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Astaxanthin-antioxidant impact on excessive Reactive Oxygen Species generation induced by ischemia and reperfusion injury

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ABSTRACT

Oxidative stress induced by Reactive Oxygen Species (ROS) was shown to be involved in the pathogenesis of chronic diseases such as cardiovascular pathologies. Particularly, oxidative stress has proved to mediate abnormal platelet function and dysfunctional endothelium-dependent vasodilatation representing a key factor in the progression of ischemic injuries. Antioxidants like carotenoids have been suggested to contribute in their prevention and treatment. Astaxanthin, a xanthophyll carotenoid produced naturally and synthetically, shows interesting antioxidant and anti-inflammatory properties. *In vivo* studies applying different models of induced ischemia and reperfusion (I/R) injury confirm astaxanthin's protective action after oral or intravenous administration. However, some studies have shown some limitations after oral administration such as low stability, bioavailability and bioefficacy, revealing a need for the implementation of new biomaterials to act as astaxanthin vehicles *in vivo*. Here, a brief overview of the chemical characteristics of astaxanthin, the carrier systems developed for overcoming its delivery drawbacks and the animal studies showing its potential effect to treat I/R injury are presented.

1. Introduction

Reactive oxygen species (ROS) refers to a variety of highly reactive molecules and free radicals derived from molecular oxygen. ROS are formed as a normal byproducts of aerobic respiration and current cellular metabolism [1]. Moderate amounts of ROS have beneficial effects on several physiological processes like the reduction of malignant pathogens, wound healing, and tissue repair processes by acting as signaling molecules [2–4]. In contrast, ROS overproduction disrupts the body homeostasis inducing oxidative tissue damage [5]. Indeed, high ROS levels leads to decreased bioavailability of nitric oxide, impairing endothelium-dependent vasodilatation thus promoting vasoconstriction [6]. These alterations occur early in the development of vascular disease [7]. Moreover, overproduction of superoxide anion radical and hydroxyl radical have been considered causative agents of severe diseases, such as arteriosclerosis and I/R injury [8–10], pathologies currently linked to increased rates of lipids peroxidation [8,11].

In the cell, these reactions are counteracted by the action of enzymatic and non-enzymatic antioxidant defenses. Tissue damage takes place when these antioxidant defenses are not sufficient to control the radicals generation [12]. Recent studies suggest the use of exogenous antioxidant supplementation with carotenoids would enhance antioxidant defenses thanks to their potential scavenging capabilities [13–17]. Astaxanthin carotenoid is known to be a potent quencher of singlet oxygen and an efficient scavenger of superoxide anion [18], and hydroxyl radical [19,20] by acting as an antioxidant. Moreover, within the cell, it can effectively scavenge lipid radicals and effectively destroys peroxide chain reactions to protect fatty acids and sensitive membranes [21,22] reducing the risk of atherosclerotic plaque formation [23,24]. Furthermore, the astaxanthin effect in the prevention and treatment of I/R pathologies *in vivo* revels its potent action as antioxidant molecule. However, astaxanthin as a highly unsaturated molecule decomposes easily when being exposed to heat, light and oxygen. Additionally, its poor water solubility, stability and bioavailability limits its appropriate oral administration and delivery *in vivo*. The implementation of new biomaterials to act as astaxanthin vectors has been attempted through various strategies. Here, a review of *in vivo* studies reporting the effect of astaxanthin supplementation to counteract ischemia/reperfusion injury will be presented, including a brief review of astaxanthin carrier's system successfully developed for overcoming delivery challenges.

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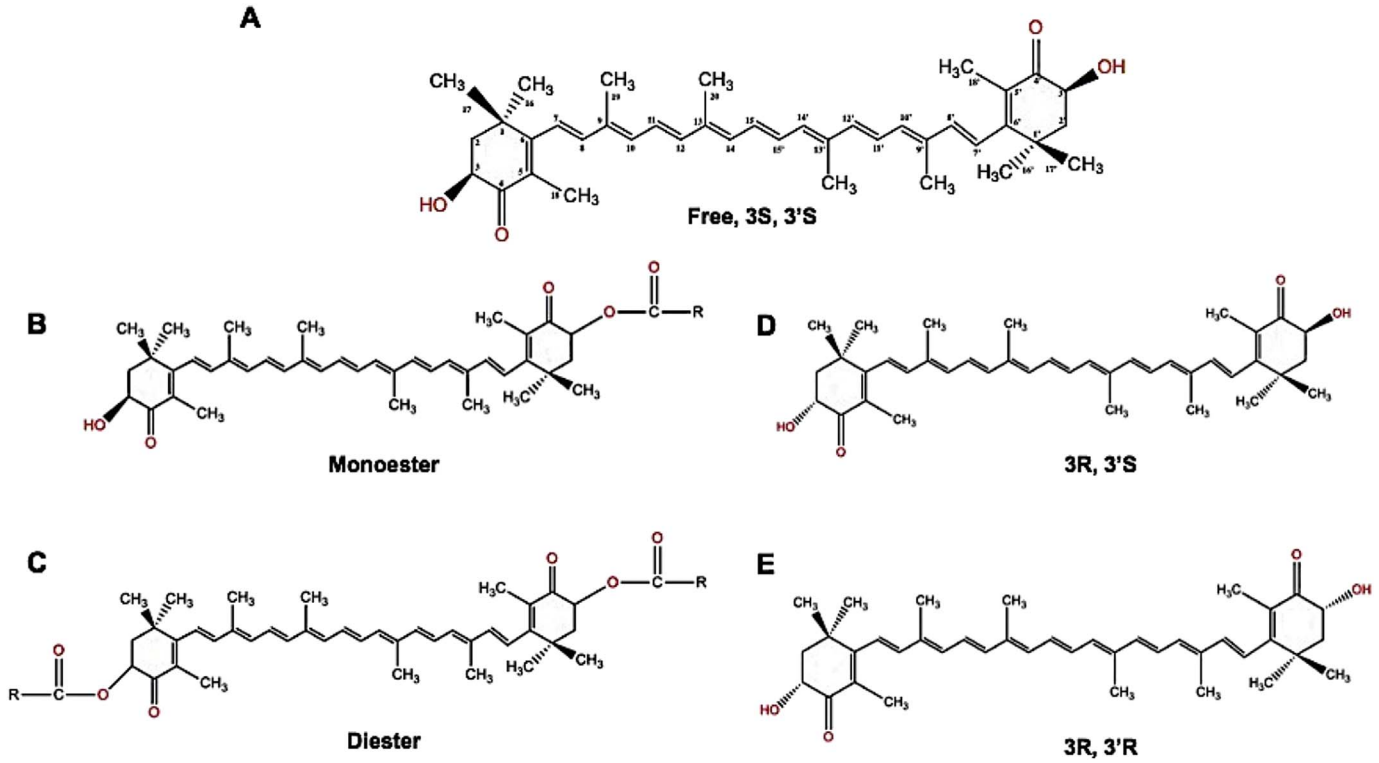

Fig. 1. (A) Structure of free astaxanthin with a numbering scheme in the stereoisomer form 3S, 3′S. Astaxanthin (B) monoester and (C) diester form. (D–E) Astaxanthin stereoisomers 3R,3′S and 3R,3′R. Natural H. pluvialis produced astaxanthin 3S,3′S containing 2% free, 90% monoester and 8% of diester, while synthetic astaxanthin exists as free form constituted by 3S,3′S, 3R,3′S and 3R, 3′R in a ratio of 1:2:1, respectively [25,43,44].

2. Astaxanthin: A powerful antioxidant molecule

2.1. Astaxanthin sources

The carotenoid astaxanthin is found in various microorganisms and marine animals, such us yeast, microalgae, salmon, krill, shrimp, complex plants and some birds [25–29]. As in general with all carotenoids, astaxanthin is not synthesized by humans and therefore requires to be ingested in the diet, seafood being the main source [30,31].

Haematococcus pluvialis (H. pluvialis), a unicellular biflagellate green microalgae, is believed to have the highest capacity to accumulate astaxanthin in nature under environmental stresses such as starvation, high salt or pH, elevated temperature, or irradiation [25,32]. Under these unfavorable conditions, microalgae modify their cellular morphology, increasing their size to become red cysts charged with ~80% of astaxanthin pigment [54] and 20% comprised a mixture of other carotenoids [33]. Due to the high astaxanthin concentration, microalgae represent the primary natural source of processed astaxanthin for human applications such as dietary supplements, cosmetics, and food and beverages [34], while the synthetic [35,36], yeast (mutated *Xanthophyllomyces dendrorhous*) [37] and bacteria sources (from *Paracoccus carotinifaciens*, an aerobic bacteria) [38] are predominantly used in the aquaculture sector [35]. Moreover, dietary supplements containing *H. pluvialis* astaxanthin have proved to be safe and accepted by the American Food and Drug Administration at daily doses of 2–12 mg per day [39,40].

2.2. Chemical characteristics

Astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione) carotenoid is a fat-soluble orange-red color pigment with the molecular formula $C_{40}H_{52}O_4$ and molar mass of 596.84 g/mol. Astaxanthin structure consists of 40 carbon atoms which contain two oxygenated β-ionone-type ring systems linked by a chain of conjugated double bonds (polyene chain). The oxygen presence in astaxanthin ionone rings in both hydroxyl (OH) and keto (C═O) groups, makes it a member of the xanthophyll carotenoid family and confers to astaxanthin a more polar nature than other carotenoids [41]. Additionally, the conjugated double bonds allow astaxanthin to act as a strong antioxidant by electron donation and by reacting with free radicals [42] (Fig. 1A).

In its free form, astaxanthin is considerably unstable and particularly susceptible to oxidation, therefore, this form is mainly produced synthetically or from yeast [43]. In nature, it is found either conjugated with proteins (e.g., salmon muscle or lobster exoskeleton) or esterified by hydroxyl reaction with one (monoester) or two (diester) fatty acids, which stabilize the molecule. Natural astaxanthin from *H. pluvialis* contains 70–90% of monoesters, about 8% of diester and 2% of free form [41,44,45] (Fig. 1B–C). A protective role against high light and oxygen radical has been attributed to astaxanthin accumulation in *H. pluvialis* [33]. The stereogenic carbons in the 3 and 3′ positions on the β-ionone moieties define astaxanthin conformation as chiral [(3S, 3′S) or (3R, 3′R)] or as meso form (3R, 3′S), with the chiral conformation the most abundant in nature [27] (Fig. 1D–E). Astaxanthin from microalgae *H. pluvialis* biosynthesizes the (3S, 3′S) isomer whereas yeast produces (3R, 3′R) isomer [41]. The synthetic source consists of isomers (3S, 3′S) (3R, 3′S) and (3R, 3′R) [27].

2.3. Extraction, storage, stability of astaxanthin

When the stress is induced, the microalgae *H. pluvialis* becomes encysted cells and accumulates high quantities of astaxanthin [46]. This growth stage is usually produced in either enclosed outdoor systems or closed indoor photo-bioreactors, which are preferred to avoid contamination by other microorganisms and to guarantee optimal and controlled growth conditions [36]. Different methods had been carried out to extract the greatest quantity of the carotenoid from *H. pluvialis* biomass by cracking the cell [33]. Some of them are based on the use of solvents [47], edible oils [48], enzymatic digestion [49], but