

Astaxanthin attenuates neurotoxicity in a mouse model of Parkinson's disease

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ABSTRACT

Background: Astaxanthin (AXT) is a natural carotenoid with diverse biological activities. Although it is best known as a potent antioxidant, recent work suggests additional mechanisms of action that have the potential to oppose the ongoing pathophysiology of Parkinson's disease (PD). For example, AXT has a putative role in modulating microglial activity and preserving mitochondrial function, thereby implicating this compound as a neuroprotective agent. Both oxidative stress and inflammation are involved in the progression of many neurodegenerative diseases. Therefore, we examined the efficacy for AXT to reduced neurotoxicity in a toxic model of PD in mice.

Methods: In this study, we used a 4-week dietary supplementation of algae derived AXT to reduce 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced dopaminergic cell death.

Results: AXT treated mice were protected against the loss of tyrosine hydroxylase (TH) staining in the substantia nigra (SN) after MPTP exposure compared to the control diet. This effect of preserved TH immunoreactivity was also observed in the striatum. Furthermore, AXT administration was able to interrupt the neuroinflammatory process known to contribute to neurodegeneration in this model.

Conclusion: We demonstrate that AXT neuroprotection was associated with attenuated microglial activation as indicated by reduced immunohistochemical detection of IBA-1 in the SN and striatum of AXT treated mice. Altogether, these studies suggest that AXT has neuroprotective property in the central nervous system against MPTP neurodegeneration.

Keywords: Astaxanthin, Neuroprotection, Neurodegeneration, Neuroinflammation, Parkinson's disease

BACKGROUND

Parkinson's disease (PD) is the second most common neurodegenerative disorder. PD is anatomically defined by the loss of dopaminergic innervation to the striatum, leading to the characteristic motor dysfunctions: bradykinesia, rigidity of the limbs and a shuffling gait. According to the Parkinson's Disease Foundation, PD is estimated to afflict 10 million people worldwide, and is thereby associated with a significant economic burden. Furthermore, PD is projected to become more prevalent as the largest sector of the US population gets older [1]. Currently, the commonly prescribed medications are based on dopamine replacement strategies. While they effectively mitigate motor symptoms temporarily, these drugs often become less effective with chronic use. The current treatment options are not adequate; there are still no medications available to stop or delay the progression of the disease. Therefore, there is still a pressing need to identify compounds capable of modulating the disease process and preventing the development of symptoms among these patients.

While the exact pathological mechanisms that initiate neurodegeneration in PD are still being investigated, it is accepted that multiple biological processes are impacted sometime during the course of the disease that likely interact with and perpetuate the pathogenesis. Both neuroinflammation and oxidative stress are thought to be involved in neurotoxic cascades and contribute to cell loss. As a result, there has been significant focus on developing therapeutic strategies to attenuate these aberrant conditions in an attempt to alleviate some of the cellular stress that causes neuronal dysfunction.

Astaxanthin (AXT) is a xanthophyll carotenoid produced primarily by the marine algae *Haematococcus Pluvialis*. AXT is currently available as a health supplement, being marketed for its antioxidant capacity; however, recent research indicates that AXT has multiple putative mechanisms of action responsible for its various health benefits [2]. This compound is being investigated in relation to multiple clinical conditions including cardiovascular disease, metabolic syndrome, and athletic performance [3-5]. Interestingly, emerging evidence suggests that the proposed biological activities of AXT precisely oppose the pathophysiology that underlies Parkinson's disease, revealing a distinct and promising therapeutic potential in the prevention or delayed onset of symptoms in PD patients.

AXT is best known for its potent antioxidant activity, reported to be much more effective than other similar compounds [6]. This is likely due to the numerous actions as an antioxidant, as it can reduce radicals by absorption, donation of electrons, and formations of adducts with the reactive species. The presence of hydroxylated ionone rings that cap both ends of the carbon backbone distinguish the molecule from other carotenoids in its class, and enables AXT energetically

favorable spanning the phospholipid bilayer of cell membranes. This orientation and chemical structure effectively protects the membrane against lipid peroxidation [3].

Many reports indicate that AXT reduces the evidence of oxidative damage [7-11]. AXT can also stimulate the expression or the activity of endogenous antioxidant enzymes, including glutathione [7, 12-14]. Glutathione is expressed in low levels in the substantia nigra, being one of the factors that renders the SN more susceptible to oxidative damage compared to adjacent brain structures [15]. It has also been shown that there is less reduced glutathione in the brains of PD patients, which further indicates the involvement of this molecule and the oxidative status of the SN to clinical presentation [16]. Elevated levels of reactive oxygen species in this region promote pathological modifications of α -synuclein. For example, nitrosylated α -synuclein that readily occurs in an oxidized environment has been shown to stimulate microglial output of pro-inflammatory compounds and is thereby associated with neuronal dysfunction and degeneration [17]. AXT may lower oxidative stress by directly neutralizing radicals and also through the modulation of antioxidant response signaling cascades.

AXT has also been suggested to modulate the immune response to various insults, which indicates that there is a potential anti-inflammatory action for this compound. Multiple investigators have demonstrated that AXT can reduce the expression of inflammatory mediators including: inducible nitric oxide synthase and nitric oxide (iNOS/NO), nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), and interleukin 6 (IL-6) [11, 18-20]. These secreted factors are released in high amounts by activated microglia and are associated with neuronal damage. A modulatory action of AXT on these microglial responses to brain insults represents a possible approach to interrupting one of the factors known to perpetuate neuronal dysfunction in the PD brain.

Interestingly, AXT has been shown to be protective in cell culture systems against the toxic compounds used to produce *in vitro* experimental models of PD. For example, Lui et al. (2009) demonstrate that AXT pretreatment effectively protects SHSY5Y cells against 6-hydroxydopamine, a commonly used toxin that degenerates the dopaminergic cells primarily as an oxidative insult. In this study, AXT attenuated neurotoxicity was associated with reduced ROS production, in addition to DNA fragmentation, cleavage of poly (ADP-ribose) polymerase, and cytosolic cytochrome C [9]. AXT pretreatments have also successfully protected PC 12 cells from 1-methyl-4-phenylpyridinium (MPP⁺) [14, 21-23]. MPP⁺ is a toxic derivative of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), another established agent used to recapitulate dopaminergic cell degeneration and is also widely used to model PD. In light of how AXT has been shown to be protective in cell culture systems against PD specific compounds, we evaluated the efficacy of AXT supplementation to prevent neurodegeneration *in vivo* at a physiological relevant dose.

In the present study, we explore the capacity for a dietary pre-treatment of AXT to protect against neuronal damage caused by the neurotoxin MPTP. C57BL6 mice (3 months) were treated with AXT for one month. These mice consumed an AXT enriched diet formulated to deliver a dose of 3mg/kg/day (treatment group-AXT). Multiple studies have reported a range of doses of AXT that were used therapeutically across various disease models; this dose was chosen in order to determine the efficacy of the physiological levels that would occur in humans taking the recommended dose of commercially available dietary supplements. Additional groups were fed

the standard grain based rodent diet (control treatment groups; CTL) or the control diet containing the inactive ingredients of the AXT supplement product to serve as the vehicle control condition (vehicle treatment group; VEH). After the month of dietary pre-treatment, half of the mice from each dietary condition received 4 injections of MPTP at 10 mg/kg intraperitoneal once per hour for 4 hours to achieve a total dose of 40mg/kg. They were then allowed to recover for 7 days before euthanasia. In this study, we show that the AXT supplemented diet protected against neurodegeneration and inflammation which typically occurs after exposure to MPTP.

METHODS

All procedures were conducted according to the National Institute of Health Guide and Use of Laboratory Animals and the University of South Florida IACUC. Male C57BL/6J mice at 3 months of age were maintained in environmentally controlled conditions of 21°C under a 12 hour light/dark cycle. The mice were allowed *ad libitum* access to food and water. However, the food was consistently monitored and replaced daily by research staff to maintain freshness.

Dietary pretreatment: Mice were treated with 3 mg/kg of Bioastin® generously supplied by Cyanotech. This is a natural astaxanthin product delivered on inert cellulose beads with trace amounts of vitamin E. For this reason, we included a separate group treated with the empty beads to serve as a vehicle control. These compounds were incorporated into the Harlan Teklad rodent diet, and delivered *ad libitum* for 1 month prior to MPTP administration. Food intake per cage and individual body weights were monitored throughout the course of treatment to ensure consumption.

MPTP administration: The MPTP-HCL (Sigma-Aldrich) was diluted in sterile saline and injected intraperitoneally. The mice received four injections at a dose of 10 mg/kg, administered once per hour four hours for a final dose of 40 mg/kg MPTP or equal volumes of sterile saline for control conditions. Mice were allowed to recover from MPTP exposure for 7 days before they were fully anesthetized with phenobarbital and transcardially perfused with phosphate buffered saline. Brains were immediately removed and one hemisphere was microdissected for areas of interest. The other hemisphere was preserved in 4% paraformaldehyde for 24 hours. The brains were then transferred to a 30% sucrose solution for cryoprotection.

Immunohistochemistry: 40 µm coronal sections were selected at a periodicity of 1 in 6 for all immunohistochemical procedures; additional sections were included both anterior and posterior to the SN to ensure thorough representation of the area of interest. Free floating sections were incubated in primary antibody (TH: Immunostar 1:1,000, IBA1: Wako Laboratory Chemicals 1:2,500, NeuN: Millipore 1:5,000) diluted in goat serum and TX-100 for 24 hours at 4°C and secondary antibody for 60 minutes at 25°. After incubation in avidin-biotin complex (Vector Labs), precipitation reactions were developed with diaminobenzidine (Sigma-Aldrich).

Quantification: Immunoreactivity was quantified using an AxioScan microscope (20X objective) and NearCYTE image analysis software (nearcyte.org). This program applies a user defined

threshold of color intensity to images of the sections and generates a ratio of positive staining within a region of interest. Our lab has previously determined that data collected with the NearCYTE software accurately reflects stereological cell counts [24].

Glutathione assay: Briefly, plasma samples isolated from whole blood were immediately diluted 1:20 in a 5% solution of sulfosalicylic acid for deproteination and preservation. All subsequent steps in this assay was performed according to manufacturer’s instructions (Arbor Assays).

GDNF ELISA: Briefly, snap frozen samples of striatum were quickly homogenized and centrifuged to collect lysates. Protein concentration was determined and 40 ug of sample was loaded into the wells while the rest of the assay was conducted according to manufacturer’s instructions (BOSTERbio).

Data Analysis: The data presented graphically as the group mean and standard error of the mean. Statistical analysis was performed using GraphPad Prism software. 2 Way ANOVAs were conducted, while Bonferroni multiple comparisons posthoc tests (unless otherwise specified) were used to further compare differences between groups.

RESULTS

AXT prevents MPTP induced neurodegeneration

As expected, we observed approximately 50% loss of tyrosine hydroxylase positive staining in the substantia nigra of control mice one week after MPTP administration. Mice that were fed a vehicle control diet demonstrated a similar TH loss as the controls. However, the AXT enriched diet was able to preserve the TH expression in this region (Figure 1).

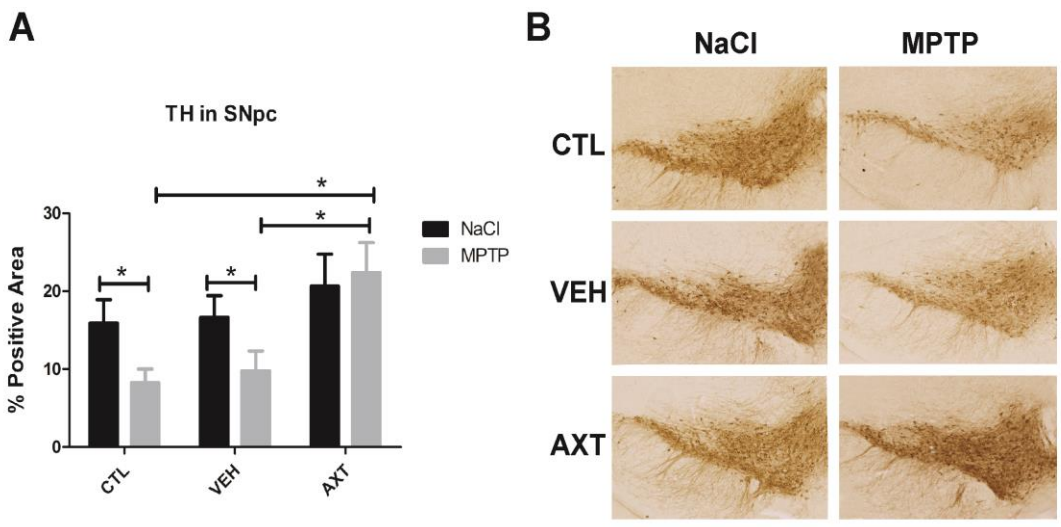


Figure 1. One month dietary supplementation with AXT preserves TH immunoreactivity in the substantia nigra pars compacta in response to MPTP. A) Bar graph of percent area of positive staining of TH relative to a user defined threshold. B) Immunohistochemistry with anti-TH antibody in the SN. 2-way ANOVA; diet effect: DF 2, F 4.458; Bonferroni posthoc p<0.05; Scale = 100 μm

Likewise, we also observed reduced positive staining for tyrosine hydroxylase in the striatum of mice that were fed the control and vehicle diets. However, mice that consumed a diet enriched with AXT were protected from this loss of TH immunoreactivity, indicating less destruction of the terminals that innervate the basal ganglia (Figure 2). It is known that MPTP treatment can directly reduce the levels and efficacy of the TH enzyme, and this deficit may be transient. Therefore, an additional immunohistochemical assessment was conducted to assess the surviving neurons in the SN. The digital image quantification of neuronal nuclei (NeuN) staining revealed that the AXT enriched diet reduced neuron loss 7 days after MPTP exposure in the SN compared to the other diets, corroborating the trends observed in TH retention (Figure 3).

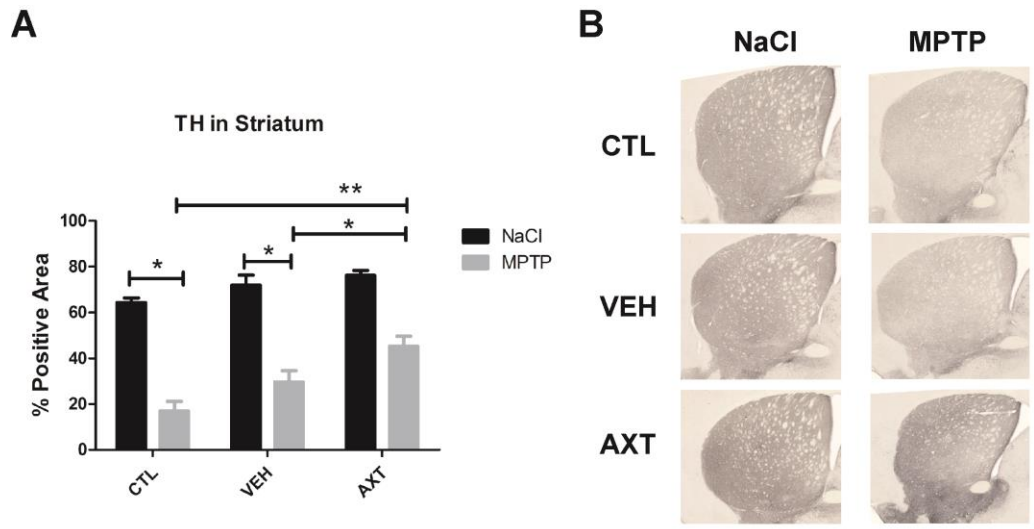


Figure 2. One month dietary supplementation with AXT reduces TH immunoreactivity loss in the striatum in response to MPTP. A) Bar graph of percent area of positive staining of TH relative to a user defined threshold. B) Immunohistochemistry with anti-TH antibody in the striatum. 2 way ANOVA, Diet effect DF 2, F 14.08 Newman-Keuls *p<0.05 **p<0.001; Scale = 200 μm

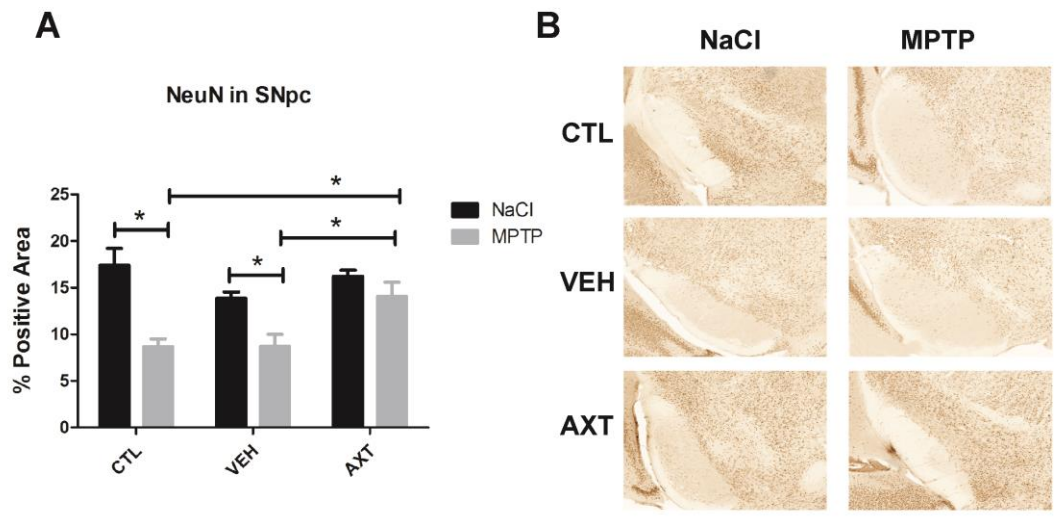


Figure 3. One month dietary supplementation with AXT preserves neurons in the SN in response to MPTP. A) Bar graph of percent area of positive staining of NeuN relative to a user defined threshold. B) Immunohistochemistry with anti-NeuN antibody in the SN. 2-way ANOVA, Diet effect DF 2, F 4.2. Tukey multiple comparison p<0.05; Scale = 200 μm

AXT alters microglial response to MPTP neurotoxicity

It has been postulated that AXT may modulate microglial function and their reactivity to various insults [18, 25, 26]. Microglial express ionized calcium-binding adaptor molecule protein 1 (IBA1) at basal levels, but are known to upregulate this molecule when exposed to certain immune stimuli; the induction of IBA1 typically indicates microglial activation. Therefore, we examined the expression of IBA1 in the striatum and SN. In this study, we demonstrate how MPTP leads to an increase of IBA1 in the striatum in the control and approaching significance in VEH treated diets. In contrast, AXT supplementation minimized the increase in expression of IBA1 (Figure 4). Interestingly, we did not observe a significant increase in IBA1 in the SN of control MPTP treated animals but we observed that AXT treatment reduced basal levels of microglial expression of IBA1 in the SN compared to control mice (Figure 5). This lower basal level of IBA1 was maintained in the MPTP treated group with AXT treatment (Figure 5).

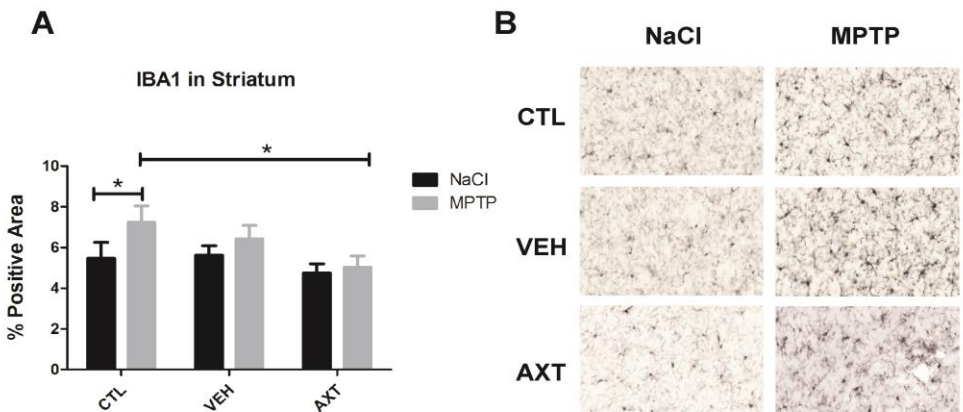


Figure 4. Dietary intervention of AXT modulates microglial response to the neurodegeneration that results from MPTP neurotoxicity. A) Bar graph of percent area with positive staining of IBA1 in the striatum relative to a user defined threshold. MPTP induced upregulation of the microglial marker IBA1 is attenuated in the striatum of mice being treated with AXT 2-way ANOVA, Diet effect DF 2, 3.3 F; Tukey’s multiple comparison (*p< 0.05); B) Immunohistochemistry with anti-IBA1 antibody in the striatum. Scale = 20 μm

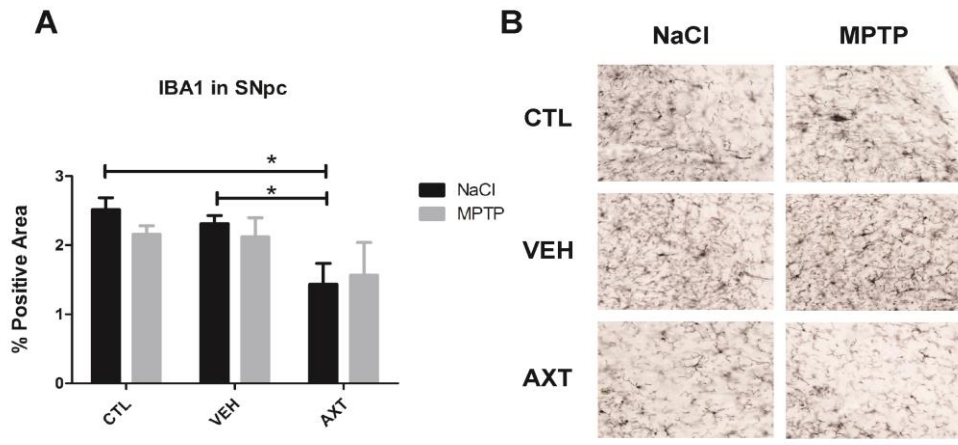


Figure 5. Dietary intervention of AXT modulates microglial response in the SN. A) Bar graph of percent area with positive staining of IBA1 relative to a user defined threshold. IBA1 in the SN is reduced with AXT treatment. 1 way ANOVA of saline treatment with Bonferroni multiple comparison (*p < 0.05); B) Immunohistochemistry with anti-IBA1 antibody in the SN. Scale = 20μm

Antioxidant effect

Oxidative stress is considered to be involved in both the pathogenesis of PD and to contribute to the neurotoxicity of MPTP [27]. Many therapeutic strategies aim to mitigate the damage caused by excessive release of reactive oxygen species. It has been suggested that AXT can increase the efficacy of endogenous antioxidant enzymes, either by increasing their expression levels or their enzymatic activity. As previously mentioned, it is well documented that AXT has an antioxidant effect and can protect biological systems, including both cell culture and whole organisms from oxidative damage, [10, 11, 14, 23, 28]. Therefore, we assessed the levels of glutathione in the plasma. In our study, one month of AXT supplementation significantly elevated the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSH). Even after exposure to MPTP, the AXT treated animals still retained a more favorable ratio of GSH to GSSH than the control mice, despite the increased output of reactive oxygen species known to occur in this model (Figure 6). In contrast, mice on the standard control diet had lower levels of GSH after MPTP exposure.

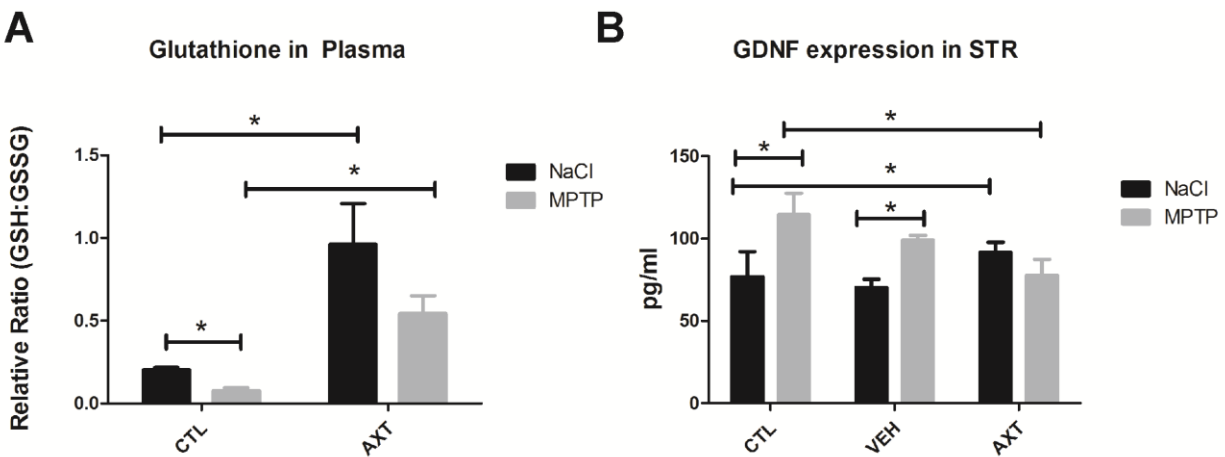


Figure 6. A) AXT increases the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG). Bar graph of the relative ratio of GSH:GSSH in plasma. AXT increases the ratio in both MPTP treated and non-MPTP animals compared to control animals. 2-way ANOVA Diet effect: DF 1, F 14.51; Bonferroni multiple comparisons ($*p<0.05$). B) MPTP exposure is associated with increased expression of GDNF in the striatum. AXT treatment suppresses the elevation of GDNF in response to MPTP. 2 way ANOVA Diet effect: DF 1, F 3.8; Bonferroni multiple comparisons ($*p<0.05$) and independent T-tests.

AXT modulates growth factor expression in the striatum

It has been shown that AXT can stimulate the expression of brain derived neurotrophic factors in various brain regions *in vivo*. We evaluated another growth factor, GDNF, because of its expression in the striatum and relevance to Parkinson's disease. GDNF is necessary for the postnatal development of dopaminergic cells within the midbrain and stimulates neurite outgrowth, facilitating integration and connectivity of the new cells. Incubating fetal grafts in GDNF also aids in the survival of transplantation into the ventral midbrain [29, 30]. GDNF has been evaluated for its therapeutic potential for supporting fiber growth and re-innervation of the striatum during the progressive cell loss in PD. Several preclinical animal studies have demonstrated that enhancing GDNF expression within the striatum can offer protection against toxic injury. For example, the viral vector driven expression of GDNF in the striatum of the

common marmoset was found to be protective against the degeneration of dopaminergic terminals caused by 6-hydroxydopamine injections. This neuroprotection was observed with GDNF levels moderately above physiological levels and was associated with improved behavioral outcomes compared to the lesioned animals [31]. In this study, we show how MPTP treated mice on either the control or vehicle control diets have higher levels of GDNF in the striatum compared to the saline injected animals (Figure 6). In contrast, we did not observe this increase in the mice that consumed the AXT enriched diet. Furthermore, AXT treatment alone seemed to elevate the expression of GDNF in the striatum compared to the control diet in the saline injected mice.

DISCUSSION

While PD is common among the elderly, there are no available medications to cure, prevent, or delay the progression of the disease. Levodopa and other routinely prescribed medications are only palliative; they are effective at restoring dopamine. However, this only addresses motor symptoms and does not prevent ongoing neurodegeneration. Therefore, elucidating disease modifying agents and developing strategies to delay or prevent the onset of symptoms are critical for the treatment of PD. AXT is a possible disease modifying agent that can interrupt various mechanisms involved in the toxic cascade of MPTP. This is noteworthy because neurotoxic mechanisms of MPTP, such as oxidative damage, mitochondrial dysfunction (complex 1 impairment), and neuroinflammation are also known to be involved in the pathogenesis of PD. Other studies have supported the therapeutic strategy of using natural compounds in the treatment of disorders with similar pathological elements of inflammation and oxidative stress [32]. Mitigating multiple pathological mechanisms is a clear advantage of an AXT based therapeutic strategy. Furthermore, this compound can be considered for use chronically as a preventative or adjuvant therapy, as there are little to no adverse effects reported to date.

AXT is known to cross the blood brain barrier and accumulate within the brain tissue in addition to other organs [33]. Multiple studies have shown that AXT may also be beneficial specifically within the central nervous system for a variety of neurological injuries and disorders. For example, AXT supplementation has been purposed to augment cognitive function and neuroplasticity something which has been recently reviewed [34]. AXT was protective in a mouse model of traumatic brain injury [35]. Ji et al (2017) demonstrated that oral AXT treatment prevented the loss of cortical neurons and the associated behavioral impairments usually detectable in mice after traumatic brain injury. Similarly, AXT administration was also able to reduce the damage in a model of subarachnoid hemorrhage; AXT decreased edema, blood brain barrier dysfunction, and oxidative damage ultimately contributed to neuronal loss observed in the control animals [28]. These recent *in vivo* reports support the therapeutic potential of AXT for neurological based diseases.

In the present study, we used astaxanthin, a naturally derived carotenoid, to mitigate the neurotoxicity using an MPTP mouse model of Parkinson's disease. Our data suggests that the mechanisms underlying AXT mediated neuroprotection are related to modulating inflammation and oxidative stress. We determined that AXT supplementation effectively protects against the loss of dopaminergic neurons in the substantia nigra and their TH enriched terminals in the striatum. We also show that dietary AXT administration altered the microglial response to MPTP

induced neurodegeneration, as IBA1 induction was not observed in the AXT treated animals. These results are consistent with previous work showing how AXT in cell culture can attenuate the toxicity of 6-hydroxydopamine and MPP+ [9, 14, 21, 23]. It also corroborates many preliminary studies that demonstrate AXT treatment may modulate the output of inflammatory cytokines. For example, AXT has been shown to reduce transcripts of proinflammatory mediators [20]. Functionally, the administration of AXT can block some inflammatory sequelae of the immunogen lipopolysaccharide injections [25] and can inhibit NF κ B translocation to the nucleus, thereby down-regulating TNF- α expression [36]. Treatments that reduce microglial activation such as fractalkine [37] or the Rho Kinase inhibitor Fasudil [38] are known to reduce the neurotoxicity of MPTP. Therefore, it is likely that the effect of AXT to reduce IBA-1 is directly related to the neuroprotection observed in this study.

We also show that AXT intake was associated with a more favorable ratio of GSH reduced glutathione to GSSH, oxidized glutathione in the plasma. Reduced glutathione indicates less oxidative stress, possibly either from increased levels or increased efficiency of glutathione to neutralize free radicals in the cellular environment. As discussed previously, glutathione status is of interest in Parkinson's disease, as the levels of this endogenous antioxidant molecule are substantially decreased within the PD brain and is thought to perpetuate oxidative damage that contributes to neuronal atrophy. Interestingly, pharmacological reduction of glutathione *in vitro* has been directly linked to nitrosylation of mitochondrial proteins leading to complex 1 deficiency. Mitochondrial dysfunction is another pathological characteristic of the PD brain [39]. Furthermore, measuring glutathione in the plasma correlates with the severity of cognitive dysfunction in Alzheimer's disease patients [40] confirming that there is a relationship between peripheral GSH levels and brain function.

The AXT modulation of glutathione observed in this experiment is consistent with other studies. Mattei et al. (2011) also demonstrates how AXT administration leads to improved antioxidant capacity including an increased ratio of GSH:GSSH in the plasma [41]. AXT was shown to increase GSH levels in the liver of animals exposed to the chemical carcinogen carbon tetrachloride (CCl₄). Kang et al. (2001) attributed the attenuation of CCL₄ induced liver toxicity to the AXT stimulation of the endogenous antioxidant system [42]. Likewise, AXT has also been shown to reduce excitotoxicity in an animal model of epilepsy. These authors demonstrate that AXT treatment reduced neuronal apoptosis and upstream oxidative damage induced by overstimulation of the amygdala. This protective effect was associated with an increase in glutathione and a decrease output of reactive oxygen species [10]. While oxidative stress plays a role in the pathogenesis of Parkinson's disease, it is not the only major biological process negatively impacted during the course of disorder. It is notable that many antioxidant compounds have failed in clinical trials. While the antioxidant capacity of AXT likely reduces some neuronal dysfunction due to minimizing rampant oxidative damage, other suggested mechanisms of action likely come together and work in a synergistic or cumulative way to reduce the neurodegeneration seen in this model.

Finally, we observed an AXT mediated effect on GDNF expression in the striatum. As previously mentioned, GDNF has been investigated as a therapeutic target in the treatment of

Parkinson's disease. Preclinical animal work supports the efficacy of GDNF based therapies as this growth factor has been shown to both be necessary for post-natal dopaminergic cell differentiation and survival in the mesencephalon, in addition to enhancing the successful integration of fetal tissue transplanted into the ventral midbrain [29, 30]. Additionally, upregulating GDNF expression above physiological levels using adeno associated viral vector effectively protected against degeneration caused by the 6-hydroxydopamine toxin and prevented the subsequent behavioral impairments [31]. It is known that growth factor expression can be stimulated by neuronal injury in effort to restore the damaged site [43]. We speculate that the increase of GDNF that we observed after MPTP exposure in our experiment was a compensatory mechanism in response to the lesion formed after the toxic insult. Likewise, it is also possible that even a subtle increase in striatal GDNF in the mice supplemented with AXT was sufficient to reduce some of the cell death caused by MPTP in the first place.

CONCLUSION

In conclusion, we have demonstrated an effect of AXT to reduce neurodegeneration in the MPTP model of PD. We show a concomitant change in the factors of inflammation and oxidative stress, suggesting these processes are associated and likely mediate the protective effect of AXT against MPTP. MPTP is a potent neurotoxin, and one caveat of this model is its rapid time course and dramatic dopaminergic cell loss does not recapitulate the slow progression of pathology seen in PD patients. Accordingly, future studies that use AXT in additional models of PD, like the viral vector mediated over expression of synuclein, will establish a role in disease progression as well as prevention.

List of Abbreviations: AXT, astaxanthin; MPP⁺, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SN substantia nigra; STR, striatum; iNOS/NO, inducible nitric oxide synthase and nitric oxide; NF κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; IL-6, interleukin 6; TH tyrosine hydroxylase; NeuN, neuronal nuclei; IBA1, ionized calcium-binding adaptor molecule protein 1; GSH, reduced glutathione; GSSH, oxidized glutathione; GDNF, glial derived neurotrophic factor; CCl₄, carcinogen carbon tetrachloride.

Competing Interests: PCB serves on the scientific advisory board for Nutrex, Hawaii, the company that provided the AXT.

Authors' Contributions: BG, CH, and LD conducted experiments, processed tissues, collected and analyzed data. BG, KN, and PCB designed experiments and interpreted data. BG, LD, KN, and PCB contributed to writing the manuscript.

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