CMLS, Cell. Mol. Life Sci. 62 (2005) 760–768
1420-682X/05/080760-09
DOI 10.1007/s00018-004-4508-y

CMLS Cellular and Molecular Life Sciences

Poly(ADP-ribosyl)ation regulation of life and death in the nervous system

D. W. Koh [a, b], T. M. Dawson [a–c] and V. L. Dawson [a–d, *]

[a] Institute for Cell Engineering, Johns Hopkins University School of Medicine, 733 N. Broadway St., Suite 711, Baltimore, Maryland 21205 (USA), Fax: +1 410 614 9568, e-mail: vdawson@jhmi.edu
[b] Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287 (USA)
[c] Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287 (USA)
[d] Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287 (USA)

Abstract. Poly(ADP-ribosyl)ation is required by multicellular eukaryotes to ensure genomic integrity under conditions of mild to moderate genotoxic stress. However, severe stress following acute neuronal injury causes overactivation of poly(ADP-ribose) polymerase-1, which results in unregulated poly(ADP-ribose) (PAR) synthesis and widespread neuronal cell death. Once thought to be a necrotic cell death resulting from energy failure, PARP-1 activation is now known to induce the nuclear translocation of apoptosis-inducing factor, which results in caspase-independent cell death. Conversely, poly(ADP-ribose) glycohydrolase, once thought to contribute to neuronal injury, now appears to have a protective role as demonstrated by recent studies utilizing gene disruption technology. Thus, the emerging mechanism dictating the fate of neurons appears to involve the regulation of PAR levels in neurons. Therefore, therapies targeting poly(ADP-ribosyl)ation in the treatment of neurodegenerative conditions such as stroke and Parkinson's disease are required to inhibit PAR synthesis and/or facilitate its degradation.

Key words. Cell death; apoptosis; poly(ADP-ribose); PARP; PARG; DNA damage; apoptosis-inducing factor; excitotoxicity.

Introduction

Poly(ADP-ribose) (PAR) is a unique nucleic acid-like biomolecule discovered over 40 years ago [1]. Resistant to DNase and RNase treatment, this biopolymer was later found to be dependent upon the coenzyme nicotinamide adenine dinucleotide (NAD^+) for its synthesis [2]. Today, we know that PAR is synthesized by the PARP family of poly(ADP-ribosyl) transferase enzymes [3], and to date, there are approximately 18 members of this family which share homology in the PARP catalytic domain [4]. Most of the fully characterized members of the PARP family are similar in that they are specifically activated by DNA strand breaks, although several members do not require DNA for activity [5, 6]. They differ in their subcellular localizations and different capacities for generating PAR. PARP-1, a nuclear enzyme selectively activated by DNA damage and the most abundant and best-understood PARP, is responsible for generating long-chained, branched PAR polymer [7]. PARP-2, PARP-3 and tankyrase, all thought to be DNA damage sentinels that facilitate DNA repair or maintain telomere length, generate short-chained PAR polymers [6, 8, 9]. The functions of the remaining putative PARP family members are not known.

Although PAR was initially found to be cleaved by phosphodiesterases to produce adenosine 5′-monophosphate (AMP) and phosphoribosyladenylate [10], the only known intracellular enzyme shown to specifically and efficiently catalyze the hydrolysis of PAR is poly(ADP-ribose) glycohydrolase (PARG), which catabolizes these polymers into free ADP-ribose [11]. Therefore, poly(ADP-ribosyl)ation can be viewed as a cyclical process beginning with PARP, which utilizes NAD^+ as substrate to transfer ADP-ribose units to protein acceptors with the subsequent release of nicotinamide [11], and concluding with PARG, which catalyzes the exo- and endoglycosidic hydrolysis of PAR into ADP-ribose [12] (fig. 1). Each PAR cycle is rapid and unique, such that the protein acceptors, includ-

* Corresponding author.

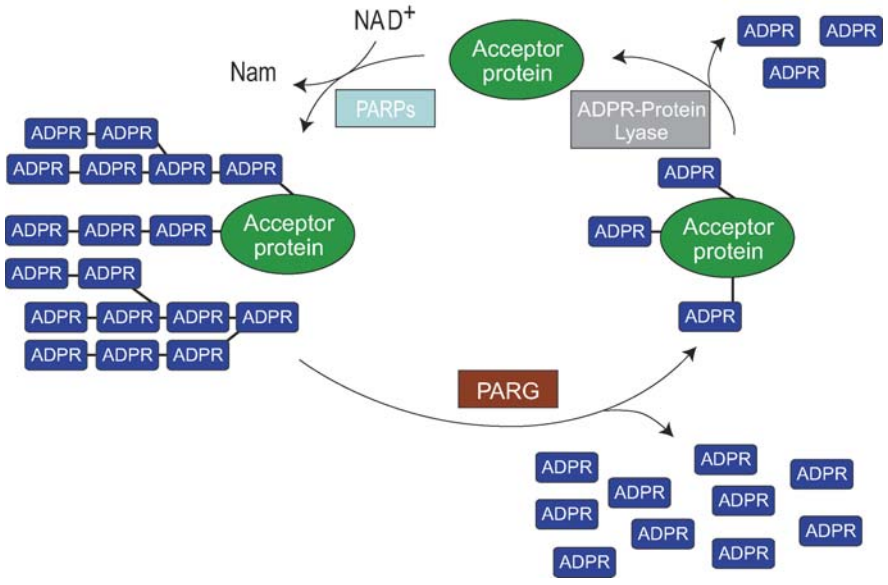

Figure 1. Poly(ADP-ribosyl)ation. Poly(ADP-ribose) (PAR) polymers are synthesized by the poly(ADP-ribosyl) transferase enzymes known as poly(ADP-ribose) polymerases (PARPs) using nicotinamide adenine dinucleotide (NAD^+) as substrate with the subsequent release of nicotinamide (Nam). Nuclear proteins are acceptors of the PAR, which results in their covalent modification. They can vary in size, with the largest containing up to 200 ADP-ribose residues with multiple branch points. However, the covalent modification of proteins by the transfer of ADP-ribose residues is only transient due to the rapid action of poly(ADP-ribose) glycohydrolase (PARG), which catalyzes the hydrolysis of these polymers into free ADP-ribose (ADPR). An ADP-ribosyl protein lyase has been proposed to remove the protein-proximal ADP-ribose residue from the acceptor protein.

ing DNA binding proteins and PARP-1 itself [11], are covalently modified, many in multiple sites, and the size and length of PAR synthesized depends both on the acceptor protein and the particular PARP isoform initiating the cycle. The hydrolysis of these polymers in all cases, however, appears to be similar since PAR is rapidly hydrolyzed by PARG once synthesized. An ADP-ribosyl protein lyase has been proposed to remove the final, protein-proximal ADP-ribose residue from the acceptor protein [13], although this protein has yet to be identified.

The proteins covalently modified with PAR or non-covalently sequestered by its highly anionic properties define the cellular processes influenced by poly(ADP-ribosyl)ation, including DNA repair and replication [14, 15], mitosis [16], transcriptional activation [17] and cell death [18–20]. Therefore, the emerging biological role for the metabolism of PAR is to maintain the fidelity of the genome [21]. The DNA damage dependence of PAR formation identified poly(ADP-ribosyl)ation as a therapeutic target in the treatment of cancer. Accordingly, PARP-1 remains a cancer drug target today, as there are several PARP-1 inhibitors in clinical trials as adjunctive therapy in several types of carcinomas [22]. However, poly(ADP-ribosyl)ation via PARP-1 is also specifically activated in neurons by nitric oxide (NO) during excitotoxicity [23], which presents an additional role for poly(ADP-ribosyl)ation in the nervous system. The therapeutic potential of targeting poly(ADP-ribosyl)ation has been expanded to include ischemic injury, diabetes, Parkinson's disease, inflammation and myocardial infarction [18–20]. This review will focus specifically on the activation and biological consequences of poly(ADP-ribosyl)ation as it pertains to the nervous system. The ability of PAR to mediate neuronal cell death through a novel pathway has elevated poly(ADP-ribosyl)ation into a unique target in the treatment for many neurological disorders.

Poly(ADP-ribosyl)ation in the nervous system

Poly(ADP-ribosyl)ation is a ubiquitous biochemical pathway, with PAR synthesis and degradation present in all mitotic and post-mitotic cells with few exceptions in mammalian organisms [3]. This holds true in the central nervous system (CNS), where PARP and PARG are present throughout the brain and spinal cord. The amount of PAR metabolism observed throughout the nervous system, as quantified by immunoblotting or immunocytochemistry, identifies the medical importance of targeting poly(ADP-ribosyl)ation for the treatment of conditions that cause neuronal damage. High levels of PARP-1 activation are seen in phagocytosing microglial cells, but not in resting microglia [24]. PARP activation is also present in macroglial cells, most notably in astrocytes. Neurons contain readily detectable PARP-1 activation following

insult, which explains the extreme sensitivity of certain brain regions to PARP-1-dependent excitotoxic cell death mediated by the neurotransmitter glutamate, such as the hippocampus, cortex and striatum [18]. Thus, providing the foundation for the ability of poly(ADP-ribosyl)ation to regulate life and death in the nervous system is this important observation.

PARP-1 and neuronal cell death

PARP-1

PARP-1 is the best understood poly(ADP-ribosyl)transferase. It is a ubiquitous nuclear enzyme with an abundance estimated at 2 million molecules per cell in the CEM lymphoblastic cell line and 200,000 molecules per cell in HeLa cells [25]. It is activated by DNA strand breaks and is stimulated by Mg^{2+} and polycations such as histones and polyamines. PARP-1 has low basal activity that is increased up to 500-fold upon activation. PARP-1 itself is the chief acceptor of PAR polymers in an automodification reaction [26], while DNA binding and processing proteins are modified to a lesser extent [27–29]. Since the K_M of PARP-1 for NAD^+ is approximately 50 μM, activation by the DNA binding domain is required for high PARP-1 catalytic activity in all cell types.

PARP-1 and cell death

The evidence for a link between poly(ADP-ribosyl)ation and cell death is unmistakable from the observation that PARP-1 is a target of caspases activated during apoptosis [30]. It is cleaved into a 24-kDa fragment containing the DNA binding domain and an 89-kDa fragment containing the catalytic and automodification domains during apoptosis [3]. The 24-kDa fragment may contribute to the irreversibility of apoptosis by blocking access of DNA repair enzymes to strand breaks [31] and thereby facilitate cellular disassembly. The cleavage of PARP-1 may also be critical in producing an 89-kDa fragment incapable of activation by DNA nicks, which suggests a possible mechanism to prevent energy depletion.

Because PARP-1 responds to DNA damage in a dose-dependent fashion, the poly(ADP-ribosyl)ation can become a very energetically expensive process. The synthesis of NAD^+, the substrate of PARP, is complex. Further, the oxido-reduction capacity of this coenzyme is required by the cell to generate ATP, the chief energy source of the cell. Accordingly, it has been proposed that massive genomic damage leads to hyperactivation of poly(ADP-ribosyl)ation, resulting in NAD^+ depletion and the cessation of energy metabolism. This leads to the loss of all energy-dependent cellular function, resulting in an acute form of pathological cell death known as necrosis [32].

PARP-1 and neurotoxicity

PARP-1 and excitotoxicity

Neuronal damage following focal ischemia is primarily caused by the activation of the N-methyl-D-aspartate (NMDA) receptors and the formation of NO, superoxide anion, which can combine to form the highly reactive peroxynitrite. The ability of glutamate to mediate this excitotoxicity via the formation of NO and the production of free radicals led to the discovery of PARP-1 activation secondary to this cascade (fig. 2). Increases in intracellular calcium via NMDA stimulation can activate a variety of enzymes, such as proteases, kinases, phospholipases and nitric oxide synthase (NOS). Formation of NO from NOS ultimately results in the formation of peroxynitrite, which activates PARP-1 and thereby initiates the poly(ADP-ribosyl)ation [23]. It was first proposed by Zhang et al. that the neurotoxicity elicited from ischemia is mediated by NO and involves the downstream activation of PARP-1 through DNA damage caused by peroxynitrite [23]. PARP-1 inhibitors were found to provide neuroprotection against excitotoxicity, and the magnitude of this neuroprotection was directly proportional to the potency of the inhibitor [23]. The most compelling evidence of this role of PARP-1 was provided by the utilization of mice displaying the PARP-1-null genotype. Primary neuronal cultures from these mice are resistant to the toxicity elicited by combined oxygen-glucose deprivation (OGD) or by neurotoxic levels of NMDA or NO generators [33]. Further, reduced infarct volume is observed following middle cerebral artery occlusion (MCAo) in the whole animal [33]. Although significant neuroprotection is afforded through the overexpression of the superoxide dismutase (SOD) or knockout of the neuronal nitric oxide synthase (nNOS) gene in mice following MCAo [34], the deletion of the PARP-1 gene provides the greatest magnitude of neuroprotection in this type of model. Thus, poly(ADP-ribosyl)ation through PARP-1 activation may be a ‘choke point’ for neuronal cell death programs.

PARP-1 and NF-κB-dependent inflammation

Other mechanisms may contribute to the ability of PARP-1 to mediate neuronal cell death. One such mechanism may be the regulation of transcriptional activation by modulating the function of NF-κB. The transcription factor NF-κB is important to many cellular responses, including DNA repair, apoptosis and immune function [35, 36]. In response to the cytotoxicity, PARP-1 null fibroblasts display lower survival rates, an inability to induce NF-κB expression and minimal NF-κB activity [37]. Two recent reports show that PARP-1 promotes NF-κB-dependent microglial activation directly through its catalytic activity [38] and a direct protein interaction with NF-κB [24]. Microglia, known as the immune cells

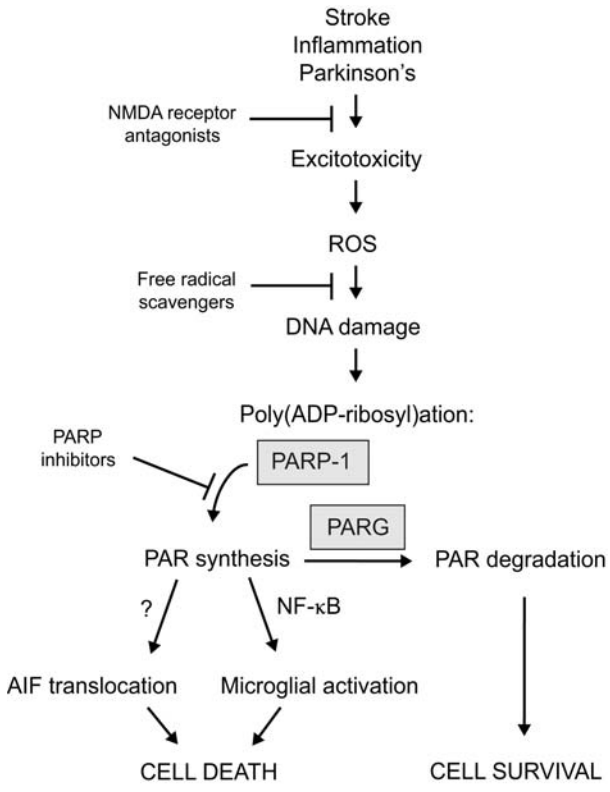

Figure 2. The role of poly(ADP-ribosyl)ation in the life and death of neurons. Primary neuronal insults, such as stroke, sets into motion many cellular signaling events. Excitotoxicity is a major trigger for secondary neuronal injury and a major pathway involved in excitotoxic cell death is DNA damage due to reactive oxygen species (ROS), namely peroxynitrite formation. This activates poly(ADP-ribosyl)ation, mainly through the nuclear enzyme poly-(adenosine diphosphoribose) polymerase-1 (PARP-1). The extent of poly(ADP-ribosyl)ation correlates with the severity of stress, and this determines the cellular response. Poly(ADP-ribosyl)ation under severe stress leads to cell death through the translocation of the death effector protein apoptosis-inducing factor (AIF) to the nucleus, which leads to DNA fragmentation and nuclear condensation. AIF function is dependent on poly(ADP-ribosyl)ation, but the mechanism of this regulation is not known. In addition, PARP-1 also contributes to neuronal cell death following primary neuronal damage due to the PARP-1/NF-κB-mediated activation and subsequent migration of microglia to the site of injury, where they release pro-inflammatory cytokines. The inhibition of PARP-1 or PAR synthesis has been shown to provide a high level of neuroprotection and thus confer cell survival. Also, recent studies utilizing the disruption of the PARG gene suggest that the degradation of PAR plays a protective role during genotoxic stress and PARG may therefore promote cell survival. Shown are the pharmacologic interventions that help reduce the amount of neuronal cell death (NMDA receptor antagonists, free-radical scavengers), but the greatest amount of neuroprotection to date has been demonstrated through PARP-1 inhibition.

of the nervous system, migrate to areas of neuronal damage and secrete cytokines and radicals noxious to neuronal tissue [38]. Therefore, the ability to regulate NF-κB activity presents a method by which PARP-1 conceivably mediates neuronal cell death.

PARP-1 and apoptosis-inducing factor

Mitochondrial mechanisms of cell death

Once thought only to have an energy-producing role as the organelle which ultimately produces ATP for the cell through oxidative phosphorylation, the mitochondrion is now considered a central cellular compartment that mediates cell death pathways. Alterations in mitochondrial function/physiology by impaired oxidative phosphorylation, generation of oxygen radicals or activation of the mitochondrial permeability transition pore promotes the release of mitochondrial death effectors, such as cytochrome *c*, Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP binding protein with low p*I*), endonuclease G (EndoG) or apoptosis-inducing factor (AIF). The effects of these death-promoting proteins can be regulated or their release can be blocked by the anti-apoptotic Bcl-2 family of proteins [39]. The cell death pathway triggered by the release of the aforementioned proteins from the mitochondrion is known as the intrinsic apoptotic pathway. This differs from the extrinsic apoptotic pathway in that it is not receptor mediated; rather, the intrinsic pathway involves the convergence of death stimuli, originating either intra- or extracellularly, that induce the release of proapoptotic proteins. AIF is one such proapoptotic protein that promotes cell death in many cell types, including neurons.

AIF

AIF is a 67-kDa flavoprotein that resides in the mitochondria inner membrane space [40]. Death stimuli cause the translocation of AIF from the mitochondria to the nucleus, where it induces DNA fragmentation, nuclear condensation and caspase-independent cell death [40]. Interestingly, AIF was purified from mouse liver mitochondria as a 57-kDa protein, which suggests some type of proteolytic processing of the full-length form during or after release from the mitochondria [40]. AIF contains no nuclease activity, but somehow activates a yet-to-be-identified endonuclease to cause DNA fragmentation. Disruption of the AIF gene in mice results in embryonic lethality, which suggests a role for AIF in embryonic development [41]. AIF shares homology with oxidoreductases in vertebrates, non-vertebrates plants and bacteria [42], while the recently solved crystal structure of murine AIF revealed that the active site structure suggests that AIF functions as an electron transferase similar to that of the bacterial ferredoxin reductases [43].

Mediation of PARP-1-dependent cell death by AIF

Translocation of AIF from the mitochondria to the nucleus is dependent on poly(ADP-ribosyl)ation, and AIF is a downstream effector in PARP-1-mediated cell death [44]. AIF fails to translocate in PARP-1 null fibroblasts following treatment with N-methyl-N′nitro-N-nitrosoguanidine

(MNNG). In addition, simultaneous with AIF translocation in wild-type cells is nuclear condensation, phosphatidylserine exposure and disruption of the mitochondrial membrane potential. These events are not prevented by utilizing the pan-caspase inhibitor z-VAD.fmk, and these events appear to precede cytochrome c release and caspase activation. Therefore, cell death mediated by the translocation of AIF is dependent on poly(ADP-ribosyl)ation, and this translocation appears to trigger a unique type of cell death that is caspase independent. How poly(ADP-ribosyl)ation, whether through NAD^+ depletion and/or PAR, or a protein modified by PAR, regulates AIF translocation or release from the mitochondria is not known.

The excitotoxicity observed following NMDA receptor stimulation in murine cortical neurons was shown to activate PARP-1 and induce AIF translocation [44]. In addition, no neuroprotection was observed, and AIF translocation was not blocked by the use of caspase inhibitors. In contrast, PARP-1 null murine cortical neurons were completely protected against the NMDA-mediated excitotoxicity, with no AIF translocation or nuclear condensation observed [44]. Therefore, our results indicate that NMDA receptor stimulation causes the activation of PARP-1 and subsequent AIF translocation, which results in neuronal cell death. The ability of poly(ADP-ribosyl)ation to regulate AIF translocation following different cytotoxic stimuli and in different cell types demonstrates the ability of PAR to act as an upstream signal following different types of genotoxic insults that determines the fate of many cell types, including post-mitotic neurons in the CNS.

PARG and neuronal cell survival

PARG

The rapid synthesis and rapid degradation of PAR is an immediate response to DNA damage. As a result, the covalent modification of proteins by PAR is only transient due to the rapid action of PARG, which suggests a closely coordinated modus operandi for the PARPs and PARG. Preliminary data in our laboratory demonstrate a mitochondrial localization of PARG. Because of the presence of a putative (NLS) [45] and (NES) [46], it should prove interesting to determine the shuttling mechanism of PARG between the mitochondria, where it housed, and the nucleus, where its catalytic activity is required. In addition, the mitochondrial localization may suggest an alternative role of this enzyme, such as the involvement in energy regulation.

Recently, an inhibitor binding site and structure-activity relationships of bovine PARG were elucidated which offer detailed insight into the active site topology and catalytic mechanism of PARG [47, 48]. However, the biological role of PARG has not been demonstrated to date. Therefore, little is known about PARG beyond its enzymatic and structural features.

The role of PARG in neuronal cell death is unknown

The putative importance of PARG in poly(ADP-ribosyl)ation suggests that it may also be a novel target, especially in the treatment of stroke. PARG provides the subsequent step in the poly(ADP-ribosyl)ation pathway, and it may provide a key role in neuronal death. It may also contribute to neurotoxicity by regenerating automodified PARP-1 through its ability to remove the PAR polymer and allow the reactivation of PARP-1. However, the biological consequences of PARG removal of PAR from all other protein acceptors are not known. Recent studies suggest that PARG has a role in neuronal cell death and that PARG inhibition is neuroprotective following excitotoxic neuronal cell death [49, 50]. However, these studies use PARG inhibitors whose specificity is questionable, or they utilize compounds that are toxic, non-selective and contain free-radical scavenging capabilities [49]. Therefore, the role of PARG in neuronal cell death and the evaluation of PARG inhibition as potential therapy have yet to be succinctly demonstrated. Thus, the lack of available cell-permeable, specific PARG inhibitors has prevented the elucidation of the role of PARG in cell death.

PARG in nervous system development

The accumulation of PAR may have dire consequences. Along these lines is the observation that the loss of PARG in *Drosophila melanogaster* leads to lethality in larval stage at the normal developmental temperature (25°C) [51]. Some PARG null mutants grow to adulthood when the developmental temperature is increased to 29°C, but they exhibit progressive neurodegeneration with reduced locomotor activity. Further, they exhibit approximately a 50% decrease in lifespan as compared to wild-type animals. The PARG null mutants show an extensive accumulation of PAR polymer, especially in the developing CNS [51]. Therefore, the proper regulation of poly(ADP-ribosyl)ation may be required for proper neuronal cell function and survival, as well as for the development of the nervous system. However, several key points remain a mystery, such as, what are the exact biochemical consequences of PAR accumulation? Does PAR accumulation induce cell death? Is PARG the only biologically relevant enzyme capable of catalyzing the hydrolysis of PAR? What is the role of poly(ADP-ribosyl)ation and PAR catabolism in neurons and the developing embryo in mammals?

Potential toxicity of PAR accumulation

The failure to degrade PAR polymer appears to have unexpected effects. Recent evidence suggests that poly(ADP-ribosyl)ation regulates transcription [17], and the failure to degrade PAR polymer may lead to transcriptional dysregulation. In fact, the absence of PARG activ-

ity leads to transcriptional dysregulation and altered circadian period length in *Arabidopsis* [52], consistent with the notion that poly(ADP-ribosyl)ation plays an important role in homeostatic cellular functions. Further, it has been proposed that inhibition of PARG would lead to permanently automodified PARP-1 and thereby lead to neuroprotection through the inhibition of PARP-1 [49, 50]. However, the lethality observed in *Drosophila* following PARG gene inactivation suggests that total PAR accumulation is toxic to cells and functional PARG is required for proper cell survival following PARP-1 activation. If indeed this is the case, then it appears that the accumulation of PAR is toxic to cells and PARG may have a protective role for cells following genotoxic stress. Therefore, PARG inhibition may actually contribute to neuronal cell death due to the dysregulation of PAR metabolism.

Therapeutic potential of targeting poly(ADP-ribosyl)ation in the nervous system: granting life in the CNS

Acute neuronal injury and neurological disorders lead to significant morbidity and mortality each year. Therapies targeting poly(ADP-ribosyl)ation for the treatment of these conditions should be designed to inhibit the ability of poly(ADP-ribosyl)ation to cause neuronal cell death. Therefore, pharmacologic therapy targeted against PARP-1 could limit neuronal cell death and grant 'life' to those neurons destined to die by PARP-1-mediated mechanisms.

Stroke and ischemia-reperfusion injury

Neuronal cell death secondary to stroke and other ischemic conditions comprises the bulk of neuronal injury, and therefore is the chief cause of the morbidity associated with these conditions. This neuronal damage is attributed, in part, to excitotoxicity due to the massive release of the excitatory neurotransmitter glutamate, which acts on NMDA receptors and ultimately activates poly(ADP-ribosyl)ation (fig. 2). The generation of free radicals at the site of injury is detrimental to tissue distal to the focal point due to reperfusion. In such conditions, inhibitors of glutamate receptors, NOS inhibitors and free-radical scavengers are neuroprotective, but the greatest neuroprotection observed to date in this type of neuronal injury model is the inhibition of PARP-1 (fig. 2). Disruption of the functional PARP-1 gene confers resistance to the neuronal cell death elicited by in vitro models of ischemia, while PARP-1 null mice are protected against in vivo neuronal injury following MCAo, an in vivo model of stroke and reperfusion injury in the whole animal [33]. Administration of PARP-1 inhibitors up to 1 h following initial insult provided significant reductions in neuronal cell death as compared to untreated controls [23]. In each case, PAR synthesis was ablated, which indicates that the effects are a result of PARP-1 inhibition.

Parkinson's disease

Parkinson's disease (PD) is a neurological disorder characterized by slowness of movement, resting tremors, rigidity and difficulty with balance [53–55]. These clinical features stem from loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) [56] and subsequent formation of Lewy bodies [57–60]. Currently, there is no therapeutic intervention that can stop the progression of PD. Genetic mutations have been found that lead to PD in familial cases (familial PD), but PD is primarily a sporadic disorder (sporadic PD) with no known etiology [57]. Experimental models used to study this more common form of PD utilize 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in animal models, which ultimately produces PD-like symptoms in the host [61]. MPTP is a neurotoxin converted to 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase. MPP^+, the active form of MPTP, is selectively transported into dopaminergic neurons and disrupts mitochondrial function via inhibition of mitochondrial complex I [57]. Energy failure ensues, which leads to glutamate accumulation and hyperstimulation of glutamate receptors. NMDA receptor antagonists provide significant protection versus MPP^+ toxicity and have shown promise as long-term therapy [62]. Due to the efficacy of these agents, excitotoxicity may play a primary role in MPTP-induced excitotoxicity, and NO activation of PARP-1 by DNA damage may be involved. PARP inhibitors, such as benzamide and 1,5-dihydroxyisoquinoline (DHIQ), significantly protect the brain from MPTP-induced decreases in NAD^+ levels [63]. PARP-1 null mice are protected from the loss of dopamine and its metabolites following MPTP exposure as compared to their wild-type littermates [64]. Further, the PARP-1 null mice show significant resistance to the loss of dopaminergic neurons in the SNpc following MPTP-induced neurotoxicity [64]. Recent studies suggest that the modus operandi of this dopaminergic neuronal loss following MPTP is PARP-1-mediated AIF translocation [65]. Therefore, poly(ADP-ribosyl)ation may be a primary cause of MPTP-induced dopaminergic neuronal cell death, possibly through AIF-mediated cell death, and inhibition of PARP activation holds promise for therapy in sporadic PD patients to ensure survival of the neurons in the SNpc.

Other disorders

Traumatic brain injury

In traumatic brain injury (TBI), DNA damage may ensue following mechanical injury due to reactive oxygen spe-

cies [66]. Further, biochemical analyses demonstrate robust AIF translocation in the ipsilateral cortex and hippocampal tissue following controlled cortical impact [67]. In these situations, PARP inhibition, demonstrated using chemical inhibitors or PARP-1 null mice, provided partial neuroprotection following injury. It is important to note that both caspase-dependent and -independent mechanisms of cell death are activated following this type of injury, which would explain the significant, but not complete, neuroprotection seen with PARP-1 inhibition.

Experimental allergic encephalomyelitis

NO overproduction, the formation of peroxynitrite and subsequent oxidative injury are detected in experimental models of allergic encephalomyelitis (EAE). Peroxynitrite scavengers and PARP inhibitors have shown efficacy in the treatment of EAE in murine models [68, 69]. Although the exact mechanism of neuroprotection afforded by PARP inhibition is unknown, preliminary evidence suggests that it may involve the inhibition of demyelination, a major feature of EAE [70].

CNS inflammation

Inflammation seems to play a crucial role in neuronal cell death following many neurological disorders, including stroke and meningitis. Following stroke or trauma, microglia become activated and migrate to the site of injury, where they release inflammatory mediators that may cause significant neuronal cell death and secondary tissue damage [71, 72]. Poly(ADP-ribosyl)ation appears to have a primary role in this process through PARP-1 activation [24]. Resting microglia has no detectable poly(ADP-ribosyl)ation, but activated and mobilized microglia have high levels of PARP-1 activation. PARP-1 regulates this microglial activation through the modulation of NF-κB-dependent integrin CD11a expression in brain tissue. Inhibition of PARP-1 by antisense oligodeoxynucleotides blocked microglial activation and provided a high level of neuroprotection from the damage secondary to brain injury [24]. Therefore, another therapeutic option for stroke is the targeting of microglia in the CNS.

Recently, PARP-1 activation was shown to play a critical role in the development of CNS complications secondary to bacterial meningitis [73]. Further, AIF translocation is observed following experimental pneumococcal meningitis [74]. PARP-1 null mice displayed resistance to the CNS complications following meningitis, including blood-brain barrier breaching and intracranial pressure increases [73]. Therefore, targeting poly(ADP-ribosyl)ation through PARP-1 may provide a method to promote CNS neuron survival and decreased morbidity following meningitis.

Concluding Remarks

In summary, poly(ADP-ribosyl)ation plays an important role in the life and death of neurons (fig. 2), and this role can be applied to a broad set of clinical applications. The underlying mechanism appears to involve NMDA receptor-mediated excitotoxicity and AIF mediation of cell death. PARP activation has an important role in neuronal cell death. PARG may modulate this role by hydrolysis of PAR, and preliminary evidence suggests that PARG is protective to the cell. It is becoming more clear that the cell death due to poly(ADP-ribosyl)ation may in fact result from a non-classical type of caspase-independent cell death rather than a necrotic-type cell death. Therefore, PAR can now be viewed as a signaling biomolecule that is involved in normal cell survival by facilitating DNA repair and maintaining genomic integrity, or in cell death by the modulation of cell death effectors and the mediation of intrinsic mitochondrial cell death. However, many questions remain, such as how poly(ADP-ribosyl)ation mediates AIF function. Does PAR have a direct function besides the modification of acceptor proteins? What is the nuclear target of AIF? How can AIF be directly targeted for therapy in neurological diseases? What is the role of the other PARP family isoforms in neuronal cell death? What neurological conditions require inhibition of caspase-independent or caspase-dependent cell death (or both) for therapeutic efficacy? Therefore, determining the complete role of poly(ADP-ribosyl)ation in the nervous system and exactly how it mediates the life and death of neurons in the CNS should provide the answers for the aforementioned questions. But based upon accumulating evidence, the therapeutic potential for targeting poly(ADP-ribosyl)ation for the treatment of various neurological diseases and disorders holds particular promise.

1 Chambon P., Weil J. D. and Mandel P. (1963) Nicotinamide mononucleotide activation of a new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. Biochem. Biophys. Res. Commun. **11:** 39–43
2 Nishizuka Y., Ueda K., Nakazawa K. and Hayaishi O. (1967) Studies on the polymer of adenosine diphosphate ribose. I. Enzymic formation from nicotinamide adenine dinuclotide in mammalian nuclei. J. Biol. Chem. **242 (13):** 3164–3171
3 de Murcia G. and Shall S., eds (2000) From DNA Damage and Stress Signaling to Cell Death: Poly ADP-Ribosylation Reactions, Oxford University Press, New York
4 Menissier de Murcia J., Ricoul M., Tartier L., Niedergang C., Huber A., Dantzer F. et al. (2003) Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. Embo J. **22 (9):** 2255–2263
5 Kickhoefer V. A., Siva A. C., Kedersha N. L., Inman E. M., Ruland C., Streuli M. et al. (1999) The 193-kD vault protein, VPARP, is a novel poly(ADP-ribose) polymerase. J. Cell Biol. **146 (5):** 917–928
6 Smith S., Giriat I., Schmitt A. and de Lange T. (1998) Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science **282 (5393):** 1484–1487

7 Juarez-Salinas H., Sims J. L. and Jacobson M. K. (1979) Poly-(ADP-ribose) levels in carcinogen-treated cells. *Nature* **282 (5740):** 740–741

8 Augustin A., Spenlehauer C., Dumond H., Menissier-De Murcia J., Piel M., Schmit A. C. et al. (2003) PARP-3 localizes preferentially to the daughter centriole and interferes with the G1/S cell cycle progression. J. Cell Sci. **116 (Pt 8):** 1551–1562

9 Amé J. C., Rolli V., Schreiber V., Niedergang C., Apiou F., Decker P. et al. (1999) PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. J. Biol. Chem. **274 (25):** 17860–17868

10 Miwa M., Saikawa N., Yamaizumi Z., Nishimura S. and Sugimura T. (1979) Structure of poly(adenosine diphosphate ribose): identification of 2′- [1-ribosyl-2″-(or 3‴-)(1″-ribosyl)]-adenosine-5′,5″,5‴-tris(phosphate) as a branch linkage. Proc. Natl. Acad. Sci. USA **76 (2):** 595–599

11 Amé J. C., Jacobson E. L. and Jacobson M. K. (2000) ADP-ribose polymer metabolism. In: From DNA Damage and Stress Signaling to Cell Death: Poly-ADP-Ribosylation Reactions, pp. 1–34, de Murcia G. and Shall S. (eds), Oxford University Press, New York

12 Brochu G., Duchaine C., Thibeault L., Lagueux J., Shah G. M. and Poirier G. G. (1994) Mode of action of poly(ADP-ribose) glycohydrolase. Biochim. Biophys. Acta **1219 (2):** 342–350

13 Oka J., Ueda K., Hayaishi O., Komura H. and Nakanishi K. (1984) ADP-ribosyl protein lyase. Purification, properties and identification of the product. J. Biol. Chem. **259 (2):** 986–995

14 D'Amours D., Desnoyers S., D'Silva I. and Poirier G. G. (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem. J. **342 (Pt 2):** 249–268

15 Dantzer F., de La Rubia G., Menissier-De Murcia J., Hostomsky Z., de Murcia G. and Schreiber V. (2000) Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1. Biochemistry **39 (25):** 7559–7569

16 Shall S. and de Murcia G. (2000) Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model? Mutat. Res. **460 (1):** 1–15

17 Hassa P. O. and Hottiger M. O. (1999) A role of poly (ADP-ribose) polymerase in NF-kappaB transcriptional activation. Biol. Chem. **380 (7–8):** 953–959

18 Yu S. W., Wang H., Dawson T. M. and Dawson V. L. (2003) Poly(ADP-ribose) polymerase-1 and apoptosis inducing factor in neurotoxicity. Neurobiol. Dis. **14 (3):** 303–317

19 Southan G. J. and Szabo C. (2003) Poly(ADP-ribose) polymerase inhibitors. Curr. Med. Chem. **10 (4):** 321–340

20 Szabo C. and Dawson V. L. (1998) Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. Trends Pharmacol. Sci. **19 (7):** 287–298

21 Jacobson M. K. and Jacobson E. L. (1999) Discovering new ADP-ribose polymer cycles: protecting the genome and more. Trends Biochem. Sci. **24 (11):** 415–417

22 Canan Koch S. S., Thoresen L. H., Tikhe J. G., Maegley K. A., Almassy R. J., Li J. et al. (2002) Novel tricyclic poly(ADP-ribose) polymerase-1 inhibitors with potent anticancer chemopotentiating activity: design, synthesis and X-ray cocrystal structure. J. Med. Chem. **45 (23):** 4961–4974

23 Zhang J., Dawson V. L., Dawson T. M. and Snyder S. H. (1994) Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. Science **263 (5147):** 687–689

24 Ullrich O., Diestel A., Eyupoglu I. Y. and Nitsch R. (2001) Regulation of microglial expression of integrins by poly(ADP-ribose) polymerase-1. Nat. Cell. Biol. **3 (12):** 1035–1042

25 Lautier D., Lagueux J., Thibodeau J., Ménard L. and Poirier G. G. (1993) Molecular and biochemical features of poly (ADP-ribose) metabolism. Mol. Cell. Biochem. **122 (2):** 171–193

26 Adamietz P. (1987) Poly(ADP-ribose) synthase is the major endogenous nonhistone acceptor for poly(ADP-ribose) in alkylated rat hepatoma cells. Eur. J. Biochem. **169 (2):** 365–372

27 Adamietz P. and Rudolph A. (1984) ADP-ribosylation of nuclear proteins in vivo. Identification of histone H2B as a major acceptor for mono- and poly(ADP-ribose) in dimethyl sulfate-treated hepatoma AH 7974 cells. J. Biol. Chem. **259 (11):** 6841–6846

28 Ruscetti T., Lehnert B. E., Halbrook J., Le Trong H., Hoekstra M. F., Chen D. J. et al. (1998) Stimulation of the DNA-dependent protein kinase by poly(ADP-ribose) polymerase. J. Biol. Chem. **273 (23):** 14461–14467

29 Wesierska-Gadek J., Schmid G. and Cerni C. (1996) ADP-ribosylation of wild-type p53 in vitro: binding of p53 protein to specific p53 consensus sequence prevents its modification. Biochem. Biophys. Res. Commun. **224 (1):** 96–102

30 Lazebnik Y. A., Kaufmann S. H., Desnoyers S., Poirier G. G. and Earnshaw W. C. (1994) Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. Nature **371 (6495):** 346–347

31 D'Amours D., Sallmann F. R., Dixit V. M. and Poirier G. G. (2001) Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. J. Cell Sci. **114 (Pt 20):** 3771–3778

32 Berger N. A. (1985) Poly(ADP-ribose) in the cellular response to DNA damage. Radiat. Res. **101 (1):** 4–15

33 Eliasson M. J., Sampei K., Mandir A. S., Hurn P. D., Traystman R. J., Bao J. et al. (1997) Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat. Med. **3 (10):** 1089–1095

34 Sampei K., Mandir A. S., Asano Y., Wong P. C., Traystman R. J., Dawson V. L. et al. (2000) Stroke outcome in double-mutant antioxidant transgenic mice. Stroke **31 (11):** 2685–2691

35 Baeuerle P. A. and Baltimore D. (1996) NF-kappa B: ten years after. Cell **87 (1):** 13–20

36 Piret B., Schoonbroodt S. and Piette J. (1999) The ATM protein is required for sustained activation of NF-kappaB following DNA damage. Oncogene **18 (13):** 2261–2271

37 Oliver F. J., Menissier-de Murcia J., Nacci C., Decker P., Andriantsitohaina R., Muller S. et al. (1999) Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice. EMBO J. **18 (16):** 4446–4454

38 Chiarugi A. and Moskowitz M. A. (2003) Poly(ADP-ribose) polymerase-1 activity promotes NF-kappaB-driven transcription and microglial activation: implication for neurodegenerative disorders. J. Neurochem. **85 (2):** 306–17

39 Hengartner M. O. (2000) The biochemistry of apoptosis. Nature **407 (6805):** 770–776

40 Susin S. A., Lorenzo H. K., Zamzami N., Marzo I., Snow B. E., Brothers G. M. et al. (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. Nature **397 (6718):** 441–446

41 Joza N., Susin S. A., Daugas E., Stanford W. L., Cho S. K., Li C. Y. et al. (2001) Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. Nature **410 (6828):** 549–554

42 Lorenzo H. K., Susin S. A., Penninger J. and Kroemer G. (1999) Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. Cell Death Differ. **6 (6):** 516–524

43 Mate M. J., Ortiz-Lombardia M., Boitel B., Haouz A., Tello D., Susin S. A. et al. (2002) The crystal structure of the mouse apoptosis-inducing factor AIF. Nat. Struct. Biol. **9 (6):** 442–446

44 Yu S. W., Wang H., Poitras M. F., Coombs C., Bowers W. J., Federoff H. J. et al. (2002) Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science **297 (5579):** 259–263

45 Lin W., Ame J. C., Aboul-Ela N., Jacobson E. L. and Jacobson M. K. (1997) Isolation and characterization of the cDNA encoding bovine poly(ADP-ribose) glycohydrolase. J. Biol. Chem. **272 (18):** 11895–11901

46 Shimokawa T., Masutani M., Nagasawa S., Nozaki T., Ikota N., Aoki Y. et al. (1999) Isolation and cloning of rat poly(ADP-ribose) glycohydrolase: presence of a potential nuclear export signal conserved in mammalian orthologs. J. Biochem. (Tokyo) **126 (4):** 748–755
47 Koh D. W., Patel C. N., Ramsinghani S., Slama J. T., Oliveira M. A. and Jacobson M. K. (2003) Identification of an inhibitor binding site of poly(ADP-ribose) glycohydrolase. Biochemistry **42 (17):** 4855–4863
48 Koh D. W., Coyle D. L., Mehta N., Ramsinghani S., Kim H., Slama J. T. et al. (2003) SAR analysis of adenosine diphosphate (hydroxymethyl)pyrrolidinediol inhibition of poly(ADP-ribose) glycohydrolase. J. Med. Chem. **46 (20):** 4322–4332
49 Ying W., Sevigny M. B., Chen Y. and Swanson R. A. (2001) Poly(ADP-ribose) glycohydrolase mediates oxidative and excitotoxic neuronal death. Proc. Natl. Acad. Sci. USA **98 (21):** 12227–12232
50 Lu X. C., Massuda E., Lin Q., Li W., Li J. H. and Zhang J. (2003) Post-treatment with a novel PARG inhibitor reduces infarct in cerebral ischemia in the rat. Brain Res. **978 (1–2):** 99–103
51 Hanai S., Kanai M., Ohashi S., Okamoto K., Yamada M., Takahashi H. et al. (2003) Loss of poly(ADP-ribose) glycohydrolase causes progressive neurodegeneration in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **101:** 82–86
52 Panda S., Poirier G. G. and Kay S. A. (2002) tej defines a role for Poly(ADP-ribosyl)ation in establishing period length of the *Arabidopsis* circadian oscillator. Dev. Cell **3 (1):** 51–61
53 Lang A. E. and Lozano A. M. (1998) Parkinson's disease. Second of two parts. N. Engl. J. Med. **339 (16):** 1130–1143
54 Lang A. E. and Lozano A. M. (1998) Parkinson's disease. First of two parts. N. Engl. J. Med. **339 (15):** 1044–1053
55 Dawson T. M. (2000) New animal models for Parkinson's disease. Cell **101 (2):** 115–118
56 Lee C. S., Schulzer M., Mak E. K., Snow B. J., Tsui J. K., Calne S. et al. (1994) Clinical observations on the rate of progression of idiopathic parkinsonism. Brain **117 (Pt 3):** 501–507
57 Dawson T. M. and Dawson V. L. (2003) Molecular pathways of neurodegeneration in Parkinson's disease. Science **302 (5646):** 819–822
58 Schapira A. H. (1996) Neurotoxicity and the mechanisms of cell death in Parkinson's disease. Adv. Neurol. **69:** 161–165
59 Mizuno Y., Sone N. and Saitoh T. (1987) Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-phenylpyridinium ion on activities of the enzymes in the electron transport system in mouse brain. J. Neurochem. **48 (6):** 1787–1793
60 Forno L. S., Langston J. W., DeLanney L. E., Irwin I. and Ricaurte G. A. (1986) Locus ceruleus lesions and eosinophilic inclusions in MPTP-treated monkeys. Ann. Neurol. **20 (4):** 449–55
61 Dawson V. L. and Dawson T. M. (1996) Nitric oxide neurotoxicity. J. Chem. Neuroanat. **10 (3–4):** 179–190
62 Turski L., Bressler K., Rettig K. J., Loschmann P. A. and Wachtel H. (1991) Protection of substantia nigra from MPP^+ neurotoxicity by N-methyl-D-aspartate antagonists. Nature **349 (6308):** 414–418
63 Cosi C., Suzuki H., Skaper S. D., Milani D., Facci L., Menegazzi M. et al. (1997) Poly(ADP-ribose) polymerase (PARP) revisited. A new role for an old enzyme: PARP involvement in neurodegeneration and PARP inhibitors as possible neuroprotective agents. Ann. N. Y. Acad. Sci. **825:** 366–379
64 Mandir A. S., Przedborski S., Jackson-Lewis V., Wang Z. Q., Simbulan-Rosenthal C. M., Smulson M. E. et al. (1999) Poly-(ADP-ribose) polymerase activation mediates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism. Proc. Natl. Acad. Sci. USA **96 (10):** 5774–5779
65 Wang H., Shimoji M., Yu S. W., Dawson T. M. and Dawson V. L. (2003) Apoptosis inducing factor and PARP-mediated injury in the MPTP mouse model of Parkinson's disease. Ann. N. Y. Acad. Sci. **991:** 132–139
66 McIntosh T. K., Juhler M. and Wieloch T. (1998) Novel pharmacologic strategies in the treatment of experimental traumatic brain injury: 1998. J. Neurotrauma **15 (10):** 731–769
67 Zhang X., Chen J., Graham S. H., Du L., Kochanek P. M., Draviam R. et al. (2002) Intranuclear localization of apoptosis-inducing factor (AIF) and large scale DNA fragmentation after traumatic brain injury in rats and in neuronal cultures exposed to peroxynitrite. J. Neurochem. **82 (1):** 181–191
68 Hooper D. C., Scott G. S., Zborek A., Mikheeva T., Kean R. B., Koprowski H. et al. (2000) Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood-CNS barrier permeability changes and tissue damage in a mouse model of multiple sclerosis. FASEB J. **14 (5):** 691–698
69 Scott G. S., Kean R. B., Mikheeva T., Fabis M. J., Mabley J. G., Szabo C. et al. (2004) The therapeutic effects of PJ34, a selective inhibitor of poly(ADP-ribose) polymerase, in experimental allergic encephalomyelitis are associated with immunomodulation. J. Pharmacol. Exp. Ther. **310:** 1053–1061
70 Virag L. and Szabo C. (2002) The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol. Rev. **54 (3):** 375–429
71 Stoll G., Jander S. and Schroeter M. (1998) Inflammation and glial responses in ischemic brain lesions. Prog. Neurobiol. **56 (2):** 149–171
72 Mabuchi T., Kitagawa K., Ohtsuki T., Kuwabara K., Yagita Y., Yanagihara T. et al. (2000) Contribution of microglia/macrophages to expansion of infarction and response of oligodendrocytes after focal cerebral ischemia in rats. *Stroke* **31 (7):** 1735–1743
73 Koedel U., Winkler F., Angele B., Fontana A. and Pfister H. W. (2002) Meningitis-associated central nervous system complications are mediated by the activation of poly(ADP-ribose) polymerase. J. Cereb. Blood Flow Metab. **22 (1):** 39–49
74 Braun J. S., Novak R., Murray P. J., Eischen C. M., Susin S. A., Kroemer G. et al. (2001) Apoptosis-inducing factor mediates microglial and neuronal apoptosis caused by pneumococcus. J. Infect. Dis. **184 (10):** 1300–1309