A potential role for astaxanthin in the treatment of bone diseases (Review)

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Abstract. Alterations in molecular signaling impair cellular functions and induce degenerative diseases. Among the factors affecting intracellular signaling pathways, oxidative stress serves an important role. Astaxanthin (3,3'-dihydroxy-β, β-carotene-4,4'-dione), a pigment found in aquatic organisms, belongs to the xanthophylls family. Astaxanthin exerts a strong antioxidant activity and is widely used in food, cosmetic and pharmaceutical industries. Oxidative stress damages bone homeostasis by producing reactive oxygen species and increasing the production of pro-resorption cytokines, such as interleukin (IL)-1, tumor necrosis factor-α and IL-6. Therefore, antioxidant molecules can counteract the negative effects of oxidative stress on bone. Accordingly, previous studies have demonstrated that supplementation of astaxanthin in bone contributes to the restoration of bone homeostasis. The present review summarizes the negative effects of oxidative stress in bone and explores the role of astaxanthin in counteracting skeletal injuries consequent to oxidative stress.

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1. Introduction

In the last few decades, the incidence of degenerative diseases, including neurodegenerative diseases, such as Alzheimer's disease, osteoporosis and osteoarthritis (OA) has increased in Western countries (1,2) These common pathologies are caused by intrinsic and extrinsic factors, such as biological, environmental and lifestyle factors (3). In addition, an increase in life expectancy, as observed in France, United Kingdom, USA and Netherlands (4), has led to a higher incidence of degenerative diseases (5). Aging is often associated with degenerative diseases caused by molecular and cellular damages (6). Therefore, throughout ageing impaired molecular signaling and disrupted cellular processes induce loss of function in tissues with a consequent physical decline (7). Nutrients that increase longevity can counteract the detrimental consequences of ageing (8) For example, astaxanthin has been demonstrated to reduce oxidative stress and apoptosis in model organisms (9,10), and thus can be considered an anti-aging antidote. This review highlights a possible role of astaxanthin in preventing or reducing the negative effects of degenerative diseases and, in particular, of pathologies related to the skeleton.

2. Skeletal degenerative diseases associated with oxidative stress

Oxidative stress and inflammation serve an important role in degenerative diseases. Reactive oxygen species (ROS) overproduction, resulting from oxidative stress, damages various biological molecules, including DNA, carbohydrates, proteins and lipids, causing alterations during cellular growth and differentiation processes, thus inducing apoptosis (7). Among degenerative diseases, bone diseases are associated with oxidative stress (11). The bone remodeling process, where old bone is replaced by newly formed bone, modulates redox state changes and increases ROS production. Defective antioxidant systems are also involved in the pathogenesis of bone loss (12). By inducing apoptosis in osteoblasts (cells with bone forming activity), ROS impair osteogenesis and mineralization. Increased osteoblastic apoptosis also promotes osteoclast-induced bone resorption, with oxidative stress further enhancing osteoclast differentiation (11,13,14).

In vitro experiments have demonstrated that H_2O_2 increases the number and activation of osteoclasts (15). In addition, as ROS are involved in the regulation of inflammation, ROS overproduction activates certain inflammatory factors such as tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) (16). TNF-α is involved in bone homeostasis regulation by activating different cellular pathways such as the upregulation of DKK1 (17) or RANKL (18), the inhibition of matrix protein genes expression as well as the stimulation of expression of genes related in the osteoclastogenesis or by inducing the resistance to 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) (19). Generally, TNF-α promotes bone resorption by stimulating osteoclastogenesis and reduces bone formation by inhibiting osteoblastogenesis (20).

Osteoclastogenesis and bone resorption are promoted by ROS under physiological and pathological conditions. In particular, ROS promote osteoclastogenesis by inducing receptor activator of nuclear factor-κB (RANK) signaling (21,22). Furthermore, oxidative stress affects fibronectin, an extracellular bone matrix component, by inducing its partial degradation, thereby impairing the proliferation and differentiation of osteoblasts (23). Oxidative stress involvement in the pathogenesis of osteoporosis has been investigated in several studies. For example, oxidative stress was demonstrated to increase bone resorption by inducing the expression of osteoclast-stimulating cytokines such as interleukin (IL)-1, TNF-α and IL-6 (23,24). In addition, postmenopausal females were prone to oxidative stress damage due to reduced levels of 17β-estradiol (E2), a hormone with antioxidant activity (25).

TNF-α is associated with the pathogenesis of various inflammatory diseases such as rheumatoid arthritis and ankylosing spondylitis. Rheumatoid arthritis is characterized by inflammatory processes that impair cartilage and bone, through TNF-α and IL-1 (26). TNF-α production in rheumatoid arthritis promotes pro-inflammatory cytokines such as IL-1 and IL-17, thereby increasing osteoclast activity and promoting bone resorption (27). TNF-α also increases the production of matrix metalloproteinases (MMPs) and aggrecanases which degrade cartilage extracellular matrix (20). Ankylosing spondylitis is a chronic form of arthritis characterized by inflammation and impairment of new bone formation (28). In particular, in ankylosing spondylitis and spondyloarthritis disorders, bone resorption and bone formation processes occur simultaneously in proximal anatomical sites (29). Treatment of patients with TNF inhibitors reduces disease symptoms, albeit without recovering bone remodeling ability (30,31).

3. Astaxanthin: Structure and properties

Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) is a ketocarotenoid pigment belonging to the family of carotenoid oxygenated derivatives, known as xanthophylls (32). Astaxanthin is a red pigment commonly found in aquatic organisms such as shrimps, crabs and Salmonidae (33) and is produced primarily by microalgae and phytoplankton (34). Microalgae are at the base of the food chain and are ingested by zooplankton and crustaceans, which are the prey of certain fish (35). The pigment is absorbed by these organisms and can accumulate in their tissue (36), giving them a distinctive orange-pink color. Tetraterpene exerts a strong antioxidant activity and is widely used in food, cosmetic and pharmaceutical industries (32).

While most commercial astaxanthin, primarily manufactured and distributed by Roche Diagnostics, is obtained from chemical synthesis (37), the greater demand for natural foods and the high cost of synthetic production have stimulated research into the production of astaxanthin from microorganisms. Consequently, this alternative source is becoming more relevant in basic research and in industrial applications, resulting in new companies and startups focusing on natural astaxanthin production (38).

The microalgae *Haematococcus pluvialis* (36) and the yeast *Xanthophyllomyces dendrorhous* (39) are currently the most promising sources of natural astaxanthin production, making them model organisms for use in industry. Natural astaxanthin produced by *H. pluvialis* exhibits greater antioxidant activity when compared with synthetic preparation (40). However, the greater costs of algae growth, production and active molecule extraction substantially limits its distribution in the international market (41).

Astaxanthin is synthesized via the carotenoid pathway, beginning with β-carotene (42), which is derived from geranylgeranyl pyrophosphate synthesis. The central long chain formed by carbon atoms linked by conjugated double bonds, alongside the two terminal rings, are responsible for the chemical characteristics and light absorption properties of astaxanthin (Fig. 1). Astaxanthin, together with other carotenoids such as canthaxanthin, exhibits various antioxidant activities, serving roles as free radical scavengers and potent quenchers of ROS and reactive nitrogen species (RNS) (43). Astaxanthin possess antioxidant activity that is 100 and x10 greater than vitamin E and β-carotene, respectively (44). It is therefore known as a superantioxidant. Unlike other antioxidant molecules, astaxanthin is free of pro-oxidative effects and produces no toxicity at high dosages (45). In addition to its antioxidant activity, astaxanthin exerts anti-inflammatory effects by inhibiting the NF-κB signaling pathway (46).

Natural astaxanthin may be associated with other compounds; for example, one or both hydroxyl groups may be esterified with different fatty acids (oleic, palmitic and stearic acid), forming a complex with proteins (carotenoproteins) or lipoproteins (carotenolipoproteins) (47) Natural astaxanthin produced in algae is always esterified, whereas its synthetic equivalent is not (48,49).

Astaxanthin has a wide range of applications in food, animal feeding supplementation (50) aquaculture (51) nutraceutical (52) cosmetic (53) and pharmaceutical industries (54). Its antioxidant activity is exploited in medicine for the prevention of or as an adjuvant in various diseases such as cancer, dermatological disorders, inflammation and cardiovascular damage, as well as for the general enhancement of the immune response (38,45).

4. Delivery of astaxanthin

Although astaxanthin possesses a strong anti-inflammatory activity (55), its use in nutraceuticals and in the medical-pharmaceutical field is limited. This is due to astaxanthin being highly unstable and easily degradable when heated. The presence of oxygen and light are therefore required for

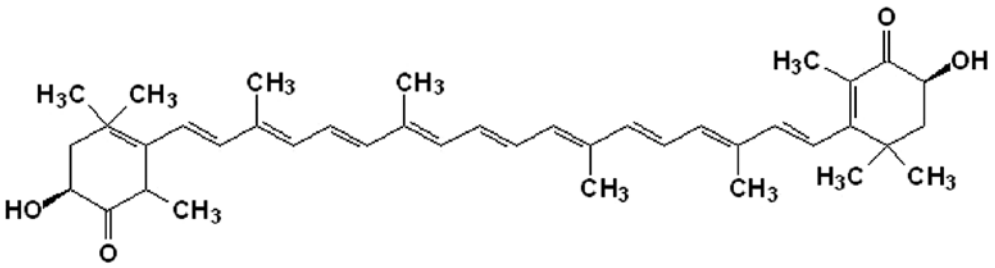

Figure 1. Chemical structure of astaxanthin.

its production, extraction and transformation, alongside additional technological processes such as mechanical and chemical treatments using solvents (45,56). The chemical structure of astaxanthin makes it insoluble in water, which leads to difficulties in intestinal tract absorption if administered orally. The insolubility of astaxanthin also makes its parenteral administration difficult without solubilisers, such as DMSO (57); however, such solubilisers would change the chemical properties of the molecule (58).

The production of astaxanthin derivatives as prodrugs has been undertaken to overcome the aforementioned difficulties (45). An alternative approach is to encapsulate astaxanthin into particles with biocompatible and biodegradable polymers, conferring water solubility and a greater resistance to degradation; thus, specially designed micro- or nano- delivery structures, such as micro- and nano-dispersion systems, are under development (59,60).

Astaxanthin formulation into nanostructures was initially employed to protect the active compound from thermal degradation and other physicochemical parameters such as pH and ionic strength (56,61). Furthermore, this innovative approach has been used to improve yield during extraction from microorganisms (62-64) or to achieve a more sustainable production process (65). Another process conferring stability to the active molecule is its encapsulation into micro- and nano-alginate beads. This method is mainly used to entrap astaxanthin oil derivatives and produce a higher stability at room temperature over time, which is important for the shelf-life of nutraceuticals and food products (66,67).

Since astaxanthin is mainly characterized by its low water solubility, novel approaches have been developed to enhance its bioavailability in cells using nanotechnology. The nanoencapsulation system most commonly used to study astaxanthin in an aqueous environment and for biomedical purposes is based on liposomes (68-70). The liposomal system preserves astaxanthin antioxidant activity as the nanoparticles used are non-toxic and cellular uptake is more efficient when compared with other delivery methods such as colloidal astaxanthin (71) or astaxanthin dissolved in dietary oils (72). Furthermore, astaxanthin can be coupled with other substances, such as vitamin E derivatives, to study their synergistic effects (73,74).

Another polymer used to encapsulate astaxanthin is chitosan, a D-glucosamine polysaccharide obtained by digestion of the chitin shells of shrimps and other crustaceans with an alkaline agent (75) Chitosan encapsulated astaxanthin results in a non-toxic and biocompatible material with antibacterial activity, which confers stability and solubility in water, and increases bioavailability of the encapsulated active compound (76). This biopolymer can be used alone or in combination with other polymers, such as proteins. For example, the chitosan and β-lactoglobulin nanoparticle complex prolongs the release of astaxanthin and improves its stability when subjected to the extreme pH conditions of the gastrointestinal tract (77). Poly(lactic-co-glycolic acid) has been approved by the Food and Drug Administration and the European Medicines Agency together with chitosan for nanoparticle synthesis (78,79). It is a biocompatible polymer that degrades into lactic and glycolic acid monomers, which enter into the Krebs cycle and are easily metabolized. The aforementioned polymers have been used recently to prepare astaxanthin loaded core-shell nanoparticles with good aggregation stability and increased bioavailability of the active compound when administered orally (60).

5. Role of astaxanthin in oxidative stress

Oxidative stress serves a central role in aging and in the development of various degenerative, neurodegenerative and inflammatory disorders, which are often the result of oxidative stress that affects mitochondrial function, disrupting cellular homeostasis (80).

Mitochondria regulate energy production at a cellular level and generate high levels of ROS. ROS produced by mitochondria are generally removed by superoxide dismutase, catalase and glutathione peroxidase (81). However, pathological disorders can induce an excessive production of mitochondrial ROS, the accumulation of which impairs mitochondria and consequently leads to cell damage (82). Damaged mitochondria exhibit morphological fragmentation. As the mitochondrial membrane potential is disrupted, their functionality is reduced. In addition, oxidative stress due to the accumulation of ROS, induces mitochondrial membrane permeabilization, leading to the activation of the intrinsic apoptotic pathway (83).

Astaxanthin scavenges singlet status oxygen molecules and free radicals, reducing lipid peroxidation (32). The ability to counteract excessive oxidation, thus causing lipidic alteration, is important in osteoporosis. It the authors' previous study, increased levels of modified lipoproteins derived from the oxidation of arachidonate-containing phospholipids (Ox-PAPCs) were identified in osteoporotic patient sera (84). In addition, it was demonstrated that Ox-PAPCs impair the osteogenic commitment of mesenchymal stem cells (MSCs) by downregulating Runt-related transcription factor 2 and upregulating adipogenic transcription factor peroxisome proliferator-activated receptor gamma 2 (84).

Mutations and disrupted enzymatic activity occurring in mitochondria are often associated with atherosclerosis (85). In particular, damaged endothelial cells result from the overproduction of mitochondrial ROS in atherosclerosis (86). Antioxidants targeting mitochondria could prevent this dysfunction to protect endothelial cells (87). Previous studies have suggested that astaxanthin reduces oxidative stress affecting the mitochondria. For example, the role of astaxanthin in maintaining mitochondrial functions by modulating their redox state has been demonstrated (88). It was also revealed that astaxanthin maintained mitochondria in a reduced state in the presence of H_2O_2 (88). Supplementation of astaxanthin to increase ATP production and to potentiate the activity of the mitochondrial respiratory chain has been proposed to counteract age-associated diseases in dogs (89). Furthermore, astaxanthin can prevent cytochrome c release due to mitochondria permeabilization, consequently inhibiting apoptosis (81). As demonstrated in lung fibrosis, astaxanthin can prevent H_2O_2-induced apoptosis and bleomycin in alveolar epithelial cells (90). Pretreatment with astaxanthin in cardiotoxicity models has been demonstrated to prevent mitochondrial fragmentation, poly-ADP-ribose polymerase and caspase activity, thus inhibiting apoptosis (91).

ROS can impair redox balance in muscle after physical activity, inducing muscle fatigue and reducing exercise performance (92). Upon supplementation, astaxanthin accumulates in the liver, kidney and muscle, where it reduces DNA and lipid peroxidation, thereby preventing further muscle damage (93). It has been suggested that astaxanthin, by reducing the accumulation of exercise-induced ROS, can improve carnitine palmitoyl transferase 1 activity, increasing fat oxidation (94,95). Astaxanthin has therefore been proposed for use in physical activity to counteract the negative effects of ROS production (96).

6. Effects of astaxanthin on bone and cartilage

Search strategy. Studies aiming to evaluate of the effects of astaxanthin on the skeletal system were collected from public databases. In particular, 80 full articles were identified by consulting the following databases: PubMed (https://pubmed.ncbi.nlm.nih.gov), Web of Science (http://login.webofknowledge.com) and Scopus (https://www.scopus.com) The search strategy employed in all databases was as follows: Astaxanthin AND bone/or mesenchymal cells/or osteoblasts/or skeletal/ or chondrocytes/or cartilage. Duplicates were removed and an independent screening of abstracts was performed for content consistency. A total of 9 papers were included.

Astaxanthin and bone. In chronic metabolic diseases, elevated levels of fatty acids impair the microenvironment of MSCs, affecting their viability and the production of certain cytokines, such IL-6, vascular endothelial growth factor and monocyte chemoattractant protein-1 (97). However, it has been demonstrated that astaxanthin supplementation protects MSCs from fatty acid induced-cell death and inflammatory effects (97). Antioxidant molecules can induce osteogenic differentiation and improve bone mineralization by counteracting oxidative stress (12,98). Accordingly, it has been demonstrated that supplementation with astaxanthin in a model of periodontitis reduced osteoclast activity and increased osteoblast number in the mandible (99). It has been revealed that the supplementation of astaxanthin improved bone quality in ovariectomized mice, an *in vivo* model of osteoporosis (100). In particular, the authors observed reduced levels of bone resorption biomarkers, such as serum calcium, inorganic phosphorus, alkaline phosphatase and tartrate-resistant acid phosphatase (TRAP), and that bone mineral density and microarchitecture were recovered after 6 weeks of astaxanthin supplementation. In addition, by performing *in vitro* experiments, the authors observed that astaxanthin affected osteoclast activation by reducing the expression of nuclear factor of activated T cells c1, dendritic cell-specific transmembrane protein, TRAP and cathepsin K (101). Furthermore, a recent study reported that in rats treated for 8 weeks with D-galactose (200 mg/kg), *H. pluvialis* biomass administration reduced bone loss (101). In particular, the authors observed that bone quality in treated rats improved via regulation of the osteoprotegerin (OPG)/RANKL pathway. This pathway regulates the crosstalk between osteoblasts and osteoclasts, consequently affecting the bone remodeling process (102). The aforementioned studies suggest the positive effects of astaxanthin in reducing bone loss. Its supplementation may therefore represent a useful tool for counteracting bone degenerative diseases.

Astaxanthin and cartilage. Previously published works have suggested positive effects of astaxanthin in OA experimental models. OA occurs due to chondrocyte dysfunction following an inflammatory state, with an overproduction of MMPs inducing the degeneration of articular cartilage (103). Two different studies have reported reduced expressions of MMP-1, MMP-3 and MMP-13 in the IL-1β stimulated chondrocytes of an *in vitro* experimental OA model treated with astaxanthin (103,104). In particular, it has been demonstrated that astaxanthin prevented MMP production by reducing the phosphorylation of p38 and ERK1/2 activated by mitogen-activated protein kinase (104), or by downregulating the expression of NF-κB and activator protein-1, which are upstream regulators of MMP production (103). The administration of astaxanthin ameliorated cartilage status by reducing MMP production in an *in vivo* OA model obtained by damaging the knee anterior cruciate ligament in rabbits (105). Therefore, the supplementation of astaxanthin can be considered a beneficial treatment to counteract OA.

7. Conclusions

Degenerative diseases and in particular bone diseases, cause physical disability and heavily impact public healthcare (106). Oxidative stress is considered an important cause of the pathogenesis of chronic and degenerative diseases. Therefore, targeted actions aimed at preventing or reducing the negative effects of oxidative stress are required. The use of natural antioxidant molecules, such as astaxanthin, may represent an effective strategy to counteract bone degenerative diseases.

To the best of our knowledge, few studies aiming to evaluate the use of astaxanthin in bone diseases have been performed thus far. The results of the present review may stimulate further investigations aimed at understanding and potentially supporting the use of astaxanthin against bone diseases.

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MTV, MP and MM conceptualized and designed the study. MTV, MP, MGR, MM and LDC acquired and interpreted the data, and wrote the manuscript. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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