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Article

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Antioxidant therapies in traumatic brain injury: a review

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Abstract: Oxidative stress constitute one of the commonest mechanism of the secondary injury contributing to neuronal death in traumatic brain injury cases. The oxidative stress induced secondary injury blockade may be considered as to be a good alternative to improve the outcome of traumatic brain injury (TBI) treatment. Due to absence of definitive therapy of traumatic brain injury has forced researcher to utilize unconventional therapies and its roles investigated in the improvement of management and outcome in recent year. Antioxidant therapies are proven effective in many preclinical studies and encouraging results and the role of antioxidant mediaction may act as further advancement in the traumatic brain injury management it may represent aonr of newer moadlaity in neurosurgical aramamentorium, this kind of therapy could be a good alternative or adjuct to the previously established neuroprotection agents in TBI.

Key words: Traumatic brain injury, oxidative stress, antioxidants

Introduction

Traumatic brain injury (TBI) still remains one of the leading causes of death and disability, worldwide (1–6). TBI constitutes a major global health and socio-economic problem with neurobehavioral sequelae contributing to long-term disability (7). The incidence of TBI progressively continues to rapid rising trends and according to predictions, the neurotrauma will continue to

representing a growing number of deaths worlwide by 2020 (8, 9).

Moderate and severe TBI produces in the greatest disability andresponsible for consumes the most resources per individual, yet magnitude of mild TBI and societal ramifications are often underestimated. Despite the label "mild", many of these injuries result in immense long-term morbidities10.

Regarding the mechanisms responsible to produce secondary damage of brain tissue following TBI, a distinction is made between primary and secondary injury mechanisms. Primary injury includes the direct effects of the mechanical energy on the brain tissue (1, 11). The secondary brain injury, which sets in at the time of injury and could continue for several weeks, is a complex neurodegenerative process, involving multiple cellular and molecular pathways, including inflammation andoxidative stress (3).

Free radical formation and oxidative damage are extensively investigated and validated as important contributors to the pathophysiology of acute central nervous system injury. The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is an early event following injury occurring within minutes of mechanical impact (12). Various mechanisms are postulated to promote free radical production, including glutamate release, intracelular calcium overload, increase in arachidonic acid and its metabolites, hemoglobin denaturation, and iron ion release (1). The production of free radicals evoked by brain injury plays a crucial role in the initiating and propagating the pathogenesis of post-traumatic secondary injury, through oxidation and nitration of the cellular membrane, proteins, and DNA (3, 13–16).

The blockade of oxidative damage seems to be a rational intervention to reduce secondary brain injury after TBI, and establishing the time course of oxidative damage provides information on a possible therapeutic window for protection against secondary brain injury (1, 17, 18). Therefore, to find pharmacological agents specifically aiming to inhibit oxidative stress and modifying the expression of inflammatory cytokines might be a critical strategy; thus, antioxidants have been a major point for consideration (19).

Related to the above, the need for news therapies for traumatic brain injury is more than evident. Therefore, this article aims to do a review about the pathophysiology of secondary injury, mainly the oxidative stress and new proposal of therapies antioxidants proposed as a treatment of traumatic brain injury.

Secondary injury mechanisms

TBI is understood to be the result of 2 phases: Initial primary neuronal injury followed by secondary insults (20). During primary injury to the head, it causes rapid deformation of brain tissue with destruction of brain parenchyma and blood vessels causing damage to cell membranes with the immediate release of intracellular contents. The initial injury event cannot be treated, only prevented (21). These effects induce secondary mechanisms which are potentially amenable to post-injury therapeutic intervention because of delayed onset and progression course over hours to days and months after the initial trauma (22).

Secondary brain injury mechanism involves a host of cellular and molecular cascades that promote cell death include: neuronal depolarization, disturbance of ionic homeostasis, glutamate excitotoxicity, generation of nitric oxide and oxygen free radicals, lipid peroxidation (LP), blood-brain barrier disruption, ischemia, cerebral edema, mitochondrial dysfunction, axonal disruption, inflammation, and apoptotic and necrotic cell death (23) (figure 1).

In addition to initiating ischemic and apoptotic cell injury cascades, primary brain injuries make injured, but salvageable, brain tissue vulnerable to secondary brain insults (SBIs). These insults are usually well-tolerated but, when occurring in an injured brain, can lead to further cell death and worsened patient outcome. Hypotension, hypoxia and hypoglycemia are all examples of SBIs in which decreased substrate delivery to an injured brain further worsens injury. On the other hand, fever, seizures and hyperglycemia are examples of SBIs in which increased metabolic demand may outstrip compensatory mechanisms and result in further injury (24).

The brain is particularly vulnerable to oxidative stress because of its high rate of oxygen consumption, intensive production of ROS, low antioxidant capacity, high level of transition metal, and polyunsaturated fatty acids (1, 4, 17, 25, 26). Oxidative stress is currently thought to be a major contributor to the secondary injury cascade following TBI by ROS/RNS-induced damage to cellular membranes and organelles by lipid peroxidation, protein oxidation and nucleotide breakdown (21).

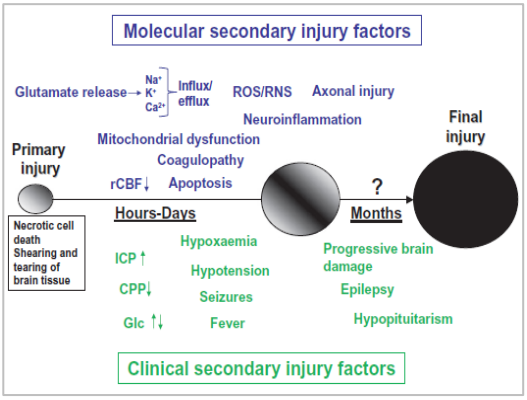

Figure 1 - Basic concept of primary and secondary injury in traumatic brain injury. From: Marklund Niklas/British Journal of Pharmacology. Simplistic illustration of major preclinical 'molecular' secondary injury factors as well as clinical secondary injury factors (named 'avoidable factors' in the neurocritical care setting). Early post-injury, glutamate releaseand ionic disturbances (Na+, Ca2+ and K+) cause an energy metabolic disturbance complicated by an early decrease in cerebral blood flow. At this time, mitochondrial disturbance is marked and a large increase in reactive oxygen/nitrogen species (ROS/RNS) is observed. Hyper - or hypocoagulation may also be present either causing microthrombosis or increased haemorrhages respectively. Neuroinflammation and axonal injury is emerging in the immediate post-injury phase. Clinically, an increased ICP and/or decreased CPP must urgently be treated and both too low and too high blood glucose levels corrected. It is also crucial that hypoxaemia and hypotension, seizures and fever is detected and treated. Chronically, marked hormone disturbance may be observed. These factors have been shown to contribute to the progression of the primary injury (indicated by the enlarging circles) and may be suitable targets for pharmacological intervention to reduce the extent of final injury. Ca2+, calcium ions; CPP, cerebral perfusion pressure; GLc, Glucose; ICP, intracranial pressure; K+, potassium; Na+, sodium; rCBF, regional cerebral blood flow

Reactive oxygen species

Reactive oxygen species (ROS) are a group of chemically reactive molecules derived from oxygen (O2) (27). ROS are formed as necessary intermediates of metal catalyzed oxidation reactions. Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell. This electron structure makes oxygen susceptible to radical formation. The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including superoxide, hydrogen peroxide, hydroxyl radical, hydroxyl ion, and nitric oxide (28).

At physiological low concentration levels, ROS function as redox messengers in intracellular signaling and regulation, whereas excess of ROS induce oxidative modification of cellular macromolecules ie. lipids, proteins, nucleic acids and carbohydrates, inhibit protein function, and promote cell death. The initial reaction generates a second radical, which in turn can react with a second macromolecule to continue the chain reaction. Among the more susceptible targets are polyunsaturated fatty acids. Abstraction of a hydrogen atom from a polyunsaturated fatty acid initiates the process of lipid peroxidation (29).

There are also some mechanisms to detoxify radicals. The key metabolic steps are superoxide dismutasa (SOD) catalysis of the dismutation of superoxide to hydrogen peroxide and oxygen and the conversion of H2O2 to 2H2O by glutathione peroxidase or to O2 + H2O by catalase (30).

Oxidative stress in traumatic brain injury

Various mechanisms are proposed, which have been described to promote free radical production, including glutamate release, intracellular calcium overload, increase in arachidonic acid and its metabolites, hemoglobin denaturation, and ionic iron release (1). Damaging reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed as unavoidable by-products of metabolism but their damaging effects are normally counteracted by endogenous anti-oxidant enzymes (e.g. catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase) and substances e.g. glutathione, metallothionein, vitamin A, vitamin C, and vitamin E) (31). Stressors arising from traumatic brain injury generate an imbalance between free radical production and the cells' antioxidant capacity, resulting in a state of oxidative stress (32).

Accumulation of ROS/RNS can result in a number of detrimental effects such as lipid peroxidation, protein oxidation, and DNA damage. Lipid peroxidation disrupts normal structure and function of lipid bilayers of the cell membrane increasing the permeability. Lipid peroxidation may ultimately result in the production of multiple aldehyde species (e.g. acrolein, malondialdehyde (MDA)) that further contribute to toxicity. Protein modifications by accumulated ROS/RNS include protein fragmentation, protein misfolding, protein–protein cross-linkages, production of protein carbonyls, and priming of oxidized proteins for proteasomal degradation (31). Alteration of different DNA structural changes including direct

modification of nucleotide bases, formation of apurinic/apyrimidinic sites, DNA single strand breaks and double strand breaks. Guanine is the most susceptible to oxidative modifications due to the fact that it has the lowest reduction potential. Hydroxyl radicals have been shown to interact with the C4, C5, and C8 positions in the imidazole ring of guanine forming of 8-hydroxyguanosine (8-OxoG). Peroxynitrite is also capable of reacting with guanine to form 8-nitroguanine. It is known that preferential target of oxidative damage are telomeres. For example, TRF2 may prevent ATM-mediated initiation of oxidative DNA damage signaling, POT1, which maintains stability of telomeric ends, is also known to inhibit another DNA damage kinase ATR. Both ATM and ATR are known to initiate apoptotic neuronal death in the context of DNA damage through activation of p53 (31).

Neuroprotection and emerging roles of Nrf2

Not all mediators induced in the peri-lesional zones necessarily contribute to celllular death. As is the case for damage-inducing pathways, protective pathways also appear to be similar if not identical in ischemic and traumatic injury. These mediators possess damage-reducing properties and represent endogenous efforts to counteract ischemic or traumatic damage and improve neuronal repair. Some of the protective mechanisms include: 1) Heat shock proteins (HSPs). They are induced early after ischemic onset and their induction prior to ischemia may confer ischemic tolerance. 2) Anti-inflammatory cytokines. Certain cytokines have anti-inflammatory capabilities such as IL-10 may be protective against ischemic damage, mainly by inhibiting production of the inflammatory cytokines. 3) Growth factors. Nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), glial derived growth factor (GDNF), basic and acidic fibroblastic growth factors (FGF) and members of the transforming growth factor super family (TGF). These growth factors are thought to confer protection mainly by interfering with apoptotic pathways and preventing apoptotic death. 4) Erythropoietin. This kidney-derived cytokine acts as a growth factor and inhibits apoptotic cell death. 5) Sex hormone. Estrogen was shown to protect the brain from experimental cerebral ischemia, likely through both vascular and neuronal cellular mechanisms. 6) Endogenous antioxidant mechanisms. Antioxidant enzymes and low-molecular-weight antioxidants are all induced in the early hours following ischemia and trauma. Such antioxidants include Mn-SOD (superoxide dismutase), extracelular SOD and Cu-Zn-SOD and also gluthatione (33).

There are few current researches showing excessive generation of oxygen free radicals after is the core pathological link leading to the nerve cell injury and apoptosis. NF-erythroid 2-related factor 2 (Nrf2) is a recently discovered nuclear factor. In physiological state, it is mainly located in the cytoplasm and forms a complex combining with cytoplasmic protein Keapl. When oxidative stress stimulus occurs, it is decoupled with Keapl through phosphorylation, transfers into nucleus, combines with antioxidant response element

(ARE) sequence, forms Nrf2/ARE pathway and then starts the gene expression of phase II detoxifying enzymes and antioxidases (H0-1, NQO1, etc) regulated by ARE. Nrf2/ARE pathway plays an important role in endogenous anti-oxidation process in vivo and can be induced by external factors (14).

Yan Wei at el. demonstrated the activation of Nrf2–ARE pathway occurs after TBI. Moreover, emerging evidence suggests that NRF2, in addition to its antioxidant functions, may also play an important role in regulating inflammation in the brain. These findings connect NRF2 not only to an elevated anti-oxidant capacity but also to expression of other types of protective proteins i.e. brain derived neurotrophic factor, the anti-apoptotic B-cell lymphoma 2, the anti-inflammatory interleukin (IL)-10, the mitochondrial transcription (co)-factors NRF-1 and peroxisome proliferator-activated receptor gamma coactivator1-alpha (PGC-1a), the iron exporter ferroportin 1, and the autophagic protein p62 (36, 34).

Nrf2 activation also protects the blood brain barrier during TBI. TBI causes a biphasic opening of the BBB. The first opening used happens within hours (acute phase) after TBI and the other secondary phase peaks 1–3 days after injury. The latter opening is associated with a loss of endothelial cells and tight junction proteins. Enhanced Nrf2 staining can be detected in the blood brain barrier following TBI (35).

It was demonstrated that the patientsdeficient (Nrf2-/-) mice exhibited poorer outcomes than the wild-type mice, while administration of tBHQ or histone deacetylase inhibitors could protect against TBI by activation of Nrf2. These evidences demonstrate that activation of the Nrf2–ARE pathway is beneficial for TBI. (16) It is now accepted that the Nrf2–ARE pathway can be regulated in many different ways (14, 16, 36).

Antioxidant therapeutic strategies

The gradual processes of secondary injury may provide doctors with so-called "golden hour" wibndowfor pharmacological based intervention, and a lot of agents and compounds targeting secondary brain injury factors are shown to be protective in experimental TBI models3. However, clinical trials for neuroprotective therapies of TBI still continue to have a high failure rate (37). Several researches have described the antioxidant mechanism of some agents, but most of them have unfortunately failed in their efforts to provide neuroprotection in moderately to severely injured TBI patients. Some of these therapies have demonstrated inadequacies include polyethyleneglycol-conjugated-SOD (PEG-SOD) and tirilazad. The non-glucocorticoid LP inhibitor tirilazad, which inhibits LP propagation reactions by membrane stabilization and scavenging LOO• was tested as a TBI therapeutic in the early 1990s. Factors contributing to the failure of the tirilazad study were the apparent inability of the agent to cross the blood–brain barrier in high enough concentrations in severely injured patients as well as gender differences in treatment outcomes and drug metabolism. On the other hand, PEG-SOD was limited in its therapeutic value based on its large size and

rather narrow therapeutic window to scavenge the short-lived primordial O2•– radical (12).

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a derivative of antipyrin and was approved as free radical scavenger. Edaravone was first reported to strongly scavenge hydroxyl radicals (OH–) produced by the Fenton reaction in vitro and to decrease lipid and L-tyrosine oxidation. Edaravone was used in other neural injury models such as spinal cord injury, TBI, and brain hemorrhage38. However, in a study conducted by Pratyush Chaudhuri, no significant difference was observed in the outcome scales between the group administered edaravon and the group not administered edaravon. On the other hand, the mortality rate was also higher in the edaravon group. The possible etiology from this investigation was the drug induced hepatic dysfunction resulting in persistently reduced serum albumin levels and thereby causing a decrease in the circulating volume (39).

Numerous experimental studies in recent years have suggested that erythropoietin (EPO) is an endogenous mediator of neuroprotection in various central nervous system disorders, including TBI (18). Erythropoietin is a glycoprotein and cytokine of 34kDa which is produced mainly by the fetal liver and the adult kidney in response to hypoxia. Erythropoietin (EPO) and the EPO receptor (EPOR), are also expressed in the brain. While EPO and EPOR are only weakly expressed in normal adult brain, expression of EPO and EPOR is greatly increased in response to different types of brain injury. Inhibition of EPO activity by the administration of soluble EPORs worsens the severity of neuronal injury, suggesting that endogenous EPO is directly involved in an intrinsic neuronal repair pathway (40).

Because of the potential thromboembolic complications caused by EPO, it may be difficult to achieve neuroprotection with EPO in TBI patients without further increasing potentially life-threatening complications. However, development of derivatives of EPO that do not bind to the classical EPOR (carbamylatederythropoietin or CEPO) or that have such a short half-life in the circulation that erythropoiesis is not significantly stimulated (asialoerythropoietin or neuroerythropoietin) have clearly demonstrated that the neuroprotective effects of EPO can be separated from the hematopoietic effects18. Specifically, the receptor complex mediating the neuroprotective effects of EPO has been reported to be associated with the common receptor (cR) subunit, also known as CD131, which is the signal-transducing component used by the granulocyte macrophage colony stimulating factor (GM-CSF), IL-3, and IL-5receptors (41).

Current promising antioxidant therapies

Currently, there is no neuroprotective agent, which demonstrated improved neurological outcomes in a large phase III clinical trial. Efforts should focus on developing novel strategies, with thorough preclinical studies and clinical trials that consider the translational barriers of a heterogeneous pathology such as TBI. The

potential use of unconventional treatments, such as antioxidant defense system reinforcement, could play a key role in the management. This reinforcement appears as a safe, low-cost and multifunctional novel therapeutic approach in TBI patients. Antioxidant therapies are effective over a long period of time, allowing for suitable use in clinical settings. Due to the encouraging preclinical results and the antioxidant drug profiles, acute antioxidant reinforcement is emerging as a highly cost-effective alternative for neuroprotection in TBI patients (42).

U-83836E

The 21-aminosteroids (lazaroids) are a new family of steroid compounds, which inhibit lipid peroxidation reactions. They are novel antioxidant agents, which have been shown to prevent free radical-mediated blood-brain barrier damage (43).

U-83836E is a second-generation lazaroid with a non-steroidal structure characterized by a ring portion of alpha-tocopherol bonded to various amine groups. Its structure enables the dual functionality of LP inhibition and scavenging LOO•, thereby making it much more effective than the endogenous scavenger vitamin E44. U-83836E treatment can attenuate post-traumatic LP in cerebral cortical tissue or mithochondria together with a preservation of aerobic respiratory function and Ca++-buffering capacity (45). More recently U-83836E has also been shown to inhibit calpain-mediated cytoskeletal degradation signifying the intricate relationship between post-traumatic LP, disruptions in neuronal Ca2+ homeostasis and calpain-mediated cytoskeletal damage (12). If U-83836E given prophylactically, enhances Na+/K+ and Mg2+/Ca2+-ATPase activities and attenuates edema in cerebral trauma in rats (46).

U-83836E also has been shown to inhibit protein nitration (3-NT) in injured cortical tissue and mitochondria even though it does not interact directly with the reactive nitrogen species peroxynitrite. Further studies to determine the pharmacokinetics of U-83836E in humans are strongly recommended, as the compound appears to inhibit a crucial step of the secondary brain injury cascade of events (42).

Resveratrol

Resveratrol (3, 4', 5-trihydroxystilbene) is a phytoalexin polyphenolic structurally related to stilbenes. It is found in two isomers, cis- (z) and trans- (E) resveratrol47. Resveratrol is present in relatively large amounts in grapes and red wine and, to a much lesser extent, in white wines (47–50). Many studies have demonstrated resveratrol has a wide range of pharmacological properties, including antioxidant, cardioprotective and anticancer effects. In addition to these beneficial actions, resveratrol also noted for its anti-inflammatory, immunomodulatory, chemopreventive and neuroprotective activities. In this respect it has been shown that resveratrol readily crosses the intact blood–brain barrier (48). Recently, resveratrol was found to be a potent neuroprotective agent against excitotoxicity, ischemia, and hypoxia, in both in vitro and in vivo models (51).

Resveratrol has demonstrated efficacious in reducing neuropathological and behavioral sequelae. Some studies have also found that

traumatic brain injury (TBI) in both adult and immature animals is amenable to treatment with resveratrol (52, 55). In rat traumatic brain injury, a single high dose of resveratrol (100 mg/kg) administerd immediately after trauma reduced brain edema and oxidative stress, and attenuated brain pathology 14 days later (49).

Many different pathways determining the possible actions of resveratrol have been proposed including anti-inflammatory properties, inhibition of NO toxicity, induction of neuroprotective enzymes such as heme oxygenase-1 (HO1), free radical scavenging, NF-kB mediated suppression of proinflammatory genes, upregulation of eNOS and VEGF, inhibition of MMP-9, and by mimicking ischemic preconditioning via the sirtuin pathway (53). A relationship exists between HO1 and NOS. The nuclear factor-erythroid-2-related factor 2 (Nrf2) regulates HO1 transcription, which acts as a scavenger of NO and an inhibitor of iNOS. It has been reported that resveratrol modulates HO1 and iNOS expression in glial cells, so HO1 can also be critical to signaling the antioxidant response of resveratrol (54).

Allicin

Allicin (which formed by the interaction of the enzyme alliinase with its substrate alliin) is responsible for most of active ingradient of garlic. Allicin treatment has showed decrease the expression of TNF-α. Some studies have reported a significant reduction of brain water content when allicin administrated 2 or 4 h after injury, but not when the administration is delayed by 8 h. Treatment with allicin can reduce brain edema, attenuate neurological deficit and inhibit TBI-induced apoptotic neuronal death (3).

Edaravone

Edaravone, a free radical scavenger, suppresses axonal injury and oxidative stress in the cortex, corpus callosum, and hippocampus 24h after injury. The neuroprotective effects of edaravone were observed in mice receiving 1.0, 3.0, or 10 mg/kg immediately after impact. With treatment 1 h after impact, axonal injury was also significantly suppressed and this therapeutic effect persisted up to 6 h after impact. Edaravone therapy protects against memory deficits following TBI mediated by suppression of traumatic axonal injury and oxidative stress (13).

Tempol

Tempol (4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-N-oxyl) is a water-soluble stable and paramagnetic nitroxyl radical or nitroxide. Tempol is a SOD mimetic, and hence an efficient scavenger of free radicals. Endogenous SOD is a much more efficient antioxidant than vitamins, since the rate constant for interaction of SOD with O2– is about 1.6–2.4x109mol L−1 s−1, whereas that of vitamin E with O2– approximates 0.59 mol L−1 s−1. Tempol is therefore not as powerful as endogenous SOD, but it is much more powerful than vitamins (55).

The neuroprotective effects of tempol is reported in both models of TBI and spinal cord injury. In the mouse Controlled cortical impact (CCI) TBI model, tempol reduced post-traumatic LP and protein nitration-induced oxidative damage, which resulted in

preserved mitochondrial bioenergetics, reduced calpain-mediated cytoskeletal damage and reduced neurodegeneration (12). Tempol showed cerebroprotective effects in terms of limiting edema formation, ameliorating blood–brain barrier disruption, and improving functional recovery. It has been concluded that the beneficial effects of tempol were because of its catalytic scavenging of superoxide radicals. More recent experiments have suggested that an important antioxidant property that may contribute significantly to the inhibition of posttraumatic oxidative damage in brain tissue involving catalytic scavenging of the PN-derived radicals ●NO2 and ●CO3 (56).

Tempol can shuttle between the nitroxide radical, the reduced hydroxylamine and the oxidized oxoammonium cation form with 1 and 2 electron transfer reactions. These are facilitated by "boat and chair" conformational changes that underlie the rapidity and catalytic nature of the reactions. The reaction of tempol with superoxide anion (O2•−) to form hydrogen peroxide (H2O2) accounts for its "superoxide dismutase (SOD) mimetic" action. Tempol is effective as a catalase-like agent in preventing the generation of•OH from H2O2 in the presence of transition metals in the Fenton reaction and it has high capacity to permeate cell membranes, the gastrointestinal tract (GIT) or the blood-brain barrier which accounts for its effectiveness after oral administration and central nervous system actions57. Previous studies have shown that tempol can decrease brain 3-NT after controlled cortical impact-induced TBI (CCI-TBI), but only if administered during the first hour. Thus, attempting to block PN formation by either inhibiting •NO synthesis or scavenging PN radicals does not appear to be a practical therapeutic approach to reducing early PN-mediated oxidative damage from a therapeutic window point of view58. However, effects of tempol remains promising and requires further investigation. In addition, tempol may be an ideal candidate for combination therapy with other neuroprotective approaches (12).

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine), a hormone secreted from pineal gland and synthesized from tryptophan or formed as the metabolic end product of serotonin, is a non-enzymatic antioxidant and neuroprotective agent. Melatonin has been shown to exert neuroprotection in several central nervous system disease models, including brain and spinal cord trauma, cerebral ischemia, subarachnoid hemorrhage, and intracerebral hemorrhage (59). Melatonin is a major molecule in protecting membrane constituents from oxidative agents. This molecule also optimizes the physiology of membrane receptors and channels as well as maintaining the shape of the cell. The effects of melatonin as a potent protective substance in post-TBI injuries have been shown in both in vivo and in vitro studies. It has been recognized that melatonin administration in both low and high doses can significantly decrease brain edema and blood-brain barrier permeability at 72 h after TBI. Moreover, this inhibitory effect is associated with improvement of neurologic scores (60).

Melatonin is also well tolerated even at supra-physiological doses (61).

Melatonin is a significant free radical scavenger and antioxidant at both physiological and pharmacological concentrations in vivo. Like other secondary metabolites, melatonin exerts antioxidant properties as a direct free radical scavenger and by stimulating antioxidant enzymes. Indeed, melatonin is able to scavenge H2O2 in a dose-dependent manner. Moreover, the biological activities of melatonin metabolites N(1)-acetyl-N(2)-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) have been described. AFMK is a potent antioxidant providing protection to DNA and lipids viaseveral mechanisms. AMK is also a potent antioxidant and inhibits prostagladin biosynthesis. Melatonin can scavenge other reactive oxygen species such as ONOO, NO and H2O2. Additionally, due to its amphiphilic structure, melatonin has no barriers of distribution and may have the advantages of having a lower side effect profile and producing fewer pharmacokinetic or phamacodynamic interactions compared to xenobiotic antioxidants (62–64).

Mammalian target of rapamycin (mTOR) pathway is an essential cellular signaling pathway involved in a number of important physiological functions like cell growth, proliferation, metabolism, protein synthesis and autophagy. Activation of microglia following TBI might results-frodue to m cell proliferation or size enlargement induced by phosphorylation and activation of mTOR pathway. In previous studies in rodents, p-mTOR, p-p70S6K and p-S6RP were markedly increased in ipsilateral cortex 24 h post-TBI in mice, suggesting activation of mTOR pathway following TBI. It has also showed that injection of melatonin repressed phosphorylation of mTOR and its downstream substrates. Furthermore, melatonin restrained the activation of microglia and decreased protein expression of IL-1b and TNF-α, thereby increasing the number of neurons in peri-contusive cortex at 24 h after TBI (65).

Melatonin has been shown to promote Nrf2 protein translocation from the cytoplasm to the nucleus and to prevent antioxidant enzyme activities decline, including superoxide dismutase and glutathione peroxidase66.

Some studies have proved combination therapy with melatonin and other therapies. The therapy with melatonin (10mg/kg) and dexamethasone (0.025mg/kg) is signigficantly able to reduce edema and brain infartions (67).

Sulforaphane

Recently, the Nrf2 signaling pathway, which is been considered an endogenous antioxidant mechanism in the cellular defense against oxidative or electrophilic stress, has generated increasing interest. The ability to induce phase 2 detoxifying genes and antioxidant enzymes at the transcriptional level could have significant advantages over more conventional approaches. Previous studies have shown that the Nrf2-ARE pathway was activated in the brain and played an important role in limiting inflammation and apoptosis after TBI (68).

Sulforaphane, a naturally occurring compound generated from cruciferous vegetables such as broccoli, is a potent inducer of antioxidant and detoxifying enzymes. As such, this compound has been suggested to provide broad protection against a variety of cellular threatseg. example, application of sulforaphane to neuron-astrocyte co-cultures protects neurons against nonexcitotoxic glutamate and hydrogen peroxide toxicity. These beneficial effects of sulforaphane have been shown to involve induction of Nrf2-driven genes (69–71).

Activation of the transcription factor NF-E2-related factor-2 (Nrf2) by sulforaphane, increase the expression of endogenous cytoprotective genes in brain tissue and microvessels. Post-injury administration of sulforaphane reduce the loss of endothelial cell markers and tight junction proteins and preserve blood–brain barrier function. These protective effects are dependent on the activity of Nrf2 (72).

In vitro studies, sulforaphane is demonstrated to disrupt the Nrf2/Keap1 interaction, leading to Nrf2 stabilization and nuclear localization and the expression of ARE-containing phase II genes, which play a major role in the detoxification of ROS produced by xenobiotics (42, 73).

Some of the effects related to the use of sulforaphane are only observed when the treatment is initiated within the first hr, but not 6 hr, post-injury (69). Several studies support the use of sulforaphane in the treatment of TBI, and the fact that Nrf2 activation may be a prime candidate for the attenuation of oxidative stress and subsequent neurotoxicity. Nevertheless, considering the narrow time window of sulforaphane use observed in preclinical studies, the success of sulforaphane in clinical settings is uncertain42.

Vitamin C and vitamin E

Vitamin C (ascorbic acid, ascorbate) is a potent watersoluble antioxidant in humans. It also behaves as an enzyme modulator, causing the up-regulation of endothelial NOS (eNOS) and down-regulation of NADPH oxidase. Antioxidant and pharmacodynamic properties allow ascorbate to protect neurons from NMDA-induced excitotoxicity and to prevent lipid peroxidation induced by various oxidizing agents, especially in combination with a-tocopherol, in cell cultures. Interestingly, there is one clinical trial of vitamin C involving in patients with severe TBI and the radiologic diagnosis of diffuse axonal injury. Patients received a total dose of 32 g of intravenous vitamin C during the first 7 days after TBI, with a maximum single dose of 10 g on the first day, resulting in a significant earlier stabilization of the perilesional edema compared with the placebo group. Although the absence of many parameters of Clinical importance and monitoring techniques may make the interpretation of these results challenging, although encouraging results regarding the use of vitamin C in humans (42).

On the other hand, Vitamin E, mainly alpha-tocopherol, is the major peroxyl radical scavenger in biological lipid phases such as cell membranes, and its antioxidant mechanism is related to the inhibition of lipid peroxidation and NADPH oxidase. One clinical trial of vitamin E has been performed in patients with

severe TBI and the radiologic diagnosis of diffuse axonal injury. For 7 days, patients received vitamin E at 400 IU/day intramuscularly, which resulted in improved clinical outcome and reduced mortality at discharge. Despite promising results, clinical trials using vitamins C and E, isolated or in combination, are needed to change current therapeutic measures in patients suffering TBI (42).

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