Age-related UV absorption of the human eye lens and its molecular background

PhD thesis

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Introduction

The human cornea absorbs most of the ultraviolet (UV) radiation, but still significant amount of UV light may reach the lens. The risk of eye diseases is related to the solar UV irradiation, especially for that of different types of cataract.

As the lens effectively absorbs both the UV-A (330-400 nm) and the UV-B (280-330 nm) radiation it is important to determine that how significantly the capsules and the different layers of the lens contribute to the total absorbance of the lens. The available data in the literature related to the absorption properties of the various layers of the lens are very limited. The UV absorbance of nucleus is higher than the outer cortex and absorbance spectra of thin lens segments with equal thickness show that the absorbance increases anteroposteriorly. On the other hand, no absorption data are available related to the posterior cortex and very little is known about the absorption capacity of the lens capsules.

The transmission spectra of human lenses of different age show that the old lens transmits less amount of light both in the visible and in the near-UV range. From the absorbance it is evident that the absorption peak at 360 nm shifts towards the shorter wavelengths with age and the band at 320 nm is less observable.

The eye lens has the highest protein content inside the human body. The crystallins are the major proteins in the lens and certainly involved in UV absorption, mainly responsible for the absorption peak at 280 nm since their spectra are very similar to that of tryptophyan (Trp). The Trp derived UV filter compounds also show higher absorption in the UV-B and -C ranges than in the UV-A and thus significantly contribute to the

UV absorption capacity of the lens. As their absorption is considerably less than the proteins, their role is mainly providing the absorption at 360 nm. The concentration of these molecules are significantly different in the cortex and in the nucleus. The levels of the protein-bound filters show age-dependent changes.

Aims of the study

In this study we intended

1. to determine the UV absorption capacity of the various parts of the lens and of the lens capsules,

2. to analyse the effect of age on the UV absorption of the different layers of the lens and of the lens capsules.

3. and to study the molecular background of the UV absorption of the anterior and posterior lenticular segments.

Material and methods

Cryostat sectioning

Human lenses (n = 44, from age of 7 to 81 years) were obtained from eyes with a post-mortem delay of maximum 6 hours. Only those lenses which showed no cataract formation upon post-mortem examinations were used for measurements. Sixty μm thick consecutive sections were cut from the lenses (n=38) on a cryostat at −18 °C. Before sectioning, the lens capsules were removed and stored in physiological saline separately. Altogether, nine 60-μm-thick sections were taken for UV absorption measurements in an anteroposterior order: two from the anterior cortex, five from the nucleus and two from the posterior cortex. For spectrophotometric measurements, the sections and the lens capsules were placed between two quartz plates with a drop of isotonic saline on both sides of the specimen.

Spectrophotometric measurements

The UV absorbance of the samples was measured by a scanning spectrophotometer. In one arm, the sample was placed and on the reference side, a drop of isotonic saline was placed between two quartz plates with a 60 μm thick ring. The absorbance was determined from the ratio of the two signals. The absorption coefficients were calculated by using the measured data.

Proteomic studies

Cryostat sections were cut from six lenses and the anterior lenticular pool of sections consisted of sections derived from the anterior cortex (group 1) and the posterior lenticular pool consisted of samples derived from the posterior cortex (group 2). These sections were used for two-dimensional gel-electrophoresis Protein spots from each gel were outlined and quantified using the Proteomweaver™ software. To identify the proteins with significantly different expression levels, a MASCOT search was performed. Modiro TM search was also performed.

Statistical analysis

The tendency between age and absorption coefficients at 280 nm and at 360 nm was characterized through the use of Pearson correlation coefficients and significance tests for correlation for each layer separately. Analysis of covariance (ANCOVA) model with factor layer and covariate age was used for further statistical analysis.

Statistical analysis to reveal between-group differences was performed by the paired Student's t-test. Bonferroni-Holm and LSD correction was applied for correction of multiple testing.

Results

Absorption spectra of the lens sections

The UV absorption of the lens sections was examined in seven different age groups: 7-19, 20-29, 30-39 years, ... and 70-81 years. In the UV-A range, each layers have almost equal average absorption coefficient values and absorption shows an increasing tendency, which continues in the UV-B range too. In the 310 nm to 240 nm range, the absorption coefficient steeply increases irrespectively of age and reaches a maximum at 280 nm. From this wavelength to 250 nm the absorption coefficient sharply decreases and then increases again. The samples, which were taken from more posterior parts of the lens, show the same characteristics, but the absorption coefficients of the posterior layers are higher. In the youngest group the increasing trend is not explicit and the absorption coefficients of sections 4-7 are almost equal and sample 9 shows very low absorption compared with the other groups. The posterior layers have higher absorption capacity in the wavelength range of 300 – 400 nm, too but the increasing tendency is not as explicit as in the case of the peak at 280 nm.

Absorption capacity of the lens capsules

The lens capsules show the same absorption characteristics as the lens sections. In the UV-A range, the absorption slightly increases towards

the shorter wavelengths. At 280 nm a sharp peak is present and the absorption increases again at wavelengths shorter than 250 nm. The posterior capsules have higher absorption capacity than the anterior ones, independently of age. The epithelial layer shows very similar absorption coefficient values as the anterior capsule in the UV-C range but it does not contribute significantly to the total absorbance due to its limited thickness.

Age-related changes

Values of the absorption coefficients taken at 280 nm of the nine sample layers of every single lens were collected. The ANCOVA model revealed a borderline significant interaction between age and layer ($p = 0.057$). Changes of coefficient values as function of age were examined for each layer. The correlation was significant in layers 6 ($R= 0.371$, $p = 0.022$), 7 ($R = 0.49$, $p = 0.002$) and 9. The same analysis was performed in the case of absorption coefficients taken at 360 nm. The ANCOVA model revealed a significant interaction between age and layer ($p < 0.01$). The correlation proved to be significant in layers 5 ($R = 0.379$, $p = 0.047$), 6 ($R = 0.614$, $p = 0.000$), and 7 ($R = 0.676$, $p = 0.000$).

Although the posterior capsules showed higher averaged absorption coefficients than the anterior ones, results of ANCOVA showed that this difference is not significant ($p = 0.198$). No correlation was found between the age and the absorption coefficients at 280 nm.

Protein expression in the anterior and posterior cortex

Altogether, five individual proteins were identified to produce significantly different levels between groups 1 and 2. High sequence coverage (max. 90.83 %) of these proteins was obtained. All peptides were identified from trypsin, chymotrypsin digestion and from treatment with proteases widely used for mass spectrometric analysis. Verification of protein expression levels from each protein spot of 2DE gels was performed using in-gel ninhydrin assay. It was found that Human glyceraldehyde-3-phosphate dehydrogenase, Beta-crystallin B2, and Beta-crystallin A3 levels were higher in the anterior lenticular pool than in the posterior one. Levels of the Human Alpha-crystallin A chain and the Human Beta-crystallin B1 were higher in the posterior pool.

Discussion

The absorbance of the different parts of the human lens and that of the capsules have been determined in the age range of 7- to 81 years. The averaged absorption spectra showed that the lens sections can absorb significantly higher levels of radiation in the UV-B and -C ranges than in UV-A range with a maximum at wavelength of 280 nm. Upon studying the absorption in the UV-A range, only fine absorption changes were observed in the case of young lenses.

It has been demonstrated that the absorption increases along the optical axis, independently of age. The maximum of the absorption spectra is higher in the case of the older lenses. The averaged absorption coefficients of the various parts of the lens indicate that at 280 nm

wavelength, the age-related changes of the posterior cortex is the most noticeable while only moderate alterations can be observed in the case of the anterior cortex. Indeed, the statistical analysis proved that only posterior layers show significant correlation with age. Considering the UV-A range, the statistical results confirmed that only the samples taken from the central and posterior nuclear region show correlation with age. The posterior capsules have slightly higher absorption capacity than the anterior ones independently of age, but significant age-related changes were not found.

It has been proved that the levels of Beta-crystallin B2, A3 and that of glyceraldehyde-3-phosphate dehydrogenase were significantly higher in the anterior part of the lens, meanwhile Alpha-crystallin A and Beta-crystallin B1 were present in higher concentration in the posterior cortex. It is clear that the differential UV-absorption of the lens is accompanied by differential protein expression levels. The age-related changes in absorption properties of the posterior parts may also be related to the age-dependent molecular changes.

Original papers related to this thesis

I. Pajer V, Tiboldi Á, Bae N, Li K, Kang SU, Hopp B, Kolozsvári L, Lubec G and Nógrádi A, The Molecular Background of the Differential UV Absorbance of the Human Lens in the 240-400 nm Range. 2013, *Photochem Photobiol*, 89: 856-863. IF: 2.684

II. Pajer V, Rárosi F, Kolozsvári L, Hopp B and Nógrádi A, Age-related absorption of the human lens in the near-ultraviolet range. 2019, *Photochem Photobiol*, php.13199. IF: 2.338 (2018)