Increase of 8-Hydroxy-2′-Deoxyguanosine in Aqueous Humor of Patients with Exudative Age-Related Macular Degeneration

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PURPOSE. Oxidative stress has been implicated as a major contributor to age-related macular degeneration (AMD). 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most abundant oxidative products of DNA damage and represents a noninvasive and sensitive biomarker of oxidative stress. The authors investigated the 8-OHdG levels in aqueous humor of patients with exudative AMD.

METHODS. Twenty-four eyes of 24 patients with active exudative AMD and 31 eyes of 31 age-matched subjects who underwent cataract surgery were enrolled. Aqueous humor samples were collected from all subjects, and the 8-OHdG levels were determined by a commercially available enzyme-linked immunosorbent assay kit. The choroidal neovascularization (CNV) subtype was classified by fluorescein angiography. The macular lesion, including CNV membrane, exudation, and retinal hemorrhage, was measured. The correlation between 8-OHdG level and the clinical features was analyzed.

RESULTS. The 8-OHdG level in the aqueous humor of AMD patients was significantly higher than it was in controls (0.581 ± 0.258 ng/mL vs. 0.251 ± 0.116 ng/mL; P < 0.001), after adjusting for age and lens status. There was no difference in the 8-OHdG levels between AMD patients with classic/predominantly classic and occult/minimally classic CNV (0.591 ± 0.262 vs. 0.566 ± 0.266 ng/mL; P = 0.639). The 8-OHdG level in aqueous humor was significantly correlated with the lesion size (ρ = 0.492; P = 0.017).

CONCLUSIONS. The 8-OHdG level in aqueous humor was higher in patients with exudative AMD, and the level was correlated with the area of macular lesion. This suggests that oxidative stress plays an important role in the disease course of AMD. (Invest Ophthalmol Vis Sci. 2010;51:5486–5490) DOI:10.1167/iovs.10-5663

Age-related macular degeneration (AMD) is one of the leading causes of irreversible blindness among patients aged 50 years or older, not only in Western countries1–3 but also in Taiwan.4 The central visual loss of AMD is attributable to degenerative or exudative changes at the macula.5 Exudative AMD is characterized by the presence of choroidal neovascularization (CNV), which accounts for approximately 75% of cases with severe central vision loss.6 Despite the high socioeconomic burden it causes, the cause of AMD is not conclusively known.

Oxidative damage has been implicated as a major contributor to the pathogenesis of AMD.1 Cigarette smoking and sunlight exposure,8–10 the two most important environmental risk factors for AMD identified by large epidemiologic studies, are related to the level of oxidative stress caused by the generation of reactive oxygen species.7,11,12 Aging, the most important inherent risk factor for AMD, is also associated with cumulative oxidative damage with decreased protection from the antioxidant enzymes.6,13 AMD patients were found to have more pronounced decreases in the protein and activity levels of serum antioxidant enzymes than were age-matched subjects.13,14 Despite all the reports, the in vivo intraocular oxidative stress status in AMD patients has remained poorly understood.

This study was undertaken to investigate the intraocular oxidative damage level in patients with exudative AMD. Among the various types of oxidative damage products, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most abundant products of oxidative DNA damage and represents a noninvasive, sensitive, and stable biomarker of oxidative stress.16 We measured the 8-OHdG concentration in the aqueous humor of patients with active exudative AMD and correlated the 8-OHdG level with the CNV subtypes and the size of macular lesions. To the best of our knowledge, this is the first study to explore the 8-OHdG level in aqueous humor of patients with exudative AMD and to correlate it with clinical features.

SUBJECTS AND METHODS

Subjects

In this prospective comparative study, we investigated the level of 8-OHdG in the aqueous humor of AMD patients with active CNV. Patients were recruited from the Retinal Clinic of Taipei Veterans General Hospital from July 2007 to July 2009. Patients who were older than 55 years and had active CNV confirmed by fluorescein angiography (FA) and who did not receive any treatment for CNV within 1 year were enrolled as the study subjects. Patients with senile cataract without any other ocular diseases who underwent cataract surgery were enrolled as the control subjects. Exclusion criteria included other ocular diseases apart from AMD and cataract, other ocular surgery apart from cataract surgery, diabetes mellitus complicated by diabetic retinopathy, malignancy, and current smoking habit (positive smoking history within 1 year). Patients with malignancy and current smokers were excluded because their serum 8-OHdG levels were higher than those of nonsmoking controls without malignancies.17–20 The Institutional Review Board for Human Research of Taipei Veterans General Hospital...
Hospital approved this study. Informed consent was obtained from each of the participants. All procedures adhered to the tenets of the Declaration of Helsinki.

All patients underwent complete ophthalmic examination, including best-corrected visual acuity, slit-lamp examination, intraocular pressure, and indirect ophthalmoscopy. Personal histories including systemic illness, smoking history, and vitamin supplementation were recorded. Patients with dense cataract that precluded detailed fundus examination before cataract operation would be reexamined after cataract operation. Fluorescein angiography was performed in all AMD patients to categorize the lesions into classic/predominantly classic CNV and occult/minimally classic CNV. Patients with polypoidal choroidal vasculopathy, diagnosed by indocyanine green angiography, were excluded from the study. Fundus photography and FA were taken with a digital fundus camera (CF-60UD; Canon, Tokyo, Japan) at 60-degree view. The area of the macular lesion, including the CNV membrane, exudation, and retinal hemorrhage, was measured with the use of an industrial computer-aided design software package (GstarCAD 2008 Professional; Starsoft.com). The measured area was converted to the actual size on the retina in proportion to the magnification of the camera and the software.

**Aqueous Humor Sampling**

Patients with exudative AMD received an intravitreal injection of either bevacizumab or triamcinolone acetonide, or both, for their active CNV. Undiluted aqueous humor (0.1–0.15 mL) was collected before intravitreal injection to prevent a surge in intraocular pressure after the injection. All injections and sample collections were performed under a standard sterilization procedure that included disinfection of the ocular surface and periocular skin with 5% povidone iodine solution. In the control subjects who underwent cataract surgery, aqueous humor was collected at the beginning of the surgery. All aqueous humor samples were collected using a 30-gauge needle connected to a tuberculin syringe. The samples were frozen immediately in liquid nitrogen and stored at −80°C until analysis.

**Quantitative Analysis of 8-OHdG**

The concentration of 8-OHdG in aqueous humor was determined by the highly sensitive 8-OHdG enzyme-linked immunosorbent assay (ELISA) kit (Japan Institute for the Control of Aging, Fukuroi, Japan) according to the manufacturer’s instructions. The detection range of 8-OHdG concentration was 0.125 to 10 ng/mL. ELISA is simpler and more efficient than the conventional high-performance liquid chromatography with electrochemical detection (HPLC-ECD) approach with respect to the equipment, analysis time, and sample volume required for quantitative analysis of 8-OHdG. Results of ELISA have been shown to correlate well with the data obtained from HPLC-ECD.21

**Statistical Analysis**

Statistical analysis was performed (SPSS for Windows, version 15.0; SPSS Inc., Chicago, IL). Data of the two groups were analyzed by the Student’s t-test for continuous variables and x² test for categorical variables. The concentration of 8-OHdG in the aqueous humor between the two groups was compared by the Student’s t-test. The correlation between the macular lesion size and the 8-OHdG level was examined by the Spearman rank correlation test. The 8-OHdG level in AMD patients with different CNV subtypes was analyzed by the Mann-Whitney U test. Multiple linear regression was performed to evaluate the factors that affected the 8-OHdG level in aqueous humor. P < 0.05 was considered statistically significant.

**RESULTS**

There were 24 AMD patients and 33 control patients enrolled consecutively throughout the study period. Two patients from the control group were excluded for further analysis because of the presence of diabetic retinopathy found after cataract extraction. Univariate analysis revealed no significant differences in age and sex ratio between the two groups (Table 1); 41.7% patients in the AMD group and 45.2% patients in the control group took multivitamin supplements (Table 1) whose antioxidant concentrations were lower than those suggested by the Age-related Macular Degeneration Study.22 The mean 8-OHdG concentration in aqueous humor was 0.581 ± 0.258 ng/mL in the AMD group, which was significantly higher than that in the control group (0.251 ± 0.116 ng/mL; P < 0.0001; Fig. 1). The difference remained highly significant under multiple linear regression analysis after adjusting the factors of age and lens status (Table 2). Age was also found as a significant factor influencing the 8-OHdG level in aqueous humor. Lens status between the two groups was significantly different because all subjects in the control group were phakic at the moment of aqueous humor collection. However, it was not a significant factor that affected the 8-OHdG level in aqueous humor after examination by multiple linear regression (Table 2). Further analysis of 8-OHdG levels in the subgroup of phakic AMD patients also revealed significantly higher concentrations than found in the controls (0.553 ± 0.285 vs. 0.251 ± 0.116 ng/mL; P = 0.006).

**Table 1.** Comparisons between Patients with Exudative AMD and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>AMD (n = 24)</th>
<th>Control (n = 31)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean ± SD</td>
<td>81.7 ± 5.9</td>
<td>78.7 ± 6.7</td>
<td>0.090</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>19:5</td>
<td>22:9</td>
<td>0.704</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>2 (8.3)</td>
<td>5 (16.1)</td>
<td>0.451</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>22 (91.7)</td>
<td>26 (83.9)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>13 (54.2)</td>
<td>14 (45.2)</td>
<td>0.591</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>11 (45.8)</td>
<td>17 (54.8)</td>
<td></td>
</tr>
<tr>
<td>Multivitamin supplements</td>
<td>10 (41.7)</td>
<td>14 (45.2)</td>
<td>0.796</td>
</tr>
<tr>
<td>Lens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phakic, n (%)</td>
<td>12 (50)</td>
<td>31 (100)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pseudophakic, n (%)</td>
<td>12 (50)</td>
<td>0 (0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IOP, mm Hg, mean ± SD</td>
<td>13.1 ± 2.8</td>
<td>13.5 ± 2.2</td>
<td>0.552</td>
</tr>
<tr>
<td>Types of CNV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic/predominantly classic, n (%)</td>
<td>14 (58.3)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Occult/minimally classic, n (%)</td>
<td>10 (41.7)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Aqueous 8-OHdG, ng/mL, mean ± SD</td>
<td>0.581 ± 0.258</td>
<td>0.251 ± 0.116</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

NA, not applicable.
Fourteen AMD patients had classic/predominantly classic CNV and 10 had occult/minimally classic CNV. There was no difference in the 8-OHdG level between these two groups (0.591 ± 0.262 vs. 0.566 ± 0.266 ng/mL; P = 0.639). Further analysis found positive correlations between the level of 8-OHdG in aqueous humor and macular lesion size (ρ = 0.492; P = 0.017; Fig. 2).

**DISCUSSION**

Oxidative stress has been associated with various ocular diseases. Increased 8-OHdG level has been observed in the trabecular meshwork of glaucoma patients, in the pterygium, in leukocytes of patients with Eales’ disease and Leber’s hereditary optic neuropathy, and in the urine of patients with Grave’s ophthalmopathy. Our study has demonstrated that the 8-OHdG level in aqueous humor was significantly higher in AMD patients and was correlated with macular lesion size.

8-OHdG is one of the most abundant forms of oxidative damage products of DNA. The modified DNA is excised and exported into serum, urine, or other extracellular fluids without further metabolism. Excretion of 8-OHdG into urine represents the average rate of oxidative damage in the whole body. The 8-OHdG level in the extracellular fluid that is isolated from the systemic circulation, such as cerebrospinal fluid (CSF) and aqueous humor, may reflect oxidative damage of the organ. The 8-OHdG level in the CSF of patients with neurodegenerative diseases was significantly higher than in the CSF of control patients, and the level was positively correlated with the duration of illness. Aquaeous humor is an intraocular fluid isolated from the systemic circulation (blood-aqueous barrier); its 8-OHdG content may reflect intraocular oxidative damage status. Moreover, the 8-OHdG concentration in the aqueous humor was positively correlated with the area of macular lesion that represented the area of retinal damage. We are unable to postulate at this stage whether the increased 8-OHdG concentration in aqueous humor is a primary event or is secondary to CNV growth. In vitro study found that oxidative stress on RPE can upregulate the secretion of vascular endothelial growth factor (VEGF), which is a potent stimulator of neovascularization. Several transcription factors that enhance expression of VEGF are also regulated by reactive oxygen species. Animal studies found more severe neovascularization growth in the mice deficient in superoxide dismutase 1 (Sod1−/−), which had increased constitutive and stimulated oxidative damage in the retina, compared with that in the Sod1+/+ mice under the stimulation of VEGF. On the other hand, retinal neovascularization may also cause retinal oxidative damage. Animal studies of the Vldlr−/− mice, which had a defective gene for VLDL receptor and developed aberrant subretinal neovascularization, showed increased oxidative stress and neuronal degeneration limited to the region with neovascularization even in the absence of clinically significant leakage or hemorrhage, and the degeneration was attenuated significantly by antioxidants. Further studies to analyze the oxidative stress level in patients with extensive early stage AMD without CNV growth may help to delineate this issue.

It is intriguing that our study found an age-dependent increase of 8-OHdG level in aqueous humor. Age-related increases in oxidative DNA damage have been found in various human organs and age-related increases in 8-OHdG level have been found in both plasma and CSF. Our study results also showed an age-related increase in oxidative DNA damage product in the intraocular fluid, providing further support that oxidative DNA damage is associated with aging.

Previous studies on human eye specimens show decreased antioxidant enzyme activity with aging, which is more pronounced in eyes with AMD, implicating the role of oxidative stress in the pathogenesis of AMD. There is an age-related decrease in metallothionein, a scavenger of hydroxyl radicals, in submacular RPE. The catalase activity of the excised RPE from human donor eye also shows an age-related decline, and the decline is more pronounced in subjects with AMD. The heme oxygenase-1 and heme oxygenase-2 immunoreactivity in the submacular RPE tends to decrease with increased age, especially in the RPE lysosomes of exudative AMD. The present study showed that the concentration of 8-OHdG in the aqueous humor was higher in patients with...

**TABLE 2.** Multiple Linear Regression of the Factors Influencing 8-OHdG Concentration in Aqueous Humor

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient ± SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups (AMD vs. controls)</td>
<td>0.255 ± 0.063</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.009 ± 0.004</td>
<td>0.027</td>
</tr>
<tr>
<td>Lens</td>
<td>0.096 ± 0.075</td>
<td>0.205</td>
</tr>
</tbody>
</table>

R² = 0.498.
exudative AMD, providing further support that oxidative damage plays an important role in AMD pathogenesis.

Aqueous humor was chosen in this study to explore intracellular oxidative damage status in AMD patients because collecting aqueous humor is an easier and less risky procedure than collecting vitreous humor. Moreover, aqueous humor can be collected readily during intravitreal injection or cataract surgery. Previous studies have demonstrated that the cytokine levels in aqueous humor are correlated with those in the vitreous humor, reflecting the activities of the retinal diseases.50–52

There are limitations to this study. The sample size was small, and other oxidative stress markers in aqueous humor were not evaluated. The amount of aqueous humor drawn was inadequate for the assay of other oxidative stress markers because 8-OHdG analysis was performed in duplicate with undiluted aqueous humor. Nevertheless, we have shown for the first time that 8-OHdG can be detected in aqueous humor and that the level is significantly higher in patients with exudative AMD than in healthy controls. Further studies with larger sample size and longitudinal follow-up are warranted to enhance our understanding of the intraocular oxidative stress status in AMD patients.

Acknowledgments

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References

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