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Review article

Aging of the crystalline lens from the viewpoint of anti-glycation and anti-oxidation.

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Abstract

Ultraviolet rays, smoking, and drugs are considered to be risk factors for presbyopia and age-related cataracts. These cause oxidative stress and glycative stress, therebyinducing disulfide bond (S-S bond) formation, followed by aggregation / denaturation and hardening of the nucleus of the lens protein, resulting in the formation of presbyopia and cataracts. Therefore, antioxidant treatment and anti-glycation treatment can be expected to have a preventive / suppressive effect on cataracts and presbyopia.

We have succeeded in delaying the progression of cataracts in the genetic cataract model, Shumiya Cataract Rat (SCR), by oral administration of an antioxidant lutein (LU) and anti-glycation water chestnut (*Trapa bispinosa* Roxb.) extract (TBE), where it was shown to increase the expression of the endogenous antioxidant protein peroxiredoxin 6 (Prdx6) and catalase in lens epithelial cells. Therefore, it can be effective in preventing cataracts to reduce not only the crystalline lens but also the whole body of oxidative and glycative stress and increase the endogenous antioxidant protein by ingesting antioxidants and anti-glycation supplements.

***KEY WORDS*:** cataract, glycative stress, oxidative stress, lutein, water chestnut (*Trapa bispinosa* Roxb.)

1. Introduction

Cataracts are the number one cause of blindness in the world and its prevalence is estimated to rise to 50 % by 2020 [1]. Cataracts are attributed to aging, ultraviolet (UV), radiation, inflammation, diabetes, smoking, steroids intake, and highmyopia. Since the lens has a poor protein turnover, the lens becomes opaque due to the accumulation of exogenous and endogenous oxidative stress and glycative stress associated with aging [2, 3]. Oxidative stress, *i.e.* free radicals, reactive oxygen species (ROS), is known to cause cell damage and protein denaturation *in vivo*. Therefore, for the prevention of cataracts, the anti-cataract effects of various supplements, phytochemicals, and functional foods have been studied for the purpose of reducing these stresses in the body and eyes [4]. This paper outlines the results of our research on the mechanism of age-related cataract development associated with oxidative stress and glycative stress, and on cataract prevention with lutein (LU) and water chestnut (*Trapa bispinosa* Roxb.) extract (TBE).

2. Changes and functions of crystallin protein in aging

About 90 % of the protein in the human lens is composed of α, β, and γ-crystallin, which plays a role in maintaining the transparency of the lens and its refraction. In order to maintain the transparency of the lens, it is necessary to correctly form the three-dimensional structure peculiar to the crystallin protein. Therefore, a molecular chaperone is present to support correctly keeping the three-dimensional structure. α-Crystallin has its chaperone activity, thus suppressing the aggregation of partially denatured α, β, and γ-crystallin, as well as other proteins, and contributes to the transparency of the lens [5]. When the structure of α-crystallin is changed by post-translational modification such as protein oxidation, glycation, deamidation, and isomerization due to the stresses, *i.e.* aging, UV exposure, its function as a molecular chaperone is reduced. Furthermore, β and γ-crystallin form large particles, abnormally aggregates, and changes to insoluble proteins, causing light scattering

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and swelling/liquefaction of fibers in the lens, resulting in the progression of cataracts. Since there is almost no protein metabolism in the lens, it is necessary to maintain the structure of α-crystallin protein normally, by keeping the chaperone function, and suppress aggregate formation in order to prevent cataracts.

Tryptophan, which is one of the constituent amino acids of proteins, *i.e.* crystallin, has an absorption maximum in the UVB region of 280 nm and is therefore affected by UV rays. Tryptophan, by absorbing UVB, changes to be kynurenine, which, under a further oxidized condition, then becomes a kynurenine derivative, 3-hydroxykynurenine. This derivative absorbs UVA and releases its light energy in the form of ROS, thus decomposing amino acids in the protein, resulting in induction of structural changes in the lens protein [6]. Pyrenoxin, an anti-cataract eye drop approved in Japan, has been reported to exert an effect of maintaining the transparency of the crystalline lens by suppressing the photooxidation process from tryptophan to N'-formylkynurenine and by competitively inhibiting the cellular membrane dysfunction, which is induced by quinoid substances. The effectiveness of pyrenoxin remains controversial. One report said that there is no scientific evidence for the anti-cataract effect of pyrenoxin [7], while another report has shown that the use of pyrenoxin for early cortical cataracts with an opacity area of 10% or less had a significant anti-cataract effect [8]. It is necessary to select a therapeutic drug in consideration of the severity and type of cataract.

3. Oxidative stress and cataracts

Oxidative stress such as free radicals and ROS, which increase with aging, causes oxidation of the crystalline protein, resulting in the formation of disulfide bonds (S-S bonds) between the protein and glutathione (GSH), thus changing to oxidized glutathione. GSHs are maintained rich in the clear lens, however with age, GSH levels become lower in the lens nucleus compared to the outer cortical region. As a result, the lens nucleus is exposed to harmful conditions such as free radicals.

This reduction in GSH promotes decreased lens antioxidant capacity, increased oxidative stress, formation of S-S bonds between proteins, protein misfolding, aggregation, and insolubilization, leading to the initiation of cataracts [9, 10]. There exist proteins such as superoxide dismutase (SOD), catalase, peroxiredoxin 6 (Prdx6), thioredoxin and glutathione peroxidase (GPX), which are called antioxidant enzymes, in the crystalline lens to eliminate oxidative stress. Vitamin E and ascorbic acid are also present in the crystalline lens as free radical scavenging substances. With aging, the decrease in these antioxidants and their activity elevates ROS production and oxidative stress, and promotes the aggregation of lens proteins. Therefore, measures are required to prevent cataracts as follows: one is the prevention of oxidative stress by avoiding UV exposure and by maintaining blood glucose; another is the elimination and suppression of oxidative stress by the maintenance of endogenous antioxidant enzyme and by the administration of exogenous antioxidants.

4. Age-related cataracts and glycative stress

It has been reported that glycation of lens protein occurs with aging [11]. Lens proteins are prone to the formation of advanced glycation endproducts (AGEs), through the formation of carbonyl compounds, by reaction with reducing sugars. AGEs usually accumulate in the crystalline lens with age, but the amount is more pronounced in cataract patients. AGEs result in the formation of high molecular weight aggregation of crystalline in the lens, followed by inducing lens opacity. AGEs are generally produced by reacting with sugars, but it is also known that kynurenine, a tryptophan oxidation product of the lens, regulates AGE synthesis by UV irradiation [11]. Furthermore, it is reported that the oxidation of ascorbic acid in the lens caused by UV rays induces AGE production, which becomes a photosensitizer, resulting in inducing ROS production and oxidative stress in the lens (***Fig. 1***) [11].

5. Cataract prevention with lutein and water chestnuts

LU and zeaxanthin (ZEA) contained in green leafy vegetables are lipid-based antioxidants that protect the crystalline lens by absorbing light in the outer layer of the retina and suppressing lipid peroxidation. Past large-scale epidemiological studies have reported that LU intake reduces the prevalence of nuclear cataracts [12, 13]. In addition, a statistically significant preventive effect was shown on nuclear cataracts in the LU and ZEA co-ingestion group in a report by Cui et al., who meta-analyzed 7 cohort studies [14], and a meta-analysis result from 14 cohort studies [15]. LU and ZEA cannot be produced in the body, therefore they must be taken from diets and supplements. Foods rich in LU include kale, paprika, spinach, parsley and other green-yellow vegetables, however depending on eating habits, it may be difficult to obtain a sufficient amount from the diet. AREDS2 (Rep # 4) reported that in the group with the lowest dietary LU and ZEA intake, the cataract surgery rate decreased due to the intake of these supplements [16]. Furthermore, the results of a meta-analysis have reported that low serum LU and ZEA levels increase the risk of nuclear cataracts [17]. It can be said that LU and ZEA are supplements that are expected to have a preventive effect on age-related cataracts.

In recent years, it has been reported that LU and TBE, which is a peel extract of water chestnut (*Trapa bispinosa* Roxb.), has a delayed effect on sugar cataracts in a diabetic rat model [18]. TBE has been biochemically reported to have anti-glycation effects, such as inhibition of AGE production [19], promotion of AGE cross-linking cleavage [19], and suppression of α-crystallin glycation [20]. Using SCR (Shumiya Cataract Rat), which is a hereditary cataract model rat that develops cataracts in about 66% due to mutation of lanosterol synthase, we reported the effect of LU and TBE administration on cataract prevention and the effect of inducing the expression of antioxidant genes in the lens [21]. The experiment consists of two designs as follows: first, LU + TBE or Control (safflower oil) was administered to a 6-week-old cataract-scheduled SCR (Cat+) and a non-cataract-free SCR (Cat−) for 4 weeks with an oral feeding needle; second, a 6-week-

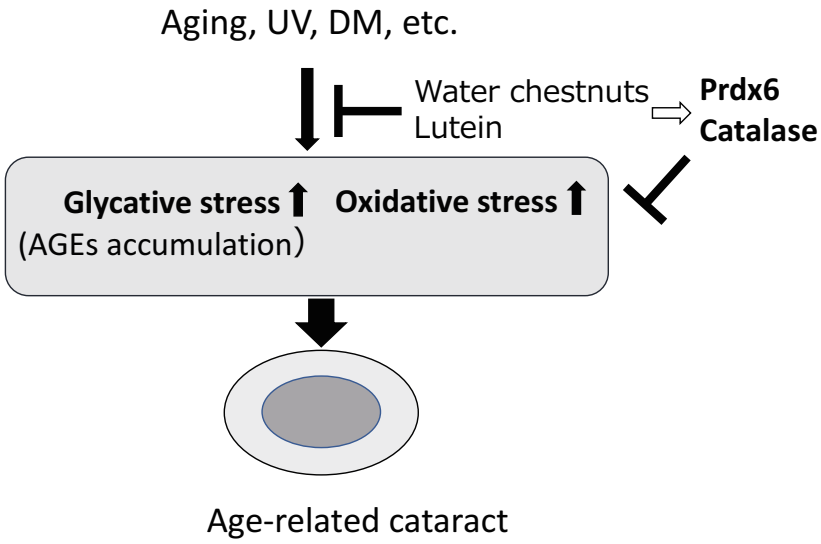

Fig. 1. **Mechanism of cataract suppression by LU and TBE.**

Due to aging, UV rays, DM, oxidative stress and glycative stress in the crystalline lens increase, and a cataract develops. LU and TBE directly suppress these stresses; furthermore, they suppress oxidative stress in the lens by inducing the endogenous antioxidant proteins Prdx6 and catalase. LU, lutein; TBE, water chestnuts (*Trapa bispinosa* Roxb.) extraxt; UV, ultraviolet; DM, diabetes mellitus; AGEs, advanced glycation endproducts.

old SCR (Cat+) was allowed to freely ingest a solid feed containing LU, TBE, LU + TBE, and a standard solid feed (Control) for 3 weeks. As a result, lens opacity appeared in SCR (Cat+) at 9-10 weeks of age, while the lens opacity in the LU + TBE-administered group was significantly reduced than that in the Control group and the TBE-only or LU-only group. This result revealed that co-administration of LU + TBE delays the onset of cataracts in SCR. Furthermore, it was demonstrated *in vivo* and *in vitro* that co-administration of LU + TBE can induce the gene expression of antioxidant enzymes as Prdx6 and catalase in lens epithelial cells. Since the co-administration of LU + TBE showed stronger effects than the single administration of LU or TBE in terms of suppressing cataract progression and inducing the expression of antioxidant genes, it is speculated that the anti-cataract effect may have been exhibited due to the synergistic action of the anti-oxidation effect of LU and the anti-glycation effect of TBE (***Fig. 1***). Therefore, LU + TBE can be expected to be effective as an anti-cataract supplement, but it is necessary to investigate the preventive effect of LU + TBE on age-related cataracts in a large-scale epidemiological study in the future.

6. Other antioxidant supplements and cataract prevention

People often take vitamin C, vitamin A, vitamin E, or β-carotene as antioxidant supplements because they reduce oxidative stress as free radical scavengers. In 15 cohort studies of vitamin C intake and cataract prevention effects, 8 studies showed that vitamin C intake reduced cataract risk, while 7 studies found no significant risk reduction. Taken together, these results showed a significant cataract prevention effect in all types of cataracts, including the nucleus, cortex, and posterior subcapsule [22]. However, there are reports that high-dose (1,000 mg) vitamin C intake acts as a prooxidant and increases the risk of cataracts [22], and that high-dose vitamin E and B increase the risk of cataracts [23, 24]. Attention is necessary to the excessive intake of the vitamin. In other words, it suggests that the action of antioxidant vitamins depends on the level of oxidative stress, and non-selective administration to individuals without excessive oxidative stress has little effect or, conversely, risks.

In addition to vitamins, there are many food factors with antioxidant capacity, and cataract prevention effects have been reported at the animal experiment level. Animal experiments and cell-culture studies have shown that cataracts were suppressed by resveratrol, a type of polyphenol that has antioxidant and anti-inflammatory effects [25], astaxanthin, a type of carotenoid [26], and curcumin, which has antioxidant effects [27].

7. Conclusion

Cataracts are associated with aging and oxidative stress glycation not only in the crystalline lens but also throughout the body. Antioxidant and anti-glycation supplements can prevent cataracts by improving the stressed condition in the crystalline lens as well as the nutritional status of the whole body. In the future, with the improvement of eating habits as the first priority, it may be better to consider taking supplements in the form of supplementing the shortage.

Conflict of interest

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