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Pathophysiologic role of oxidative stress-induced poly(ADP-ribose) polymerase-1 activation: focus on cell death and transcriptional regulation

K. Erdélyi[a], E. Bakondi[a], P. Gergely[a], C. Szabó[b] and L. Virág[a,*]

[a] Department of Medical Chemistry, Research Center for Molecular Medicine, University of Debrecen, Bem tér 18/B, 4026 Debrecen (Hungary), e-mail: lvirag@dote.hu
[b] Inotek Pharmaceutical Corp., 100 Cummings Center Suite #419E, Beverly, Massachusetts 01915 (USA)

Abstract. PARP-1 is a nuclear enzyme activated by DNA breaks. Activated PARP-1 cleaves NAD into nicotinamide and ADP-ribose and polymerizes the latter covalently coupled to nuclear acceptor proteins. Poly(ADP-ribosyl)-ation has been implicated in the regulation of a diverse array of cellular processes ranging from DNA repair, chromatin organization, transcription, replication to protein degradation. On the 'dark side' of poly(ADP-ribosyl)ation, PARP-1 activation has been shown to contribute to tissue injury in shock, diabetes, myocardial or cerebral ischemia reperfusion and various forms of inflammation, as proven by pharmacological studies as well as experiments utilizing PARP-1 knockout animals. To our current knowledge, two mechanisms are responsible for the beneficial effects of PARP inhibitors in inflammatory, neurodegenerative and ischemia-reperfusion-based diseases: (i) inhibition of cell death caused by over-activation of PARP-1; (ii) inhibition of inflammatory signal transduction and production of inflammatory mediators. Here we review the possible regulatory mechanisms (e.g. calcium signaling, metabolism, density-dependent signaling, kinase cascades) of the PARP-1-mediated cell death pathway and discuss recent developments shedding new light on the complex role of PARP-1 in the regulation of the expression of inflammatory mediators.

Key words. Poly(ADP-ribose) polymerase; cytotoxicity; calcium signal; necrosis; apoptosis; mitochondria; DNA damage; peroxynitrite

Introduction

Poly(ADP-ribose) polymerase 1 (PARP-1), the 'founding member' of the PARP enzyme family is a nuclear enzyme responsible for the bulk of poly(ADP-ribosyl)ation [attachment of NAD^+-derived, long, branching $(ADP\text{-}ribose)_n$ polymers to acceptor proteins such as PARP-1 itself, histones, DNA repair proteins and transcription factors] following DNA damage [1–3]. By conferring negative charges to acceptors, this covalent protein modification alters the physico-chemical properties of the modified target proteins and thus regulates (inhibits) their function. In addition, protein-protein interactions between PARP-1 and an increasing set of partner proteins may also influence important cellular processes ranging from facilitation of transcription (e.g. NFκB, p53-mediated gene activation) to DNA repair [4]. It is a subject of debate whether or not PARP enzymatic activity is required for this latter type of regulation exerted by PARP-1 [5]. The third mechanism proposed to be responsible for the regulatory effects of poly(ADP-ribosyl)ation is polymer signaling. $(ADP\text{-}ribose)_n$oligomers are generated by poly-(ADP-ribose) glycohydrolase (PARG) responsible for the rapid degradation of poly(ADP-ribose). Liberated polymers can non-covalently but rather strongly bind to certain proteins in a sequence-specific manner [6–9]. Poly(ADP-ribose) binding motives contain basic and hydrophobic amino acids, and non-covalent poly(ADP-ribose) binding may also alter the function of the modified proteins.

* Corresponding author.

Through one or more of the above-detailed three mechanisms, PARP-1 exerts a wide array of biological activities ranging from enhancing base excision DNA repair, maintenance of genomic integrity, replication, transcription and proteasomal protein degradation [10]. In light of this vital role of PARP-1 in various fundamental cellular processes, it may appear surprising that inhibition of PARP activity proved beneficial in many disease models [10]. What these models have in common is two features: (i) a severe oxidative stress leading to very intense DNA breakage and PARP activation and (ii) an inflammatory component characterized by inflamatory cell migration and production of cytokines. By consuming NAD^+, the substrate of PARP-1, overactivation of the enzyme may deplete the cells from their energy metabolites and thus may execute the cells by necrosis [10]. This PARP-1-mediated cellular suicide mechanism first described by Nathan Berger [11] provided a feasible explanation for the deleterious consequences of PARP activation and the beneficial effects of PARP inhibition as observed in stroke and myocardial ischemia-reperfusion injury. On the other hand, an increasing body of evidence suggests that PARP-1 is part of the NFκB-driven transcriptosome and thus contributes to the synthesis of inflammatory mediators [5, 12–14]. Here we review the possible control points of the PARP-mediated suicidal pathway and discuss the role of PARP-1 in the production of inflammatory mediators.

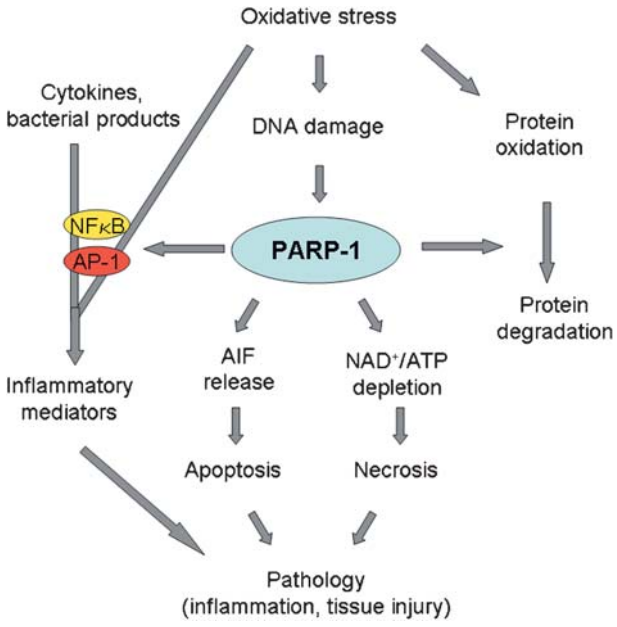

Figure 1. The central role of PARP-1 in oxidative stress-related pathology. Oxidative-stress-induced DNA strand breakage triggers the activation of PARP-1, leading to AIF release from the mitochondria and AIF-mediated, caspase-independent apoptotic cell death NAD/ATP consumption and consequent necrotic cell death. Oxidative stress also stimulates activation of redox-sensitive transcription factors such as NFκB and AP-1, key regulators of inflammatory cytokines and chemokines. Poly(ADP-ribosyl)ation is also required for the proteasomal degradation of oxidatively damaged proteins.

PARP-1 and cell death

Several lines of evidence suggest that poly(ADP-ribosyl)-ation enhances DNA repair [15–17] and thus promotes cell survival. However, when the intensity of DNA damage exceeds the capacity of the repair machineries, then high ADP-ribose turnover is considered as an NAD^+/ATP-depleting futile cycle killing the cells by energetic catastrophy [10, 11, 18]. Thus, the vulnerability of severely DNA-damaged cells arises from the lack of control mechanisms which would downregulate PARP activity under conditions of severe, unrepairable DNA damage. This, however, does not mean that PARP-1 is not regulated. The following mechanisms are known or are likely to affect PARP activation and PARP-1-mediated cell death:

1) Regulation of PARP-1 expression

PARP-1 protein occurs in a high copy number in most cells of multicellular eukaryotes with the notable exception of terminally differentiated granulocytes [19]. DNA damage induces a rapid increase (within a few minutes) in cellular PARP activity. Thus the regulation of PARP takes place mainly at the activation of preexisting PARP-1 enzyme, rather than at the level of PARP-1 gene expression. Nonetheless, it has been reported that PARP-1 expression can be stimulated. For example, Liu et al. [20] demonstrated that PARP messenger RNA (mRNA) increased in the dentate gyrus (part of the hyppocampus) of gerbil brains 4 h after 10 min of global ischemia, and returned to basal levels 8 h after ischemia. Kainic acid injection also induced a marked elevation in PARP mRNA level selectively in the dentate gyrus of rat brains 1 h following the injection, and returned to basal levels 4 h after the injection [20]. Of note, the dentate gyrus is resistant to both ischemia and to kainate-induced seizure-related damage. Increased expression of PARP-1 mRNA may either represent an adaptive response to chronic stress situations aiming at preparing the cells for dealing with constant DNA damage or may be associated with the proliferative response of dentate granule cells. This latter scenario is also supported by other findings reporting increased PARP-1 expression in phytohemagglutinin-induced lymphocyte proliferation and in proliferating hepatocytes during liver regeneration [21, 22]. Whether changes in PARP-1 mRNA level are also accompanied by elevated PARP-1 protein content and activity and whether elevated PARP-1 protein levels affect the cells' ability to cope with DNA damage remains to be investigated.

2) Calcium signal

Of the wide array of genotoxic and PARP-activating stimuli, oxidative stress and ultraviolet (UV) radiation are also known to elevate intracellular calcium levels [23]. The sources of elevated [Ca^{2+}_i] may include both intracellular stores (e.g. endoplasmic reticulum) and the extracellular compartment. The calcium signal contributes to cell death in various models, including oxidative stress-induced cytotoxicity [23]. Buffering intracellular calcium by cell-permeable chelators [BAPTA-AM, EGTA-AM, Quin-2-AM] has been shown by us to protect thymocytes and other cell types from oxidative stress-induced apoptotic and necrotic cell death [24, 25]. These chelators targeted an early step of the cytotoxic pathway as indicated by their inhibitory effects on all death parameters measured. Interestingly, these chelators abolished oxidative stress-induced PARP activation in thymocytes with no effect on the activity of the purified PARP-1 enzyme [25]. Moreover, the chelators likely inhibit a step upstream from PARP activation and DNA damage, as single strand breakage was also blocked by these agents [25]. The calcium signal is proximal to the mitochondrial perturbations, because the calcium chelators (similarly to PARP inhibition [26]) efficiently blocked the collapse of mitochondrial membrane potential, secondary superoxide generation and mitochondrial membrane damage. These findings are consistent with reports from Cantoni's lab proving that many oxidative species do not directly break DNA. Instead they proposed that oxidative agents primarily target mitochondria and induce the mitochondrial production of secondary radicals in a calcium-dependent manner [27, 28].

Furthermore, calcium signal also plays a role in a fast signal-induced activation of PARP-1. Homburg et al. [29] have demonstrated increased poly(ADP-ribosyl)ation in neuronal cells depolarized either by increased extracellular [K] or by pulsed electric stimulation. Depolarization was accompanied by a rise in [Ca^{2+}_i], the chelation of which by BAPTA-AM abolished PARP activation. This fast calcium-mediated PARP activation could also be elicited by IP_3 (inositol 1,4,5 triphosphate) stimulation of isolated nuclei. The uniqueness of these findings was that PARP-1 activation occurred in the absence of detectable DNA breakage. Moreover, they further supported the existence of a connection between calcium signaling and PARP activation. From a recent report from Kun's and Ordahl's laboratory, it appears that calcium's effect on PARP activity is not as indirect as one may assume from the above detailed publications [30]. They showed that despite previous thinking, PARP-1 activity may be directly modulated by divalent cations. Although neither 3 mM Ca^{2+} nor 3 mM Mg^{2+} have affected PARP-1 activity, the combination of the two ions increased PARP-1 activity. This finding underscores the importance of the composition of the ionic milieu in the regulation of PARP-1 activity.

3) Metabolic status and cellular microenvironment

A high degree of variability in the intensity of genotoxic stress-induced PARP activation and cell death could be observed between different cell types. The question has arisen what factors may determine the cellular response to genotoxic stress. Several such factors, including cellular metabolic state, the cells' microenvironment as well as external stimuli, should be considered as susceptibility modifying factors.

The importance of cellular metabolism is underscored by observations that cells capable of utilizing the glycolytic pathway are resistant to nitric oxide(NO)-induced cell death. Almeida et al. [31] have reported differential sensitivity of neuronal cells and astrocytes to NO. Whereas NO inhibited oxygen consumption and triggered mitochondrial depolarization and cell death in neurons, astrocytes responded to NO-induced inhibition of oxygen consumption by mobilizing glucose from their glycogen stores, resulting in preservation of cellular ATP stores, mitochondrial hyperpolarisation and resistance to cell death. The higher sensitivity of neuronal cultures was due to the lack of glycogen stores and the inability to utilize the glycolytic pathway. Recently, the same group also showed that the mechanism by which NO stimulates glycolysis in astrocytes involves activation of phosphofructo-2-kinase in a protein kinase A-dependent manner [32]. The hypothesis that glycolytic metabolism renders cells less sensitive to genotoxic stimuli is also supported by findings from Swanson's lab. They found that fueling the tricarboxylic acid cycle by utilizable substrates such as oxaloacetate or pyruvate protects both neurons and astrocytes from PARP-mediated cell death induced by the alkylating agent MNNG (N-methyl-N′-nitro-N-nitrosoguanidine) [33]. Thus, cells may protect themselves from the deleterious consequences of ATP-depleting stimuli, including genotoxic, PARP-activating noxa.

The microenvironment may also have an impact on the outcome of genotoxic stress. In our previous work we have demonstrated a remarkable resistance of high-density HaCaT cell cultures to peroxynitrite, hydrogen peroxide or superoxide-induced cytotoxicity [24]. Moreover, these agents failed to induce PARP activation and caspase activation in high-density cultures as compared to subconfluent ones. Recently, similar findings have been reported in primary mouse fibroblasts treated with different DNA-damaging agents such as cisplatin, campthotecin or 4-NQO (4-nitroquinoline 1-oxide; generator of bulky DNA adduct) [34]. In these experiments, defective p53 activation resulting from decreased p53 stabilization was identified as the mechanism underlying the resistance of high-density cultures [34]. Given the functional relathionship between PARP-1 and p53, it would be interesting to investigate whether these abnormalities in p53 activation are related to inhibition of PARP activation in high-density cultures. Moreover, the signaling route link-

ing high-density to decreased p53 stabilization also needs to be established.

4) Reversible protein phosphorylation

may represent an additional mechanism regulating DNA damage-induced PARP activation and cell death. Protein kinases and phosphatases mediate a variety of cellular responses, including the oxidative stress response. Whereas some are known to protect against cell death, others are considered mediators of cytotoxicity. For example exposure of SH-SY5Y neuroblastoma cells to peroxynitrite caused a transient increase in MAP kinase activity, and resulted in cell death which could be blocked by PD98059, a selective inhibitor of MAP kinase kinase [35]. (Of note, deficient MAP kinase activation may also explain the resistance of high-density cultures to oxidative stress [36].) On the other hand, the G-protein-coupled receptor-inositol-1,4,5-triphoshate (PI3) kinase pathway was proposed to counteract peroxynitrite toxicity in primary rat astrocytes [37]. Although kinases are known to regulate various steps of the apoptotic response, it cannot be excluded that their apoptosis-modifying effect is mediated, at least in part, via modulation of PARP activation. Indeed, PARP-1 has been shown to serve as substrate for protein kinase C (PKC) [38]. Phosphorylation of PARP-1 by PKC inhibits DNA binding and activation of PARP-1 [39]. Therefore it is plausible to assume (and is also supported by our unpublished observations) that stimuli causing PKC activation may lead to PARP inhibition and mitigation of DNA damage-induced cell death. Moreover, PARP-1 interacts with DNA-dependent protein kinase (DNA-PK) [40]. PARP-1 has also been shown to be phosphorylated by DNA-PK, resulting in downregulation of enzymatic activity [41]. Whether other kinases also phosphorylate PARP-1 and whether these modifications affect enzymatic activity of the enzyme remains to be seen.

5) Automodification

PARP-1 automodifies itself in an intermolecular autopoly(ADP-ribosyl)ation reaction. In fact, PARP-1 is one of the main acceptors of the $(ADP\text{-}ribose)_n$ polymer. As automodification results in PARP-1 inhibition, degradation of the polymer by PARG may be required to reactivate PARP-1 by removing the inhibitory polymers from the enzyme. Thus, reactivated PARP may carry out multiple poly(ADP-ribosyl)ation cycles, which may cause cytotoxicity via NAD^+/ATP depletion. Thus far, this hypothesis has only been tested in pharmacological experiments utilizing tannins (e.g. gallotannin and nobotanin B) as PARG inhibitors. These naturally occuring PARG inhibitory compounds provided cytoprotection in two PARP-mediated cytotoxicity systems: N-methyl-D-aspartate (NMDA)-stimulated neurons [42] and oxidatively-stressed HaCaT cells [43], as well as in a mouse stroke model [42]. It has also been shown that these tannins lead to accumulation of poly(ADP-ribose) in the cells [42–44]. Recently, using two not commercially available non-tannin PARG inhibitors (GPI 16552 and GPI 18214), significant protection has been reported in animal models of stroke [45] and zymozan-induced multiple organ failure [46]. As these beneficial effects were very similar to the ones obtained previously with PARP inhibitors, it was proposed that PARG inhibition may break the futile, energy-consuming poly(ADP-ribose) cycle by maintaining the inactive, auto-poly(ADP-ribosyl)ated form of PARP-1. This hypothesis was challenged by recent findings from Miwa's laboratory reporting that at the normal development temperature of 25 °C, PARG-deficient *Drosophila* mutants display lethality in the larval stages [47]. At higher temperatures (29 °C) one-fourth of the mutants progressed to the adult stage but showed progressive neurodegeneration with reduced locomotor activity and a short lifespan. (Of note, in zebrafish embryos PARG mRNA expression is most pronounced in the caudal primary motoneurones [48], a finding that may explain neurodegeneration in PARG-deficient conditions.) Similarly, PARG-deficient mice generated in Valina Dawson's laboratory proved embryonic lethal. PARG knockout mice lacking only the full-length isoform but not the truncated ones generated in Wang's laboratory were viable and fertile [49]. These mice, like the PARP-1 knockout mice, proved hypersensitive to alkylating agents and ionizing radiation and, unlike the PARP-1 knockout mice, were also susceptible to streptozotocin-induced diabetes and endotoxic shock [49]. At the moment it would be too premature to draw conclusions regarding the role of PARG in cell death either from the pharmacological experiments or from data obtained with the knockout/mutant mice/*Drosophila*. Tannin-based PARG inhibitors are known to have various additional (e.g. antioxidant) effects which may be responsible for the beneficial in vivo effects. On the other hand, we should not rely solely on knockout/mutant studies either, as they reflect a situation in which the PARG protein is missing which may have different effects from PARG inhibition. One should not exclude the possibility that, similarly to PARP-1, PARG may also regulate cellular functions via protein-protein interactions with yet unidentified partner molecules rather than via enzymatic activity. In order to see clearer in this issue, potent and specific PARG inhibitors, molecular tools (antisense and inhibitory RNAs) and a systematic analysis of knockout cells are clearly needed.

PARP-1 and the production of inflammatory mediators

Until 1999, the 'suicide hypothesis' was considered a key mechanism explaining the protective effects of PARP

Table 1. Poly(ADP-ribosyl)ation regulates transcription factor activation.

Transcription factor	Model/inhibitor	Stimulus	Observation	Reference
NFκB	in vitro	N/A	poly(ADP-ribosyl)ation inhibits NFκB p50/p65 DNA binding activity	[59]
NFκB	in vitro	N/A	PARP-1 binds NFκB better when not modified, Non-poly-(ADP ribosyl)ated PARP-1 is able to specifically bind NFκB-p50 and to block its sequence-specific DNA binding; the modification results in upregulation of gene expression.	[13]
NFκB	PARP$^{-/-}$ fibroblast	TNFα , H_2O_2	decreased NFκB activation, but normal nuclear translocation	[14]
	macrophages	LPS	decreased NFκB activation	
NFκB	PARP$^{-/-}$ fibroblast	TNFα, MNNG, H_2O_2 , LPS	decreased κB-dependent gene expression in PARP$^{-/-}$ cells but no effect of 3-AB on the κB-dependent gene expression	[51]
	PARP$^{+/+}$ fibroblast +3AB	TNFα, LPS		
	HeLa	TNFα	p65/p50 interacts with PARP-1	
NFκB	mice macrophage and fibroblast inhibitor	LPS	decreased DNA binding activity	[60]
NFκB	rat lymphocytes + PHE, BZD	PMA/ ionomycin	decreased DNA binding activity	[61]
NFκB	lung epithelial cell (A549) 3AB	H_2O_2	decreased DNA binding activity and IL8 gene expression	[62]
	PARP$^{-/-}$ fibroblast			
NFκB	mice liver PJ34	LPS	decreased NFκB activation	[63]
			activation of PI3-kinase-AKT/protein kinase B cytoprotective pathway	
NFκB	rat colitis	TNBS	decreased DNA binding activity	[64]
	+3AB, DHQ			
NFκB–p50	in vitro	N/A	PARP-1 acts as an essential coactivator of NFκB in concert with p300 to facilitate the formation and subsequent activation of the pre-initation complex in a stimulus-dependent manner	[65]
NFκB	PARP$^{-/-, +/+}$ murine heart	TNFα	defective NFκB transcriptional activation in the knockout cells	[66]
	endothelial cell		several NFκB-dependent genes were dramatically inhibited in the absence of PARP, while the expression of other NFκB-dependent genes was upregulated in PARP deficient cells	
NFκB, AP-1	mice liver, spleen, lung 4HQN	LPS	4HQN decreased NFκB and AP-1 activation	[57]
			4HQN activated the PI-3-kinase/Akt pathway and downregulated p38 and Erk1/2 MAP kinases	
AP-1	rat lymphocytes + PHE, BZD	PMA/ionomycin	decreased DNA binding activity	[61]
AP-1	rat colitis +3AB, DHQ	TNBS	decreased DNA binding activity	[64]

Table 1. (continued)

Transcription factor	Model/inhibitor	Stimulus	Observation	Reference
AP-1	PARP$^{-/-}$ fibroblast	$ONOO^-$, H_2O_2, IL-1β	the PARP-1 knockout phenotype or INH_2BP treatment inhibits the DNA binding of AP-1	[55]
	+ INH_2BP		increased basal and peroxynitrite-stimulated JNK activity and c-jun phosphorylation in knockout cells	
			reduced hydrogen peroxide- or IL-1-induced MEK4 phosphorylation in knockout cells (no effect of INH_2BP)	
NFκB, AP-1 Oct-1, SP-1, Stat-1, YY-1	PARP$^{-/-}$ glial cell	TNFα, LPS	decreased DNA binding activity of all tested transcription factors in PARP-1$^{-/-}$ cells	[52]
	+ DPQ		DPQ had no effect on LPS-induced NFκB DNA binding	
ATF-2 CREB p65	PARP$^{-/-}$ and PARP$^{+/+}$ glial cells	LPS or TNFα IL-1β	defective activation of ATF-2, CREB and NFκB p65 in knockout cells	[58]
			defective p38 MAP kinase activation in the cytokine-stimulated knockout glial cells but not in LPS-stimulated knockout primary macrophages	
	DPQ		DPQ had no effect on p38 activation in the glial cells	

INH_2BP, 5-iodo-6-amino-1,2-benzopyrone; PHE, 6(5H)-phenanthridione; BZD, benzamide; PI3 kinase, phosphatidylinositol 3-kinase; DPQ, 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1-(2H)-isoquinolinone.

inhibition in various oxidative stress-induced diseases. Our understanding of the role of PARP in these diseases has been changed by de Murcia's observation identifying PARP-1 as a coactivator of NFκB, the key inflammatory transcription factor [14]. In most cells, Rel/NF-kB is present as a latent, inactive, IκB (inhibitor κB)-bound complex in the cytoplasm. When a cell receives any of a multitude of extracellular signals, phosphorylation of IκB induces proteasomal degradation of the inhibitor. In turn, NF-κB rapidly enters the nucleus and activates gene expression by binding to 9–10 bp DNA sites (called κB sites) as dimers. All vertebrate Rel proteins can form homodimers (e.g. p50-p50, p65-p65 and c-Rel-c-Rel) or heterodimers (e.g. p50-p65), except for RelB, which can only form heterodimers (e.g. p50-RelB, p65-RelB) [50]. De Murcia's group identified deficient NFκB activation as the underlying mechanism of the endotoxin resistance of PARP$^{-/-}$ mice [14]. In tumor necrosis factor α (TNFα)-treated fibroblasts, IκB degradation and nuclear translocation of p65 was found unaltered in PARP$^{-/-}$ cell whereas DNA binding of p65 was markedly reduced. Thus it was proposed that PARP-1 is required for NFκB-mediated transactivation. Subsequent work by Hassa and Hottiger [51] has demonstrated that DNA binding and catalytic activity of PARP-1 are not required for the NFκB coactivator function. They proposed that PARP-1 physically interacts with the NFκB-p50, resulting in enhanced transactivation. In apparent contradiction with this model, Chang and Alvarez-Gonzalez found that non-poly-ADP-ribosylated PARP-1 can bind to NFκB-p50+consensus oligonucleotide complex [13]. However, addition of NAD^+ to the reaction mixture resulted in dissociation of PARP-1 from NFκB-p50 in a 3-aminobenzamide-inhibitable manner. These latter data suggest that PARP-1 enzyme activity regulates the interaction between PARP-1 and NFκB-p50. However, it is not yet clear, which polymer acceptor proteins are involved in the regulation of PARP-1-NFκB binding, NFκB itself or other costimulatory proteins such as HMG-I (high mobility group I protein). The requirement for PARP-1 enzymatic activity for the NFκB coactivator function is also in line with the anti-inflammatory effect of PARP inhibitors, as reflected by suppressed production of inflammatory mediators in animals treated with PARP inhibitors. Interestingly, the requirement of PARP activity for the transcriptional regulatory role of PARP-1 appears to largely depend on the inflammatory signal as well as on the cell type [52]. Nonetheless, the exact nature of the PARP-1-NFκB connection remains to be elucidated.

PARP-1 has also been implicated in the regulation of AP-1-driven transcriptional activity. The redox-sensitive transcription factor AP-1 is composed of a mixture of heterodimeric protein complexes derived from the Fos and Jun families, including c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB and JunD [53, 54]. AP-1 is induced by multiple stimuli, including the presence of serum, growth factors, phorbol esters and oncogenes. AP-1 heterodimers bind to DNA on a serum response element with the 5′-TGA(C/G)TCA-3′ sequence. Jun proteins can form transcriptionally active homodimers or heterodimers with CREB/ATF members to bind the CRE element. Phosphorylation of AP-1 family members by kinases is

required for transactivation activity. AP-1 is regulated at the level of both *jun* and *fos* gene transcription and by posttranslational modifications of their gene products. Synthesis of c-Fos and the *trans*-activating capacity of c-Jun and c-Fos have been shown to be regulated by the mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinases (ERKs), Jun N-terminal kinases (JNKs) and Fos-regulating kinase (FRK), respectively.

AP-1 activation is mediated mainly by phosphorylation of c-Jun by JNK [53, 54]. Zingarelli's group reported alterations in AP-1 activation in oxidatively stressed or interleukin (IL)-1-treated murine PARP-1 knockout fibroblasts [55]. They found decreased AP-1 DNA binding in PARP$^{-/-}$ cells after peroxynitrite, hydrogen peroxide or IL-1 stimuli. Furthermore, increased basal JNK activity and c-Jun phosphorylation were found to accompany these changes. Later, in a myocardial reperfusion injury model, the same group reported that cardioprotection observed in PARP$^{-/-}$ mice was associated with a reduction of the phosphorylative activity of JNK and, subsequently, reduction of the DNA binding of the signal transduction factor AP-1 [56]. Microarray analysis revealed that expression of several AP-1-dependent genes of proinflammatory mediators and heat shock proteins was altered in PARP-1$^{-/-}$ mice [56].

These interesting novel findings have implicated PARP-1 in upstream events of inflammatory signaling. Recent data from Sümegi's group provided further support for the role of PARP-1 in upstream signaling events [57]. They found that PARP inhibitors increased survival and suppressed inflammation in endotoxin shock. These beneficial effects were accompanied by decreased MAP kinase activation. MAP kinases (ERK1/2, p38 and JNK) are known to mediate lipopolysaccharide (LPS)-induced inflammation [57]. Treatment of animals with the PARP inhibitor 4-hydroxyquinazoline decreased phosphorylation (i.e. activation) of p38 and ERK1/2 but not JNK in a tissue-specific manner. Shortly thereafter, Ha has reported deficient p38 signaling in immune-stimulated PARP-1 deficient (PARP-1$^{-/-}$) microglial cells and fibroblasts but not in PARP-1$^{-/-}$ macrophages [58]. These findings support the hypothesis that PARP-1 regulates MAP kinase activation by a yet unidentified mechanism. As MAP kinases are crucial signal transducers in some signaling pathways but are dispensible for others, these findings may explain stimulus dependency of PARP-1's effect on NFκB activation.

The transcriptional co-activator role of PARP-1 in NFκB- and AP-1-mediated transcription is consistent with previous findings reporting inhibition of expression of various key molecules of the immune response and inflammation such as major histocompatibility (MHC)-II molecules, inducible NO synthase, adhesion molecules and collagenase in PARP-inhibited or knockout cells. Nonetheless, it must be noted that the function of PARP-1 and poly(ADP-ribosyl)ation as a regulator of inflammatory signal transduction and transactivation shows marked cell-type and stimulus dependency. Therefore, care should be taken to avoid generalization of findings and to evaluate experimental findings in the context of the actual work. An interesting issue to be investigated will be the effect of PARG on the transcriptional regulatory role of PARP-1. A cohort of data in the literature supports potent anti-inflammatory effects of gallotannin. Our unpublished data also indicate that gallotannin exerts a very potent inhibitory effect on inflammatory signal transduction leading to downregulation of a high number of inflammatory mediators. Whether or not these effects are due to PARG inhibition or result from PARG-unrelated pathways requires further investigation.

Conclusions

Inhibition of poly(ADP-ribose) polymerases provides remarkable therapeutic benefits in various acute, often life-threatening diseases (e.g. reperfusion injury, septic and hemorrhagic shock, stroke) as well as in chronic inflammations (e.g. arthritis, experimental allergic encephalomyelitis, asthma). These beneficial effects likely result from improvement of cellular energetic status leading to cell survival and from inhibition of inflammatory signal transduction leading to suppressed expression of inflammatory mediators. The actual contribution of these two mechanisms to the effect of PARP inhibitors in various disease models may likely differ to a great extent. Future investigations will aim at revealing further details on the role of poly(ADP-ribosyl)ation in oxidative stress-related pathologies and should also identify potential risks associated with short-term or long-term PARP inhibitory treatments.

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