Lipid Peroxidation in Aqueous Humor Obtained from Primary Open-Angle Glaucoma Patients and Pseudoexfoliative Glaucoma Patients

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Introduction

Lipid peroxidation is a well-defined mechanism of cellular damage in animals, and lipid peroxides are unstable indicators of oxidative stress in cells. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. 4-hydroxynonenal (4-HNE) has been shown to be capable of binding to proteins and forming stable adducts, and is termed an advanced lipid peroxidation end product. 4-HNE can cause both the structural and functional change of oxidized proteins. In this study, we investigated oxidative stress in the aqueous humor (AH) of glaucoma patients.

Purpose

4-hydroxynonenal (4-HNE) is one of the aldehydic secondary products of lipid peroxidation, which are generally accepted markers of oxidative stress. Oxidative modification of lipids can be induced during aging and in certain disease conditions. It was reported that primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (PEG) were caused by oxidative damage to trabecular meshwork (TM) cells. The purpose of the present study was to examine oxidative stress in the anterior chamber of the eye as a measure of concentrations of 4-HNE in AH specimens obtained from glaucoma patients.

Methods

1) AH samples were directly obtained at the beginning of surgery without blood contamination.
2) The amount of protein in 1ml of each sample was quantified by bicinchoninic acid assay (BCA).
3) The samples were diluted using phosphate buffered saline (PBS) to adjust the amount of protein in each sample to 10μg/ml.
4) Each sample underwent an HNE-BSA standard assay in duplicate.
5) 100μl of the 10μg/ml protein samples or reduced HNE-BSA standards were added to a well-protein binding plate and incubated overnight at 4°C.
6) The wells were washed 3X with 200μl of PBS per well.
7) 200μl of the assay dilution was added to each well and incubated for 1 hour at room temperature (RT).
8) The wells were wash 3X with 200μl of 1X washing buffer with a thorough aspiration between each wash.
9) 100μl of dilute anti-HNE-His antibody was added to all wells and incubated for 1 hour at RT.
10) The wells were washed 4X as described above.
11) 100μl of diluted secondary antibody-HRP conjugate was added to all wells and incubated for 1 hour at RT.
12) The wells were washed 6X as above.
13) 100μl of substrate solution was added to each well, including the blank wells, and then incubated at RT.
14) When the color changed, the enzyme reaction was stopped by adding 100μl of reaction-stopping solution to each well.
15) The absorbance of each well was read on a microplate reader using 450nm as the primary wavelength.
16) Oxidative stress in the AH specimens was then evaluated by quantifying the 4-HNE-His protein adducts by enzyme immunoassay.

Results

Characteristics of the subjects

<table>
<thead>
<tr>
<th>ages (y.o.)</th>
<th>60-69</th>
<th>70-79</th>
<th>80-89</th>
</tr>
</thead>
<tbody>
<tr>
<td>POAG</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>PEG</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

• The mean 4-HNE concentrations in the AH specimens obtained from the POAG patients were 1.35μg/ml in patients 60-70 years of age, 0.83μg/ml in patients 70-80 years of age, and 0.82μg/ml in patients 80-90 years of age.
• The mean 4-HNE concentrations in the AH specimens obtained from the PEG patients were 0.89μg/ml in patients 60-70 years of age, 1.35μg/ml in patients 70-80 years of age, and 0.64μg/ml in patients 80-90 years of age.
• The mean 4-HNE concentrations in the AH specimens obtained from the controls were 0.92μg/ml in controls 60-70 years of age and 1.15μg/ml in controls 70-80 years of age.
• In the POAG patients and the PEG patients, the mean concentrations in AH did not significantly vary from that observed in the controls.

Discussion

It was reported that mitochondrial damage in the TM occurs in cases of POAG and PEG as the general consideration. However, there was no difference of lipid peroxidation in AH among the POAG patients, the PEG patients, and the controls in this research. This result shows that mitochondrial damage in the TM is caused by intrinsic factors such as reactive oxygen species from mitochondria, vasoactive cytokines and intracellular iron rather than extrinsic factor.

Conclusions

The findings of this study showed that there was no significant difference of lipid peroxidation in the anterior chamber among POAG patients, PEG patients, and control subjects, regardless of age distribution.

References