Comparison of the in vitro permeation of different ophthalmic drugs through a human cornea construct (HCC) and human donor corneas

S. Döhring, S. Reichl, C.C. Müller-Goymann

Institut für Pharmazeutische Technologie, Technische Universität Carolo-Wilhelmina zu Braunschweig, Mendelssohnstrasse 1, D-38106 Braunschweig

Introduction

Transcorneal in vitro permeation studies of ophthalmic drugs were normally performed with either excised animal corneas or latterly organotypic corneal equivalents. The usefulness of bovine, porcine and human cornea constructs in comparison to excised bovine [1] and porcine [2,3] corneas has already been demonstrated. The aim of the present study was to compare permeation data and morphological structure of the human cornea construct with those from excised human cornea.

Experimental methods

Materials

Glucanex® 0.5 % from Alcon-Pharma (D-Freiburg) containing 0.5 % betafenac hydrochloride and Bovocarn® 5.2 % from Dr Wetzler (D-Ochsen) containing 2 % pilocarpine hydrochloride are commercial aqueous eye drop solutions. HC 0.02 % OK is an aqueous solution of 0.02 % hydrocorosine: 0.5 % sodium diclofenac and 0.5 % timolol maleate are also used as aqueous isotonic solution. Human donor corneas were obtained from the MHH and UKE cornea banks (D-Hannover, D-Hamburg). The donor age ranged from 35 to 90 years.

Human cornea construct - HCC

The cornea is a multilayered tissue and consists of three different cell types. Thus the HCC was constructed step-by-step in Transwell® (D-Fernwald) cell culture inserts as described before [3]. Immortalized human corneal endothelial cells (HENC) were seeded onto a polycarbonate filter covered with a layer of collagen and grown to confluence. A collagen gel matrix containing native stromal fibroblasts was then cast atop the confluent endothelial cell layer. Immortalized human corneal epithelial cells (CEPI) were seeded onto the contracted collagen lattice and grown submerged to confluence, then lifted to the air liquid interface for additional two weeks. In this time a multilayered epithelium was performed.

Scanning electron microscopy studies

The surface morphology of the reconstructed cornea and an excised porcine cornea was studied by SEM Stereoscan 250 (GB-Cambridge). Samples were prepared according to Fujita [4].

Permeation studies

Permeation studies were carried out in modified Franz cells at 37 °C. Five different drug formulations as described in Materials were used as donors. The receiver solution contained isotonic phosphate buffered saline pH 7.4. Analysis was performed by HPLC with different methods according to [3,5,6]. The permeation profiles of model drugs were determined by plotting the amounts [µg/cm²] of drug permeated through excised cornea or HCC versus time [min]. The permeation coefficient P was calculated as fluxing concentration from the linear ascent of the permeation curves.

Results and discussion

Figure 1 shows the epithelium and stroma of a human donor cornea, 35 days after excision and storage. A clearly structured epithelium consisting of four to five cell layers, which is the main barrier for ophthalmic drugs, is visible. The micrograph of HCC section shows also a tightly packed multilayered epithelium of seven to nine cell layers grown on stroma equivalent (Fig. 2). The cultivated epithelium resembles closely the corneal epithelium found in a human cornea in vivo but in the case of reconstructed epithelium it is difficult to distinguish the basal cells from the wing cells. However, the topmost cells (superficial cell layer) appears flattened as it is also the case in vivo.

Materials were used as donors. The receiver solution contained isotonic solution. Human donor corneas were obtained from the MHH and UKE cornea banks (D-Hannover, D-Hamburg). The donor age ranged from 35 to 90 years.

Human donor corneas

Althought the epithelium of the HCC is somewhat thicker, the structure is very similar to the human cornea.

SEM experiments show that the endothelial and epithelial surfaces of HCC resemble those from original in terms of junctions and micropvice (Fig. 3). The superficial epithelial cells of HCC (Fig. 3C) form tight sheets and typical structures such as microvilli and micropvice are visible, but not as much as in original porcine cornea (Fig. 3A). The characteristic hexagonal form of endothelial cells and junctional complexes between neighbours appear well developed in HCC (Fig. 3D). Although the epithelium of the HCC is somewhat thicker, the structure is very similar to the human cornea.

In order to investigate the potential of HCC as an in vitro model for corneal permeation studies and for prediction of ocular drug absorption into human eye, the permeation of five different ophthalmic model drugs was evaluated and compared with that via excised human donor cornea. The resulting permeation coefficients P were expressed as mean ± standard deviation and are shown in Table I. The permeability of HCC in comparison to human donor cornea is slightly increased for pilocarpine hydrochloride and betafenac hydrochloride and slightly decreased for hydrocorosine. For timolol maleate and sodium diclofenac equal permeabilities could be found. Such a very small difference between the HCC and original corneas makes this human cornea construct a promising tool for prediction of ocular drug absorption into human eye.

Conclusion

In the present study a three-dimensional human cornea equivalent including all three different cell types could be developed, which resembles the human cornea in morphological structure and permeation data.

Acknowledgment

We would like to thank Nestec (CH-Lausanne) as the source of human corneal epithelial cell line CEP. Dr. Meyer from the MHH cornea bank (D-Hannover) and Dr. Bednarz from the UKE cornea bank (D-Hamburg) for the supply of human corneas and immortalized endothelial cells (HENC).

References


