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Astashine Capsule: A potential chemopreventive antioxidant provides promising approach in cancer therapy

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ABSTRACT

A number of substances naturally occurring in foodstuffs, particularly antioxidant compounds in plant products, have shown promise as potential chemopreventive agents. Among these phytonutrients, the yellow, orange and red carotenoid pigments have recently sparked much interest. In epidemiological studies, vegetable and fruit consumption has consistently been associated with reduced incidence of various cancers, and dietary carotenoid intake from these sources has similarly been correlated with reduced cancer risk. However, several recent large-scale intervention trials failed to find any chemopreventive effect of long-term supplementation with β-carotene, the most abundant dietary carotenoid.

Several naturally occurring carotenoids other than β-carotene have exhibited anticancer activity, and are being considered further as potential chemopreventive agents.

Among these carotenoids, the red pigment astaxanthin is of particular interest in health management due to its unique structural and chemical properties. Based on these facts, A Super Antioxidant ASTASHINE Capsules has been developed by R&D Centre, Lactonova Nutripharm (P) Ltd, HYDERABAD. The present paper Reviews the Role of ASTASHINE CAPSULES in the evidence for Anticarcinogenic behavior with an emphasis on the chemopreventive activities of astaxanthin.

INTRODUCTION

There are clear links between human cancers and diet [1, 2]. By some estimates, dietary risk factors rank higher than tobacco usage and much higher than pollution or occupational hazards in their association with cancer deaths [3]. In addition to avoidance of tobacco smoke and carcinogenic food items, regular intake of chemopreventive compounds is a promising approach for reducing cancer incidence [3, 4]. A number of substances naturally occurring in foodstuffs, particularly antioxidant compounds in plant products, have shown promise as potential chemopreventive agents [3-6]. Among these phytonutrients, the yellow, orange and red carotenoid pigments have recently sparked much interest. In epidemiological studies, vegetable and fruit consumption has consistently been associated with reduced incidence of various cancers, [5-7] and dietary carotenoid intake from these sources has similarly been correlated with reduced cancer risk [8-10]. However, several recent large-scale intervention trials failed to find any chemopreventive effect of long-term supplementation with β-carotene, the most abundant dietary carotenoid [11-13]. Several naturally occurring carotenoids other than β-carotene have exhibited anticancer activity, [14-17] and are being considered further as potential chemopreventive agents. Among these carotenoids, the red pigment astaxanthin is of particular interest in health 2

management due to its unique structural and chemical properties [18-20].

Antioxidants and cancer prevention

The higher eukaryotic aerobic organisms, including human beings, cannot exist without oxygen, yet oxygen represents a danger to their very existence due to its high reactivity. This fact has been termed the .paradox of aerobic life [21]. A number of reactive oxygen species are generated during normal aerobic metabolism, such as superoxide, hydrogen peroxide and the hydroxyl radical [22]. In addition, singlet oxygen can be generated through photochemical events (such as in the skin and eyes), and lipid peroxidation can lead to peroxyl radical formation [22].

These oxidants collectively contribute to aging and degenerative diseases such as cancer and atherosclerosis through oxidation of DNA, proteins and lipids [21-23] Antioxidant compounds can decrease mutagenesis, and thus carcinogenesis, both by decreasing oxidative damage to DNA and by decreasing oxidant-stimulated cell division [22]. The human body maintains an array of endogenous antioxidants such as catalase and superoxide dismutase; however, exogenous dietary antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E) and carotenoids play important roles in reducing oxidative damage as well, [21-23] and their serum levels have the potential to be manipulated [23].

Fruits, vegetables and carotenoids

Human epidemiological studies have revealed a protective effect of vegetable and fruit consumption for cancers of the stomach, esophagus, lung, oral cavity and pharynx, bladder, endometrium, pancreas, colon and rectum, breast, cervix, ovary and prostate [24-26] A variety of compounds found in these foods have known bioactive mechanisms and are suspected as anticancer agents; these include vitamins C and E, flavonoids, isothiocyanates, phytosterols, selenium, folic acid, dietary fiber, protease inhibitors, isoflavones, indoles, carotenoids and others [1, 25]. The carotenoids are a group of approximately 600 naturally-occurring pigments with diverse biological functions [27]. In plants and algae, carotenoids serve both photosynthetic and photo protective roles; in animals, carotenoids are effective chain-breaking antioxidants and singlet oxygen quenchers, and some also serve as precursors for retinoid (vitamin A) [28]. Some carotenoids also appear to have effects on cell communication and proliferation in animals [29]. Because animals cannot synthesize carotenoids de novo, they must obtain them from dietary sources [30].

The β-carotene hypothesis

In a landmark 1981 paper, Peto and colleagues posed the provocative question, Can dietary beta-carotene materially reduce human cancer rates? [31]. their focus on this particular carotenoid was largely due to its known bioactivity (as provitamin A), emerging information on its antioxidant properties and its abundance in common fruits and vegetables. These authors suggested that although an inverse correlation of dietary β-carotene intake and cancer incidence 4 was evident, a genuine protective effect of β-carotene could not be verified without controlled trials [31]. Three large human intervention trials were initiated to test the β-carotene hypothesis in the mid-1980s; the results from these trials were disappointing. Not only did β-carotene supplementation offer no significant protection from lung and other cancers, it actually increased lung cancer risk among smokers in two of the trials [11-13].

It has been suggested that these negative results should not have been wholly unexpected. Rather than individual agents, the total diet and all its constituents need to be considered in determining nutrient factors related to cancer risk incidence [32]. A diet rich in fruits and vegetables provides a suite of phytonutrients, including some 40-50 carotenoids and their metabolites [33]. Which may themselves have chemopreventive potential [34, 35]. Biological antioxidants, including carotenoids and vitamins C and E, are known to act synergistically through radical repairing and other mechanisms [36-40]. An individual antioxidant, in high doses by itself, may yield undesirable effects not realized in combination with other antioxidants at normal biological doses [41]. In the case of β-carotene, although it normally functions as an antioxidant, it exhibits prooxidant effects at high concentration and especially at high oxygen tension [42, 43].

Supplementation with high doses of this carotenoid therefore has the potential to enhance oxidation in the lungs, especially when radicals from tobacco smoke are present [44, 45]. Thus, in considering the potential role of carotenoids in cancer prevention, we must not look at β-carotene as a

supplement in isolation, but consider multiple dietary carotenoids and their various interactions within biological systems [5].

Dietary carotenoids other than β-carotene

Despite the presence of 40 or more naturally occurring carotenoids in the human diet, only a handful of carotenoids are commonly detected in human plasma and tissues, along with several of their isomers and various metabolites [33]. The most common of these dietary carotenoids are three hydrocarbon carotenoids (carotenes): α-carotene, β-carotene and lycopene, and three oxycarotenoids (xanthophylls): lutein, zeaxanthin and β-cryptoxanthin [33, 46]. Intake of these compounds is principally through consumption of fruits and vegetables; the xanthophylls, astaxanthin, on the other hand, are obtained principally from seafood such as salmon and shrimp.

Astaxanthin occurs in these animals naturally, but it also occurs in farmed fish, shellfish and poultry as a result of its use as a feed additive [47, 48]. Astaxanthin is therefore an occasional component of the human diet in most populations, but can be more significant in populations that regularly consume such foodsm [49]. Canthaxanthin, another potentially important xanthophyll, is also not generally considered a dietary carotenoid, but may be included in the human diet through its widespread use as a coloring agent in foods and animal feeds [50, 51]. The structures of these eight important carotenoids are given in Figure 1. Among them, only α-carotene, β-carotene and β-cryptoxanthin can be converted to vitamin A in humans [51]. Nevertheless, all of these dietary carotenoids have demonstrated some anticarcinogenic activity in animal experiments [49, 51-53].

Lycopene

Tomatoes and tomato-based products are the major dietary sources for the red carotenoid lycopene, although other plant sources exist, such as watermelon, grapefruit and guava [54]. Lycopene is a very efficient biological singlet oxygen quencher [55] and has exhibited tumor suppressive properties on animal and human cells in vitro and on mice in vivo [56, 57]. Lycopene is found at high concentrations in the human prostate [58] and epidemiological studies have revealed strong negative correlations between lycopene intake and prostate cancer risk [26, 59] and have implicated lycopene as a factor in the prevention of several additional types of cancer and other human diseases [60].

Lutein and zeaxanthin

Lutein and zeaxanthin are yellow xanthophyll carotenoids common in green and yellow vegetables. Lutein is obtained primarily from leafy green vegetables such as spinach and kale, while orange peppers are rich in zeaxanthin [49]. These carotenoids accumulate in the macular region of the human retina, and are believed to play important roles in protecting the retina from photo oxidative damage [61-63]. In cancer chemoprevention, a high intake of lutein and zeaxanthin has been correlated with a lower incidence of lung cancer in humans [64, 65] and lutein has exhibited antimutagenic effects in vitro [66]. Lutein has also demonstrated an ability to inhibit carcinogenesis in rat colons [67] and in the lungs of mice, [68] and inhibits mammary tumor growth in mice [69] and in human cell cultures [70] by regulating apoptosis. Similarly, zeaxanthin has been shown to reduce the formation of liver tumors in mice [71].

α- carotene and β-cryptoxanthin

Serum levels of the two other major carotenoids in the human diet, α-carotene and β- cryptoxanthin have been inversely correlated with the incidence of human cervical cancer [72]. In addition, dietary intake of β-cryptoxanthin is associated with reduced risk for lung cancer [65]. Carrots and pumpkin are good sources of α-carotene, while β-cryptoxanthin is abundant in red bell peppers, papayas and tangerines [49, 73]. In studies with mice, α-carotene has been demonstrated to have a potent preventive action against lung, skin and liver carcinogenesis [14]. Similarly, β-cryptoxanthin is effective at inhibiting skin tumor formation in mice [68, 71].

Canthaxanthin, astaxanthin and others

Because it is not a significant dietary carotenoid, epidemiological data on canthaxanthin in disease prevention is lacking. However, it has exhibited potential anticancer properties in vitro and in animal models. Canthaxanthin can suppress proliferation of human colon cancer cells, [74] protect mouse embryo fibroblasts from transformation75 and protect mice from mammary and skin tumor development [17, 76]. Canthaxanthin has also proved effective at inhibiting both oral and colon carcinogenesis in rats [77, 78]. Although it is a potent antioxidant, the

chemopreventive effects of canthaxanthin may also be related to its ability to up-regulate gene expression, resulting in enhanced gap junctional cell-cell communication [79, 80]. The chemopreventive effects of canthaxanthin may also be related to its ability to induce xenobiotic metabolizing enzymes, as has been demonstrated in the liver, lung and kidney of rats [81, 82]. Unfortunately, canthaxanthin overuse as a .sunless. Tanning product has led to the appearance of crystalline deposits in the human retina [83]. Although these retinal inclusions are reversible [84] and appear to have no adverse effects, [83] their existence has prompted caution regarding intake of this carotenoid.

Several other naturally occurring carotenoids that are not considered significant in the human diet have shown potential as cancer chemopreventive agents. These include neoxanthin, fucoxanthin, phytofluene, ζ-carotene, phytoene, crocetin, capsanthin, peridinin and astaxanthin [52, 53, 85]. The xanthophyll astaxanthin is a powerful antioxidant and has great potential for reducing human disease processes related to oxidative damage [49].

PROPERTIES OF ASTAXANTHIN

Structure and forms

Like all carotenoids, astaxanthin (3, 3.-dihydroxy-β, β-carotene-4, 4-dione) is derived from a central phytoene .backbone of 40 carbon atoms linked by alternating single and double bonds. This structure is useful in energy transfer and dissipation and gives carotenoids their characteristic colors. As with all the dietary carotenoids except lycopene, the phytoene chain is terminated on either end by ionone rings. The presence of oxygen-containing functional groups on these rings classifies astaxanthin among the xanthophylls. These hydroxyl and keto groups allow astaxanthin to be esterified and also render it more polar than related carotenoids [20]. Astaxanthin has a number of geometric (Z) isomers, and also is optically active, having three possible stereoisomers [47].

In nature, astaxanthin is usually found either conjugated to proteins (as in the flesh of salmon or in the lobster carapace), or esterified with fatty acids (as in Haematococcus pluvialis microalgae) [20]. In contrast, synthetic astaxanthin is produced in the free form. Synthetic, algae based and yeast-based (from Xanthophyllomyces dendrorhous) astaxanthin are distinct in their stereoisomeric compositions as well [48]. Synthetic astaxanthin, as well as all three significant natural sources (Haematococcus, Xanthophyllomyces and extracted crustacean shells), are used widely as feed additives [48, 86]. Human dietary astaxanthin supplements derived from these three natural sources have also been marketed in recent years [20, 48].

Antioxidant potential

Astaxanthin has demonstrated strong antioxidant behavior in a variety of in vitro studies. In organic solutions, astaxanthin is a potent quencher of singlet oxygen [87-89] an effective inhibitor of peroxyl radical-dependent lipid peroxidation [809-91] and an efficient peroxyl radical-trapping compound [92, 93]. Both synthetic astaxanthin and a commercial Haematococcus algae extract were shown to be excellent scavengers of hydroxyl radicals and superoxide anions when introduced in DMSO to aqueous solutions (as shown in Figure 2) [94]. These antioxidant properties of astaxanthin extend to model membrane systems and cultured animal cells. Astaxanthin and several other carotenoids inhibited peroxyl radical-mediated lipid peroxidation in liposomal [91, 95] and microsomal [96-98] systems and in large unilamellar vesicles [99]. Similarly, astaxanthin was among the carotenoids found to be effective at quenching singlet oxygen [100] and at inhibiting photosensitized oxidation [101] in unilamellar liposomes. Astaxanthin was superior to β-carotene and lutein in its ability to protect rat kidney fibroblasts from UVA light-induced oxidative stress [102]. Astaxanthin also offered in vitro protection from chemically-induced oxidation to cultured chicken embryo fibroblasts, [103] rat blood cells and mitochondria, [89] human lymphoid cells [104] and human low-density lipoprotein (LDL) [105].

The antioxidant behavior of astaxanthin has been demonstrated in vivo as well. In Haematococcus algae, astaxanthin is accumulated as part of a stress response, and is believed to protect cellular DNA from photodynamic damage [106]. This carotenoid also protects lipids from peroxidation in trout [107] and salmon [108]. In chicks, astaxanthin supplementation suppressed the formation of lipid peroxides in the plasma [95]. Significant biological antioxidant effects have been observed in vitamin E-deficient rats fed an astaxanthin-rich diet; these include protection of mitochondrial function [109] and inhibition of peroxidation of erythrocyte membranes [89, 109]. In two independent studies, lipid peroxidation in the serum and liver of

astaxanthin-fed rats treated with carbon tetrachloride was significantly inhibited relative to rats fed a control diet [97, 110]. Similar protection from peroxidation was afforded by astaxanthin to the serum, liver, kidney, spleen and brain of rats exposed to cobalt-60 irradiation [97]. In an ex vivo study of human volunteers, dietary supplementation for 14 days with esterified astaxanthin extracted from krill significantly extended the lag time for chemically-initiated LDL oxidation [105]. This effect appeared to be dose-dependent: supplementation at 3.6, 14.4 or 21.6 mg astaxanthin per day produced significant differences from the control group, while 1.8 mg per day did not produce a significant effect [105].

The interactions of carotenoids with free radicals are complex, and depend on factors such as the structure of the carotenoid, the nature of the radical species, the composition of the surrounding matrix, the presence of other oxidants and antioxidants, and the concentrations of the radicals, carotenoids and oxygen. All of these factors need to be taken into account to explain the uniquely effective antioxidant properties of astaxanthin.

The radical quenching properties of carotenoids lie not only in the conjugated polyene chain but in the functional groups as well [111]. The xanthophylls therefore have inherently different antioxidative properties from the carotenes. For example, astaxanthin and canthaxanthin are inherently poor antioxidants. when compared with β-carotene in electron transfer reactions with radicals, [112] yet the opposite is true in reactions that involve the formation of carotenoid-radical adducts [113].

Moreover, the overall antioxidant properties of carotenoids reflect not only their ability to scavenge radicals, but also on the reactivity of carotenoid radicals or carotenoid-radical adducts that are formed in the process of radical quenching [114]. Astaxanthin, for example, is the most difficult carotenoid to reduce to its radical cation; [115] the β-carotene radical cation, on the other hand, is more easily formed via electron transfer, [112-114] and is itself long-lived and capable of oxidizing protein components such as tyrosine and cysteine [115, 116]. In contrast, carotenoid-radical adducts formed with astaxanthin or canthaxanthin decay quickly to stable products [113].

Astaxanthin therefore has the advantage of being an effective radical quencher in some reactions while not itself being converted into a damaging radical species in others. In addition, when compared with other carotenoids, the astaxanthin radical cation is the most easily reduced; [117] hence, if the astaxanthin radical cation should form, it can easily be converted back to the stable carotenoid via electron transfer from vitamin E, with which it reacts at a higher rate than do the other carotenoids [112].

The position, concentration and orientation of carotenoids within membranes may strongly influence both the structure and dynamics of the lipid bilayer and the antioxidant properties of the carotenoids in membrane systems [118-120]. Polar carotenoids such as zeaxanthin and astaxanthin may span the bilayer, where they tend to stabilize and rigidify the lipid membrane, while nonpolar carotenoids such as β-carotene are more likely to remain completely within the bilayer [121-123]. In the case of astaxanthin, intermolecular hydrogen bonds likely form with phospholipids in the membrane, anchoring the carotenoid molecule like a rivet; at the same time, intra molecular hydrogen bonding between the keto and hydroxyl groups of individual astaxanthin molecules can increase their hydrophobicity and thus keep them within the bilayer [123]. It has been suggested that roughly equal amounts of intra- and intermolecular hydrogen-bonded astaxanthin can exist simultaneously in a membrane, hence allowing for both scavenging of lipid peroxyl radicals within the membrane and interception of reactive oxygen species at the membrane surface [123]. Astaxanthin molecules spanning the bilayer may also be involved in a hypothesized mechanism in which they trap alkoxyl radicals within the hydrophobic core of the membrane and transport the unpaired electron up the polyene chain to the lipid-water interface where it reacts with aqueous vitamin C, yielding stable products in the lipid phase and an ascorbyl radical in the water phase [124].

Mechanisms such as these may explain the highly potent anti peroxidative activity of this carotenoid in lipid membranes. The concentrations of carotenoids and the level of oxygen they are exposed to can also influence their antioxidant activities. At low oxygen partial pressures, diverse carotenoids effectively inhibit in vitro oxidation reactions, and their antioxidative abilities increase with increasing carotenoid concentration [40, 42]. As oxygen levels are increased, however, their antioxidant potential typically decreases [40, 42]. Certain carotenoids, notably β-carotene but also lycopene, exhibit unusual behavior; beyond a threshold carotenoid concentration, they actually decrease in antioxidant

ability with increasing carotenoid concentration, and this effect is further exacerbated at high oxygen levels m [42, 43, 125, 126]. This prooxidant behavior of β-carotene appears to be related to its degradation products and their potential to be involved in radical chain reactions [125] and may help to explain the unexpected increase in lung cancer deaths among smokers supplemented with this carotenoid [41, 45]. The xanthophylls zeaxanthin, canthaxanthin and especially astaxanthin are considered pure antioxidants because they exhibit little or no prooxidative behavior even at high carotenoid concentration and high oxygen tension [125, 126].

Astaxanthin as a potential cancer preventative

Because astaxanthin has not typically been identified as a major carotenoid in human serum, information on its epidemiology in human health is lacking. Salmon, the principal dietary source of astaxanthin, is an important component of the traditional diets of Eskimos and certain coastal tribes in North America; these groups have shown unusually low prevalence of cancer [127, 128]. This low cancer incidence has been attributed to the high levels in salmon of certain fatty acids, notably eicosapentaenoic acid (EPA) [128] yet it is possible that astaxanthin has played a role in cancer chemoprevention among these peoples as well. Regardless, the existing data on the potential for astaxanthin to directly prevent cancer is limited to in vitro cell culture studies and in vivo studies with rodent models.

Cell culture studies

Methyl cholanthrene-induced (Meth-A) mouse tumor cells grown in an astaxanthin supplemented medium had reduced cell numbers and lower DNA synthesis rates 1-2 days post incubation than control cultures [129]. Similarly, astaxanthin inhibited murine mammary tumor cell proliferation by up to 40%, in a dose-dependent fashion, when included in the culture medium [130].

In addition, of eight carotenoids tested, astaxanthin was the most effective at inhibiting the invasion of rat ascites hepatoma cells in culture [131]. The growth of human cancer cell lines has also been inhibited by astaxanthin in vitro. Two human colon cancer cell lines were significantly less viable than control cultures after a four-day incubation with astaxanthin, although a stronger effect was seen from α-carotene, β-carotene or canthaxanthin.74 Also, a weak effect of astaxanthin on human prostate cancer cell viability has been noted, but in this case neoxanthin and fucoxanthin appeared to be much more effective.85 On the other hand, significant inhibition of androgen-induced proliferation of human prostate cancer cells was recently demonstrated in the presence of either astaxanthin or lycopene [132]. Exposure to UVA radiation is believed to be the primary causative agent in skin tumor pathogenesis; both synthetic astaxanthin and an astaxanthin-rich algal extract gave significant protection from UVA-induced DNA damage to human skin fibroblasts, melanocytes and intestinal CaCo-2 cells in culture [133].

Rodent model studies

In studies with BALB/c mice, dietary astaxanthin inhibited the growth of transplanted Meth-A tumor cells in a dose-dependent fashion [129]. In a related study, Meth-A tumor cell growth was inhibited when dietary astaxanthin supplementation was started at one and three weeks prior to tumor inoculation, but not when supplementation was begun at the same time as tumor inoculation [134]. These results suggest that astaxanthin may inhibit tumor development in the early stages but not in the later stages of progression [134]. In other studies with mice, astaxanthin supplementation reduced transplanted mammary tumor growth17 and suppressed spontaneous liver carcinogenesis [71]. Dietary consumption of egg yolks containing astaxanthin inhibited benzo (a) pyrene-induced mouse fore stomach neoplasia [135] and sarcoma -180 cell-induced mouse ascites cancer [136]. In addition, dietary astaxanthin inhibited the accumulation of potentially tumor-promoting polyamines in the skin of vitamin A-deficient hairless mice after exposure to UVA and UVB irradiation [137].

A series of studies on cancer chemoprevention by natural and synthetic substances in mice and rats revealed several carotenoids, including astaxanthin, as effective antitumor agents [138]. In one of these studies, dietary astaxanthin was found to significantly reduce both the incidence and proliferation of chemically-induced urinary bladder cancer in mice [139]. In two related studies, the incidence and proliferation of chemically-induced cancers of the oralcavity [78] and colon [77] were significantly reduced in astaxanthin-supplemented rats relative to control rats. Astaxanthin has shown effectiveness against the initiation of liver carcinogenesis in rats.

An astaxanthin-supplemented diet reduced the number of DNA single-strand breaks and the number and size of liver pre neoplastic foci induced in rats by aflatoxin B1 [140, 141].

Dietary astaxanthin also reduced metastatic nodules and lipid peroxidation in the livers of rats treated with restraint stress [142, 143]. Although the above studies all point to potent anticarcinogenic effects of astaxanthin in vivo, a few studies have offered less compelling results. For example, in one study of chemically-induced hepatocarcinogenesis in rats, dietary astaxanthin had no effect on the development of pre neoplastic liver foci while lycopene produced a significant reduction in foci [144]. Similarly, activation of pim-1 gene expression (which is involved in regulating cell differentiation and apoptosis) was stimulated in lutein-fed but not in astaxanthin-fed mice [145].

Finally, one in vivo dietary astaxanthin study has reported negative results; dietary supplementation with either β-carotene or astaxanthin exacerbated carcinogenic expression in the skin of hairless mice after UV irradiation [146].

Possible mechanisms of action

The proposed mechanisms of action in the cancer chemopreventive actions of carotenoids can be grouped into three major categories: carotenoids can act as potent biological antioxidants, as enhancers of immune system function and as regulators of gene expression [147]. Astaxanthin is expected to function through each of these mechanisms in living systems.

Antioxidation

Several recent examples testify to the effectiveness of astaxanthin in the prevention and treatment of oxidative cell and tissue damage in vivo. Dietary astaxanthin limits exercise-induced muscle damage in mice, [148] protects β-cell and renal function in diabetic mice [149, 150] and both retards and ameliorates retinal damage from photic injury in rats [151]. An algal extract containing astaxanthin was similarly found to attenuate selenite-induced cataract formation in the eyes of rat pups [152]. Inflammation is believed to be a major contributor to carcinogenesis, through several mechanisms including the production of free radicals by inflammatory cells [153]. Astaxanthin has been found effective at reducing the severity of several inflammatory conditions in rodents and humans.

Gastric inflammation associated with infection by Helicobacter pylori bacteria was reduced in mice fed astaxanthin-containing algal meal [154] or algal cell extract [155, 156]. Astaxanthin was also shown to have a dose-dependent ocular anti-inflammatory effect on lipopolysaccharide induced uveitis in rats [157]. Two small, randomized, placebo-controlled trials were recently conducted on human volunteers to assess the effect of supplementation with an astaxanthin-rich algal extract on symptoms associated with the inflammatory diseases rheumatoid arthritis (RA) and carpal tunnel syndrome (CTS) [158, 159]. The results revealed that astaxanthin significantly relieved pain and improved performance in patients with RA; [158] the results on CTS patients were similar but statistically insignificant [159]. Although other mechanisms may be at work, the antioxidant properties of astaxanthin likely contribute to its ability to prevent and/or treat these various conditions, and thereby potentially reduce cancer risk.

Immuno modulation

It is well established that carotenoids can have an enhancing effect on immune function, and that such immune enhancement may be manifested independently of their pro vitamin A activity or antioxidant potential [160, 161]. Carotenoids appear to have specific immune functions that may enhance immunity to cancer cells [160]. Astaxanthin in particular has exhibited numerous immune-enhancing activities both in vitro and in vivo. In cell culture experiments, astaxanthin stimulated proliferation of mouse thymocytes and spleen cells, stimulated immunoglobulin production of murine spleen cells, and enhanced the release of interleukin-1α and tumor necrosis factor-α from murine peritoneal adherent cells [162]. Similarly, production of antibodies in response to T-dependent antigens and other stimuli are enhanced by astaxanthin in mice in vitro and in vivo [163-167].

Astaxanthin also enhanced in vitro immunoglobulin production by human peripheral blood mononuclear cells in response to antigens [168]. Phytohemagglutinin-induced splenocyte proliferation and lymphocyte cytotoxic activity were stimulated in mice fed astaxanthin [169] while dietary astaxanthin was able to delay symptoms of proteinuria and lymphadenopathy in autoimmune-prone mice [170].

Similar immune responses in astaxanthin-fed mice have been noted when this carotenoid was used to

reduce the inflammatory symptoms of H. pylori infections [155, 156]. Moreover, immune enhancement has been observed when astaxanthin was fed to tumor-inoculated mice. For example, Meth-A tumor inoculated mice developed significantly higher cytotoxic Tlymphocyte activity and interferon-γ production by tumor-draining lymph node and spleen cells when fed an astaxanthin-supplemented diet relative to those fed a control diet; in parallel with these observations, a significant inhibition of tumor growth in the astaxanthin-fed mice was noted [129, 134]. Taken together, these studies of the ability of astaxanthin to stimulate immune responses both in vitro and in vivo suggest that the immune enhancing properties of this carotenoid may play an important role in its ability to function as a cancer chemopreventive agent.

Gene regulation and other mechanisms

Other unexpected biological functions of carotenoids have been recently demonstrated that appear to be independent of their pro vitamin A and antioxidant activities [79]. Effective cell communication through gap junctions is deficient in many human tumors, and its restoration tends to decrease tumor cell proliferation [171]. Several retinoids and carotenoids are now known to enhance gap junctional communication between cells, and the enhancement by carotenoids is well correlated with their ability to inhibit neoplastic transformation in mouse embryo fibroblasts [29, 171, 172]. This stimulation of gap junctional communication occurs as a result of a dose-dependent increase in the connexin 43 protein, via up-regulation of the connexin gene [29, 79, 171]. Interestingly, while β-carotene enhanced connexin 43 expression in murine fibroblasts, it did not do so in murine lung epithelial cells; this observation may at least in part explain why β-carotene is ineffective in chemoprevention of lung cancer [173]. It is not known if astaxanthin has an up-regulating effect on connexin 43, but the closely related carotenoid canthaxanthin has shown a strong stimulatory effect on gap junctional communication between mouse embryo fibroblasts [80, 172].

Another regulatory function of carotenoids is the induction of xenobiotic-metabolizing enzymes (XME); by enhancing the production of these enzymes, carotenoids may help to prevent carcinogenesis by stimulating the detoxification of carcinogenic compounds. A number of studies have demonstrated such regulation by carotenoids, especially astaxanthin and canthaxanthin, in the liver of rats. Specific enzymes that were induced by astaxanthin and canthaxanthin included P4501A1 and 1A2, and CYP1A1and 1A2, which are involved in the metabolism of such potential carcinogens as polycyclic aromatic hydrocarbons, aromatic amines and aflatoxin [81, 140, 141, 174]. These two xanthophylls also induced selected P450 enzymes in rat lung and kidney tissues, but not in the small intestine [82]. XME induction by astaxanthin is not only enzyme-specific and tissue-specific, but varies between species as well; different mechanisms appear to be at work in Swiss mice [175] and in human hepatocytes [176] than in rat liver.

Several additional regulatory mechanisms have been described involving astaxanthin that may underlie its anticarcinogenic effects. These include a regulatory influence of astaxanthin on transglutaminases in UV-irradiated hairless mice, [137] an inhibitory effect of astaxanthin and other carotenoids on metabolic activation of specific mutagens in bacteria, [177] and an induction of apoptosis by astaxanthin in murine mammary tumor cells.130 Furthermore, inhibition of the enzyme 5α-reductase by astaxanthin may explain its anti-proliferative effect on human prostate cancer cells, [178] and selective inhibition of DNA polymerases by astaxanthin and retinoids may result in reduced human gastric cancer cell growth [179]. Finally, direct blocking of nitric oxide synthase activity appears to be the mechanism by which astaxanthin reduces lipopolysaccharide induced inflammation in rats [157].

Safety and metabolism of dietary astaxanthin

Astaxanthin is not known to present any special health risk to humans. Astaxanthin is a natural, albeit minor component of the human diet through consumption of salmon, trout, and various crustaceans, and has been used as a dietary supplement at least since 1999 [20]. The most common source of astaxanthin used in these supplements is an extract of Haematococcus pluvialis microalgae. Numerous acute and repeated-dose toxicity studies in mice, rats and humans have demonstrated the lack of toxicity of the whole algal biomass [180]. Moreover, the extract has recently undergone a 13-week repeated-dose toxicity study in rats, [181] as well as an 8- week randomized, double-blind, placebo-controlled clinical safety trial of 35

human volunteers; [182] no safety concerns were raised by either of these studies.

Despite the existing evidence attesting to the safety of dietary astaxanthin, little is known about the bioavailability and metabolism of this carotenoid in humans. Several steps are involved in the assimilation of carotenoids by mammals, including transfer from the food matrix, transfer to lipid micelles in the small intestine, uptake by intestinal mucosal cells, transport to the lymph system and eventually, deposition of the carotenoid or its metabolites in specific tissues [183, 184]. A number of factors can influence the progression of these steps, including the nature of the food matrix [184, 185] the structure of the carotenoid (including potential esterification and the nature of its isomeric composition), [183-187] the presence of other carotenoids [184, 188] and the amount and types of lipids in the diet [189-191]. Overall, human metabolism of astaxanthin should be somewhat similar to that of the other xanthophylls, but subtle differences are expected.

Astaxanthin absorption and metabolism has been fairly well researched in birds, crustaceans and especially fish, [192] but only a handful of studies report on its uptake and metabolism in humans and other mammals. In rat hepatocytes, astaxanthin was metabolized into two racemic compounds: 3-hydroxy-4-oxo-β-ionone and its reduced form, 3-hydroxy-4-oxo-7, 8- dihydro-β-ionone [193]. Both of these metabolites were also produced from astaxanthin in cultured human hepatocytes and in the plasma of human volunteers who ingested synthetic astaxanthin; however in these systems, two additional metabolites, 3-hydroxy-4-oxo-β-ionol and 3-hydroxy- 4-oxo-7, 8-dihydro-β-ionol, were produced as well [176]. In terms of absorption, human volunteers ingesting a very large dose (100 mg) of synthetic astaxanthin readily incorporated this carotenoid into plasma lipoproteins to a considerable degree, and reached maximum plasma concentrations of astaxanthin in about 7 hours [194]. All isomers of astaxanthin were incorporated, but there was a selective enrichment of the Z-isomers relative to all-E astaxanthin in the plasma [194]. The bioavailability of astaxanthin demonstrated in the above study was in contrast to the lack of astaxanthin detected in the plasma of human subjects who ingested an astaxanthin-containing salmon meal [195]. It is likely that the serum astaxanthin concentration achieved from this 500 g of salmon was below the detection limit of the assay, both because the salmon contained only 1.5 mg of astaxanthin, and because the salmon also contained canthaxanthin [195] which could potentially have interfered with astaxanthin uptake [188]. The bioavailability of both free and esterified astaxanthin was also examined in healthy male volunteers who ingested a single 40 mg dose of this carotenoid in one of several different formulations; the results demonstrated an enhancement of astaxanthin bioavailability in humans when incorporated into lipid-based formulations [196]. It has been shown as well that the type of oil used influences astaxanthin bioavailability; in rats, astaxanthin assimilation was better when the carotenoid was introduced in olive oil than when it was introduced in corn oil [191].

To date, no human bioavailability or metabolism studies have been reported that have utilized relevant dietary dosages of astaxanthin (4-12 mg daily is typically recommended by supplement manufacturers), nor has serum astaxanthin been tracked in humans undergoing longer-term (weeks-months) supplementation with this carotenoid.

SUPPLEMENT FACTS

Presentation

60 capsules

Usage

As a food supplement combination of antioxidants to improve health and vitality.

Contra-indications

Product is contra-indicated in persons with Known hypersensitivity to any component of the product hypersensitivity to any component of the product.

Recommended usage

- Adults: two capsules per day along with food.
- “Do not exceed the recommended daily dose”

Administration

Taken by oral route at any time with food.

Precautions

Food Supplements must not be used as a substitute for a varied and balanced diet and a healthy lifestyle. This Product is not intended to diagnose, treat, cure or prevent any diseases. Do not exceed the recommended daily dose.

Warnings

If you are taking any prescribed medication or has any medical conditions or have any medical conditions (seizures) under age group 17 year always consults doctor or health care practitioner before taking supplements.

Side effects

Mild side effects like nausea, headache and vomiting in some individuals have been reported.

Storage

- Store in a cool, dry and dark place
- Keep out of reach of children

SUMMARY & CONCLUSION

A diet rich in fruits and vegetables is an important factor for the chemoprevention of a number of human cancers. Such a diet is rich in carotenoids, yet consumption of a wide variety of vegetables can have a greater bearing on the risk of specific cancers than intake of any specific carotenoids or total carotenoids [197]. The whole of the diet must be considered, including the various dietary carotenoids and other anticarcinogenic compounds [198, 199]. It is becoming increasingly clear that relevant dietary dosages of a mixture of carotenoids are more likely to yield beneficial effects in cancer chemoprevention than high doses of a single carotenoid like β- carotene [200]. Astaxanthin has exhibited potent antioxidant, immune modulating and enzyme inducing properties, all of which suggest a potential role for this carotenoid in the prevention of cancer. Moreover, its unique structural properties and its lack of prooxidant activity make it a prime candidate for further investigation in this area of human health. More research is needed on the absorption and metabolism of this promising anticancer agent in humans, and on its interactions with other carotenoids and vitamins in the human system.

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