UV Absorbance of the Human Cornea in the 240- to 400-nm Range

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PURPOSE. To determine the UV absorbance of the corneal layers (epithelium, Bowman layer, stroma) in the 240- to 400-nm range.

METHODS. Consecutive slices (100 μm) of human cadaveric corneas were cut, and the UV absorbance of each sample was determined in a scanning spectrophotometer. In some cases the epithelium was scraped off and its absorbance measured separately.

RESULTS. The investigation of the UV-B absorption of consecutive corneal slices revealed evidence that UV-B absorbance is 1.8 times higher in the anterior 100 μm of the human cornea than in the posterior layers. The UV absorbance of the posterior layers was uniform, showing no further structural dependence. The epithelium and Bowman layer are both effective absorbers of UV-B radiation.

CONCLUSIONS. These results suggest that the anterior corneal layers are particularly important in preventing damage by UV-B radiation. (Invest Ophthalmol Vis Sci. 2002;43:2165–2168)

A series of recent studies has shown that exposure of the cornea to ultraviolet irradiation (UV-IR) induces pathologic changes in its structure. Acute exposure of the cornea to UV radiation results in formation of photo-ophthalmia, whereas chronic, repeated exposure of the eye results in keratopathies that affect the epithelium and the anterior part of the corneal stroma. However, a specular microscopy study indicated that the corneal endothelium also reacts to UV radiation. Chronic UV exposure experienced by welders results in endothelial pleomorphism.

Altogether, the cornea absorbs most of the UV-B radiation. Therefore, the cornea is exceptionally sensitive to the damage induced by UV-B exposure. There are only a limited number of studies concerning the absorption of UV radiation in different layers of the cornea. In 1948, Kinsey found that most UV radiation shorter than 290 nm is absorbed in the corneal epithelium. In contrast, a study by Schive et al. showed no difference in UV absorption between the epithelium and the stroma.

It is well established that a significant amount of UV radiation may penetrate the cornea, reach the deeper ocular structures, and induce ocular light damage. Exposure of the cornea to suprathreshold or repeated subthreshold UV-IR may cause irreversible damage. It has become evident in recent years that removal of the most anterior layers of the cornea makes the internal structures of the eye more sensitive to damage induced by UV-IR. This finding is particularly important, because photorefractive keratectomies have recently become routine ophthalmic procedures. In its classic form, the surgery begins by scraping off the epithelium, and then the Bowman layer and some of the underlying stroma are removed by laser keratectomy, thus leaving the remainder of the stroma unprotected against UV-IR at least temporarily.

The purpose of the present study was to determine the UV-A (330–400 nm), UV-B (280–330 nm), and UV-C (240–280 nm) absorbance of the corneal layers and thus reveal the importance of the structural elements of the cornea in the protection against excessive UV radiation.

MATERIALS AND METHODS

Human corneal rims (n = 20) were obtained from cadaveric eyes within 6 hour after death. The human samples were used with the approval of the Human Ethics Committee of University of Szeged, Albert Szent-Györgyi Medical Centre, and the study was conducted in accordance with the provisions of the Declaration of Helsinki for experimentation involving human tissue. The eyeballs were transported and stored in a humid chamber at 4°C until the corneas were removed. The corneas were flattened, and 100-μm-thick sections were cut on a freezing microtome. Sectioning started from the internal surface. If the first slice of the cornea was uneven, it was removed, and only the uniform slices were used for measurements. The thickness of corneal samples was determined with a microscope (depth resolution, 2 μm; model BX50; Olympus, Tokyo, Japan) immediately after sectioning. The sections were then stored in cold physiological saline.

Special care was taken to preserve the epithelium on the outer surface of the cornea. In case of three corneas, the epithelium was first carefully scraped off with a surgical blade, and the remainder was sectioned as described. All the corneas were investigated within 12 hours after death.

The dependence of the UV absorbance (ABS) of these slices on the wavelength was measured by a scanning spectrophotometer (model UV-2101 UV-VIS; Shimadzu, Kyoto, Japan). To avoid light scattering, the samples were placed between two quartz glass plates with a drop of physiological saline on both sides of the specimen. On the reference side, only a drop of physiological saline was placed between the glass plates. This device contains two spectral lamps (halogen lamp for 900–340 nm, and deuterium lamp for 340–190 nm wavelength ranges), a double monochromator to select the proper wavelengths, a sample chamber including measuring and reference beam paths, and a photomultiplier to detect and amplify the measuring and reference light intensities. The spectrophotometer was connected to a computer. The absorbance is defined by the following equation:

\[ I = I_0 10^{-\text{ABS}} \Rightarrow \text{ABS} = \lg \left( \frac{I}{I_0} \right) \]

where \( \lg (I/I_0) \) corresponds to the logarithm of the incident light intensity divided by the transmitted light intensity with 10 as the base. ABS is not a characteristic material constant; however, the absorption coefficient \( (\alpha) \) of the investigated sample can be calculated using the following relation.
The absorption coefficient of the stroma and that of the uppermost layer (including epithelium, Bowman layer, and some of the stroma) of the cornea as a function of wavelength in the range of 240 to 400 nm. Note the higher absorption coefficient of the uppermost layer in the UV-B and -C ranges. The absorption coefficient curve represents the average of measurements of 20 different samples. The SE was no greater than 10%.

The important UV-A, -B, and -C wavelength ranges are also indicated in this figure. It can be seen that the absorption coefficients of both the Bowman layer and the epithelium were several times higher in UV-B and -C ranges than that of the stroma.

From Figure 2 we calculated the normalized absorption coefficients of the epithelium and Bowman layer compared with the coefficient of the stroma (Fig. 3). The absorption coefficient of the epithelium and Bowman layer are approximately 2 to 7.5 times higher than that of the stroma below 300 nm of wavelength.

Figure 4 demonstrates the calculated intensity changes of the incident light in the different corneal layers at three UV wavelengths. The intensity of the incident light on the surface of the cornea corresponds to 100%. For example, at 280 nm it can be seen that this decreases to 19.9% on the epithelium and Bowman layer interface. The intensity behind the Bowman layer is only 4.8%. This means that the epithelium and Bowman layer transmit only approximately one twentieth of the 280-nm light intensity to the stromal surface. Finally, the remaining intensity of the transmitted light after the stroma was not more than 10% after paraform embedding, we took relative values compared with the native thickness of the corneal slices.

**RESULTS**

Figure 1 shows the absorption coefficient of the stroma and that of the uppermost layer (including epithelium, Bowman layer, and some of the stroma) of the cornea as a function of wavelength in the range of 240 to 400 nm. The absorption coefficient shown on Figure 1 represents the average of measurements on 20 separate samples. It appears that the absorption coefficient of the most superficial composite layer is significantly higher than that of the pure stromal layers. Because this composite layer consists of the epithelium, the Bowman layer, and some stroma, the first two of these are thought to be responsible for the higher absorbance. We decided to determine the absorption coefficient of each corneal layer separately. In case of three corneas, we scraped off the epithelium (depth of epithelium \(d_1 = 30 \mu m\)) as an intact layer and measured the absorbance of this intact layer only (Fig. 2). Because we could not separate the Bowman layer from the underlying stroma, a 100-\(\mu m\)-thick corneal section was cut, that contained the whole Bowman layer and some stroma. We measured the absorbance of this section, determined the thickness of the Bowman layer \(d_0 = 15 \mu m\) and of the corneal stroma \(d_a = 85 \mu m\). Because the absorbance of the whole corneal section containing Bowman layer and stroma, the thickness of the different parts, and the absorption coefficient of stroma were known, the absorption coefficient of the Bowman layer (Fig. 2) could be calculated as

\[
I = I_0 10^{-\alpha d} = I_0 e^{-(\alpha d_0 + \alpha d_a)} \Rightarrow \frac{\alpha}{d_0} = \frac{\text{ABS} \ln 10 - \alpha d_a}{d_0}
\]

where \(d\) is the thickness of the sample and \(\ln e = 0.434\).

After the measurements, the samples were fixed in 7% buffered paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin, and the thickness of the epithelium and Bowman layer was determined. Because the degree of shrinkage was less than 10% after paraffin embedding, we took relative values compared with the native thickness of the corneal slices.

**FIGURE 1.** The absorption coefficient of the stroma and that of the uppermost layer (including epithelium, Bowman layer, and some of the stroma) of the cornea as a function of wavelength in the range of 240 to 400 nm. Note the higher absorption coefficient of the uppermost layer in the UV-B and -C ranges. The absorption coefficient curve represents the average of measurements of 20 different samples. The SE was no greater than 10%.

**FIGURE 2.** Dependence of the absorption coefficient of the different corneal layers on the wavelength. The important UV-A, -B, and -C wavelength ranges are also indicated.

**FIGURE 3.** The absorption coefficient of epithelium and Bowman layer divided by the absorption coefficient of the stroma as a function of wavelength. The absorption coefficients of epithelium and Bowman layer are approximately 2 to 7.5 times higher than that of the stroma below 300 nm wavelength.
than 0.0055%. In the cases of the longer wavelengths, this decrease is not so dramatic, but it is still significant in the UV range. The central corneal thickness of 570 μm was taken to be an accepted reference value. It is demonstrated that the epithelial and Bowman layers absorb relatively high amounts of ultraviolet radiation compared with their relatively low thickness.

**DISCUSSION**

We report the structural dependent UV absorbance of various layers of the human cornea between 240 and 400 nm of wavelength. We believe that our measurements correspond to the UV absorbance in vivo, because we used fresh, intact samples, and cutting and further processing was made as quickly as possible. We found that the epithelium and Bowman layer have significantly higher absorption coefficients than that of the stroma in UV spectra shorter than 310 nm. It appears, according to our calculations, that the whole thickness of the human corneal stroma is responsible for approximately 70% to 75% of the UV absorbance in this range. The rest of UV irradiation is absorbed more or less equally by the epithelium and Bowman layer, although the latter always absorbs slightly less than the epithelium. Moreover, the various stromal layers show no further difference in UV absorbance, although the fine structure of the corneal stroma markedly changes toward the deeper corneal layers.

To understand the role of different corneal layers in the UV filtering, their absorbance is shown as the function of wavelength in Figure 5. It can be seen clearly that the stroma has the most significant absorbance in the cornea. However, our absorption measurements showed that this is not due to its high absorption coefficient but its thickness, which is more than 10 times larger than that of the other layers. Figure 6 shows the transmission of epithelium, Bowman layer, stroma, and the whole cornea as a function of wavelength. On the basis of these data it could be argued that the stroma is responsible for the UV filtering of cornea by its thickness. The UV filtering ability of the epithelium and Bowman layer may be due to their special molecular composition and higher dry mass content of the Bowman layer, resulting in a higher absorption coefficient.

The molecular components of the Bowman layer are very similar to those of the stroma, and such a difference was unexpected. The absorbance of Bowman layer was calculated indirectly, supposing that the underlying stroma has the same absorbance characteristics as the other parts of the stroma. We produced specimens in which the first stromal layer just underlying the Bowman layer was cut and measured, and this layer showed no difference in absorbance compared with other stromal slices. Because the absorption coefficient of the Bowman layer is up to eight times greater than that of corneal stroma, it acts as an effective filter in UV radiation.

The significant absorbance of the cornea in the UV-B and -C spectra appears to be due to high amounts of tryptophan residues in the proteins of the stroma and the high ascorbic acid content of the epithelium. Recent data suggest that the high amount of ascorbic acid alone in the epithelium could absorb 77% of the incident radiation at wavelengths likely to be dangerous. This study was not intended to determine the molecular components of the Bowman layer that could be responsible for the increased absorbance. However, the fine structural organization of the Bowman layer, such as the arrangement of collagen fibers and extracellular molecules, is different from that of the stroma, and this may be one factor.
determining the higher absorption coefficient of the Bowman layer. It is important to call attention to the point that removal of the anterior layers (epithelium and Bowman layer) makes the eye more susceptible to ocular light damage than removal of a considerable thickness of the corneal stroma. Because UV-B is mainly absorbed in the cornea, the absence of the important anterior layers may lead to corneal and lens damage in case of excessive UV-B irradiation. Earlier studies suggested that Bowman layer may play a protective role against development of subepithelial haze in humans.15,16 Photorefractive keratectomy (PRK) may lead to the irreversible loss of the Bowman layer and thus may sensitize the eye to ocular light damage. As a consequence, one complication of deep photorefractive ablation may be stromal haze formation.16,17

Laser-assisted subepithelial keratectomy (LASEK), a modified PRK offer the advantages of both PRK and LASIK, because in this method the epithelial flap is placed back onto the ablated stroma and acts as a biological therapeutic lens,17 whereas in LASIK both the corneal epithelium and Bowman layer are saved. Recent data show that LASEK appears superior to LASIK, because it may prevent the corneal flap- and interface-related problems of LASIK.18

Both LASEK and LASIK are less likely to induce stromal haze than PRK, but the underlying cellular mechanisms are unknown. It can be argued that the presence of the near intact epithelium after LASEK or epithelium and Bowman layer after LASIK effectively reduces the initial cellular damage to the corneal stroma. Whatever the mechanism of cellular events after laser ablation methods, it is suggested that corneas freshly operated on should be carefully protected from direct exposure to UV-irradiating sources, no matter whether keratectomy or keratomileusis was applied.

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References