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Review Article

Spirulina Rising: PhyCB, Treg Induction, and AST

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Abstract

Phycocyanin (PhyCB), derived from specific microalgae, has demonstrated a potential to inhibit or diminish the adverse effects of oxidative stress and NOX in mice models. Treg induction and astaxanthin (AST) are vital factors to consider in further evaluating the bioactive and relevant pathways of discouraging disease and encouraging health. Treg cells play a vital role in inhibiting and regulating autoimmune disorders. PhyCB may replicate the Treg inductive activity of the biliverdin-bilirubin pathway. AST is a natural antioxidant that may be beneficial to mitochondrial membranes and other cellular membranes; thus, opposing oxidative stress to the mitochondria and the adverse production of NOX. AST has been shown to protect mitochondria from oxidative damage in several cell culture studies. AST has been found to promote a more selective utilization of fat during exercise, which with PhyCB, should prove useful for endurance in athletes, or for those who want to use exercise training to lose weight or maintain proper weight. Also, AST can have a favorable impact on metabolic syndrome. The concurrent administration or supplementation of PhyCB and AST displays promise in treating or preventing a wide array of human disorders.

Keywords: *Astaxanthin; Microalgae; Omega-3 Fatty Acid DHA; Phycocyanobilin; Phyconutrients; Spirulina; Treg Cells*

Abbreviation

AST: Astaxanthin; ATP: Adenosine Triphosphate; DHA: Docosahexaenoic Acid; HO-1: Heme Oxygenase-1; NOX: Nox Reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase; O_2: Oxygen; PhyCB: Phycocyanobilin

Introduction

The oral administration of phycocyanin (PhyCB) or whole Spirulina has shown the potential to prevent or treat many human conditions caused by oxidative stress and the overproduction of bilirubin-inhibited NOX. PhyCB or whole Spirulina could be used to eliminate or ameliorate such conditions. However, other pathways and factors should be considered regarding the use of specific microalgae for therapeutic purposes or as supplemental food sources for humans, such as Treg induction and astaxanthin (AST), respectively.

Discussion

Treg induction: an independent target for bilirubin and PhyCB

The inhibition of NOX may not be the only crucial physiological role for bilirubin. Several studies have suggested that heme oxygenase-1 (HO-1) and its product bilirubin may promote the formation of a type of regulatory immune cells known as Treg cells (regulatory T cells). Treg cells play a vital role in preventing and controlling autoimmune disorders [1–4]. It is unlikely that NOX inhibition is responsible for this effect; superoxide produced by immune cells (macrophages) is required for the effective induction of Treg cells [5,6]. Moreover,

lymphocytes (of which Treg cells are a subclass) do not express NOX activity. Thus, the impact of bilirubin on Treg induction presumably reflects an interaction of bilirubin with some molecular target other than NOX. The nature of this target remains to be defined. Some scientists suspect that this target may be biliverdin reductase, the enzyme that converts biliverdin to bilirubin [7]. Biliverdin reductase has a range of cellular functions independent of its ability to convert biliverdin to bilirubin [8].

Treg induction could be helpful in managing autoimmune disorders. Injections of bilirubin are therapeutic in the mouse model of multiple sclerosis, and experimental autoimmune encephalomyelitis [9,10]. Conceivably, the Treg inductive activity and the antioxidant activity of bilirubin play a role in this favorable reaction. Treg cells help to prevent the rejection of transplanted organs that are not a perfect genetic match. Dr. Fritz Bach, a pioneer of transplant immunology, has demonstrated that biliverdin administration can prevent rejection of mismatched heart transplants in rats. The induction of Treg activity is a possible reason for this protective effect [11,12].

Could PhyCB replicate the Treg inductive activity of the biliverdin-bilirubin pathway? Cuban scientists reported that the incubation of lymphocytes with phycocyanin in cell culture influenced the conversion of these lymphocytes into Treg cells [13]. Also, these scientists showed that phycocyanin injections protected mice from the autoimmune syndrome, experimental autoimmune encephalomyelitis, previously reported in response to bilirubin administration. Phycocyanin prevented the syndrome, if it was administered before the injection of the agent that triggers it. The Treg inductive activity of phycocyanin in cell culture reflects the lymphocytes' ability to assimilate phycocyanin and degrade it, liberating the PhyCB—that then mimics the bilirubin's physiological role in Treg induction. Thus, PhyCB may have potential for preventing and treating various autoimmune conditions as it combines potent antioxidant activity with the ability to promote Treg formation. Similarly, PhyCB may have value in transplant medicine—although the impact of Spirulina or its components on transplant tolerance has not yet been studied adequately.

There are further considerations regarding Spirulina. Spirulina's cell wall polysaccharides have specific immune-stimulantory effects, that could prove counterproductive in the treatment of autoimmune disorders or transplant medicine [14–16]. If so, PhyCB-enriched extracts of Spirulina rather than whole Spirulina might be more appropriate under such circumstances.

Treg activity is not uniformly beneficial. There is considerable evidence that many cancers recruit Treg cells that protect them from rejection by the body's immune system [17]. However, the antioxidant activity of PhyCB might have direct growth-retardant effects on specific tumors and suppress other mechanisms (myeloid-derived suppressor cells) that tumors use to evade immune rejection [18–20]. When whole Spirulina is administered, the cell wall polysaccharides might exert immunostimulant effects, that could aid tumor rejection [16]. Hence, it might be more appropriate for cancer patients to use whole Spirulina rather than PhyCB-enriched extracts.

Further research in rodent cancer models, and ultimately human clinical studies, will be necessary to determine whether there is a role for PhyCB or Spirulina in cancer treatment, and to determine which Spirulina preparation is most appropriate to use. The administration of Spirulina with drugs that are known to suppress Treg induction, might prove effective in treating specific cancers [21,22].

Astaxanthin (AST): nature's prime cell membrane protector

Mitochondria are primary loci of oxidative stress in human cells. The low natural rate of superoxide production by healthy mitochondria can increase dramatically when the membranes of mitochondria are damaged. Oxidative stress is the most common mechanism that damages these membranes. When damaged by the oxidative stress induced by activated NOX, mitochondria can become pernicious sources of superoxide [23,24]. (Conversely, the oxidative stress produced by damaged mitochondria can often boost NOX activity by inducing increased synthesis of NOX subunits.) Thus, an antioxidant that is very effective for controlling oxidative damage to mitochondrial membranes should help prevent excessive mitochondrial superoxide production.

Astaxanthin (AST)— "x" is pronounced "z"—is a natural antioxidant for mitochondrial membranes and other cellular membranes. AST is a phytochemical produced by specific types of microalgae. AST is an oxygenated derivative of beta-carotene; such derivatives are known

as xanthophylls and include the compounds lutein and zeaxanthin, that function to protect the retina from light-induced oxidative stress. AST appears to have the highest antioxidant activity of any of the xanthophylls. Like other xanthophylls, it is a poorly water-soluble, linear molecule. It tends to integrate into cellular membranes perpendicular to the plane of the membrane, with the oxygenated portions of the molecule protruding into the cellular fluid. (AST appears to have been "designed" by nature to fit perfectly into such membranes.) These oxygenated portions of the molecule are capable of donating electrons and are mainly responsible for AST's high antioxidant activity. AST is a superior antioxidant as it is more oxygenated than other xanthophylls [25–28]. The interior region of AST, situated within membranes, also shares the ability of other xanthophylls to scavenge a specific antioxidant type, known as singlet oxygen. Singlet oxygen is generated by the interaction of UV light with specific photosensitizing compounds in cells.

Xanthophylls are typically found in photosynthetic organisms that require high-light exposure to survive, and in a portion of the human retina, the macula, that receives the most intense light exposure. Some microalgae are abundant in AST. Strains of *Haematococcus pluvialis* can contain up to 1–3% AST by dry weight and are cultivated as a source of AST used in animal feeds and human nutraceutical supplements [29–31]. AST is found in animals that consume AST-rich algae, directly or indirectly, and in many crustaceans. (AST is the source of the pink color in salmon and flamingoes.) A synthetic source of AST has been developed as an additive in fish food (in one application, to make farmed salmon a pink color). However, the synthetic version is not entirely natural in structure: it is racemic, containing an unnatural form that is a mirror image of the natural form. This synthetic version of AST is not approved for use in human nutrition.

Most humans consume little AST in their diets (unless they consume an abundance of salmon or shrimp); however, humans can absorb AST efficiently and distribute it to their tissues. It is not known if AST can function as efficiently as lutein or zeaxanthin to protect the retina from light-induced oxidative stress. Unique transport mechanisms concentrate lutein and zeaxanthin in the retinal macula photoreceptors. It has not been determined if these mechanisms work comparably well in concentrating AST in the macula. In mice, dietary AST is assimilated into the retina, which can help retinal photoreceptors survive specific stresses. Thus, AST might serve as a retinal antioxidant in humans [31–34].

AST appears to be more effective than other natural antioxidant studied thus far for protecting cellular membranes from oxidative damage—decidedly more effective than vitamin E. When cells are exposed to AST, a high proportion of AST is incorporated eventually into the mitochondrial membranes. Many of the favorable effects of AST supplementation, observed in rodent and clinical studies, are likely attributable to its ability to prevent structural damage to mitochondria subjected to oxidative stress [35–38]. Not only do these biocharacteristics of AST influence the mitochondria to function efficiently in producing sources of useable biochemical energy (ATP), but they also prevent the sorts of damage to mitochondria that can induce mitochondria into potent sources of superoxide. Hence, AST serves a dual purpose in protecting cellular membranes from oxidative damage and inhibiting mitochondrial superoxide production that gives rise to oxidant stress. (These factors indicate the prospect of a joint administration of PhyCB and AST to control the two primary sources of cellular oxidative stress, NOX and mitochondria.

AST's potential as a mitochondrial antioxidant has been demonstrated by a number of studies showing that the preadministration of AST (or of a synthetic water-soluble derivative of AST that can be administered by intravenous infusion and is converted to AST in cells) provided protection from a particular type of tissue injury, known as ischemia-reperfusion damage [39–41]. When tissues are subjected to a temporary cut-off of blood flow (ischemia), the inner membranes of mitochondria are prone to oxidative damage. When circulation is restored (if thrombolytic therapy is used to clear a blocked artery after a heart attack or stroke), the renewed availability of oxygen to the tissue leads to an intense burst of superoxide production by the damaged mitochondria, often resulting in severe tissue damage. Much of the tissue damage and death that result from a heart attack or stroke occur after the circulation is restored to the affected tissue. AST is one of the most effective agents known for preventing this type of damage, providing it is preadministered (before the temporary cut-off of the blood flow). This result has been demonstrated in rats, rabbits, and dogs when coronary arteries have been temporarily occluded. The extent of heart muscle damage that resulted from such experimentation was reduced by up to 70% in animals pretreated with AST.

In rats, this compound protected the brain and the liver from ischemia-reperfusion damage [41,42]. Oral administration of phycocyanin was shown to counteract ischemia-reperfusion damage in rodents, perhaps because NOX becomes activated under these circumstances [44–46]. The tissue damage caused by sleep apnea or sickle cell disease may be similar in origin (ischemia-reperfusion), as a temporary deprivation of oxygen in affected tissues is responsible for such damage. Thus, AST and PhyCB might be helpful in these disorders.

The ability to protect mitochondria from oxidative damage was demonstrated in several cell culture studies [35–38]. One of these studies suggested that AST has the potential for preventing Parkinson's disease and other types of neurodegeneration associated with oxidative stress. A more recent mouse study showed that AST pretreatment is markedly protective in the leading model for Parkinson's disease [47]. In another study in diabetic mice, oral AST decreased the fibrotic kidney damage (glomerulosclerosis) caused by hyperglycemia [35,48]. Also, AST preserved the function of beta cells in genetically-obese mice prone to diabetes [49].

The only study to examine the impact of AST on cholesterol-induced atherosclerosis, found that AST failed to protect cholesterol-fed rabbits [50]. This finding contrasts with the marked efficacy of PhyCB in this regard and likely reflects that NOX, rather than mitochondria, is the chief source of oxidative stress in vascular cells exposed to excessive levels of cholesterol-rich LDL particles. However, mouse studies revealed that AST has the potential for promoting leanness. When mice are allowed to run on treadmills, presupplementation with AST enhances their endurance and promotes a more selective utilization of fat for fuel [51,52]. The increase in endurance presumably reflects, in part, a sparing of glycogen stores, so that it takes longer for running mice "to hit the wall" when glycogen is depleted. When mice were allowed to run five times weekly for four weeks, the AST-supplemented mice eventually expressed 16% less body fat (epididymal fat) than those mice not supplemented. Similarly, when mice were fed a high-fat diet inducing obesity, the AST-supplemented mice gained less weight (despite comparable calorie intake) and burned fat at a higher rate throughout the day than the non-AST-supplemented mice [53].

AST's proclivity to promote a more selective utilization of fat during exercise is consistent with Spirulina [54]. It could be interpreted that the oxidative stress generated in muscle by prolonged exercise might selectively damage the capacity of mitochondria to utilize fat for fuel. In one AST-mouse study, researchers found that the enzyme that is rate-limiting for fatty acid uptake into mitochondria was oxidatively modified in exercised mice. However, the long-term administration of AST alleviated the extent of this oxidative damage [52]. Thus, the combination of PhyCB and AST may prove favorable for endurance athletes (in whom fat is a major fuel) and in people who exercise to attain or maintain weight control.

AST has not been studied in rodent models of liver disease (although it did decrease liver fat levels in mice on a high-fat diet). There is considerable evidence that mitochondrial damage may be responsible for the hepatic oxidative stress that characterizes and promotes tissue damage in non-alcoholic fatty liver disease, the leading cause of liver failure in the United States [55–57]. AST in conjunction with other antioxidant measures, may prove valuable in treating this disorder.

First clinical trials with AST

Historically, as there were so few adequately controlled clinical studies with AST, it was difficult to ascertain what dose to recommend, or to conclude that AST had the practical potential for promoting human health. Most rodent studies have utilized doses that would be decidedly expensive if the appropriate doses were extrapolated to humans. However, recent clinical studies with orally administered AST suggested that intakes as low as 12–20 mg daily may have worthwhile antioxidant activity in humans. The most significant of these clinical studies was a double-blind controlled trial in which healthy subjects, with moderately elevated triglyceride levels, received daily doses of 0, 6, 12, or 18 mg AST for 12 weeks [58]. The 12 mg and 18 mg doses were found to exert markedly favorable and statistically significant effects on metabolic syndrome parameters triglycerides fell by about 25%, whereas the protective hormone adiponectin rose by about 20% and HDL increased modestly. A trend in the reduction in systolic blood pressure was noted, but did not achieve statistical

significance. These findings confirm previous uncontrolled clinical studies suggesting that comparable intakes of AST have a favorable impact on metabolic syndrome parameters [59,60].

Since rodent studies suggest that oxidative stress in fat cells is the underlying cause of metabolic syndrome [61,62], a reasonable interpretation of these findings is that the long-term administration of adequate amounts of AST can suppress oxidant stress in fat cells, presumably by preserving the mitochondrial structure. Similar benefits have been reported in human diabetics supplemented with Spirulina (8 grams daily) [63]. These findings suggest that NOX and mitochondria contribute decidedly to oxidative stress in hypersaturated fat cells.

An earlier double-blind, placebo-controlled study (in which 16 mg AST was administered daily for three months to men being treated for infertility) was associated with improved sperm function and a notable increase in pregnancy rates in women [64]. Thus, 12–20 mg per day AST has shown the potential to confer clinically relevant antioxidant protection at a dose that is reasonably affordable.

Conclusion

Phycocyanobilin may replicate the biliverdin-bilirubin pathway effects on Treg induction. Astaxanthin, acting as an antioxidant, could be beneficial to mitochondrial membranes and other cellular membranes by opposing oxidative stress and the adverse production of NOX. By targeting the two primary sources of oxidative stress in most human disorders and the aging process, the joint administration of PhyCB and AST has the potential for promoting health and healthful aging. These two super antioxidants, PhyCB and AST, are derived from microalgae.

Conflict of Interest Statement

The authors declare that this paper was written in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest, at the time of its research and writing. Subsequently, Dr. Mark F. McCarty has become co-inventor and co-owner of U.S. and E.U. patents on the use of PhyCB oligopeptides as nutraceuticals, and holds an E.U. patent on the use of PhyCB for the prevention and control of diabetic glomerulosclerosis.

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Supplementary Note

Parts of this paper were previously made available in a booklet entitled, *A Guide to Health-Protective Microalgal Phyconutrients*, posted on Capitalife, Inc. (USA) website, and used with permission.

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