Antioxidant Therapies for Traumatic Brain Injury

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Summary: Free radical-induced oxidative damage reactions, and membrane lipid peroxidation (LP), in particular, are among the best validated secondary injury mechanisms in preclinical traumatic brain injury (TBI) models. In addition to the disruption of the membrane phospholipid architecture, LP results in the formation of cytotoxic aldehyde-containing products that bind to cellular proteins and impair their normal functions. This article reviews the progress of the past three decades in regard to the preclinical discovery and attempted clinical development of antioxidant drugs designed to inhibit free radical-induced LP and its neurotoxic consequences via different mechanisms including the $O_2^{\cdot -}$ scavenger superoxide dismutase and the lipid peroxidation inhibitor tirilazad. In addition, various other antioxidant agents that have been shown to have efficacy in preclinical TBI models are briefly presented, such as the LP inhibitors U83836E, resveratrol, curcumin, OPC-14177, and lipoic acid; the iron chelator deferoxamine and the nitroxide-containing antioxidants, such as α-phenyl-tert-butyl nitrone and tempol. A relatively new antioxidant mechanistic strategy for acute TBI is aimed at the scavenging of aldehydic LP byproducts that are highly neurotoxic with "carbonyl scavenging" compounds. Finally, it is proposed that the most effective approach to interrupt posttraumatic oxidative brain damage after TBI might involve the combined treatment with mechanistically complementary antioxidants that simultaneously scavenge LP-initiating free radicals, inhibit LP propagation, and lastly remove neurotoxic LP byproducts. **Key Words:** Traumatic brain injury, lipid peroxidation, oxidative damage, antioxidants.

INTRODUCTION

At present, there are no Food and Drug Administration pharmacological therapies that have been approved for the acute treatment of traumatic brain injury (TBI) patients that are conclusively proven to mitigate the often devastating neurological effects of their injuries. Nevertheless, the possibility of an effective treatment is based on the fact that even though some of the neural injury is due to the primary mechanical events (i.e., shearing of nerve cells and blood vessels), the majority of post-traumatic neurodegeneration is due to a pathochemical and pathophysiological cascade of secondary events occurring during the first minutes, hours, and days after the injury that exacerbates the damaging effects of the primary injury. Arguably, one of the most validated "secondary injury" mechanisms revealed in experimental TBI studies involves oxygen radical-induced oxidative damage to lipids, proteins, and nucleic acids. This review briefly outlines the key sources of reactive oxygen species (ROS), including their derived highly reactive free radicals, the mechanisms associated with their neural damage and the past, present, and future of pharmacological antioxidants that should be able to produce a clinically demonstrable neuroprotective effect, if properly applied.

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OXIDATIVE DAMAGE IN TBI

Superoxide radical

The first body of work showing a role of oxygen radicals in acute TBI pathophysiology was conducted by Kontos and Povlishock[1] and Kontos and Wei[2] who demonstrated an almost immediate post-injury increase in brain microvascular superoxide radical ($O_2^{\cdot -}$) production associated with compromise of autoregulatory function in fluid percussion TBI models. These early investigators also demonstrated that scavengers of $O_2^{\cdot -}$ decrease the post-traumatic superoxide levels and protect against the loss of microvascular autoregulatory competency. Within the injured nervous system, a number of possible sources of $O_2^{\cdot -}$ may be operative during the first minutes and hours after injury including: the arachidonic acid cascade (i.e., prostaglandin synthase and 5-lipoxygenase activity), enzymatic or autoxidation of biogenic

amine neurotransmitters (e.g., dopamine, norepinephrine, 5-hydroxytryptamine), "mitochondrial leak," xanthine oxidase activity, and the oxidation of extravasated hemoglobin. Activated microglia and infiltrating neutrophils and macrophages provide additional sources of $O_2^{\cdot -}$ at later time points.

Superoxide, which is formed by the single electron reduction of oxygen, may act as either an oxidant or reductant. Although $O_2^{\cdot -}$ itself is reactive, its direct reactivity toward biological substrates in aqueous environments is relatively weak. Moreover, once formed, $O_2^{\cdot -}$ undergoes spontaneous dismutation to form hydrogen peroxide (H_2O_2) in a reaction that is markedly accelerated by the enzyme superoxide dismutase (SOD): $O_{2-}^{\cdot -} + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$.[3] In solution, $O_2^{\cdot -}$ actually exists in equilibrium with the hydroperoxyl radical ($HO_2^{\cdot}$): $O_2^{\cdot -} + H^+ \rightarrow HO_2^{\cdot}$, which is considerably more lipid soluble and a far more powerful oxidizing or reducing agent. Since the pKa of the $O_2^{\cdot -}/HO_2^{\cdot}$ is 4.8, as the pH of a solution falls (i.e., tissue acidosis), the equilibrium between $O_2^{\cdot -}$ and $HO_2^{\cdot}$ shifts in favor of $HO_2^{\cdot}$, which is much more reactive than $O_2^{\cdot -}$, particularly toward lipids.

Iron and hydroxyl radical

The CNS is an extremely rich source of iron and its regional distribution varies in parallel with the sensitivity of various regions to oxidative damage.[4] Under normal circumstances, low molecular weight forms of redox-active iron are maintained at extremely low levels. In plasma, the iron transport protein transferrin tightly binds iron in the Fe^{+++} form. Intracellularly, Fe^{+++} is sequestered by the iron storage protein ferritin. Although both ferritin and transferrin have a very high affinity for iron at neutral pH and effectively maintain iron in a noncatalytic state,[3] both proteins readily give up their iron at pH values of 6.0 or less, which is a level of acidosis that has been shown to be reached in the injured brain. Once iron is released from ferritin or transferring, it can actively catalyze oxygen radical reactions. Therefore, within the traumatized brain, where pH in injured areas is typically lowered, conditions are favorable for the potential release of iron from storage proteins.[3] In the case of ferritin, its iron can also be released by reductive mobilization by $O_2^{\cdot -}$.

A second source of catalytically active iron is hemoglobin. Hemorrhage resulting from mechanical trauma provides an obvious source of hemoglobin. Although hemoglobin itself has been reported to stimulate oxygen radical reactions, it is more likely that iron released from hemoglobin is responsible for hemoglobin-mediated oxidative damage.[5,6] Iron is released from hemoglobin by either H_2O_2 or by lipid hydroperoxides (LOOH; see as follows), and this release is further enhanced as the pH falls to 6.5 or below. Therefore, hemoglobin may catalyze oxygen radical formation and LP either directly or through the release of iron by H_2O_2, LOOH and/or acidic pH.

Free iron or iron chelates participate in free radical reactions at two levels. The autoxidation of Fe^{++} results in the formation of $O_2^{\cdot -}$[3]: $Fe^{++} + O_2 \rightarrow Fe^{+++} + O_2^{\cdot -}$. Second, Fe^{++} is also oxidized in the presence of H_2O_2 to form hydroxyl radical (·OH) (Fenton reaction): $Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + \cdot OH + OH^-$. Using the salicylate trapping method, a rise in brain ·OH levels has also been documented in a mouse diffuse and rat focal TBI models by the senior author[7,8] and others.[8–10] As with the previously discussed work of Kontos and Povlishock[1] and Kontos and Wei,[2] the cerebral microvasculature appears to be the initial source of post-traumatic ·OH production.

Chemistry of lipid peroxidation and target mechanisms for its pharmacological inhibition

The most studied mechanism of oxidative damage in models of TBI concerns free radical-induced lipid peroxidation (LP). The process of LP is presented in FIG. 1 in the context of the ·OH-induced peroxidation of the LP-susceptible arachidonic acid (AA), which is highly enriched in brain cell membranes. **"**Initiation" of LP occurs when a radical species such as ·OH reacts with and removes an allylic carbon (carbon surrounded by adjacent double bonds) and extracts a hydrogen and its single electron from AA ($AA + R\cdot \rightarrow AA\cdot + RH$). In the process, the initiating radical is quenched by receipt of an electron (hydrogen) from the polyunsaturated AA. This, however, converts the AA into a lipid or "alkyl" radical (AA·). This sets the stage for a series of **"**propagation**"** reactions, which begins when the alkyl radical takes on a mole of oxygen creating a lipid peroxyl radical (AA-OO·; $AA\cdot + O_2 \rightarrow$ AA-OO·). The peroxyl radical then reacts with a neighboring AA within the membrane and steals its electron forming a lipid hydroperoxide (AA-OOH) and a second alkyl radical (AA·; AA-OO· + AA → AA-OOH + AA·).

Once LP begins the propagation phase, iron may participate in driving the process as lipid hydroperoxides are decomposed by reactions with either ferrous iron (Fe^{++}) or ferric iron (Fe^{+++}). In the case of Fe^{++}, the reaction results in formation of a lipid alkoxyl radical (AA-O·; AA-OOH + $Fe^{++} \rightarrow$ AA-O· + OH^- + Fe^{+++}). If, however, the reaction involves Fe^{+++}, the AA-OOH is converted back into a lipid peroxyl radical (AA-OO·; AA-OOH + $Fe^{+++} \rightarrow$ AA-OO· + Fe^{++}). Both of the reactions of AA-OOH with iron have acidic pH optima causing them to be augmented by tissue acidosis. Either alkoxyl (AA-O·) or peroxyl (AA-OO·) radicals arising from AA-OOH decomposition by iron can initiate so-called lipid hydroperoxide-dependent LP resulting in "chain

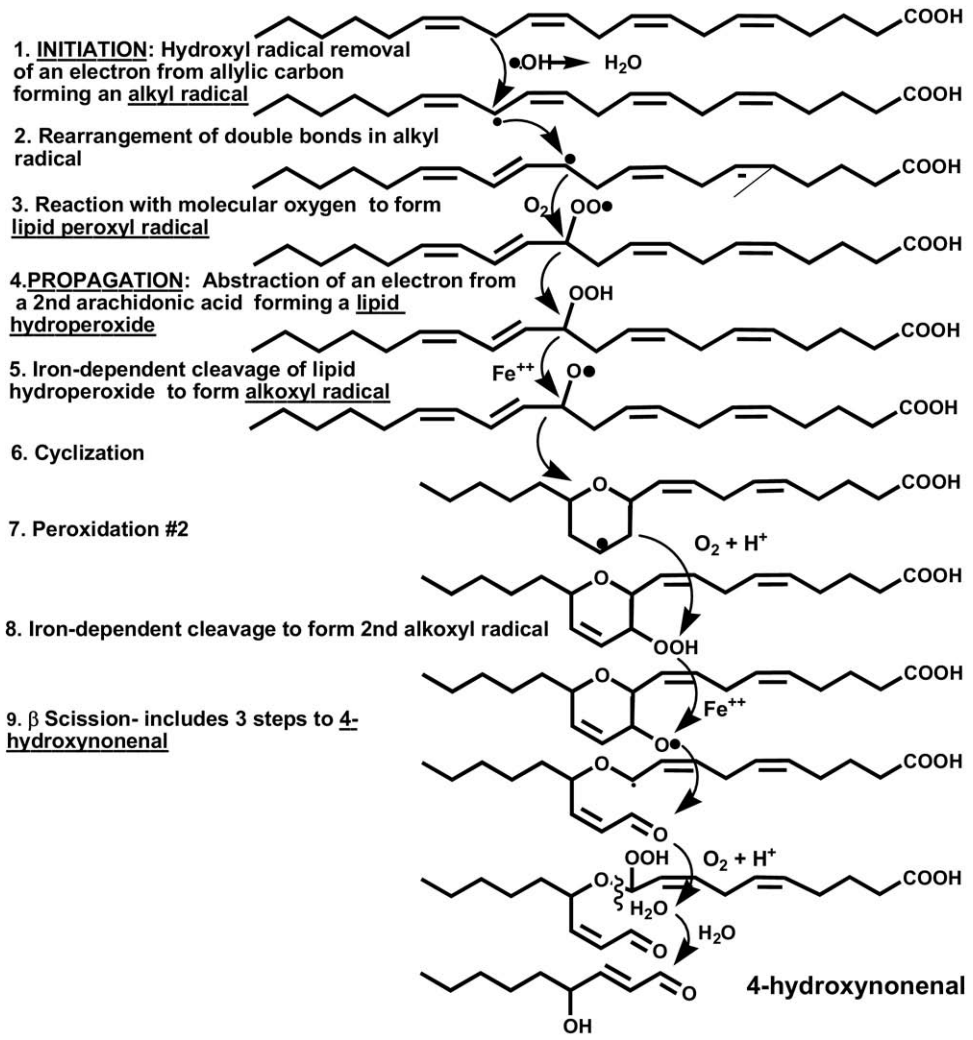

FIG. 1. Chemistry involved in the initiation, propagation, and termination reactions of arachidonic acid during lipid peroxidation with the resulting formation of the aldehydic end-product 4-hydroxynonenal (4-HNE).

branching" reactions: (AA-OO· + AA → AA-OOH + AA· or AA-O· + AA → AA-OH +AA·).

Ultimately, the LP process leads to "fragmentation" or "scission" reactions in which the peroxidized AA breaks down to give rise to the neurotoxic aldehydes 4-hydroxynonenal (4-HNE) or 2-propenal (acrolein). The 4-HNE (as well as acrolein) produces neurotoxicity by binding to basic amino acids, such as lysine or histidine, as well as sulfhydryl-containing cysteine residues in cellular proteins as illustrated in FIG. 2. The resulting chemical modifications have been shown to inhibit the function of a variety of structural and enzymatic cellular proteins.

Other forms of oxidative damage

The central nervous system is exquisitely sensitive to LP because of its high content of peroxidation-susceptible lipids, such as AA, linoleic acid, linolenic acid, and docosahexaenoic acid and the high levels of iron. Although LP disrupts the normal phospholipid architecture of cellular and subcellular organellar membranes, end-products of LP, most notably 4-HNE and acrolein, can bind to proteins, modifying their structure and compromising function. However, primary radical-mediated oxidative damage can also occur in proteins. For instance, iron-catalyzed, ·OH mechanisms can target certain basic amino acids (e.g., lysine, arginine, histidine) leading to the formation of "protein carbonyl" moieties. Another form of protein oxidative damage involves the oxidation of cysteine sulfhydryl groups, which can lead to the formation of abnormal disulfide bridges.

Nucleic acids, both DNA and RNA, are also susceptible to oxidative modification by inorganic and organic (i.e., lipid) radicals. In addition to potentially compromising DNA replication, transcription, and mRNA translation, DNA oxidative damage also triggers DNA repair mechanisms that can greatly stress cellular function and survival. One such mechanism concerns the activation of poly ADP ribose polymerase whose action can lead to

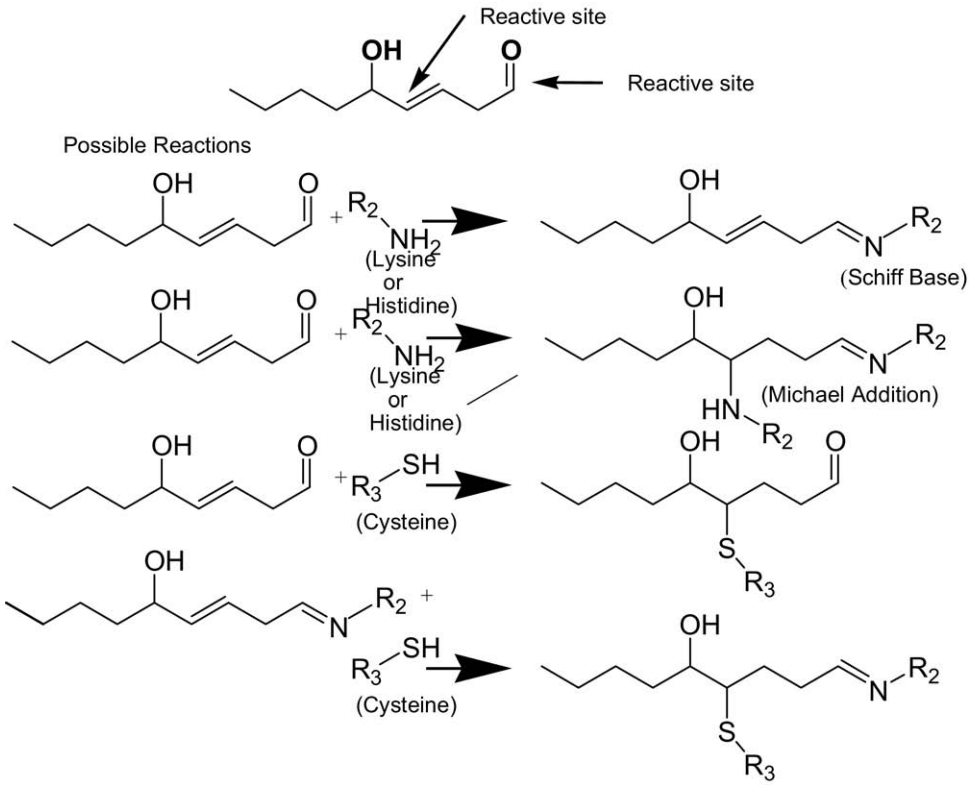

FIG. 2. Chemical reactions of 4-hydroxynonenal (4-HNE) with amino acids that lead to impairment of protein structure and function.

severe depletion of cellular stores of ATP. In addition, DNA-protein cross-linking can occur (e.g., thymine-tyrosine).[3] However, compared with the numerous studies that have documented post-traumatic LP and protein oxidative damage in TBI models, very little examination of nucleic acid oxidation has occurred.

Peroxynitrite

Nearly 20 years ago, Beckman[11] introduced the theory that the principal ROS involved in producing tissue injury in a variety of neurological disorders is the "reactive nitrogen species" peroxynitrite (PN; $ONOO^-$), which is formed by the combination of nitric oxide synthase (NOS)-generated ·NO radical and $O_2^{\cdot -}$: $O_2^{\cdot -} + \cdot NO \rightarrow ONOO^-$. Since that time, the biochemistry of PN has been in large part defined. PN-mediated oxidative damage is actually caused by PN decomposition products that possess potent-free radical characteristics. These are formed in one of two ways. The first involves the protonation of PN to form peroxynitrous acid (ONOOH), which can undergo homolytic decomposition to form the highly reactive nitrogen dioxide radical ($\cdot NO_2$) and OH; ($ONOOH \rightarrow \cdot NO_2 + \cdot OH$). Perhaps more important physiologically, PN will react with carbon dioxide (CO_2) to form nitrosoperoxocarbonate ($ONOOCO_2$), which can decompose into $\cdot NO_2$ and carbonate radical ($\cdot CO_3$); ($ONOOCO_2 \rightarrow \cdot NO_2 + \cdot CO_3$).

Each of the PN-derived radicals (·OH, $\cdot NO_2$ and $\cdot CO_3$) can initiate LP cellular damage by abstraction of an electron from a hydrogen atom bound to an allylic carbon in polyunsaturated fatty acids or cause protein carbonylation by reaction with susceptible amino acids (e.g., lysine, cysteine, arginine). In addition, $\cdot NO_2$ can nitrate the third position of tyrosine residues in proteins; 3-NT is a specific footprint of PN-induced cellular damage. Peroxynitrite-mediated protein nitration can involve the initial oxidation of a tyrosine moiety by a lipid peroxyl or alkoxyl radical, followed by nitration by $\cdot NO_2$.

The implication of PN in post-TBI pathophysiology is derived from four lines of evidence. First of all, all three NOS isoforms (endothelial, neuronal, and inducible) are known to be up regulated during the first 24 h after TBI in rodents.[12–14] Second, several laboratories have shown that the acute treatment of injured mice or rats with NOS inhibitors can exert a neuroprotective effect and/or improve neurological recovery.[15–22] Third, biochemical footprints of PN-mediated damage have been documented in rodent TBI paradigms, including an increase in 3-NT levels[15,21] and ADP ribosylation (evidence of poly ADP ribose polymerase activation). Fourth, the notion that these markers of PN-mediated damage are pathophysiologically important is supported by the finding that the NOS inhibitor L-NAME can lessen the accumulation of 3-NT in in-

jured brains[15,21] at the same doses that improve neurological recovery.[23]

MECHANISMS FOR PHARMACOLOGICAL INHIBITION OF OXIDATIVE DAMAGE IN TBI

Based on this outline of the steps involved in oxygen radical-induced oxidative damage, and LP in particular, a number of potential mechanisms for its inhibition are apparent, which fall into three categories. The first category includes compounds that inhibit the initiation of LP and other forms of oxidative damage by preventing the formation of ROS or reactive nitrogen species. For instance, NOS inhibitors, as previously discussed, exert an indirect antioxidant effect by limiting ·NO production and thus PN formation. However, they also have the potential to interfere with the physiological roles that ·NO is responsible for including antioxidant effects, which are due its important role as a scavenger of lipid peroxyl radicals (e.g., AAOO· + ·NO →AAOONO).[24] Another approach to blocking post-traumatic radical formation is the inhibition of the enzymatic (e.g., cyclooxygenase, 5-lipoxygenases) AA cascade during which the formation of $O_2^{\cdot -}$ is produced as a byproduct of prostanoid and leukotriene synthesis. Kontos and Wei,[2] Kontos,[25] and Hall and coworkers[26,27] have shown that cyclooxygenase inhibiting nonsteroidal anti-inflammatory agents (e.g., indomethacin, ibuprofen) are vasoprotective and neuroprotective in TBI models.

A second indirect LP inhibitory approach involves chemically scavenging the radical species (e.g., $O_2^{\cdot -}$, ·OH, $\cdot NO_2$, $\cdot CO_3$) before they have a chance to steal an electron from a polyunsaturated fatty acid and thus initiate LP. The use of pharmacologically-administered SOD represents an example of this strategy. Another example concerns the use of the nitroxide antioxidant tempol, which has been shown to catalytically scavenge the PN-derived free radicals $\cdot NO_2$ and $\cdot CO_3$.[28] In either case, a general limitation to these first two approaches is that they would be expected to have a short therapeutic window and would have to be administered rapidly to have a chance to interfere with the initial post-traumatic "burst" of free radical production that has been documented in TBI models.[2,29] Although it is believed that ROS, including PN production, persists several hours after injury, the major portion is an early event that peaks in the first 60 minutes after injury, making it clinically impractical to pharmacologically inhibit, unless the antioxidant compound is already "on board" when the injury occurs, or it is available for administration immediately thereafter.

In contrast, indirect-acting antioxidant mechanisms, the third category, involves stopping the "chain reaction" propagation of LP once it has begun. The most demonstrated way to accomplish this is by scavenging lipid peroxyl (LOO·) or alkoxyl (LO·) radicals. The endogenous scavenger of these lipid radicals is alpha tocopherol or vitamin (Vit) E, which can donate an electron from its phenolic hydroxyl moiety to quench LOO·. However, the scavenging process is stoichiometric (1 Vit E can only quench 1 LOO·), and in the process, Vit E loses its antioxidant efficacy and becomes Vit E radical (LOO· + Vit E → LOOH + Vit E·). Although Vit E· is relatively unreactive (i.e., harmless), it also can not scavenge another LOO· until it is reduced back to its active form by receiving an electron from other endogenous antioxidant reducing agents, such as ascorbic acid (Vit C) or glutathione. Although this tripartite LOO· antioxidant defense system (Vit E, Vit C, glutathione) works fairly effectively in the absence of a major oxidative stress, numerous studies have shown that each of these antioxidants is rapidly consumed during the early minutes and hours after TBI. Thus, it has been recognized for a long time that more effective pharmacological LOO· and LO· scavengers are needed. Furthermore, it is expected that compounds that could interrupt the LP process after it has begun would be able to exert a more practical neuroprotective effect (i.e., possess longer antioxidant therapeutic window).

A second approach to inhibiting the propagation of LP reactions is to chelate free iron, either ferrous (Fe^{++}) or ferric (Fe^{+++}), which potently catalyzes the breakdown of LOOH, an essential event in the continuation of LP chain reactions in cellular membranes. The prototypical iron-chelating drug, which chelates Fe^{+++}, is the bacterially (streptomyces pilosus)-derived tri-hydroxamic acid compound deferoxamine.

NEUROPROTECTIVE EFFECTS OF PHARMACOLOGICAL ANTIOXIDANTS

TBI clinical trial results with polyethylene glycol-conjugated-SOD, tirilazad, and dexanabinol

During the past 25 years, there has been an intense effort to discover and develop pharmacological agents for acute treatment of TBI. This has included multiple compounds that possess free radical scavenging/antioxidant properties, including polyethylene glycol-conjugated superoxide dismutase (PEG-SOD), the LP inhibitor tirilazad,[30–32] and more recently the mixed glutamate antagonist/antioxidant compound dexanabinol.[33] However, each of these trials was a therapeutic failure in that no overall benefit has been documented in moderate and severe TBI patient populations, which was the primary goal in each case. These failures can be attributed to several factors. Perhaps most importantly, the preclinical assessment of compounds destined for acute TBI trials has often been woefully inadequate in regard to the definition of neuroprotective dose-response relationships,

pharmacokinetic-pharmacodynamic correlations, therapeutic window, and optimum dosing regimen and treatment duration. However, a number of other issues related to the design of the clinical trials are also believed to be involved.[32] The following sections briefly review the TBI histories of PEG-SOD and tirilazad. Dexanabinol (HU211) is not discussed further because it is a mixed glutamate antagonist/antioxidant compound that was studied very little in preclinical TBI paradigms prior to being the subject of clinical development for that indication.

PEG-SOD

As mentioned earlier, the earliest studies of free radical scavenging compounds in TBI models were carried out with Cu/Zn SOD based on the work of Kontos and Povlishock[1] and Kontos and Wei[2] and Kontos[25] who showed that post-traumatic microvascular dysfunction was initiated by $O_2^{\cdot -}$ generated as a byproduct of the arachidonic acid cascade, which is massively activated during the first minutes and hours after TBI. Their work showed that administration of SOD prevented the post-traumatic microvascular dysfunction. This led to clinical trials in which the more metabolically stable polyethylene glycol (PEG)-conjugated SOD was examined in moderate and severe TBI patients when administered within the first 8 h after injury. Although an initial small phase II study showed a positive trend, subsequent multicenter phase III studies failed to show a significant benefit in terms of increased survival or improved neurological outcomes.[34] Although many explanations for these negative results may be postulated, one reason may be that a large protein like SOD is unlikely to have much brain penetrability and therefore its radical scavenging effects may be limited to the microvasculature. A second reason may be that attempting to scavenge the short-lived inorganic radical $O_2^{\cdot -}$ may be associated with a very short therapeutic window, as previously suggested. Indeed, the time course of measurable post-traumatic ·OH formation in the injured rodent brain has been shown to largely run its course by the end of the first hour after TBI.[9,29] A more rational strategy would be to inhibit the LP that is triggered by the initial burst of inorganic radicals. A comparison of the time course of LP with that of post-traumatic ·OH shows that LP reactions continue to build beyond the first post-traumatic hour[9] and may continue for 3 to 4 days.[35] Despite the failure of PEG-SOD in human TBI, experimental studies have shown that transgenic mice that over-express Cu/Zn SOD are significantly protected against post-TBI pathophysiology and neurodegeneration.[36–40] This fully supports the importance of post-traumatic $O_2^{\cdot -}$ in post-traumatic secondary injury, despite the fact that targeting this primordial radical is probably not the best antioxidant strategy for acute CNS injury compared with trying to stop the downstream LP process that is initiated by the early increases in $O_2^{\cdot -}$, ·OH, $\cdot NO_2$, and $\cdot CO_3$.

Tirilazad

Consistent with that rationale, the 21-aminosteroid LP inhibitor tirilazad (aka U74006F) was discovered, which inhibits free radical-induced LP by a combination of LOO· scavenging and a membrane-stabilizing action that limits the propagation of LP reactions between an LOO· and an adjacent polyunsaturated fatty acid. The protective efficacy of tirilazad has been demonstrated in multiple animal models of acute TBI in mice,[41] rats,[42] and cats.[43] Although the compound is largely localized in the microvascular endothelium, the post-traumatic disruption of the BBB is known to allow the successful penetration of tirilazad into the brain parenchyma, as noted earlier.[44] Other mechanistic data derived from the rat-controlled cortical impact and the mouse-diffused concussive head injury models have definitively shown that a major effect of tirilazad is to lessen post-traumatic BBB opening.[9,44]

Tirilazad was taken into clinical development in the early 1990s, and after a small phase II dose-escalation study that demonstrated the safety of the drug in TBI patients, tirilazad was evaluated in two phase III multicenter clinical trials for its ability to improve neurological recovery in moderately and severely injured closed TBI patients. One trial was conducted in North America and the other was in Europe. In both trials, TBI patients were treated within 4 h after injury with either vehicle or tirilazad (2.5 mg/kg intravenously every 6 h for 5 days). The North American trial was never published due to a major confounding imbalance in the randomization of the patients to a placebo or the tirilazad in regard to injury severity and pre-treatment neurological status. In contrast, the European trial had much better randomization balance and has been published.[30] The results failed to show a significant beneficial effect of tirilazad in either moderate (GCS = 9–12) or severe (GCS = 4–8) patient categories. However, a post hoc analysis showed that moderately-injured male TBI patients with traumatic SAH has significantly less mortality after treatment with tirilazad (6%) as compared with a placebo (24%; $p < 0.026$). In severely injured males with tSAH (tSAH), tirilazad also lessened mortality from 43% in placebo treated to 34% ($p < 0.071$).[30,45] This result is consistent with the fact that this compound is also highly effective in animal models of SAH.[7] Nevertheless, additional trials would have been required to establish the neuroprotective usefulness of tirilazad in certain human TBI subgroups and to gain Food and Drug Administration approval in the United States. However, the sponsoring company Pharmacia & Upjohn opted not to continue the development of the compound for TBI, although it was successfully approved and marketed for use in aneurysmal SAH in several western European and Aus-

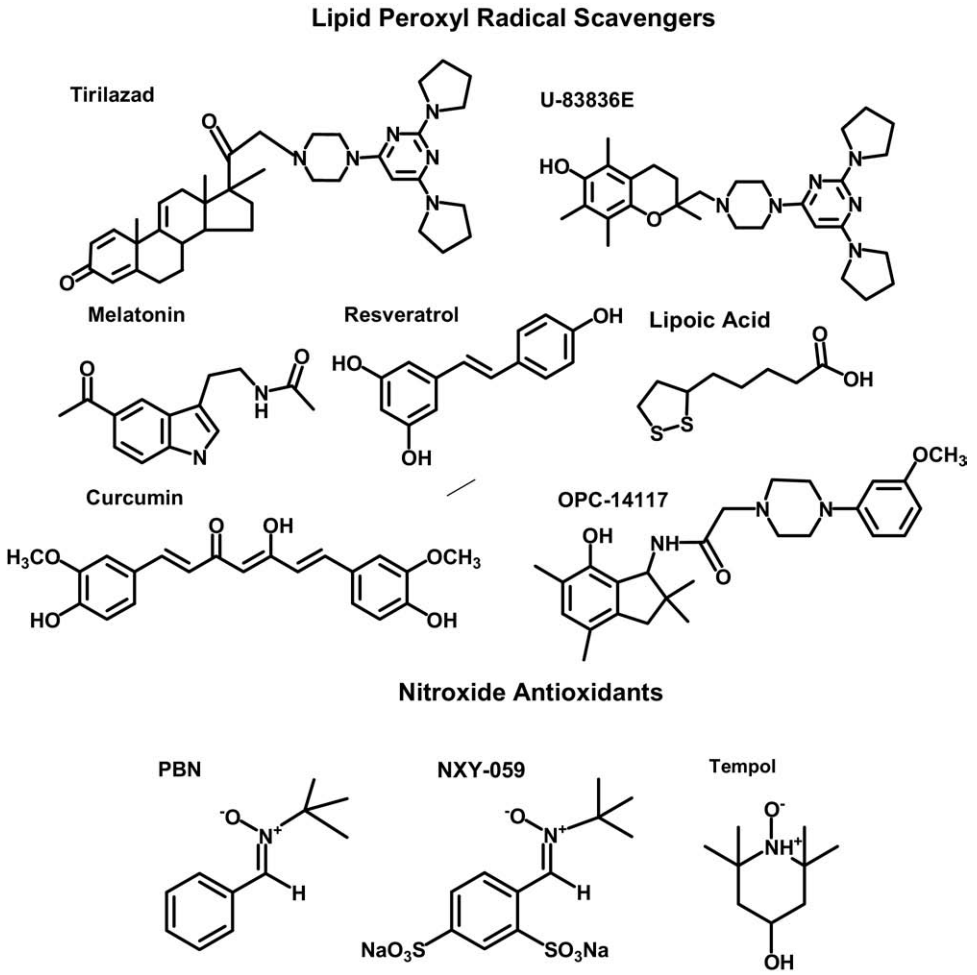

FIG. 3. Chemical structures of lipid peroxyl radical scavenging and nitroxide-containing antioxidants shown to be neuroprotective in traumatic brain injury (TBI) models.

tralian countries based on its demonstrated efficacy in phase III SAH trials.[46,47]

Effects of direct and indirect-acting lipid peroxidation inhibitors

In addition to tirilazad, several other LP inhibitors have been reported to be effective neuroprotectants in TBI models. These include the lipid peroxyl radical (LOO·) scavenging 2-methylaminochromans U-78517F and U-83836E,[48] the pyrrolopyrimidine U-101033E,[48,49] OPC-14117,[50] and the naturally-occurring LOO· scavengers curcumin,[51,52] and resveratrol,[53,54] the indoleamine melatonin,[21,55–58] and last, the endogenous antioxidant lipoic acid (FIG. 3).[59] In the case of curcumin and resveratrol, these are potent LOO· scavengers due to their possession of multiple phenolic hydroxyl groups that can donate electrons to LOO· radicals. Melatonin also has LOO· scavenging capability,[60] but in addition appears to react with PN.[61,62] Lipoic acid may also have LOO· scavenging effects, but these are more likely to be indirect via the regeneration (i.e., re-reduction) of other endogenous electron-donating antioxidants, including Vit E, glutathione, and Vit C.

Among these LP inhibitors, the most potent and effective LOO· scavenging LP inhibitors yet discovered is the 2-methylaminochroman compound U-83836E which combines the LOO· scavenging antioxidant chroman ring structure of Vit E with the bis-pyrrolopyrimidine moiety of tirilazad. The phenolic chroman antioxidant moiety can be re-reduced by endogenous ascorbic acid (Vit C) or glutathione after it has donated its phenolic electron to an initial LOO· making it able to quench a second and then a third LOO·, and so on. The bis-pyrrolopyrimidine moiety, on the other hand, can also scavenge multiple moles of LOO·, but by a true catalytic mechanism.[49,63] Thus, U-83836E, is a dual functionality LOO· scavenger that is understandably more effective than either Vit E, tirilazad,[63] and possibly the other naturally-occurring LOO· scavengers, such as curcumin, resveratrol, melatonin, and lipoic acid. Furthermore, U-83836E possesses a high degree of lipophilicity endowing it with a high

affinity for membrane phospholipids in which LP takes place. Recent studies from the authors' laboratory in the mouse- controlled cortical impact TBI model have shown that U-83836E is able to reduce post-traumatic LP and protein nitration, and preserve mitochondrial respiratory function in injured cortical tissue and mitochondria.[64]

Nitroxide antioxidants and peroxynitrite scavengers

In addition to the lipid peroxyl (LOO·) radical scavengers, the neuroprotective effects of a family of nitroxide-containing antioxidants have also been examined in experimental TBI models. These are sometimes referred to as "spin-trapping agents," and include α-phenyl-tert-butyl nitrone and its thiol analog NXY-059 and tempol (see bottom of FIG. 3). Both alpha-phenyl-tert-butyl nitrone and tempol have been shown to be protective in rodent TBI paradigms.[65,66] As mentioned earlier, tempol has been shown by the author and colleagues to catalytically scavenge PN-derived $\cdot NO_2$ and $\cdot CO_3$,[28,67] and to reduce post-traumatic oxidative damage (both LP and protein nitration), preserve mitochondrial function, decrease calcium-activated, calpain-mediated cytoskeletal damage, and reduce neurodegeneration in mice subjected to a severe controlled cortical impact-induced focal TBI.[68] Another laboratory has reported that tempol can reduce post-traumatic brain edema and improve neurological recovery in the rat contusion injury model.[69,70] However, the neuroprotective effect of tempol administered alone is associated with a therapeutic window of an hour or less in the mouse-controlled cortical impact TBI model. Moreover, tempol is not effective at directly inhibiting LP in the latter model.[68]

Effects of the iron chelator deferoxamine

The prototype iron chelator deferoxamine that binds ferric (Fe^{+++}) iron, and thereby would lessen the catalytic effects of iron on LP, has also been reported to have beneficial actions in preclinical TBI or TBI-related models.[71,72] However, deferoxamine is hindered by its lack of brain penetration and rapid plasma elimination rate. To partially counter the latter limitation, a dextran-coupled deferoxamine has been synthesized that has been reported to significantly improve early neurological recovery in a mouse diffuse TBI model.[73] Much of this activity, however, is probably due to microvascular antioxidant protection because of limited brain penetrability. Another caveat to the iron-chelation antioxidant neuroprotective approach that is at least relevant to the ferric iron chelators, such as deferoxamine, is that they can cause a pro-oxidant effect in that their binding of Fe^{+++} can actually drive the oxidation of ferrous to ferric iron, which can increase superoxide radical formation in the process ($Fe^{++} + O_2 \rightarrow Fe^{+++} + O_2^{\cdot -}$).

Carbonyl scavenging as an approach to inhibit 4-HNE and acrolein binding to proteins

As pointed out earlier (FIG. 2), the LP-derived aldehydic (carbonyl-containing) breakdown products 4-HNE and acrolein have a high affinity for binding to selected protein amino acid residues including histidine, lysine, arginine, and cysteine. These modifications have been shown to inhibit the activities of a variety of enzymatic proteins.[3] Several compounds have been recently identified that are able to antagonize this "carbonyl stress" by covalently binding to reactive LP-derived aldehydes. For instance, D-penicillamine has been demonstrated to form an irreversible bond to primary aldehydes. We have previously demonstrated that penicillamine is able to scavenge PN[74] and to protect brain mitochondria from PN-induced respiratory dysfunction in isolated rat brain mitochondria.[75] This latter action was observed along with an attenuation of 4-HNE-modified mitochondrial proteins.[75] The PN scavenging action of penicillamine, along with its carbonyl scavenging capability may jointly explain our previous findings that acutely administered penicillamine can improve early neurological recovery of mice subjected to moderately severe concussive TBI.[76]

More recently, it has been demonstrated that a variety of hydrazine (-NH-NH_2)-containing compounds such as the anti-hypertensive agent hydralazine, the anti-depressant phenelzine, and the anti-tubercular agent iproniazid can react with the carbonyl moieties of 4-HNE or acrolein, which prevents the latter from binding to susceptible amino acids in proteins.[77] Consistent with this effect being neuroprotective, others have shown that hydralazine inhibits either compression or acrolein-mediated injuries to ex vivo spinal cord.[78] Hydralazine, which is a potent vasodilator would be difficult to administer in vivo after either spinal cord injury or TBI, in which hypotension is already a common pathophysiological problem. However, other hydrazine-containing compounds, such as phenelzine and iproniazid, do not compromise blood pressure as readily as hydralazine, and have a long history of clinical use, although never having been examined in acute neurotrauma models. 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.[77] Such an effect could be associated with a favorable neuroprotective therapeutic window.

RATIONALE FOR COMBINATION ANTIOXIDANT TREATMENT OF TBI

Antioxidant neuroprotective therapeutic discovery directed at acute TBI has consistently been focused on attempting to inhibit the secondary injury cascade by pharmacological targeting of a single oxidative damage

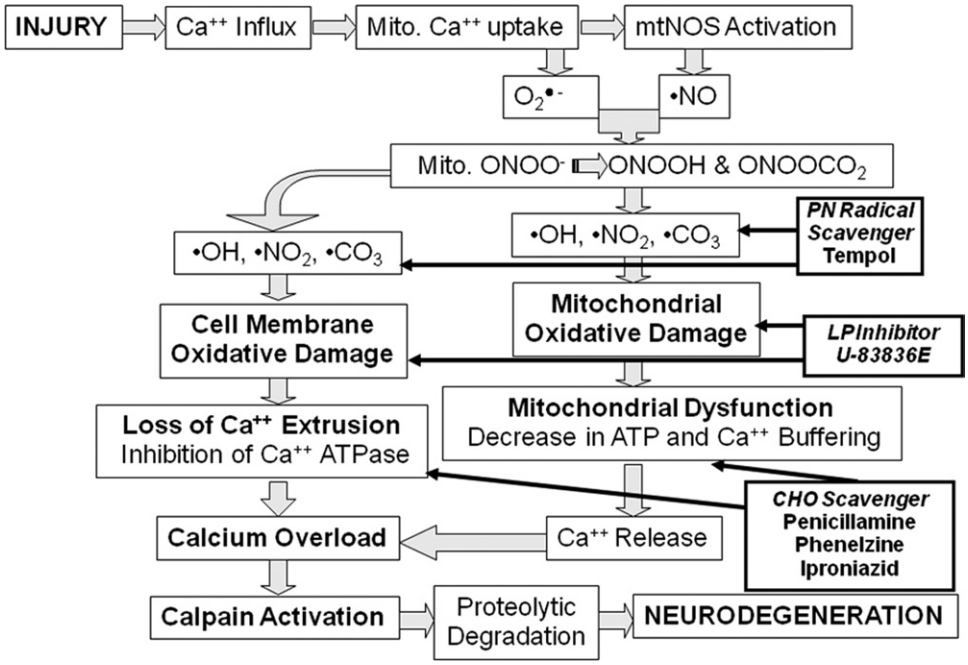

FIG. 4. Rationale for combination antioxidant therapy for traumatic brain injury (TBI). Injury triggers an increase in cytoplasmic Ca^{++} via voltage-dependent and glutamate receptor-operated channels. The increase in intracellular Ca^{++} initiates activation of cytoplasmic calpain. Mitochondrial Ca^{++} uptake (buffering) stresses the mitochondria and contributes to mitochondrial dysfunction. Specifically, Ca^{++} uptake by the mitochondria leads $O_2^{\cdot -}$ leakage from the electron transport chain and activation of Ca^{++}-activated mitochondrial nitric oxide synthase (NOS). The $O_2^{\cdot -}$ and NO· combine to form the potent reactive nitrogen species PN, which is able to in turn give rise to the highly reactive nitrogen dioxide (·NO_2), hydroxyl (·OH), and carbonate (·CO_3) radicals that cause oxidative damage to the mitochondria, as well as other cellular structures due to PNs large diffusion radius. When this becomes severe, there is a decrease in the mitochondrial ATP production and membrane potential ($\Delta\Psi$). This leads to catastrophic mitochondrial failure (mitochondrial permeability transition [MPT]) and the dumping of mitochondrial Ca^{++} into the cytoplasm where it exacerbates cytoplasmic calpain activation and proteolysis of a cytoskeletal proteins and other substrates. The combination of the antioxidant tempol, which catalytically reacts with PN-derived radicals with a chain-breaking lipid peroxidation (LP) inhibitor such as U-83836E or a carbonyl (CHO) scavenging compound that should produce a better neuroprotective effect than any of these compounds alone.

mechanism. As presented previously, these efforts have included either enzymatic scavenging of superoxide radicals with SOD[34] or inhibition of LP with tirilazad.[30] Although 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 the post hoc subgroup analysis suggests that the microvascularly localized tirilazad may have efficacy in moderate and severe TBI patients with tSAH.[30] Although there are many reasons that have been identified as possible contributors to the failure, one logical explanation has to 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.

In addition to the antioxidant strategy of scavenging the initiating radicals and stopping the propagation of LP reactions in the injured brain tissue, recent work has shown that carbonyl scavenging compounds can also act to protect cellular proteins from the binding of neurotoxic LP-derived aldehydes. Thus, we are presently exploring the neuroprotective efficacy of three of the prototypes of this new class of compound alone and in combination with the PN-radical scavenging tempol and/or the LP-inhibiting U-83836E. FIG. 4 summarizes the overall rationale for a multi-mechanistic antioxidant therapy for TBI. 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 with the window for the individual strategies. If these theoretical combinatorial benefits are confirmed in preclinical TBI models, then this should greatly enhance the chance of neuroprotective success in future clinical trials in contrast to previous failures with single antioxidant agents.

Acknowledgments: Portions of the work reviewed in this article were supported by funding from 5R01 NS046566, 5P30 NS051220, and 5P01 NS58484 and from the Kentucky Spinal Cord & Head Injury Research Trust.

REFERENCES

1. Kontos HA, Povlishock JT. Oxygen radicals in brain injury. Cent Nerv Syst Trauma 1986;3:257–263.
2. Kontos HA, Wei EP. Superoxide production in experimental brain injury. J Neurosurg 1986;64:803–807.
3. Halliwell B, Gutteridge, JMC. Free Radicals in biology and medicine, 3rd ed. Oxford: Oxford University Press; 2008.

4. Zaleska MM, Floyd RA. Regional lipid peroxidation in rat brain in vitro: possible role of endogenous iron. Neurochem Res 1985;10: 397–410.
5. Sadrzadeh SM, Graf E, Panter SS, Hallaway PE, Eaton JW. Hemoglobin. A biologic fenton reagent. J Biol Chem 1984;259: 14354–14356.
6. Sadrzadeh SM, Eaton JW. Hemoglobin-mediated oxidant damage to the central nervous system requires endogenous ascorbate. J Clin Invest 1988;82:1510–1515.
7. 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;28:221–268.
8. Hall ED, Braughler JM. Free radicals in CNS injury. Res Publ Assoc Res Nerv Ment Dis 1993;71:81–105.
9. 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.
10. Globus MY, Alonso O, Dietrich WD, Busto R, Ginsberg MD. Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. J Neurochem 1995;65:1704–1711.
11. Beckman JS. The double-edged role of nitric oxide in brain function and superoxide-mediated injury. J Dev Physiol 1991;15:53–59.
12. Cobbs CS, Fenoy A, Bredt DS, Noble LJ. Expression of nitric oxide synthase in the cerebral microvasculature after traumatic brain injury in the rat. Brain Res 1997;751:336–338.
13. Rao VL, Dogan A, Bowen KK, Dempsey RJ. Traumatic injury to rat brain upregulates neuronal nitric oxide synthase expression and L-[3H]nitroarginine binding. J Neurotrauma 1999;16:865–877.
14. Gahm C, Holmin S, Mathiesen T. Temporal profiles and cellular sources of three nitric oxide synthase isoforms in the brain after experimental contusion. Neurosurgery 2000;46:169–177.
15. Mesenge C, Charriaut-Marlangue C, Verrecchia C, Allix M, Boulu RR, Plotkine M. Reduction of tyrosine nitration after N(omega)-nitro-L-arginine-methylester treatment of mice with traumatic brain injury. Eur J Pharmacol 1998;353:53–57.
16. Mesenge C, Verrecchia C, Allix M, Boulu RR, Plotkine M. Reduction of the neurological deficit in mice with traumatic brain injury by nitric oxide synthase inhibitors. J Neurotrauma 1996;13: 209–214.
17. Wada K, Alonso OF, Busto R, et al. Early treatment with a novel inhibitor of lipid peroxidation (LY341122) improves histopathological outcome after moderate fluid percussion brain injury in rats. Neurosurgery 1999;45:601–608.
18. Wada K, Chatzipanteli K, Busto R, Dietrich WD. Role of nitric oxide in traumatic brain injury in the rat. J Neurosurg 1998;89: 807–818.
19. Wada K, Chatzipanteli K, Busto R, Dietrich WD. Effects of L-NAME and 7-NI on NOS catalytic activity and behavioral outcome after traumatic brain injury in the rat. J Neurotrauma 1999;16:203–212.
20. Wallis RA, Panizzon KL, Girard JM. Traumatic neuroprotection with inhibitors of nitric oxide and ADP-ribosylation. Brain Res 1996;710:169–177.
21. Mesenge C, Margaill I, Verrecchia C, Allix M, Boulu RG, Plotkine M. Protective effect of melatonin in a model of traumatic brain injury in mice. J Pineal Res 1998;25:41–46.
22. Wada K, Chatzipanteli K, Kraydieh S, Busto R, Dietrich WD. Inducible nitric oxide synthase expression after traumatic brain injury and neuroprotection with aminoguanidine treatment in rats. Neurosurgery 1998;43:1427–1436.
23. Mesenge C, Verrecchia C, Allix M, Boulu RR, Plotkine M. Reduction of the neurological deficit in mice with traumatic brain injury by nitric oxide synthase inhibitors. J Neurotrauma 1996;13:11–16.
24. Hummel SG, Fischer AJ, Martin SM, Schafer FQ, Buettner GR. Nitric oxide as a cellular antioxidant: a little goes a long way. Free Radic Biol Med 2006;40:501–506.
25. Kontos HA. Oxygen radicals in CNS damage. Chem Biol Interact 1989;72:229–255.
26. Hall E. Beneficial effects of acute intravenous ibuprofen with inhibition of thromboxane A2 synthetase or 5-lipoxygenase. CNS Trauma 1986;2:75–83.
27. Hall E. Beneficial effects of acute intravenous ibuprofen on neurological recovery of head injured mice: Comparison of cyclooxygenase inhibition of thromboxane A2 synthetase or 5-lipoxygenase. CNS Trauma 1986;2:75–83.
28. Carroll RT, Galatsis P, Borosky S, et al. 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (Tempol) inhibits peroxynitrite-mediated phenol nitration. Chem Res Toxicol 2000;13:294–300.
29. Hall ED, Andrus PK, Yonkers PA. Brain hydroxyl radical generation in acute experimental head injury. J Neurochem 1993;60: 588–594.
30. 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.
31. Langham J, Goldfrad C, Teasdale G, Shaw D, Rowan K. Calcium channel blockers for acute traumatic brain injury. Cochrane Database Syst Rev 2000:CD000565.
32. Narayan RK, Michel ME, Ansell B, et al. Clinical trials in head injury. J Neurotrauma 2002;19:503–557.
33. Maas AI, Murray G, Henney H 3rd, et al. Efficacy and safety of dexanabinol in severe traumatic brain injury: results of a phase III randomised, placebo-controlled, clinical trial. Lancet Neurol 2006; 5:38–45.
34. Muizelaar JP, Kupiec JW, Rapp LA. PEG-SOD after head injury. J Neurosurg 1995;83:942.
35. Du L, Bayir H, Lai Y, et al. Innate gender-based proclivity in response to cytotoxicity and programmed cell death pathway. J Biol Chem 2004;279:38563–38570.
36. 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;12:815–824.
37. 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;85:885–891.
38. Lewen A, Matz P, Chan PH. Free radical pathways in CNS injury. J Neurotrauma 2000;17:871–890.
39. 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;95:732–744.
40. Gladstone DJ, Black SE, Hakim AM. Toward wisdom from failure: lessons from neuroprotective stroke trials and new therapeutic directions. Stroke 2002;33:2123–2136.
41. Hall ED, Yonkers PA, McCall JM, Braughler JM. Effects of the 21-aminosteroid U74006F on experimental head injury in mice. J Neurosurg 1988;68:456–461.
42. 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.
43. 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;52:365–375.
44. 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–S442.
45. Lyons WE, George EB, Dawson TM, Steiner JP, Snyder SH. Immunosuppressant FK506 promotes neurite outgrowth in cultures of PC12 cells and sensory ganglia. Proc Natl Acad Sci U S A 1994;91:3191–3195.
46. 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;84:221–228.
47. 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;90:1018–1024.
48. Hall ED. The mouse head injury model: utility in the discovery of acute cerebroprotective agents. In: Stoo T, ed. Central nervous system trauma research techniques. Boca Raton, FL: CRC Press, 1995:213–233.

49. Hall ED, Andrus PK, Smith SL, et al. Neuroprotective efficacy of microvascularly-localized versus brain-penetraiting antioxidants. Acta Neurochir Suppl 1995;66:107–113.
50. Mori T, Kawamata T, Katayama Y, et al. Antioxidant, OPC-14117, attenuates edema formation, and subsequent tissue damage following cortical contusion in rats. Acta Neurochir Suppl (Wien) 1998; 71:120–122.
51. Sharma S, Zhuang Y, Ying Z, Wu A, Gomez-Pinilla F. Dietary curcumin supplementation counteracts reduction in levels of molecules involved in energy homeostasis after brain trauma. Neuroscience 2009;161:1037–1044.
52. Wu A, Ying Z, Gomez-Pinilla F. Dietary curcumin counteracts the outcome of traumatic brain injury on oxidative stress, synaptic plasticity, and cognition. Exp Neurol 2006;197:309–317.
53. Ates O, Cayli S, Altinoz E, et al. Neuroprotection by resveratrol against traumatic brain injury in rats. Mol Cell Biochem 2007;294: 137–144.
54. Sonmez U, Sonmez A, Erbil G, Tekmen I, Baykara B. Neuroprotective effects of resveratrol against traumatic brain injury in immature rats. Neurosci Lett 2007;420:133–137.
55. Beni SM, Kohen R, Reiter RJ, Tan DX, Shohami E. Melatonin-induced neuroprotection after closed head injury is associated with increased brain antioxidants and attenuated late-phase activation of NF-kappaB and AP-1. FASEB J 2004;18:149–151.
56. Cirak B, Rousan N, Kocak A, Palaoglu O, Palaoglu S, Kilic K. Melatonin as a free radical scavenger in experimental head trauma. Pediatr Neurosurg 1999;31:298–301.
57. Ozdemir D, Tugyan K, Uysal N, et al. Protective effect of melatonin against head trauma-induced hippocampal damage and spatial memory deficits in immature rats. Neurosci Lett 2005;385: 234–239.
58. Ozdemir D, Uysal N, Gonenc S, et al. Effect of melatonin on brain oxidative damage induced by traumatic brain injury in immature rats. Physiol Res 2005;54:631–637.
59. Toklu HZ, Hakan T, Biber N, Solakoglu S, Ogunc AV, Sener G. The protective effect of alpha lipoic acid against traumatic brain injury in rats. Free Radic Res 2009;43:658–667.
60. Longoni B, Salgo MG, Pryor WA, Marchiafava PL. Effects of melatonin on lipid peroxidation induced by oxygen radicals. Life Sci 1998;62:853–859.
61. Zhang H, Squadrito GL, Pryor WA. The reaction of melatonin with peroxynitrite: formation of melatonin radical cation and absence of stable nitrated products. Biochem Biophys Res Commun 1998; 251:83–87.
62. Zhang H, Squadrito GL, Uppu R, Pryor WA. Reaction of peroxynitrite with melatonin: A mechanistic study. Chem Res Toxicol 1999;12:526–534.
63. Hall ED, Braughler JM, Yonkers PA, et al. U-78517F: a potent inhibitor of lipid peroxidation with activity in experimental brain injury and ischemia. J Pharmacol Exp Ther 1991;258:688–694.
64. Mustafa AG, Singh IN, Carrico KM, Hall ED. Mitochondrial protection after traumatic brain injury by scavenging lipid peroxyl radicals. J Neurotrauma 2009;26:A93.
65. Awasthi D, Church DF, Torbati D, Carey ME, Pryor WA. Oxidative stress following traumatic brain injury in rats. Surg Neurol 1997;47:575–582.
66. Marklund N, Clausen F, Lewen A, Hovda DA, Olsson Y, Hillered L. alpha-Phenyl-tert-N-butyl nitrone (PBN) improves functional and morphological outcome after cortical contusion injury in the rat. Acta Neurochir (Wien) 2001;143:73–81.
67. Bonini MG, Mason RP, Augusto O. The mechanism by which 4-hydroxy-2,2,6,6-tetramethylpiperidene-1-oxyl (tempol) diverts peroxynitrite decomposition from nitrating to nitrosating species. Chem Res Toxicol 2002;15:506–11.
68. Deng-Bryant Y, Singh IN, Carrico KM, Hall ED. Neuroprotective effects of tempol, a catalytic scavenger of peroxynitrite-derived free radicals, in a mouse traumatic brain injury model. J Cereb Blood Flow Metab 2008;28:1114–1126.
69. Zhang R, Shohami E, Beit-Yannai E, Bass R, Trembovler V, Samuni A. Mechanism of brain protection by nitroxide radicals in experimental model of closed-head injury. Free Radic Biol Med 1998;24:32–40.
70. Beit-Yannai E, Zhang R, Trembovler V, Samuni A, Shohami E. Cerebroprotective effect of stable nitroxide radicals in closed head injury in the rat. Brain Res 1996;717:22–8.
71. Long DA, Ghosh K, Moore AN, Dixon CE, Dash PK. Deferoxamine improves spatial memory performance following experimental brain injury in rats. Brain Res 1996;717:109–117.
72. Gu Y, Hua Y, Keep RF, Morgenstern LB, Xi G. Deferoxamine reduces intracerebral hematoma-induced iron accumulation and neuronal death in piglets. Stroke 2009;40:2241–2243.
73. Panter SS, Braughler JM, Hall ED. Dextran-coupled deferoxamine improves outcome in a murine model of head injury. J Neurotrauma 1992;9:47–53.
74. 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;83:243–254.
75. Singh IN, Sullivan PG, Hall ED. Peroxynitrite-mediated oxidative damage to brain mitochondria: protective effects of peroxynitrite scavengers. J Neurosci Res 2007;85:2216–2223.
76. Hall ED, Kupina NC, Althaus JS. Peroxynitrite scavengers for the acute treatment of traumatic brain injury. Ann N Y Acad Sci 1999;890:462–468.
77. Galvani S, Coatrieux C, Elbaz M, et al. Carbonyl scavenger and antiatherogenic effects of hydrazine derivatives. Free Radic Biol Med 2008;45:1457–1467.
78. 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;104:708–718.