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Excessive hydrogen peroxide enhances the attachment of amyloid β_{1-42} in the lens epithelium of UPL rats, a hereditary model for cataracts

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ABSTRACT

Several studies have reported that hydrogen peroxide (H_2O_2) is related to the toxicity of amyloid β (Aβ), and that the accumulation of Aβ in the lenses of humans causes lens opacification. In this study, we investigate the accumulation of $A\beta_{1-42}$ in the lenses of UPL rats, which then leads to lens opacification. In addition, we demonstrate the effect of disulfiram eye drops (DSF), a potent radical scavenger, on $A\beta_{1-42}$ accumulation in the lenses of UPL rats. The H_2O_2 levels in 46- to 60-day-old UPL rat lenses are significantly higher than in normal rats, and the $A\beta_{1-42}$ levels in 53- and 60-day-old UPL rats are also increased only in lens epithelium containing capsules (capsule-epithelium), not in the lens cortex and nucleus. However, no increases in amyloid precursor protein (APP), β- or γ-secretase mRNA were observed in lenses of the corresponding ages. It has been thought that $A\beta_{1-42}$ that accumulates in the lenses of UPL rats is actually produced in another tissue containing neuronal cells, such as brain or retina. $A\beta_{1-42}$ levels in the brain and retina rise with aging, and the levels of APP, β- and γ-secretase mRNA in the retinas of 53-day-old UPL rats with opaque lenses are significantly higher than in 25-day-old UPL rats with transparent lenses. In contrast to the results in retinas, the levels of APP, β- and γ-secretase mRNA in the brains of 25- and 53-day-old UPL rats are similar. On the other hand, in an *in vitro* study, $A\beta_{1-42}$ attachment in the lens capsule-epithelium of UPL rats was found to increase in H_2O_2. In addition, in an *in vivo* study, the inhibition of H_2O_2 by DSF was found to attenuate the increase in $A\beta_{1-42}$ in the lens capsule-epithelium of 60-day-old UPL rats. Taken together, we hypothesize that excessive H_2O_2 in the lens enhances the attachment of $A\beta_{1-42}$ in the lens capsule-epithelium of UPL rats, and that the instillation of DSF has the ability to attenuate the attachment of $A\beta_{1-42}$ by inhibiting H_2O_2 production in lens. These findings provide significant information that can be used to design further studies aimed at developing anti-cataract drugs.

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1. Introduction

Cataracts represent a disease of increasing lens opacity, and numerous factors have been implicated in their etiology including genetic factors, diabetes, smoking, nutrition, the cumulative effect of X-rays, ultraviolet irradiation, and alterations in both endocrine and enzymatic equilibria (Sallman and Locke, 1951; Iwata, 1986; Garland, 1990; Rasi et al., 1992; Ye and Zadunaisky, 1992; Cekic and Bardak, 1998; Dilsiz et al., 2000). The calcium ion (Ca^{2+}) content of cataractous lens in human cortical cataracts and rodents becomes elevated, and elevated Ca^{2+} in the lenses of rodents has been found to activate calpain, a Ca^{2+}-dependent protease. The increased degradation of lens proteins, such as crystallin proteins, could result in an opaque lens (Duncan and Bushell, 1975; Shearer et al., 1992; Dilsiz et al., 2000). On the other hand, there have been several reports that hydrogen peroxide (H_2O_2) can increase the accumulation and toxicity of amyloid β (Aβ) peptide in neuronal cells, such as in the retina and brain, and that Aβ enhances oxidative stress (Tamagno et al., 2002, 2008; Melov et al., 2005; Tong et al., 2005; Shen et al., 2008; Oda et al., 2010). In addition, the enhanced levels of Aβ in the brain and retina shift into the blood, and Aβ also accumulates in the lens. Aβ accumulation in the human lens causes lens opacification (Goldstein et al., 2003; Meehan et al., 2004; Moncaster et al., 2010; Jun et al., 2012). On the other hand, in contrast to the results of Goldstein et al. (2003) and Michael et al. (2013) reported the absence of Aβ in cortical cataracts of donors

Abbreviations: ADAM10, A disintegrin and metalloprotease domain protease 10; Aβ, amyloid β; APP, amyloid precursor protein; BACE1β, site APP cleaving enzyme; Ca^{2+}, calcium ion; DDC, diethyldithiocarbamate; DMSO, dimethyl sulfoxide; DSF, disulfiram; GSH, glutathione; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H_2O_2, hydrogen peroxide; HPβCD, hydroxypropyl-β-cyclodextrin; HPMC, hydroxypropylmethylcellulose; iNOS, inducible nitric oxide synthase; LPO, lipid peroxide; PS, presenilin; SDS, sodium dodecyl sulfate.

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with and without Alzheimer's disease; therefore, whether or not Aβ accumulates in human cataracts is currently controversial. Aβ peptides vary in length from 39 to 43 amino acid residues, and are produced by the sequential proteolytic processing of amyloid precursor protein (APP) by β-secretase (β site APP cleaving enzyme, called BACE1, Vassar et al., 1999) and γ-secretase (a presenilin complex, PS1 and PS2, Takasugi et al., 2003). BACE1 is a membrane-bound aspartic protease that is the rate-limiting enzyme in Aβ production from APP. APP can be cleaved by α-secretase within the Aβ domain to generate non-amyloidogenic soluble APPα. The A disintegrin and metalloprotease domain protease 10 (ADAM10) is the major protease for the α-cleavage of APP (Zheng and Koo, 2006; Kukar et al., 2008; Wong, 2008). However, the factors and mechanisms for the accumulation and toxicity of Aβ in the lens remain obscure. Therefore, it is necessary to elucidate the relationship between H_2O_2 and Aβ accumulation in lenses in an *in vivo* study.

For *in vivo* studies aimed at clarifying the relationship between H_2O_2 and Aβ accumulation in the lens, the selection of the experimental animal is very important. The UPL rat is a dominant hereditary cataract model derived from Sprague-Dawley rats. Opacification of the lenses of UPL rats starts at 35–42 days of age, and by 50 days of age, the lenses are almost entirely opaque. The incidence of cataracts in adult UPL rats is 100% (Tomohiro et al., 1996, 1997). Previous investigations have revealed that oxidized glutathione concentrations in the lenses of UPL rats are increased, and that reduced glutathione values are decreased (Nabekura et al., 2003). The proteolyses of some crystallins and cytoskeletal proteins are enhanced in the lenses of UPL rats (Tomohiro et al., 1996, 1997). Ca^{2+} levels in the lenses of UPL rats rise markedly with aging as compared with normal rats, and the autolytic product of calpain is also detected in the lenses of UPL rats (Tomohiro et al., 1997). Our previous reports also showed that the increase in Ca^{2+} content in the lenses of UPL rats is inhibited by the oral administration of disulfiram (DSF), a powerful antioxidant (Nagai and Ito, 2007). We have reported the mechanism of Ca^{2+} enhancement in the lenses of the UPL rats as excessive nitric oxide *via* inducible nitric oxide synthase (iNOS) causing mitochondrial damage that results in a decrease in ATP production and an increase in Ca^{2+}-ATPase activity. The decrease in ATP content causes a decrease in Ca^{2+}-ATPase function resulting in an increased Ca^{2+} content in the lens. The elevation in the lens Ca^{2+} level leads finally to lens opacification (Nagai and Ito, 2007). Thus, some of the changes observed in the biological characteristics of UPL rat lenses may correspond to those of human cortical cataracts. Therefore, these rats provide a useful model for studies to reveal the mechanism of senile cataract development, as well as for the development of anti-cataract drugs.

In the present study, we examined whether UPL rats can be used to study Aβ accumulation and toxicity, and investigated the relationship between H_2O_2 and Aβ accumulation in the lenses of UPL rats. Furthermore, the preventive effects of DSF eye drops (Nagai and Ito, 2007; Nagai et al., 2008) on the accumulation of Aβ were also examined in the lenses of UPL rats.

2. Materials and methods

2.1. Animals and materials

Non-cataractous lenses (normal) and cataractous lenses from UPL rats were used in this study. The UPL rats were provided by Meijo University (Aichi, Japan), housed under standard conditions (12 h/d fluorescent light (07:00–19:00), 25 °C room temperature), and allowed free access to a commercial diet (CE-2, Clea Japan Inc., Tokyo, Japan) and water. All procedures were performed in accordance with the Association for Research in Vision and Ophthalmology resolution on the use of animals in research. DSF was kindly donated by Ouchi Shinko Chemical Industrial Co., Ltd. (Tokyo, Japan). Hydroxypropyl-β-cyclodextrin (HPβCD, average molar substitution, 0.6; average MW, 1380) was a gift from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Hydroxypropylmethylcellulose (HPMC) was provided by Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Benzalkonium chloride was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Rat β Amyloid (42) ELISA Kit was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The hydrogen peroxide colorimetric detection kit was provided by ENZO Life Sciences, Inc. (Philadelphia, USA). LPO Assay Kit (BIOXYTECH® LPO-586™) was obtained from OXIS International, Inc. (Portland, OR, USA). All other chemicals used were of the highest purity commercially available.

2.2. Preparation of eye drops containing the DSF/HPβCD inclusion complex

HPβCD was added to saline containing 0.005% benzalkonium chloride and DSF, and then HPMC was added to this solution. The mixtures were stirred for 24 h in the dark at room temperature, and filtered through a Minisart CE (pore size of 0.20 μm, Costar Co. MA, USA). DSF eye drops were prepared in the following compositions: 0.25% DSF eye drops (0.25% DSF, 3.0% HPβCD and 0.1% HPMC), 0.5% DSF eye drops (0.5% DSF, 5.0% HPβCD and 0.1% HPMC).

2.3. Instillation of DSF eye drops

UPL rats, 18 days old, were randomly divided into three groups. Two groups were administered DSF eye drops (0.25% or 0.5%), and the third group received saline. Thirty microliters of 0.25% or 0.5% DSF eye drops or saline were instilled into the eye twice a day (9:00 AM and 7:00 PM) beginning when the rats were 18 days of age. The eyes were kept open for about 1 min to prevent the eye drops from being washed out.

2.4. Image analysis of cataract development in UPL rats

The experiment was performed as described by Nagai and Ito (2007). The pupils of UPL rats were dilated by the instillation of 0.1% pivalephrine (Santen Pharmaceutical Co., Osaka, Japan) without anesthesia. Changes in the transparency of the lenses were monitored using an EAS-1000 equipped with a CCD camera (Nidek, Gamagori, Japan). The outline of the lens image was determined by selecting 4 points on the image, and then the transparent area within the outline and defining level were set automatically by the software. The total area of opacity of the lenses, expressed as pixels, was calculated based on the following equation:

$$\text{Pixels within opacity(pixel)} = \text{pixels within outline} - \text{pixels within transparent area}$$

2.5. Measurement of H_2O_2 levels

UPL rats were euthanized with pentobarbital sodium [120 mg/kg intraperitoneally (i.p.)], and the eyes were removed and the lenses isolated for the determination of H_2O_2 levels using a hydrogen peroxide colorimetric detection kit (ENZO Life Sciences, Inc., Philadelphia, USA) according to the manufacturer's instructions. Briefly, the lenses were homogenized in 200 μl phosphate buffer (20 mmol/l, pH 7.4), and centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatants (50 μl) were mixed with the hydrogen peroxide color reagent (100 μl), and incubated for 30 min at room temperature. The absorbance was measured with a microplate reader (BIO-RAD, CA, USA) at 540 nm. H_2O_2 levels in lenses are expressed as μmol/g of protein.

2.6. Measurement of $A\beta_{1-42}$ levels

Blood was obtained from the vena cava of living rats anesthetized by injection of pentobarbital sodium (0.6 mg/kg), after which the rats were euthanized by injection of a lethal dose of pentobarbital sodium into the vena cava, and the eyes and brain were removed. The aqueous humor was collected from the eyes through a 29 gauge injection needle, and the lenses and retinas were isolated. The epithelium containing capsules (capsule-epithelium) was carefully removed and separated from the nucleus and cortical portions. The cortex was also separated, and the remaining lens other than the capsule-epithelium and cortex was used as the nucleus. The samples were homogenized in 100–500 μl diethylamine solution (0.2% diethylamine and 50 mM NaCl), and centrifuged at 100,000 × *g* for 1 h at 4 °C. The pellets were dissolved in guanidine hydrochloride (Gu-HCl) solution (5 M Gu-HCl in 50 mM Tris-buffered saline, pH 8), and used for the measurement of soluble and insoluble amyloid β_{1-42} ($A\beta_{1-42}$) (Shimazawa et al., 2008). The aqueous humor and blood were centrifuged at 5000 × *g* for 15 min at 4 °C, and the supernatants (100 μl) were used for the measurement of total $A\beta_{1-42}$ levels (both of soluble and insoluble $A\beta_{1-42}$). $A\beta_{1-42}$ levels were measured using a Rat β Amyloid (42) ELISA Kit according to the manufacturer's instructions (Wako, Osaka, Japan). Briefly, monoclonal antibodies specific for rat $A\beta_{1-42}$ were pre-coated onto microplates; standards and samples were pipetted into the wells; and the microplates were incubated at 4 °C for 15 h. The wells were then washed to remove unbound materials, after which HRP-conjugated antibody solutions and, finally, substrates were added to the wells. The enzyme reactions yielded blue products that turned yellow when the stop solution was added. The absorbance was measured with a microplate reader (BIO-RAD, CA, USA) at 450 nm.

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