REVIEW

Targeting the mitochondrial permeability transition pore in traumatic central nervous system injury

Joe E. Springer*, Pareshkumar Prajapati, Patrick G. Sullivan
Spinal Cord and Brain Injury Research Center, Department of Neuroscience, University of Kentucky, Lexington, KY, USA

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Abstract

The mitochondrion serves many functions in the central nervous system (CNS) and other organs beyond the well-recognized role of adenosine triphosphate (ATP) production. This includes calcium-dependent cell signaling, regulation of gene expression, synthesis and release of cytotoxic reactive oxygen species, and the release of cytochrome c and other apoptotic cell death factors. Traumatic injury to the CNS results in a rapid and, in some cases, sustained loss of mitochondrial function. One consequence of compromised mitochondrial function is induction of the mitochondrial permeability transition (mPT) state due to formation of the cyclosporine A sensitive permeability transition pore (mPTP). In this mini-review, we summarize evidence supporting the involvement of the mPTP as a mediator of mitochondrial and cellular demise following CNS traumatic injury and discuss the beneficial effects and limitations of the current experimental strategies targeting the mPTP.

Correspondence to:
Joe E. Springer, Ph.D., jspring@uky.edu.

orcid:
0000-0001-9611-8107 (Joe E. Springer)

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Introduction

The ability of cells in the central nervous system (CNS) to survive and maintain a functional level of homeostasis following a traumatic injury depends on a number of critical factors. At a relatively gross level, the type of insult (vascular, structural, *etc.*) as well as the severity and proximity of cells to the injury site are obvious factors. However, many pathophysiological events occur at the subcellular and molecular levels that ultimately determine cellular demise or recovery. These events have been well studied and include, but are not limited to, glutamate excitotoxicity, Ca^{2+} overload, inflammation, free radical mediated oxidative damage, and a loss of mitochondrial bioenergetics. Some of these secondary injury events are intimately interconnected, which is one rationale for developing neuroprotective therapeutic strategies targeting cell death signaling pathways at the molecular and cellular level.

The Mitochondrial Permeability Transition

Our research has focused on characterizing changes in mitochondrial function after spinal cord (SCI) and traumatic brain (TBI) injuries (Sullivan et al., 2005; McEwen et al., 2011). This is based on well-documented observations that mitochondria play a critical role in determining cellular fate, and compromised mitochondrial function is a prominent feature in both SCI and TBI. Under physiological conditions, mitochondria exhibit a high transmembrane potential generated by the proton pumping components of the respiratory electron transport system. This transmembrane potential is a driving force in the phosphorylation of adenosine diphosphate (ADP) and sequestering Ca^{2+} from the cytosol. However, following SCI or TBI, mitochondria rapidly become dysfunctional resulting in a loss of cytosolic Ca^{2+} buffering capacity due to influx of massive pathophysiological levels of Ca^{2+} through glutamate receptor subtypes, free radical mediated oxidative damage to mitochondrial complex proteins, and a subsequent compromise in bioenergetic capacity. This process becomes insidious as the loss of energy production further reduces the ability of adenosine triphosphate (ATP)-dependent Ca^{2+} channels to regulate Ca^{2+} cytosolic levels. Prolonged Ca^{2+} overload can push mitochondria to the next pathophysiological stage- induction of the mitochondrial permeability transition (mPT) state due to mPT pore (mPTP) formation (Halestrap and Brenner, 2003). The mPT state uncouples respiration from ATP production and by this point recovery of mitochondrial function is limited at best. Cell survival is then compromised when sufficient numbers of mitochondria undergo mPT. Given the importance of mitochondria in cellular function, inhibiting formation of the mPTP is a strategy for limiting cell death in both SCI and TBI. In this perspective article, we summarize what is known about the mPTP and discuss the possible pros and cons of targeting the mPTP as a therapeutic strategy in CNS injury.

The mPTP is a multiprotein mega-channel complex spanning both the inner and outer mitochondrial membranes, essentially allowing for communication of small molecules between the matrix and cytosol. The structural components of the pore are not well understood and the subject of continuing debate. Initial studies suggested that the pore was made up of the voltage-dependent anion channel (VDAC) located on the outer mitochondrial membrane, the adenine nucleotide translocator (ANT) on the inner mitochondrial membrane, and cyclophilin-D (Cyp-D) in the matrix (Halestrap and Brenner, 2003). However, follow-up genetic deletion studies revealed that ANT and VDAC are not required for mPTP formation, which may be explained, in part, by the presence of different isoforms of these putative pore components (Bernardi et al., 2015). Despite these observa-

Table 1 Experimental studies testing the efficacy of CsA or NIM811 in the treatment of TBI

Study	TBI model/species	Route	Dose/time	Outcome
Okonkwo et al. (1999)	IA/rat	i.t.	10 mg/kg CsA/30 minutes pre-injury	Decreased calpain-induced neurofilament compaction and cytokeletal damage
Okonkwo and Povlishock (1999)	IA/rat	i.t.	10 mg/kg CsA/30 minutes pre-injury	Preserves mitochondrial morphology and prevents axonal failure
Büki et al. (1999)	IA/rat	i.t.	10 mg/kg CsA/30 minutes post-injury	Decreases in neurofilament compaction, calpain activity and amyloid precursor protein (APP) accumulation
Scheff and Sullivan (1999)	CCI/rat and mice	i.p.	FK506: 40 or 20 mg/kg 5 minutes post-injury + 24 hours post-injury. CsA: 20, 40, or 150 mg/kg 5 minutes pre-injury	Improvement in cortical sparing with CsA but not FK-506
Sullivan et al. (1999)	CCI/rat	i.p.	20 mg/kg CsA 15 minutes post-injury	Preservation of mitochondrial function and decreased free radical production.
Fukui et al. (2003)	CCI/rat	i.v.	20 or 35 mg/kg CsA 1 hour infusion at 30 minutes post-injury	No significant change in brain water content
Signoretti et al. (2004)	IA/rat	i.t. and i.v.	(1) i.t. 10 mg/kg 30 minutes post-injury (2) i.v. 20 mg/kg 30 minutes post-injury (3) i.v. 35 mg/kg 30 minutes post-injury	Adenosine triphosphate (ATP) levels restored in i.t. and 35 mg/kg i.v. CsA administration groups
Gabbita et al. (2005)	CCI/rat	i.p.	20 mg/kg CsA 15 minutes + 24 hours post-injury	Significant decrease in hippocampal tau protein degradation
Turkoglu et al. (2010)	Contusion/rat	i.p./cortical implant	20 mg/kg CsA at time of injury 10 mg/kg CsA in microsphere formulation	Implantation of microsphere formulation improves mitochondrial integrity
Colley et al. (2010)	FPI/rat	i.p.	20 mg/kg CsA 15 minutes or 1 hour post-injury	Significant neuroprotection for myelinated axons.
Sullivan et al. (2011)	CCI/rat	i.p./pump	20 mg/kg CsA/i.p./1–8 hours post + pump 10 mg/kg/day	Neuroprotection was observed when CsA started within 8 hours post-injury
Readnower et al. (2011)	CCI/rat	oral	NIM811: 5–40 mg/kg/15 minutes and 24 hours post-injury NIM811: 10 mg/kg/15 minutes and 24 hours post-injury	NIM811 (10 mg/kg) improves mitochondrial function, and reduces oxidative at 6 hours post-injury, and improves cognition 10 days post-injury
Kilbaugh et al. (2011)	CCI/rat IA/piglet	i.p.	Rat: 20 mg/kg/CsA/15 minutes post-injury Piglet: 20 mg/kg/CsA/5 minutes and 12 hours post-injury	CsA improves mitochondrial function, preserves cerebral blood flow, and limits neuropathology
Dixon et al. (2016)	FPI/CCI/PBBI/rat	i.v.	CsA:10 or 20 mg/kg 15 minutes and 24 hours post-injury	No protective/functional effect of CsA on any measure
Kulbe et al. (2017)	CCI/rat	i.p.	20 mg/kg/CsA/15 minutes post-injury	Improve synaptic and non-synaptic mitochondrial respiration
Kulbe et al. (2018)	CCI/rat	i.p./pump	CsA: 20 mg/kg/CsA/15 minutes post-injury + pump: 10 mg/kg/day/3 days Phenelzine (PZ): 10 mg/kg/CsA/15 minutes post-injury + pump: 10 mg/kg/day/3 days	PZ and CsA together enhance neuroprotection

CCI: Controlled cortical impact; TBI: traumatic brain injury; CsA: cyclosporin A; FPI: fluid percussion injury; IA: inertial acceleration; i.p.: intraperitoneal; i.t.: intrathecal; i.v.: intravenous; PBBI: penetrating ballistic-like brain injury.

tions, there is a general consensus that Cyp-D functions as a major contributor to mPTP formation.

Inhibiting mPTP Formation in TBI and SCI

Cyclosporine A (CsA) is an immunosuppressant that inhibits mPT by binding to Cyp-D. It has been shown repeatedly to be neuroprotective in TBI, although similar effects in SCI remain controversial based on conflicting reports. This differential efficacy of CsA in TBI versus SCI may be related to properties inherent to mitochondria in the respective CNS regions. Moreover, it is difficult to examine a wide dose range of CsA due to its potent side effects and potential toxicity. In fact, doses of CsA above 20 mg/kg were, for the most part, ineffective in providing neuroprotection following TBI. Given these caveats, our group started investigating the use of NIM811, a Cyp-D binding CsA derivate lacking any immunosuppressive properties and having relatively minimal toxicity (Waldmeier et al., 2002). Our early studies provided evidence that NIM811 reduces oxidative damage while improving mitochondrial function and tissue sparing following TBI or SCI (McEwen et al., 2007; Mbye et al., 2008). The outcome of these studies provided strong evidence that NIM811 and CsA exhibited neuroprotective effects by inhibiting mPTP formation. A recent report by our group revealed a dose dependent effect of post-injury NIM811 treatment in experimental SCI (Springer et al., 2018). Interestingly, we observed that a low dose of NIM811 significantly improved locomotor recovery, while higher doses significantly increased tissue sparing and reflexive bladder control but not recovery of locomotor function. The reasons for these dose-related differences are not clear at this time, although it can be suggested that conducting a follow-up study examining additional doses in the range used in this study might prove revealing.

As stated above, there is strong evidence that targeting the mPTP has therapeutic potential in the treatment of TBI and

Table 2 Experimental studies testing the efficacy of CsA or NIM811 in the treatment of traumatic SCI

Study	SCI model/ species	Route	Dose/time	Outcome
Diaz-Ruiz et al. (1999)	Contusion/rat	i.p.	CsA: 2.5 mg/kg every 12 hours for 1 or 3 days	CsA significantly decreased lipid peroxidation and improved motor scores on the Tarlov scale.
Rabchevsky et al. (2001)	Contusion/rat	i.p./pump	CsA: 20 mg/kg/15 minutes post-injury + 2.5 mg/kg/day pump	CsA had no effect on any outcome measure.
Ibarra et al. (2003)	Compression/ rat	Oral, i.p.	CsA: 5 mg/kg/12 hours starting 48 hours before injury until study endpoint. 2.5 mg/kg i.p. first 3 days after injury.	CsA inhibited demyelination and death of descending rubrospinal tract axons, and also improved motor recovery (BBB scale).
McEwen et al. (2007)	Contusion/rat	i.p.	NIM811: 40 mg/kg/15 minutes pre-injury	NIM811 significantly improved mitochondrial function and decreased mitochondrial free radical production.
Ibarra et al. (2007)	Transection/ rat	Oral	CsA: 2.5 mg/kg 6 hours and 12 hours post followed by 5 mg/kg daily until study endpoint	CsA increased axonal growth and GAP-43 expression, but axons were not functional and there was no behavioral recovery.
Ravikumar et al. (2007)	Contusion/rat	Oral	NIM811:20 mg/kg/1, 12, and 24 hours post-injury	NIM811 significantly reduced cytochrome c and fragmented DNA, and increased spared gray and white matter.
Springer et al. (2018)	Contusion/rat	Oral	NIM811: 10, 20, or 40 mg/kg at 1, 12, and 24 hours post-injury	NIM811 significantly improved open field locomotor performance (10 mg/kg). 20 and 40 mg/kg significantly improved return of reflexive bladder control and tissue sparing.
Chen et al. (2018)	Contusion/rat	Pump	CsA: 4 mg/kg/daily for 7, 14, or 21 days	CsA decreased apoptosis and cytokine levels. Preserves mitochondrial morphology and prevents diffuse axonal injury.

SCI: Spinal cord injury; CsA: cyclosporin A; i.p.: intraperitoneal; BBB: Basso Beattie Bresnahan.

SCI and numerous studies have examined this hypothesis with mixed results. We conducted a PubMed search to identify published studies investigating CsA or NIM811 in either TBI or SCI and the results of this search are summarized in **Tables 1** and **2**, respectively. What is clear from this analysis is that the many labs found CsA or NIM811 to be effective in the treatment of various TBI-related outcomes across a range of doses, routes of administration and injury models. NIM811 was reported to be effective in one TBI study while all but two studies (see Fukui et al. (2003) and Dixon et al. (2016) in **Table 1**) reported structural, physiological, or functional efficacy with CsA treatment. It is interesting to point out that these two negative outcome studies used an intravenous route of delivery, while the positive outcome studies employed intrathecal, subcutaneous or intraperitoneal routes of administration. Given the pharmacokinetics, clearance rates, *etc*., associated with different routes of administration, it is possible to speculate that an intravenous route may not be optimal. One of these studies (Fukui et al., 2003) recommended that another route of administration be considered as there was no effect of intravenous CsA administration at least in terms of reducing brain edema. In addition, we have avoided an intravenous route of administration in our TBI and SCI studies due to the relative toxicity of the vehicle. However, the results of a prospective randomized clinical trial indicate a good safety profile when using intravenous CsA administration in severe TBI patients (Mazzeo et al., 2009).

CsA treatment in compression, contusion, or transection models of SCI has been reported to be either effective or ineffective in promoting functional recovery and tissue sparing (see **Table 2**). For example, work from Ibarra and colleagues report that CsA reduces lipid peroxidation, increases survival of rubrospinal tract axons, and improves functional recovery, while Rabchevsky et al. (2001) report no effect of CsA treatment on any outcome measure including functional recovery, tissue sparing. The reason for the discrepancies is not entirely clear, but may be related to differences in the injury models, injury severities, dosing, and routes of administration. We also demonstrated that the Cyp-D and oxidative stress levels are higher in uninjured spinal cord relative to cortex, and that it takes a higher dose of CsA to inhibit mPTP in mitochondria isolated from spinal cord compared to cortex (Sullivan et al., 2004). This is an important observation as our recent and previous studies have focused exclusively on NIM811, which can be used at higher doses than CsA. These studies demonstrate that NIM811 treatment increases mitochondrial function, spinal cord tissue sparing, and functional recovery in a contusion injury model (**Table 2**).

Future Considerations

One caveat of comparing CsA efficacy is the fact that many negative reports go unpublished making it difficult to ascertain the true impact of CsA treatment in TBI and SCI. Regardless, given the detrimental side effects of CsA and its vehicle, as well as its immunosuppressive properties, we support the idea of targeting mPTP in both TBI and SCI using reformulated CsA and non-immunosuppressive CsA derivatives such as NIM811. However, some effort should go into understanding the impact of targeting mPTP formation in cells that are significantly compromised and may not survive without intervention. There is evidence that mitochondrial dysfunction persists well beyond the acute stages following TBI and SCI, and it is not clear whether rescuing compromised mitochondria in minimally functioning cells is beneficial. For example, the benefit of CsA administration seems to follow an inverted U-shape highlighting the need

for robust therapeutic window data in preclinical studies. It also is not clear what cellular energy demands are placed on surrounding cells in order to maintain minimally functioning cells that might die off in the absence of mPTP targeted drugs. Finally, the current mPTP drugs do not exhibit any cell specificity, which might prove detrimental if treatment promotes survival and life span of pro-inflammatory and destructive microglia, neutrophils, macrophages, and astrocytes.

In conclusion, there is compelling evidence that targeting the mPTP with CsA or NIM811 limits secondary injury and promotes functional recovery after TBI and SCI. Future studies examining the therapeutic potential of this strategy will rely, in part, on 1) identification of other CsA analogues or other compounds that selectively target the mPTP, 2) replication of existing studies using identical methodologies, and 3) examination of mPTP targeted drugs in other species and other injury models in which mPTP is known to occur.

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