ORIGINAL ARTICLE

Spectral transmission of the pig lens: Effect of ultraviolet A + B radiation

Transmission spectrale du cristallin du porc : l’effet du rayonnement ultraviolet A + B

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Spectral transmission; Pig lens; Lens transmission; Ultraviolet radiation

Summary
Objective. — To determine the spectral transmission curve of the crystalline lens of the pig. To analyse how this curve changes when the crystalline lens is irradiated with ultraviolet A + B radiation similar to that of the sun. To compare these results with literature data from the human crystalline lens.

Procedures. — We used crystalline lenses of the common pig from a slaughterhouse, i.e. genetically similar pigs, fed with the same diet, and slaughtered at six months old. Spectral transmission was measured with a Perkin-Elmer Lambda 35 UV/VIS spectrometer. The lenses were irradiated using an Asahi Spectra Lax-C100 ultraviolet source, which made it possible to select the spectral emission band as well as the intensity and exposure time.

Results. — The pig lens transmits all the visible spectrum (95%) and lets part of the ultraviolet A through (15%). Exposure to acute UV (A + B) irradiation causes a decrease in its transmission as the intensity or exposure time increases: this decrease is considerable in the UV region.

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Conclusions. — We were able to determine the mean spectral transmission curve of the pig lens. It appears to be similar to that of the human lens in the visible spectrum, but different in the ultraviolet. Pig lens transmission is reduced by UV (A + B) irradiation and its transmission in the UV region can even disappear as the intensity or exposure time increases. An adequate exposure intensity and time of UV (A + B) radiation always causes an anterior subcapsular cataract (ASC).

Résumé


Procédures. — Nous avons utilisé les cristallins du porc commun d’abattoir, c’est-à-dire des porcs génétiquement similaires, avec le même régime alimentaire et abattus à six mois. La transmission spectrale a été mesurée à l’aide d’un spectrophotomètre Perkin-Elmer Lambda35 UV/VIS. Les cristallins ont été irradiés en utilisant la source d’ultraviolets Asahi Spectra Lax-C100, qui nous a permis de choisir la bande d’émission de spectre aussi bien que l’intensité et le temps d’exposition.

Résultats. — Le cristallin du porc transmet tout le spectre visible (95%) et laisse passer une partie de l’ultraviolet A (15%). L’exposition aux radiations UV (A + B) provoque une diminution de la transmission à mesure qu’augmentent l’intensité de la radiation ou le temps d’exposition. Cette diminution est considérable dans la région UV.

Conclusions. — Nous avons pu déterminer la courbe de transmission spectrale moyenne du cristallin de porc. Elle s’avère être semblable à celle du cristallin humain dans le spectre visible, mais différente dans la région des ultraviolets. Dans ce dernier cas, la transmission se réduit pour les UV (A + B), et peut même disparaître dans la région UV en fonction de l’augmentation du temps d’exposition ou de l’intensité. Une intensité et un temps d’exposition de rayons UV (A + B) suffisants provoquent toujours une cataracte sous-capsulaire antérieure dans le cristallin du porc.

Introduction

Numerous studies state that ultraviolet radiation (UV) is one of the factors that most influences the loss of transparency of the crystalline lens (cataracts) in both humans and animals [1—12]. Several studies with rats [5—11] and rabbits [13] show the influence UV radiation bears on the development of cataracts.

For different reasons, swine ophthalmology has historically received little attention [14]. Consequently, there are few studies on the porcine eye in the literature and fewer focusing on its optical properties. However, currently a greater interest has emerged in the study of the eye of the pig because it is used as a model in investigation [15—17] since it does not have the ethical and economic restrictions of other species and it shares many similarities with the human eye. The porcine eye is phylogenetically close to the human eye, since, for example, it does not have a tapetum lucidum, it has holangiotic retinal vascularisation, it has cones on the external retina, and the thickness, shape and size of the sclera is similar to that of the human lens [18]. Thus, some inferences may be made to the human lens.

In the human eye, it is complicated to determine the influence of the UV radiation precisely on the development of opacities as it is difficult to isolate its effect from other factors that can also bear an influence. Nonetheless, some epidemiologic [1,2] studies have been performed in humans with a view to analysing the effect that exposure to sunlight, especially ultraviolet B (UVB) radiation, has on crystalline lens opacification. Sunlight is the principal source of ultraviolet radiation for most of the world’s population. This fact now has considerable interest in the era of ozone depletion and potential for even greater exposure to UVB. The Chesapeake Bay Waterman Study [1] was the first investigation to develop a detailed model of personal ocular exposure to UVB, and correlate it with a detailed standardised system for cataract assessment. An increased risk of cortical opacities was found with increasing average annual ocular UVB exposure. Likewise, the Beaver Dam Eye Study [2] reveals a significant correlation between exposure to UVB radiation and cortical opacities.

Deduction from animal data to the human situation is always questionable. However, it is the only option for development of empirically based safety recommendations for avoidance of cataract after exposure to UV radiation.

The use of the porcine eye as a research model involves studying its specific characteristics, therefore diverse studies on the pig eye have been performed [18—21]. Regarding the action of UV radiation on the pig lens, Oriowo et al. [22] analysed in vitro the action spectrum for acute UV
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Materials and methods

Pig lens

We used the lenses of freshly slaughtered common pigs from an industrial abattoir for our experiments. The pigs were slaughtered at six months of age and the eyes were removed at the same time, stowed in suitable recipients in saline solution, and sent to our laboratory five or six hours afterwards where the lenses were then removed. Therefore, all the eyes we used belonged to genetically similar pigs that were fed the same diet and that were slaughtered at the same age: thus, our study was made up of a sample of homogeneous lenses.

Particular care was taken in the lens removal process when detaching the lenses from the zonules to avoid damaging the capsules. The lenses were then submerged in saline solution and subsequently placed in specifically designed lens holders.

Spectral transmission measurements

The transmission curves were obtained by using a Perkin-Elmer Lambda 35 UV/VIS spectrometer (Perkin-Elmer Inc. Shelton, CT, USA). This apparatus can measure the spectrum from 200 nm onwards, which means that spectral transmissions in ultraviolet A (UVA), ultraviolet B (UVB), and part of ultraviolet C (UVC) are accurately determined (precision is up to 1 nm). The air was taken as a reference to measure transmittance. The measurements correspond to the total transmission of the crystalline lens, so the apparatus was equipped with an integrating sphere. A suitable lens holder was designed and constructed to place the sample (lens) directly in front and covering the complete entrance hole of the integrating sphere of the spectrophotometer. This lens holder also made it possible to position it directly in the appropriate position to be irradiated by the UV radiation source and subsequently replace it in the spectrophotometer for the following measurement. All of this is achieved without having to touch the lens, which could undergo deterioration if continually handled.

Ultraviolet radiation

We used a Xenon lamp, Asahi Spectra Lax-C100 (Asahi Spectra Co. Ltd. Japan), which can be tuned to different wavelengths to irradiate the lenses with ultraviolet light; its output beam was directed by optical fibre. The UV beam was focused on the anterior surface of the lens and irradiated the whole surface of the lens: it was possible to regulate the intensity. An electronic shutter was used to control the exposure time. After positioning the mirrors and appropriate filters, we selected the 300–400 nm band, which corresponds to UVA radiation (315–380 nm) plus part of UVB radiation (280–315 nm). In this region of the spectrum, the emission curve of the Xenon lamp is more or less similar to that of the sun [24–26].

In our study, we used two radiation intensities, 10 and 20 j/cm² and three exposure periods, 5, 10 and 20 minutes. The intensities used, without being exaggeratedly high, were strong enough for the exposure periods to produce variations in the spectral transmission of the irradiated lenses.

Photographs of lenses

To show the effect of the UV radiation on the lenses, we take different photographs of lenses by the slit-lamp technique using an orange background for more clarity. First, we photographed a freshly removed porcine lens, then the lens was irradiated at an intensity of 20 j/cm² for twenty minutes and finally the exposure time was half an hour at the same intensity.

Data analysis

We used the IBM SPSS 19 statistics program for statistic analysis of the results.

Results

We measured the transmission of twelve crystalline lenses, randomly selected, to establish the baseline. The Kolmogorov-Smirnov test confirmed that the variable follows a normal distribution (P > 0.50 for any wavelength). As the standard deviations were small for all values of wavelength (λ), we investigated if three measurements would be enough to establish a transmission curve. Fig. 1 shows the mean and the confidence interval from three spectral transmission curves of three porcine lenses. In fact, there was no significant difference between the twelve and three measurements and the significance P < 0.30 was for any λ in the
curve. In view of the results, we considered the measurement of three lenses was sufficient, in our study, to obtain a reliable determination. Thus, all the curves shown in this report are always the mean of three lenses.

To be continued, we investigated the evolution with time of the crystalline lens transmission, time ranges from 0 to 60 minutes in order to control a possible deterioration of the lens in the time used for measuring. The standard deviation (SD) in these curves takes values between 0.1 and 2.6 which are similar to or lower than those SD obtained from the variability between elements, that is, from the twelve curves measured before.

Fig. 2 shows how the transmission spectrum of the pig lens varies when it is irradiated with UV (A + B) at an intensity of 10 j/cm² for a period of 5 (T5), 10 (T10), and 20 (T20) minutes of exposure. There was no significant difference between the lens that was not irradiated (curve T0) and T5 at any wavelength (P > 0.20). The difference was statistically significant, from 320 nm onwards, between T0 and T10 and also between T0 and T20 (P < 0.05 throughout the entire wavelength).

Fig. 3 shows the same as Fig. 2, but for an intensity of 20 j/cm². The difference is very significant (P < 0.01) from 320 nm onwards, between T0 and T20 curves. When we compare T0 with T5 and also T0 with T10, the difference between the curves is significant, from 320 nm onwards, with P < 0.05.

**Discussion**

Spectral transmission of the common pig lens

The spectral transmission curve of the common pig lens (Fig. 1) shows that this lens lets the whole visible spectrum through with practically no attenuation (about 95% transmission). However, from the 400 nm wavelength onwards and towards the short wavelengths, an abrupt decline in transmission commences which stops at about 370 nm; from then on the transmission remains constant at about 15% up to 320 nm where it drops abruptly. This wavelength can be considered as the cut-off point since transmission at 315 nm is virtually zero.

Effect of A + B ultraviolet radiation on pig lens transmission

Fig. 2 shows how the spectral transmission varies, depending on the exposure period at a radiation intensity of 10 j/cm². Two data can be observed in this graph.

Visitable spectrum (380—780 nm)

In the visible region, transmission decreases with exposure time almost evenly. Nevertheless, there is no significant difference between T0 and T5 at any wavelength for five minutes exposure. After ten minutes exposure the decrease, in the same interval, is 10%, again evenly, and after twenty minutes 16%. The fall is slightly more pronounced for the three exposure times for the 450 nm wavelength. However, from 420 nm onwards the decrease in transmission is accentuated as the wavelength decreases, but only for exposure periods of ten and twenty minutes. At the edge of the visible spectrum (approximately at 380 nm) such drops are about 40% for both ten and twenty minutes of exposure.

Ultraviolet spectrum (300—380 nm)

In the ultraviolet area, the decrease in lens transmission is much more pronounced than in the visible spectrum. Specifically, for exposure periods of ten and twenty minutes, this decrease is 45% at the 350 nm wavelength and about 60% at 320 nm; it is 100% at 300 nm, which means that its transmission at that wavelength is zero.
Fig. 3 also shows how the spectral transmission varies depending on the exposure period, but at a radiation intensity of 20 j/cm². This graph illustrates, in both the visible region of the spectrum and the ultraviolet, the same behaviour as in the previous case for five- and ten-minute exposure periods, i.e., an even decrease in transmission in the visible area, and a much more pronounced decrease in the UV area. Nonetheless, for a twenty-minute exposure period the decrease was much more pronounced in the entire spectrum, particularly in the blue and ultraviolet areas, which indicates the presence of opacities that block the transmission of light.

In addition, Figs. 2 and 3 show that roughly the same result is obtained by irradiating the lens for ten minutes at an intensity of 10 j/cm² as by irradiating it for five minutes, but at an intensity of 20 j/cm². The same occurs when the lens is irradiated for twenty minutes at an intensity of 10 j/cm² as when it is irradiated for ten minutes but at an intensity of 20 j/cm². This means that the effect brought about by ultraviolet radiation on the pig lens, at the time intervals and intensities used, chiefly depends on the total energy received by the lens, in other words, the product of the intensity by the exposure time (commonly defined like reciprocity Bunsen-Roscoe law).

Finally, it must be stressed that in all the cases, the decrease of the transmission in the UV area is much greater. This means that when the lens is irradiated with UV radiation, it becomes more opaque to UV radiation itself, i.e. it filters this radiation more, in many cases eliminating it.

**Formation of opacities**

Fig. 4 depicts different photographs of lenses taken by the slit-lamp technique using an orange background for more clarity. Fig. 4a shows a freshly removed porcine lens. Fig. 4b shows a porcine lens irradiated at an intensity of 20 j/cm² for twenty minutes in which a low density anterior subcapsular cataract (ASC) can already be seen (white arrow). Fig. 4c exhibits a dense ASC cataract produced when the exposure time was half an hour and the radiation intensity was 20 j/cm². It is worth mentioning that in our experience the cataract produced by UV (A+B) radiation was always ASC. This result is similar to that of Mody et al. [9], who found that all guinea pig eyes exposed to UVR-B developed cataract in the anterior subcapsular region.

**Comparison with the human lens**

Comparison of these results with the data of the human lens is always debatable and the following facts must be kept in mind:

- Two different species are being compared;
- The UV irradiation applied was acute. In other words, intense in a short period of time, while in the human case the irradiation took place at a low intensity over years.

The positive aspects are that:

- The porcine lens is very similar to the human lens and the pig eye is currently being used as an experimental model;
- The lenses used in this experiment were all young and practically the same as they came from genetically similar pigs that were fed the same diet, lived in the same environment, and were all slaughtered at six months of age. Thus, the modifications in the spectral transmission of the lens that could occur due to the UV radiation were exclusively due to the UV radiation.

If we compare the curve that shows the spectral transmission of the porcine lens (Fig. 1) with that of the human lens [28,29], we can see (Fig. 5) that in the region of the visible spectrum the transmission is practically identical; nonetheless, it is different in the ultraviolet region. The
porcine lens transmits throughout the entire ultraviolet A area (315–380 nm) approximately 15% and completely filters all the radiations below 315 nm approximately, i.e. it does not let UVB (280–315) or UVC (100–280) through. By contrast, the human lens hardly transmits at all below 380 nm, i.e. besides UVB and UVC radiation it also filters UVA radiation. It only presents a small window of approximately 10% transmission centred at 320 nm (Fig. 5, human, 4 ½ years old) at birth, which decreases with age until virtually disappearing.

In our study, an intense and prolonged exposure of the porcine lens to UV (A+B) radiation always produced an anterior subcapsular cataract. However, in humans [1,2] an increased risk of cortical opacity was found with increasing average annual ocular UVB exposure. In this aspect, it is difficult to make a direct comparison due to the experimental differences, which is why we only conclude that an acute exposure to UV (A+B) radiation of the porcine lens in vitro always produces an ASC cataract as it does when the guinea pig eye is irradiated with UVB [9].

As can be seen in Figs. 2 and 3, exposure to UV radiation causes a decrease in transmission in general, but it is greater in the short wavelengths and more in the UV; it is much greater as the intensity and exposure time increase and, in many cases, it reaches zero transmission. This means that as we irradiate the lens with UV, it becomes ever more opaque precisely to the UV, even filtering it entirely.

To conclude:
• the porcine lens presents a similar spectral transmission to that of the human lens in the visible spectrum, but different in the ultraviolet area;
• exposure of the porcine lens to UV (A+B) intense radiation and in a short time always produces an anterior subcapsular cataract as it does when the guinea pig eye is irradiated with UVB. In humans, however, epidemiologic studies provide relative evidence that a greater exposure to UVB sun radiation increases the risk of cortical opacities appearing;
• exposure of porcine lens to intense UV (A+B) radiation darkens it very slightly. Aged human lenses (over 60 years) progressively go yellow;
• the transmission the porcine lens presents in the UV area disappears progressively when it is irradiated precisely with UV radiation.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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