REVIEW ARTICLE

Oxidative Stress Following Traumatic Brain Injury: Enhancement of Endogenous Antioxidant Defence Systems and the Promise of Improved Outcome

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Abstract

Background: *Management of brain injury can pose enormous challenges to the health team. There are many studies aimed at discovering or developing pharmacotherapeutic agents targeted at improving outcome of head-injured patients. This paper reviews the role of oxidative stress in neuronal loss following traumatic brain injury and presents experimental and clinical evidence of the role of exogenous antioxidants as neuroprotectants.*

Method: *We reviewed published literature on reactive oxygen species and their role in experimental and clinical brain injuries in journals and the Internet using Yahoo and Google search engines.*

Results: *Traumatic brain injury causes massive production of reactive oxygen species with resultant oxidative stress. In experimental brain injury, exogenous antioxidants are useful in limiting oxidative damage. Results with clinical brain injury are however more varied.*

Conclusion: *Oxidative stress due to excessive generation of reactive oxygen species with consequent impairment of endogenous antioxidant defence mechanisms plays a significant role in the secondary events leading to neuronal death. Enhancement of the defence mechanisms through the use of exogenous antioxidants may be neuroprotective, especially if the agents can penetrate cell membranes, are able to cross the blood-brain barrier and if they are administered within the neuroprotective time window.*

Keywords: *Antioxidants; Oxidative stress; Outcome; Pathophysiology; Reactive oxygen species; Traumatic brain injury*

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Introduction

Traumatic Brain Injury (TBI) is a worldwide problem[1], and its management can pose enormous challenges to the medical team as well as significantly stretch hospital resources (including manpower, facilities, etc). It is predominantly a "disease" of young persons and has assumed such immense proportions that the World Health Organisation (WHO) has placed it as a 21st century epidemic equivalent only to malaria and HIV/AIDS[2]. The last few decades have witnessed tremendous advances in virtually every aspect of TBI, but perhaps the area where this has been most remarkable is in the understanding of the pathophysiological processes attending the injury. Though many questions remain unresolved, research studies have shed much light on the accompanying events. It is now believed that even though the initial injury, which may be focal or diffuse, may produce neuronal damage or death, it also sets off a cascade of other events including the effects of hypoxia, release of endogenous excitatory amino acids (such as glutamate), production of pro-inflammatory substances as well as the generation of free radicals[1,3].

Experimental studies have shown that one of the key participants in the events leading to secondary brain damage is oxidative stress (OS), which is believed to be the common pathway for diverse brain disorders[4]; and it is thought that defining its role in central nervous system injury could help with the development of novel, clinically applicable therapies[5]. Thus, several of the ongoing research efforts aimed at discovering measures targeted at improving the outcome of patients following TBI are the direct consequences of the recognition of the possible role of oxidative stress in its pathophysiology.

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This brief review deals with the sources and nature of reactive oxygen species, their role in traumatic brain injury and the endogenous antioxidant defence mechanisms. It also summarises new pharmacological strategies some of which are at various stages of clinical trial, and presents experimental and clinical evidence of the role of exogenous antioxidants as neuroprotectants in traumatic brain injury. The need for more clinical trials is highlighted.

Free Radicals and Oxidative Stress

Free radicals are highly reactive compounds which are mostly generated during cellular respiration and normal metabolism[6]. Their possession of unpaired electrons in their outer shell causes them to be more reactive than their corresponding non-radicals. This is because they act as electron acceptors, and essentially "steal" electrons from other molecules and thereby modify their chemical structures – an action which is referred to as oxidation[7].

Free radicals are liberated from a variety of sources, including inflammatory cells, dysfunctional mitochondria and excitotoxic mechanisms which are stimulated by increased glutamate and aspartate concentrations[8]. Superoxide radical is the most commonly occurring cellular free radical and it is produced when an oxygen molecule gains one electron from another compound[6].

There is a vast amount of evidence implicating oxygen-derived free radicals as well as other high-energy oxidants as playing mediatory roles in diverse pathological conditions[6,9-14]. They have also been shown to be key contributors in the secondary events following brain injury[7,15-17]; and the formation of the oxygen radical, superoxide anion, is believed to be one of the final events of several metabolic pathways in the cascade which leads to delayed neuronal death after traumatic or ischaemic brain injury[18]. Cellular injury caused by reactive oxygen species (including free radicals, peroxides and oxygen ions), is believed to occur as a result of oxidation of polydesaturated fatty acids in lipids, oxidation of amino acids in proteins, damage of the DNA and inactivation of specific enzymes by oxidation of co-factors[19].

Oxygen free radicals are produced continuously in tissues, and even though they are considered to be destructive agents, they can sometimes be useful. It is believed that they act as secondary messengers in intracellular signalling cascades which are responsible for the induction and maintenance of the oncogenic phenotype of cancer cells. They may also function as anti-tumourigenic species[20].

Normally, a state of equilibrium exists between tissue oxidant and antioxidant activities[21]. This balance can, however, be upset as a result of excessive free radical generation, depletion of endogenous antioxidants or failure to repair oxidative injury induced by reactive oxygen species. The resultant condition is referred to as oxidative stress (OS)[11,22,23]. Enhanced oxidative stress associated with increased production of free radicals has been implicated in the disruption of neuronal homeostasis induced by traumatic brain injury[24-29].

Traumatic brain injury (TBI) leads to massive production of reactive oxygen species (ROS), which in turn mediate further damage via various cellular molecular pathways, including damage to cellular components like lipids, proteins and nucleic acids6,[30-33] with neuronal death occurring as a result of impairment of cellular calcium homeostasis, tissue acidosis, and oxidative stress[34]. OS is also thought to, at least in part, initiate or influence autophagy – a process whereby cellular components are digested by enzymes within the same cell[35]. Experimental data indicate that the destructive oxidative events following TBI reach their peak within the first 24 hours of injury, and that the brain damage that occurs as a result of the impact can be the cause of death or irreversible permanent disabilities in affected patients[36]. The degree of oxidative injury in clinical TBI is, however, not yet completely understood[5].

Endogenous Antioxidant Defence Mechanisms

Antioxidants are compound whose actions include: inhibition of the formation of reactive oxygen species and binding of metal ions needed

for catalysis of their generation, and scavenging of reactive oxygen species or their precursors. They inhibit other oxidation reactions by being oxidized themselves. Endogenous antioxidant scavenger mechanisms help to defend cells against oxidative injury[37-39]. Two major groups of these compounds are recognised: antioxidant enzymes (such as superoxide dismutase, catalase and glutathione peroxidise), and low molecular weight antioxidants (LMWA) The latter group contains directly acting agents (such as ascorbic acid, lipoic acid, uric acid, α-Tocopherol, ubiquinol and carotenoids) and indirectly acting antioxidants (e.g. chelating agents)[5,6].

The high rate of oxidative metabolism in the normal brain and its elevated levels of polyunsaturated lipids (which are the target of lipid peroxidation) render it particularly vulnerable to oxidative stress[19,40,41]. Oxidative damage is, however, prevented by the actions of endogenous antioxidants. Free radicals, especially superoxide and some non-radicals, (e.g. hydrogen peroxide), can be generated in such large quantities that the endogenous protective enzyme systems are overwhelmed[42]. When there is brain trauma, the regulatory mechanisms are also progressively compromised and they may either be eliminated altogether or even be converted to become pro-oxidant[5,6,33,43]. Environmental as well as genetic factors are thought to play some role in the reduction in the endogenous antioxidant defence system and thereby contribute to the evolution of oxidative stress[6]. Experimental studies have shown that enhancing this endogenous antioxidant defence mechanism may be neuroprotective during injury.

Exogenous Antioxidants

There are several research studies on the use of exogenous antioxidants in TBI. Most of these are hinged on the belief that the free radicals (which are by-products of increased neurotransmitter activity), are the agents actually responsible for neuronal death[40]. A number of therapeutic trials based the ability of antioxidants to scavenge free radicals have been attempted in both experimental[44,45] and clinical TBI[46].

It is thought that antioxidants that would effectively cross the blood-brain barrier and penetrate into the damaged tissue may help to maintain the redox status of the neurons, decrease ROS-associated neuronal damage and thus reduce neurological impairment and disability[47].

Furthermore, to be effective, such antioxidant should be given within the neuroprotective "time window", which is the time interval between the trauma event and irreversible neuronal loss. When given during this period, they may be expected to significantly reduce or prevent cerebral damage[6,48]. However, majority of the currently available antioxidants are not able to cross the blood-brain barrier from the systemic circulation, thereby limiting their efficacy as neuroprotectants.

Animal studies have shown that antioxidant administration interrupts the sequence of brain injury responses[15,18,49]. Results with clinical TBI have, however, been more varied[6]. In some studies involving TBI patients, the exogenous antioxidants were reported to have no beneficial effects. Young et al carried out a randomized, parallel, placebo-controlled, third-party-blind, multicenter trial of pegorgotein (a scavenger of oxygen-derived free radicals), with the aim of evaluating its effects on neurologic outcome of patients with severe closed head injury. They reported that even though pegorgotein was well tolerated at 3 months after injury, there was no significant statistical difference in neurologic outcome between the pegorgotein and the placebo-treated groups. However, there was no increase in the mortality or disability states among the patients given pegorgotein[50].

Other studies have shown that the neutralisation of ROS by endogenous or exogenous antioxidants has a protective effect on brain tissue. The study by Shohami et al demonstrates that the brain responds to ROS by increasing low molecular weight antioxidants and that among head-injured patients, the degree of the response correlates well with clinical recovery: the greater the response, the more favourable the outcome[15].

Similarly, in a randomised controlled Phase II trial to evaluate the safety and efficacy of polyethylene glycol-conjugated superoxide dismutase (PEG-SOD) in severely head-injured patients, Muizelaar et al reported significantly improved outcome in the groups of patients treated with PEG-SOD as compared with their placebo-treated groups[18]. It thus appears from these reports that there might some promise in the use of exogenous antioxidants in situations where the natural defence mechanisms are compromised.

Uric acid (UA), which is one of the most abundant aqueous antioxidants in humans is thought to account for as much as two-thirds of all free radical scavenging capacity in plasma (being found to be particularly effective in quenching hydroxyl, superoxide and peroxynitrite radicals)[51]. Experimental data have shown that local uric acid concentrations are significantly increased in acute traumatic brain injury[52]; and that early elevation of UA, during or shortly after TBI and other cerebral disorders mediated by free radicals, could confer significant protection against the occurrence of neurological deficits[53].

Ascorbic acid is known to be a very effective free radical scavenger. It is not synthesised in humans and as such, it must be obtained from dietary sources. Even though its exact functions are not known, it is believed that ascorbic acid can neutralise reactive oxygen species such as hydrogen peroxide which are produced during TBI[54]. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidise.

Melatonin is also a very potent antioxidant and direct free radical scavenger that is particularly efficacious because of its ability to penetrate cell membranes as well as cross the blood-brain barrier. It protects against oxidative damage at the level of cells, tissues, organs and organisms. Its antioxidative mechanisms seem different from those of the classical antioxidants in that whereas the latter undergo redox cycling and thus have the potential to promote oxidation as well as prevent it, melatonin does not and as such, it does not promote oxidation.

Melatonin has been referred to as a terminal (or suicidal) antioxidant because once oxidized, it cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals[43,55-57].

Another group of antioxidants that has received tremendous attention during the last few years are the cannabinoids. These include compounds which have similar structure with the psychoactive components in cannabis, as well as agents which activate the cannabinoid receptor system in the body. Researchers have shown that the cannabinoids are effective neuroprotectants in experimental animals. Some of them, such as the synthetic cannabinoid, dexanabinol (HU-211), are at various stages of clinical trials. The results have so far been promising. In a prospective, randomised, double-blind, placebo-controlled, multicenter study including six Israeli neurosurgical intensive care units, dexanabinol was shown to be safe and well tolerated in severely headed-injured patients. The treated patients achieved significantly better intracranial pressure/cerebral perfusion pressure control. The authors also observed a trend towards faster and better neurologic outcome. While confirming the strong cerebroprotective effect of dexanabinol, the study by Shohami et al also showed a therapeutic window of about 4 hours[3,58].

Dexanabinol is a promising drug which does not produce psychotrophic or other adverse side effects associated with some of the earlier drugs of its type[59]. Though the neuroprotective effect of the cannabinoids is thought to be due to their strong antioxidant property, some researchers consider that this attribute alone is unlikely to account for all of the protection. It is thought that an additional mechanism whereby they provide their protective effect is by the inhibition of the glutamate system in the brain thereby dampening neural activity with consequent reduction of excitotoxicity.

Conclusion

Traumatic brain injury remains a significant problem worldwide. Oxidative stress plays a significant role in neuronal death following injury due to the progressive compromise of endogenous antioxidant defence systems. The neutralisation of reactive oxygen species by endogenous or exogenous antioxidants has a protective effect on the brain.

Consequently, the discovery and development of antioxidant agents is one of the most promising approaches in the search for more effective treatment of TBI. Several potentially useful agents have been identified, and even though the effect of many of them in clinical TBI is still controversial, some have shown some promise. More clinical trials are however needed to resolve some of the ongoing controversy.

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