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Poly(ADP-Ribose) Metabolism in Brain and Its Role in Ischemia Pathology

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Abstract The biological roles of poly(ADP-ribose) polymers (PAR) and poly(ADP-ribosyl)ation of proteins in the central nervous system are diverse. The homeostasis of PAR orchestrated by poly(ADP-ribose) polymerase-1 (PARP-1) and poly(ADP-ribose) glycohydrolase (PARG) is crucial for cell physiology and pathology. Both enzymes are ubiquitously distributed in neurons and glia; however, they are segregated at the subcellular level. PARP-1 serves as a "nick sensor" for single- or double-stranded breaks in DNA and is involved in long and short patch base-excision repair, while PARG breaks down PAR. The stimulation of PARP-1 and PAR formation can activate proinflammatory transcription factors, including nuclear factor kappa B. However, hyperactivation of PARP-1 can result in depletion of NAD/ATP, and in PAR-dependent mitochondrial pore formation leading to release of apoptosis inducing factor and cell death. The role of PAR as a death signaling molecule in brain ischemia–reperfusion and inflammation as well as the effect of gender and aging is presented in this review. Modulating the PAR level through pharmacological or genetic intervention on PARP-1/PARG activity and gene expression should be a valuable way for neuroprotective strategy.

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Introduction

A variety of reactive oxygen (ROS)/nitrogen species released during recirculation after brain ischemia leads to oxidation of macromolecules including DNA. The central nervous system (CNS) is particularly sensitive to oxidative stress and DNA damage. The cells of adult human brain consume approximately 20% of oxygen utilized by the body, although the brain comprises only 2% of the body weight. The active metabolism in the brain leads to high rate of ROS production [1]. The brain is comprised of both actively dividing (glia) and terminally differentiated cells (neurons), which differ in their capacity to quench various stressors and to repair damage. For example, astrocytes protect neurons against the toxicity of ROS by supplying glutathione precursors to neurons and by pyruvate release [2–5]. Although the CNS is well-equipped for limiting the entry of potentially neurotoxic compounds to the brain via the blood–brain barrier, the high oxygen and glucose demand makes CNS particularly susceptible to perturbations in the delivery of these essential molecules [6]. If the supplies are disrupted for only a brief period of time, the cascade of events that ensue can wreak havoc on cells during reperfusion, including those cells forming the blood–brain barrier [7]. Post-ischemic reperfusion injury is multifactorial with key precipitating events being the release of glutamate, activation of *N*-methyl-D-aspartate (NMDA) receptors, and production of nitric oxide (NO), which can subsequently react with superoxide anion (O_2^-) to form peroxynitrite ($ONOO^-$) [8]. The injury that accompanies reperfusion is largely caused by generation

of reactive oxygen (e.g., O_2^-; $^{\bullet}OH$) and nitrogen species (e.g., $ONOO^-$; $NO_2^{\bullet -}$), which damage DNA, and ultimately lead to hyperactivation of poly(ADP-ribose) polymerase-1 (PARP-1; EC 2.4.2.30).

PARP-1 is a 116-kDa protein that functions as a DNA nick sensor and nuclear target for various signaling pathways. This protein contains three primary functional domains: an N-terminal DNA-binding domain (46 kDa), including a nuclear localization signal, a central auto modification domain (16 kDa), and a C-terminal catalytic domain (55 kDa). The DNA binding domain contains two zinc-finger motifs that facilitate binding to both single and double-stranded DNA breaks. The functional roles of PARP-1 are largely mediated by its activation after binding to damaged DNA. Upon binding, PARP-1 activity increases rapidly, up to 500 fold, and cleaves β-nicotinamide adenine dinucleotide (β-NAD^+) into ADP-ribose and nicotinamide. Linear and branched polymers of ADP-ribose (PAR) are synthesized and covalently attached to more than 40 different acceptor proteins, including histones and PARP-1 itself. The highly negative charge of PAR leads to electrostatic repulsion between DNA and histones. Under normal conditions, PARP-1 acts as a key protein in genome stability by detecting DNA strand breaks and nicks and by being involved in DNA repair machinery [9]. The recent findings of Cohen-Armon [10] presented an alternative mechanism of PARP-1 stimulation through direct interaction with phosphorylated extracellular signal regulated kinase (ERK-2). This pathway is not related to DNA damage and PARP-1 binding to DNA. Moreover, ERK-2 induced PARP-1 activation amplifies ERK signals through transcription factor ELK 1 and its target gene c-fos. These data indicated that PARP-1 through Raf-MEK-ERK-cfos signaling cascade might be involved in growth, differentiation, and proliferations of cells. However, increased activation of PARP-1 can lead to inflammation [11]. PARP-1 activates proinflammatory transcription factors through different mechanisms, including protein–protein interaction, and PAR or poly(ADP-ribosyl)ation. The severe injuries such as ischemia–reperfusion can hyperactivate PARP-1 leading to an energy crisis in the cells [12–18].

PAR/PARP//PARG and Mitochondria—Nucleus Cross Talk

During PAR-mediated cell death, the enhanced consumption of β-NAD^+ impairs glycolysis and oxidative metabolism in the mitochondria, leading to ATP depletion [18–20] and to the following key events: (1) PAR-dependent specific mitochondrial permeability transition or pore formation and membrane depolarization, (2) release of apoptosis-inducing factor (AIF), (3) nuclear translocation of AIF, and (4) DNA degradation. However, the exact signals involved in the nuclear-mitochondrial cross talk are unclear. PAR has been shown to act as a signaling molecule that induces cell death following hyperactivation of PARP-1 [21]. Sequential activation of PARP-1, calpains, and BAX is also reported to be essential in AIF-mediated programmed cell death [22]. These authors found that, once activated, PARP-1 mediates mitochondrial AIF release and necrosis, through a mechanism requiring calpains but not caspase and not cathepsins. They observed that single ablation of the BAX but not other proapoptotic protein as for example BAK prevented AIF release and cell death evoked by alkylating DNA damage. This data presented that BAX was indispensable for the highly regulated necrosis. The last study of Wang et al. [23] demonstrated that calpain activation was not required for AIF translocation from mitochondria in PARP-1-dependent cell death.

Xu et al. [24] reported that hyperactivation of PARP-1 in mouse embryonic fibroblasts treated with the DNA alkylating agent *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG) required c-Jun N-terminal kinase-1 (JNK-1) activation for steps 1, 2, and 3 described in the beginning of this chapter. Based on these findings, Alano and Swanson [25] speculated that JNK-1 activation may play a role in nuclear export of PAR. The in vivo half-life of PAR is less than 1 min. The dynamic process of synthesis and hydrolysis of PAR is performed by PARP and primarily poly(ADP-ribose) glycohydrolase (PARG; EC 3.2.1.143). Catabolism is also performed by ADP-ribosyl protein lyase, although to a much lesser extent, and by three novel proteins possessing PARG activity [26, 27]. PARG cleaves ribose–ribose bonds of both linear and branched portions of PAR, whereas ADP-ribosyl protein lyase cleaves the protein proximal ADP-ribose monomer [28]. Recently, it was demonstrated that PARG was required for the global repair of single-strand DNA breaks and that PARP-1 and PARG accelerated single-strand break repair in concert with one another [29]. The authors further confirmed that XRCC1, a scaffolding protein involved in single-strand break repair, was negatively regulated by PARG, meaning PARG prevented accumulation of XRCC1 at sites of DNA damage and facilitated its disassembly once repair was completed. The tissue distribution of PARG parallels to PARP-1. Both enzymes are detected in glia and neurons. However, differences in the subcellular distribution of these enzymes have been reported. Poitras et al. [30] found that, in the brain, endogenous PARG was associated with the outer mitochondrial membrane and confirmed that the subcellular segregation of PARP-1 and PARG required PARG to translocate to the nucleus upon PARP-1 activation. Recently, Whatcott et al. [31] reported that specific isoform of

PARG was targeted to the mitochondrial matrix. According to the authors, the identification of a PARG isoform as a component of the mitochondrial matrix raises several interesting possibilities concerning mechanism of nuclear–mitochondrial cross talk and PAR polymer metabolism within mitochondria. Moreover, they suggested [31] that mitochondrial PARG may play protective role in preventing inappropriate release of AIF or could promote AIF release by generating free ADP-ribose that has been shown to activate membrane calcium channels. The data of Meyer et al. [32] indicated that PARG gene shares a promotor with a gene encoding a protein TIM 23 involved in import of proteins into mitochondria. Poitras et al. [30] described spatial and functional relationships of PARG and PARP-1. It was found that these enzymes, responsible for PAR metabolism and several cell function processes, were evenly distributed throughout the brain. While PARP-1 in neurons is localized exclusively in the nucleus, PARG is mainly found in the mitochondrial fraction under unstimulated conditions together with manganese superoxide dismutase and cytochrome C. However, in contrast to neurons, PARG is presented in different subcellular compartments in other mammalian cells [30]. The co-localization of PARG with cytochrome C in brain has been confirmed using confocal microscopy. A short 63 kDa isoform of PARG was also presented in primary neuronal culture; however, Poitras et al. [30] observed only the full-length isoform in the brain. In other mammalian cells the full-length $PARG_{110}$ (kDA) is localized in the nucleus, $PARG_{103}$ and $PARG_{99}$ in cytoplasm, but $PARG_{60}$ was found in cytoplasm and mitochondria. Moreover, $PARG_{85}$ and $PARG_{74}$, both catalytic active fragments liberated after caspase-3 action, have been detected in cytoplasm [33]. It is important to underline that most of the PARG activity in unstimulated brain cells is localized in mitochondria and cytoplasm. During pathology, when DNA is damaged, PARG is translocated to the nucleus. It was observed that NMDA receptor-dependent PARP-1 activation led to PARG translocation into the nucleus. PARG overexpression reduces PARP-1 dependent NMDA excitotoxicity. Due to potential role of PARG in pathophysiology, the several inhibitors of this enzyme are under investigation [33–35]. The events described in this chapter are presented on Fig. 1.

PAR in Brain Ischemia Reperfusion–Injury

Although the role of PAR in cell physiology and pathology has been intensively investigated for the last few years, the molecular mechanism by which PAR is involved in maintenance of genomic stability, regulation of transcription, energy metabolism, and cell death is not well understood. The last study of Ahel et al. [36] indicated the important mechanism for PAR function in chromatin remodeling. A chromatin remodeling enzyme amplified in liver cancer 1 (ALC1) also known as CHD1 interacts with PAR and catalyzes PARP-1 stimulated nucleosome sliding. The details results of this study provide new insights into mechanisms by which PAR regulates DNA repair. However, the last studies were focused on the role of PAR in cell death. Alteration of PAR homeostasis has been indicated recently to be important for cell pathology. The regulation of PAR level and the activity of PARP-1 are crucial in the function of many transcription factors that mediate microglial gene transcription during reperfusion after ischemic insult. Nuclear factor kappa B (NF-κB) and p53 have a specific domain that interacts with PAR [37]. NF-κB requires PARP-1 as a coactivator in systemic inflammatory responses [38]. The recent data demonstrated that PARP-1 modulates the hypoxia inducible factor-1 (HIF-1) in vivo. Immediate post-hypoxic HIF-1 alpha accumulation was significantly higher in the presence of PARP-1 [39]. This data indicated that PARP-1/PAR have an important regulatory role in the in vivo response of brain HIF-1 to hypoxia–reoxygenation. Brain ischemia leads to excessive biosynthesis of PAR polymer that exerts toxicity to neurons and other cells. Degradation of PAR by PARG prevents PAR-induced cell death [21]. The authors demonstrated that NMDA receptor excitotoxicity and NMNG-induced cell death were significantly reduced or abolished by decreasing PAR level or by PARG overexpression. Moreover, in an in vivo model of focal cerebral ischemia significantly reduced infarct volume was observed in mice overexpressing PARG. Analysis of the cell death in brain ischemia pathology induced by overstimulation of PARP-1 indicated that not only β-NAD^+/ATP depletion was responsible for cell death, but probably the most important was the action of PAR on transcription factors and mitochondria. The alteration of mitochondrial permeability by PAR and then AIF release and its nuclear translocation seem to be crucial for cell death described as parthanatos [40, 41]. The results of Dawson's group suggest toxic action of PAR itself [21, 40, 41]. They found that isolated PAR and supernatants containing PAR induced the release of AIF from mitochondria. Andrabi et al. [21] presented that highly complex and long-chain PAR polymers were more toxic in comparison to the shorter PAR molecule. Polymer of 16 ADP-ribose residues elicited a small amount of cell death; however, a polymer of >60 ADP-ribose units induced more than 80% of cell death [21]. The recent study has reported the development of circulating IgG antibodies against PAR in the serum of patients with Alzheimer disease [42]. This phenomenon may also occur in human

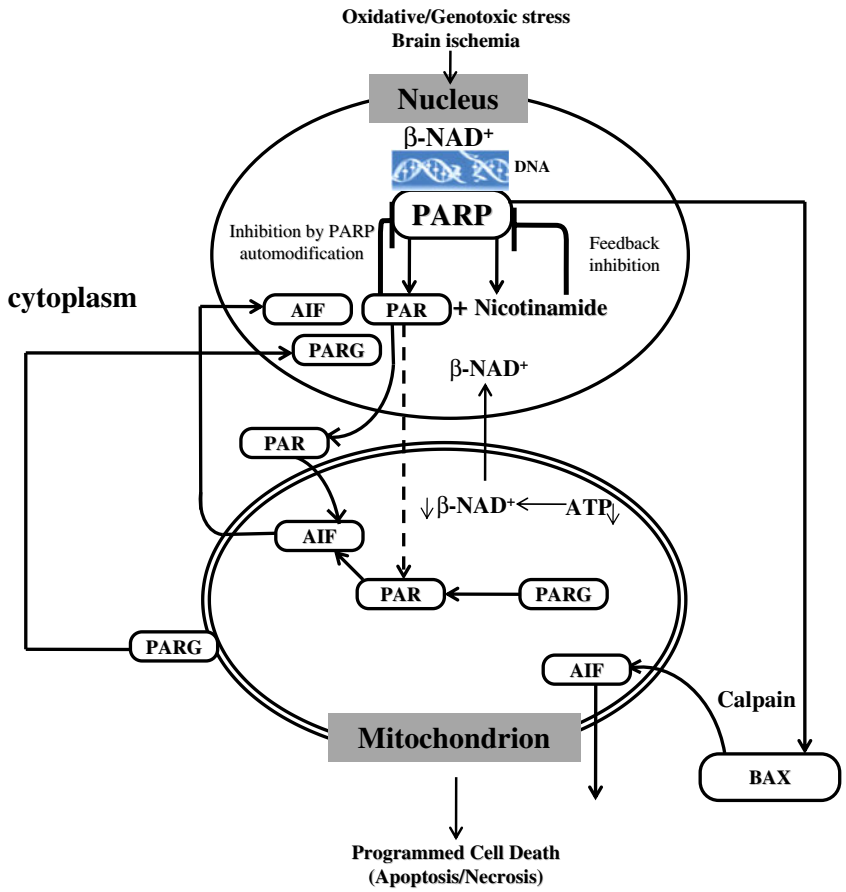

Fig. 1 PAR/ PARP and PARG in mitochondria-nucleus cross talk in brain. The nuclear enzyme PARP-1 use βNAD^+ as a substrate. The products of PARP activity are PAR and nicotinamide, which is responsible for the feedback inhibition of PARP. This DNA bound enzyme is also inhibited by auto poly(ADP-ribosyl)ation. PAR is degraded by PARG to ADP-ribose. The overactivation of PARP leads to enhancement of PAR synthesis that during brain ischemia can be release from nucleus and may lead to mitochondria permeability and AIF release and its translocation into nucleus. The excessive PARP activity may also induce translocation of PARG into nucleus. PARP may also evoked BAX and calpain-dependent AIF release. PARG in mitochondrial matrix could be involve in the regulation of PAR level and AIF release

brain ischemia and may be important for diagnosis and prognosis of ischemic pathology. In human stroke, PARP activation and PAR formation are biphasic. During the early stage of stroke (up to 24 h) the activation of PARP occurs in neuronal cells. In the later stage (3–4 days after stroke), the PARP activity was shown in immune/inflammatory cells [3, 43].

Effect of PARP and PARG Inhibition in Brain Ischemia

Recently, several new regulators of PARP-1 and PARG have been found. Among others: are kinases (including ERK), purines, polyamines, vitamin D_3, theophylline, caffeine metabolites, and tetracycline antibiotics [10, 44, 45]. The data of Hamby et al. [46] indicated that inhibitor of PARP-1 (PJ-34) suppressed inflammation and neuronal cell death in CA1 layer of hippocampus when administered 8 h after transient (10 min) forebrain ischemia. Animals treated with PJ-34 showed a significant inhibition of microglia/macrophage activation. These data indicated that administration of PARP-1 inhibitor at delayed time points after transient forebrain ischemia–reperfusion had a large protective effect of neuronal death in CA1 layer of the hippocampus and a significant reduction in inflammatory response in adult rats. The overactivation of PARP promotes inflammation and cell death [47, 48]. Microglia require several hours to become fully activated, and a PARP-1 inhibitor could potentially offer a long therapeutic window. PARP-1 inhibition before global cerebral ischemia preserves the functionality of the blood–brain barrier with reduced edema. PJ-34 decreases the loss of the tight junction protein occludin after an ischemic episode. In addition, neutrophil infiltration of the cortex was lowered after PJ-34 treatment [7]. Our previous data indicated also a protective effect of PARP-1 inhibitor on brain edema. PARP-1 inhibitors protected the brain against swelling, not only of neurons but also of astrocytes and pericytes. 3-AB decreased swelling of mitochondria and Golgi apparatus evoked by ischemia [16, 49]. Alano and coworkers [44] showed that some common antibiotics of the tetracycline class such as doxycycline or minocycline were relatively potent inhibitors of PARP-1. Neuronal β-NAD^+ depletion and PAR formation were blocked by 100 nM minocycline [44]. The study presented the neuroprotective effect of minocycline not only in focal but also in

global forebrain ischemia. Currently, minocycline is in clinical trials for stroke patients. The anti-inflammatory effect of minocycline is also connected with PARP-1 function as a coactivator of NF-κB. The work of Kauppinen and Swanson [50] demonstrated that PARP-1 interaction with NF-κB promoted microglial metalloproteinase-9 release and neurotoxicity. It was found that many cytokines (TNF-α, MIP-1α, IL-1β, IL-6, IL-8, and INF-γ), inducible nitric oxide synthase (iNOS), and adhesion molecules were marginally induced in the absence of PARP-1 not only in brain where PARP is responsible for over 97% of poly(ADP-ribosyl)ation but also in the other organs [51–54]. Anti-inflammatory and antiapoptotic effects of PARP-1 inhibitors were also described by other groups [45, 47, 55–57]. Pharmacological inhibition of PARP-1 decreased plaque formation in atherosclerosis. Chronic treatment with PJ-34 markedly decreased E-selectin, P-selectin, VCAM-1, and iNOS protein levels [58]. In most studies, the protective effects against stroke or ischemic episodes were observed when PARP-1 inhibitors were used before the beginning of a pathology process. However, papers presented by Strosznajder et al. [16, 49, 59] and Hamby et al. [46] showed that the ameliorating effects of PARP-1 inhibitors were visible even when the inhibitors were applied after an ischemic episode, which is more realistic scenario. Our results with PJ-34 indicated also, that this potent PARP-1 inhibitor significantly augmented the ameliorating effect of pre-conditioning (2 min nonlethal ischemia, 48 h before the main ischemic episode) on neuronal survival [17]. Our data indicated that 3-AB prevented AIF translocation to nucleus and led to overexpression of Bcl-2 protein level [57]. To better understand the role of AIF in cell death, Harlequin (Hq) mice with about 80% reduction of AIF expression were used in the experiments [60]. The authors showed that Hq mice were resistant to death induced by PAR (parthanatos). These animals had reduced lesions after excitotoxicity and stroke. The other studies confirmed the role of AIF in cell death also propagated by BAX-dependent and BAX-independent or BID-mediated mechanism [61–63]. Our data presented the cytoprotective effect of carvedilol (β-adrenoreceptor antagonist) in brain ischemia–reperfusion injury and suggested that this effect was connected with suppression of PARP activity [64]. Moreover, carvedilol prevented β-NAD^+ depletion after ischemic–reperfusion insult [64]. It is known now that PARP-1 inhibition leads not only to neuronal cell protection but also has a positive effect on integrity of the blood–brain barrier and protects brain and endothelial blood vessel cells against inflammation [14, 65, 66]. The data in Table 1 show the effect of PARP-1 inhibitors in animal models of brain ischemia. In contrast, currently available PARG inhibitors display poor specificity and poor blood–brain barrier penetration. The studies on brain ischemia yielded inconsistent results. Wei et al. [35] presented that intranasal administration of PARG inhibitor profoundly decreased ischemic brain injury. Lu et al. [34] demonstrated that an inhibitor of PARG could ameliorate ischemic brain damage and suggested PARG as a new therapeutic target for treatment of ischemic injury. The novel PARG inhibitor GPI 16552 provided significant protection even as a post-ischemic treatment [34]. According to Li et al. [78], PARG and PARP inhibitors display a similar time window when used to treat ischemia. PARG-blocking by gallotannin and nobotannin counteract cell death in the in vitro model induced by pro-oxidative factors including H_2O_2 and NMDA [79, 80]. However, selective disruption of mouse $PARG_{110}$ increases the cell damage measured 24 h after middle cerebral artery occlusion [81]. The limitations of PARG inhibitors and knockouts clearly show that further work is needed to clarify the role of this enzyme under pathological conditions.

Effect of Aging and Gender on PARP-1 and PAR Formation

The data presented in this review were obtained using male adult animals. The question arises how PAR metabolism is affected by aging and gender.

PARP-1 activity and PAR formation are significantly modulated by environmental conditions during life span, including accompanying infections and other types of oxidative or genotoxic stress. Until now, little is known on PARP-1 activity and its role in the brain during healthy aging where a lot adaptive/protective processes are activated.

The study of Shibata et al. [82] demonstrated that PARP-1 deficiency caused an increase of point mutations in the brain by aging. These results support the idea that PARP-1 is involved in suppressing imprecise repair of endogenous DNA damage, leading to deletion mutation in brain during aging. Our previous study [83, 84] indicated that, depending on housing conditions (under or without barrier), PARP activity was increased or decreased in the aged hippocampus. Our data showed the significantly higher PARP-1 activity in hippocampus and brain cortex of pathogen-free aged animals comparing to adult animals [84]. The alteration of PARP-1 during aging has different dynamics in particular brain regions. Moreover, PARP-1 activity enhanced by aging in the hippocampus is less or not sensitive for further alteration by additional stress conditions evoked by Fenton reaction or MNNG. These agents significantly stimulated PARP activity in adult hippocampus [84]. In the aged brain, NMDA receptor stimulation had no effect on PARP-1 activity (that has already been increased by aging), while

Table 1 Neuroprotection by pharmacological inactivation of PARP-1 in animal models of brain ischemia

Animal model	Inhibitor	Dose (mg/kg b.w.)	Mode of administration	Effect of PARP-1 inhibition	Ref.
Focal cerebral ischemia					
Rat	Cilostazol	30	Twice orally	Reduction of infarct size, nuclear AIF translocation and apoptosis after 2 h MCAO with 24 h reperfusion	[67]
	DPQ	5–40	i.p., 2 h before ischemia	Reduction of infarct volume (up to 20 mg/kg dose)	[68]
	3-AB	10	Microdialysis, after ischemia	Reduction of infarct volume after 90-min MCAO; attenuation of NMDA-induced glutamate elevation	[69]
	3-AB	1–30	Intra-ventricular, 30 min before ischemia	Decrease of infarct volume in a dose dependent manner after MCAO	[70]
	3-AB	30	i.p. 30 min post-MCAO, combined with 3 mg/kg z-VAD-fmk	Improvement over single-modality treatment of MCAO	[71]
	3-AB	15 and 55	Half of the dose injected i.p. 0.5 h before onset and half injected i.v. at the end of MCAO	Decrease of infarct volume, improvement the neurological outcome and motor function up to 28 days after 2-h MCAO	[72]
	3-AB	250	i.p 15 min before or after reperfusion	Significant decrease in the volume of damaged tissue after 90-min. MCAO—only if administered before reperfusion	[73]
Mouse	3-AB	20–320	i.p. 15 min before reperfusion	Neuroprotection, up to 30% decrease in the infarct volume, improvement of the neurological score 24 h after 15-min left MCAO. Maximal effects at 40 mg/kg; highest doses ineffective	[74]
Global cerebral ischemia					
Rat	PJ34	15	i.p. 8 h after reperfusion and then in 12 h intervals for 7 days	Near-complete inhibition of microglia/macrophage activation and an 84% reduction in CA1 neuronal death after 10 min forebrain ischemia	[46]
	Minocycline	45 (first day) +22,5 (next 13 days)	i.p., twice/day, starting from 24 h after ischemia combined with lateral intracerebral ventricular infusion of flavopiridiol	Synergistic neuro-protection with flavopiridiol 2 and 10 weeks after 10-min four-vessel occlusion, but does not fully inhibit impairment of CA1 synapses	[75]
Gerbil	3-AB	30	i.v., directly after ischemia	Protected >60% of CA1 neurons against death after 3-min. but not 10-min ischemia; decreased forebrain edema after both times	[49]
	DPQ, Benzamide	10–30 (DPQ) or 60, 160 (benzamide)	i.p 2 h before and 2 h after ischemia or 0–360 min during reperfusion	No improvement of the survival of CA1 neurons 7 days after 5-min bilateral carotid artery occlusion	[76]
Mouse	Minocycline	90 (first day) +45 (next 2 days)	i.p., immediately after BCCAO	Specific protection in hippocampus (CA1) and striatum after 30-min bilateral common carotid artery occlusion	[77]

in the adult hippocampus, a significant reaction was observed [85]. In adult male animals, PARP-1 inhibitors exerted several protective effects at cellular and subcellular level [16, 59, 86]. The enhanced activity of PARP-1 in the brain during healthy aging should not be inhibited because of its involvement in DNA repair and genome stability. However, during aging, the immune system is very often disturbed, and inflammation processes through PARP-NF-κB interactions may affect cellular functions leading to cells death. Mabley et al. [87] reported that inhibition of PARP had protective effect by reducing inflammatory mediators and mortality exclusively in male but not in female mice. Moreover, female mice produced less inflammatory mediators compared to the male mice, and they were more resistant to endotoxin-induced mortality when compared to males. To understand the mechanism of this gender specificity and the role of PARP, the effect of ovariectomy was investigated, and it was indicated that this strategy only partially reversed the protection observed in female mice. Pretreatment of male mice with 17-β-estradiol reduced PARP activity and injury evoked by endotoxin (lipopolysaccharide). On the basis of the in vitro study, Mabley et al. [87] suggested that estrogen itself was not responsible for the gender differences but the interaction of estrogen receptor alpha with PARP and complex formation that binds to DNA preventing the recognition of DNA strand breaks by PARP. Moreover, it was found that inhibition of neuronal NO synthase and PARP-1 protected neurons against ischemic damage only in males, while it enhanced ischemic injury in females [88, 89]. Then, Lang and McCullough [90] suggested that divergent cell death pathways are activated after brain ischemic insult in male and female. According to their new concept, the mechanism and outcome of cerebral ischemic injury are influenced strongly by level and availability of sex steroids in the brain. The caspase-mediated neuronal death pathway may occur in female in contrast to male where PAR/AIF signaling is involved in cell death [90]. The further study of Yuan et al. [91] presented that deletion of PARP-1 led to significant decrease in PAR formation and AIF translocation in males and females, but ischemic damage was reduced only in males. Estrogen had no protective effect in this study. Their following studies using AIF-deficient male and female Harlequin mice demonstrated that the neuroprotective effect of this gene deletion occurred only in males. The last data of Liu et al. [92] showed that apoptosis in the female mouse brain reperfused after middle cerebral artery occlusion (MCAO) was dependent on caspase activation. Females treated with the caspase inhibitor (Q-VD-OPh) showed significantly reduced infarct volume and improved neurological scores after MCAO without any significant protection in males. The release of cytochrome C and nuclear caspase-8 level were increased in females after stroke [92]. Moreover, also the effect of minocycline has been found to be sex-dependent [93]. Epidemiological studies indicated that ischemic events occurred with greater frequency in men versus women until advance age. Stroke increases significantly in women after menopause suggesting that sex hormones are mainly responsible in this pathology. Figure 2 presents the signaling pathway evoked by ischemia–reperfusion, which, as consequence, leads to activation of inflammation and to cognitive decline. Gender and age are marked as significant modulatory factors of this pathway.

General Summary

The data presented in this review underline the novel important role of poly(ADP-ribosyl)ation as a life/death signaling pathway playing a crucial function in cross talk between nucleus and mitochondria in brain ischemic pathology.

Several new regulators of PAR homeostasis have been recently found and described. The presented data indicated that ischemic death pathways were sexually dimorphic, and the pharmacological substances used to reduce PARP activa-

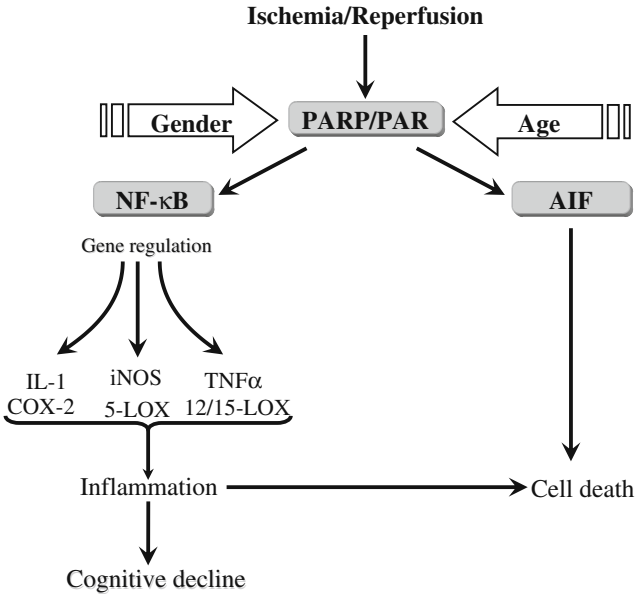

Fig. 2 The role of PARP/PAR in brain ischemia pathology. Effect of age and gender. In brain ischemia pathology, PAR/AIF death pathway occurs mainly in adult male. In adult female, the PARP-1/NF-κB inflammatory pathways are less expressed compared to male. It is suggested that the caspase/cytochrome C signaling is responsible for cell death in adult females. Aging affects the molecular targets as PARP/PAR in nucleus and AIF in mitochondria

tion may have limited benefits to females. Age- and gender-dependent regulations of PARP-1 and PARG may be key factors in the creation of a therapeutic strategy against brain ischemia–reperfusion injury.

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