Chapter 9
Pharmacological Therapies for Concussions

Edward D. Hall, Eugene Park, and Andrew J. Baker

Introduction

Pharmacotherapy in broad terms may target underlying biological processes or target symptom complexes such as headache. This chapter explores potential therapies aimed at the underlying biological processes. There is a spectrum of symptoms, signs, and imaging findings that co-varies with force intensity in traumatic brain injury (TBI). It is therefore reasonable to consider that the pathophysiology of concussion includes processes that are seen in moderate and severe TBI – but to a lesser extent. Therefore, the first section of this chapter explores the recent history of the inroads into the pharmacological therapies aimed at moderate and severe TBI as well as mild TBI and concussion with the assumption of relevance related to common mechanisms between severity categories.

The pharmacological approach to concussion can be framed as targeting secondary injury mechanisms and/or targeting symptoms associated with concussion and persistent concussion syndrome (PCS). As we understand concussion and PCS better, and the role of comorbidities that are either pre-existent or precipitated, the approach also includes the active identification of clinical entities that are amenable to evidence-based interventions. Of course, the goal is to achieve a clinical pathological correlation, as well as an understanding of the pathophysiology and its

E. D. Hall (✉)
Spinal Cord & Brain Injury Research Center (SCoBIRC), University of Kentucky College of Medicine, Lexington, KY, USA
e-mail: edhall@uky.edu

E. Park
Keenan Research Centre, Li Ka Shing Knowledge Institute at St. Michael's Hospital, Toronto, ON, Canada

A. J. Baker
Departments of Critical Care and Anesthesia, St. Michael's Hospital, Unity Health Toronto and University of Toronto, Toronto, ON, Canada

T. A. Schweizer, A. J. Baker (eds.), *Tackling the Concussion Epidemic*,
https://doi.org/10.1007/978-3-030-93813-0_9

precision mitigation in concussion. Unfortunately, there is generally a lack of evidence-based direction to guide treatments [1–3]. Furthermore, while there is evidence following concussions/mild traumatic brain injury (mTBI) of persisting cellular changes from laboratory-supported research, this is largely in the context of the absence of evident anatomical injury or lesions by basic clinical imaging [4–6] adding to the challenges of clinical research. This chapter, therefore, provides a description of recent history of the development of a pharmacologic approach to the secondary cellular mechanisms, largely in the context of moderate or severe TBI. This narrative arises from the assumption that the traumatic etiology in concussion points to a shared pathophysiology and pharmacologic potential. The chapter ends with a brief survey of the pharmacological approaches for treating symptoms of neurotransmitter dysfunction and concussion-related comorbidities, while recognizing the importance of a multi-dimensional approach, as discussed in many chapters of this book.

Pharmacological Approaches to Limit Neurodegenerative Mechanisms: Antioxidant Therapies for Concussions/Mild TBI and Moderate/Severe TBI

Traumatic brain injury (TBI) involves a "primary" mechanical insult to the brain, which initiates a rapidly evolving, "secondary" biochemical injury. This biochemical "cascade" that is responsible for the post-TBI neurodegenerative events that take place during the first minutes, hours, and days after injury may initiate progressive vascular, neuronal, and glial degeneration resulting in permanent mild, moderate, or severe neurological disability or death. TBI researchers have documented that the "secondary" injury process begins with the injury-induced depolarization of excitatory glutamate-releasing neurons, which causes excessive release of the brain's principal excitatory neurotransmitter glutamate. This results in overactivation of the N-methyl-D-aspartate (NMDA) receptor on downstream neurons, which causes "excitotoxic" intra-neuronal accumulation of calcium (Ca^{++}) and sodium (Na^{+}) [7, 8]. The increases in intracellular Ca^{++} activate the proteolytic enzymes calpain I and II, leading to progressive degradation of cytoskeletal neurofilament proteins (e.g., α-spectrin). Brain cellular mitochondria, in addition to their principal bioenergetic functions (electron transport and ATP synthesis) perform mitochondrial "buffering" of intracellular Ca^{++}. However, as the intra-mitochondrial matrix Ca^{++} accumulation builds to an extreme level, where it is above the cytoplasmic Ca^{++} "set point", it causes mitochondrial membrane potential ($\Delta\psi$)-dependent electron transport, ATP synthesis, and mitochondrial Ca^{++} buffering to fail, resulting release of previously buffered intra-mitochondrial Ca^{++} [9, 10].

Pathophysiological studies in male CF-I mice [11, 12], and Sprague-Dawley rats [13, 14] using controlled cortical impact (CCI)-induced TBI paradigms, demonstrated a gradually increasing formation of reactive oxygen species (ROS) and

reactive nitrogen species (RNS) which initiate progressive lipid peroxidation (LP) that begins during the first post-TBI minutes and slowly increases over the first 4–12 hours. Beyond that time window, any opportunity for effective pharmacological LP inhibitory neuroprotection has closed. Nevertheless, our neuroprotective drug studies have shown that certain LP targeting antioxidants possess a clinically practical therapeutic window that ranges between 4 and 12 hours in both moderate and severe rodent and human TBI studies.

While ROS-/RNS-induced LP-mediated brain damage may be less intense in Glasgow Coma Score (GCS 13–15) "mild" TBI/concussion patients, recent studies have shown that ROS-/RNS-induced LP occurs in single "mild" TBI models (concussions) and more so in "repetitive concussions" that are allowed to occur within a "return-to-play" period limited 3 days since. Thus, pharmacological inhibition of LP appears to be a promising neuroprotective strategy in concussive TBIs which constitute ~75–80% of the acute TBI patient population.

Biochemistry of Free Radical-Induced Lipid Peroxidation, Protein Oxidation, and Carbonylation

One of the most extensively validated "secondary injury" mechanisms revealed in experimental TBI studies is the early post-traumatic increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) that cause oxygen radical-induced oxidative damage to brain cellular lipids and proteins [15, 16]. This chapter outlines the key sources of ROS, RNS, and their highly reactive (i.e., rapidly oxidizing) free radicals, the pathophysiological mechanisms associated with oxidative neural damage, and, most importantly, pharmacological antioxidants that have been shown to produce neuroprotective actions that limit ROS-/RNS-initiated neurodegeneration.

Superoxide Radical

The primordial oxygen free radical that comes from several pathophysiological sources involves the single electron (e^-) reduction of an oxygen molecule (O_2) to produce the superoxide radical (O_2^-). Superoxide can be generated from several sources; one of the main sources is $O_2^{\bullet -}$ leakage from complex I of the mitochondrial electron transport chain in Ca^{++}-overloaded brain mitochondria. However, $O_2^{\bullet -}$ is considered by many free radical chemists to be a modestly reactive radical, but nevertheless one that can react with other molecules to give rise to more reactive, and thus more damaging, radical species. The reason that $O_2^{\bullet -}$ is only modestly reactive is that it can act as either an oxidant that is capable of stealing an electron from another oxidizable molecule or as a reductant by which it donates its unpaired

electron to another radical species, thus acting as an antioxidant. However, if $O_2^{\bullet -}$ reacts with a proton (H^+) to form a hydroperoxyl radical ($HO^{\bullet}_2$), this results in a superoxide form that is much more likely to trigger LP (i.e., to act as an electron thief). This is more likely to occur in injured tissue where tissue acidosis is present that favors the predominance of $HO^{\bullet}_2$.

Superoxide Dismutase, Pro-oxidant Effects of Iron, and Tissue Lactic Acidosis

One of the most important endogenous antioxidants is the enzyme superoxide dismutase (SOD) which rapidly catalyzes the dismutation of O_{2-} into H_2O_2 and oxygen. At low pH, O_{2-} can dismutate spontaneously. The formation of highly reactive oxygen radicals, which have unpaired electron(s) in their outer molecular orbitals, and the propagation of LP chain reactions are fueled by non-radical ROS, which do not have unpaired electron(s) but are chemically reactive. For example, OH· radicals are generated in the iron-catalyzed Fenton reaction where ferrous iron (Fe^{2+}) is oxidized to form the highly reactive OH· in the presence of H_2O_2 ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH-$). Superoxide, acting as a reducing agent (i.e., an electron-donating antioxidant), can donate its unpaired electron to ferric iron (Fe^{3+}), cycling it back to the ferrous state (Fe^{2+}, via the Haber-Weiss reaction $O_{2-} + Fe^{3+} \rightarrow Fe^{2+} + O_2$). This sets up additional Fe^{2+}-catalyzed Fenton reactions and increased production of OH·.

Under physiological conditions, iron is tightly regulated by its transport protein transferrin and the storage protein, ferritin, both of which bind the ferric (Fe^{3+}) form of iron. However, this reversible bond of transferrin and ferritin with ferric iron decreases with declining pH (below pH 7). Tissue acidosis is known to occur in the traumatized CNS and to cause the release of iron and initiation of iron-dependent oxygen radical production and LP. A second source of iron comes from hemoglobin released during injury-induced brain tissue hemorrhage.

Peroxynitrite and Its Highly Reactive Radicals

Although $O_2^{\bullet -}$ is much less reactive than OH· radical, its reaction with nitric oxide (NO.) radical forms the highly reactive RNS peroxynitrite (PN: $ONOO_-$). This reaction ($O_{2-} + NO. \rightarrow ONOO-$) occurs with a very fast rate constant which out-competes mitochondrial manganese SOD's ability to convert $O_2^{\bullet -}$ into H_2O_2. Subsequently, at physiological pH, $ONOO^-$ will largely undergo protonation to form peroxynitrous acid (ONOOH) or it can react with carbon dioxide (CO_2) to form nitrosoperoxycarbonate ($ONOOCO_3^-$). The ONOOH can break down to form highly reactive nitrogen dioxide ($NO^{\bullet}_2$) and hydroxyl radical ($ONOOH \rightarrow NO^{\bullet}_2 + OH\cdot$). Alternatively, the $ONOOCO_3^-$ can decompose into $NO^{\bullet}_2$ and carbonate radical ($CO^{\bullet}_{-3}$)

($ONOOCO_3^- \rightarrow NO^{\bullet}_2 + CO^{\bullet}_{-3}$). Each of these PN-derived radicals ($NO^{\bullet}_2$, OH·, and $CO^{\bullet}_{-3}$) are highly reactive and able to initiate and propagate LP neurodegeneration with diffusion rate-limited speed [15, 16].

Lipid Peroxidation and the Formation of Highly Reactive Protein Carbonyls

Increased production of reactive free radicals (i.e., "oxidative stress") in the injured brain has been shown to cause oxidative damage to cellular lipids and proteins leading to functional compromise and cell death in both the cerebral microvascular and brain parenchymal compartments. Extensive study has confirmed that a major form of radical-induced oxidative damage involves ROS/RNS radical attack on brain cell membrane polyunsaturated fatty acids (PUFAs) that triggers the process of LP that is characterized by three distinct steps: *initiation*, *propagation*, and *termination and carbonylation* [17].

Initiation

LP is initiated when one of the highly reactive oxygen radicals (hydroxyl radical, OH·; nitrogen dioxide radical, $NO^{\bullet}_2$; and carbonate radical, $CO^{\bullet}_{-3}$) reacts with polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA), linoleic acid (LA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA), all of which have multiple allylic carbons that are susceptible to LP. Initiation of LP begins when the reactive radicals steal a hydrogen atom of a PUFA from its associated electrons from one of their allylic carbons. The basis for the susceptibility of the allylic carbon(s) of the PUFAs to having of its allylic carbon electrons stolen by a highly electrophilic free radical is that the carbon is surrounded by two relatively electrophilic double bonds which act to pull one of the electrons from the allylic carbon. Consequently, a reactive free radical can easily pull the hydrogen electron off the allylic carbon because its commitment to pairing with the carbon electron has been weakened by the surrounding electronegative double bonds. This results in the original radical being quenched while the polyunsaturated fatty acid (L) becomes a lipid radical ($L^{\bullet}$).

Propagation

Subsequently, LP propagation begins when the unstable $L^{\bullet}$ reacts with O_2 to form a lipid peroxyl radical ($LOO^{\bullet}$). The $LOO^{\bullet}$ in turn abstracts a hydrogen atom from an adjacent PUFA, yielding a lipid hydroperoxide (LOOH) and a second $L^{\bullet}$, which sets off a series of LP propagating "chain" reactions in the brain microvascular, neuronal, and glial cells.

Termination and Carbonylation

The LP propagation reactions are terminated in the third step when the substrates (i.e., peroxidizable PUFAs) become depleted and a lipid radical reacts with another radical to yield potentially neurotoxic non-radical, but highly reactive, aldehydic end products referred to as carbonyls. Two highly reactive and neurotoxic carbonyls of LP are 4-hydroxynonenal (4-HNE) and acrolein, both of which have been well characterized in both TBI and spinal cord injury (SCI) experimental models [13–16, 18]. The LP-derived 4-HNE and acrolein covalently bind to proteins, mainly the basic amino acids (lysine, histidine, arginine) by either Schiff base or Michael addition reactions, which alter the structural and functional properties of brain proteins.

Contribution of Lipid Peroxidative Damage to Mitochondrial Failure, Intracellular Calcium Overload, and Activation of Calcium-Dependent Proteolytic Enzymes and Neurodegeneration

The impact of ROS/RNS production is heightened when oxygen radicals amplify other secondary injury pathways creating a continuous cycle of neuronal ion imbalance, Ca^{++} buffering impairment, mitochondrial dysfunction, glutamate-induced excitotoxicity, and microvascular disruption. One example of ROS-induced ionic disruption arises from LP-induced damage to the plasma membrane ATP-driven Ca^{2+} pump (Ca^{++}-ATPase) and Na^{+} pump (Na^{+}/K^{+}-ATPase), which contributes to increases in intracellular Ca^{++} concentrations, mitochondrial dysfunction, and additional ROS production. Both Ca^{++}-ATPase and Na^{+}-/K^{+}-ATPase disruptions result in further increases in intracellular Ca^{++} and Na^{+} accumulation, respectively [15], the latter causing reversal of the Na^{+}/Ca^{++} exchanger which further exacerbates intracellular Ca^{++} [19, 20]. As already noted above, PN, formed from mitochondrial Ca^{++} overload, also contributes to post-TBI mitochondrial dysfunction. Specifically, nitric oxide (NO·), formed from mitochondrial nitric oxide synthase (mNOS), in turn reacts with $O_2^{\bullet -}$ to produce the highly toxic PN, which impairs mitochondrial respiratory function (electron transport and ATP synthesis) and Ca^{2+} buffering capacity via its derived free radicals. Indeed, increased PN-derived 3-NT and 4-HNE have been detected during the time of mitochondrial dysfunction and correlated with respiratory [13, 14] and Ca^{2+} buffering impairment [21].

Lipid peroxidation-derived neurotoxic aldehydes (carbonyls) 4-HNE or acrolein have been shown in neuronal or astrocytic cultures or in synaptosomes to impair glutamate uptake and to inhibit mitochondrial function [22–25]. On the other hand, glutamate-induced excitotoxic damage in synaptosomal or neuronal cultures is attenuated by pharmacological LP inhibition, confirming that oxidative damage is a promoter of glutamate excitotoxicity [7, 8].

Phase II and III Clinical Trial Results of PEG-SOD and Tirilazad Mesylate: Initial Validation of Antioxidant Neuroprotection in TBI Animal Models and Moderately Severe Human TBI

Polyethylene Glycol (PEG)-Conjugated Superoxide Dismutase (SOD): Scavenging the Primordial Superoxide Radical

The initial studies of free radical scavenging compounds in TBI models were carried out with Cu^{++}/Zn^{++} SOD in the collaborative work of Kontos, Wei, and Povlishock at the Medical College of Virginia. These investigators showed that post-traumatic cerebral microvascular dysfunction was initiated by $O_2^{\bullet -}$ generated as a by-product of TBI-triggered activation of the arachidonic acid cascade which begins during the first minutes and hours after TBI [26–28]. Their pioneering experimental work in rodent and feline TBI models demonstrated that administration of Cu^{++}/Zn^{++} SOD prevented post-traumatic free radical–induced disruption of cerebrovascular autoregulatory dysfunction.

This work led to phase II and III clinical trials in which the more metabolically stable polyethylene glycol (PEG)-covalently conjugated bovine Cu^{++}/Zn^{++} SOD (PEG-SOD; generic name: pegorgotein; trade name Dismutec®) was examined in moderate and/or severe TBI patients. The PEG modification of SOD had been shown by the pharmaceutical sponsor Sterling-Winthrop to not modify the SOD activity, but rather to dramatically increase its in vivo metabolic stability, which increased the half-life of SOD activity in rats from 8 minutes to over 30 hours with PEG-SOD [29].

An initial phase II double-blinded dose-response study, conducted at two centers (the Medical College of Virginia and the University of Maryland Shock Trauma Center), showed a positive trend in humans as well [30]. Specifically, 104 patients with severe TBI were randomized to either PEG alone or one of three PEG-SOD dose groups (2000, 5000, or 10,000 Units/kg administered as a single i.v. bolus) within 4 hours after TBI. Glasgow Outcome Scale (GOS)-assessed outcome at 3 and again at 6 months, in 91 and 93 patients, respectively, showed that at 3 months, 44% of the PEG-treated patients were either in a persistent vegetative state or had died, while only 20% of the patients who received the 10,000 Units/kg PEG-SOD dose were in those bad outcome groups ($p < 0.03$) compared to placebo. At 6 months post-TBI, these figures were reduced from 36% for PEG-treated patients to only 21% for PEG-SOD, respectively ($p < 0.04$). Differences in outcome between PEG and either of the two lower PEG-SOD dose groups were not statistically significant.

These encouraging phase II placebo-controlled clinical results carried out in two highly experienced trauma centers (Medical College of Virginia and the University of Maryland) inspired a subsequent phase III, 29 trauma center study that randomized 463 “severe” TBI patients randomized to either PEG or 10,000 or 20,000 PEG-SOD Units/kg i.v. administered within 8 hours after severe TBI. Disappointingly,

neither PEG-SOD dose, administered within the 8-hour post-TBI time window, showed a significant benefit in terms of increased survival or improved neurological outcomes in "severe" TBI. This implies that the PEG-SOD antioxidant neuroprotective therapeutic efficacy window may be limited to the 4-hour post-TBI time frame. However, in the 10,000 Units/kg PEG-SOD-treated patients, there was a significantly lower incidence of adult respiratory distress syndrome (ARDS) in the 10,000 Units/kg PEG-SOD patients compared to the PEG-treated TBI patients ($p < 0.015$) [31]. Unfortunately, PEG-SOD trials in TBI patients were discontinued by the corporate sponsor Sterling-Winthrop, and questions of whether repeated, rather than single PEG-SOD, dosing would be more effective were never resolved and PEG-SOD passed into pharmacological history.

Despite the failure of PEG-SOD in human TBI, experimental studies have shown that transgenic mice that overexpress Cu^{++}/Zn^{++} SOD are significantly protected against post-TBI pathophysiology and neurodegeneration [32–36]. This fully supports the importance of post-traumatic $O_2^{\bullet -}$ in post-traumatic secondary injury, despite the fact that targeting this primordial radical, which is only at its highest level during the first few hours after TBI, may not be the best antioxidant target for severe TBI compared to interrupting the ROS-/RNS-initiated LP process, which, as our recent rodent TBI studies show, does not peak in intensity until 4–12 hours after TBI [13, 14].

21-Aminosteroid Tirilazad Mesylate – Membrane Stabilization + Scavenging of Lipid Peroxyl Radicals

Consistent with targeting LP as probably the dominant mechanism involved in post-TBI oxidative damage, the 21-aminosteroid LP inhibitor tirilazad mesylate, trade name Freedox®, was discovered to potently inhibit free radical-induced, iron-catalyzed LP by a combination of catalytic LOO· scavenging along with a membrane-stabilizing action that limits the propagation of LP reactions between an LOO· and an adjacent polyunsaturated fatty acid [37]. The protective efficacy of tirilazad was demonstrated in multiple animal models of acute TBI in mice [38], rats [39], and cats [40]. While this highly lipophilic compound is largely localized in the microvascular endothelium, the early post-traumatic disruption of the BBB allows the penetration of tirilazad into the brain parenchyma [41]. Nevertheless, experimental data derived from the rat controlled cortical impact, and the mouse diffuse concussive head injury models have shown that a major effect of tirilazad is to lessen post-traumatic microvascular damage, as evidenced by attenuation of blood–brain barrier (BBB) opening [41, 42].

Nearly coincident with the PEG-SOD phase II and III TBI trials, the LP inhibitor tirilazad mesylate was taken into clinical development in the early 1990s. Following a phase II dose-escalation study that demonstrated the drug's safety in TBI patients,

in two phase III multi-center clinical trials, the ability of tirilazad mesylate to improve neurological recovery in moderately and severely injured TBI patients was evaluated. One trial was conducted in North America and the other in Europe, Australia, and South Africa. In both trials, TBI patients were treated randomly within 4 hours after TBI with tirilazad (10 mg/kg i.v. q6h for 5 days) or its aqueous vehicle (as a placebo). However, the North American trial was never published due to a major confounding imbalance in the blinded randomization of the moderate and severe patients to placebo or tirilazad with regard to injury severity and pre-treatment neurological status.

In contrast, the parallel European-Australasian-South African phase III moderate/severe TBI trial that enrolled 1120 moderate (GCS 9–12) and/or severe (GCS 4–8) TBI patients showed much better randomization balance between the placebo- and tirilazad-treated patients. Additionally, the principal investigator of this study, Dr. Larry Marshall, Chairman of the Department of Neurosurgery at UC San Diego and a leading TBI expert, urged us to include computerized axial tomography (CT) confirmation of traumatic SAH (49.7% of placebo treated and 50.4% of tirilazad treated), which was published in the *Journal of Neurosurgery* [43].

A post-hoc analysis showed that tirilazad-treated, moderately injured (GOS 9–12) male TBI patients with traumatic subarachnoid hemorrhage (tSAH) had a significantly lower incidence of 6-month mortality after treatment with tirilazad (7.1%) compared to placebo (25.0%, $p < 0.042$). Also in severely injured males with tSAH, tirilazad also lessened mortality from 42.5% in placebo-treated to 33.3% ($p < 0.026$). Additionally, 6-month post-TBI GOS favorable outcome was increased by 21%, from 60% in placebo-treated to 81.5% in tirilazad-treated moderate tSAH patients, albeit not significantly ($p < 0.13$).

The borderline significance of this tirilazad mesylate improvement in favorable outcome in tSAH patients would have needed to be replicated, had Pharmacia & Upjohn and tirilazad mesylate (Freedox®) survived the rampant "merger mania" of the decade of the 1990s. Nevertheless, from a scientific point of view, this result is consistent with the fact that tirilazad is also highly effective in reducing SAH-induced brain edema and cerebral vasospasm in multiple animal models of aneurysmal SAH [37] and in humans with aneurysmal SAH [44, 45] and traumatic SAH [43].

Current Enthusiasm for Antioxidant Neuroprotective Drug Discovery for Concussion (GCS 13–15)

Two recently published review articles have strongly encouraged mild TBI/concussion preclinical and clinical investigators toward an increased focus on the pharmaceutical investigation of various antioxidants for their neuroprotective utility. One of those reviews has stated that *"Of the several biochemical changes that occur in a patient's brain following a concussion, an increase in reactive oxygen species (ROS) is of particular concern"* [46].

A prominent and highly productive group of Italian TBI investigators state in their recent review the following evaluation concerning the history of "*Antioxidant Therapies in Traumatic Brain Injury*" research and development**.**

> A large number of studies have evaluated the efficacy of antioxidant administration to decrease TBI-associated damage in various animal models and in a limited number of clinical trials. Points of weakness of preclinical studies are represented by the large variability in the TBI model adopted, in the antioxidant tested, in the timing, dosages and routes of administration used, and in the variety of molecular or neurocognitive parameters evaluation. The analysis of the very few clinical studies does not allow strong conclusions to be drawn on the real effectiveness of antioxidant administration to TBI patients [47].

Later in their review, the authors state their view that the post-concussion antioxidant neuroprotective therapeutic window, which they believe, is limited to only 3 hours post-concussion and believe that antioxidant administration for concussions should be started shortly following admission.

> According to what is stated above, sports-related concussions are a type of TBI in which prevention might effectively be applied either by modifying rules of those sports disciplines at higher risk of concussion, or in preventively treating athletes with drugs capable of inhibiting specific molecular pathways activated by concussions. It should also be taken into account that drug treatments might be helpful in allowing safer return of athletes to play. In this light, few studies have been carried out to evaluate the effects of the administration of antioxidants prior to concussion in reducing molecular changes and symptoms associated with concussion [47].

The views of Di Pietro et al. [47] leave the reader initially uncertain about their enthusiasm for post-concussion antioxidant efficacy and for the therapeutic practicality of post-TBI antioxidant neuroprotection. However, their view is certainly reasonable about trying to pharmacologically intercept the highly reactive, hydrogen peroxide (H_2O_2)-derived hydroxyl radical (OH·) or the peroxynitrite-generated nitrogen dioxide ($NO^{\bullet}_2$), both of which peak within the concussed mouse brain within first 15 minutes post-injury as evidenced by 4-HNE or 3-NT immunostaining [48]. Logically, for prophylactic pharmacological neuroprotective treatment to be widely accepted for pre-treatment of competitive athletes at risk for concussions, antioxidant pre-treatments need to be safe and devoid of stimulant or depressant neuropharmacology.

Accordingly, in their review, Di Pietro et al. [47] highlight several "nutraceuticals," including ascorbic acid (vitamin C), N-acetyl-cysteine (a glutathione (GSH) congener), flavonoids, resveratrol, α-tocopherol (vitamin E), coenzyme $Q_{10,}$ carotenoids (natural products that possess antioxidant and anti-inflammatory properties), and omega 3 fatty acids including docosahexaenoic acid (DHA).

Concerning DHA as a potentially approvable prophylactic antioxidant neuroprotective agent, a recent study evaluated the effects of pretreatment of 81 Division I American football athletes who were recruited and randomly administered 2.4 or 6 g/day of DHA/day. The football players, during the 189-day season of the study, were randomly serum-sampled for neurofilament light (NFL) levels, as a measure of concussion-induced axonal injury. Surprisingly, the lowest DHA daily dose (2 g/day) produced the best effect in serum NFL levels suggesting that DHA

administered at higher doses may possess a biphasic/U-shaped dose-response curve in regard to the axonal protective effects of DHA [49].

Newer Multi-mechanistic Pharmacological Approaches for Antioxidant Neuroprotection That May Be Parenterally or Orally Administrable for Early Treatment of Mild TBI/ Concussion (GCS 13–15)

Pharmacological Nrf2-Antioxidant Response Element (ARE) Activation Enhancement

The body's endogenous antioxidant defense system is largely regulated by the nuclear factor E2-related factor 2/antioxidant response element (Nrf2/ARE) signaling pathway at the transcriptional level [50, 51]. Recent work has revealed that following controlled cortical impact TBI in mice there is a progressive activation of the Nrf2-ARE system in the traumatically-injured brain, as evidenced by an increase in HO-1 mRNA and protein that peaks at 72 hours after TBI. However, this effect does not precede, but rather is coincident with the post-injury increase in LP-related 4-HNE [52]. Therefore, it is apparent that this endogenous neuroprotective antioxidant response needs to be pharmacologically sped up and increased in magnitude if it is to be capable of exerting meaningful acute post-TBI neuroprotection. Two nutraceutical Nrf2 activators that have been shown to speed up Nrf2/ARE expression and provide effective neuroprotective actions in TBI models are sulforaphane and carnosic acid.

Sulforaphane

Administration of the natural product sulforaphane, a Nrf2/ARE signaling activator found in high concentrations in broccoli, significantly reduced contusion volume and increased post-SCI coordination. These positive outcomes were a result of sulforaphane-induced increases in Nrf2, glutamine, and decreases in inflammatory cytokines, IL-1b and TNFα [53]. The mRNA levels of Nrf2-regulated antioxidant enzymes heme oxygenase (HO-1) and NADPH:quinone oxidoreductase-1 (NQO1) are upregulated 24 hours post-TBI [54]. In contrast, Nrf2 knockout mice were found to be susceptible to increased oxidative stress and neurologic deficits following TBI compared to their wild-type counterparts [55].

Sulforaphane is also neuroprotective in various animal models of TBI specifically reducing cerebral edema and oxidative stress and improving BBB function and cognitive deficits [56]. Studies by Chen and coworkers [57] demonstrated increased cortical expression of Nrf2 and HO-1 in the rat SAH model. However, treatment with sulforaphane further increased the expression of Nrf2, HO-1, NQ01,

and glutathione S-transferase-α1 (GST-α1), resulting in the reduction of brain edema, cortical neuronal death, and motor deficits.

Carnosic Acid in Moderately Severe TBI

Another Nrf2-ARE activating natural product, carnosic acid, from the herb rosemary, has been shown to more effectively induce this antioxidant defense system than the prototype Nrf2-ARE activator sulforaphane [58]. This is because the parent carnosic acid, which contains a di-phenolic catechol moiety, is capable of scavenging LOO· radicals, making it in part, a typical electron-donating LP-inhibiting antioxidant. Additionally, the catechol of carnosic acid is metabolically converted to a carnosic acid quinone which is responsible for activating the Nrf2/ARE signaling pathway. Thus, carnosic acid is a dual mechanism antioxidant with combined electron-donating properties and Nrf2/ARE-activating activity.

It has been shown that administration of carnosic acid to non-TBI mice is able to significantly increase the resistance of isolated cortical mitochondria, harvested 48 hours later, to the respiratory depressant effects of 4-HNE applied in vitro together with a decrease in 4-HNE modification of mitochondrial proteins [11] Subsequent studies, with a single 1 mg/kg i.p. dose of carnosic acid administered to mice at 15 minutes after controlled cortical impact TBI, demonstrated preserved mitochondrial respiratory function along with a reduction in the level of LP-mediated damage in mitochondria harvested from the injured cortex at 24 hours after TBI [59]. Furthermore, carnosic acid's antioxidant effects were still apparent when its post-TBI administration was delayed until 8 hours post-injury in terms of an attenuation of the neurotoxic LP-derived 4-HNE and 3-NT in the injured cortical tissue together with a decrease in Ca^{2+}-activated, calpain-mediated, neuronal cytoskeletal degradation. Regarding the latter neuronal protective effect, a decrease in 48-hour cytoskeletal degradation was also shown to occur, even with a post-TBI treatment delay of 8-hours post-TBI (Fig. 9.1).

Carnosic Acid in Repetitive Mild TBI (rmTBI)

Recent studies by Dash and colleagues [60] have demonstrated that carnosic acid is able to improve neurological recovery in a mouse repetitive mild concussion TBI (rmTBI) model. Each mouse received 3 concussions, each 72 hours apart. At 30 minutes after each TBI, the mice received a 1 mg/kg i.p. dose of carnosic acid. In the rm-TBI paradigm, carnosic acid (1 mg/kg i.p dose) was shown to improve spatial learning and memory beginning at 15 days after the last injury [60].

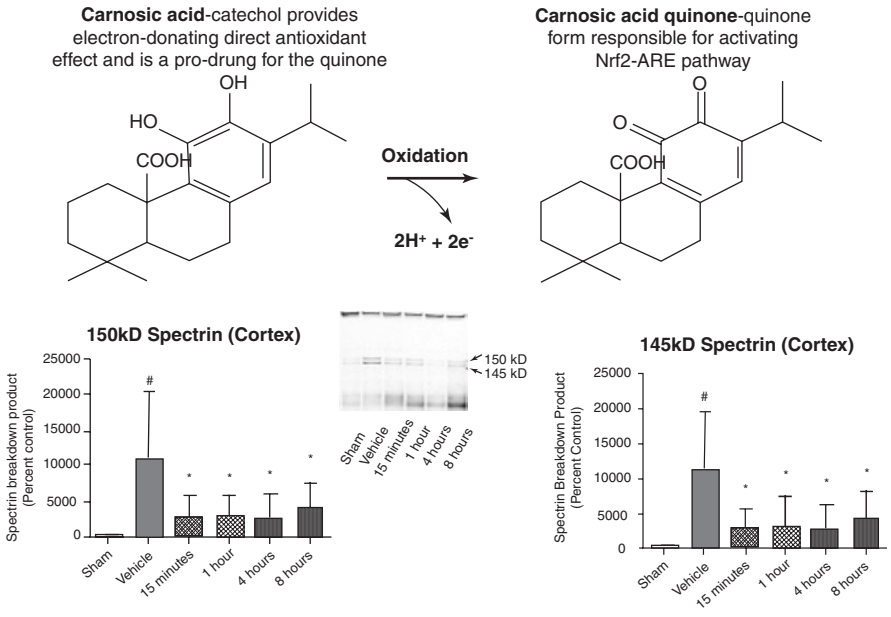

Fig. 9.1 *Top*: Multi-mechanistic antioxidant carnosic acid (CA): Although the parent drug CA (left) possesses direct radical scavenging activity due to its electron-donating diphenolic hydroxyls, its ability to activate the Nrf2-ARE pathway requires its oxidation to the more electrophilic CA ortho-quinone (right) species which is facilitated by conditions of oxidative stress. Both CA and the CA quinone are highly brain penetrable. *Bottom*: Delayed administration of CA provides a clinically relevant therapeutic window. The administration of CA (1 mg/kg i.p.) was delayed to either 15 minutes, 1 hour, 4 hours, or 8 hours post-injury for the initial i.p. injection followed by a booster injection at 24 hours post-injury. At 48 hours post-injury, ipsilateral cortical tissue was collected for Western blot analysis of α-spectrin breakdown products as an indication of cellular cytoskeletal degradation. All delayed time points (15 minutes, 1 hour, 4 hours, and 8 hours) were significantly decreased ($p < 0.05$) as compared to vehicle for both the 150 kD (caspase and calpain derived) and 145 kD (calpain specific) breakdown products. Analyzed by one-way ANOVA followed by Student Newman-Keuls post-hoc test. * = $p < 0.05$. Error bars represent +/− SD. $n = 8–10$ per group. (Reprinted from Miller et al. [53]. With permission from Elsevier)

Mitochondrial Protection by Scavenging of Lipid Peroxidation-Derived Protein Carbonyls 4-Hydroxynonenal (4-HNE) and Acrolein

Penicillamine

We have previously demonstrated that D-penicillamine is able to scavenge the RNS peroxynitrite (PN) [61] and to protect brain mitochondria from PN-induced respiratory dysfunction in isolated rat brain mitochondria [62]. D-penicillamine has also been documented to form an irreversible (covalent) bond to primary aldehydes

enabling the drug to scavenge neurotoxic LP-derived carbonyl compounds such as 4-HNE and acrolein [63]. Consistent with that mechanism of action, D-penicillamine has been shown to attenuate the levels of 4-HNE-modified mitochondrial proteins following exposure of isolated brain mitochondria to 4-HNE [12]. The PN scavenging action of D-penicillamine, along with its carbonyl scavenging capability, may jointly explain our previous findings that acutely administered D-penicillamine can improve early neurological recovery of mice subjected to moderately severe concussive TBI [64].

Phenelzine

Another FDA-approved hydrazine-containing drug phenelzine, long used for certain depressive patients, does not seem to compromise arterial blood pressure as readily as hydralazine. Accordingly, a very recently published paper has shown that phenelzine administration to rats subjected to acute contusion SCI mitigated post-SCI neuropathic pain, reduced motor deficits, and improved spinal cord tissue sparing [65]. Earlier studies have demonstrated neuroprotective efficacy in a rodent ischemia-reperfusion stroke model, which was attributed to reducing "aldehyde load" in the stroke-injured brain [66]. In vitro studies have documented the ability of phenelzine to protect isolated rat brain mitochondria from the respiratory depressant effects of 4-HNE together with a concentration-related attenuation of the accumulation of 4-HNE-modified mitochondrial proteins. Subsequent in vivo studies in the rat controlled cortical impact TBI model have found that a single 10 mg/kg subcutaneous dose of phenelzine can also reduce early (3 hours) post-traumatic mitochondrial respiratory failure, as well as cortical lesion volume at 14 days post-injury [67]. More recently, we have observed that phenelzine is able to protect isolated mitochondria from respiratory functional depression and carbonyl modification of mitochondrial proteins following application of the more highly reactive aldehyde acrolein [68].

To better define the optimal neuroprotective use of phenelzine, additional in vivo TBI studies shown in Fig. 9.2 have demonstrated that repeated dosing with phenelzine over a 60-hour post-TBI period is able to reduce LP-derived mitochondrial carbonylation and bioenergetic failure at its 72-hour peak, along with a reduction in cortical lesion volume that is greater than that seen with only a single early dose. This makes sense since the adequate carbonyl-scavenging drug levels logically need to be maintained during the 72-hour long time course of post-traumatic generation of LP-derived neurotoxic aldehydes [68]. Subsequent in vivo TBI experiments revealed that in addition to preserving mitochondrial bioenergetics out to 72-hours post-TBI, phenelzine administration was able to significantly improve intraneuronal calcium homeostasis, to maintain mitochondrial membrane potential, and, thereby, to partially protect neuronal cytoskeletal integrity [69].

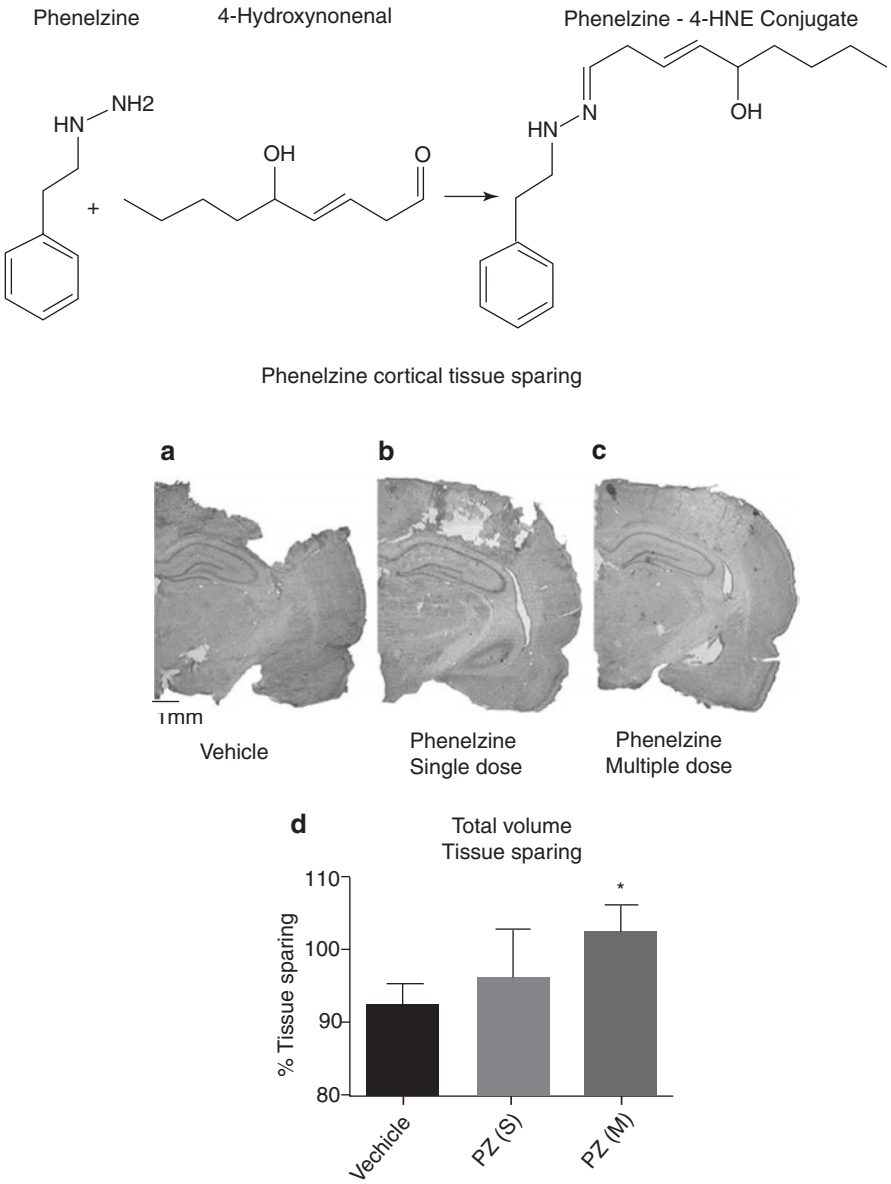

Fig. 9.2 Covalent reactivity of the hydrazine side chain of phenelzine with the lipid peroxidation-derived carbonyl 4-hydroxynonenal. (**a**) Vehicle (0.9% saline)-treated rat brain injected 15 minutes after TBI. All groups (vehicle, PZ(s), PZ(m)) were euthanized 72 hours after first injection. (**b**) Phenelzine single dose (PZs)-treated animal, injected with a single dose of PZ, 15 minutes after injury at 10 mg/kg. (**c**) Brain of PZ-treated rat with a multiple dosing paradigm (PZm): single subcutaneous injection of PZ 15 minutes after injury, followed by maintenance dosing of 5 mg/kg every 12 hours thereafter. (**d**) Percent of tissue sparing followed by either vehicle (saline), PZ(s), or PZ(m) treatment did not exhibit a statistically significant amount of cortical tissue sparing when compared to vehicle. However, PZm significantly increased the total volume of spared cortical tissue. One-way ANOVA ($F = 8.5$, $df = 2,20$, $p < 0.002$) followed by Dunnett's post-hoc test. * = $p < 0.05$ compared to vehicle. Error bars represent mean ± SD; $n = 7–8$ rats per group. (Reprinted from Cebak et al. [68]. With permission from Mary Ann Liebert, Inc.)

Hydralazine

More recently, it has been documented that a variety of FDA-approved hydrazine ($NH\text{-}NH_2$)-containing compounds, including the anti-hypertensive agent hydralazine and the anti-depressant phenelzine, can covalently react with the carbonyl moieties of 4-HNE or acrolein which prevents their covalent binding to susceptible amino acids in proteins [70]. Most impressive is the fact that the application of hydrazines can rescue cultured cells from 4-HNE toxicity even when administered after the 4-HNE has already covalently bound to cellular proteins [70]. Consistent with this effect being neuroprotective, Shi and colleagues at Purdue University have shown that hydralazine inhibits either compression- or acrolein-mediated injuries to rat spinal cord tissue [71]. However, a concern about hydralazine is that it is a potent arterial vasodilator that has long been used for interruption of hypertensive crises. Thus, hydralazine might potentially worsen post-TBI hypotension and decrease arterial perfusion in the injured brain and/or spinal cord that might offset hydralazine carbonyl scavenging neuroprotective effects. However, Shi and coworkers, in their rat SCI studies with hydralazine, have documented that the neuroprotective dose they are using in SCI models (5 mg/kg i.p.) does not have significant hypotensive effects, and the achieved levels in spinal cord tissue are sufficient to reduce the post-SCI accumulation of the neurotoxic aldehyde acrolein [72].

Combinatorial Antioxidant Neuroprotection

Antioxidant neuroprotective therapeutic discovery directed at acute TBI has consistently 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 [29–31] or inhibition of LP with tirilazad [43]. While each of these strategies alone has shown protective efficacy in multiple animal models of TBI, phase III clinical trials with either compound failed to demonstrate a statistically significant positive effect, although post-hoc subgroup analysis showed that the microvascularly localized tirilazad may have efficacy in moderate and severe TBI patients with tSAH [43]. While many reasons have been identified as possible contributors to the failure, one logical explanation has to do with the possible need to interfere at multiple points in the oxidative damage portion of the secondary injury cascade, either simultaneously or in a phased manner, to achieve a clinically demonstrable level of neuroprotection. To begin to address this hypothesis, we are currently exploring the possibility that reducing posttraumatic oxidative damage more completely and less variably might be achievable by combined treatment with two mechanistically complimentary antioxidant compounds. Figure 9.3 summarizes the theoretical rationale for a multi-mechanistic antioxidant therapy for TBI, whether in concussions and mild, moderate, or severe TBI. It is anticipated that the combination of two or more antioxidant mechanistic strategies may improve the extent of

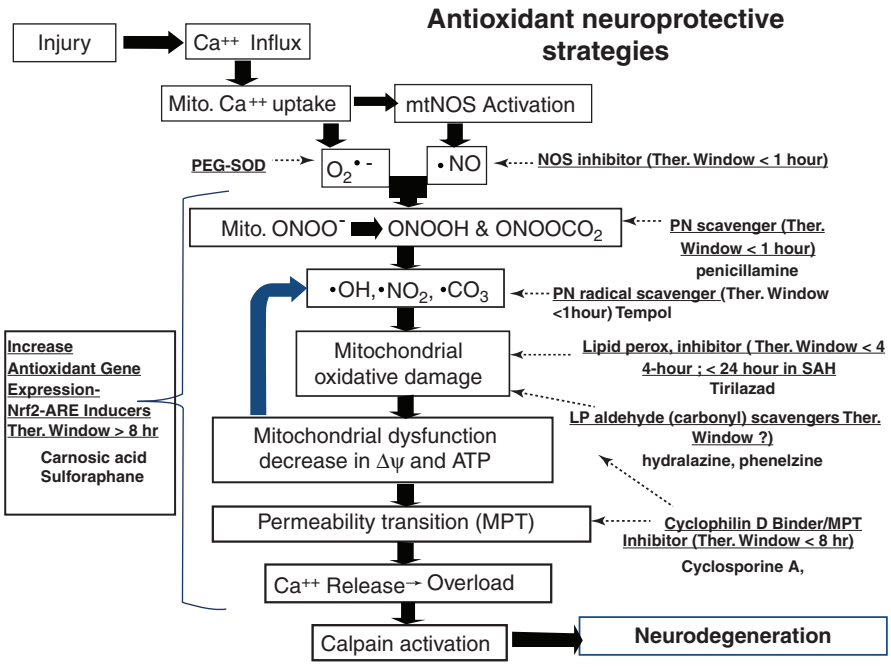

Fig. 9.3 Rationale for the combination of two or more antioxidant strategies to achieve a more effective and consistent (i.e., less variable) neuroprotective effect in the injured brain

neuroprotective efficacy and lessen the variability of the effect obtained with single antioxidant strategies.

Preliminary data, shown in Fig. 9.4 (top), suggests that a combination treatment that includes a peroxynitrite radical scavenger Tempol with an LP inhibitor U-83836E in mice subjected to controlled cortical impact TBI is more effective in reducing 48-hour calpain-mediated neurofilament damage (i.e., α-spectrin breakdown). In parallel, experiments (Fig. 9.4 (bottom)) showed that the same treatment combination reduced 7-day post-TBI cortical tissue damage. In the case of both parameters, the variability of the data is reduced to approximately half of that seen in the parallel groups treated with either of the two drugs alone.

Temporal Window of Metabolic Brain Vulnerability to Repeat Concussions

Regarding concussions (GCS 13–15), arguably one of most important considerations concerning recovery is whether this victim is part of a TBI group that is likely to have frequent repetitive concussions. This scenario most commonly applies to high school, college, or professional athletes who participate (particularly as

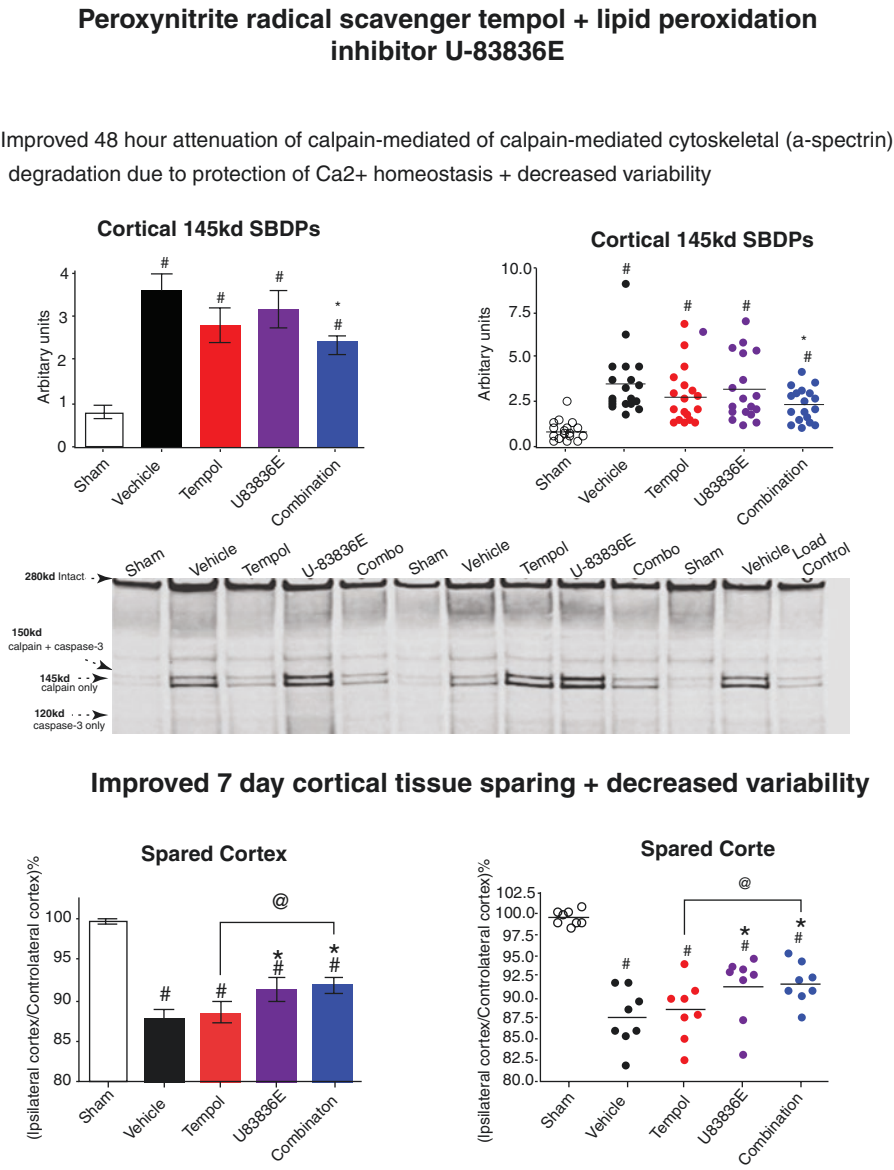

Fig. 9.4 *Top*: Comparison of the effects of the PN radical scavenger Tempol and the lipid peroxidation inhibitor U-83836E alone and in combination (each administered at 15 minutes post-TBI) on 48 hours calpain-mediated cortical neuronal cytoskeletal α-spectrin degradation (SBDP = spectrin breakdown products), measured by Western blot, at its 48 hours post-injury peak in the mouse controlled cortical impact TBI model ($N = 18$ male mice/group; # = $p < 0.05$ compared to sham, uninjured mice) and on 7 day cortical tissue sparing ($N = 8$/group; # = $p < 0.05$ compared to sham, *$p < 0.05$ compared to vehicle group). Values = mean ± standard deviation. *Bottom*: Comparison of the effects of the PN radical scavenger Tempol and the lipid peroxidation inhibitor U-83836E alone and in combination on 7 day post-TBI cortical tissue sparing (@ = $p < 0.05$ compared to Tempol). For both neuroprotective parameters, it is apparent that Tempol and U- 83836E result in as much as a 50% decrease in variability. (Adapted from Hall et al. [123]. With permission from Elsevier)

"starters") in rough and tumble contact sports (e.g., football, rugby, ice hockey, soccer), and are likely to sustain repeat concussions during one or more seasons, or in frontline military personnel who are at risk for repetitive concussions from frequent exposure to explosions from mortar or artillery fire during their deployments.

Accordingly, the important question concerns how frequently athletes or military personnel sustain concussions, and how long should they be removed from athletic competition, or combat, to allow for adequate rest and recovery between their repetitive concussions. Fortunately, we currently live in a time when the prevailing opinion is that if an athlete, or frontline soldier, sustains a concussion, they need to be relieved from duty for a period of time before they are allowed to return to either athletic competition or combat. While it is uncertain whether the needed rest period to enable adequate post-concussion recovery is different between rodents and humans, it seems likely that post-TBI oxidative and nitrosative damage time courses in acutely brain-injured rodents and probably humans are similar.

Vagnozzi et al. (2007) determined that there is a temporal window of brain vulnerability recovery in rats undergoing repeat mTBIs with the optimal timing for the second impact being delayed until after at least 5 days following the last impact. Specifically, radical-induced lipid peroxidation (LP), measured by accumulation of malondialdehyde (MDA), does not die down completely until oxidative and nitrosative-induction of LP has returned to its nearly normal level at least 5 days post-TBI. This is the first study to show the existence of oxidative and nitrosative stresses in repeat TBIs, and their neuroprotective modulation by the time interval between two concussive episodes separated by a post-concussion recovery period to allow time for repair from post-TBI free radical-induced LP.

Pharmacological Approaches for Treating Post-concussion Symptoms

Both neurocognitive and neuropsychiatric sequelae following concussion and mild TBI (mTBI) likely arise with alterations to the usually physiologically controlled balances in neurotransmitters [73–75]. Common targets in the treatment of concussion include addressing neurotransmitter imbalances in dopamine, serotonin, the cholinergic system, and the noradrenergic system. Dopamine (DA) plays numerous roles in cognition, attention, executive function, and memory. The dopaminergic response to TBI is complex owing to the numerous areas of DA activity including the prefrontal cortex [76], hippocampus[77], and striatum [78]. DA receptors, D1 and D2, are differentially expressed throughout these brain structures [79, 80] and also fluctuate in expression levels in response to injury [81]. There is evidence that genetic variability in DA receptors can influence outcome after concussion [82]. Several animal studies support a role for targeting DA dysregulation as a potential pharmacotherapeutic target following TBI [83]. DA receptor agonists have been evaluated clinically in the treatment of concussion with varying measures of

success. Bromocriptine, a D2 agonist, has demonstrated some benefit in working memory in concussed patients, while Pergolide, a D1/D2 agonist, had even greater effect [84]. Other clinically evaluated DA agonists also include amantadine, which has shown to improve cognitive processing and functional improvement [85]. Amantadine facilitates the presynaptic release of DA while inhibiting its uptake, effectively increasing the concentration and duration of its neurotransmitter effects [86, 87]. Amantadine is also a weak NMDA receptor antagonist that may confer some limited neuroprotection following trauma [87].

Post-traumatic headache is one of the most frequently reported symptoms following concussion and can persist for months after injury [88–90]. Metoclopramide is a dopamine and serotonin receptor antagonist that has been shown to reduce headaches [91, 92], but there is no evidence to support a rationale for its use in mTBI. The antagonism of DA runs counter to several studies in which agonism of DA improves symptom reporting following concussions.

The cholinergic system drives sensory processing [93], attention [94], sleep [95], arousal [96], and memory [97]. To date only one randomized control study [98] and one open-label study [99] have examined modulation of the cholinergic pathways with some success. In the RCT trial, galantamine did not confer an improvement in primary outcome (cognitive symptoms) but was associated with an improvement in secondary outcomes (episodic memory) relative to placebo [98]. Galantamine, a competitive inhibitor of acetylcholinesterase, prevents the breakdown of acetylcholine, thereby increasing the synaptic presence and duration of the neurotransmitter [100]. Given the extensive axonal projections of dopaminergic [101] and cholinergic neurons [102] to major structures throughout the brain, and the seemingly beneficial effects of pharmacological modulation of these pathways, it seems logical that future pharmacological initiatives should address concussion from the perspective of axonal injury in these neuronal subpopulations.

Extrapolation of potential drug effectiveness in concussion is based on the treatment of other neurodegenerative disorders, psychiatric conditions, or more severe forms of brain trauma. Many pharmacological agents have pleiotropic effects across a variety of receptors and signaling systems and in this regard target numerous pathways simultaneously. Methylphenidate, for example, is a compound used in the treatment of attention deficit hyperactivity disorder (ADHD) [103] through targeting dopaminergic and noradrenergic pathways by increasing the synaptic concentration. This is achieved by inhibiting uptake of serotonin and dopamine in the synaptic cleft [104]. Its application to treat impairments in executive function and depression demonstrates some promising potential in TBI [105]. However, this recent study by Al-Adawi et al. is hampered by similarly recurring pitfalls in many pharmacological TBI trials. These include open-label applications (i.e., no double-blinded experimental design or control group), small sample size, as well as a heterogeneity of injury severities. The multimodal activity of pharmacological compounds and the interpretation of their effects on concussion outcome are complicated by the complexity of the brain. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) which is commonly used in the treatment of depression, also demonstrates effectiveness in motor recovery after stroke, improved memory and

cognition in Alzheimer's disease patients, a decrease in tremor severity in Parkinson's patients, and improved outcome in a host of other neurological disorders including multiple sclerosis and epilepsy [106]. Similarly, clinical trials with other SSRIs including sertraline [107, 108] and citalopram [109] have also demonstrated improvements in the treatment of depression. Animal studies point to numerous pathways of activation by these compounds including effects on plasticity through BDNF upregulation [110] and neurogenesis [111]. In animal studies, fluoxetine's effects on depression also suggest a role in reducing neuroinflammation [112]. While imaging of neuroinflammation in mBTI patients has recently been demonstrated [113], understanding the role of inflammation in development of post-concussive symptoms and neurodegenerative diseases such as chronic traumatic encephalopathy (CTE) is in its infancy. Collectively, pharmacotherapies may influence both cognitive and psychiatric outcomes simultaneously. Determining the interdependence of these systems remains a challenge in study design.

The use of cannabidiol (CBD) has gained recent attention as a potential treatment for concussion and mild TBI. Patients in the acute post-concussive phase report reduced symptom severity scores with cannabis use [114]. CBD receptors are expressed widely throughout the brain. Two receptors have been identified to date, cannabinoid receptor 1 and receptor 2 (CB1 and CB2, respectively) 115. CB1 is mainly expressed in axons and synaptic terminals while CB2 is highly expressed in microglia [116]. CBD has been implicated in several in vivo and in vitro studies to influence various aspects of cellular signaling including activity of the blood–brain barrier, dopaminergic agonism, neurogenesis, neuroprotection and immune modulatory effects [117]. It is also known to interact with a variety of receptors in the CNS with implications for modulation of symptoms following concussion. Among these, the serotonin receptor, 5HT1A, has a high affinity for CBD as an agonist and has potential roles in reducing pain, anxiety, and headaches [118]. Similarly, CBD is a weak agonist for the vanilloid receptor, TRPV1, a ligand-gated ion channel involved in nociception and is expressed pre-synaptically on afferent neurons and sensory ganglia [119].

Neuropsychiatric conditions have been studied through several large, randomized control trials of various drugs including amantadine [120], rivastigmine [121], and sertraline [108]. These compounds have demonstrated effects on irritability, cognitive impairment, and depression, respectively. In all cases, however, the placebo treatment groups also demonstrate a considerable effect on outcome. In the amantadine trial, an improvement in irritability outcomes with drug treatment was also paralleled by a significant effect in the placebo group. This potentially masked the true biological effects of amantadine [120]. The rivastigmine trial demonstrated some benefit on cognitive outcome. However, inclusion criteria for TBI patients with GCS ranges from 3 to 15 imply heterogeneous pathophysiologies across injury severities which further complicates the interpretation of rivastigmine's actions at a mechanistic level. In all these studies, the significant contribution of placebo effects on improving outcome compared to medicated groups demonstrates the importance of psychological and neurobiological input on pharmacotherapeutic activity [122].

References

1. McCrory P, Meeuwisse W, Dvorak J, et al. Consensus statement on concussion in sport-the 5(th) international conference on concussion in sport held in Berlin, October 2016. Br J Sports Med. 2017;51:838–47.
2. Comper P, Bisschop SM, Carnide N, Tricco A. A systematic review of treatments for mild traumatic brain injury. Brain Inj. 2005;19:863–80.
3. Marshall S, Bayley M, McCullagh S, et al. Updated clinical practice guidelines for concussion/mild traumatic brain injury and persistent symptoms. Brain Inj. 2015;29:688–700.
4. Kamins J, Giza CC. Concussion-mild traumatic brain injury: recoverable injury with potential for serious sequelae. Neurosurg Clin N Am. 2016;27:441–52.
5. Giza C, Greco T, Prins ML. Concussion: pathophysiology and clinical translation. Handb Clin Neurol. 2018;158:51–61.
6. Barkhoudarian G, Hovda DA, Giza CC. The molecular pathophysiology of concussive brain injury – an update. Phys Med Rehabil Clin N Am. 2016;27:373–93.
7. Monyer H, Hartley DM, Choi DW. 21-Aminosteroids attenuate excitotoxic neuronal injury in cortical cell cultures. Neuron. 1990;5:121–6.
8. Pellegrini-Giampietro DE, Cherici G, Alesiani M, Carla V, Moroni F. Excitatory amino acid release and free radical formation may cooperate in the genesis of ischemia-induced neuronal damage. J Neurosci. 1990;10:1035–41.
9. Nicholls DG. Mitochondrial calcium function and dysfunction in the central nervous system. Biochim Biophys Acta. 2009;1787:1416–24.
10. Nicholls DG. Brain mitochondrial calcium transport: origins of the set-point concept and its application to physiology and pathology. Neurochem Int. 2017;109:5–12.
11. Miller DM, Singh IN, Wang JA, Hall ED. Administration of the Nrf2-ARE activators sulforaphane and carnosic acid attenuates 4-hydroxy-2-nonenal-induced mitochondrial dysfunction ex vivo. Free Radic Biol Med. 2013;57:1–9.
12. Singh IN, Sullivan PG, Deng Y, Mbye LH, Hall ED. 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. 2006;26:1407–18.
13. Hill RL, Kulbe JR, Singh IN, Wang JA, Hall ED. Synaptic mitochondria are more susceptible to traumatic brain injury-induced oxidative damage and respiratory dysfunction than non-synaptic mitochondria. Neuroscience. 2018;386:265–83.
14. Hill RL, Singh IN, Wang JA, Hall ED. Time courses of post-injury mitochondrial oxidative damage and respiratory dysfunction and neuronal cytoskeletal degradation in a rat model of focal traumatic brain injury. Neurochem Int. 2017;111:45–56.
15. Bains M, Hall ED. Antioxidant therapies in traumatic brain and spinal cord injury. Biochim Biophys Acta. 2012;1822:675–84.
16. Hall ED, Vaishnav RA, Mustafa AG. Antioxidant therapies for traumatic brain injury. Neurotherapeutics. 2010;7:51–61.
17. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem. 1995;41:1819–28.
18. Hamann K, Shi R. Acrolein scavenging: a potential novel mechanism of attenuating oxidative stress following spinal cord injury. J Neurochem. 2009;111:1348–56.
19. Rohn TT, Hinds TR, Vincenzi FF. Ion transport ATPases as targets for free radical damage. Protection by an aminosteroid of the Ca2+ pump ATPase and Na+/K+ pump ATPase of human red blood cell membranes. Biochem Pharmacol. 1993;46:525–34.
20. Rohn TT, Hinds TR, Vincenzi FF. Inhibition of Ca2+-pump ATPase and the Na+/K+-pump ATPase by iron-generated free radicals. Protection by 6,7-dimethyl-2,4-DI-1-pyrrolidinyl-7H-pyrrolo[2,3-d] pyrimidine sulfate (U-89843D), a potent, novel, antioxidant/free radical scavenger. Biochem Pharmacol. 1996;51:471–6.
21. Sullivan PG, Krishnamurthy S, Patel SP, Pandya JD, Rabchevsky AG. Temporal characterization of mitochondrial bioenergetics after spinal cord injury. J Neurotrauma. 2007;24:991–9.

22. Keller JN, Mark RJ, Bruce AJ, et al. 4-Hydroxynonenal, an aldehydic product of membrane lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes. Neuroscience. 1997;80:685–96.
23. Keller JN, Pang Z, Geddes JW, et al. Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. J Neurochem. 1997;69:273–84.
24. Lovell MA, Xie C, Markesbery WR. Acrolein, a product of lipid peroxidation, inhibits glucose and glutamate uptake in primary neuronal cultures. Free Radic Biol Med. 2000;29:714–20.
25. Springer JE, Azbill RD, Mark RJ, Begley JG, Waeg G, Mattson MP. 4-hydroxynonenal, a lipid peroxidation product, rapidly accumulates following traumatic spinal cord injury and inhibits glutamate uptake. J Neurochem. 1997;68:2469–76.
26. Kontos HA. Oxygen radicals in CNS damage. Chem Biol Interact. 1989;72:229–55.
27. Kontos HA, Povlishock JT. Oxygen radicals in brain injury. Cent Nerv Syst Trauma. 1986;3:257–63.
28. Kontos HA, Wei EP. Superoxide production in experimental brain injury. J Neurosurg. 1986;64:803–7.
29. Muizelaar JP. Clinical trials with Dismutec (pegorgotein; polyethylene glycol-conjugated superoxide dismutase; PEG-SOD) in the treatment of severe closed head injury. Adv Exp Med Biol. 1994;366:389–400.
30. Muizelaar JP, Marmarou A, Young HF, et al. Improving the outcome of severe head injury with the oxygen radical scavenger polyethylene glycol-conjugated superoxide dismutase: a phase II trial. J Neurosurg. 1993;78:375–82.
31. Muizelaar JP, Kupiec JW, Rapp LA. PEG-SOD after head injury. J Neurosurg. 1995;83:942.
32. Chan PH, Epstein CJ, Li Y, et al. Transgenic mice and knockout mutants in the study of oxidative stress in brain injury. J Neurotrauma. 1995;12:815–24.
33. Gladstone DJ, Black SE, Hakim AM, Heart, Stroke Foundation of Ontario Centre of Excellence in Stroke Recovery. Toward wisdom from failure: lessons from neuroprotective stroke trials and new therapeutic directions. Stroke. 2002;33:2123–36.
34. Lewen A, Matz P, Chan PH. Free radical pathways in CNS injury. J Neurotrauma. 2000;17:871–90.
35. Mikawa S, Kinouchi H, Kamii H, et al. Attenuation of acute and chronic damage following traumatic brain injury in copper, zinc-superoxide dismutase transgenic mice. J Neurosurg. 1996;85:885–91.
36. Xiong Y, Shie FS, Zhang J, Lee CP, Ho YS. Prevention of mitochondrial dysfunction in post-traumatic mouse brain by superoxide dismutase. J Neurochem. 2005;95:732–44.
37. Hall ED, McCall JM, Means ED. Therapeutic potential of the lazaroids (21-aminosteroids) in acute central nervous system trauma, ischemia and subarachnoid hemorrhage. Adv Pharmacol. 1994;28:221–68.
38. Hall ED, Yonkers PA, McCall JM, Braughler JM. Effects of the 21-aminosteroid U74006F on experimental head injury in mice. J Neurosurg. 1988;68:456–61.
39. McIntosh TK, Thomas M, Smith D, Banbury M. The novel 21-aminosteroid U74006F attenuates cerebral edema and improves survival after brain injury in the rat. J Neurotrauma. 1992;9:33–46.
40. Dimlich RV, Tornheim PA, Kindel RM, Hall ED, Braughler JM, McCall JM. Effects of a 21-aminosteroid (U-74006F) on cerebral metabolites and edema after severe experimental head trauma. Adv Neurol. 1990;52:365–75.
41. Hall ED, Yonkers PA, Andrus PK, Cox JW, Anderson DK. Biochemistry and pharmacology of lipid antioxidants in acute brain and spinal cord injury. J Neurotrauma. 1992;9(Suppl 2):S425–42.
42. Smith SL, Andrus PK, Zhang JR, Hall ED. Direct measurement of hydroxyl radicals, lipid peroxidation, and blood-brain barrier disruption following unilateral cortical impact head injury in the rat. J Neurotrauma. 1994;11:393–404.
43. Marshall LF, Maas AI, Marshall SB, et al. A multicenter trial on the efficacy of using tirilazad mesylate in cases of head injury. J Neurosurg. 1998;89:519–25.

44. Kassell NF, Haley EC Jr, Apperson-Hansen C, Alves WM. Randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in Europe, Australia, and New Zealand. J Neurosurg. 1996;84:221–8.
45. Lanzino G, Kassell NF. Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage. Part II. A cooperative study in North America. J Neurosurg. 1999;90:1018–24.
46. Kim K, Priefer R. Evaluation of current post-concussion protocols. Biomed Pharmacother. 2020;129:110406.
47. Di Pietro V, Yakoub KM, Caruso G, et al. Antioxidant therapies in traumatic brain injury. Antioxidants (Basel). 2020;9(3):260.
48. Hall ED, Detloff MR, Johnson K, Kupina NC. Peroxynitrite-mediated protein nitration and lipid peroxidation in a mouse model of traumatic brain injury. J Neurotrauma. 2004;21:9–20.
49. Oliver JM, Jones MT, Kirk KM, et al. Effect of docosahexaenoic acid on a biomarker of head trauma in American football. Med Sci Sports Exerc. 2016;48:974–82.
50. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu Rev Pharmacol Toxicol. 2007;47:89–116.
51. Zhang DD. Mechanistic studies of the Nrf2-Keap1 signaling pathway. Drug Metab Rev. 2006;38:769–89.
52. Miller DM, Wang JA, Buchanan AK, Hall ED. Temporal and spatial dynamics of nrf2-antioxidant response elements mediated gene targets in cortex and hippocampus after controlled cortical impact traumatic brain injury in mice. J Neurotrauma. 2014;31:1194–201.
53. Wang X, de Rivero Vaccari JP, Wang H, et al. Activation of the nuclear factor E2-related factor 2/antioxidant response element pathway is neuroprotective after spinal cord injury. J Neurotrauma. 2012;29:936–45.
54. Yan W, Wang HD, Hu ZG, Wang QF, Yin HX. Activation of Nrf2-ARE pathway in brain after traumatic brain injury. Neurosci Lett. 2008;431:150–4.
55. Hong SC, Goto Y, Lanzino G, Soleau S, Kassell NF, Lee KS. Neuroprotection with a calpain inhibitor in a model of focal cerebral ischemia. Stroke. 1994;25:663–9.
56. Dash PK, Zhao J, Orsi SA, Zhang M, Moore AN. Sulforaphane improves cognitive function administered following traumatic brain injury. Neurosci Lett. 2009;460:103–7.
57. Chen G, Fang Q, Zhang J, Zhou D, Wang Z. Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage. J Neurosci Res. 2011;89:515–23.
58. Satoh T, Kosaka K, Itoh K, et al. Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1. J Neurochem. 2008;104:1116–31.
59. Miller DM, Singh IN, Wang JA, Hall ED. Nrf2-ARE activator carnosic acid decreases mitochondrial dysfunction, oxidative damage and neuronal cytoskeletal degradation following traumatic brain injury in mice. Exp Neurol. 2015;264:103–10.
60. Maynard ME, Underwood EL, Redell JB, et al. Carnosic acid improves outcome after repetitive mild traumatic brain injury. J Neurotrauma. 2019;36:2147–52.
61. Althaus JS, Oien TT, Fici GJ, Scherch HM, Sethy VH, VonVoigtlander PF. Structure activity relationships of peroxynitrite scavengers an approach to nitric oxide neurotoxicity. Res Commun Chem Pathol Pharmacol. 1994;83:243–54.
62. Singh IN, Sullivan PG, Hall ED. Peroxynitrite-mediated oxidative damage to brain mitochondria: protective effects of peroxynitrite scavengers. J Neurosci Res. 2007;85:2216–23.
63. Wood PL, Khan MA, Moskal JR. Mechanism of action of the disease-modifying anti-arthritic thiol agents D-penicillamine and sodium aurothiomalate: restoration of cellular free thiols and sequestration of reactive aldehydes. Eur J Pharmacol. 2008;580:48–54.
64. Hall ED, Kupina NC, Althaus JS. Peroxynitrite scavengers for the acute treatment of traumatic brain injury. Ann N Y Acad Sci. 1999;890:462–8.
65. Chen Z, Park J, Butler B, et al. Mitigation of sensory and motor deficits by acrolein scavenger phenelzine in a rat model of spinal cord contusive injury. J Neurochem. 2016;138:328–38.

66. Wood PL, Khan MA, Moskal JR, Todd KG, Tanay VA, Baker G. Aldehyde load in ischemia-reperfusion brain injury: neuroprotection by neutralization of reactive aldehydes with phenelzine. Brain Res. 2006;1122:184–90.
67. Singh IN, Gilmer LK, Miller DM, Cebak JE, Wang JA, Hall ED. 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. 2013;33:593–9.
68. Cebak JE, Singh IN, Hill RL, Wang JA, Hall ED. Phenelzine protects brain mitochondrial function in vitro and in vivo following traumatic brain injury by scavenging the reactive carbonyls 4-hydroxynonenal and acrolein leading to cortical histological neuroprotection. J Neurotrauma. 2017;34:1302–17.
69. Hill RL, Singh IN, Wang JA, Hall ED. Effects of phenelzine administration on mitochondrial function, calcium handling, and cytoskeletal degradation after experimental traumatic brain injury. J Neurotrauma. 2019;36:1231–51.
70. Galvani S, Coatrieux C, Elbaz M, et al. Carbonyl scavenger and antiatherogenic effects of hydrazine derivatives. Free Radic Biol Med. 2008;45:1457–67.
71. Hamann K, Nehrt G, Ouyang H, Duerstock B, Shi R. Hydralazine inhibits compression and acrolein-mediated injuries in ex vivo spinal cord. J Neurochem. 2008;104:708–18.
72. Park J, Zheng L, Marquis A, et al. Neuroprotective role of hydralazine in rat spinal cord injury-attenuation of acrolein-mediated damage. J Neurochem. 2014;129:339–49.
73. Kmieciak-Kolada K, Felinska W, Stachura Z, Majchrzak H, Herman ZS. Concentration of biogenic amines and their metabolites in different parts of brain after experimental cerebral concussion. Pol J Pharmacol Pharm. 1987;39:47–53.
74. Kline AE, Yu J, Massucci JL, Zafonte RD, Dixon CE. Protective effects of the 5-HT1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin against traumatic brain injury-induced cognitive deficits and neuropathology in adult male rats. Neurosci Lett. 2002;333:179–82.
75. Kosari-Nasab M, Shokouhi G, Azarfarin M, Bannazadeh Amirkhiz M, Mesgari Abbasi M, Salari AA. Serotonin 5-HT1A receptors modulate depression-related symptoms following mild traumatic brain injury in male adult mice. Metab Brain Dis. 2019;34:575–82.
76. Trujillo P, van Wouwe NC, Lin YC, et al. Dopamine effects on frontal cortical blood flow and motor inhibition in Parkinson's disease. Cortex. 2019;115:99–111.
77. McNamara CG, Dupret D. Two sources of dopamine for the hippocampus. Trends Neurosci. 2017;40:383–4.
78. Howe MW, Tierney PL, Sandberg SG, Phillips PE, Graybiel AM. Prolonged dopamine signalling in striatum signals proximity and value of distant rewards. Nature. 2013;500:575–9.
79. Sawaguchi T, Goldman-Rakic PS. D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science. 1991;251:947–50.
80. Levey AI, Hersch SM, Rye DB, et al. Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. Proc Natl Acad Sci U S A. 1993;90:8861–5.
81. Lan YL, Li S, Lou JC, Ma XC, Zhang B. The potential roles of dopamine in traumatic brain injury: a preclinical and clinical update. Am J Transl Res. 2019;11:2616–31.
82. Abrahams S, McFie S, Lacerda M, et al. Unravelling the interaction between the DRD2 and DRD4 genes, personality traits and concussion risk. BMJ Open Sport Exerc Med. 2019;5(1):e000465.
83. Bales JW, Kline AE, Wagner AK, Dixon CE. Targeting dopamine in acute traumatic brain injury. Open Drug Discov J. 2010;2:119–28.
84. Flashman LA, McDonald BC, Ford JC, et al. Differential effects of pergolide and bromocriptine on working memory performance and brain activation after mild traumatic brain injury. J Neurotrauma. 2021;38:225–34.
85. Reddy CC, Collins M, Lovell M, Kontos AP. Efficacy of amantadine treatment on symptoms and neurocognitive performance among adolescents following sports-related concussion. J Head Trauma Rehabil. 2013;28:260–5.

86. Farnebo LO, Fuxe K, Goldstein M, Hamberger B, Ungerstedt U. Dopamine and noradrenaline releasing action of amantadine in the central and peripheral nervous system: a possible mode of action in Parkinson's disease. Eur J Pharmacol. 1971;16:27–38.
87. Mizoguchi K, Yokoo H, Yoshida M, Tanaka T, Tanaka M. Amantadine increases the extracellular dopamine levels in the striatum by re-uptake inhibition and by N-methyl-D-aspartate antagonism. Brain Res. 1994;662:255–8.
88. Seifert TD, Evans RW. Posttraumatic headache: a review. Curr Pain Headache Rep. 2010;14:292–8.
89. Blume HK, Vavilala MS, Jaffe KM, et al. Headache after pediatric traumatic brain injury: a cohort study. Pediatrics. 2012;129:e31–9.
90. Faux S, Sheedy J. A prospective controlled study in the prevalence of posttraumatic headache following mild traumatic brain injury. Pain Med. 2008;9:1001–11.
91. Bresee N, Aglipay M, Dubrovsky AS, et al. No association between metoclopramide treatment in ED and reduced risk of post-concussion headache. Am J Emerg Med. 2018;36:2225–31.
92. Friedman BW, Babbush K, Irizarry E, White D, John GE. An exploratory study of IV metoclopramide+diphenhydramine for acute post-traumatic headache. Am J Emerg Med. 2018;36:285–9.
93. Minces V, Pinto L, Dan Y, Chiba AA. Cholinergic shaping of neural correlations. Proc Natl Acad Sci U S A. 2017;114:5725–30.
94. Sarter M, Hasselmo ME, Bruno JP, Givens B. Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. Brain Res Brain Res Rev. 2005;48:98–111.
95. Xu M, Chung S, Zhang S, et al. Basal forebrain circuit for sleep-wake control. Nat Neurosci. 2015;18:1641–7.
96. Szymusiak R. Magnocellular nuclei of the basal forebrain: substrates of sleep and arousal regulation. Sleep. 1995;18:478–500.
97. Bertrand D, Wallace TL. A review of the cholinergic system and therapeutic approaches to treat brain disorders. Curr Top Behav Neurosci. 2020;45:1–28.
98. McAllister TW, Zafonte R, Jain S, et al. Randomized placebo-controlled trial of methylphenidate or galantamine for persistent emotional and cognitive symptoms associated with PTSD and/or traumatic brain injury. Neuropsychopharmacology. 2016;41:1191–8.
99. Tenovuo O. Central acetylcholinesterase inhibitors in the treatment of chronic traumatic brain injury-clinical experience in 111 patients. Prog Neuro-Psychopharmacol Biol Psychiatry. 2005;29:61–7.
100. Razay G, Wilcock GK. Galantamine in Alzheimer's disease. Expert Rev Neurother. 2008;8:9–17.
101. Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. Trends Neurosci. 2007;30:194–202.
102. Li X, Yu B, Sun Q, et al. Generation of a whole-brain atlas for the cholinergic system and mesoscopic projectome analysis of basal forebrain cholinergic neurons. Proc Natl Acad Sci U S A. 2018;115:415–20.
103. Abikoff H, Hechtman L, Klein RG, et al. Symptomatic improvement in children with ADHD treated with long-term methylphenidate and multimodal psychosocial treatment. J Am Acad Child Adolesc Psychiatry. 2004;43:802–11.
104. Volkow ND, Ding YS, Fowler JS, et al. Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. Arch Gen Psychiatry. 1995;52:456–63.
105. Al-Adawi S, Al-Naamani A, Jaju S, et al. Methylphenidate improves executive functions in patients with traumatic brain injuries: a feasibility trial via the idiographic approach. BMC Neurol. 2020;20:103.
106. Mostert JP, Koch MW, Heerings M, Heersema DJ, De Keyser J. Therapeutic potential of fluoxetine in neurological disorders. CNS Neurosci Ther. 2008;14:153–64.
107. Fann JR, Uomoto JM, Katon WJ. Sertraline in the treatment of major depression following mild traumatic brain injury. J Neuropsychiatry Clin Neurosci. 2000;12:226–32.

108. Ashman TA, Cantor JB, Gordon WA, et al. A randomized controlled trial of sertraline for the treatment of depression in persons with traumatic brain injury. Arch Phys Med Rehabil. 2009;90:733–40.
109. Rapoport MJ, Chan F, Lanctot K, Herrmann N, McCullagh S, Feinstein A. An open-label study of citalopram for major depression following traumatic brain injury. J Psychopharmacol. 2008;22:860–4.
110. Yan L, Xu X, He Z, et al. Antidepressant-like effects and cognitive enhancement of coadministration of Chaihu Shugan San and fluoxetine: dependent on the BDNF-ERK-CREB signaling pathway in the hippocampus and frontal cortex. Biomed Res Int. 2020;2020:2794263.
111. Levy MJF, Boulle F, Emerit MB, et al. 5-HTT independent effects of fluoxetine on neuroplasticity. Sci Rep. 2019;9:6311.
112. Kosari-Nasab M, Shokouhi G, Ghorbanihaghjo A, Abbasi MM, Salari AA. Anxiolytic- and antidepressant-like effects of Silymarin compared to diazepam and fluoxetine in a mouse model of mild traumatic brain injury. Toxicol Appl Pharmacol. 2018;338:159–73.
113. Ebert SE, Jensen P, Ozenne B, et al. Molecular imaging of neuroinflammation in patients after mild traumatic brain injury: a longitudinal (123) I-CLINDE single photon emission computed tomography study. Eur J Neurol. 2019;26:1426–32.
114. Lawrence DW, Foster E, Comper P, et al. Cannabis, alcohol and cigarette use during the acute post-concussion period. Brain Inj. 2020;34:42–51.
115. Mackie K. Cannabinoid receptors: where they are and what they do. J Neuroendocrinol. 2008;20(Suppl 1):10–4.
116. Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F. CB2 receptors in the brain: role in central immune function. Br J Pharmacol. 2008;153:240–51.
117. Singh J, Neary JP. Neuroprotection following concussion: the potential role for cannabidiol. Can J Neurol Sci. 2020;47:289–300.
118. Elliott MB, Ward SJ, Abood ME, Tuma RF, Jallo JI. Understanding the endocannabinoid system as a modulator of the trigeminal pain response to concussion. Concussion. 2017;2:CNC49.
119. Benitez-Angeles M, Morales-Lazaro SL, Juarez-Gonzalez E, Rosenbaum T. TRPV1: structure, endogenous agonists, and mechanisms. Int J Mol Sci. 2020;21:3421.
120. Hammond FM, Sherer M, Malec JF, et al. Amantadine effect on perceptions of irritability after traumatic brain injury: results of the amantadine irritability multisite study. J Neurotrauma. 2015;32:1230–8.
121. Silver JM, Koumaras B, Chen M, et al. Effects of rivastigmine on cognitive function in patients with traumatic brain injury. Neurology. 2006;67:748–55.
122. Polich G, Iaccarino MA, Kaptchuk TJ, Morales-Quezada L, Zafonte R. Placebo effects in traumatic brain injury. J Neurotrauma. 2018;35:1205–12.
123. Hall ED, Wang JA, Miller DM, et al. Newer pharmacological approaches for antioxidant neuroprotection in traumatic brain injury. Neuropharmacology. 2019;145(B):247–58.