Ocular Cell Culture Models

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Drug Discovery and Development Technology Center, DDTC

• located in the Faculty of Pharmacy, University of Helsinki
• multidisciplinary research center
• develops novel tools drug discovery and development processes
• 7 research groups
**OCULAR KINETIC BARRIERS**

Anterior barriers & delivery

Posterior barriers & delivery

**Clinical reality:**
* anterior delivery for anterior targets
* intraocular injections & systemic delivery for posterior targets
1) **TOPICAL ADMINISTRATION: CORNEAL ABSORPTION, eydops**

ONLY ANTERIOR TARGETS ARE REACHED

corneal epithelium is the rate-limiting barrier
Human corneal epithelial cell model (HCE) as alternative to rabbit experiments?

**Physical Barrier:**
- morphology
- transepithelial electric resistance
- paracellular permeability
- transcellular permeability

**Biochemical Features**
- enzymes
- active transporters
- efflux pumps
- inflammatory mediators

**AIM:** mimick corneal epithelium barrier
**Morphology of the HCE Model**

**Cell culture method:**
- 1 week normal culture
- 2-3 culture at air-medium interface

Tight junctions are formed
Cells differentiate
GROWTH CONDITIONS AFFECT HCE BARRIER

Assessment of TER

TER (Ω x cm²)

- polyester
- polyester/collagen
- polyester/collagen/fibroblasts
- polycarbonate
- polycarbonate/collagen
- polycarbonate/collagen/fibroblasts
- polyester/collagen/laminin

Mannitol permeability (10⁻⁶ cm/sec)

- no air-lift
PARACELLULAR PERMEABILITY

- paracellular pore size and porosity analysis from PEG-data (20 PEG oligomers)
  - pore size
    - HCE 8.7 Å
    - rabbit cornea 7.3 Å
  - pore number (10⁶ / cm²)
    - HCE 11.9
    - rabbit cornea 12.5
EFFECT OF LIPOPHILICITY ON PERMEABILITY (HCE MODEL VS RABBIT CORNEA)

permeability vs log D

HCE MODEL
RABBIT CORNEA
CORRELATION OF PASSIVE PERMEABILITY IN CORNEA AND HCE MODEL

\[ R^2 = 0.9687 \]
Active Transporters
- generally poorly known in cornea
- expression of efflux proteins at mRNA level in HCE model differs from human cornea
- HCE model shows higher expression than the normal cornea
Prediction of Ocular Absorption with HCE Model and Kinetic Models

Simulation model:
1) cell data
2) kinetic parameters
In vivo prediction:

Simulation model (STELLA program was used)

From

Cell culture experiment:

\[ C_{T,CO} = k_D \times V_{Donor} \]

Literature
In vivo prediction:
Timolol data
(25 μl of 0.5% eye drop)

Simulation
\[ C_{T,CO} = 2.0 \, \mu l/min \]
\[ C_{T,CO} = 1.3 \, \mu l/min \]

Literature
Lee et al. 1991
Järvinen et al. 1992
**HCE Model: Current Status, Pros**

+ anatomical barrier OK
+ passive diffusion similar with rabbit cornea
+ predicts timolol absorption in vivo
+ immortalized cells
+ human cell model
+ applicable to corneal gene delivery experiments (good in vitro - in vivo correlation)
+ may replace some rabbit experiments
HCE MODEL:
CURRENT STATUS, CONS &
UNKNOWN
— good barrier maintained only for 3-5 days
— active transporters and efflux pumps deviate and sparsely characterized
— formulation effects unknown
— absorption prediction of new drugs?
IMPORTANCE OF POSTERIOR SEGMENT DRUG DELIVERY

AGE RELATED MACULAR DEGENERATION

animal models / 3 R ?
2) **BLOOD-RETINA BARRIER**

**RETINAL PIGMENT EPITHELIUM (RPE)**

RPE PERMEABILITY? RPE CELL CULTURE MODEL?
**RPE Permeability Experiments**

Blood retina barrier

- In vitro in diffusion cells
- Bovine RPE-choroid
- Integrity controlled
  1) TEER
  2) Potential difference
Hydrophilic and large molecules

RPE is the major barrier compared to sclera
Drug transport in RPE

Cell culture model

ARPE-19 human cell line

Differentiates on filter

Active transport in RPE is poorly known

CHOROID

RPE

RETINA

Efflux-pump

Drug transporters
ARPE-19 cell culture model

**membrane** $R(\Omega \cdot \text{cm}^2)$

<table>
<thead>
<tr>
<th>ARPE-19 cells</th>
<th>80-110</th>
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<tbody>
<tr>
<td>Bovine RPE-choroid</td>
<td>90-153 (Pitkänen et al, 2005)</td>
</tr>
<tr>
<td>Human RPE-choroid</td>
<td>79 (Quinn and Miller, 1992)</td>
</tr>
<tr>
<td>ARPE-19 with BRE or bFGF</td>
<td>140-150</td>
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</tbody>
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differentiation markers (Q-PCR) RPE65, bestrophin, CLARB)

- microvilli
- tight junctions
Permeation of Carboxyfluorescein Across ARPE-19 Cell Model
Expression of efflux proteins in ARPE-19 model (Q-PCR)
Conclusions

Cell culture models are useful in ocular drug delivery studies.

Corneal models
1) HCE is realistic model for passive diffusion in the cornea.
2) Active transport in cornea and HCE model not characterised., but seem to differ.
3) HCE model data can be used in combination with computational models.

RPE models
1) ARPE-19 cells form physical barrier similar to bovine RPE.
2) Active transport in RPE and ARPE model are not yet characterised.

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