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The gender-differentiated antioxidant effects of a lutein-containing supplement in the aqueous humor of patients with senile cataracts

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ABSTRACT

Antioxidant supplements are expected to decrease oxidative damage and prevent ocular diseases. In this study, changes in the anti-oxidative ability and oxidative status in the aqueous humor before and after intake of a lutein-containing supplement were measured. Forty patients who all had identical grades of cataracts in both eyes were included. The aqueous humor was collected as pre-intake samples during cataract surgery. Ocuvite + Lutein®, an antioxidant supplement, was administered orally beginning the day after surgery. Six weeks later, the aqueous humor was collected as a post-intake sample during cataract surgery of the opposite eye. To determine the anti-oxidative ability, the levels of superoxide ($O_2^{\bullet -}$) scavenging activity were measured. To determine the oxidative status, the levels of hydrogen peroxide (H_2O_2) and total amount of hydroperoxides (TH, including H_2O_2 and peroxides of lipids, proteins, and nucleic acids) were measured. In post-intake samples, the $O_2^{\bullet -}$ scavenging activities were significantly higher in both genders ($p < 0.05$). The levels of H_2O_2 were significantly higher ($p < 0.01$) while the levels of TH were significantly lower ($p < 0.01$) only in females. The level of H_2O_2 was significantly negatively correlated to the TH in the post-intake samples of both genders ($r = -0.50$ and $p < 0.05$ for males; $r = -0.59$ and $p < 0.01$for females) while the level of H_2O_2 was significantly positively correlated to the $O_2^{\bullet -}$ scavenging activity in both pre- and post-intake aqueous humor in females only ($r = 0.66$ and $p < 0.01$ for pre-intake samples, $r = 0.71$ and $p < 0.01$ for post-intake samples). After the introduction of the antioxidant supplement, $O_2^{\bullet -}$ scavenging activity increased while H_2O_2 levels remained the same in males, suggesting that scavenging rates are proportional. In postmenopausal females, the superoxide scavenging activity also increased, however unlike in males, the H_2O_2 levels also increased meaning H_2O_2 was not completely scavenged. H_2O_2 is a non-free radical and can be excreted from the aqueous humor to prevent further oxidation of lipids, proteins, and nucleic acids, which was confirmed by the low levels of TH in post-intake samples. Antioxidant supplements are suggested to be effective in reducing oxidation in the aqueous humor by different mechanisms in both genders.

1. Introduction

Cataracts have been reported to be responsible for 51% of blindness worldwide, which represents approximately 20 million people according to the statistics of the World Health Organization. Cataract is not only one of the most important causes of blindness in developing countries but also one of the important problems in developed countries due to the large portion of medical expenses spent on treating cataracts. According to the statistics of the Ministry of Health, Labour and Welfare of Japan in 2010, nearly 40% of medical expenses in ophthalmology were related to the treatment of cataracts among patients over 65 years of age. It is important to determine the causes while considering the prevention of cataracts. Oxidation is reported as an important cause of cataracts (Obara, 1995; Lin, 1996; Truscott, 2005) and antioxidants are presumed to be the key for preventing cataracts. The results of both in vitro and animal studies support the hypothesis that nutrients with antioxidant capabilities, such as ascorbate (Blondin et al., 1986), and carotenoids (Kim et al., 2008; Gao et al., 2011) prevent oxidative damage in lens tissues. Epidemiological studies also reported decreased prevalence of cataract with dietary antioxidant nutrients, such as vitamin C (Vit C; Taylor et al., 1991;

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Abbreviations

$O_2^{\bullet-}$	superoxide
H_2O_2	hydrogen peroxide
TH	total amount of hydroperoxides
Vit C	vitamin C
Vit E	vitamin E
SOD	superoxide dismutase
GSH	reduced glutathione
G6PDH	glucose-6-phosphate dehydrogenase
18S	18S ribosomal RNA
Zn	zinc
Se	selenium
Cu	copper
Cu, Zn-SOD	Cu, Zn-superoxide dismutase
Gpx	glutathione peroxidase

Yoshida et al., 2007; Tan et al., 2008) and vitamin E (Vit E; Christen et al., 2008).

Among antioxidant nutrients, lutein is a noteworthy antioxidant that is proven to be effective in preventing age-related macular degeneration (Age-related Eye Disease Study Group, 2001). However, the results of epidemiological studies on the effects of lutein on human cataracts have been inconclusive; some clearly support the effects of lutein (Lyle and Mares-Perlman, 1999; Chasan-Taber et al., 1999; Christen et al., 2008; Moeller et al., 2008) while others support the effects of lutein, but not in a statistically significant way (Brown et al., 1999; Gale et al., 2001; Delcourt et al., 2006; Vu et al., 2006). Confounding factors, such as individual differences, may interfere with the investigation of the effects of this antioxidant. In this study, we attempted to control the confounding factors that may occur while investigating the relationship between antioxidant nutrients and cataracts. We investigated the changes in oxidative status in the aqueous humor before and after intake of a lutein-containing supplement, Ocuvite + Lutein®, measuring samples from patients who had binocular cataracts of identical grades. By investigating the same patient and administering a determined dose of antioxidant supplement, the individual differences and the dosage of nutrients can be controlled. Moreover, we measured the oxidative status in the aqueous humor as it has been reported that lens epithelial cells are the primary targets of oxidation in the aqueous humor due to the fact the epithelial surface of the lens is in contact with the aqueous fluid (Spector, 1995).

This is the first study to investigate the changes in oxidative status in the aqueous humor of patients who had an identical grade of binocular cataracts after the intake of a lutein-containing supplement. The different effects of antioxidant supplementation between genders were also investigated.

2. Methods

The composition of the antioxidant supplement, Ocuvite + Lutein®, used in this study is described in Table 1.

Forty patients, 18 male patients (70.8 ± 7.6 years old) and 22 female patients (69.8 ± 6.7 years old), undergoing binocular cataract surgery were included. The patients were included in this study after giving informed consent according to the tenets of the Declaration of Helsinki. Approval from the institutional human experimentation committee was also granted. Although the grades of cataracts from patient to patient varied, this study included only patients who had identical types of lens opacity and identical grades of cataracts in each of their eyes in accordance to the Lens Opacities Classification System III (Chylack et al., 1993). The distribution in types and grades of cataracts were shown in Table 2. Patients with ocular diseases other than cataract, such as retinopathy, or patients with systemic diseases, such as diabetes, were not included.

Between 0.1 ml and 0.15 ml of the aqueous humor was collected during cataract surgery (as the pre-intake samples). Three tablets (the recommended daily dosage) of Ocuvite + Lutein®, the antioxidant supplement, were administered orally every day beginning the day after surgery. Six weeks later, samples of the aqueous humor were collected during cataract surgery for the other eye (as the post-intake samples). Both the pre-intake and post-intake samples were filled with nitrogen gas, immediately frozen after collection and stored at −40 °C until measured.

As an indicator of the antioxidant status in the aqueous humor, the levels of superoxide ($O_2^{\bullet-}$) scavenging activity were measured using the NBT reduction method of which the details are described in our previous report (Obara, 1995). This method measures the spectral absorbance using xanthine oxidase which reacts to total $O_2^{\bullet-}$ scavengers, including superoxide dismutase (SOD), L-ascorbic acid, and reduced glutathione (GSH). 1.0 ml of xanthine oxidase was added to the mixture of aqueous humor sample and colorimetric reagent. Once the reaction with the quenching reagent had finished, a spectral absorbance at 560 nm was measured (Fig. 1). As indicators of the oxidative status, the levels of hydrogen peroxide (H_2O_2), which is a following product of $O_2^{\bullet-}$ scavenging, and the total amount of hydroperoxides (TH) were measured. H_2O_2 was measured using a colorimetric method which measures the yellow color produced when oxidized titanium reacts with H_2O_2 (Patti and Bonet-Maury, 1953). 80 μl of oxidized titanium was added to 80 μl of aqueous humor sample and a spectral absorbance at 410 nm was measured (Fig. 2). The TH, including H_2O_2 and the peroxides of lipids, peptides, proteins, nucleic acids and nucleotides, was investigated using a method modified from the dROMs test (Cesarone et al., 1999). The colorimetric pigment of N,N-diethyl p-phenylenediamine, which reacts with all hydroperoxides (−OOH), was used to measure the total amount of oxidation products

Table 1
The composition of Ocuvite + Lutein®.

Substance	Amount
Lutein	6.0 mg
vitamin C	300.0 mg
vitamin E	60.0 mg
vitamin B_2	3.0 mg
β-carotene	1200.0 μg
Naicin	12.0 mg
zinc	9.0 mg
selenium	45.0 μg
copper	0.6 mg
manganese	1.5 mg

Table 2
The distribution of patients classified in types and grades of cataracts.

Cataract	Males (no.)	Females (no.)
C3 NO_2 NC2	1	0
C3 NO_3 NC3	7	3
C4 NO_2 NC2	3	9
C4 NO_3 NC3	9	9

In the Lens Opacities Classification System III (Chylack et al., 1993), the opacities are classified into C1 to C5 based on the area % covered by the cortical cataract, NO_1 to NO_6 and NC1 to NC6 based on the nuclear opalescence and the nuclear color.