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REVIEW ARTICLE

Neuroprotective mechanisms of astaxanthin: a potential therapeutic role in preserving cognitive function in age and neurodegeneration

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Abstract Astaxanthin (AXT) is a carotenoid with multiple health benefits. It is currently marketed as a health supplement and is well known for its antioxidant capacity. Recent evidence has emerged to suggest a broad range of biological activities. The interest in this compound has increased dramatically over the last few years and many studies are now applying this molecule across many disease models. Results from the current research are beginning to come together to suggest neuroprotective properties including anti-inflammatory, anti-apoptotic, and antioxidant effects, as well as the potential to promote or maintain neural plasticity. These emergent mechanisms of actions implicate AXT as a promising therapeutic agent for neurodegenerative disease. This review will examine and extrapolate from the recent literature to build support for the use of AXT in mitigating neuropathy in normal aging and neurodegenerative disease.

Keywords Astaxanthin · Aging · Neuroprotection · Neural plasticity · Microglial function

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Introduction

Aging is a primary risk factor for the development of many diseases including neurodegenerative diseases. Aging leads to numerous physiological changes associated with loss of tissue integrity and loss of organ function. In the brain, the age-related anatomical and physiological changes can compromise cognitive functions including memory, attention, executive function, and perception, with high variability (Arvanitakis et al. 2016; Glisky 2007; Wahl et al. 2016). Although the exact molecular mechanisms through which aging of the brain ultimately leads to significant changes in some cognitive domains and neurodegenerative diseases are still being elucidated, it is widely accepted that increased inflammation, mitochondrial dysfunction, disrupted calcium homeostasis, and elevated oxidative stress within the brain all contribute to neurodegeneration (Wang and Michaelis 2010). Incidents of neurodegenerative disease are projected to increase as the largest sector of the population grows older and this would result in a greater economic burden. Therefore, developing strategies to manage these deleterious effects of aging is a high priority in efforts to preserve cognitive function and

hinder the development of neurological pathologies in an aging population.

Carotenoids are a large class of compounds synthesized in plants and certain photosynthetic microorganisms. Many carotenoids are directly involved in photosynthesis, while others are produced as a means of protecting these species from photooxidation and related damage. They are typically red, orange, or yellow pigments thus found in fruits and vegetables of these colors as well as leafy greens. Many carotenoids have been identified in nature; however, far less are consumed and reach detectable levels in serum and tissues, and only some of which can be converted to vitamin A in humans. Carotenoids are further subdivided into carotenes and xanthophylls; the latter are disguised by the presence of oxygen at the end of polyene chain. Carotenoids have been intensely investigated for their role in human health, and it is suggested that certain carotenoids can generally promote health and reduce risk of developing various diseases reviewed here (Kaulmann and Bohn 2014; Woodside et al. 2015).

One carotenoid that has gained significant interest in recent years is astaxanthin (AXT). AXT, a xanthophyll carotenoid, is being investigated individually in a broad range of clinical applications, including cardiovascular health, metabolic syndrome, treatment of gastric ulcers, and cancer, all of which have elements of inflammation and/or oxidative stress in their pathogenesis (Ambati et al. 2014). AXT is already approved as a dietary supplement and is widely available commercially. To date, there are no significant adverse effects attributed to AXT supplementation, indicating that this is both a cost-effective and relatively safe compound. It has also been shown to cross the blood-brain barrier and is detectable in the brain tissue. These characteristics make AXT a desirable candidate for further investigation to elucidate its therapeutic potential.

Reactive oxygen species (ROS) have important roles in normal brain function, energy production, and redox-sensitive signaling pathways. Appropriate redox states are usually maintained by many endogenous antioxidant mechanisms that exist in order to protect against excessive production of ROS and ensuing tissue damage (Koutsilieri et al. 2002). However, the weakening of the antioxidant defense system such as the loss of superoxide dismutase (SOD), catalase, and glutathione is associated with aging (Haider et al. 2014). All of these features of aging contribute to a state of oxidative stress in the brain, where the organ cannot combat the deleterious effects of ROS and damage to proteins, lipids, and nucleotides accumulates promoting cellular dysfunction and subsequent cognitive impairment (Finkel and Holbrook 2000; Koutsilieri et al. 2002). Focusing on the development of antioxidant-based therapies to modulate central nervous system (CNS) pathologies has been of great interest in recent history. While there is a plethora of empirical evidence that antioxidants can be an effective treatment in preliminary studies, it is important to note that this strategy has been largely unsuccessful when translated in clinical trials. The failure of this antioxidant centric approach may be explained, in part, by the multifaceted pathology of neurodegenerative disorders. While oxidative stress is a common denominator implicated by numerous reports, there are many other factors contributing to neuronal dysfunction in aging and disease, as mentioned above. In this regard, AXT is an interesting compound, as it has multiple proposed biological activities, including the capacity to augment the endogenous antioxidant defense mechanisms such as SOD and heme oxygenase-1 (HO-1).

There is strong evidence demonstrating that AXT can calm microglial activation and suppress the output of cytotoxic substances. During inflammation, microglia will release nitric oxide, among other factors, when stimulated. Nitric oxide will interact with superoxide to form peroxynitrite, an aggressive reactive oxygen species that will damage proteins, as well as lipids and DNA (Barros et al. 2014; Jenner and Olanow 1996). The ability for AXT to attenuate microglial activation and the release of pro-inflammatory cytokines is an important mechanism of action for protecting neuronal integrity, especially with age, as it is established that there is increased inflammation in the brains of aged organisms (Satoh et al. 2009a).

Recently, there has been emerging evidence that AXT can promote neurogenesis and plasticity. Neurogenesis is now widely accepted to occur throughout adulthood, primarily in two regions of the brain: the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Because the hippocampus is essential for learning and memory, neurogenesis likely plays a role in these cognitive processes. Although the precise mechanisms of regulation have yet to be elucidated, promoting neurogenesis has been associated with improved behavioral performance on hippocampal-dependent tasks and this may be a prominent mechanism through which AXT supplementation can augment cognitive function.

Because many diseases are caused by more than one pathology, the traditional drug discovery paradigm is shifting away from addressing single pathological mechanisms to multiple therapeutic targets. As a result, more attention is being paid to finding multipotent agents that can surpass the therapeutic effects of selective drugs. Because oxidative stress plays an important role in the pathogenesis of many diseases, continued interest exists in the identification and development of novel antioxidants that prevent free radical damage. However, there are many cases of natural antioxidants that show efficacy for various diseases, but further investigations fail to show that the antioxidant properties can account for compound's presumed efficacy. The misattribution of mechanism of action to only known antioxidant properties can lead to a common error in translational research known as the "tomato effect" whereby a failed attempt to prove an agent's putative mechanism of action leads to a premature conclusion that the agent simply has no real therapeutic effects at all. In order to better understand the mechanism of action of natural compounds typically classified simply as "antioxidants" that are efficacious for complex diseases in which oxidative stress may be present, but not the only significant pathogenic mechanism, the multiple pharmacological effects should be considered and investigated more broadly.

This emerging evidence suggests that AXT may be a useful compound in delaying or ameliorating cognitive impairment associated with normal aging or alleviating the pathophysiology of various neurodegenerative diseases. In this review, we will explore the recent literature supporting these putative neuroprotective effects of AXT.

Astaxanthin

AXT is naturally produced by various microorganisms including microalgae *Chlorella zofingiensis*, *Chlorococcum sp.*, red yeast *Phaffia rhodozyma*, and the marine *Agrobacterium aurantiacum* (Yuan et al. 2002). The richest natural source for AXT is the marine algae *Haematococcus pluvialis* (Boussiba 2000; Boussiba et al. 1999). When these organisms are exposed to environmental stressors, they synthesize AXT as a cellular protectant. AXT, due to its dark red-orange pigment, is responsible for giving the color to crustacean shells and the flesh of the salmonids and other fish species that ingest AXT as a food source; thus, seafood is an abundant dietary source of AXT (Guerin et al. 2003; Kidd 2011). However, in order to achieve a 4–20-mg dose of AXT through diet alone, one would have to consume 600 to 2000 g of salmonoid fish (Seabra and Pedrosa 2010); therefore, it is more realistic and effective to take AXT as a supplement to maintain appreciable levels of this compound. AXT has been approved as a dietary supplement in the USA since 1999. AXT is now widely available and commercially advertised for an array of health benefits. AXT is certified as generally regarded as safe (GRAS) by the food and drug administration, a classification that requires both extensive information on its consumption in humans and a plethora of data from strict, high-quality scientific assessment to determine its safety.

AXT is digested and absorbed similar to lipids and other carotenoids, although the bioavailability is heavily influenced by other dietary components. When administered orally, a higher proportion is absorbed when taken with a meal or delivered in an oil-based formulation (Odeberg et al. 2003). After release from the food matrix, AXT is believed to accumulate in the lipid droplets within the gastric juices and then incorporate into micelles when they encounter bile acids, phospholipids, and lipases in the small intestine. The micelles thought to passively diffuse into the plasma membrane of enterocytes. AXT, like the more polar xanthophylls, is transported in the circulation by high-density lipoprotein (HDL) and low-density lipoprotein (LDL), after being liberated from chylomicrons that are formed in the intestinal cells (Furr and Clark 1997). It has been reported that after a 100-mg/kg dose, plasma concentrations peak to 1 μg/ml at approximately 9 h after dosing. It is taken up into many tissues, including the brain, but primarily accumulates in the liver (Choi et al. 2011).

Like other carotenoids, the chemical structure of AXT includes a long carbon chain with conjugated double bonds, but AXT is unique that it contains two hydroxylated ionone rings at either end of the lipophilic portion of the molecule that associates with the polar heads of the phospholipids (Kidd 2011). This configuration and size of AXT allow it to become vertically integrated through the phospholipid bilayer as the functional groups of the AXT structure are energetically favorable in this orientation (Guerin et al. 2003; Kidd 2011). This feature precisely positions the molecule so that it can interfere with lipid peroxidation. In this regard, AXT is especially adept at protecting the integrity of cell membranes.

Natural vs synthetic astaxanthin

AXT is a common additive in animal diets used to enhance the coloration of farmed fish species and increase their marketability. Currently, 95% of AXT is generated synthetically using petrochemicals due to cost-efficiency for mass production (Panis and Carreon 2016). However, AXT is becoming increasingly popular in the human health sector and is driving the need for higher quality AXT production. The comparability of synthetic and naturally derived AXT is currently debated, and safety issues have arisen regarding the use synthetic AXT for human consumption (Capelli et al. 2013).

Natural AXT is produced from algae, yeast, and crustacean byproduct. Natural AXT occurs as a mono-/di-ester or as carotenoproteins/carotenolipoproteins by conjugating to proteins/lipoproteins (Kidd 2011; Yuan et al. 2011). On the other hand, synthetic AXT is often produced as a non-esterified monomer-free form (Higuera-Ciapara et al. 2006). These two forms of AXT yield different stereoisomers that putatively impact the stability and, therefore, antioxidant function (Higuera-Ciapara et al. 2006). While natural AXT is mainly found as (3S, 3'S) isomers, synthetic AXT consists of different isomer configurations that consist of (3S, 3'S), (3R, 3'S), and (3R, 3'R) in ratio of 1:2:1 (Østerlie et al. 2000). The efficacy of natural and synthetically derived AXT has received increasing attention and is currently under debate. As it stands now, there is some evidence to suggest that natural AXT has a higher oxygen radical absorbance capacity (ORAC) value (approximately three times higher) than that of the synthetically produced AXT (Naguib 2000; Nguyen 2013).

Recently, the efficiency of the different isomers were directly compared to *Caenorhabditis elegans* (Liu et al. 2016). Interestingly, the natural (3S, 3'S) AXT isomer reduced ROS the most and increased SOD3 gene expression, suggesting that natural AXT may be a more effective antioxidant than synthetic AXT. Régnier et al. (2015) compared antioxidant activity of natural AXT from *Haematococcus pluvialis* and synthetic AXT using human endothelial cells. In their trolox equivalent antioxidant capacity (TEAC) assay, oxygen radical antioxidant capacity (ORAC), and reactive oxygen species (ROS) scavenging activity, overall antioxidant capacity of natural AXT was significantly higher over synthetic AXT. Capelli et al. (2013) have also reported significantly stronger antioxidant capacity of the natural AXT compared to the synthetic and claimed that dosage for synthetic AXT needs to be 20 to 30 times higher to obtain similar level of antioxidant power.

Differences shown in between the natural and synthetic AXT could be due to the differences in stereoisomer composition in natural and synthetic AXT. However, it is important to note that microorganisms used for AXT harvest also produce other carotenoids including β-carotene, canthaxanthin, and lutein, although in much lower quantities. AXT extracts derived from natural sources may contain small traces of other bioactive compounds, whereas synthetic astaxanthin does not (Capelli et al. 2013). It is difficult to determine if the trace amounts of other carotenoids play a role in these studies; however, at such low concentrations, it is less likely that they make a significant contribution.

Taken together, the data discussed above suggests that the biological activity of natural AXT may be significantly better than synthetic AXT. Interest in using high-quality AXT supplements for health benefits has been increasing steadily; the apparent disparity in efficacy between these two sources will be an important consideration in moving this compound toward medical applications or adjuvant therapy for management of disease symptoms. However, more comprehensive studies will be needed to better characterize the quality and safety of natural and synthetic AXT in relation to each other before one's superiority can be confirmed.

Neuroprotective properties of AXT

AXT reduces oxidative stress

The antioxidant activity of AXT is perhaps its best-known health benefit; it has been marketed as a nutritional supplement due to its potent antioxidant capacity for years. This antioxidant effect is of interest because the brain is already susceptible to higher amounts of oxidative stress due to high metabolic activity, presence of readily oxidized compounds like catecholamine neurotransmitters, and the polyunsaturated fatty acids that comprise cell membranes; thus, oxidative stress can harm macromolecules and lead to neuronal dysfunction over time. Further, oxidative stress is both a feature of normal aging and is an element that appears in many disease conditions. AXT is a potent antioxidant with a biological activity many times higher than that of alpha-tocopherol and beta-carotene (Ambati et al. 2014). It has

suggested that this powerful antioxidant effect is due to the ketone-bearing ionone rings by stabilizing radicals more effectively synergistically with polyene backbone (Jackson et al. 2008). AXT exerts its antioxidant activity through various mechanisms including absorbing free radicals into the polyene chain, by donating an electron, or by forming chemical bonds with reactive species. This antioxidant versatility is a characteristic of AXT and sets this molecule apart from other carotenoids. Not surprisingly, there is abundant empirical support demonstrating the ability for AXT to reduce ROS in vitro (Liu et al. 2009b), and more recent reports have recapitulated these early findings in animal models as described in Table 1.

AXT treatment is frequently associated with reduced markers of oxidative damage (Liu et al. 2009a; Lu et al. 2015; Park et al. 2013); however, its mechanisms of action extend far beyond its ability to directly scavenge free radicals. There is significant support that AXT may increase the levels of or promote the activity of endogenous antioxidant enzymes including superoxide dismutase and catalase. This observation is relevant to neurodegeneration and protecting cognitive function with age, as it is observed that efficiency of these molecules decrease with age (Haider et al. 2014; Puertas et al. 2012). The efficacy of SOD, catalase, and other antioxidant mechanisms are particularly useful in protecting brain tissue from the damaging effects of ROS. It has been reported that AXT supplementation can also stimulate the expression of thioredoxin reductase (TrxR), HO-1, and nuclear factor erythroid-related factor 2 (NRF-2), which are known to be associated with robust cellular protection from oxidative stress in vivo (Guerin et al. 2003). Al-Amin et al. (2015a) performed a thorough investigation of major antioxidant enzymes across major brain structures in mice treated with AXT (2 mg/kg for 1 month). In agreement with the previous findings, these authors also report that AXT treatment increased the activity of SOD and catalase, as well as increased the level of reduced glutathione (GSH). It was also observed that AXT treatment decreased evidence of lipid peroxidation, indicated by lower levels of malondialdehyde (MDA) and of advanced protein oxidation product (APOP) in areas including the frontal cortex, hippocampus, cerebellum, and striatum. Taken together, these findings illustrate that multiple endogenous antioxidant molecules are targeted by AXT.

Therapeutic potential of AXT in neurodegenerative diseases

Although there is support for an interaction between AXT and endogenous antioxidant mechanisms, many of the early studies that report this effect have been conducted in disease models of the periphery. While studies using AXT intervention have increased steadily over the last few years, there are still limited publications that have investigated this effect in the CNS. However, extrapolating from the existing research, the evidence does indicate that therapeutic effect is occurring in the CNS. Promoting the efficacy of antioxidant enzymes seems to be a mechanism of action of AXT and this has some important implications for a role in neuroprotection in the brain. Maintaining the function of these enzymes has important contribution to normal aging, neurodegenerative diseases, and brain injury (Stranahan and Mattson 2012). Perhaps even more interestingly, many of these studies that have described antioxidant capacity of AXT within experimental models of specific diseases and have also supplied data that is beginning to elucidate additional biological activities of AXT and further implicates this compound as a neuroprotective agent in the context of neurodegenerative diseases.

AXT has been shown to be protective across various models of Parkinson's disease (PD) (Grimmig et al. 2016). AXT effectively attenuated the toxicity of 6-hydroxydopamine (6OHDA) and docosahexaenoic acid hydroperoxide by reducing ROS, protein carbonylation, cytochrome C release, and mitochondrial membrane potential, ultimately inhibiting apoptosis in SH-SY5Y human neuroblastoma cells (Liu et al. 2009a). Interestingly, when the subcellular location of AXT was evaluated, it was found that AXT accumulated predominately in the mitochondrial and cellular membrane subcellular fractions. This suggests that AXT can be incorporated into these neuronally derived cells in vitro and may specifically modulate mitochondrial function. AXT has also been shown to reduce n-methyl-4-phenylpyridinium iodide (MPP+) toxicity in PC12 cells (Ye et al. 2012). MPP+ is the toxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a well-established and commonly used toxic model of Parkinson's disease; this cell line shares features of dopaminergic cells that are selectively targeted both in PD and by this neurotoxin. Additionally, similar results were observed in an in vivo experiment where AXT

Table 1 Neuroprotective effects of AXT in vivo

Neuroprotective effects	Main outcomes	Dose	Duration	Species	Route	References
Anti-oxidative	Decreased oxidative stress makers and increased antioxidant enzyme activities in different regions of the brain including the frontal cortex, hypothalamus, striatum, pariental cortex, hippocampus, and cerebellum in young and old animals	2 mg/kg	4 weeks	Mouse	Oral	Al-Amin et al. 2015a
	Reduced neuronal damage, decreased oxidative stress, and apoptosis in hippocampus	75 mg/kg	Once	Rat	I. P	Lu et al. 2015
	Decreased serum triglyceride and cholesterol levels and increased antioxidant capacity. Improvement in passive avoidance learning performance	0.6 g/kg	180 days	Rat	Oral	Komaki et al. 2015
	Attenuated oxidative stress caused by subarachnoid hemorrhage	0.1 and 0.01 nM/L 25 and 75 mg/kg	Twice	Rat and rabbit	I.C.V. injection, oral	Zhang et al. 2014c
	Reduced apoptosis after subarachnoid hemorrhage, modulation of Akt/Bad signaling cascade	20 μL of 0.1 mM solution	Once	Rat	I.C.V. injection	Zhang et al. 2014b
	Protected brain from edema, BBB distruption, apoptosis, and neurological dysfunction. Alleviated oxidative stress by increasing HO-1 expression and mRNA expression of HO-1, NAD (P)H, NQO-1, GST-a1, through Nrf2 pathway	0.1 and 0.01.nM/L 27 and 75 mg/kg	Once	Rat	I.C.V. injection	Wu et al. 2014
	Reduced ROS and apoptosis in vitro and reduced neurodegeneration in vivo	30 mg/kg	28 days	Mouse	I.P.	Lee et al. 2011
	Increased antioxidant capacity, GSH/GSSG in plasma and decreased in oxidative stress markers in forebrain	1 mg/kg	45 days	Rat	Oral	Mattei et al. 2011
	Inhibited neural cell death in CA1 region of hippocampus by modulating apoptosis induced by ischemia	30 mg/kg	Once	Rat	I.P.	Lee et al. 2010
	Reduced infarct volume and decreased neuronal injury induced by middle cerebral artery occlusion	20, 50, and 80 mg/kg	Twice	Rat	Intragastric	Lu et al. 2015
	Reduced oxidative stress, glutamate release, and apoptosis induced by ischemia	20 μL of 0.1 mM per rat (250-300 g)	Once	Rat	I.C.V. injection	Shen et al. 2009
Anti-inflammation	Rescued performance in Morris water maze and extended neuronal survival in CA3 in hippocampus and frontal cortex, possibly via NF kB and TNFa modulation	25 mg/kg	10 weeks	Mouse	oral	Zhou et al. 2015
	Decreased MDA, but increased in GSH and SOD levels in hippocampus and cerebral cortex.	10,20, and 40 mg/kg	5 days	Rat	I.P.	Xu et al. 2015

Table 1 (continued)

Neuroprotective effects	Main outcomes	Dose	Duration	Species	Route	References
	Decreased activity of eNOS and iNOS, pro-inflammatory cytokine levels (NFkB, TNFa, IL-6 and IL-1ß), capase 3 and 9 mRNA expressions. Increased expression of AKT and PI3K in hippocampus and cerebral cortex. Improved performance in Morris water maze test					
	Decreased lL1ß and increased 1 L10 in hippocampus and/or cerebellum in aged female animals. Also showed similar effect only in hippocampus in aged male animals	10 mg/kg	6 weeks (5 days/week)	Rat	Oral	Balietti et al. 2016
	Neuroprotection against subarachnoid hemmorrhage induced brain damage by NFkß downregulation and suppression of inflammatory cytokines	0.1 and 0.01 nM/L 25 and 75 mg/kg	Once	Rats	I.C.V. injection	Zhang et al. 2014a
Misc.	Prenatal valproic acid incuced autistic behaviors were attenuated by AXT injection in offspring	2 mg/kg	4 weeks	Mouse	I.P.	Al-Amin et al. 2015b
	Improved spatial memory	0.26, 1.3 and 6.5 mg/kg	30 days	Mouse	Oral	Zhang et al. 2007
	Improved performance in Morris water maze at high doses against ischemia-induced spatial memory deficits	55, 550 mg/kg	Once	mouse	Oral	Hussein et al. 2005
Promoting neural plasticity	Promote hippocampal neurogenesis in dose-dependent manner and improved performance with Morris water maze test at 0.5% dose. Enchanced neurogenesis and spatial memory-related markers	0.02, 0.1 and 0.5% (*w*/w)	16 weeks	Mouse	Oral	Yook et al. 2016
	Ameliorated oxidative stress, hippocampal damage, and restored BDNF levels after D-galactose-induced brain aging	0.02% of 30 g daily feed intake	3 times/week for 8 weeks	rat	Oral	Wu et al. 2014
	Ethanol-induced high cortical spreading depression levels were reduced by AXT dose dependently	2.5, 10 and 90 μg/kg/d	18 days	Rats	Oral	Abadie-Guedes et al. 2008

administration successfully rescued loss of tyrosine hydroxylase in the substantia nigra and striatum of mice treated for a month with MPTP (Lee et al. 2011).

AXT has also been successful at reducing neurotoxicity in cell culture models of Alzheimer's disease (AD). PC12 cells were protected from cytotoxicity induced by the amyloid β fragments (Chang et al. 2010). Wang et al. (2010) replicated the previous findings and attributed the involvement of extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling and the downstream activation of HO-1 on observed neuroprotection from the amyloid beta peptides. AXT ultimately reduced apoptotic-related mediators caspase 3 and Bax in PC12 and SH-SY5Y cells, respectively. Lobos et al. provide further support of the AXT neuroprotection against amyloid toxicity. These authors used AXT to protect primary hippocampal neurons from amyloid-β-induced generation of ROS and calcium dysregulation (Lobos et al. 2016). The preliminary findings from these PD and AD centric experiments report various end points that promote neuronal survival, highlighting the multipotent action of AXT.

AXT also attenuated the neurotoxicity of chronic exposure to aluminum chloride in 4-month-old swiss albino mice. Forty-two days of AXT treatment resulted in proteins, lipids, and reduced glutathione. This reduced oxidative stress was also associated with the preservation of cognitive function demonstrated by the improved performance on the radial arm water maze, an indicator of spatial memory. Interestingly, the deposition of heavy metals including aluminum is associated with the pathology of multiple neurodegenerative diseases. These results suggest another therapeutic mechanisms for the intervention in neuropathy and preserving neuronal function (Al-Amin et al. 2016a).

In addition, in the amygdala kindling model of epilepsy, AXT lowered seizure activity and reduced neuronal loss in CA3 region of hippocampus of the supplemented rats (Lu et al. 2015). These major findings were associated with reduced oxidative damage (MDA and ROS output) and reduced caspase 3 expression with a concomitant decreased release of mitochondrial cytochrome C into cytosol. These observations suggest that AXT may be neuroprotective based not only on the capacity to buffer oxidative stress but also indicates an additional action of interfering with mitochondrial-mediated apoptosis occurring in this model and ultimately attenuating neuron loss.

Al-Amin et al. (2015b) demonstrate that AXT administration during early developmental period rescued some of the autistic features and behavior deficits induced by prenatal valproic acid. The same group (Al-Amin et al. 2016b) also observed that prenatal lipopolysaccharide (LPS) exposure-induced behavioral abnormalities and oxidative stress were alleviated with 6 weeks of AXT oral administration in their adult offspring. These two reports suggest that AXT may play an important role in maintaining an optimal environment and redox homeostasis during critical periods of brain development in the face of CNS insults.

Implications for hippocampal neurogenesis and plasticity

Neurogenesis and plasticity significantly decrease with age (Shetty et al. 2013), a trend that also reflects a concomitant cognitive decline among some people. Efforts to rescue this deficit are associated with improved behavioral outcomes in hippocampal-dependent tasks and restored cognitive function (Sahay et al. 2011). It is known that neurogenesis and hippocampal plasticity can be stimulated (and even negatively regulated) by extrinsic factors including environment, exercise, and diet (Thangthaeng et al. 2016). For example, our lab has successfully used dietary supplements to maintain progenitor cell proliferation and neurogenesis in aged animals (Acosta et al. 2010; Bachstetter et al. 2010; Bickford et al. 2015), supporting the possibility for natural compounds to maintain cognitive function through modulation of neurogenesis.

There is emerging evidence that suggests AXT may promote neurogenesis and plasticity. Neural progenitor cells (NPCs) are a self-renewing stem cell population that can give rise to new neurons that later become integrated into existing circuits of the hippocampus to replace degenerating cells. NPC proliferation is known to slow with aging (Shetty et al. 2013), and promoting stem cell proliferation in the DG is regarded as a strategy for maintaining the regenerative capacity of the hippocampus and is associated with preserving cognitive function. It has been reported that AXT can increase proliferation of neural precursor cells in vitro. Kim et al. (2010a) show that the application of AXT in culture increased the proliferative and colony-forming capacity of neural stem cells in a time- and dose-dependent manner and observed an increase in proliferation-related genes like cyclin-dependent kinase

2 (CDK2). It has demonstrated that not only can AXT treatment promote cell replication but can also be directly protect NPCs when exposed to an oxidative insult (of 0.3 mM H_2O_2) and limit the subsequent apoptotic cascade (Kim et al. 2009). As stated above, oxidative damage is known to increase with age, and AXT may help restore oxidative stress in the aging hippocampus. These in vitro observations of cell proliferation have recently been corroborated in young adult mice (11 weeks) treated for 4 weeks with an AXT-supplemented diet (Yook et al. 2016). This study reported an increased immunohistochemical labeling of bromodeoxyuridine (Brdu) in cells in the DG of the AXT-treated mice, indicating that the AXT-enriched diet was able to stimulate cell division in the SGZ. These trends of AXT-augmented neurogenesis were also associated with improved performance on the Morris water maze (Hussein et al. 2005; Xu et al. 2015; Yook et al. 2016; Zhang et al. 2007), a spatial learning task mediated by the hippocampus. This data is particularly exciting as it demonstrates that reported AXT effects both in culture and in the brains of young animals can lead to functional behavioral outcomes and discernable improvement on cognition.

Various reports implicate cell growth and differentiation pathways are likely stimulated by AXT treatment. A few studies report the capacity for AXT to modulate ERK signaling, further supporting a possible role for AXT in cell growth or differentiation and neurogenesis. ERK signaling pathway is classically described as part of the mitogen-activated protein kinase (MAPK) superfamily, a highly studied and conserved group of kinases with numerous downstream receptor tyrosine kinases and mitogens. Also, this cell signaling cascade is complex; ERK and related proteins are important regulatory molecules that interact with other pathways to integrate signals, leading to various end points depending on the biological system (Kim et al. 2010b). Although it is difficult to interpret how this signaling cascade is modulated in an intact organism (in contrast to isolated cells in culture), this observation has some interesting implications for protecting cognitive function through age. ERK is capable of regulating other pathways, but specifically in neurons, it is necessary for the induction of long-term potentiation. Further, ERK signaling is involved in many hippocampal functions and therefore has significant implications for learning and memory. For example, blocking ERK signaling can impair recall after water maze training and impair the associative learning in an odor avoidance task. These intricacies have not yet been fully elucidated in regards to how AXT is modulating ERK signaling as a regenerative or neuroprotective mechanism. While ERK is known to be involved in neurogenesis, there is also some indication that AXT-induced activation of the ERK pathway may facilitate the release of NRF2, allowing the protein translocate to the nucleus and increase transcription of antioxidant defense mechanisms like HO-1. This upregulation of HO-1 was attributed to the cytoprotection from oxidative stress caused by exposure to amyloid beta, indicating another mechanism through which AXT can reduce oxidative stress and protect the CNS (Li et al. 2013). AKT, PI3K, and MEK are other commonly reported cell signaling cascades that are affected by AXT treatment. These proteins are involved in major signal transduction pathways with known roles in cell growth and differentiation. Xu et al. (2015) describe changes in these AKT-related molecules and attributed this pathway to mediate improved performance on the Morris water maze.

BDNF is decreased with age and is associated with reduced hippocampal volume and corresponding impaired spatial memory in aged humans (59–80 years old) (Wibrand et al. 2013; Wu et al. 2014). BDNF is an important nerve growth factor that not only promotes neurogenesis but also has a reported role in modulating synaptic transmission. While the upregulation of AKT or ERK could be the underlying mechanism driving stem cell proliferation in the hippocampus, AXT may also be influencing synaptic plasticity through inducing an upregulation in BDNF (Wibrand et al. 2013; Wu et al. 2014). Moreover, BDNF can facilitate LTP through ERK-dependent mechanisms (Peng et al. 2010). When ERK is activated by BDNF, it can lead to the transcription of genes needed for synaptic plasticity and neurogenesis (Stranahan and Mattson 2012; Wu et al. 2014). To date, there is little discussion of either of these processes being influenced by AXT treatment, although both synaptic plasticity and neurogenesis decrease with age and are associated with loss of cognitive function (Jenner and Olanow 1996).

Anti-inflammatory activities and modulation of microglial activity

Many recent studies have generated data that lends support of the putative anti-inflammatory effect of AXT. Increased inflammation is a characteristic of the

aged brain and is also a pathological feature of many neurodegenerative diseases. Much of the early evidence has shown that AXT has the capacity to modulate the immune response or reduce inflammation associated with maladies of the periphery including gastric ulcers and activity of T and B lymphocytes (Chew et al. 2011; Guerin et al. 2003; Kim et al. 2005; Otton et al. 2010). One exciting trend that has emerged from the current literature is the indication that AXT can specifically modulate microglial function, also described in Table 1 (Balietti et al. 2016; Choi et al. 2008; Kim et al. 2010b). Microglia are the resident macrophages of the brain and are intimately involved in the CNS immune responses. In what is commonly referred to as the "un-activated state," microglia will continuously scan their microenvironment with highly motile processes, facilitating the detection of potential homeostatic disturbances. These cells are rapidly activated when they encounter various danger signals such as invading pathogens or cellular damage and respond to these threats by releasing pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, and NO (Kettenmann et al. 2011). While this response is initially effective at neutralizing the threat, sustained microglial activation can become harmful to the CNS due to the cytotoxic nature of these pro-inflammatory molecules. This necessary immune response becomes dysregulated with age; microglia from the aged CNS are referred to as "primed" and are characterized by elevated basal output of pro-inflammatory factors even without an immune stimulus. Not only do these primed cells become hyper-reactive, increasing the output of inflammatory mediators in response to successive stimulation, but also become insensitive to the regulatory signals that terminate activation of microglia. These age-related alterations of normal microglial responses lead to chronically elevated levels of cytotoxic compounds that impair the otherwise healthy neural tissue (Ekdahl et al. 2003; Lynch 2009). It is known that microglial activation and inflammation increase with age and correlate to decreased neurogenesis and therefore cognitive function.

It has been reported that AXT treatment can reduce the expression and release of IL-6, Cox-2, and iNos/nitric oxide in a microglia stimulated with the bacterial antigen lipopolysaccharide in vitro (Choi et al. 2008; Kim et al. 2010b). Similarly, downregulation of IL-6 was observed in the transformed BV2 cell line, as well as primary macrophages from the periphery. These trends were recapitulated in an animal model of diabetic encephalopathy where they demonstrated that AXT treatment could reduce the neuropathology and cognitive impairments (Xu et al. 2015). These authors reported that AXT-treated mice improved their performance in the Morris water maze, associated with reduced NF-κB and neurodegeneration in the frontal cortex and hippocampus. Park et al. (2009) show that dietary treatment of AXT was able to lower the expression of several inflammation-related transcripts. Complement component 1q chain A (Cq1A), Ctss (cathepsin, an enzyme that facilitates MHC II antigen presentation), and glial acidic fibrillary protein (GFAP) are all known to increase with age and indicate a pro-inflammatory environment. The suppression of this age-related positive fold change is suggestive of a capacity for AXT to prevent or stall the shift to a pro-inflammatory environment that commonly occurs in aged organisms. Balietti et al. (2016)) show that 6 weeks of oral administration of AXT (10 mg/kg) was sufficient to modulate the expression of certain cytokines in the brains of aged rats. These authors examined both males and females and report divergent results in cytokine levels across brain regions of each gender. It was observed that AXT treatment reduced IL-1β in the hippocampus and cerebellum of aged female rats. In contrast, an increase of IL-10 was detected in the female cerebellum, while IL-10 was increased in the male hippocampus, showing that AXT supplementation may alter the cytokine activity differently among each gender. It is important to note that these authors reported increase in anti-inflammatory factors as well. The capacity for AXT to increase or rescue the expression of anti-inflammatory mediators like IL-10 and IL-4 may be just as important for the restoration of the trophic or repair functions of microglia that are blunted in the context of aging and neurodegenerative disease.

It should also be noted that chronically activated microglia are also a major source of reactive oxygen species. Given that AXT has significant anti-oxidative properties (discussed above), it may be possible that a significant effect of AXT on microglia is due to reductions in oxidative stress and damage, which results in a neuroprotective mechanisms (Koutsilieri et al. 2002). However, it seems that AXT is potentially having a dual

modulation of microglia by inhibiting both increased oxidation and pro-inflammatory activation of microglia.

Clinical trials

Some preliminary work has begun to examine the cognitive effects of AXT supplementation on humans. One trial tested male subject ages 50–69 that had symptoms of mild cognitive impairment. They were treated with AXT doses of 20 mg/day for 12 weeks. The subjects displayed improvements in performances on the CogHealth and P300 cognitive tests when compared to their own baseline data (Satoh et al. 2009b). The CogHealth battery is a standardized, computerized, and card-based design that assesses multiple domains of cognition. The P300 test uses auditory elements for recognition and association and is a better indication of selective attention and processing (Polich et al. 1986).

Similar results were observed in a controlled and double-blinded clinical trial that used low and high doses (6 and 12 mg/day) for 12 weeks, with male and female subjects ages 45–64. These subjects were also tested using CogHealth as well as the Groton Maze Learning Test (GMLT). The GMLT is a computer-based maze learning test that uses repeated trials to allow for the assessment of learning. By the end of the study, AXT-treated subjects had improved significantly, making fewer errors in the maze test in both low and high doses. The high-dose group also demonstrated faster reaction times in the CogHealth tests (Katagiri et al. 2012).

Another randomized, controlled, and double-blinded clinical study administered 6 and 12 mg doses to 50- to 69-years-old subjects (Nakagawa et al. 2011). After 12 weeks of diet, AXT levels and phospholipid hydroperoxides (PLOOH) level were measured from both plasma and erythrocytes. PLOOH is a lipid hydroperoxide that is produced as a result of phospholipid oxidation. In this study, plasma and erythrocyte levels of PLOOH were significantly decreased in both low- and high-dose groups compared to the control group. This finding maybe significant because it has been suggested that elevated erythrocyte membrane oxidation is associated with neurodegenerative diseases (Bosman et al. 1991; Giavarotti et al. 2013; Goodall et al. 1994; Kawamoto et al. 2005; Kawamoto et al. 2013; Kosenko et al. 2013).

Concluding remarks

AXT displays health-promoting effects in various diseases. The emerging research data in regard of the possible neuroprotective effects of AXT is particularly exiting due to the steadily increasing population with aging and neurodegenerative diseases. Elevated oxidative stress, inflammation, and diminished neurogenesis are all features of normal aging and neurodegenerative diseases. Besides the well-known antioxidant effect, AXT offers neuroprotection against normal aging and neurodegeneration by promoting neurogenesis through modulating microglial activity and important signaling molecules such as ERK, AKT, and BDNF in vitro and in vivo, therefore improving cognitive functions. Moreover, the efficacy of AXT in cognitive function in aged population is also shown in several human clinical studies, indicating possible neuroprotective effects. It is promising that AXT may be a safe and potent neuroprotective agent. Yet, there are far more to be discovered on how AXT exerts the protective effects in CNS.

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Compliance with ethical standards

Conflict of interest PCB is a member of the scientific advisory board for Nutrex, Hawaii; RDS was awarded funding from manufactures of AXT supplements.

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