P 219

DISTRIBUTION OF INTEGRINS IN THE RABBIT LENS EPITHELIUM

B. Briat, J. M. Legeais, D. Thevenin, G.Renard and Y.Pouliquen.
Department of Ophthalmology, Inserm U 86, Hôtel-Dieu Hospital, Paris, France;

Purpose : Cell-cell and cell-substratum interactions are mediated through different families of receptors in particular integrins. These cell receptors influence many physiological processes including cellular proliferation and differentiation which are implicated in the secondary cataract development. The current study reports the distribution of the integrins expressed at the cell surface in a normal epithelium, an epithelium derived from postoperative capsular opacification and in a tissue-cultured rabbit lens epithelium.
Methods : The distribution of integrins was determined by immunohistochemical study using monoclonal antibodies α_1, α_2, α_5, α_6, α_v, β_1 and β_4. The noncultured epithelial cells derived from normal lens frozen sections and also from postoperative capsular opacification frozen sections. The cultured lens epithelial cells were isolated and grown in tissue-culture from primary culture to third passage, studied without and with confluence in each passage. The expression was also evaluated on Lab-Tek chamber slide uncoated and another coated with collagen I, IV, laminin and fibronectin.
The normal cell surface expression of integrins and the modulation with disease (secondary cataract), different passages, confluence and culture chamber slide coated with extracellular matrix proteins were evaluated.
Results : The data indicated that the non cultured normal lens epithelium has a variety of distinct integrin subunits including α_1, α_2, α_5, α_6, α_v, β_1 and β_4. Furthermore, we determined that the epithelium cells from postoperative capsular opacification showed a variation of this expression. When the cells were placed in culture, the integrin pattern changed, according to the different passages, confluence and chamber slide coated or not.
Conclusions : This is the first report of integrin pattern on lens epithelium cells. The expression of integrin subunits change according to the cells come from non-cultured epithelium (normal or pathological) or from different stages of cultured epithelium. The present results suggest that cell-substratum interactions, mediated through integrins, may play a critical role in the development of secondary cataract.

P 220

HEPARIN MODIFIES PROLIFERATIVE ACTIVITY OF CULTURED BOVINE LENS EPITHELIAL CELLS STIMULATED BY LYMPHOCYTE-CONDITIONED MEDIUM

WUNDERLICH K., ERB C., KNORR M., Völker M. AND THIEL H.-J.
University Eye Clinic Tuebingen (FRG)

Purpose. We have previously demonstrated that lymphokines, which are secreted by activated lymphocytes, stimulate lens epithelial cell (LEC) proliferation in vitro. Treatments proposed to date for the prevention of secondary cataract have shown limited efficacy or have not been satisfactory due to ocular toxicity. Since we have shown that heparin enhances cell proliferation of FGF-activated LEC in vitro, we investigated the influence of heparin on the stimulating activity of lymphocyte-conditioned medium (LCM).
Methods. Phythemeagglutinin-stimulated human lymphocytes were cultured for 24h in RPMI-1640-Medium supplemented with 10% fetal calf serum (FCS). Afterwards lymhocytes were centrifuged and the super-natant was used as LCM. Bovine LEC, obtained by encymatic disaggregation, were cultured in LCM in the presence and absence of 50 µg/ml heparin. LEC cultured in RPMI-1640 with 10% FCS served as control. After 4 days LEC were detached by trypsin/EDTA treatment, diluted in 0.9% NaCl-solution and cell number was determinded by using a cell counter. **Results.** LEC cultured in LCM revealed a strong increase in cell proliferation when compared to the control medium. Population doublings per day (PD/d) was 1.1±0.25 (mean value±SD) for the controls and 3.2±0.4 for LEC cultured in LCM. Addition of 50 µg/ml heparin to LCM reduced LEC proliferation to 65.4%±8.8% of viable cells with a PD/d of 2.1±0.3. **Conclusion.** The antiproliferative activity of heparin on LEC cultured in lymphocyte-conditioned medium suggests that heparin is a valuable tool for the modification of lens epithelial cell proliferation in vitro.

P 221

PROLIFERATIVE EFFECT OF HYALURONIC ACID ON CULTURED BOVINE LENS EPITHELIAL CELLS

KNORR M., ERB C., WUNDERLICH K., KRUSE FE. and THIEL H.-J.
University Eye Clinic, Tübingen (Germany)

Purpose: Hyaluronic acid (HA) is widely used in intraocular ophthalmic surgery because of its unique viscoelastic properties. Since HA is known to be a component of the extracellular matrix and has been shown to play an important role as an extracellular signal for various cell types (e.g., corneal epithelial cells), we evaluated the proliferative effect of HA on cultured bovine lens epithelial cells (BLEC) under serum-free (SFC) and serum-containing (SCC) culture conditions (10% FCS).
Methods/Results: Cell numbers were determined after 6 days in culture. Stimulation of BLEC with HA in concentrations ranging from 50 to 1000 µg/ml led to a dose-dependent increase in cell proliferation with an EC_{50} of 101,6 µg/ml under SFC and an EC_{50} of 152,4 µg/ml under SCC. Maximum increase of cell number (obtained with 1000 µg/ml HA) was 44% under SFC and 23,9% under SCC.
Conclusions: Comparison of proliferative capacity of HA under SFC and SCC suggests that HA and serum-derived mitogens act independently. Our data indicate that HA is a potent proliferative stimulus for BLEC and that it may influence ocurrence and extent of posterior capsule opacification after extracapsular cataract surgery.

P 222

Age-related changes in the mechanical properties of the human anterior lens capsule.

Susanne Krag[1], Troels T. Andreassen[2], Thomas Olsen[1].
[1]Department of Ophthalmology, Aarhus University Hospital.
[2]Department of Connective Tissue Biology, Aarhus University.

Purpose
To investigate the influence of age on the biomechanical properties of the anterior lens capsule.

Method
Lens capsules from 69 human donor eyes aged 7 months to 98 years were included in the study. Mechanical testing was carried out on tissue rings prepared from the central part of the anterior lens capsule by means of photoablation technique. The mean diameter of the rings was 3.1 mm and the width was approximately 100 µm. The capsular rings were slipped over two pins and stretched at a constant rate until rupture. Load and deformation were recorded continuously. Strain values were obtained by expressing deformation values in percent of the initial circumference of the capsular rings.

Results
Load and deformation showed a nonlinear relationship. Extensibility (maximum strain) (1), maximum tensile strength (2) and maximum elastic stiffness (load increment per unit strain)(3) of the capsular rings showed a highly significant negative association with age ($p < 0.001$). The slope of regression was -0.50 ± 0.03 %/year (1), -0.38 ± 0.03 mN/year (2) and -0.81 ± 0.05 mN/year (3) respectively. However, the elastic stiffness in the physiological function range (0-10% strain) showed a highly significant positive association with age up to the age 50 (slope of the regression: 0.15 ± 0.01 mN/year). After this age there was no significant correlation with age.

Conclusion
The maximum mechanical strength of the anterior lens capsule is significantly reduced with age. However, in the physiological function range the elastic stiffness of the anterior lens capsule seems to increase with age, which may be a factor in the development of presbyopia.