

C6 ECV304 in vitro

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Characterization of immortalized ECV304 cells cocultured with astrocytes as a cell-based blood brain barrier model for in vitro cellular biopharmaceutics studies

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Abstract: Drug targeting to the brain is one of the most challenging areas of biopharmaceutical investigations due to the presence of the blood-brain barrier (BBB). Up until now, no cell culture models were shown to represent a well-characterized in vitro BBB model to be utilized for drug delivery and targeting to the brain. Cell lines derived from brain endothelium lack the ability to generate a restrictive barrier function despite possessing some characteristics of BBB such as carrier-mediated transporters. Among cell lines used as in vitro cell-based BBB models, we aimed to evaluate the most studied cell line (ECV304 cell line) for its bioelectrical properties and permeability characteristics upon treatments with astrocytic factors (using rat glioma C6 cells) in the absence or presence of various modulators including extra-cellular matrix and some other tight junction modulators (i.e, cAMP elevators, retinoic acid, dexamethasone, and γ -linolenic acid). Significant ($p < 0.05$) increase in trans-endothelial electrical resistance (TEER) values was only observed for ECV304 cells cocultured with astrocytes and treated with cAMP elevators ($110 \Omega \cdot \text{cm}^2$), however such TEER is not comparable to that of in vivo ($1500-2000 \Omega \cdot \text{cm}^2$). ECV304 cells cocultured with astrocytes and treated with cAMP elevators displayed the highest discrimination for permeability coefficients of trans-cellular marker propranolol ($25 \times 10^{-6} \text{ cm/sec}$) and para-cellular marker sucrose ($11.3 \times 10^{-6} \text{ cm/sec}$). The permeability coefficient ratio of propranolol over sucrose was 2 which is much lower than that of primarily isolated porcine brain microvascular endothelial cells. ECV304 cells failed to generate restrictive para-cellular barrier, thus it is not suggested to be used as an in vitro BBB model for drug screening although its usefulness has been shown for carrier-mediated transport studies.

Keywords: Blood-brain barrier (BBB), Cell Models, Cellular Biopharmaceutics, Cellular Uptake, ECV304.

() ECV304
C6 ECV304
(Tight (cAMP ECV304 junction)
C6 ECV304 cAMP
×) cAMP C6 ECV304
(/ ×)
ECV304
ECV304

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b.End3

(blood-brain barrier)

b.End3

(Polyoma middle T oncogene) .()

b.End3

(brain drug targeting)

.()

P-glycoprotein (P-gp)

in vitro

ECV304

ECV304
(umbilical cord)

(immortalized)

(T24 cells)

.()

in vivo

.()

.()

)

(

ECV304

OptiPhase HiSafe3™ (C6)
 () Fisher Scientific Chemicals C6
 C6 ECV304 : (Cell lines) (Rat glioma C6 cells)
 (ECACC)

: ECV304
 () M199
 () ()
 (%)
 : C6
 () DMEM (Dulbecco's :
 () (25 mM Modified Eagle's Medium) DMEM
 (%) FBS Trypsin-EDTA M199 HEPES

ECV304 (Heat-inactivated bovine serum)
 C6 (Foetal Bovine Serum) FBS
 ECV304 (Invitrogen)
 (A) Rat-tail collagen type I ()
 ECV304 (25 and 75 () (Roche)
) (Transwell-Clear™ T-flask)
 (C6 filter inserts, pore size 0.4µm, surface area
 (B) ACM Cellagen disk™ 0.3318 cm²
) (Corning Costar)
 Astrocyte -Conditioned Media) :(Modulatores)

(Cyclic AMP analogue: 8- CPT-cAMP
 (4-chlorophenylthio)-adenosine 3', 5'-cyclic
 ECV304 - monophosphate)
 (Astrocyte Co-Culture) C6 Ro20-1724 ()
 ECV304 (Calbiochem) Forskolin
 C6 (Apical side) ()
 (Basal side) [³H] propranolol)
 (C) ACC ([¹⁴C] sucrose) (hydrochloride
 ECV304 () Amersham Life Science

C6

(confluently) (D) ACC-D

C6

() (/) ECV304

()

CPT-cAMP cAMP)

() Ro20-1724 () ACM (

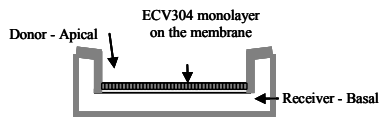
() Forskolin

CPT-cAMP Ro20-1724 CPT-cAMP ECV304

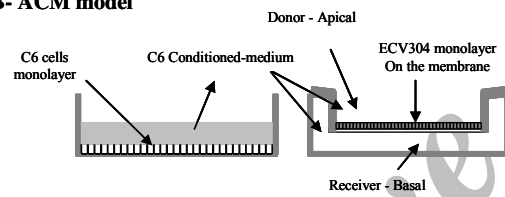
Forskolin %

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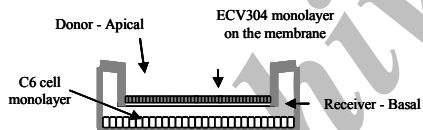
A- ECV304 alone



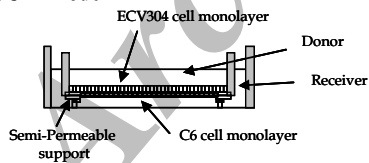
B- ACM model



C- ACC model



D- ACC-D model



(A) ECV304 . ECV304

(B) (Astrocyte Conditioned-Media,(ACM)
(Astrocyte Co-Culture, (ACC))

C6 (D) (C)

ACM

C6

(ACC, ACC-D)

(extra-cellular matrix)

(rat-tail collagen coated)

(uncoated)

% /

$$\frac{dM}{dt} = P.A.C_d :$$

P
A ()
C_d ()
()
() t

ECV304
World Endohm™ electrode
() Precision Instrument
ECV304
([C])
([H])

MiniTab13

/ p

(apical side)

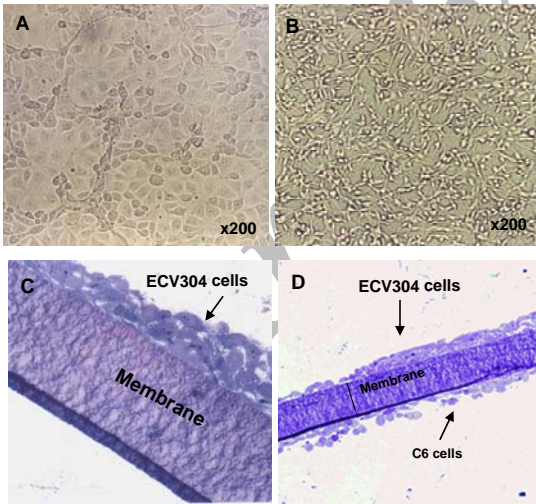
[C]

ECV304

[H]

B A

C6



(basal)

OptiPhase-HiSafe3™

liquid scintillation

Perkin-Elmer Life

counting

[C]

() Sciences

([H])

ECV304

C6 ECV304

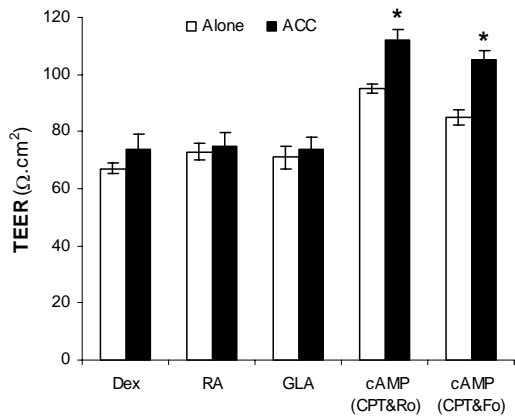
B A

ECV304

D C

()

C6

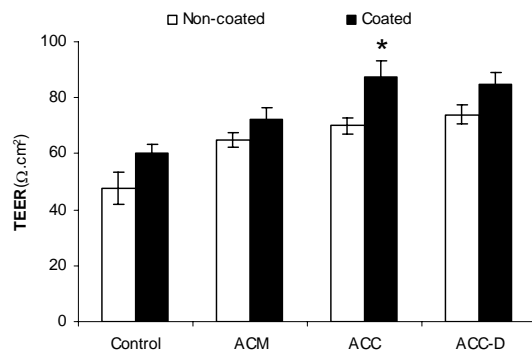


C6

ECV304

(ACC)

(Dex) (/) (RA)
) (GLA)
) CPT-cAMP : cAMP
) Forskolin () Ro20-1724 ()
 (p<0.05)
 (ACC) (ECV304)
 t-test
) (/)
)
 CPT- cAMP ()
) Ro20-1724 () cAMP
 () Forskolin ()



ACM .ECV304

ECV304

ACC-D ACC

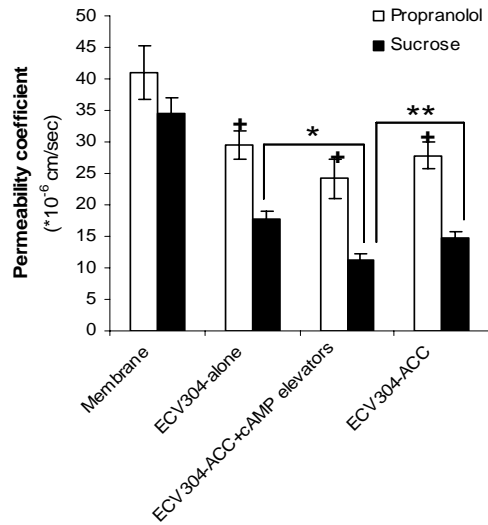
ECV304

(p<0.05)

t-test

ECV304
 ([C])
 ([H])

ACC
 cAMP
 Ro20-1724 CPT-cAMP



ACC
 cAMP
 ECV304
 ([C])
 ([H])

(ACC) (ECV304)

(p<0.05)

T-test

(Immortalized)

Species	Identification	Immortalization technique	Ref.
Human	BB19	E6E7 genes of human papilloma virus	(17)
	SV- HCEC	pSV3-neo, coding for the SV40 large T antigen gene	(18)
	HBEC- 51		(19)
Bovine	SV-BEC	pSV3 neo., expressed nuclear SV 40 large T antigen gene	(20)
	t-BBEC-117	Transfection with the SV40 large T antigen	(21)
	BBMEC		
Rat	CR3	SV40 large T gene under the control of the human vimentin promoter	(22)
	GP8.39	Temperature sensitive SV40 large T immortalized	(23)
	RBE4	Transfection with a plasmid containing the E1A adenovirus gene	(24)
	GPNT		(25)
	RBEC1		(26)
Mouse	TR-BBB	Temperature-sensitive simian virus 40 large T-antigen	(27; 28)
	TM-BBB1-5	Temperature-sensitive simian virus 40 large T-antigen gene	(29)
	b.End3, b.End5	Polyoma middle T oncogene	(30)
Porcine	TM-BBB	Temperature-sensitive simian virus 40 large T-antigen	(27)
	PBMEC/C1-2	pRNS-1, encoding for the small and large T-antigens of SV 40, lipofection	(31)

(;) ECV304
()
() ECV304
(;) cAMP
ECV304 ()
(T24)
ECV304
(Cellular intercommunication)
()
() Ramsohoye
ECV304
()
(p>0.05) ()
() ECV304
(p<0.05) cAMP
ACC ECV304 (ECV304-
() () Scism .9)
() Scism
ECV304
ECV304

()

.(;) Galla .(;)
b.End3

(ZO) cAMP in vivo .()
(Occludin) .() ECV304
[C]
(p<0.05)
: [C]
ACC () / ×
) / × cAMP
() × ACC ()
. ECV304
([H])
(p>0.05)

ECV304

)
()
()

cAMP
 ECV304 ECV304
 ECV304 Astrocyte
 cAMP
 ()
)
 ()
 ()
 ()
 Astrocyte

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