

ADULT AND DEVELOPING CORNEAL STROMA AS REVEALED BY RAPID-FREEZING AND DEEP-ETCHING

HIRSCH M. PRENANT G. and POULIQUEN Y.
INSERM U.86, Hôtel Dieu, Paris (France)

Purpose. The objective was to determine the supramolecular organization of the corneal stroma extracellular matrix (ECM) and the changes which occurred during the acquisition of transparency. **Methods.** Mature corneas and chicken embryo corneas were used. Samples were frozen against a metal block cooled at -180°C , transferred on a cold stage of a freeze-etch unit, cleaved, etched and rotary replicated in vacuum. **Results.** Mature corneas displayed type I collagen fibrils with high and low molecular density regions and microfibrillar subunits of 8nm in diameter. Interfibrillar bridges with globular entities linked adjacent fibrils. Filaments with globules were found running along the fibrils. Networks of beaded microfilaments with 100-110nm periodicity were often encountered. These macromolecular assemblies were suggestive of proteoglycans, type IX and type VI collagens, respectively. The secondary developing stroma showed disordered arrays of collagen fibrils and interfibrillar bonds which organized during the maturation. Sheets of closely associated fine, long filaments, resembling type IV collagen were encountered. The beaded microfilaments were also present. **Conclusion.** Used in conjunction with other approaches, this method should offer a very useful aid in the understanding of the interactions of ECM macromolecules in the corneal stroma.

P 152

THE ROLE OF XANTHINE OXIDASE IN THE ANTERIOR EYE SEGMENT IRRADIATED WITH UVB RAYS

ČEJKOVÁ J.¹ and LOJDA Z.²

¹ The Laboratory of Biochemistry and Histochemistry, Institute of Experimental Medicine, Academy of Sciences of Czech Republic, Praha (CR)

² Institute of Histology and Embryology, 1st Faculty of Medicine, Charles University, Praha (CR)

Purpose of this study is to detect histochemically the activity of xanthine oxidase (an enzyme belonging among oxidases generating reactive oxygen species [ROS]) in the rabbit anterior eye segment repeatedly irradiated with UVB rays.

Methods : Rabbit eyes were repeatedly irradiated with UVB rays (312 nm, 1 x daily for one till 5 days from a distance of 0.2 m with UV lamp, 6W). After the killing of animals the anterior eye segments were examined histochemically for the activity of xanthine oxidase, catalase, acid glycosidases and some lysosomal proteases (Dipeptidylpeptidase I, II). Activities of these enzymes were compared with those in untreated eyes.

Results : During the irradiation of the eyes with UVB rays (from day 1 to day 5) the activity of xanthine oxidase was gradually increasing in the corneal epithelium, endothelium and lens epithelium (together with activities of acid glycosidases and lysosomal proteases). In contrast, the activity of catalase was decreasing and afterwards lost in the same cell layers of the cornea and lens.

Conclusions : From the increased activity of xanthine oxidase (an enzyme generating ROS), increased activities of acid glycosidases and lysosomal proteases (enzymes associated with the degradation of glycoproteins) and the decreased and afterwards lost activity of catalase (an enzyme cleaving ROS) it is suggested that xanthine oxidase participates in the generation of ROS (and the damage of the anterior eye segment evoked by them) after the irradiation of the eye with UVB rays.

Supported by the grant No. A5039501 from GA of Academy of Sciences of CR and by the grant No.2711-3 from GA of the Ministry of Health (CR).

PREVENTION OF THE EYE AGAINST THE DAMAGING EFFECT OF UV RAYS BY SOFT CONTACT LENSES WITH UV ABSORBERS ČEJKOVÁ J.¹, LABSKÝ J.² AND VACÍK J.²

¹ The Laboratory of Biochemistry and Histochemistry, Institute of Experimental Medicine, Academy of Sciences of Czech Republic, Praha (CR)

² The Laboratory of Contact Lenses, Institute of Macromolecular Chemistry, Academy of Sciences of Czech Republic, Praha (CR)

Purpose of this study is to examine a suitable absorber of UV rays bound in contact lenses for the protection of the eye against the effect of UV rays.

Methods : Following UV absorbers were chemically bound in contact lenses (HEMA, hydroxyethyl methacrylate, 38% water content; HEMA-acetate, vinylpyrrolidone 2-acetyl hydroxyethyl methacrylate, 68% water content) : UV absorber NO 1 (4-methacryloyloxy-2-hydroxybenzophenone) and NO II (4-methacryloyloxyethoxy-2-hydroxybenzophenone). The transmittance of these lenses was measured (UV rays from 220 to 365 nm) using various concentrations of UV absorbers. The rabbit eyes with contact lenses containing UV absorbers (and also with contact lenses in which UV absorbers were absent) were irradiated with UV rays of various wavelength (UVA [365 nm], UVB [312 nm], UVC [254 nm]) repeatedly for one till 10 days (1 x daily for 5 min). The anterior eye segments were examined macroscopically and histochemically (the detection of various enzymes). The eyes without contact lenses (and similarly irradiated) were also examined.

Results : Eyes irradiated without contact lenses (particularly with UV rays below 320 nm) were seriously damaged. This was prevented using contact lenses with UV absorbers. Best results were obtained with UV absorber NO II in HEMA-acetate; 2 % concentration of absorber completely eliminated UV rays at the area 200-350 nm. The eyes covered with contact lenses with this absorber were completely protected against the damaging effect of UV rays.

Conclusions : UV rays particularly belong 320 nm cause severe damage of the anterior eye segment. Contact lenses with suitable UV absorber protected eyes against the damaging effect of UV rays.

Supported by the grant No.304/93/0393 from GA of CR.

P 154

EFFECTS OF CONTACT LENS USE TO CORNEAL EPITHELIAL BARRIER FUNCTION BY OBJECTIVE FLUOROPHOTOMETER

TAKAHASHI T¹⁾, ENOMOTO Y¹⁾, ITOI M¹⁾, ITOH N¹⁾, NAKAYASU K¹⁾, AND KANAI A¹⁾, KOBUCHI T²⁾, AND NISHIMAKI K²⁾

1) Department of Ophthalmology, Juntendo University School of Medicine, (Japan) 2) SEED Co. Ltd. (Japan).

Purpose: We assessed the effect of the contact lens (CL) use to the corneal epithelial barrier function.

Methods: The corneal epithelial barrier function was studied in 51 subjects (Male;23, Female;28 , Age; 22.7 ± 5.6 yrs), classified into 4 groups : the eyes using no CL (NCL, 11 eyes), the polymethylmethacrylate CL (PMMA CL, 11 eyes), the rigid gas permeable CL (RGPCL, 21 eyes), and the soft CL (SCL, 8 eyes). The eyes with corneal diseases were excluded from this study. The values of the fluorescence from the cornea were measured before and 30 mins after the instillation of 3 ul of 0.5 % fluorescein solution (washing the eye after the instillation) by the objective fluorophotometer. The fluorescein concentration in the cornea (FC) was obtained by subtracting the value of fluorescein before the instillation from its after the instillation and calculating.

Results: There was no significant difference of the age between in each groups. The FC in PMMA CL and RGPCL was significantly higher than its in NCL (each $p < 0.05$), but there was no significant difference between NCL and SCL.

Conclusion: These results suggest that the contact lens use impairs the corneal epithelial barrier function and the impairment might be mainly caused by the mechanical contact of CL rather than the hypoxia of the corneal epithelium.