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REVIEW

The Role of the Reactive Oxygen Species Scavenger Agent, Astaxanthin, in the Protection of Cisplatin-Treated Patients Against Hearing Loss

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Abstract: Emerging evidence of significant hearing loss occurring shortly after cisplatin administration in cancer patients has stimulated research into the causes and treatment of this side effect. Although the aetiology of cisplatin-induced hearing loss (CIHL) remains unknown, an increasing body of research suggests that it is associated with excessive generation of intracellular reactive oxygen species (ROS) in the cochlea. Astaxanthin, a xanthophyll carotenoid, has powerful anti-oxidant, anti-inflammatory, and anti-apoptotic properties based on its unique cell membrane function, diverse biological activities, and ability to permeate the blood-brain barrier. In this review, we summarize the role of ROS in CIHL and the effect of astaxanthin on inhibiting ROS production. We focus on investigating the mechanism of action of astaxanthin in suppressing excessive production of ROS.

Keywords: astaxanthin, oxidative stress, cisplatin, hearing loss

Introduction

Cisplatin, an effective antineoplastic agent commonly used in clinical practice, has many serious adverse effects including nephrotoxic, neurotoxic, and ototoxic effects. These life-long disabling adverse effects are strongly associated with the dosage, frequency, and duration of cisplatin treatment. Cisplatin-induced hearing loss (CIHL), which is permanent and mostly bilateral, can negatively affect academic development and social integration, especially in children.[1] To the best of our knowledge, cisplatin ototoxicity has not been studied in detail, and the mechanisms responsible for the degeneration of cochlear structures are not completely understood.

Emerging evidence indicates that excessive production of reactive oxygen species (ROS) contributes to cisplatin ototoxicity. Mechanistically, cisplatin ototoxicity is associated with the absence of glutathione (GSH) and the inhibition of glutathione peroxidase (GSH.Px) and glutathione reductase activities because cisplatin can covalently bind to the sulfhydryl groups of anti-oxidant enzymes, causing enzyme inactivation.[2] Increased lipid peroxidation in the cochlea inhibits essential cellular enzymes and membrane transporters, thereby disturbing ion channel function. Increased ROS production eventually results in apoptosis and necroptosis, supporting the hypothesis that ROS play a crucial role in cisplatin ototoxicity and suggesting that inhibiting ROS production could be beneficial for protecting the cochlea and reversing hearing loss.

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Astaxanthin is a red carotenoid agent with potent anti-oxidant properties that can scavenge singlet oxygen and free radicals. These properties confer astaxanthin with anti-inflammatory and immunomodulatory activities, protective effects against neuronal damage, anti-aging and anti-cancer activities, and the ability to inhibit cell membrane peroxidation. The anti-oxidant activity of astaxanthin is 10-fold greater than that of zeaxanthin, lutein, canthaxanthin, and β-carotene, and 100-fold greater than that of α-tocopherol.[3] Growing evidence suggests that astaxanthin inhibits the development of oxidative stress-associated diseases and mitochondrial dysfunction.[4] Moreover, powerful permeation of the blood-brain barrier (BBB) allows astaxanthin to act as a potent neuroprotective agent in mammals.

The use of cisplatin is limited by its ototoxicity and nephrotoxicity. Methods to increase diuresis, such as hydration, have the potential to reduce its nephrotoxicity. However, there are currently no effective FDA-approved treatments for ototoxicity. We reviewed the evidence supporting the ability of astaxanthin to inhibit ROS generation and prevent mitochondrial dysfunction and neurodegeneration. Based on this assessment, we hypothesized that astaxanthin may be effective for the prevention and treatment of CIHL. In this review, we focus on the following topics: (1) The mechanisms underlying cisplatin ototoxicity; (2) astaxanthin-based therapies for diseases related to excessive ROS production; (3) astaxanthin biochemistry and bioactivity; and (4) downstream pathways of astaxanthin contributing to the inhibition of ROS generation.

Mechanisms of Cisplatin Ototoxicity

An increasing body of research suggests that cisplatin ototoxicity is related to cellular hypersensitivity, although the precise cellular and molecular mechanisms remain unclear. Our understanding of the role of cisplatin in ototoxicity is limited; however, research suggests that cisplatin uptake plays a crucial role. A recent study detected residual platinum in the cochleae of mice and cancer patients receiving cisplatin chemotherapy months-to-years after the treatment.[5]

Cisplatin Transportation

Cisplatin is a square planar complex of a bivalent platinum cation with two cis chloride ligands and two cis ammonia ligands.[6] The complex was originally assumed to enter cells by passive diffusion because its uptake is concentration-dependent and non-saturable.[7] However, subsequent studies showed that copper transporter 1 (CTR1),[8,9] organic cation transporter 2 (OCT2),[10] mechanotransduction (MET)[11] and copper-extruding P-type ATPases (ATP7A and ATP7B)[12] coordinate the cellular uptake of cisplatin. Although there may be other channels involved in cisplatin transportation, they have yet to be identified.[13–16]

CTR1, a high-affinity copper transporter, is highly expressed in outer hair cells, inner hair cells, stria vascularis, and spiral ganglion neurons,[8] and contributes to drug entry and cell apoptosis.[17] CTR1 is a major entry route for cisplatin in hair cells, and it can enhance the cytotoxicity and cellular uptake of cisplatin in cells and in mouse.[8] Coactivity of both CTR1 and OCT2 may lead to secondary damage in the stria vascularis and spiral ganglion.[8] Knockout of CTR1 in yeast was reported to increase cisplatin resistance and decrease the intracellular concentration of cisplatin.[18] Although increased expression of CTR1 may affect the intracellular concentration and distribution of cisplatin, it does not affect the ability of cisplatin to target DNA.[19]

OCTs belong to the solute carrier (SLC) 22A family,[20] and three isoforms (OCT1–3) have been identified, which are mainly expressed in the kidneys and liver.[21–23] OCT2 plays a key role in cisplatin transportation and is also expressed in the organ of Corti and stria vascularis.[10,24] In OCT1/2 double-knockout (KO) mice, cisplatin shows no ototoxicity and only mild nephrotoxicity.[10] OCT2 variants were also reported to impede CIHL in children and adult, which highlights its key role in cisplatin transportation in the cochlea.[25]

The MET channel is a nonselective cation channel that allows calcium and other ions to cross the membrane.[18] In zebrafish, CIHL is associated with functional MET channels, and inhibition of MET channels by quinine or EGTA has protective effects against CIHL.[11] Furthermore, zebrafish mutants lacking MET channels are resistant to CIHL.[11] These studies suggest that MET channels contribute to the entry of cisplatin into hair cells.

A small increase in the expression of the copper transporter ATP7A resulted in resistance to clinically available platinum drugs in human 2008 ovarian carcinoma cells that may be attributed to ATP7A binding and sequestration of platinum compounds.[26] The HSC-4-R, OSC-19-R, and HOC313-R cell lines have acquired resistance to cisplatin that is related to ATP7B overexpression.[27] Both ATP7A and ATP7B are expressed in the organ of Corti, stria vascularis and spiral ganglion neurons.[28] Inhibition of Na^+-K^+-ATPase reduces cisplatin accumulation, suggesting the involvement of other transporters in cisplatin resistance.[29]

It has been suggested that dysfunction of the LRRC8A and LRRC8D subunits of heteromeric volume-regulated

anion channels (VRAC) can lead to cisplatin resistance in KCP-4 human epidermoid cancer cell line and human lung adenocarcinoma cells.[30,31] In 2015, Planells-Cases reported that the absence of VRAC is related to cisplatin resistance in haploid KBM7 cells based on genome-wide loss-of-function screening.[32] However, the role of VRAC in cisplatin resistance is still unclear. Overexpression of multidrug resistance protein 2 (MRP2) increases the efflux of cisplatin in hepatocellular carcinoma and oesophageal squamous cell carcinoma.[33,34]

After entry into the cell, cisplatin undergoes aquation to form $[Pt(NH_3)_2Cl(OH_2)]^+$ and $[Pt(NH_3)_2(OH_2)_2]^{2+}$ (Figure 1). The complex interacts with various reactive groups, blocking DNA replication and transcription and resulting in the inhibition of DNA repair and cell cycle progression.[7] Platinum-DNA adducts found in the hair cells of the cochlea and the marginal cells of the stria vascularis contribute to the inhibition of cell proliferation.[7] DNA damage caused by the formation of DNA adducts induces apoptosis initiated by activation of p53, which activates apoptotic proteins such as Bax or inhibits anti-apoptotic members of the Bcl-2 family[35] (Figure 1). Cisplatin-induced DNA damage in the cochlea may interfere with signal transduction pathways, including the v-akt murine thymoma viral oncogene homologue (AKT), v-abl Abelson murine leukaemia viral oncogene homologue 1 (c-ABL), p53, and mitogen-activated protein kinase/c-Jun N-terminal kinase/extracellular signal–regulated kinase (MAPK/JNK/ERK) pathways.[7,36]

ROS in CIHL

Normally, the maintenance of cochlea function requires high metabolic activity in areas such as the stria vascularis, spiral ligament, and spiral prominence, leading to leakage of electrons from the mitochondrial respiratory chain, which react with oxygen (O_2) to produce superoxide ($O_2^-\bullet$). Environmental stimuli increase oxidative stress in

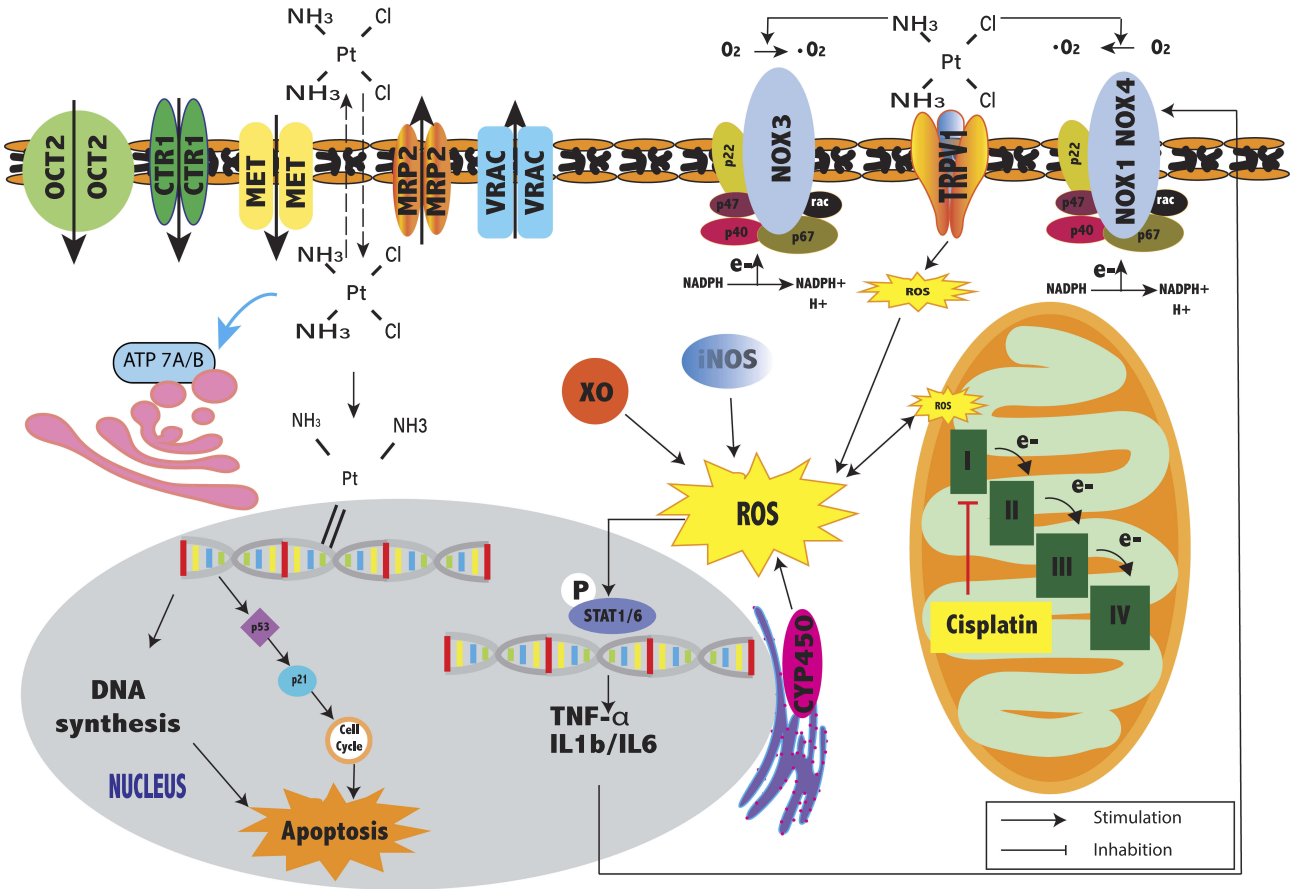

Figure 1 Schematic of the proposed mechanism of cisplatin transportation and the generation of ROS in CIHL. Cisplatin is transported into cochlear cells by membrane transporters, including copper transporter 1 (CTR1), organic cation transporter 2 (OCT2), and mechanoelectrical transduction (MET), and is excluded by copper-extruding P-type ATPases (ATP7A and ATP7B), multidrug resistance protein 2 (MRP2), and volume-regulated anion channels (VRAC). Cisplatin induces ROS production in the inner ear via NADPH oxidase (NOX), xanthine oxidase (XO), cytochrome P450 (CYP450), induced nitric oxide synthase (iNOS), and disturbances in the mitochondrial electron transport chain.

Abbreviations: IL, interleukin; STAT1, signal transducer and activator of transcription 1.

the cochlea in association with increased metabolic activity. The metabolic requirements of the cochlea lead to vulnerability to hypoxic events and ischemia-reperfusion injury.[37] Cisplatin promotes the generation of ROS by stimulating enzyme systems or deactivating anti-oxidant systems.[38,39] This is supported by decreased cochlear GSH and anti-oxidant enzyme activity in rats treated with cisplatin.[40] This decrease could result from covalent binding of cisplatin to anti-oxidant enzymes (e.g., superoxide dismutase and catalase), loss of metal cofactors, depletion of anti-oxidant enzymes by increased ROS, and depletion of cochlear anti-oxidant enzyme cofactors such as GSH and NADPH, which protect against the toxicity of ROS and allow the regeneration of GSH.[41]

NADPH oxidases (NOXs) are membrane-bound, multisubunit enzyme complexes that face the extracellular space and transfer electrons across the plasma membrane from NADPH to molecular oxygen, generating superoxide in the cell.[42] Inactivate NOX is present in an unassembled form in which p22phox and gp91phox are present in the membrane, whereas p47phox, p67phox, and p40phox exist in the cytosol. When NOX is activated by phosphorylated p47phox, the cytosolic subunits translocate to the membrane and convert the oxidase into an assembled and active form that can transfer electrons from the substrate to O2, forming O2•−.[43] Some members of the NOX family may be responsible for the bulk of ROS generation in cochlea cells.[13,44,45] NOX3-dependent ROS generation may be a target of cisplatin, as indicated by evidence showing that knockdown of NOX3 by trans-tympanic delivery of siRNA attenuates cisplatin ototoxicity in rats.[44,46] NOX3-derived ROS can activate signal transducer and activator of transcription 1 and 6 (STAT1 and STAT6), increasing the production of the pro-inflammatory cytokines tumour necrosis factor alpha (TNFα), interleukin (IL)-1b, and IL-6.[47,48] These pro-inflammatory mediators exacerbate CIHL by increasing the activity of NOX isoforms including NOX1 and NOX4.[45] In addition, NOX3 expression can be suppressed by silencing the transient receptor potential vanilloid 1 (TRPV1) channel, which acts as a key factor contributing to ROS generation in cochlear hair cells.[13] siRNA-mediated silencing of STAT1 abolishes cisplatin-induced p53 activation, consistent with a study showing that STAT1 regulates cisplatin-induced hair cell death.[48,49] These findings suggest that the TRPV1 and NOX3 signalling pathways may be associated with STAT1, resulting in inflammation and cisplatin-induced hair cell death leading to hearing loss. Increased NOX2 expression in the cochlea increases ROS production in outer hair cells of the basal turn, which is an important factor associated with the vulnerability of outer hair cells.[50]

The xanthine/xanthine oxidase (XO) system is another active ROS-generating system found in the cochlea that contributes to both superoxide and hydrogen peroxide generation. XO is a xanthine oxidoreductase isoform that catalyses the reduction of O_2 into $O_2\bullet^-$ and H_2O_2. Its activity may be enhanced by ROS derived from mitochondria and NOX.[51] Inhibition of XO in a rat model administered ebselen, a GSH.Px mimetic, decreases cisplatin ototoxicity and nephrotoxicity.[52] Depletion of intracellular ATP transforms adenine nucleotides into hypoxanthine and xanthine, which are substrates of XO.[53] This is proposed as a principal mechanism underlying oxidative injury.

Disturbance of the mitochondrial electron transport chain system by cisplatin increases ROS generation concomitant with a decrease in the mitochondrial membrane potential (MMP), an indicator of mitochondrial malfunction.[54,55] Disruption of Complex I function resulting in elevated ROS production underlies the destruction of sensory hair cells in the cochlea caused by cisplatin.[56] In addition, ROS-mediated mitochondrial dysfunction can suppress the amplification of intracellular ROS generation, resulting in hair cell apoptosis and necrosis.[57]

Evidence supports an association between the toxic effects of cisplatin and cytochrome P450 (CYP450) enzymes.[58] However, there is little research on the relation between CYP450 and CHIL. CYP450 is an important source for the generation of ROS and catalytic iron in cisplatin-induced cytotoxicity of the LLC-PK1 cells.[59] Liu found that CYP450 subfamily members (CYP2e1) play a pivotal role in cisplatin-induced nephrotoxicity and apoptosis related to the generation of ROS.[60] CYP2e1 null mice showed significantly functional and histologic protection against renal injury and decrease of apoptosis by cisplatin.[60] Increasing CYP2E1 enhances the toxicity induced by cisplatin in liver injury models both in vitro and in vivo, and the mechanism might associate with accumulating production of ROS and oxidative stress.[58] E47 HepG2 cells, overexpress human CYP2E1, were sensitive to cisplatin because of an earlier activation of ERK.[61] These results suggest that the generation of ROS by cisplatin in the cochlea is associated with CYP450.

Induced nitric oxide synthase (iNOS) and direct NO generation can be observed in the inner ear (including the spiral ligament, modiolus, spiral limbus, supporting cells, nerve fibres, stria vascularis, hair cells, and spiral ganglion neurons) in response to cisplatin administration.[62–64] ROS/reactive nitrogen species (RNS), as intracellular second

messengers, play a pivotal role in ototoxicity. ROS/RNS can activate downstream signalling pathways, regulate gene expression, and interfere directly with lipids, DNA, RNA, metal cofactors and proteins. Nitrosative stress can react with and suppress ROS generation, overpowering anti-oxidant defence capacities within the inner ear, triggering the pro-apoptotic pathway, and leading to outer hair cell death.[65,66]

Although our understanding of the mechanism by which ROS exert their effects in the cochlea is still incomplete, it is well established that oxidative stress can cause DNA damage. It is also evident that the DNA damage caused by ROS may be repaired via several pathways, including base excision repair (BER),[67–69] mismatch repair (MMR),[70] and nucleotide excision repair (NER).[71] The molecule 8-oxoguanine (8-oxo-G) is well known to cause DNA damage by creating a gap in the DNA, which can be repaired by the enzyme 8-oxoguanine glycosylase (8-OGG1) in mitochondria and nucleus.[72] Chronic exposure to a low dose of H_2O_2 induces a DNA protective response in C2C12 cells by activating transcription factors that enhance the expression of DNA repair enzymes.[73,74] An increase in poly-ADP-ribose transferase 1 (PARP1), an enzyme that recruits DNA repair proteins, has been detected in a mouse model of CHIL, and its inhibition is associated with decreased cisplatin-induced cell death.[75] HEI-OC1 cells exposed to cisplatin can promote and initiate DNA repair, but cannot prevent or reverse DNA damage,[76] possibly because of the absence of some DNA repair pathways in mitochondria and BER can only cope with minor damage or process single nucleotides in the mitochondria.[72]

Thus, the cisplatin complex leads to reciprocal activation of ROS generation, NOX, XO, mitochondria, CYP450, MMP, and iNOS as well as pro-inflammatory signalling, suggesting that cisplatin initiates a series of vicious cycles (Figure 1). In turn, ROS enhance lipid peroxidation and DNA damage, finally leading to auditory cell death.

Astaxanthin-Based Therapies for Diseases Related to ROS

Oxidative stress leads to disease conditions that result from increased production of ROS or depletion of the anti-oxidant enzyme system. Mitochondrial disturbance is often involved in the onset of oxidative stress-associated diseases, because the mitochondria are responsible for energy generation and are important sources of ROS. Astaxanthin, which has strong anti-oxidant activity and the ability to maintain metabolic efficiency, is a potent anti-oxidant with the potential to target several health conditions.[77]

Because nervous system tissues show intense metabolic aerobic activity, rich irrigation with blood vessels, and are abundant in unsaturated fats and iron, they are particularly susceptible to oxidative damage.[78] Substantial evidence supports the hypothesis that oxidative stress and impaired mitochondrial efficiency can be causative or at least ancillary factors in the pathogenesis of major neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's, and amyotrophic lateral sclerosis.[79–82] Anti-oxidants can improve mitochondrial redox potential, which is a key target of ROS and free radical production.[83] A number of studies have shown that diets high in anti-oxidants can reduce these associated risks.[84] Synthesized docosahexaenoic acid-acrylated astaxanthin diesters can relieve oxidative stress and inflammasome activation in patients with AD.[85] Astaxanthin inhibits the generation of intracellular ROS and protects against 1-methyl-4-phenyl-pyridinium (MPP+)-induced cytotoxicity in a cellular model of PD, protecting SH-SY5Y cells and substantia nigra neurons from apoptosis in a PD model mouse.[80,86] H_2O_2-stimulated mouse neural progenitor cells pre-treated with astaxanthin show suppression of apoptosis, resulting in cell proliferation.[87] Recent rat models show that astaxanthin can ameliorate aluminium-induced impaired memory performance.[88] According to studies of rats fed with natural astaxanthin, astaxanthin can cross the BBB in mammals, and its anti-oxidant potential may extend beyond the BBB, allowing it to act as a potent neuroprotective agent.[89] In a murine model of ischemic stroke, pre-treatment with astaxanthin decreased ROS production, lipid peroxidation, and cerebral infarction and promoted locomotor function recovery.[90] Astaxanthin was therefore suggested as a promising candidate neuroprotective agent in mammals.[91]

Elevated oxidative stress associated with ROS/RNS and chronic inflammation can aggravate cardiovascular diseases. Emerging in vitro- and in vivo-based evidence indicates that astaxanthin can reduce ROS, RNS, lipid peroxidation, and inflammatory signalling, and activate anti-oxidant enzymes in the heart.[92–96] Investigations of the effect of astaxanthin on the myocardium of ischemic mice demonstrated that astaxanthin can significantly prevent ischemic myocardial injury by alleviating mitochondrial impairment in mitochondria with increased ROS expression, mitochondrial depolarization, and swelling.[97] Astaxanthin promoted recovery of mitochondrial integrity and blocked mitochondria-mediated apoptosis in a homocysteine-induced cardiotoxicity model

resulting from overexpression of ROS, loss of MMP, and fragmentation of mitochondria.[98] In addition, astaxanthin balanced the expression of Bcl-2 family proteins, thereby suppressing mediators of mitochondrial apoptosis such as PARP1 and caspases.[98] Studies also suggest that dietary astaxanthin has beneficial effects during chemotherapy in BALB/c mice, not only because it can counteract induced oxidative stress, but also because it has cardioprotective effects.[94] Epidemiological and clinical data indicate that dietary anti-oxidants might protect against cardiovascular disease, reducing the risk of atherosclerosis by decreasing plasma LDL-cholesterol oxidation.[99]

Astaxanthin may be more effective than vitamin E for preventing and treating non-alcoholic steatohepatitis in mice and humans.[100] It protects the liver against acetaminophen hepatotoxicity by alleviating hepatocyte necrosis, blocking ROS generation, inhibiting oxidative stress, and reducing apoptosis.[101] Astaxanthin accumulates in the liver, especially in the microsomal and mitochondrial fractions of liver tissues.[102] These studies indicate the potential of astaxanthin for the treatment of oxidative stress-related liver and heart diseases.

Astaxanthin also has shown biological activity in dermatology clinical trials, promoting skin health and achieving effective skin cancer chemoprevention.[103] Randomized double-blinded, controlled studies reported that astaxanthin can ameliorate skin wrinkles, elasticity, texture, and viscoelasticity affected by aging and associated with oxidative metabolism and subsequent ROS production.[104–107] A recent study using a rodent model of deep burns revealed that astaxanthin protects against early progression of a burn wound by reducing ROS-induced inflammation and apoptosis.[108] Moreover, it was reported that astaxanthin powerfully accelerates wound recovery in mice.[109]

In addition to its strong antioxidative effect, there is increasing evidence to show that astaxanthin can inhibit the growth of several types of cancer.[110] Studies have shown that ROS may be involved in cancer initiation, progression, and proliferation by acting as messengers of the oxidative stress cascade, which can be inhibited by anti-oxidants such as astaxanthin.[110,111] In the hamster buccal pouch carcinogenesis model, it was shown that astaxanthin prevents the growth of oral cancer by suppressing cell proliferation and inducing apoptosis.[112] In 1,2-dimethyl hydrazine-induced rat colon carcinoma and rat hepatocellular carcinoma CBRH-7919 cells, pre-treatment with astaxanthin exerted anti-cancer effects by inducing cellular apoptosis.[113,114] Astaxanthin also enhanced apoptosis of leukaemia K562 cells and interfered with cell cycle progression.[115] In ovarian cancer SKOV3 cells, astaxanthin combined with human serum albumin could be a candidate treatment via arresting the cell cycle in the G1 phase and inducing apoptosis.[116]

These positive effects have been attributed to the ability of astaxanthin to scavenge free radicals, quench singlet oxygen, and inhibit lipid peroxidation. The effect of astaxanthin on protecting cellular membranes against oxidation is related to the protection of the inner part and external surface.[117–119] Anti-oxidants are indispensable for maintaining cellular health by protecting cellular components against oxidative stress and suppressing ROS-induced inflammation. The effects of anti-oxidants are evident in inflammation-related clinical conditions, such as Crohn's disease, asthma, and exercise-induced muscle damage.[120,121] In 2000, Bennedsen reported that dietary astaxanthin helped reduce the symptoms of ulcerative disease and gastric inflammation.[122] Because of these effects, astaxanthin should be considered for the treatment of various ROS-related diseases.

Astaxanthin Biochemistry and Bioactivity

Astaxanthin is the main carotenoid pigment produced in marine animals, and it is present in many types of seafood, such as salmon, trout, shrimp, and lobster. Astaxanthin has three natural stereoisomers [(3S, 3′S), (3R, 3′R), and (3R, 3′S)], which vary in the configuration of the two hydroxyl groups on the molecule (Figure 2). The presence of the hydroxyl and keto groups on each ionone ring confer unique features, including conversion into an ester, and providing greater anti-oxidant activity and more polar properties than other carotenoids.[3,123] Astaxanthin can preserve the integrity of cell membranes by penetrating bilayers and protecting the redox state and functional integrity of mitochondria.[124]

Astaxanthin is present in natural sources as an ester of fatty acids or as a conjugate of proteins in foods. Astaxanthin is absorbed into enterocytes through passive diffusion, and is incorporated into chylomicrons to be transferred to the liver. This natural product is then incorporated into low-density lipoprotein and high-density lipoprotein and transported via the circulation. Moreover, the bioavailability of astaxanthin is enhanced when it is taken with dietary lipids.[123] Astaxanthin does not turn into vitamin A in the human body; therefore, it is nontoxic if given orally. Even at low concentrations astaxanthin is effective because of its polar features, which optimize the rate and extent of absorption.[125] The only study on humans confirmed the bioavailability of

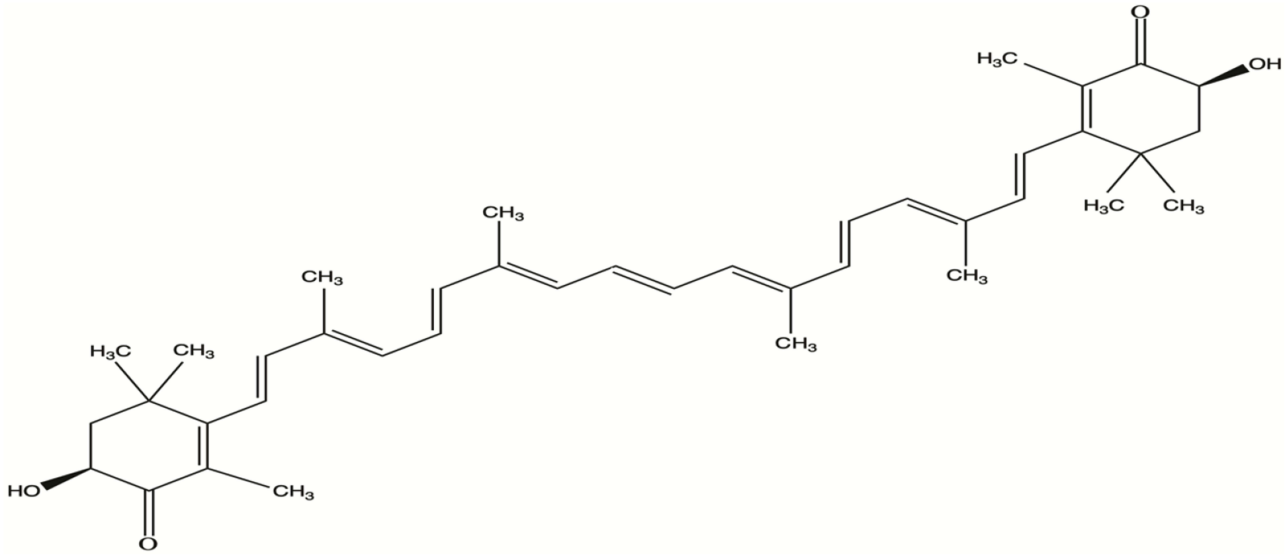

Figure 2 Chemical structure of astaxanthin (3,3′-dihydroxy-β, β- carotene-4,4R′-dione).

astaxanthin administered at a single high dose of 100 mg, and demonstrated that is it transported to the plasma by lipoproteins.[126] Astaxanthin can be used as a dietary supplement for human, animal, and fishery consumption. The FDA has validated the use of astaxanthin for food colouring (or colour additive) and different applications in animal and fish food.

Downstream Pathways of Astaxanthin Against the Generation of ROS

Although the anti-oxidant properties of astaxanthin play a crucial role in its biological activity, the concentrations achieved in the blood that protect cells against oxidative injury are well below those required to scavenge free radicals directly. To explain this discrepancy, it has been suggested that astaxanthin increases resistance to oxidative stress by activating signalling pathways associated with cell survival (Figure 3).

MAPK Pathway

Astaxanthin may help to prevent neurotoxicity by promoting the activation of the AKT/cyclic AMP-responsive element binding protein and ERKs, and blocking the activation of p38 MAPKs, which play pro-apoptotic roles, whereas ERKs have anti-apoptotic roles.[127] This is consistent with the observation that astaxanthin decreases ROS production and inflammatory cytokines to inhibit apoptosis and autophagy, which may be related to inactivation of components of the MAPK family, such as p38 MAPK, JNK and ERK, in a model of hepatic ischemia reperfusion injury.[128] In addition, inhibition of the TNF-α-mediated JNK signal pathway and phosphorylation of ERK and p38 MAPK are involved in the process of hepatocyte necrosis induced by acetaminophen.[101] In a mouse model of smoke-induced impairment of cognitive function, astaxanthin protects the brain against neuroinflammation, synaptic plasticity impairment, and oxidative stress in the cortex and hippocampus by inhibiting p38 MAPK.[129] In human umbilical vein endothelial cells (HUVECs), however, Western blot analysis revealed that astaxanthin significantly upregulated p-ERK without affecting p38 MAPK. Although expression of p-Akt was also increased, it was not significant.[130] These results suggest that astaxanthin protects cells against oxidative stress-induced apoptosis.

Nrf2/ARE Pathway

Hydroxypropyl-β-cyclodextrin-astaxanthin (CD-A) can lead to the dissociation of nuclear factor-erythroid 2-related factor 2 (Nrf2) from Keap1, which allows Nrf2 to translocate to the nucleus and bind to anti-oxidant response elements (AREs), thereby inducing an endogenous anti-oxidant response caused by heme oxygenase-1 (HO-1) and NAD(P)H Quinone Dehydrogenase 1 (NQO1).[131] HO-1 is responsible for many oxidative and cytoprotective functions, and NQO1 has anti-inflammatory effects and can prevent the reduction of quinone.[132,133] Astaxanthin pre-treatment significantly increased the

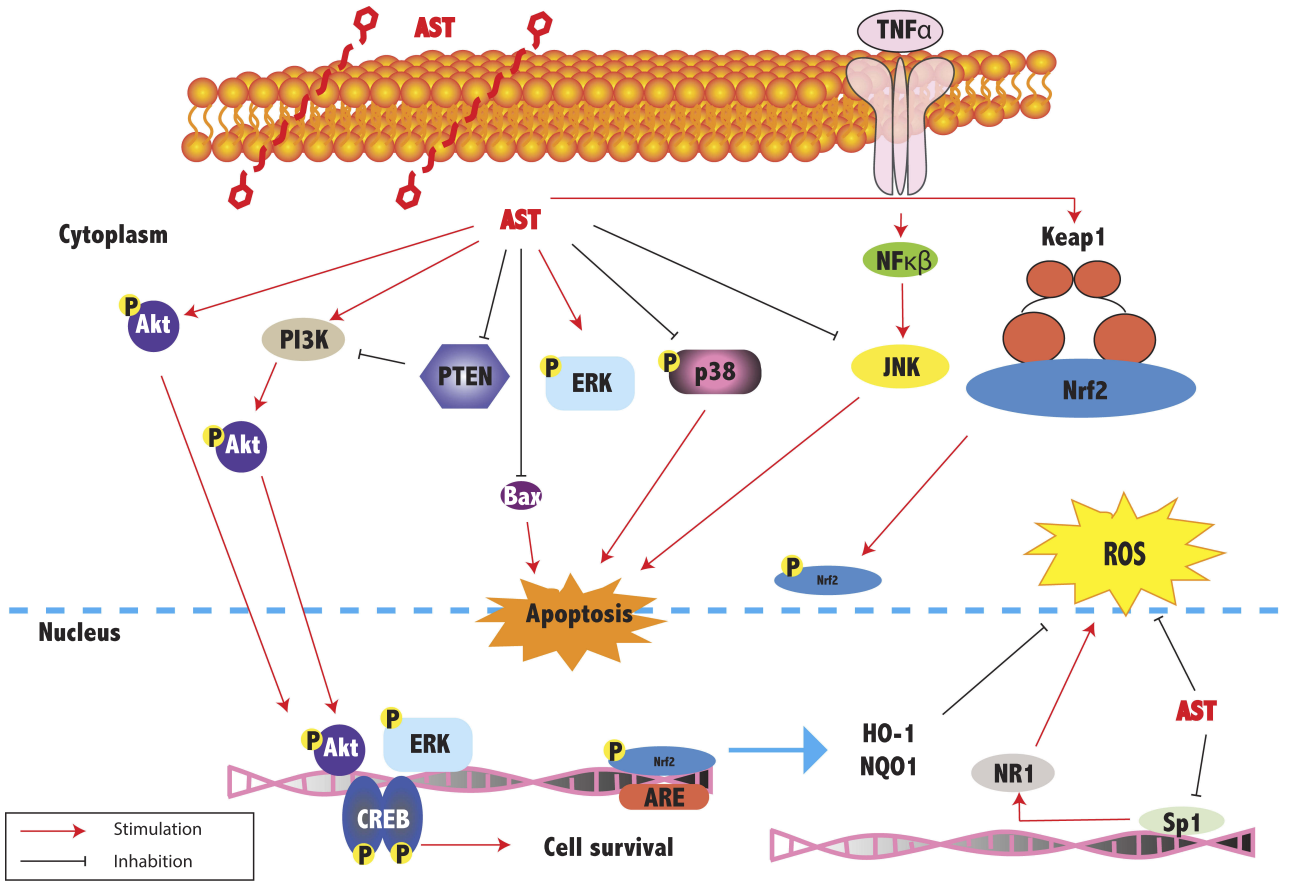

Figure 3 Proposed mechanism by which astaxanthin inhibits ROS and apoptosis. Astaxanthin inhibits apoptosis and scavenges ROS, and modulates various intracellular pathways, predominantly MAPK, PI3K/Akt and Nrf2/ARE. It can also activate the specificity protein 1 (Sp1)–NMDA receptor subunit 1 (NR1) signalling pathway, which leads to cell death.
Abbreviations: AST, astaxanthin; Akt, v-akt murine thymoma viral oncogene homologue; ARE, anti-oxidant response element; CREB, CAMP-responsive element binding protein; ERK, extracellular signal–regulated kinases; JNK, c-Jun N-terminal kinase; Keap1, cytosolic inhibitor of Nrf2; Nrf2, nuclear factor-erythroid 2-related factor 2; NF-κβ, nuclear factor-kappa B subunit; NQO1, NADH Quinone Dehydrogenase1; PTEN, phosphatase and tensin homolog.

expression of Nrf2, HO-1, and NQO1 mRNA, exerting a protective effect against brain injuries. This was demonstrated in a cerebral ischemia rat model, rat hepatocytes, the human retinal pigment epithelial cell line ARPE-19, and early brain injury in a prechiasmatic cistern model of subarachnoid haemorrhage.[131,134,135] In HUVECs, astaxanthin activates the Nrf-2/ARE signalling pathway by developing small amounts of ROS, whereas knockdown of Nrf-2 by siRNA inhibits HO-1 mRNA expression.[130] However, the direct molecular targets responsible for induction of the Nrf2/HO-1/NQO1 pathway remain undefined, as astaxanthin has an indirect anti-oxidant protective effect against ROS.

PI3K/AKT Pathway

Previous studies indicate that cell survival is affected by intracellular ROS generation through the modulation of the phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinase (PI3K)/AKT pathway.[136] Astaxanthin protects against isoflurane-induced neuroapoptosis in a rat model, as indicated by decreased brain damage, inhibition of caspase-3 activity, and upregulation of the PI3K/AKT pathway.[137] Zuluaga recently reported that the generation of ROS induced by stressors (AAPH and t-BuOOH, which are free radical donors that generate a burst of ROS) upregulates PTEN gene expression, which causes cellular apoptosis by deactivating AKT.[138] Conversely, astaxanthin treatment significantly suppressed PTEN expression and reduced both eNOS and Bax gene expression in endothelial cells under oxidative stress.[138] Astaxanthin can activate the PI3K/Akt pathway, protecting against H_2O_2-induced oxidative stress through the Nrf2/ARE pathway in ARPE-19 cells.[139]

Astaxanthin activates the specificity protein 1 (Sp1) and NMDA receptor subunit 1 (NR1) signalling pathway, inhibiting the upregulation and nuclear transfer of Sp1

resulting from MPP+-induced production of intracellular ROS and cytotoxicity in PC12 cells.[140]

Conclusion

Astaxanthin possesses ROS scavenging and anti-oxidant activities, and thus inhibits oxidative stress-induced mitochondrial dysfunction and ROS production in cells caused by various stimuli. We propose that astaxanthin treatment might be a viable approach for the effective mitigation and prevention of CIHL associated with ROS. Astaxanthin may be an excellent candidate for treating CHIL, as it is a safe nutrient with no toxicity when consumed with food, and it has the ability to pass through the BBB because of its lipid solubility. Future studies should investigate the protective properties and underlying mechanisms of astaxanthin, which may contribute to the use of astaxanthin as an otoprotective agent.

The otoprotective effects of astaxanthin have been examined in a zebrafish model, although research into its otoprotective effects is limited.[141] Future studies should focus on the pharmaceutical potential and effects of astaxanthin for the treatment of hearing loss, particularly because astaxanthin can be easily absorbed, and when metabolized, it may have greater biological activity than its free form.[3] Future studies should include a therapeutic time window, reliability of drug administration routes, and the optimal dosages of astaxanthin. The design of clinical trials to assess the potential of astaxanthin for the treatment of ototoxicity is warranted based on evidence showing its safety. In addition, the efficacy of astaxanthin for the treatment of neurological diseases should promote clinical trials of this compound for the treatment of other diseases.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval for publication, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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