Lipid peroxidation in brain or spinal cord mitochondria after injury

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Abstract Extensive evidence has demonstrated an important role of oxygen radical formation (i.e., oxidative stress) as a mediator of the secondary injury process that occurs following primary mechanical injury to the brain or spinal cord. The predominant form of oxygen radical-induced oxidative damage that occurs in injured nervous tissue is lipid peroxidation (LP). Much of the oxidative stress in injured nerve cells initially begins in mitochondria via the generation of the reactive nitrogen species peroxynitrite (PN) which then can generate multiple highly reactive free radicals including nitrogen dioxide ($\cdot NO_2$), hydroxyl radical ($\cdot OH$) and carbonate radical ($\cdot CO_3$). Each can readily induce LP within the phospholipid membranes of the mitochondrion leading to respiratory dysfunction, calcium buffering impairment, mitochondrial permeability transition and cell death. Validation of the role of LP in central nervous system secondary injury has been provided by the mitochondrial and neuroprotective effects of multiple antioxidant agents which are briefly reviewed.

Keywords Oxidative stress · Traumatic brain injury · Spinal cord injury · Mitochondria · Lipid peroxidation · Antioxidant

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Introduction

The feasibility of an effective neuroprotective treatment for acute traumatic brain injury (TBI) or spinal cord injury (SCI) is based upon the fact that even though some of the neural injury is due to the primary mechanical events (i.e., stretching, twisting, compressing or shearing of nerve cells and blood vessels), the majority of posttraumatic neurodegeneration is due to a pathomolecular and pathophysiological secondary cascade that occurs during the first minutes, hours and days after injury which exacerbates the damaging effects of the primary injury. One of the most validated "secondary injury" mechanisms revealed in experimental TBI and SCI studies involves oxygen radical-induced lipid peroxidative (LP) damage to brain cell lipids and proteins (Hall 2011a; Hall et al. 2010). The cellular mitochondrion has been convincingly demonstrated to be a critically important source, and target, of oxidative stress and damage within the injured central nervous system. This mini-review summarizes the evidence for this scenario and the neuroprotective effects of mitochondrially-targeted pharmacological antioxidants.

Generation of reactive oxygen species, reactive nitrogen species and highly reactive free radicals The cascade of posttraumatic oxygen radical reactions begins in response to rapid elevations in intracellular Ca^{2+} immediately following the primary mechanical injury to the brain or spinal cord with the single electron (e^-) reduction of an oxygen molecule (O_2) to produce superoxide radical ($O_2^{\cdot-}$) which is considered to be a modestly reactive primordial radical that can potentially react with other molecules to give rise to much more reactive, and thus more potentially damaging radical species. The reason that $O_2^{\cdot-}$ is only modestly reactive is that it can act as either an oxidant by stealing an electron from another oxidizable

molecule or it can act as a reductant by which it donates it's unpaired electron to another radical species (i.e., an electron-donating antioxidant).

Although $O_2^{•-}$ itself is less reactive than •OH radical, its reaction with nitric oxide (•NO) radical forms the highly reactive oxidizing agent, peroxynitrite (PN, ONOO-). This reaction ($O_2^{•-} + •NO \rightarrow ONOO-$) occurs with a very high, diffusion-limited rate constant. Subsequently, at physiological pH, ONOO- can either undergo protonation to form peroxynitrous acid (ONOOH) or it can react with carbon dioxide (CO_2) to form nitrosoperoxocarbonate ($ONOOCO_2$). The ONOOH can break down to form highly reactive nitrogen dioxide(•NO_2) and •OH ($ONOOH \rightarrow •NO_2 + •OH$). Alternatively, the $ONOOCO_2$ can decompose into •NO_2 and carbonate radical (•CO_3) ($ONOOCO_2 \rightarrow •NO_2 + •CO_3$).

Lipid peroxidation Increased production of reactive free radicals (i.e., "oxidative stress") in the injured brain or spinal cord has been shown to cause "oxidative damage" to cellular lipids and proteins leading to functional compromise and possibly cell death in both the microvascular and brain parenchymal compartments (Hall 2011b; Hall et al. 2010). The major form of radical-induced oxidative damage involves oxidative attack on cell membrane polyunsaturated fatty acids triggering the process of lipid peroxidation (LP) which has three distinct chemical phases: initiation, propagation and termination. The initiation of LP is triggered when a highly reactive (i.e., electron-seeking) oxygen radical (e.g., •OH, •NO_2, •CO_3) reacts with membrane polyunsaturated fatty acids such as arachidonic acid, linoleic acid, eicosapentaenoic acid or docosahexaenoic acid resulting in disruptions in cellular and membrane integrity. Specifically, initiation of LP begins when a highly "electrophilic" radical steals the hydrogen electron from an allylic carbon of the peroxidizable polyunsaturated fatty acid. The allylic carbon is susceptible to free radical attack because it is surrounded by two relatively electronegative double bonds which tend to pull one the carbon electrons away from the hydrogen electron it is paired with. Consequently, a reactive free radical has an easy time pulling the hydrogen electron off of the carbon because the commitment of the carbon electron to staying paired with it has been weakened by the surrounding double bonds. This reaction results in the original radical being quenched while the polyunsaturated fatty acid (L), becomes a lipid radical (L•) due to its having lost an electron.

In the subsequent propagation step, the unstable L• reacts with O_2 to form a lipid peroxyl radical (LOO•). The LOO• in turn abstracts a hydrogen atom from an adjacent polyunsaturated fatty acid yielding a lipid hydroperoxide (LOOH) and a second L•, which sets off a series of propagation "chain" reactions. These propagation reactions are terminated in the third step when the peroxidizable substrate becomes depleted and/or a lipid radical reacts with another radical or radical

scavenger to yield relatively stable non-radical end products. One of those end products that is often used to measure LP is the 3 carbon-containing malondialdehyde (MDA) which is mainly a stable non-toxic compound that when measured represents an LP "tombstone". In contrast, two highly toxic aldehydic products of LP are 4-hydroxynonenal (4-HNE) or 2-propenal (acrolein) both of which have been well characterized in experimental brain or spinal cord injury models (Bains and Hall 2012; Hall et al. 2010; Hamann and Shi 2009). These latter two aldehydic LP end products can covalently bind to basic amino acids (e.g., lysine, histidine, arginine, cysteine) in cellular proteins, altering their structure and functional properties.

Protein carbonylation and nitration In addition to LP. Free radicals can cause various forms of oxidative protein damage. A major mechanism involves carbonylation by reaction of various free radicals with susceptible amino acids. The protein carbonyls thus formed are measurable through immunoblotting after derivatization of the carbonyl groups with diphenylhydrazine (DNPH); widely known as the "carbonyl assay". It should be noted that the carbonyl assay also picks up protein carbonyls that are present due to covalent binding of LP-derived 4-HNE and acrolein to cysteine residues, in addition to those resulting from direct free radical-induced amino acid oxidation. Thus, as a result, the carbonyl assay is as much an indirect index of LP as it is of primary direct protein oxidation.

Secondly, •NO_2 can nitrate the 3 position of tyrosine residues in proteins forming 3-nitrotyrosine (3-NT) which is a specific footprint of PN-induced cellular damage. Similarly, lipid peroxyl radicals (LOO•) can promote tyrosine nitration by producing initial oxidation (loss of an electron) which would enhance the ability of •NO_2 to nitrate the phenyl ring (Mustafa et al. 2010). Multiple commercially available polyclonal and monoclonal antibodies are available for immunoblot of immunohistochemical measurement of proteins that have been nitrated by PN. Thus, in summary, LP can cause cellular damage by destroying the integrity of the phospholipid bilayer as well as membrane-localized proteins by modification by carbonylation, nitration and/or binding of aldehydic LP end products such as 4-HNE or acrolein.

Role in lipid peroxidation and related protein oxidation in mitochondrial dysfunction Mitochondrial oxidative stress and resulting dysfunction plays an especially critical role in the post-traumatic cell death cascade in the injured brain (Lifshitz et al. 2003; Singh et al. 2006; Sullivan et al. 1999; Xiong et al. 1997a) or spinal cord (Azbill et al. 1997; Sullivan et al. 2007). The initiator of this mitochondrial homeostatic dysfunction involves a rapidly evolving post-injury intracellular accumulation of Ca^{++} ions which causes the mitochondria to try to buffer (sequester) the excess Ca^{++} which in turn

causes mitochondrial respiratory dysfunction and lessened oxidative phosphorylation(Lifshitz et al. 2003; Singh et al. 2006; Sullivan et al. 1998; Xiong et al. 1997a) and Ca^{++} buffering capacity (Lifshitz et al. 2003; Sullivan et al. 1999) and mitochondrial failure due to mitochondrial permeability transition (MPT) (Sullivan et al. 2002, 2005). In addition to the potential activation of mitochondrial caspase-dependent and caspase-independent cell death cascades, this mitochondrial failure causes a disruption of synaptic function, and indeed synaptic mitochondria are more susceptible than non-synaptic mitochondria (Sullivan et al. 1998).

The disruption of respiratory function is preceded by, or at least coincident with, an increase in mitochondrial free radical production and/or free radical-triggered LP-mediated oxidative damage (Azbill et al. 1997; Singh et al. 2006; Sullivan et al. 2007). Evidence has accumulated which shows that a particularly important oxidant that is being formed by injured brain of spinal cord mitochondria is peroxynitrite (PN) (Bao and Liu 2002, 2003; Deng et al. 2007; Sullivan et al. 2007; Xiong et al. 2007; Xiong and Hall 2009). The relationship of PN generation to mitochondrial dysfunction in the injured spinal cord has been recently documented by studies that have shown that the timing of post-SCI mitochondrial dysfunction (i.e., respiratory and Ca^{++} buffering impairment) is preceded by an increase in PN-induced 4-HNE (i.e., LP), 3-NT (i.e., protein nitration) and 4-HNE and protein carbonyl content in mitochondrial proteins. Nitric oxide and a mitochondrial nitric oxide synthase (NOS) isoform (mtNOS) have been shown to be present in mitochondria (Lopez-Figueroa et al. 2000; Zanella et al. 2002). Exposure of mitochondria to Ca^{++}, which is known to cause them to become dysfunctional, leads to PN generation which in turn triggers mitochondrial Ca^{++} release (i.e., limits their Ca^{++} uptake or buffering capacity) (Bringold et al. 2000). In vitro application of each of the three PN forms, $ONOO^-$, ONOOH and $ONOOCO_2$, have been found to deplete mitochondrial antioxidant stores and to cause protein nitration, which is the hallmark of PN oxidative damage (Valdez et al. 2000). The loss of mitochondrial function and the increase in oxidative damage markers, including 3-NT, is antagonized by early in vivo post-TBI (Deng et al. 2007) or post-SCI (Xiong and Hall 2009) treatment with the PN radical scavenger tempol. Furthermore, exposure of non-injured mitochondria to PN quickly leads to respiratory dysfunction that is reduced by tempol pretreatment along with a reduction in nitration of mitochondrial proteins (Xiong et al. 2009).

Additionally, the neurotoxic LP end products 4-HNE or acrolein are also able to produce a concentration-related attenuation of the respiratory function of normal brain or spinal cord mitochondria (Vaishnav et al. 2010). In those experiments, acrolein was observed to be 10x more potent than 4-HNE. Furthermore, spinal cord mitochondria were found to be significantly more susceptible than brain mitochondria. However, scavenging of these carbonyl-containing compounds

with the carbonyl scavenging compound phenelzine has been shown to attenuate mitochondrial respiratory depression along with a reduction in the levels of aldehyde-modified mitochondrial proteins (Singh et al. 2013).

Attenuation of posttraumatic lipid peroxidation-induced mitochondrial dysfunction by antioxidant agents Consistent with the above discussion of the role of mitochondrial membrane LP in the posttraumatic secondary injury that follows acute TBI and SCI, several pharmacological compounds with antioxidant properties have been shown to be neuroprotective in preclinical neurotrauma models. For a more in-depth presentation of the neuroprotective effects of various antioxidant agents in the traumatized brain and spinal cord the reader is directed to three recent review articles from our laboratory (Bains and Hall 2012; Hall 2011a; Hall et al. 2010). In the current mini-review, we will only discuss those compounds, shown in Fig. 1, for which there is clear evidence that their neuroprotective effects are mediated by a direct protection of injured mitochondria from LP and related oxidative damage.

The first compound is the pyrrolopyrimidine compound U-101033 which is a highly potent and selective LP inhibitor which possesses excellent blood brain barrier and neuronal penetration and has been shown to be neuroprotective in ischemic stroke and TBI models (Hall et al. 1997). In the controlled cortical impact TBI model in rats, U-101033, administered in two i.v. boluses at 5 min and 2 h post-injury; produced a significant protection of mitochondrial respiration and calcium homeostasis 6 h and 14 days after TBI (Xiong et al. 1997b). In a second study, the mitochondrial protection was enhanced if the antioxidant U-101033 was combined with the N-type calcium channel blocker SNX-111 supporting the notion that posttraumatic mitochondrial respiratory dysfunction is due to a combination of LP and disruption of Ca^{++} buffering capacity (Xiong et al. 1998). A second highly lipophilic and selective LP inhibitor, the 2-methylaminochroman U-83836 (Hall et al. 1991), has similarly been documented to reduce mitochondrial respiratory failure and to maintain Ca^{++} buffering capacity in the controlled cortical impact TBI paradigm together with a reduction in the 4-HNE and 3-NT content of mitochondrial proteins (Mustafa et al. 2010). This effect is associated with an attenuation of Ca^{++} mediated proteolytic degradation of brain axonal neurofilaments, and the therapeutic window (i.e., post-injury treatment delay) for this neuroprotective action extends out to 12 h after TBI (Mustafa et al. 2011).

A third class of antioxidant drugs that are currently being explored in acute neurotrauma models are actually a collection of compounds from various pharmacological classes that each contain a hydrazine moiety ($-NH-NH_2$), that enables them to scavenge the neurotoxic LP-derived aldehydes 4-HNE and acrolein by covalently reacting with their carbonyl functional group. The first

Fig. 1 Chemical structures of direct and indirect-acting LP-inhibiting compounds shown to be mitochondrial protective and neuroprotective in SCI and/or TBI models. Detailed explanation of mechanisms for the lipid peroxyl radical (LOO•) scavengers U-83836 and U-101033 are presented in references (Bains and Hall 2012; Hall et al. 1991; Singh et al. 2013). The carbonyl scavenging chemistry of phenelzine is explained in reference (Singh et al. 2013). For an understanding of the mechanism by which cyclosporine A (and NIM811) inhibit mitochondrial permeability transition (MPT), see references (Mbye et al. 2008; Sullivan et al. 1999, 2005)
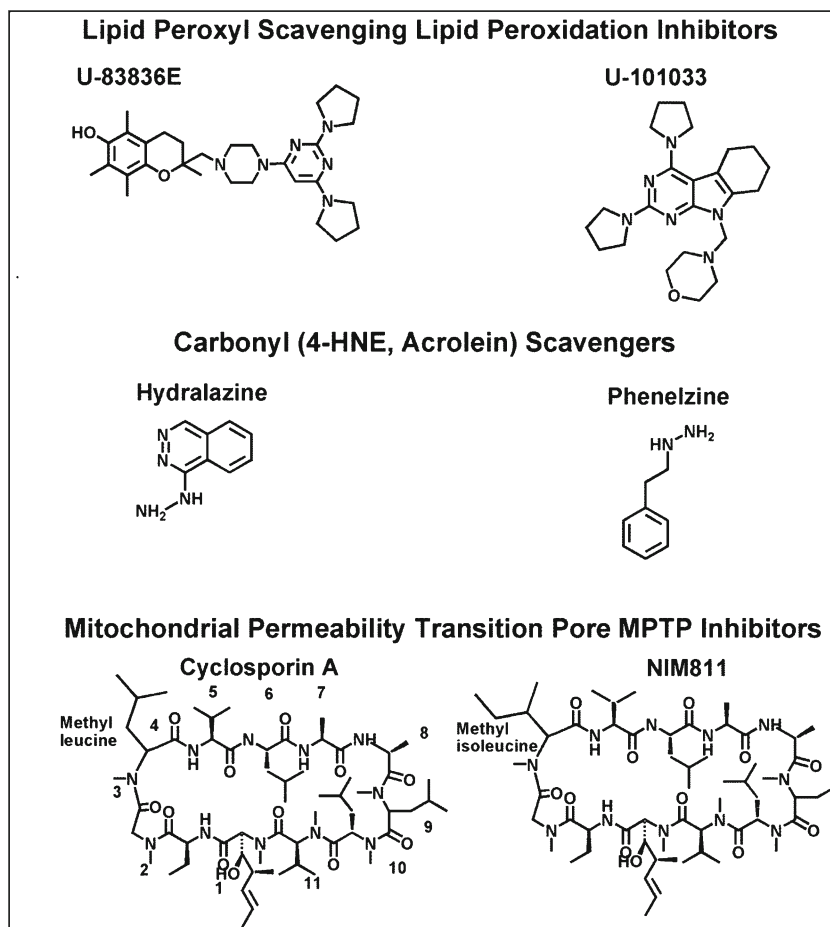
of these, hydralazine, is a vasodilator that has long been used for hypertension. Shi and colleagues have published multiple papers showing it neuroprotective effects in models of SCI (Hamann et al. 2008a, b; Hamann and Shi 2009). Our laboratory has been studying the effects of another hydrazine-containing compound phenelzine, which has long been used as an antidepressant and more recently documented to react with 4-HNE and acrolein (Galvani et al. 2008). Our recently published results have reported the ability of the hydrazine-containing phenelzine to protect isolated rat brain mitochondria from the respiratory depressant effects of 4-HNE together with a concentration-related attenuation of 4-HNE modified mitochondrial proteins (Singh et al. 2013). Subsequent in vivo studies in the rat controlled cortical impact TBI model have found that a single 10 mg/kg s.c. dose of phenelzine can also reduce early (3 h) posttraumatic mitochondrial respiratory failure as well as reducing cortical lesion volume at 14 days post-injury. Further experiments are ongoing to determine dose–response, therapeutic window and optimum treatment regimen.

The fourth and final neuroprotective compound to be mentioned in this review is cyclosporin A, long used as

a T cell-directed immunosuppressive agent for prevention of organ graft rejection, an action mechanistically related to its inhibition of calcineurin. It is actually not a scavenger of either reactive radicals or aldehydes, but rather has been shown to attenuate mitochondrial failure in the acutely injured brain by inhibition of the formation of the mitochondrial permeability transition pore (MPTP) thus preventing Ca^{++} overload induced MPT. However, it has been repeatedly observed that cyclosporine A inhibition of MPT in TBI models secondarily lessens mitochondrial free radical formation and decreases the further accumulation of 4-HNE and 3-NT together with a histological neuroprotection in terms of decreased lesion volume with a therapeutic window as long as 8 h (Mbye et al. 2008; Okonkwo et al. 1999; Okonkwo and Povlishock 1999; Sullivan et al. 1999, 2000, 2011). This effect is totally unrelated to immunosuppression since the non-immunosuppressive cyclosporin A analog, NIM811, is able to duplicate the posttraumatic mitochondrial and neuroprotective effects (Mbye et al. 2008, 2009). Lastly, a phase 2 dose escalation and safety study in 40 severe TBI patients has suggested that cyclosporin A treatment initiated within the

first 8 h and continued for 3 days may improve their functional recovery (Hatton et al. 2008). However, additional clinical trials are needed to determine the reliability of this effect.

The future: Is it time to investigate combination antioxidant therapies? Antioxidant neuroprotective therapeutic discovery directed at acute TBI has previously been focused upon attempting to inhibit the secondary injury cascade by pharmacological targeting of a single oxidative damage mechanism. As presented above, these efforts have included either enzymatic scavenging of superoxide radicals with SOD (Muizelaar et al. 1995) or inhibition of LP with tirilazad (Marshall et al. 1998). While each of these strategies has shown protective efficacy in animal models of TBI, phase III clinical trials with either compound failed to demonstrate a statistically significant positive effect, although post hoc subgroup analysis suggests that the microvascularly localized tirilazad may have efficacy in moderate and severe TBI patients with traumatic subarachnoid hemorrhage (Marshall et al. 1998). While many reasons have been identified as possible contributors to the failure, one explanation concerns the possible need to interfere at multiple points in the oxidative damage secondary injury process in order to achieve a clinically demonstrable level of neuroprotection. Thus, we are presently exploring the neuroprotective efficacy of the lipid peroxyl radical scavenger U-83836E, the carbonyl scavenger phenelzine and the MPT inhibitor cyclosporin A. It is anticipated that the combination of two or three antioxidant mechanistic strategies may improve the extent of neuroprotective efficacy, lessen the variability of the effect and possibly provide a longer therapeutic window of opportunity compared to the window for the individual strategies. If these theoretical combinatorial benefits are confirmed in preclinical TBI models this may enhance the chance of neuroprotective success in future clinical trials.

References

Azbill RD, Mu X, Bruce-Keller AJ, Mattson MP, Springer JE (1997) Impaired mitochondrial function, oxidative stress and altered antioxidant enzyme activities following traumatic spinal cord injury. Brain Res 765(2):283–290

Bains M, Hall ED (2012) Antioxidant therapies in traumatic brain and spinal cord injury. Biochim Biophys Acta 1822(5):675–684. doi:10.1016/j.bbadis.2011.10.017

Bao F, Liu D (2002) Peroxynitrite generated in the rat spinal cord induces neuron death and neurological deficits. Neuroscience 115(3):839–849

Bao F, Liu D (2003) Peroxynitrite generated in the rat spinal cord induces apoptotic cell death and activates caspase-3. Neuroscience 116(1):59–70

Bringold U, Ghafourifar P, Richter C (2000) Peroxynitrite formed by mitochondrial NO synthase promotes mitochondrial Ca^{2+} release. Free Radic Biol Med 29(3–4):343–348

Deng Y, Thompson BM, Gao X, Hall ED (2007) Temporal relationship of peroxynitrite-induced oxidative damage, calpain-mediated cytoskeletal degradation and neurodegeneration after traumatic brain injury. Exp Neurol 205(1):154–165

Galvani S, Coatrieux C, Elbaz M, Grazide MH, Thiers JC, Parini A et al (2008) Carbonyl scavenger and antiatherogenic effects of hydrazine derivatives. Free Radic Biol Med 45(10):1457–1467. doi:10.1016/j.freeradbiomed.2008.08.026

Hall ED (2011a) Antioxidant therapies for acute spinal cord injury. Neurotherapeutics 8(2):152–167. doi:10.1007/s13311-011-0026-4

Hall ED (2011b) Antioxidant therapies for acute spinal cord injury. Neurotherapeutics 8(2):152–167. doi:10.1007/s13311-011-0026-4

Hall ED, Braughler JM, Yonkers PA, Smith SL, Linseman KL, Means ED et al (1991) U-78517F: a potent inhibitor of lipid peroxidation with activity in experimental brain injury and ischemia. J Pharmacol Exp Ther 258(2):688–694

Hall ED, Andrus PK, Smith SL, Fleck TJ, Scherch HM, Lutzke BS et al (1997) Pyrrolopyrimidines: novel brain-penetrating antioxidants with neuroprotective activity in brain injury and ischemia models. J Pharmacol Exp Ther 281(2):895–904

Hall ED, Vaishnav RA, Mustafa AG (2010) Antioxidant therapies for traumatic brain injury. Neurotherapeutics 7(1):51–61. doi:10.1016/j.nurt.2009.10.021

Hamann K, Shi R (2009) Acrolein scavenging: a potential novel mechanism of attenuating oxidative stress following spinal cord injury. J Neurochem 111(6):1348–1356. doi:10.1111/j.1471-4159.2009.06395.x

Hamann K, Durkes A, Ouyang H, Uchida K, Pond A, Shi R (2008a) Critical role of acrolein in secondary injury following ex vivo spinal cord trauma. J Neurochem 107(3):712–721. doi:10.1111/j.1471-4159.2008.05622.x

Hamann K, Nehrt G, Ouyang H, Duerstock B, Shi R (2008b) Hydralazine inhibits compression and acrolein-mediated injuries in ex vivo spinal cord. J Neurochem 104(3):708–718. doi:10.1111/j.1471-4159.2007.05002.x

Hatton J, Rosbolt B, Empey P, Kryscio R, Young B (2008) Dosing and safety of cyclosporine in patients with severe brain injury. J Neurosurg 109(4):699–707. doi:10.3171/JNS/2008/109/10/0699

Lifshitz J, Friberg H, Neumar RW, Raghupathi R, Welsh FA, Janmey P et al (2003) Structural and functional damage sustained by mitochondria after traumatic brain injury in the rat: evidence for differentially sensitive populations in the cortex and hippocampus. J Cereb Blood Flow Metab 23(2):219–231

Lopez-Figueroa MO, Caamano C, Morano MI, Ronn LC, Akil H, Watson SJ (2000) Direct evidence of nitric oxide presence within mitochondria. Biochem Biophys Res Commun 272(1):129–133

Marshall LF, Maas AI, Marshall SB, Bricolo A, Fearnside M, Iannotti F et al (1998) A multicenter trial on the efficacy of using tirilazad mesylate in cases of head injury. J Neurosurg 89(4):519–525

Mbye LH, Singh IN, Sullivan PG, Springer JE, Hall ED (2008) Attenuation of acute mitochondrial dysfunction after traumatic brain injury in mice by NIM811, a non-immunosuppressive cyclosporin A analog. Exp Neurol 209(1):243–253

Mbye LH, Singh IN, Carrico KM, Saatman KE, Hall ED (2009) Comparative neuroprotective effects of cyclosporin A and NIM811, a nonimmunosuppressive cyclosporin A analog, following

traumatic brain injury. J Cereb Blood Flow Metab 29(1):87–97. doi:10.1038/jcbfm.2008.93

Muizelaar JP, Kupiec JW, Rapp LA (1995) PEG-SOD after head injury. J Neurosurg 83(5):942

Mustafa AG, Singh IN, Wang J, Carrico KM, Hall ED (2010) Mitochondrial protection after traumatic brain injury by scavenging lipid peroxyl radicals. J Neurochem 114(1):271–280. doi:10.1111/j.1471-4159.2010.06749.x

Mustafa AG, Wang JA, Carrico KM, Hall ED (2011) Pharmacological inhibition of lipid peroxidation attenuates calpain-mediated cytoskeletal degradation after traumatic brain injury. J Neurochem 117(3):579–588. doi:10.1111/j.1471-4159.2011.07228.x

Okonkwo DO, Povlishock JT (1999) An intrathecal bolus of cyclosporin A before injury preserves mitochondrial integrity and attenuates axonal disruption in traumatic brain injury. J Cereb Blood Flow Metab 19(4):443–451

Okonkwo DO, Buki A, Siman R, Povlishock JT (1999) Cyclosporin A limits calcium-induced axonal damage following traumatic brain injury. Neuroreport 10(2):353–358

Singh IN, Sullivan PG, Deng Y, Mbye LH, Hall ED (2006) Time course of post-traumatic mitochondrial oxidative damage and dysfunction in a mouse model of focal traumatic brain injury: implications for neuroprotective therapy. J Cereb Blood Flow Metab 26:1407–1418

Singh IN, Gilmer LK, Miller DM, Cebak JE, Wang JA, Hall ED (2013) Phenelzine mitochondrial functional preservation and neuroprotection after traumatic brain injury related to scavenging of the lipid peroxidation-derived aldehyde 4-hydroxy-2-nonenal. J Cereb Blood Flow Metab 33(4):593–599. doi:10.1038/jcbfm.2012.211

Sullivan PG, Keller JN, Mattson MP, Scheff SW (1998) Traumatic brain injury alters synaptic homeostasis: implications for impaired mitochondrial and transport function. J Neurotrauma 15(10):789–798

Sullivan PG, Thompson MB, Scheff SW (1999) Cyclosporin A attenuates acute mitochondrial dysfunction following traumatic brain injury. Exp Neurol 160(1):226–234. doi:10.1006/exnr.1999.7197

Sullivan PG, Thompson M, Scheff SW (2000) Continuous infusion of cyclosporin A postinjury significantly ameliorates cortical damage following traumatic brain injury. Exp Neurol 161(2):631–637

Sullivan PG, Keller JN, Bussen WL, Scheff SW (2002) Cytochrome c release and caspase activation after traumatic brain injury. Brain Res 949(1–2):88–96

Sullivan PG, Rabchevsky AG, Waldmeier PC, Springer JE (2005) Mitochondrial permeability transition in CNS trauma: cause or effect of neuronal cell death? J Neurosci Res 79(1–2):231–239

Sullivan PG, Krishnamurthy S, Patel SP, Pandya JD, Rabchevsky AG (2007) Temporal characterization of mitochondrial bioenergetics after spinal cord injury. J Neurotrauma 24(6):991–999

Sullivan PG, Sebastian AH, Hall ED (2011) Therapeutic window analysis of the neuroprotective effects of cyclosporine A after traumatic brain injury. J Neurotrauma 28(2):311–318. doi:10.1089/neu.2010.1646

Vaishnav RA, Singh IN, Miller DM, Hall ED (2010) Lipid peroxidation-derived reactive aldehydes directly and differentially impair spinal cord and brain mitochondrial function. J Neurotrauma 27(7):1311–1320. doi:10.1089/neu.2009.1172

Valdez LB, Alvarez S, Arnaiz SL, Schopfer F, Carreras MC, Poderoso JJ et al (2000) Reactions of peroxynitrite in the mitochondrial matrix. Free Radic Biol Med 29(3–4):349–356

Xiong Y, Hall ED (2009) Pharmacological evidence for a role of peroxynitrite in the pathophysiology of spinal cord injury. Exp Neurol 216:105–114

Xiong Y, Gu Q, Peterson PL, Muizelaar JP, Lee CP (1997a) Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. J Neurotrauma 14(1):23–34

Xiong Y, Peterson PL, Muizelaar JP, Lee CP (1997b) Amelioration of mitochondrial function by a novel antioxidant U-101033E following traumatic brain injury in rats. J Neurotrauma 14(12):907–917

Xiong Y, Peterson PL, Verweij BH, Vinas FC, Muizelaar JP, Lee CP (1998) Mitochondrial dysfunction after experimental traumatic brain injury: combined efficacy of SNX-111 and U-101033E. J Neurotrauma 15(7):531–544

Xiong Y, Rabchevsky AG, Hall ED (2007) Role of peroxynitrite in secondary oxidative damage after spinal cord injury. J Neurochem 100(3):639–649

Xiong Y, Singh IN, Hall ED (2009) Tempol protection of spinal cord mitochondria from peroxynitrite-induced oxidative damage. Free Radic Res 43(6):604–612. doi:10.1080/10715760902977432

Zanella B, Calonghi N, Pagnotta E, Masotti L, Guarnieri C (2002) Mitochondrial nitric oxide localization in H9c2 cells revealed by confocal microscopy. Biochem Biophys Res Commun 290(3):1010–1014