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Review Article

Combined Therapy Potential of Apocynin and Tert-butylhydroquinone as a Therapeutic Agent to Prevent Secondary Progression to Traumatic Brain Injury

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Abstract

Traumatic brain injury is caused by physical collision (primary injury). It changes the brain's biochemistry and disturbs the normal brain function such as memory loss and consciousness disturbance (secondary injury). The severity can be measured with the Glasgow Coma Scale. The secondary injury will cause oxidative stress that leads to the nervous cells death, so treatment is needed before it gets worse. Primary injury results in excess of reactive oxidative stress (ROS) which is known from NADPH oxidase 2 (Nox2). Excessive ROS is deadly to the nerve cells. Excessive ROS will activate nuclear factor erythroid 2-like 2 (Nrf2). Nrf2 will bind to antioxidant response elements, to protect multi organs against ROS, including this brain injury. However, this does not last long, so it requires handling excess ROS. *Apocynin* can inhibit the activation of Nox2, and reduce the neuron injuries in the hippocampus. It also protects the tissues from oxidative stress. While Nrf2 can be activated by *tert-butylhydroquinone*, to protect cells. The combination may reduce the secondary brain injury, improve the neurologic recovery, cognitive function, and reduce the secondary cortical lesion.

Keywords: *Apocynin, NADPH oxidase 2, nuclear factor erythroid 2-like 2, tert-butylhydroquinone, traumatic brain injury*

Background

Traumatic brain injury (TBI) is a type of head injury that leads to biochemical changes in the brain, such as neuron cell death, neurological dysfunction, and inflammation in the nervous system. TBI has various etiology, severity, sign, and symptoms.[1,2] TBI happened due to physical contact or vigorous movement toward the head that causes a disturbance in brain normal physiological function, but not all physical contact may lead to TBI. TBI severity could be measured from mild, moderate-to-severe with different signs and symptoms. Signs and symptoms that are caused by TBI could vary from decreased consciousness, headache, head swelling or hemorrhage, neck stiffness, coma, and death.[3,4]

TBI is classified as primary injury and secondary injury. Primary injury is a type of injury that causes direct mechanical damage. Recent therapy targets the prevention of secondary damage. Secondary injury is an injury that shown up by changes in biomolecular and physiological changes that could lead to further damage. Secondary injury determines further patients' prognosis during the recovery period.[5] A need for preventive actions to prevent the worsened condition from secondary trauma and lead to worsened patients' prognosis.[6] A secondary TBI could occur from minutes to days after the incident of primary TBI, where biomolecular changes occur. This period would be the target of medical intervention to prevent progressive damage and increase functional recovery after TBI.[7] If during this period, the proper treatment cannot be delivered, there are probability where neuron depolarization activity will increase, leading to excess reactive oxidative species (ROS) and worsen the prognosis and condition.[8]

TBI is one of the major health problems that still cause a high rate of permanent disability, even death globally. TBI incident occurs from 150 to 250 cases from 100,000

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people globally annually. From those numbers, 10% of the cases are severe cases, 10% are moderate cases, and 80% are mild cases. In the United States, TBI occurred in 1.7 million people annually. Therefore, this marks TBI as the third deadliest cause of death within death by injury. Age groups that are susceptible to TBI are 0–4 years old, 15–19 years old, and above 65 years old.[9] According to a meta-analysis by Peeters *et al*.,[10] the incidence rate of TBI in Europe is 262 by 100,000 populations annually, with an average death rate 10.5 by 100,000 populations.

Several disturbances that often occur in TBI are a cognitive deficiency and motoric dysfunction where patients might lose their memory and having the inability to receive new memory process. Due to this day, there is no effective therapy in increasing functional recovery except conservative therapy (e.g., surgery) and routine medical follow-up, which could cause a high expenditure. Therefore, development in the therapeutic agent will bring benefit clinically and economically.[11]

Apocynin is a substance that is isolated from medical herb Picrorhiza kurroa that has a neuroprotective effect that protects neuron cell from damage caused by over ROS concentration.[12] *Tert-butylhydroquinone* (tBHQ) is a synthetic phenolic antioxidant with hydrophobic property and mainly used as a food preservative, has a strong antioxidant effect and protects the cell from damage caused by acute toxicity and oxidative damage.[13,14] Chandran *et al*.[15] reported that a combination therapy from *apocynin* and tBHQ had been proved giving neurological benefit significantly compared to monotherapy each of the substances.

This paper aimed to contribute about the usage of combination therapy of *apocynin* and tBHQ that has a significant effect and could minimalize damage effect from ROS from injury, especially in secondary injury by TBI in the future.

Traumatic Brain Injury

TBI is identified with injury on the head that causes by direct physical contact.[4] TBI could cause unconsciousness, amnesia, neurologic changes, skull fracture, intracranial trauma lesion, or even death. TBI severity usually measured using the Glasgow Coma Scale (GCS).[4,16] In general, the GCS level could determine the severity of TBI, which are mild with score 13–15, moderate with score 9–12, and severe with score 3–8.[17] Another examination that could be conducted and examined are from patients' unconsciousness, amnesic period, intracranial pathology imaging with contrast radiology examination, and pupil reaction.[16]

The incident of TBI in children under 5 years old usually occurred by falling and traffic accidents. In 5–14 years old children, the injury usually happens due to sports or recreational activities. Teenager above 15 years old incident usually happens caused by traffic accident and physical fight. Another incident of TBI usually happened caused by abuse.[16] Older people, female, low economic status, and has a history of mental disorder could worsen patients' prognosis and lead to chronic effect after TBI.[18]

Pathophysiology

TBI could be classified into primary injury and secondary injury. Primary injury is caused by direct physical contact that could affect macroscopic brain lesions such as swelling, hematoma, bruising, and diffuse axonal injury (DAI). DAI is an axonal injury in brain white matter that progressively widespread and could lead to total damage that causes cognitive, behavioral, and motoric disturbance for a long period.[15,19]

Secondary injury is a biochemical cascade that involves oxidative stress, glutamate excitotoxicity, and nervous system inflammation that could lead to neuronal death. Oxidative stress, the imbalance between reactive oxygen species (ROS) level and antioxidant, is the main contributor to secondary injury pathophysiology that could be known from NADPH oxidase 2 (Nox2).[19] The secondary injury could cause hypotension, hypoxia, ischemia, and inflammation. This is because the primary injury disturbs the integrity of the plasma membrane, followed by disturbance of ion balance in the plasma membrane, which in turn activates depolarization and trigger over the release of neurotransmitter. This effect could lead to an accumulation of calcium ion and cause mitochondria dysfunction.

Calcium ion accumulation also increases ROS production by activating CaMKII that would activate Nox2 as the main contributor to ROS.[15,19] Nox2 contains b558 cytochrome that connected with Nox2 membrane. This cytochrome has several cytosolic subunits (p22phox, gp91phox, p47phox, p40phox, p67phox, and GTPase). These cytosolic subunits will translocate to the membrane and connected with b558 cytochrome, where during this translocation process, ROS will be produced.[20]

ROS overproduction would lead to structural dysfunction, including in mitochondria and protein that could affect membrane pores. This damage is known as lipoperoxidation. This condition also would cause DNA mutation due to fragmentation and would increase neutrophil infiltration. In general, exceeding ROS would cause neuron cell death.[18] Several studies have shown that Kelch-like erythroid-derived CNC-homology factor-associated protein 1 (Keap 1) would activate nuclear factor erythroid 2-like 2 (Nrf2) to mobile from cytoplasm to nucleus due to exceeding ROS.[17]

Keap 1 is a type of inhibitory protein that functions as substrate connector that involved in Nrf2 compartment and degradation.[21] Nrf2 will translocate to the nucleus, and conduct gene transcription by heterodimerization

with several transcription regulatory proteins that bind antioxidant response element (ARE) and function as a protective gene. Heme oxygenase 1 and NADPH are included in ARE that induced phase II detoxification enzyme production and an antioxidant gene that would function in cell protection from further damage caused by ROS. The Nrf2-are pathway could protect several tissues such as lungs, liver, kidneys, central nervous system, and digestive tissues from oxidative stress.[14,21-23] Even so, direct mechanism of Nrf2 toward oxidative stress damage caused by TBI remain unclear [Figure 1].[24]

The Current Traumatic Brain Injury Therapy Method

The current method in treating TBI according to several guidelines are management and monitoring of blood pressure, intracranial pressure, brain perfusion pressure, and advance monitoring and therapy such as decompression craniectomy, cerebrospinal fluid drainage, and hyperosmolar therapy. These managements expected outcome is to decrease negative result probability and an increase in positive result probability, that lead to changes in the recovery period.[25]

Up to this day, there is a lot of clinical trials that aim to upgrade the quality of TBI patients therapy, but there is no trial that has given the impact to increase functional recovery for general TBI populations. Therefore, there is a need to improve therapy agents and to plan new approach plan that could improve motoric, sensory, and cognitive aspects that would increase TBI patients' quality of life.[8]

Apocynin and *Tert-butylhydroquinone* as a Treatment in Traumatic Brain Injury

Apocynin functions to inhibit Nox2 in systemic neutrophil, macrophages, and endothelium through translocation p47phox subunit inhibition. From several studies, it is found that *apocynin* has a neuroprotective effect by reducing Nox2 activation, protecting against neuronal injury in hippocampus, improving spatial cognitive function, and protecting tissues against oxidative stress.[26-28] Nox2 is proved to be expressed in the brain cortex and hippocampus after TBI that lead to nerve cell death and functional disorders. Research by Feng *et al*.,[28] has shown that morphological changes were characterized by increased neurons in cortex and hippocampus in the rat after TBI. This study also shown that Nox2 is expressed in hippocampus 24 h after TBI, where the expression will increase from 12 to 48 h after TBI. *Apocynin* as a treatment agent not only reduces the expression of Nox2 but also slows down morphological changes and increases the survival of neurons.

The tBHQ has antioxidant effects to improve the stability of Nrf2 through ubiquitin inhibition, which mediated by Keap 1. Research by Li *et al*. showed that the administration of tBHQ could suppress the brain inflammation and reduce secondary damage due to TBI.[29,30] The Nrf2-ARE plays important role in protection pathway. Keap1 and β transduction which contains E3 ubiquitin ligase act as an adapter for cullin 3 ubiquitin ligase that regulate Nrf2, to facilitate the degradation of proteasome. The tBHQ has improve Nrf2 to increase regulation of xenobiotic metabolic enzymes, antioxidants, DNA repair enzymes, and act as inflammatory effect.[31]

Combination therapy between *apocynin*, which serves to inhibit the formation of ROS and tBHQ which activate Nrf2 could improve better recovery in neurological. This combination of therapy is important to use in multifactorial diseases such as TBI to achieve better nervous system protection. Research by Chandran *et al*., has shown that combination therapy between *apocynin* and tBHQ was more effective in improving neurological recovery and could reduce secondary cortical lesions after TBI. This result was achieved in the first dose of *apocynin* and tBHQ being given after 2 h of TBI [Figure 2].[15]

Clinical Effect of *Apocynin* and *Tert-butylhydroquinone* as a Treatment in Traumatic Brain Injury

According to a study by Ferreira *et al*., subcutaneous administration of *apocynin* at a dosage of 5mg/kg for 30 min and 24 h after injury has proved that this therapy provides protection against memory recognition damage in 7 days after nerve injury. The result showed that brain injury could induce an increase of inflammation in the cortex, with inflammatory sign such interleukin-1b (IL-1b) ($F(1,44) = 8,41$; $P < 0.001$), tumor necrosis factor-α (TNF-α) ($F(1,44) = 9,62$; $P < 0.01$), Nox ($F(1,44) = 33,66$; $P < 0.001$), and water content ($F(1,32) = 62,56$; $P < 0.001$). Giving *apocynin* can significantly reduce the increase of Nox ($F(1,44) = 8,11$; $P < 0.01$), IL-1b ($F(1,44) = 4,23$; $P < 0.05$), and TNF-α ($F(1,44) = 4,50$; $P < 0.05$), but not significant in reducing the volume of brain lesions ($F(1,32) = 1,32$; $P > 0.05$).[32]

According to the research by Lu *et al*., tBHQ at a dose of 50 mg/kg in 24 h after TBI can increase the protein of Nrf2, which is essential in protecting the nervous system. TBI increases Nrf2 formation by 5% as a resistance mechanism to excessive ROS, which by administering tBHQ can increase Nrf2 formation up to 12% compared to those without treatment ($P < 0.05$). Furthermore, the administration of tBHQ can also reduce the volume of brain lesions.[13]

Chandran *et al*. reported a combination therapy between *apocynin* (10 mg/kg) and tBHQ (25 mg/kg) that administered 5 min within 2 h after TBI and a second dose at 24 h through intraperitoneal injection decreases motor deficits, increases memory function, decreases volume brain lesions, and increases the number of neurons in the

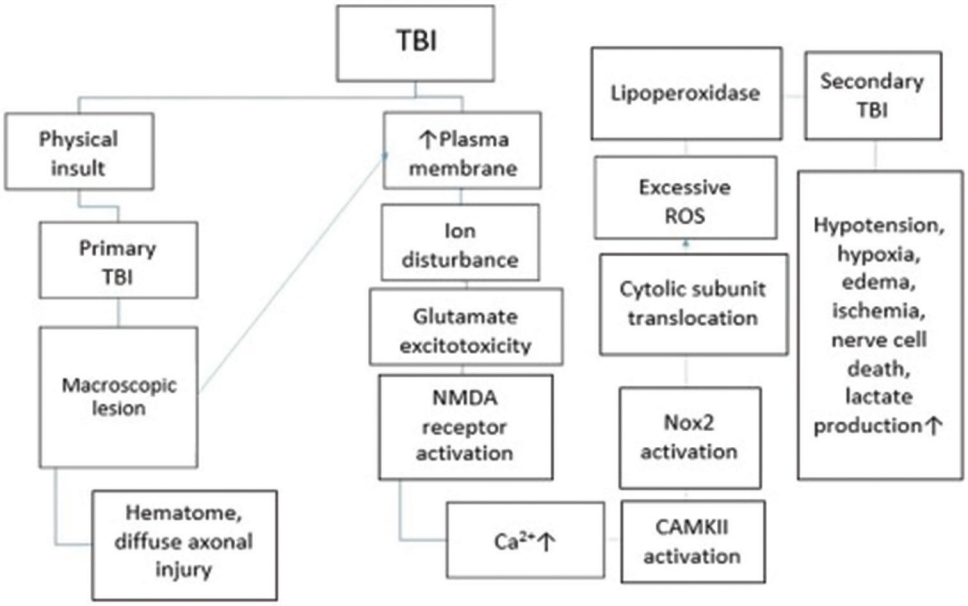

Figure 1: Pathophysiology of traumatic brain injury. TBI: Traumatic brain injury, NMDA: N-methyl-D-aspartate, Ca2+: Calcium, CaMKII: Calmodulin-dependent protein kinase type II, Nox2: NADPH oxidase, ROS: Reactive oxygen species

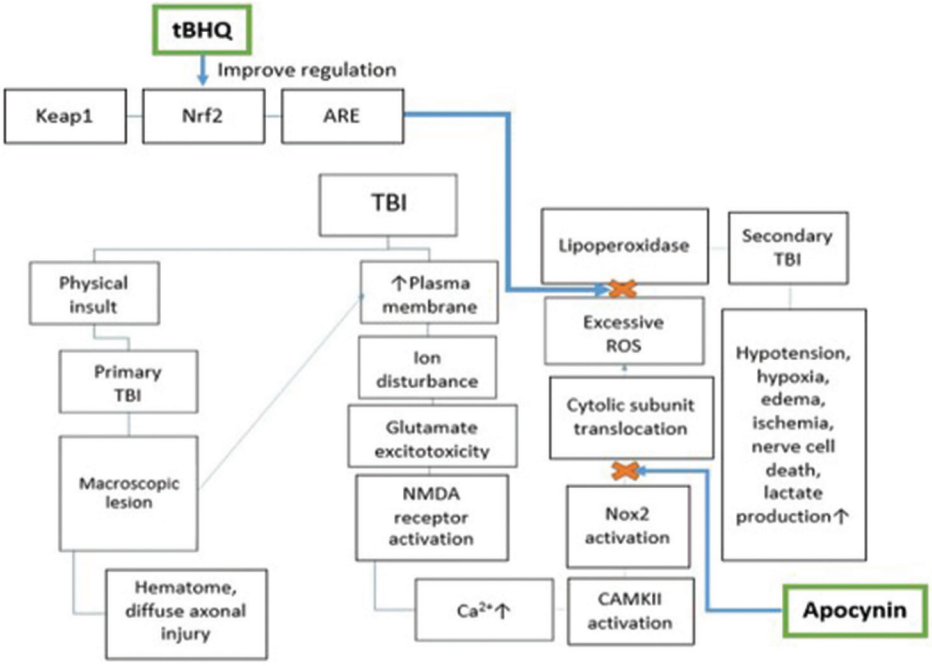

Figure 2: Mechanism of apocynin and tert-butylhydroquinone in traumatic brain injury

hippocampal CA1 and CA2 with $P < 0.05$ compared to those without treatment.[15]

Benefits and Weakness in Using Combination Therapy

Combined therapy between *apocynin* and tBHQ is expected to provide great benefits for human because *apocynin* is an organic compound that comes from plants and tBHQ is a food additive substance with a high antioxidant effect.[33,34] However, there may be some limitations in giving this combined therapy such as inefficient drug response or side effect in humans due to dose differences. Hence, more research is needed on combination therapy in this secondary brain injury therapy. Furthermore, more comprehensive and broader studies and researches of these two agents need to be done because not many literatures are available to be reviewed.[15]

Conclusion

Primary brain injury is caused by direct contact to the patient's head, and the secondary brain injury is the later effect from the primary injury. Most treatments are focused on to treat secondary brain injury. Currently, no therapy could recover cognitive and motoric function effectively.

A secondary brain injury will activate Nox2 and produces ROS that can cause cell death. Because of the ROS, the body will respond by activates Nrf2 that will bind to ARE and produce a protective gene. Even so, this reaction cannot hold effectively because of the ROS might escalate quickly. In this situation, *apocynin* is needed to inhibit ROS formation.

Apocynin therapy proved to give a neuroprotective effect by reducing the activation of Nox2, while tBHQ has an antioxidant effect that may increase Nrf2 to protect the cells. The combination of *apocynin* and tBHQ can give better neurologic effects, such as improving the neurological recovery and reducing the lesion volume.

Suggestion

This therapy combination can be a big positive impact on the medical world because the characteristics and material of the therapy are organic. It is important to do further research about the side effects and the limitation of this treatment, in the hope of raising it effectively. There are a few publications about this therapy, so it needs more observation. Coordination between the governments, practitioners, and the worldwide community is needed to develop this therapy against secondary brain injury.

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Conflicts of interest

There are no conflicts of interest.

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