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REVIEW

Drug targets for traumatic brain injury from poly(ADP-ribose)polymerase pathway modulation

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The deleterious pathophysiological cascade induced after traumatic brain injury (TBI) is initiated by an excitotoxic process triggered by excessive glutamate release. Activation of the glutamatergic N-methyl-D-aspartate receptor, by increasing calcium influx, activates nitric oxide (NO) synthases leading to a toxic production of NO. Moreover, after TBI, free radicals are highly produced and participate to a deleterious oxidative stress. Evidence has showed that the major toxic effect of NO comes from its combination with superoxide anion leading to peroxynitrite formation, a highly reactive and oxidant compound. Indeed, peroxynitrite mediates nitrosative stress and is a potent inducer of cell death through its reaction with lipids, proteins and DNA. Particularly DNA damage, caused by both oxidative and nitrosative stresses, results in activation of poly(ADP-ribose) polymerase (PARP), a nuclear enzyme implicated in DNA repair. In response to excessive DNA damage, massive PARP activation leads to energetic depletion and finally to cell death. Since 10 years, accumulating data have showed that inactivation of PARP, either pharmacologically or using PARP null mice, induces neuroprotection in experimental models of TBI. Thus TBI generating NO, oxidative and nitrosative stresses promotes PARP activation contributing in post-traumatic motor, cognitive and histological sequelae. The mechanisms by which PARP inhibitors provide protection might not entirely be related to the preservation of cellular energy stores, but might also include other PARP-mediated mechanisms that needed to be explored in a TBI context. Ten years of experimental research provided rational basis for the development of PARP inhibitors as treatment for TBI.
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Abbreviations: 3-AB, 3-aminobenzamide; 4HNE, 4-hydroxynonenal; AG, aminoguanidine; AIF, apoptosis-inducing factor; AMPAR, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid receptor; Ca^{2+}, calcium; CBF, cerebral blood flow; CSF, cerebrospinal fluid; GPx, glutathione peroxidase; KO, knockout; L-NAME, Nω-nitro-L-arginine-methylester; L-NIL, L-N(6)-(1-iminoethyl) lysine hydrochloride; Na^{+}, sodium; NMDAR, N-methyl-D-aspartate receptor; NOS, nitric oxide synthase; PARP, poly(ADP-ribose)polymerase; SOD, superoxide dismutase; TBI, traumatic brain injury

With a global incidence of traumatic brain injury (TBI) generally reported as ≈200 in 100 000 with a mortality of 20 per 100 000, neurotrauma is a major public health challenge. TBI remains one of the leading causes of death and disability in industrialized countries and its incidence increases in developing countries (Reilly, 2007). Over the last two decades, understanding of the complex pathobiology of TBI has improved significantly. However, despite numerous studies on animal models of TBI searching for therapeutic strategies, no neuroprotective therapy is currently available for human traumatized patients (Bramlett and Dietrich, 2004).

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TBI promotes focal injuries resulting from direct loadings. Focal injuries to the brain account for one half of all severe head injuries. Diffuse injury also occurs after TBI and is most often caused by inertial forces such as translational and/or rotational acceleration (LaPlaca *et al*., 2007). The pathophysiology of TBI is complicated and involves both primary and secondary insults. Primary injury to the brain can be induced by numerous mechanisms, such as brain contusion, shearing and stretching of the brain tissue caused by motion of the brain structures relative to the skull and haematoma. Secondary injury development includes complex biochemical and physiological processes that are initiated by the primary insult and manifest over a period of hours to days and even months. Animal models commonly used in TBI research have been developed and used to experimentally mimic some aspects of the behavioural, tissular and cellular consequences of human

TBI in order to understand post-traumatic pathophysiology (Cernak, 2005).

This report will outline the involvement of nitric oxide (NO), oxidative and nitrosative stresses, and poly(ADP-ribose)polymerase (PARP) in the pathophysiology of TBI. Because NO and oxidative stress-mediated toxicities are triggered by excitotoxicity, the role of glutamate will be briefly examined.

Excitotoxicity: initiator of the deleterious cascade of cell death

The deleterious pathophysiological cascade induced after TBI is initiated by an excitotoxic process triggered by excessive glutamate release. Glutamate is the major excitotoxic neurotransmitter in the mammalian central nervous system, but it is also a potent neurotoxin that can kill nerve cell. In traumatized patients, a correlation has been demonstrated between bad outcome and important increase in glutamate (Bullock *et al.*, 1998; Zhang *et al.*, 2001). Increased levels of extracellular glutamate following head injury causes overstimulation of both metabotropic and ionotropic receptors that may result in secondary events leading to neuronal cell death. Activation of metabotropic receptors causes mobilization of calcium (Ca^{2+}) from internal stores. Three types of ionotropic glutamatergic receptors are activated: N-methyl-D-aspartate (NMDA) receptor, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid receptor (AMPAR) and kainate receptor. When activated, AMPAR leads to the entry of Na^+ in cell contributing to membrane depolarization. In addition, intracellular water increases inducing a cell swelling also called cytotoxic oedema. When activated, NMDA receptor promotes the entry of Na^+ and Ca^{2+} in cell contributing to membrane depolarization and to activation of Ca^{2+}-dependent enzymes (Yi and Hazell, 2006). Studies using glutamatergic receptors antagonists showed neuroprotective effects in experimental models of TBI, demonstrating the deleterious role of excitotoxicity (Faden *et al.*, 1989; McIntosh *et al.*, 1990; Shapira *et al.*, 1990; Faden, 1993; Allen *et al.*, 1999). Calcium overload can trigger many downstream neurotoxic cascades, including the uncoupling mitochondrial electron transfer from ATP synthesis, and the activation and overstimulation of enzymes, such as calpains, protein kinases, endonucleases and NO synthases (NOS) (Farkas and Povlishock, 2007).

Involvement of NOS in TBI sequelae

Experimentally, TBI induces the activation of NOS leading to the production of NO in brain (Yamanaka *et al.*, 1995; Sakamoto *et al.*, 1997; Muralikrishna Rao *et al.*, 1998; Cherian *et al.*, 2000; Ahn *et al.*, 2004). NO production has been also demonstrated in cerebrospinal fluid (CSF) and in brain tissue after human TBI (Clark *et al.*, 1996; Uzan *et al.*, 2001; Hlatky *et al.*, 2002; 2003; Silberstein *et al.*, 2002; Toczylowska *et al.*, 2006) and the end–products of NO, nitrate/nitrite, levels are strongly correlated with TBI severity (Hlatky *et al.*, 2002). Interestingly, injured patients who died had less nitrate/nitrite than the survivors. Particularly, it has been shown that NO can combine to cellular thiols compounds leading to the formation of S-nitrosothiols (Foster *et al.*, 2003; Gow *et al.*, 2004). Bayir *et al.* (2002; 2003) demonstrate that increase in S-nitrosothiols is correlated with intracranial pressure decrease, suggesting a neuroprotective role of S-nitrosothiols following TBI. NO can be synthetized by three isoforms of NOS: NOS1 (neuronal NOS) and NOS3 (endothelial NOS) that are both constitutive and Ca^{2+}-dependent enzymes. The third one is NOS2 (inducible NOS) that produces large quantity of NO in inflammatory situations. The involvement of NOS has been quite well established in TBI pathophysiology, even if each isoform plays a different role. The first study demonstrating the role of NOS in TBI has been published by Mésenge *et al.*, (1996). Indeed, post-treatment with Nω-nitro-L-arginine-methylester (L-NAME), a non-selective NOS inhibitor, decreases the post-traumatic neurological deficit with a therapeutic window of opportunity of 60 min. The same results have been observed with 7-nitroindazole, a selective NOS1 inhibitor (Mésenge *et al.*, 1996). In addition, pretreatment with this inhibitor decreases the brain lesion volume following TBI (Wada *et al.*, 1999). Intracerebral injection of NOS1 antiserum reduced blood–brain barrier permeability and cerebral oedema (Sharma *et al.*, 2006). All these data demonstrate the deleterious role of NOS1.

Inhibition of all NOS isoforms is not desirable considering the vasodilator role of NO produced by NOS3 and involved in the regulation of vascular tone. Indeed, treatment with L-arginine, the substrate of NOS, reduces the brain lesion volume and this is associated with NOS3 activation and improved cerebral blood flow (CBF) (DeWitt *et al.*, 1997; Wada *et al.*, 1998a; 1999; Cherian *et al.*, 1999). In addition, $NOS3^{-/-}$ mice have a decreased CBF compared with wild-type animals (Hlatky *et al.*, 2003), and NOS3 is necessary to induce beneficial vascular effect of L-arginine after TBI (Hlatky *et al.*, 2003). Considering these data, NO produced by NOS3 plays a beneficial role after TBI.

If the roles of NOS1 and NOS3 are quite well understood, the one of NOS2 is still controversial in TBI. Some studies suggest that early after TBI, effects of NOS2 may be detrimental as aminoguanidine (AG), a selective NOS2 inhibitor, has been shown to reduce neuronal cell death following TBI (Wada *et al.*, 1998b; Görlach *et al.*, 2000; Stoffel *et al.*, 2000; 2001). In addition, $NOS2^{-/-}$ mice have a reduced motor and cognitive deficits and brain lesion volume than those of wild-type animals after cryogenic TBI (Jones *et al.*, 2004). More recently, three different selective NOS2 inhibitors, AG, L-N(6)-(1-iminoethyl) lysine hydrochloride (L-NIL) and 1400W reduce motor deficits and brain lesion 24 and 72 h after TBI (Jafarian-Tehrani *et al.*, 2005; Louin *et al.*, 2006). So these data argue in the deleterious role of NOS2 in the early phase after brain trauma. However, several reports have suggested potential beneficial effects of NOS2, particularly at delayed time points after injury. Indeed, Sinz *et al.* (1999) have shown that $NOS2^{-/-}$ mice present more important cognitive deficits than wild-type mice at 17–21 days after TBI. In addition, NOS2 knockout (KO) mice have been demonstrated to have greater loss of brain levels of ascorbate, an endogen antioxidant, compared with wild-type animals at 72 h after TBI (Bayir *et al.*, 2005). Moreover, treatment with two selective

inhibitors of NOS2, AG and L-NIL increases post-traumatic cognitive deficits at 21 days after injury (Sinz *et al.*, 1999). Treatment with L-arginine 48 h after TBI, that is, when NOS2 is activated, induces neuroprotective effects (Cherian *et al.*, 2003). As TBI decreases CBF (Bouma *et al.*, 1991; Marion *et al.*, 1991) and NO is a powerful cerebral vasodilator, two studies examined the role of NOS2-derived NO on CBF after TBI. Animals receiving antisense NOS2 oligodeoxynucleotides exhibited an exacerbation of the CBF reduction after TBI (Steiner *et al.*, 2004) and NOS2 KO mice showed a reduced recovery of CBF 72 h after TBI in hippocampus, thalamus and amygdala/piriform cortex, which are structures outside the contusion (Foley *et al.*, 2008). Taken together, NOS2 appears to mediate detrimental effects early after TBI while mediating beneficial actions at more delayed time points.

Involvement of oxidative and nitrosative stresses in TBI

The glutamate-mediated increase in intracellular Ca^{2+} also activates several free radical pathways, including the conversion of xanthine dehydrogenase to xanthine oxidase, NOS, the phospholipase A2-cycloxygenase pathway and mitochondria, leading to post-traumatic oxidative stress. The oxidative stress situation is defined as an imbalance between free radical production and endogen antioxidant systems. Brain is particularly vulnerable to oxidative stress as it contains low levels of antioxidant systems and high concentrations of iron that can catalyse the production of free radicals, and as it is rich in unsaturated fatty acids that are targets for lipid peroxidation (Margaill *et al.*, 2005). In humans, TBI increases plasma and CSF levels of malonedialdehyde, a marker of lipid peroxidation, as early as 2–3 h that persists at least until 7 days after injury (Cernak *et al.*, 2000; Cristofori *et al.*, 2001; Bayir *et al.*, 2002). In addition, superoxide dismutase (SOD) activity decreases at 24 h and during 7 days after severe TBI (Cernak *et al.*, 2000).

After experimental TBI, hydroxyl radicals OH and superoxide anions O_2^- increase early after injury (Hall and Braughler, 1993; Fabian *et al.*, 1998). In addition, production of OH is strongly correlated with lipid peroxidation and post-traumatic cerebral oedema (Nishio *et al.*, 1997). Endogen antioxidants enzymatic systems, such as catalase and glutathione peroxidase (GPx), have increased activities between 3 and 7 days following TBI (Goss *et al.*, 1997). If oxidative stress has been shown in brain after TBI, brain trauma also induces whole-body oxidative stress (Shohami *et al.*, 1999; Pratico *et al.*, 2002). There is compelling evidence supporting the role of oxidative stress in post-traumatic sequelae. Indeed, antioxidant strategies aiming at decreasing oxidative stress reduced post-traumatic neurological deficits, cerebral oedema and brain lesion volume: PEG-SOD, an antioxidant enzyme (Hamm *et al.*, 1996); melatonine mimicking GPx (Mésenge *et al.*, 1998a); OPC-14117 (7-hydroxy-1-[4-(3-methoxyphenyl)-1-piperazinyl]acetylamino-2,2,4,6-tetramethylindan), a scavenger of superoxide anions (Aoyama *et al.*, 2002); phenyl-tert-butylnitrone, a free radical scavenger (Lewen *et al.*, 2001; Marklund *et al.*, 2001); mesylate tirilazad, an inhibitor of lipid peroxidation (Hall *et al.*, 1988), and SOD1 (Mikawa *et al.*, 1996) or SOD3 (Pineda *et al.*, 2001) transgenic mice. In addition, KO mice for GPx1 present an increased neuronal cell death after TBI compared with wild-type animals (Flentjar *et al.*, 2002).

NO-mediated toxicity has been shown to involve peroxynitrite. Indeed, superoxide anions could combine with NO to generate peroxynitrite anions. Peroxynitrite is a strong oxidant that can directly react with tyrosine of proteins producing nitrotyrosine (Pacher *et al.*, 2007). The development of antibodies that recognize nitrotyrosine provided a major impetus to the study of peroxynitrite and nitrotyrosine is now widely used as a marker of nitrosative stress. Mésenge *et al.* (1998b) have shown an increase in nitrotyrosine in brain tissue between 4 and 24 h in a model of diffuse TBI. In a model of focal TBI, nitrotyrosine is present as early as 30 min after TBI and persists at least during 72 h (Besson *et al.*, 2003a). Several studies have shown 3-nitrotyrosine staining in infiltrating polymorphonuclear neutrophils, microvascular endothelium and neurons throughout the cytoplasm of both cell bodies and the proximal portions of axons and dendrites (Whalen *et al.*, 1999; Hall *et al.*, 2004; Bayir *et al.*, 2005). Treatment with L-NAME, a non-selective NOS inhibitor, decreases nitrotyrosine levels after TBI, demonstrating the involvement of NO in peroxynitrite formation (Mésenge *et al.*, 1998b). The deleterious role of peroxynitrite is supported by reports demonstrating the beneficial effects of peroxynitrite scavengers such as penicillamine, penicillamine methyl-ester (Hall *et al.*, 1999), and tempol (Deng-Bryant *et al.*, 2008), and the peroxynitrite decomposition catalyst FP15 (Lacza *et al.*, 2003) in reducing neuronal injury and improving neurological recovery following TBI.

Although not a free radical in nature, peroxynitrite is much more reactive than its parent molecules NO and O_2^- (Pacher *et al.*, 2007). The half-life of peroxynitrite is short but sufficient to cross biological membranes and allow significant interactions with most critical biomolecules (Pacher *et al.*, 2007). Peroxynitrite anions attack protein components, such as transition metal centres and amino acids with cysteine oxidation, tyrosine nitration and tryptophan, methionine and histidine oxidation. In many cases, nitration of antioxidant enzymes leads to their inactivation. In particular, it has been shown recently that SOD2 extracted from brains of mice and humans after TBI demonstrates both a significant increase in tyrosine nitration and a dramatic decrease in its enzymatic activity (Bayir *et al.*, 2007). Furthermore, deficiency of NOS1 but not NOS2 and NOS3 attenuates SOD2 nitration after experimental TBI (Bayir *et al.*, 2007). So nitration of SOD2 is likely a consequence of peroxynitrite within the intracellular milieu of neurons rather than activated inflammatory cells. Nitration and inactivation of SOD2 could lead to self-amplification of oxidative stress in the brain progressively enhancing peroxynitrite production and secondary damage. Peroxynitrite anions also trigger lipid peroxidation in membranes, liposomes and lipoproteins. Several intermediates products of lipid peroxidation including isoprostanes and 4-hydroxynonenal (4HNE) serves as an indirect marker of peroxynitrite effects on lipids. Lipid peroxidation has been demonstrated with 4HNE staining in experimental models of diffuse and focal TBI (Hall *et al.*, 2004; Chen *et al.*, 2007). Last

but not the least, peroxynitrite can alter DNA by introducing oxidative damage on guanine producing 8-oxoguanine. It may also attack DNA generating strand breaks. The formation of peroxynitrite-mediated DNA breakage represents one of the most deleterious aspects of peroxynitrite anions as they represent the trigger for the activation of the nuclear DNA repair enzyme, PARP.

PARP: executioner of cell death

PARP is a constitutive nuclear enzyme present in eukaryotes. It belongs to an expanding family of 17 members implicated in such physiological processes as DNA repair, maintenance of genomic integrity, gene transcription, cell division and apoptosis (Schreiber *et al.*, 2006). PARP-1 (EC 2.4.2.30), also known as poly(ADP-ribose) synthetase, the founding member of the superfamily, is the major isoform present in the nucleus. It has been the most extensively studied as it is responsible for most of the poly(ADP-ribose) formation in health and disease.

At the beginning of PARP research, strategies aiming at studying approaches to inhibit PARP have been carried out almost exclusively by scientist working on cancer research. Enhanced PARP expression and/or activity have been demonstrated in several tumour cell lines. This may allow tumour cells to withstand genotoxic stress and increase their resistance to DNA-damaging agents. Interestingly, inhibition of PARP sensitizes tumour cells to cytotoxic therapy, including temozolomide, platinums, topoisomerase I inhibitors and radiation (Ratnam and Low, 2007). Thus inhibition of PARP could enhance the anti-tumour effect of radiation or chemotherapy. In this order, PARP inhibitors are being developed for clinical use both alone and as chemosensitizers in combination with chemotherapy. Several clinical studies are conducted to evaluate the safety and effectiveness of the combination of PARP inhibitor with chemotherapy (de la Lastra *et al.*, 2007; Lewis and Low, 2007; Ratnam and Low, 2007).

PARP is also known to have an essential role in other various diseases (Pacher and Szabo, 2008). When activated in response to oxidative and nitrosative stress-induced DNA strand breaks, PARP initiates an energy-consuming cycle by transferring ADP-ribose units from NAD to a set of nuclear acceptor proteins, including histones, several chromatin-binding proteins and PARP itself (Hassa *et al.*, 2006). In order to study the role of PARP in pathological conditions, numerous inhibitors of PARP have been synthetized (Southan and Szabó, 2003; Jagtap & Szabó, 2005) and three different KO mice for PARP-1 have been generated (Wang *et al.*, 1995; de Murcia *et al.*, 1997; Masutani *et al.*, 2000). Using these pharmacological tools, it has been shown that PARP mediates necrotic cell death in response to excessive DNA damage under pathological conditions. This process results in rapid depletion of intracellular NAD, in a loss of ATP as it is used to synthesize new NAD, and finally to cell death. It was first suggested by Nathan Berger more than two decades ago (Berger, 1985) and this 'PARP suicide hypothesis' was demonstrated *in vitro*. Oxidative stress induced *in vivo* in brain has been demonstrated to promote PARP activation, which contributes to neuronal cell death (Besson *et al.*, 2003b). Additionally, PARP inhibitors have been shown to prevent *in vitro* neuronal cell injury from glutamate (Cosi *et al.*, 1994), NMDA (Mandir *et al.*, 2000), direct NO (Wallis *et al.*, 1993) and NO donors (Zhang *et al.*, 1994). So these results demonstrate a role of PARP in glutamate-mediated excitotoxicity. As this deleterious cascade occurs in TBI, massive DNA breakage has been reported after TBI (Rink *et al.*, 1995; Colicos and Dash, 1996; LaPlaca *et al.*, 1999; Satchell *et al.*, 2003), and peroxynitrite and poly(ADP-ribosyl)ation co-localize in areas of cell death in traumatic brain tissues (Besson *et al.*, 2003a). PARP is markedly activated as early as 30 min after TBI and its activation persists for 72 h after TBI (LaPlaca *et al.*, 1999; Besson *et al.*, 2003a; Satchell *et al.*, 2003) Tyrosine nitration and PARP activation are both found to be persistently increased compared with normal brain, with relative peaks seen at 8 and 72 h (Satchell *et al.*, 2003). As poly(ADP-ribose) glycohydrolase rapidly degrades polymers of ADP-ribose (Davidovic *et al.*, 2001), this suggests that ADP-ribosylation, that is, PARP activation, is a prolonged phenomenon. This pattern of PARP activation is likely related to the continuing presence of peroxynitrite in the lesioned brain tissue. It is also conceivable that a massive early DNA breakage, which remains unrepaired for prolonged periods of time, is responsible for the prolonged pattern of PARP activation. Cleavage of PARP by caspase-3, occurring only 7 days after TBI (LaPlaca *et al.*, 1999), is one of the mechanism to inactivate PARP, thus preserving cell energy stores required during apoptosis. Finally taken together, these data indicate that, following brain trauma, NO leads to peroxynitrite formation, via its combination with superoxide anion, which in turn induces DNA strand breaks rendering PARP active.

After TBI, PARP is present in human brain pericontusional tissue in neurons either in the nucleus exclusively or in both nuclear and cytoplasmic compartments (Ang *et al.*, 2003). Moreover, PARP is activated by brain trauma as poly(ADP-ribose)-modified proteins are increased at 24 h and until 48 h in CSF of pediatric TBI patients (Fink *et al.*, 2008). Mitochondrial proteins belonging to electron transport chain complexes, such as cytochrome c reductase and oxidase, F_1F_0ATPase β subunit, HSP60, were found to be proteins poly-ADP-ribosylated (Lai *et al.*, 2008). Post-traumatic PARP activation is also associated to tissue NAD decrease in cortex and hippocampus (Lai *et al.*, 2008). These data suggest that poly(ADP-ribosyl)ation on electron transport chain function may impair mitochondrial respiration contributing, at least partly, to PARP-mediated energy failure. Two PARP inhibitors, INH2BP (5-iodo-6-amino-1,2-benzopyrone) (Satchell *et al.*, 2003) and INO-1001 (Clark *et al.*, 2007), prevent NAD depletion and improve post-TBI deficits, demonstrating PARP-mediated energy failure as a contributor to the pathological sequelae of TBI. *In vitro* PARP inhibition protects hippocampal slices against percussion-induced loss of CA1 pyramidal cell-evoked response (Wallis *et al.*, 1996). Whalen *et al.* (1999) showed that motor and cognitive deficits of mice submitted to TBI are less severe when the PARP-1 gene is inactivated. The prototypical PARP inhibitor, 3-aminobenzamide (3-AB), and other benzamide derivatives induce neuroprotective effects on the neurological deficit and the brain lesion after closed head injury in mice (Mésenge *et al.*, 1999) and after TBI

induced by fluid percussion (Besson *et al.*, 2003a). Delaying the treatment of PARP inhibition relative to the TBI produces a therapeutic window of opportunity of 2 h (Mésenge *et al.*, 1999). GPI-6150 (1,11b-dihydro-[2H]benzopyrano[4,3,2-de]isoquinolin-3-one), another PARP inhibitor, reduces the injured area in the very early phase (24 h) after TBI (LaPlaca *et al.*, 2001). Two water-soluble PARP inhibitors, PJ34 [N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(N,N-dimethylamino)acetamide, HCl] and INO-1001 induce neurological recovery-promoting effects in a model of TBI caused by fluid percussion in rat (Besson *et al.*, 2005). Indeed, repeated treatment with PJ34 and INO-1001 decreases the neurological deficit 3 days after injury and these protective effects still persist at 7 days post injury (Besson *et al.*, 2005). It is important to emphasize that the protective effect of PARP inhibition on neurological function is a lasting one, which remains significant even 7 (Besson *et al.*, 2003a; 2005) or 21 days (Satchell *et al.*, 2003) after TBI. These data strengthen the hypothesis that PARP activation promotes deleterious effects on the neurological consequences in the early and late phases after TBI. Finally, a NOS1 inhibitor, 3-bromo-7-nitroindazole, significantly decreases the production of poly(ADP-ribose) in damaged cerebral cortex after cryogenic lesion, demonstrating that TBI-induced PARP activation depends, at least in part, on prior activation of NOS1 (Hortobagyi *et al.*, 2003).

Pharmacological inhibition of PARP activation may also be a viable approach for improving the outcome of stem cell transplantation. This can be achieved by two ways. First, it may make the host tissue environment more receptive for a graft. Second, it may directly protect the transplanted cells from necrosis induced by peroxynitrite. Indeed, treatment with the PARP inhibitor PJ34 improves the neurological score after cryogenic lesion, and increases it even further following stem cell transplantation. High peroxynitrite production coincides with the loss of the majority of the grafted cells, and inhibition of the PARP activation cascade increases the number of surviving cells (Lacza *et al.*, 2003). This PARP inhibition approach may ultimately lead to an optimized grafting strategy.

In cerebral ischaemia studies in which different PARP inhibitors were used and full dose–response curves were obtained, the protection provided by PARP inhibitors diminishes when the dose of agent is increased; that is, a bell-shaped dose–response is observed (Takahashi *et al.*, 1997). Consistent with this observation, similar data have been noticed with 3-AB in a model of TBI (Mésenge *et al.*, 1999). The beneficial effects of PJ34 and INO-1001, two potent PARP inhibitors, on the neurological score have been seen despite no significant benefit on brain lesion volume, suggesting that the protective effect by PARP inhibition could not be attributed to salvaging significant amounts of lesioned tissue (Besson *et al.*, 2005). This observation has been also noticed in both pharmacological inhibition and genetic intervention in models of TBI induced by controlled cortical impact (Whalen *et al.*, 1999; Satchell *et al.*, 2003) and cold injury (Lacza *et al.*, 2003), indicating that functional improvement does not always correlate with the extent of brain damage. In addition, more complete inhibition of PARP with INH2BP impairs spatial memory acquisition independent of injury, and is associated with ribosylation of 14-3-3gamma, a protein implicated in learning and memory (Satchell *et al.*, 2003). As the catalytic domain is very similar in PARP members, PARP inhibitors are not isoenzyme-specific but block total PARP activity, thus demonstrating the overall role of PARP in TBI (Figure 1). These observations suggest that complete and non-selective inhibition of all PARP isoforms may produce some potential adverse effects, and that partial inhibition may be a more desirable approach. All PARP family members ribosylate target proteins and use NAD, but PARP-1 and -2 are the only family members known to be activated by DNA strand breaks (Schreiber *et al.*, 2002). Even if both enzymes have overlapping and redundant functions (Ménissier de Murcia *et al.*, 2003), PARP-2 has been thought to be less active isoform contributing only 5–10% of total PARP activity in response to DNA damage (Amé *et al.*, 1999; Schreiber *et al.*, 2002). PARP-2 KO mice have been generated (Ménissier de Murcia *et al.*, 2003) and very recently, Pellicciari *et al.* (2008) have synthetized selective PARP-2 inhibitors. These two pharmacological

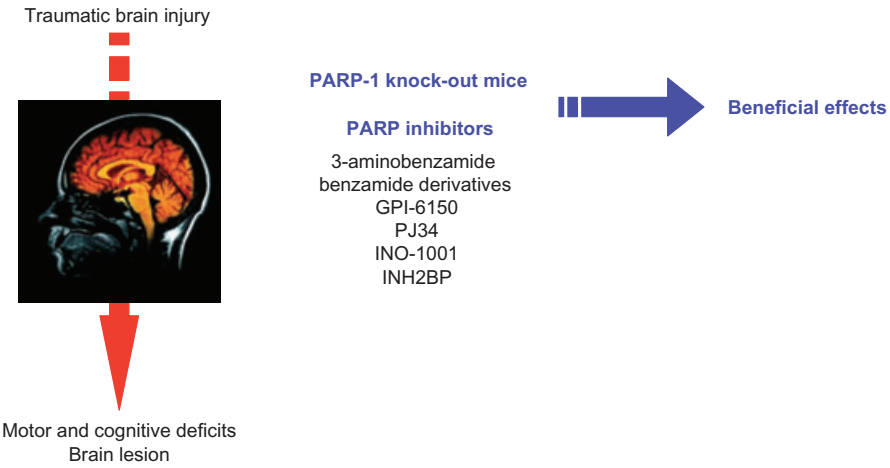

Figure 1 Poly(ADP-ribose) polymerase (PARP) strategies evaluated on deleterious consequences induced by traumatic brain injury. GPI-6150, 1,11b-dihydro-[2H]benzopyrano[4,3,2-de]isoquinolin-3-one; INH2BP, 5-iodo-6-amino-1,2-benzopyrone; PJ34 N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(N,N-dimethylamino)acetamide, HCl.

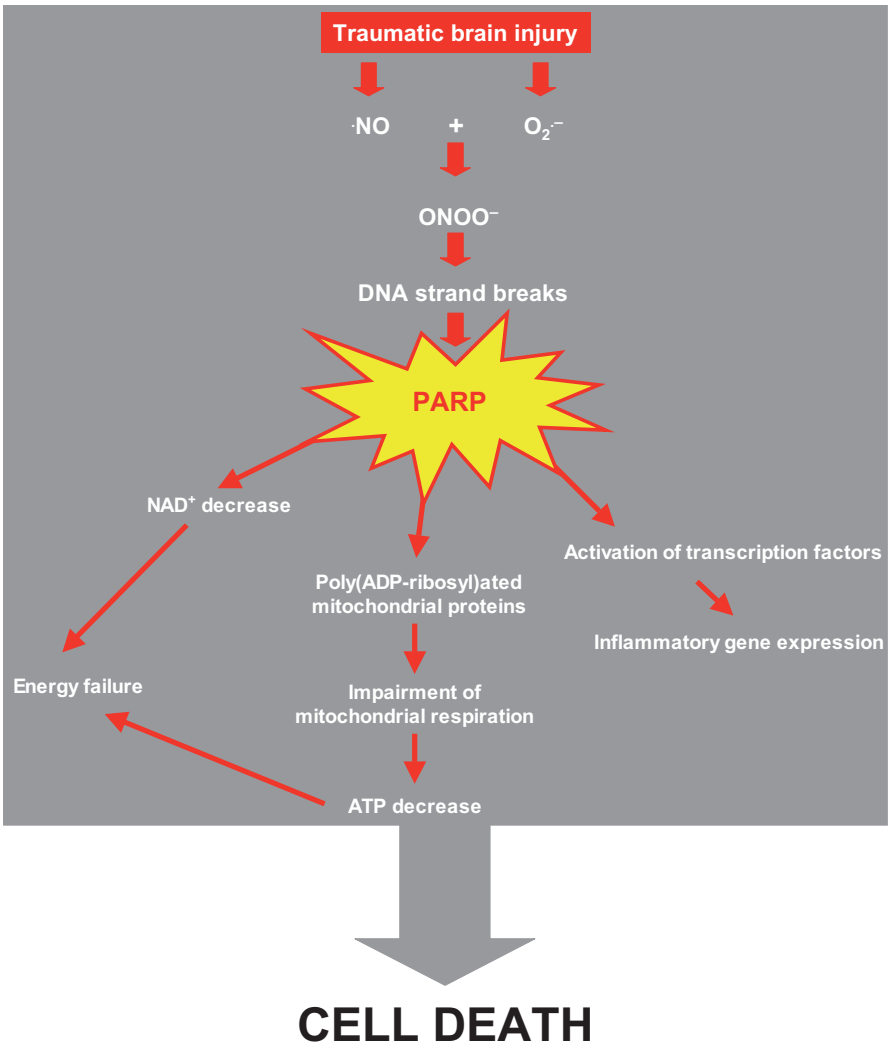

Figure 2 The nitric oxide-oxidative and nitrosative stress–Poly(ADP-ribose) polymerase (PARP) pathway in traumatic brain injury.

approaches will further help in the understanding of the role of PARP-2 in TBI. In addition, it will be very interesting to further evaluate the only effect of PARP-1 inhibition.

Deleterious mechanisms of PARP activation toxicity in brain are multiple (Figure 2). First, PARP activation mediates cell death. When DNA is severely damaged, PARP is massively activated resulting in high consumption of NAD and ATP and finally necrosis. Second, PARP regulates inflammation as it acts also as a co-activator of the transcription factor nuclear factor-kappa B resulting in the synthesis of pro-inflammatory mediators. In addition, PARP is able to directly poly-ADP-ribosylate other transcription factors including STAT and activator protein-1 and -2 (Kauppinen, 2007). By this way, inhibition of PARP has been shown to mediate many anti-inflammatory effects in various inflammatory diseases (Szabó, 2006) and acute brain injuries including stroke (Koh *et al.*, 2004; Haddad *et al.*, 2006). Moreover, PAR synthesis induced by PARP promotes translocation of apoptosis-inducing factor (AIF) from mitochondria to the nucleus, subsequent DNA fragmentation and caspase-independent programmed cell death (Andrabi *et al.*, 2006; Yu *et al.*, 2006). As TBI induces neuroinflammation (Ray *et al.*, 2002) and AIF translocation (Zhang *et al.*, 2002), one can ask whether PARP inhibition may promote beneficial effects by interacting theses mechanisms.

General conclusion

Research in the area of NO-oxidative and nitrosative stresses–PARP cascade has led to a better understanding of the pathophysiology of TBI. The mechanisms by which PARP inhibitors provide protection might not entirely be related to the preservation of cellular energy stores, but might also include other PARP-mediated mechanisms that needed to be explored in the context of TBI. The marked beneficial effects of PARP inhibitors in different animal models of TBI suggest that PARP inhibitors can be exploited to treat this important cause of mortality. It is necessary to point out that PARP participates in DNA repair, many global cellular functions (Hassa *et al.*, 2006) and memory formation (Satchell *et al.*, 2003). Partial inhibition may be a safer approach for the TBI treatment than

complete PARP inhibition. The advantage of PARP inhibitors as agents for the treatment of TBI includes the short duration of treatment, which is an important safety concern when inhibiting an enzyme that regulates nuclear integrity. Thus the future clinical use of PARP inhibitors for acute life-threatening indications, such as TBI, seems to be well justified and very promising.

Conflics of interest

None.

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