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Original article

Administration of antioxidant compounds affects the lens chaperone activity and prevents the onset of cataracts

Yosuke Nakazawa[a,*], Noriaki Nagai[b], Nana Ishimori[a], Jun Oguchi[a], Hiroomi Tamura[a]

[a] Faculty of Pharmacy, Keio University, Tokyo, 105-8512 Japan
[b] Faculty of Pharmacy, Kindai University, Osaka, 577-8502, Japan

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ABSTRACT

To prevent lens opacification and cataract formation, the lens contains α-crystallin, which has been shown to function as a molecular chaperone that maintains the correct folding of other proteins. Oxidative stress is known to be an important factor in the initiation and progression of a cataract. So far, several antioxidant compounds have been reported to prevent cataracts *in vivo* and *in vitro*. This stress also triggers α-crystallin modifications and alters its chaperone activity. However, few studies have examined the relationship between the consumption of antioxidant compounds and lens chaperone activity. To elucidate the effect of antioxidants on lens chaperone activity, antioxidants were administered to a selenite-induced cataract model of rats. The chaperone activity in lens water-soluble fraction was measured using aldehyde dehydrogenase. All antioxidant treatment groups, except decaffeinated coffee treatment, had less severe central opacities and lower stage cataracts than control groups. The chaperone activity was weaker in lens of selenite cataract rats, but antioxidant compounds and coffee treatment can prevent the chaperone activity decreasing, but not decaffeinated coffee. These results suggested that the treatment with antioxidant compounds could prevent cataract formation by the maintenance of the chaperone activity in water-soluble lens proteins. Thus, this study describes the development of an anticataract drug target for lens chaperone activity.

1. Introduction

The quality of our vision is critically dependent on the ability of the transparent tissues in the front of the eye to correctly focus light onto the retina at the back of the eye. This is illustrated by the fact that loss of transparency in the lens, known as a cataract, is the most common cause of blindness in the world today [1]. It is well known that oxidative stress plays an important role in the initiation and progression of a cataract, and active type of oxygen and nitrogen species in the eye are implicated in the onset of cataract [2,3]. According to these reports, dietary antioxidants may be useful in the prevention and/or mitigation of cataracts.

The lens contains a high concentration of crystallins, which are water-soluble proteins that constitute up to 90% of the total proteins in the lens [4]. There are three different crystallin isoforms in mammals: α-, β-, and γ-crystallin. α-Crystallin consists of two 20-kDa subunits, αA-crystallin and αB-crystallin, which exist in the lens as a polydisperse multimeric protein [5]. Structurally, α-crystallin has been shown to function as a molecular chaperone that maintains the correct folding of other proteins and thus prevents protein aggregation, formation of light scattering elements, lens opacification, and cataract formation [6]. Molecular chaperone activity plays an important role in the *in vivo* maintenance of lens transparency because lens proteins are long-lived and have negligible turnover. It has been reported that α-crystallin chaperone activity is affected by several posttranslational modifications (PTM), including oxidation, racemization, and deamidation [7–9]. Some antioxidant compounds were reported to inhibit the PTM of α-crystallin and protect the chaperone activity *in vivo* [10,11]. Therefore, it can be hypothesized that the treatment with antioxidant compounds may be a key factor in the maintenance of the chaperone activity of α-crystallin and the prevention of cataracts.

We used a subcutaneous injection of sodium selenite (Na_2SeO_3) to induce bilateral nuclear cataracts in rat pups. Previously, α-crystallin in selenite-induced cataract lenses was modified by oxidative stress, which decreased the chaperone activity of this protein [11]. Among the various available models used, the selenite cataract model is one of the most commonly used experimental models to evaluate anti-cataract agents [12].

Abbreviations: ALDH, aldehyde dehydrogenase; AMD, :age-related macular degeneration; DHA, dehydroascorbic acid; GSH, reduced glutathione; VEGF, vascular endothelial growth factor

* Corresponding author.
E-mail address: nakazawa-ys@pha.keio.ac.jp (Y. Nakazawa).

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In this study, in order to explore the methods by which antioxidant compounds can affect the chaperone activity of lens proteins and maintain lens transparency, we administered various antioxidant compounds in animal models of cataracts and evaluated the effects of these compounds on chaperone activity and cataract mitigation.

2. Materials and methods

2.1. Materials

Sodium selenite (Na_2SeO_3), ALDH, 1,10-phenanthroline, olive oil, rutein, zeaxanthin, hesperetin, quercetin, ascorbic acid, cyanidin chloride, caffeine, and pyrocatechol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). β-Carotene and α-tocopherol were purchased from Sigma-Aldrich Co. (St. Louis, MO). Dark-roasted coffee beans (Columbian Arabica) were purchased from Nakaya coffee (Tokyo, Japan). Decaffeinated coffee beans were purchased from Starbucks Coffee Japan (Tokyo, Japan).

2.2. Animals

Sprague-Dawley (SD) rat pups (13-days postnatal) and their mother were obtained from Sankyo Labo Service Corporation (Tokyo, Japan). They were housed under standard conditions (12 h/day fluorescent light, 25 °C ± 5 °C controlled room temperature), and allowed free access to water and a balanced commercial rat chow (CE-2, Clea Japan, Inc., Tokyo, Japan). The rats were sacrificed by an overdose of isoflurane inhalation (Wako Pure Chemical Industries). The Keio University Animal Research Committee approved all animal procedures performed in this study and all procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in research.

2.3. Selenite-induced cataracts

The rat pups were randomly divided into groups of 6–8 pups (Table 1). The rats in Groups 1 and 9 were subcutaneously injected with phosphate-buffered saline (PBS) and those in the other groups were injected with sodium selenite (Na_2SeO_3) at 20 μmol/kg body weight at 13 days old (day 0).

Table 1
Experimental groups.
Thirteen-day-old Sprague-Dawley rats were randomized into 16 groups. PBS was injected into Groups 1 and 9, and sodium selenite (Se) was injected into other groups on day 0. Four hours after PBS or selenite injection, and on two subsequent days (days 0, 1, and 2), antioxidant or vehicle treatment was administered to each group. (S.C.: Subcutaneous, P.O.; Per Os.).

Group	Challenge	Antioxidant	Administration route
Group 1	PBS	Vehicle (oil)	S.C.
Group 2	Se	Vehicle (oil)	S.C.
Group 3	Se	Lutein	S.C.
Group 4	Se	Zeaxanthin	S.C.
Group 5	Se	Hesperetin	S.C.
Group 6	Se	Quercetin	S.C.
Group 7	Se	β-Carotene	S.C.
Group 8	Se	α-Tocopherol	S.C.
Group 9	PBS	Vehicle (PBS)	P.O.
Group 10	Se	Vehicle (PBS)	P.O.
Group 11	Se	Ascorbic acid	P.O.
Group 12	Se	Cyanide	P.O.
Group 13	Se	Coffee	P.O.
Group 14	Se	Decaffeinated coffee	P.O.
Group 15	Se	Caffeine	P.O.
Group 16	Se	Pyrocatechol	P.O.

Thirteen-day-old Sprague-Dawley rats were randomized into 16 groups. PBS was injected into Groups 1 and 9, and sodium selenite (Se) was injected into other groups on day 0. Four hours after PBS or selenite injection, and on two subsequent days (days 0, 1, and 2), antioxidant or vehicle treatment was administered to each group. (S.C.: Subcutaneous, P.O.; Per Os.).

2.4. Treatment with antioxidant compounds

The water-insoluble antioxidant compounds, rutein, zeaxanthin hesperetin, quercetin, anthocyanin, β-carotene, and α-tocopherol, were dissolved in 7% ethanol and 93% olive oil solution. Four hours before the selenite injection on day 0 the rats were subcutaneously injected with compounds as follows: Group 2 (selenite treatment), 100 μL of vehicle (olive oil-ethanol mixture); Group 3 (selenite-rutein treatment), 100 μL rutein (10 μmol/kg body weight); Group 4 (selenite-zeaxanthin treatment), zeaxanthin (10 μmol/kg); Group 5 (selenite-hesperetin treatment), hesperetin (0.4 μg/kg); Group 6 (selenite-quercetin treatment), quercetin (3 μg/kg); Group 7 (selenite-β-carotene treatment), β-carotene (100 μmol/kg); Group 8 (selenite-α-tocopherol treatment), α-tocopherol (100 μmol/kg). These injections were repeated once per day for the following two days (on days 1 and 2).

The water-soluble antioxidant compounds, ascorbic acid, cyanidin, caffeine, and pyrocatechol, were dissolved in pure water to produce final concentrations of 10 mM, 1 mM, 5.4 mM, or 34 μM, respectively [13]. Coffee or decaffeinated coffee extract was prepared by pouring 140 mL hot pure water (95 °C) onto 8 g of coffee bean or decaffeinated coffee grounds. Rats in Group 10 (selenite treatment only), Group 11 (selenite-ascorbic acid treatment), Group 12 (selenite-cyanidin treatment), Group 13 (selenite-coffee treatment), Group 14 (selenite-decaffeinated coffee treatment), Group 15 (selenite-caffeine treatment), or Group 16 (selenite-pyrocatechol treatment) received 200 μL PBS, ascorbic acid, cyanidin, coffee extract, decaffeinated coffee extract, caffeine, or pyrocatechol, respectively, via a feeding tube 4 h before the selenite injection, then once per day for two more days (on days 0, 1, and 2).

On day 6 (when the rats were 19-days old), the rat lenses were observed and classified by cataract stage based on the scale of 1–6 reported by Ishimori et al. [13]. After classification of the cataracts, the rats were euthanized and lens chaperone activity was analyzed.

2.5. Chaperone activity measurements

Each rat lens was homogenized in four equivalent volumes of PBS and centrifuged at 20,000*g* for 20 min at 4 °C. The supernatant proteins were collected as water-soluble proteins and protein concentrations were measured using the Bradford Protein Assay kit (Bio-Rad, Hercules, CA, USA). The chaperone activity was determined using heat-aggregated ALDH to measure the light scattering of ALDH (ΔA_{360}/60 min). The water-soluble lens proteins (8 mg/mL) were mixed with an equal volume of 1 mg/mL ALDH in 50 mM sodium phosphate buffer (pH = 7.0) containing 100 mM NaCl. ALDH was aggregated by the addition of 100 μM 1,10-phenanthroline at 42 °C. Chaperone activity was monitored by the measurement of light scattering at 360 nm using an Infinite M200 microplate reader (Tecan Co, Männedorf, Switzerland).

2.6. Statistical analyses

All results were presented as the mean ± standard error. Statistical analysis was performed by using a one-way analysis of variance with Tukey's multiple comparison test for post hoc multiple comparisons.

3. Results

3.1. Effects of antioxidant compounds on selenite-induced cataract formations

To explore the effects of antioxidant compounds on the onset of cataracts, a rat model of selenite-induced cataracts, with or without