).
135. Faro, R. *et al.* Myocardial protection by PJ34, a novel potent poly(ADP-ribose) synthetase inhibitor. *Ann. Thorac. Surg.* **73**, 575–581 (2002).
136. Szabó, G. *et al.* Poly(ADP-ribose) polymerase inhibition reduces reperfusion injury after heart transplantation. *Circ. Res.* **90**, 100–106 (2002).
137. Goldfarb, R. D. *et al.* Protective effects of a novel, potent inhibitor of poly(adenosine 5'-diphosphate-ribose) synthetase in a porcine model of severe bacterial sepsis. *Crit. Care Med.* **30**, 974–980 (2002).
138. Mabley, J. G. *et al.* Anti-inflammatory effects of a novel, potent inhibitor of poly(ADP-ribose) polymerase. *Inflamm. Res.* **50**, 561–569 (2001).
139. Pacher, P., Liaudet, L., Mabley, J., Komjati, K. & Szabó, C. Pharmacologic inhibition of poly(adenosine diphosphate-ribose) polymerase may represent a novel therapeutic approach in chronic heart failure. *J. Am. Coll. Cardiol.* **40**, 1006–1016 (2002).
140. Pacher, P. *et al.* The role of poly(ADP-ribose) polymerase activation in the development of myocardial and endothelial dysfunction in diabetes. *Diabetes* **51**, 514–521 (2002).
141. Pacher, P. *et al.* Endothelial dysfunction in aging animals: the role of poly(ADP-ribose) polymerase activation. *Br. J. Pharmacol.* **135**, 1347–1350 (2002).
142. Iwashita, A. *et al.* Neuroprotective effects of a novel poly(ADP-ribose) polymerase-1 inhibitor, 2-[3-[4-(4-chlorophenyl)-1-piperazinyl] propyl]-4(3H)-quinazolinone (FR255595), in an *in vitro* model of cell death and in mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *J. Pharmacol. Exp. Ther.* **309**, 1067–1078 (2004).
143. Iwashita, A. *et al.* A novel and potent PARP-1 inhibitor, 5-chloro-2-[3-(4-phenyl-3, 6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone (FR247304), attenuates neuronal damage *in vitro* and *in vivo* models of cerebral ischemia. *J. Pharmacol. Exp. Ther.* **310**, 425–436 (2004).
144. Iwashita, A. *et al.* A new poly(ADP-ribose) polymerase inhibitor, 2-(4-chlorophenyl)-5-quinoxalinecarboxamide (FR261529), ameliorates methamphetamine-induced dopaminergic neurotoxicity in mice. *J. Pharmacol. Exp. Ther.* **310**, 1114–1124 (2004).
145. Kamanaka, Y. *et al.* Neuroprotective effects of ONO-1924H, an inhibitor of poly(ADP-ribose) polymerase (PARP), on cytotoxicity of PCl2 cells and ischemic cerebral damage. *Life Sci.* **76**, 151–162 (2004).
146. Pellicciari, R. *et al.* Towards new neuroprotective agents: design and synthesis of 4H-thieno[2,3-c] isoquinoline-5-one derivatives as potent PARP-1 inhibitors. *Farmaco* **58**, 851–858 (2003).
147. Chiarugi, A. *et al.* Novel isoquinolinone-derived inhibitors of poly(ADP-ribose) polymerase-1: pharmacological characterization and neuroprotective effects in an *in vitro* model of cerebral ischemia. *J. Pharmacol. Exp. Ther.* **305**, 943–949 (2003).
148. Nakajima, H., Kakui, N., Ohkuma, K., Ishikawa, M. & Hasegawa, T. A newly synthesized poly(ADP-ribose) polymerase inhibitor. 2-methyl-3,5,7,8-tetrahydrothipyrano[4,3-d]pyrimidine-4-one (DR2313): pharmacological profiles, neuroprotective effects, and therapeutic time window in cerebral ischemia in rats. *J. Pharmacol. Exp. Ther.* **312**, 472–481 (2005).

149. Ferraris, D. *et al*. Design and synthesis of poly ADP-ribose polymerase-1 inhibitors. 2. Biological evaluation of aza-5[H]-phenanthridin-6-ones as potent, aqueous-soluble compounds for the treatment of ischemic injuries. *J. Med. Chem.* **46**, 3138–3151 (2003).
150. Ferraris, D. *et al*. Design and synthesis of poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors. Part 3: In vitro evaluation of 1,3,4,5-tetrahydro-benzo[c][1,6]- and [c][1,7]-naphthyridin-6-ones. *Bioorg. Med. Chem. Lett.* **13**, 2513–2518 (2003).
151. White, A. W. *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.* **46**, 3138–3151 (2003).
152. Canan-Koch, S. S. *et al.* Novel tricyclic poly(ADP-ribose) polymerase-1 inhibitors with potent anticancer chemopotentiating activity: design, synthesis, and X-ray cocrystal structure. *J. Med. Chem.* **45**, 4961–4974 (2002).
153. Skalitzky, J. D. *et al.* Tricyclic benzimidazoles as potent poly(ADP-ribose) polymerase-1 inhibitors. *J. Med. Chem.* **46**, 210–213 (2003).
154. Calabrese, C. R. *et al.* Identification of potent nontoxic poly(ADP-ribose) polymerase-1 inhibitors: chemopotentiation and pharmacological studies. *Clin. Cancer Res.* **9**, 2711–2718 (2003).
155. Tikhe, J. G. *et al.* Design, synthesis, and evaluation of 3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-ones as inhibitors of poly(ADP-ribose) polymerase. *J. Med. Chem.* **47**, 5467–5481 (2004).
156. Curtin, N. *PARP-1: A New Target for Cancer Treatment*. Cancer Research UK Scientific Yearbook 52–54 (2002–03).
157. Farraris, D. *et al.* Design and synthesis of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. Part 4: biological evaluation of imidazobenzodiazepines as potent PARP-1 inhibitors for treatment of ischemic injuries. *Bioorg. Med. Chem.* **11**, 3695–3707 (2003).
This paper describes imidazobenzodiazepines and their effects in streptozotocin-induced diabetes and focal cerebral ischaemia.
158. Zimmermann, K., Portmann, R. & Rigel, D. Indoloquinazolinones. US Patent 0199505 A (2003).
159. Miknyoczki, S. J. *et al.* Chemopotentiation of temozolomide, irinotecan, and cisplatin activity by CEP-6800, a novel poly(ADP-ribose) polymerase inhibitor. *Mol. Cancer Ther.* **2**, 371–382 (2003).
160. Jagtap, P. G., Baloglu, E., van Duzer, J. H., Szabó, C. & Salzman, A. Substituted indeno[1,2-c]isoquinoline derivatives and methods of use thereof. US Patent 6,828,319 (2004).
161. Komjati, K. *et al.* Poly(ADP-ribose) polymerase inhibition protect neurons and the white matter and regulates the translocation of apoptosis-inducing factor in stroke. *Int. J. Mol. Med*. **13**, 373–382 (2004).
162. Woolley, S. M. *et al*. Role of poly (ADP) ribose synthetase in lung ischemia–reperfusion injury. *J. Heart Lung Transplant*. **23**, 1290–1296 (2004).
163. Xiao, C. Y. *et al.* Poly(ADP-ribose) polymerase promotes cardiac remodeling, contractile failure and translocation of apoptosis-inducing factor in a murine experimental model of aortic banding and heart failure. *J. Pharmacol. Exp. Ther.* **3/2**, 891–898 (2005).
164. Farivar, A. S. *et al.* Intratracheal poly (ADP) ribose synthetase inhibition ameliorates lung ischemia reperfusion injury. *Ann. Thorac. Surg.* **77**, 1938–1943 (2004).
165. Murthy, K. G., Xiao, C. Y., Mabley, J. G., Chen, M. & Szabó, C. Activation of poly(ADP-ribose) polymerase in circulating leukocytes during myocardial infarction. *Shock* **21**, 230–234 (2004).
166. Li, F., Drel, V. R., Szabó, C., Stevens, M. J. & Obrosova, I. G. Low dose PARP inhibitor-containing combination therapies reverse early experimental diabetic neuropathy. *Diabetes* (in the press).
167. Iwashita, A. *et al.* Discovery of quinazoline and quinoxaline derivatives as potent and selective poly(ADP-ribose)polymerase-1/2 inhibitors. *FEBS Lett.***579**, 1389–1393 (2005).
168. Popoff, I. *et al.* Antisense oligonucleotides to poly(ADP-ribose) polymerase-2 ameliorate colitis in interleukin-10-deficient mice. *J. Pharmacol. Exp. Ther.* **303**, 1145–1154 (2002).
169. Bryant, H. E. *et al*. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434**, 913–917 (2005).
170. Farmer, H. *et al*. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434**, 917–921 (2005).

Acknowledgements
Work in the area of PARP and the pathogenesis of various diseases was supported by grants from the US National Institutes of Health to C.S. and P.J. The authors thank G. Graziani of the University of Rome for helpful discussions.

Competing interests statement
The authors declare competing financial interests. See Web version for details.

Online links

DATABASES
The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ATF2 | ICAM1 | IL-6 | PARP1 | PARP2 | TNF-α | WRN | XRCC1
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
atherosclerosis | Parkinson disease

FURTHER INFORMATION
Inotek Pharmaceuticals: http://www.inotekcorp.com
Northern Institute for Cancer Research:
http://www.ncl.ac.uk/nicr/research/trials/med/melanoma.htm
Access to this interactive links box is free online.