Astaxanthin Attenuates Oxidative Damage in Retina by Potentiation of the Antioxidant Capacity in Experimental Model of Diabetic Retinopathy

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Abstract

Background: Astaxanthin is a natural carotenoid, which is belonged to the Xanthophylls family. Previously, the powerful antioxidant properties of this compound have been demonstrated in several biological environments.

Objectives: As overproduction of reactive oxygen species (ROS) has a critical role in the pathophysiology of diabetic retinopathy (DR), we aimed to evaluate the effects of astaxanthin on the antioxidant capacity of retina in experimental model of DR.

Methods: Forty eight rats were randomly divided into four groups (n=12 for each group); normal, diabetic, and two treated normal and diabetic groups. Diabetes was induced by a single intravenous injection of streptozotocin (50 mg/kg). Rats with blood glucose >500 mg/dl were selected as diabetic animals. Treatment groups were treated with astaxanthin (20 mg/kg/day, orally) for six weeks. At the end, the eyeballs were removed under deep anesthesia and then the retinal tissues were quickly frozen for assessment of the glutathione and malondialdehyde contents as well as superoxide dismutase (SOD) activity.

Results: Malondialdehyde levels of the retina in diabetic rats increased significantly (16%), whereas the glutathione levels decreased (85%), (P<0.05). Astaxanthin significantly increased the glutathione content of retina in treated diabetic rats by 77% compared to untreated rats (P<0.05). Diabetes also increased the activity of SOD in retina. Treatment with astaxanthin enhanced the SOD activity both in treated normal and diabetic rats (P<0.05).

Conclusion: Our findings reveal that astaxanthin can decrease oxidative damage of retina by improving the antioxidant capacity in diabetic rats, which may ultimately delay the appearance of DR.

Keywords: Antioxidant capacity, Astaxanthin, Diabetes mellitus, Diabetic retinopathy, Hyperglycemia

1. Background

Diabetic retinopathy (DR) is one of the main complications of diabetes, which leads to vision loss in diabetic patients (1). According to the WHO (World health organization) report, the prevalence of DR will be increased worldwide due to diabetes epidemic, and thus, it is predicted that the number of people at risk of blindness will be double till the year 2030 (2). Experimental and clinical findings have been evidently demonstrated that overproduction of oxygen free radicals and oxidative stress during chronic uncontrolled diabetes plays a crucial role in the pathogenesis of DR (3,4). As the highest quantities of polyunsaturated fatty acids, glucose oxidation and oxygen uptake in the retina, this tissue is more vulnerable to oxidative damage compared to other tissues (5-7). It has been reported that chronic hyperglycemia increases the generation of reactive oxygen radicals (ROS) in retina by several pathways, including activation of polyol, protein kinase C (PKC) and hexosamine pathways as well as advanced glycation end product (AGE) formation (8,9). Activations of pro-oxidant enzymes (such as NADPH-oxidase) are other pathways that diabetes induces oxidative stress by them (10). Moreover, previous findings have demonstrated that the antioxidant defense system of retina, which neutralizes oxygen free radicals, is weakened in diabetes (8,9). In the mammalian cells, this defense system is the first line of defense against the adverse effects of free radicals. Superoxide dismutase (SOD) is the main antioxidant enzyme in retina that neutralizes superoxide anion to hydrogen peroxide. It is well known that the levels and the activity of SOD change during diabetes (11). Additionally, glutathione is the non-enzymatic antioxidant that the levels of it decline in retina during diabetes (12,13).

Astaxanthin is a natural carotenoid that is belonged to the Xanthophylls family. This compound is present in some microorganisms in the high quantities such as Haematococcus Pluvialis (14). Several studies have been demonstrated the capability of astaxanthin for protection of brain, central nervous function, cellular health and overall health in several pathological states (15-20). Previously, the antioxidant effects of astaxanthin have been reported in experimental and clinical studies (14,21). This compound is located between phospholipids molecules in cells membranes, which help to protect both sides of the cells against adverse effects of various free radicals. Astaxanthin is able to...
ameliorate the enzymatic antioxidant profile of salivary gland as well as the redox imbalance in lymphocytes in experimental model of diabetes (15,16). According to the previous findings, astaxanthin has been proposed as a novel potential treatment against oxygen free radicals and oxidative stress, and also for inflammation in several cardiovascular diseases (14). Astaxanthin treatment has been also demonstrated that protects the retinal photoreceptors of rats against the damaging effects of UV-light (22). Moreover, astaxanthin improves mitochondrial function such as respiratory function and membrane potentials (23,24).

2. Objectives

Because of weakening the retina antioxidant capacity, oxidative damage plays a crucial role in the pathophysiology of DR. Thus, we aimed to examine the possible protective effects of astaxanthin against hyperglycemia-induced retina damage in the rat model of DR through potentiation of the enzymatic and non-enzymatic antioxidant defense systems during uncontrolled diabetes.

3. Methods

3.1. Animals

In the present study, forty eight male Wistar rats (6-7 weeks old) weighting 190–225 g were used. The rats were obtained from the Experimental Animal Center of Baqiyatallah University of Medical Sciences, Iran. All protocols of the study were approved by the Bioethics Committee of Animals from the University of Baqiyatallah medical sciences that followed the NIH guidelines for use and care of animals. Rats were housed in the standard situations, including 12 hours light/dark cycle, humidity of 40%-60%, temperature of 23±4°C, and free access to food and water throughout the study.

3.2. Induction of diabetes mellitus

In the present study, diabetic rats were used to achieve the animal model of diabetic retinopathy (10). Diabetes was induced by a single intravenous injection of streptozotocin at dose of 50 mg/kg body weight (dissolved in cold normal saline) through the lateral tail vein (8). Streptozotocin (STZ) was purchased from Sigma Aldrich, USA. On the fifth day after streptozotocin injection, diabetes was confirmed by determination of blood glucose and the rats with blood glucose levels above 500 mg/dL were selected as diabetic animals.

3.3. The protocols of the experiment

To perform the study, the rats were randomly divided into four groups of equal numbers (12 rats in each group) as follows: normal group (normal healthy rats that used as normal control), astaxanthin-treated normal rats (normal healthy rats that received orally astaxanthin at a dose of 20 mg/kg/day for six weeks), diabetic control (diabetic rats that used as diabetic control) and astaxanthin-treated diabetic rats (diabetic rats that received orally astaxanthin at a dose of 20 mg/kg/day for six weeks). The dose of astaxanthin (Viva Labs, Manufactured Number 1-800-921-8482, USA) was selected according to the previous studies (15,16).

Blood glucose was measured at the begging and termination of the study by a glucometer (ACCU-CHEK, Germany). After termination of the study (six weeks) the blood glucose was checked. Then, the rats were deeply anesthetized and the eyeballs were removed and the retinal tissues were quickly washed with cold PBS (phosphate buffer saline) at pH 7.4, and then frozen (using liquid nitrogen) and stored at -80°C.

3.5. Tissue Preparation

The retinal tissues were homogenized with special lysis buffer containing 20 mM TrisHcl pH 7.4, 50 mM NaCl, 1mM ethylene-diamine tetra acetic acid (EDTA), 0.5mM Triton *100, Sodium deoxycholate 0.5%, Sodium dodecyl sulfate (SDS) 0.1%. The homogenized solutions were sonicated at temperature of 4°C and then for thirty minutes placed on ice. Afterwards, they were centrifuged at 10000 * g for 10 minutes at 4°C to remove nuclear particles and tissue pieces. A part of the supernatant was consumed for the determination of total protein concentration by using Bradford method. The supernatants were allocated for determination of glutathione (GSH) and malondialdehyde (MDA) contents as well as superoxide dismutase (SOD) activity.

3.6. Glutathione (GSH) content of retina

The method of Tietz was used to determine the GSH contents of retinal tissues (25). 100 µl of supernatant was mixed with 10 µl of 5% sulfosalicylic acid then centrifuged for 1 minute (10000 rpm). GSH content in the supernatant was determined as follows: 100 µl of the protein-free supernatant of the cell lysate, 810 µl of 0.3 mM Na2HPO4 and 90 µl of 0.04% 5, 5’-dithiobis-(2-nitrobenzoic acid) (DTNB) in 0.1% sodium citrate. The absorbance of DTNB was monitored at 412 nm for 5 minutes. A standard curve of GSH was produced and sensitivity of measurement was determined to be between 1 and 100 µM. The GSH content of retina was eventually calculated as nMol/mg protein.

3.7. Superoxide dismutase (SOD) activity of retina

SOD activity of the retinal tissues was measured according to the ability of SOD to prevent the decline of nitroblue tetrazolium (NBT) by superoxide using Winterbourn method (26).

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In the present study, all values are expressed as mean±SEM. The analyses of data between groups were performed using the nonparametric tests of Kruskal–Wallis and Mann–Whitney. Also, the paired sample t-test was used for the analyses of data within groups. All states, P<0.05 was considered to be statistically significant.

4. Results

4.1. Blood glucose

As shown in Table 1, induction of diabetes noticeably increased blood glucose in diabetic rats (554±5 mg/dL) compared to normal animals (126±2 mg/dL, P<0.001). Blood glucose of diabetic rats did not change during the test and the mean value of blood glucose was 580±5 mg/dL at the termination of the experiment. Treatment with astaxanthin also did not change the mean values of blood glucose in normal and diabetic rats during the test and these values were 126±2 mg/dL and 581±5 mg/dL for astaxanthin-treated normal and diabetic rats, respectively.

4.2. Malondialdehyde (MDA) content

The levels of malondialdehyde (MDA) in the retinal tissues are shown in figure 1 at the end of the experiment. Treatment with astaxanthin for six weeks did not change the MDA contents of retinal tissues in treated normal rats (2.37±0.10 nMol/mg protein) compared to non-treated normal animals (2.36±0.10 nMol/mg protein). The MDA contents of retinal tissues were considerably increased in diabetic rats (2.86±0.15 nMol/mg protein) compared to normal animals (P<0.05), whereas the mean value of MDA content at retinal tissues of astaxanthin-treated diabetic rats was lower than diabetic control group (2.64±0.12 nMol/mg protein) and had no significant difference compared to normal group.

4.3. Superoxide dismutase (SOD) activity

As shown in figure 2, the activity of superoxide dismutase in the normal retinal tissues was 2.62±0.37 mU/mg protein. Astaxanthin significantly increased the superoxide dismutase activity in treated normal rats (11.47±3.21 mU/mg protein) compared to untreated normal group (P<0.05). The activity of superoxide dismutase was significantly higher in diabetic control group (15.52±1.36 mU/mg protein) compared to normal group (P<0.05). Moreover, astaxanthin increased the superoxide dismutase activity of treated diabetic rats (19.17±1.56 nMol/mg protein).

4.4. Glutathione (GSH) content

The glutathione levels of the retinal tissues are shown in figure 1 at the end of the experiment. Treatment with astaxanthin for six weeks did not significantly change the glutathione levels of retinal tissues in treated normal rats (130±22 pMol/mg protein) compared to non-treated normal animals (212±72 pMol/mg protein). The glutathione levels of retinal tissues significantly decreased in diabetic rats (29±4 pMol/mg protein) compared to normal.

| Table 1. Effects of astaxanthin on blood glucose (mg/dL) of normal and diabetic rats after six weeks treatment |
|------------------------|------------------------|------------------------|------------------------|------------------------|
|                        | Normal                | Normal & Astaxanthin   | Diabetes               | Diabetes & Astaxanthin |
| Beginning (day 1)      | 126±2                 | 124±2                  | 554±5*                 | 582±5*                 |
| Termination (day 42)   | 125±22                | 126±2                  | 580±5*                 | 581±5*                 |

All values are presented as mean±SEM

* As significant difference compared with normal group (P<0.001)
5. Discussion

Previous studies have shown the higher antioxidant properties of astaxanthin, including scavenging of superoxide anion, hydrogen peroxide and hydroxyl radicals (14,21). According to the experimental and clinical studies, astaxanthin is able to ameliorate the oxidative damage of several tissues such as brain, heart and reproductive organs in pathological conditions (15-20,29). The present study showed, in diabetic rats increased the index of oxidative damage (increased MDA levels) in the retinal tissues after six weeks significantly. The glutathione levels of retina also reduced significantly in diabetic rats that have uncontrolled hyperglycemia. On the other hand, astaxanthin-treated diabetic rats revealed a decrease in the MDA levels of retina after six weeks treatment. Treatment with astaxanthin in diabetic rats for six weeks also potentiated the antioxidant capacity of retina through an increase in the activity of superoxide dismutase and glutathione levels of retina.
The results of present study indicate that the levels of oxidative damage were significantly increased in the retinal tissues of diabetic rats (increased MDA levels). MDA has been demonstrated as an early biomarker of the lipid peroxidation and oxidative stress (30). Based on previous findings, an increase in the levels of MDA in diabetic patients (as an index of oxidative damage) has been reported in the blood and several tissues (31). Because of high polyunsaturated fatty acids and oxygen uptake, the retina is more susceptible to oxidative damage compared to other tissues (30,32). Additionally, it has been repeatedly demonstrated that the antioxidant capacity of retina is weakened during chronic diabetes, which has a key role in the abolishing of ROS (8,9).

In accordance with these results, our findings revealed that the glutathione content of retina was decreased in diabetic rats. Yoshida et al. demonstrated that the intracellular content of glutathione decreases during diabetic conditions because the activity of γ-glutamyl cysteine ligase, the rate-limiting enzyme for glutathione synthesis, decreases in diabetes (33). Moreover, it is well known that the levels and activity of SOD change during diabetes (11). In the present study, the activity of SOD in the retinal tissues of diabetic rats significantly increased compared to the normal animals. This finding is in accordance with other findings that have shown the oxidative stress through overexpression of SOD in mitochondria may prevent the accumulation of ROS in diabetic mice (6). Therefore, it is suggested that increased retinal activity of SOD in diabetic rats is a compensatory response against oxidative stress.

According to our results, astaxanthin decreased the oxidative damage of the retinal tissue in treated diabetic rats (decreased MDA levels). Astaxanthin has an individual molecular structure making it as a powerful antioxidant to scavenge the various free radicals, including superoxide anion, hydroxyl and hydrogen peroxide radicals (34–36). It has been demonstrated that astaxanthin decreases the apoptosis of retinal ganglion cells in mice probably by inhibition of ROS generation and oxidative stress (10). Astaxanthin by antioxidative property is also a new potential treatment for cardiovascular diseases (14). Our findings also indicated that astaxanthin increased the activity of SOD in treated normal and diabetic rats. SOD is the main antioxidant enzyme in retina that neutralizes superoxide anion to hydrogen peroxide. Moreover, the results of present study revealed that astaxanthin increased the glutathione content of retinal tissue in treated diabetic rats. Glutathione scavenges hydroxyl radical and singlet oxygen directly and is also a cofactor of several detoxifying enzymes against oxidative stress such as glutathione peroxidase (37). Therefore it is proposed that astaxanthin potentiates the antioxidant capacity of retinal tissue of diabetic rats, which in turn, can decrease the ROS accumulation and oxidative stress of retina during diabetes. Our results also showed that astaxanthin did not change the blood glucose of diabetic rats. Thus, these findings confirm the direct antioxidant properties of astaxanthin for protection of retina against adverse effects of diabetes-induced oxidative damage.

It is concluded that astaxanthin, with antioxidative properties, protects the retinal tissue against uncontrolled diabetes-induced retinopathy by increment of the antioxidant capacity of retina. Also, our findings are suggesting the effective therapeutics of this compound for reduction of the retina damage during diabetes.

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Conflicts of interest

The authors report no declarations of interest.

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