Reconstituted epithelial corneal tissues for evaluation of drug delivery

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Abstract

One possible approach to reduce the use of animals in the evaluation of the ocular drug delivery is that to use cell cultures as model of tissues. In this study are compared two different reconstituted epithelial corneal tissues, a homemade artificial corneal epithelium (Reconstituted Rabbit Corneal Epithelium) and a commercial human corneal epithelial model (COR-100 EpiCorneal™, MatTek), for permeation experiments of three drugs with different physical chemical properties: timolol maleate, cyclosporin A, and a newly synthesized compound, *MAGL 17.b*. The collected data show that corneal epithelial models are not sufficient to simulate the complexity of the corneal barrier and the presence of a layer simulating the stroma may be necessary to approach the structure of native cornea.

Introduction

In the last few decades, new approaches have been used in research for the treatment of a large number of diseases, including the nanotechnologies, in order to prepare formulations/technologies for drug delivery. This great development of medical technologies has produced a significant increase of the number of animals used in research protocols, leading to an increasing demand to replace the *in vivo* methods with *in vitro* models. One possible approach to reduce the use of animals in the evaluation of drug delivery is that to use cell cultures as model of tissues. This approach is applied also in ocular field to study the biopharmaceutical behaviour of a lot of new drug delivery systems that have been developed to allow the drug to reach the different districts of the eye. Aim of this study was to compare different reconstituted epithelial corneal tissues for evaluation of ocular drug delivery: i) a homemade artificial corneal epithelium (Reconstituted Rabbit Corneal Epithelium, RRCE)[1] and ii) a commercial human corneal epithelial model (COR-100 EpiCorneal™, MatTek).[2]

Materials and Methods

The rabbit corneal epithelial (RCE) cell line was obtained from the European Cell Culture Collection (N° 95081046, ECACC). To prepare RRCE, cells with passage numbers 12-16 were seeded onto specific polyester membranes (surface area 1.0 cm^2 and pore size 0.4 μm; Snapwell®, Costar) at a concentration of 8 x 10^4 cells/cm^2 and were grown at 37 °C in a humidified atmosphere containing 5% CO_2 with growth medium both in the apical and basolateral compartment, for 8 days until the cells had become confluent and were stratified. By removing the medium from the apical compartment, the cells were kept exposed to air for further 7 days changing daily the medium in the basolateral compartment up to obtain the reconstituted epithelium.

Permeation experiments through RRCE, COR-100, and rabbit cornea

For drug permeation experiments, the Snapwell® devices containing RRCE and the freshly excised rabbits' corneas were fitted into appropriated perfusion chambers,[3,4] consisting of two compartments (donor and receiving) separated by the tissue while COR-100 was tested into its native support (Transwell® plate).

The drugs employed in the study were timolol maleate (TM), cyclosporin A (CyA), and a newly synthesized compound, *MAGL 17.b*. The physical chemical properties of the different molecules are listed in Table 1. The donor phases of the permeation experiments were aqueous solutions containing drug plus i) permeation enhancers (surfactants) and ii) nanostructured systems as nanomicelles.

Trans-epithelial electrical resistance

The trans-epithelial electrical resistance (TEER) was measured during RCE cell growth using an EVOM epithelial voltohmmeter to evaluate the integrity of the cell layers and their tightness. TEER was also always measured before and at end of each permeation experiment to verify the integrity of the membranes.

Results

The results of permeation experiments were reported as apparent permeability coefficient (P_{app}) defined by the equation:

$$P_{app} = (dQ/dt)/(AC_0)$$

where A and C_0 are the exposed membrane surface area and the initial drug concentration, respectively. P_{app} was calculated from slopes of linear plots of amount of drug in the receiving chamber (Q) *vs* time (t) at the steady-state.

The close similarity of P_{app} values obtained with RRCE and isolated corneas in permeation experiments with TM (the most hydrophilic among the drugs tested) points to the analogy of barriers to drug permeation present in the two substrates. When the lipophilic properties of the molecule increase the stroma becomes the barrier limiting the permeation through the cornea and the epithelial models become inadequate to simulate the ocular barrier and a

Table 1. Physical chemical properties of the different molecules.

Drug	MW (Da)	LogP o/w	Vehicle
TM	432	1.4	surfactants
CyA	1202	2.8-3.6	nanomicelles
MAGL 17.b	343	3.4	surfactants

corrective value should be introduced. From the toxicity point of view, in every case a decrease of the TEER values measured before and after trans-epithelial permeation experiments was evident, regardless of the type of product used, indicating some degree of suffering of the reconstituted epithelial corneal tissues caused by the test conditions.

Conclusions

The collected data show that corneal epithelial models are not sufficient to simulate the complexity of the corneal barrier and the presence of a layer simulating the stroma may be necessary to approach the structure of native cornea.

Moreover, the epithelial models were more sensitive than native cornea to the permeation experiment conditions.

References

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