

Neuroprotective Effect of Astaxanthin in the Animal Model of Spinal Cord and Traumatic Brain Injury: A Review

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ABSTRACT

Astaxanthin (AST), (family xanthophylls), is a food colorant used for animal, fish and nutritional supplements in humans. It has antioxidant activity. Brain and spinal cord injuries need neuroprotective treatment. The AST protects neuronal apoptosis and promotes regeneration. It quenches the free radicals to inhibit lipid peroxidation of neurons and promotes antioxidant enzyme production. Additionally the AST possesses anti-inflammatory property and prevents brain edema.

Key words: Astaxanthin, Spinal cord injury, Traumatic brain injury, Neuroprotection, antioxidant, anti-inflammatory.

ASTAXANTHIN

The AST is a red fat soluble pigment belonging to carotenoids.^[1] The AST has been approved by the US Food and Drug Administration (USFDA) as food colorant in animal and fish feed whereas European Commission has permitted natural AST as a food dye.^[2] AST possesses powerful antioxidant properties because of its multiple double bonds containing polyene chain which absorb free radicals during oxidative stress.^[3] Its antioxidant potency is 10 times stronger than other carotenoids, viz. zeaxanthin, lutein, canthaxanthin, and β -carotene and 100 times than those of α -tocopherol.^[4] The natural sources of AST are algae, yeast, salmon, trout, krill, shrimp, crayfish and microorganisms. Commercially AST is obtained from *Phaffia* yeast, *Haematococcus* and chemical synthesis.^[2] The FDA has approved AST from *H. pluvialis* for direct human consumption.^[5] The AST is being investigated for its

antioxidant and anti-inflammatory role in the pathogenesis of cardiovascular disease, metabolic syndrome, gastric ulcer, cancer, diabetes mellitus, brain injury, subarachnoid haemorrhage, and cognitive impairment.^[2]

Human brain is highly susceptible to oxidative stress because of various reasons like higher consumption of oxygen & glucose leads to higher free radicals generation, major component of brain is made up of lipids which is highly susceptible for lipid peroxidation, low endogenous antioxidant defence mechanism etc.^[6-8] Oxidative stress is a well-established causative factor for various neurodegenerative disorders and ageing.^[9] AST can act as a neuroprotective agent during pathogenesis of various neurological disorders because of its unique structure which enable it to readily cross the blood brain barrier to execute its action.^[10] In this review article we shall be discussing neuroprotective mechanism of AST in

the spinal cord and traumatic brain injury (TBI).

Spinal cord injury (SCI)

The spinal cord acts as a communicating centre between brain and peripheral nerves that enter the cord. It also produces spinal reflexes. Whenever the spinal cord is lacerated / macerated by a sharp penetrating force, contused / compressed by a blunt force or infarcted by a vascular insult, it begins a neurological damage in the spinal cord that is normally called “primary injury”. A “secondary injury” proceeds after primary mechanical one and occurs at the time course of minutes to weeks and leads to further neurological damage.^[11,12] The secondary events consist of vascular changes including haemorrhage, vasospasm, thrombosis, loss of autoregulation, disruption of blood brain barrier and infiltration of inflammatory cells which leads to edema, necrosis and ischemia. Secondary events include lipid peroxidation, free radicals and cytokine production, neuronal apoptosis, disturbance of ionic balance, glutamate excitotoxicity and inflammation.^[12] Final event, called chronic phase comprises of white matter demyelination, gray matter dissolution, connective tissue deposition and reactive gliosis that lead to glial scar formation which prevent axonal growth through it^[12] Research on SCI has focused on protection, regeneration and rehabilitation of the neuronal and supporting tissue and the recommended pharmacotherapy is a high-dose methylprednisolone intravenously, which has limited efficacy and serious adverse effect.^[13] A low level of antioxidant after SCI indicates antioxidant therapy may have promising result to prevent secondary damage and promote functional recovery of neurons.^[11] The major portion of lipid in spinal cord makes it vulnerable for free radical attack and the lipid peroxidation process.^[14]

A study on rat model of compressive spinal cord injury with single dose AST (80mg/kg ip after 5 mins of SCI) evaluated the role of

Neurotrophin 3 (NT-3).^[15] The NT-3 enhances the regenerative sprouting of the transected corticospinal tract, promotes cell survival and inhibits neuronal apoptosis.^[16]

There was an increased expression of NT-3 mRNA as well as NT-3 protein which was down regulated after SCI.

Spinal cord contusion injury in rats and effect of intrathecal injection of AST were evaluated.^[17] Animals treated with AST were presented with higher Basso, Beattie, Bresnahan (BBB) score compared to vehicle treated, indicating improved functional recovery after SCI. The AST treatment significantly decreased the Bax/Bcl-2 ratio, Bax/ β -actin and Cleaved capase-3, compared with the injury and vehicle treated groups. Contusion injury induced-demyelination and reduction in the number of motor neurons were also significantly preserved in AST treated animals. This indicates that AST possesses neuroprotective affects by showing anti-apoptotic and pro-survival effect on neurons, reducing demyelination and tissue damage and improving functional recovery after SCI.

Traumatic brain injury

Traumatic brain injury (TBI) is a major cause of mortality and morbidity.^[18] It is categorised as mild, moderate and severe on the basis of severity of TBI like duration of loss of consciousness, post-trauma amnesia Glasgow Coma Scale Altered mental state.^[19] Out of the total number of TBI, mild traumatic brain injury (MTBI) represents 70-90%. Motor vehicle collisions and falls are the main causes of MTBI which is common with men.^[18] Pathogenesis of TBI consists of primary and secondary events leading to temporary or permanent neurological deficit. Primary external impact of brain is directly related to primary injury like neuronal cell death and neurological dysfunction whereas secondary injury can happen from minutes to days from the primary impact. Secondary injury induces molecular, chemical, metabolic and gene related changes which is responsible

for further cerebral damage. Secondary events consist of cerebral ischemia, hypoxia, loss of ionic homeostasis, increased release of inflammatory mediators, oxidative stress and release of excitatory neurotransmitters that lead to increased intracellular calcium. Intracellular calcium activates caspases, calpases enzymes and free radicals which initiate apoptosis cascade of neuronal cells. This degradation of neuronal cells is associated with disruption of BBB, cerebral edema and cognitive deficit.^[20-22]

Cerebral edema and the ensuing increased intracranial pressure is the leading cause of high morbidity and mortality following TBI. There are two types of edema: cytotoxic and vasogenic, in which vascular edema is the main contributor to the process of brain edema.^[23] Cytotoxic edema is associated with failure of ATP-dependent Na⁺/K⁺-pumps during energy shortage conditions like cerebral ischemia, anoxic-ischemic encephalopathy and severe TBI. Vasogenic edema occurs when the BBB becomes leaky, permitting an influx of plasma constituents from the vasculature into the extracellular space.^[23] Studies suggest that aquaporins (AQPs) and Na⁺-K⁺-2Cl⁻ co-transporters (NKCCs), expressed in glial cells in brain, play a critical role in brain edema and maintaining cellular ionic homeostasis.^[24,25] Aquaporins (AQP) are a family of water channels which regulate water movement according to the osmotic gradient. The AQP4 is the predominant isoform expressed on astrocytes which gets up-regulated following TBI.^[26] The TBI induces expression of AQP4 and NKCC1 mRNA as well as its protein.^[27] The inappropriate activation of NKCC1 would result to cell swelling and tissue edema while its inhibition protects neurons from ischemic injury and limits acidosis-induced glial swelling.^[28]

A controlled cortical impact (CCI) model was used to demonstrate neuroprotective effect of AST following TBI.^[27] Neurological deficit was evaluated by Garcia scoring system which was

significantly reduced post CCI in all the mice but administration of AST (100mg/kg) 30 min post CCI markedly improved the behavioural outcome. Significant improvements were seen on rota rod test in AST treated group. Morris water maze (MWM) test was performed to evaluate spatial memory in which mice showed shorter latencies to find hidden platform in AST treated group.

The mRNA expression of AQP4 and NKCC1 and their protein was markedly elevated after CCI but there was significant reduction in AST treated group. The AST significantly attenuated TBI induced cerebral oedema and protected BBB disruption in dose dependent manner (25, 50,100 mg/kg). Evans blue extravasation in the CCI group was markedly increased due to BBB disruption whereas AST treated group had significantly lower extravasation. This demonstrated dose dependant neuroprotective effect of AST by improving behavioural outcome, prevented BBB disruption and overexpression of AQP4 and NKCC1, thus decreases cerebral oedema.

Neuroprotective action of AST after mild traumatic brain damage has been demonstrated.^[29] Neurological Severity Score (NSS) was calculated by performing ten different tasks to evaluate motor ability, balancing, and alertness where score zero was for healthy uninjured animals to a maximum of 10 in impaired mice. After TBI, spontaneous recovery decreased NSS value with time; however, the recovery of AST treated animals was faster. Sensorimotor coordination and motor learning performance scores significantly increased in AST treated group at day 3 after TBI but due to spontaneous recovery, the differences became insignificant from day 7 on rota rod test. The AST treated group exhibited greater visual, non-spatial and spatial cognitive performance during object recognition test (ORT) and Y maze test respectively. The AST treated mice had greater preference exploring a novel object compared to a familiar object in ORT whereas there were increased number of

entries into the new arm in Y maze test on day 7 and 28. These results indicate that AST has beneficial effects on cognitive deficit and recovery following TBI. Nissl stained brain sections conformed decreased apoptosis and increased neuronal densities in cerebral cortex in AST treated group. The AST also reduces cerebral infarct volume induced by TBI. Neurotrophic factor BDNF, axonal regeneration marker GAP 43 and synaptic vesicle-associated protein Synapsins; all play a critical role in synaptic vesicle exocytosis and synaptogenesis. These proteins were significantly decreased after TBI whereas AST administration partially restored their levels. Synaptophysin (SYP) a synaptic vesicle protein involved at neurotransmitter release as well as in synaptic plasticity and cognition^[29,30] was also decreased after injury and restored after AST administration. The AST treatment was associated with a reduced cortical lesion volume, neurodegeneration and neuronal apoptosis while stimulatory effect on neurotrophic factors, synaptogenesis, neural plasticity and survival of neurons.

DISCUSSION

The AST is a potent antioxidant having multiple mechanisms of action to protect neurons. AST involved in promoting cell survival and attenuation of neuronal apoptosis through NT-3 pathway which belongs to a neurotrophin family.^[15] It has been demonstrated that NT-3 enhances the regenerative sprouting of the transected corticospinal tract but not BDNF or nerve growth factor (NGF).^[16] Pro-apoptotic protein Bax gets inserted into mitochondrial membrane and induces outer membrane permeabilization whereas regulatory protein Bcl-2 directly interacts with Bax and inhibits the cascade pathway of apoptosis which include release of cytochrome C along with other apoptotic factors from the mitochondria into the cytosol and activation of caspase-3.^[31] The AST treatment significantly decreases the Bax/Bcl-2 ratio, Bax/ β -actin and cleaved capase-3 which is

important for mitochondrial membrane stabilization and inhibition of neuronal apoptosis.^[17] TBI induces expression of AQP4 and NKCC1 on glial cells in the brain which leads to cerebral edema^[24,25,27] AST protects BBB disruption and prevent formation of cerebral edema by down regulating overexpression of AQP4 and NKCC1 on astrocyte.^[27] Cognitive impairment due to brain injury also has been reversed by AST which was observed during morris water maze test, object recognition test (ORT) and Y maze test.^[17,22] NSS and BBB scores were also improved in AST treated rats.^[17,22] Various proteins, factors and markers related to growth and maintenance of neurons like BDNF, GAP 43, synapsins and Synaptophysin are upregulated by AST, to protect and regenerate neurons after injury.^[22]

Thus AST protects brain, BBB, cerebral edema, neuronal apoptosis and other secondary events caused by SCI & TBI by various pathways and mechanism. These effects and mechanism of neuroprotection of AST; is yet to be explored even deeper in context with human to utilise its full potential in clinical aspect.

CONCLUSION

These studies support the claim of neuroprotection by AST but they are still inadequate to support the same in humans. The AST can be the answer for many neurodegenerative disorders if we explore it as a potential drug.

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