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A NEW BBB MODEL: TESTING SPECIFIC FUNCTIONS

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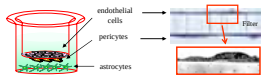
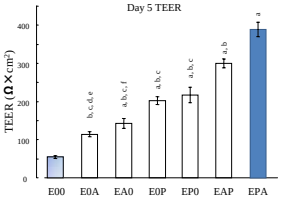
Introduction

1

A new innovative *in vitro* BBB model has been developed using primary cultures of three main cell types of the BBB, rat brain capillary endothelial cells (RBEC), pericytes and astrocytes. In order to develop an easily transportable ready-to-use model, the BBB kit™ (EPA), different conditions for freezing the system as a whole have been tested.

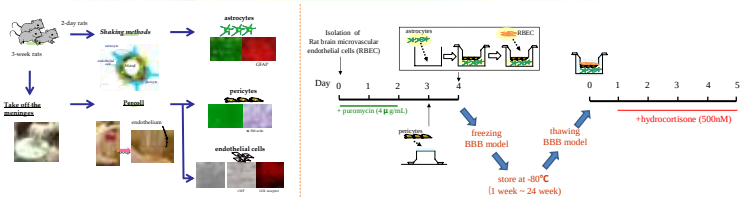




$^{a}p < 0.01$ vs. E00 $^{b}p < 0.01$ vs. EPA $^{c}p < 0.01$ vs. EAP
$^{d}p < 0.01$ vs. EP0 $^{e}p < 0.01$ vs. E0P $^{f}p < 0.05$ vs. EP0

cell culture

2



Rat cerebral endothelial cells

The microvessels were isolated from 3-week old Wistar rats according to the method of Deli MA et al. Primary culture of cerebral endothelial cells and constructed BBB model were maintained in DMEM/F12 supplemented with 10% plasma-derived bovine serum (PDS) , 1.5 ng/mL basic fibroblast growth factor (bFGF), 100 μg/mL heparin and insulin (5 μg/ml), transferrin (5 μg/ml), sodium selenite (5 ng/ml) (insulin-transferrin-sodium selenite media supplement), without cAMP and its analogs.

Rat cerebral pericytes

Pure cultures of rat cerebral pericytes were obtained by a prolonged, 2-week culture of isolated brain microvessel fragments, that contain pericytes beside endothelial cells.

Rat cerebral astrocytes

Rat cerebral astrocytes were obtained from neonatal Wistar rats. Meninges were removed and cortical pieces mechanically dissociated in astrocyte culture medium (DMEM supplemented with 10 % fetal bovine serum).

Barrier function & drug permeability assay

3

Transendothelial electrical resistance (TEER)

TEER was measured by EVOM resistance meter (World Precision Instruments). TEER depends on the voltage between electrodes across RBEC monolayer, which reflects an amount of ionic molecule flux through RBEC monolayer.

Transcellular transport and paracellular transport

Permeability of drugs across RBEC monolayer was determined as previously described (Kis et al., 2001).

$$\frac{1}{PS_e} = \frac{1}{PS_{total}} - \frac{1}{PS_{mem}}$$

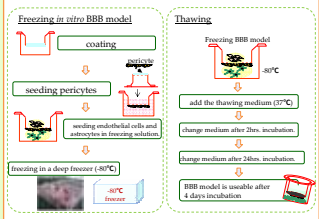
$$P_e\,(cm/min) = \frac{PS_e}{A}$$

•Kis B, Deli MA, Kobayashi H, Ábrahám CS, Yanagita T, Kaiya H, Isse T, Nishi R, Gotoh S, Kangawa K, Wada A, Greenwood J, Niwa M, Yamashita H, Ueta, Y (2001) Adrenomedullin regulates blood–brain barrier functions in vitro. Neuroreport 12:4139-4142

Freezing & Thawing

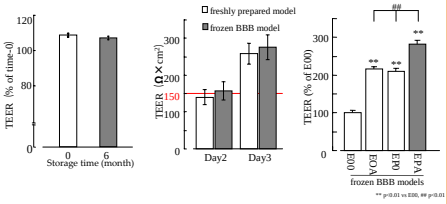
4

The BBB kit was frozen in a programmable freezer with a cooling rate of 1 ℃/min until it reached -50 ℃. The kits were stored at -80 ℃ up to 6 months. After melting (day 0), triple co-cultures were incubated in culture medium (10% PDS in DMEM/F-12) at 37 ℃.



Result 1-1 ~Freezing & Thawing~

5



** p<0.01 vs E00, ## p<0.01

- The frozen BBB kit can be stored at -80℃ for up to 6 months.
- The BBB kit had the high level of TEER, with values being no different from those of freshly prepared model.

Result 1-2 ~Freezing & Thawing~

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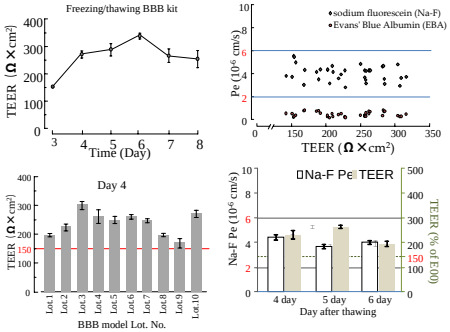

- TEER of RBEC monolayer, indicating the tightness of interendothelial tight junctions (TJs), was gradually increased to the level of 250 Ω×cm² at day 4, and reached a plateau until 8 day.
- We found no changes in endothelial permeability coefficients (Pe) for sodium fluorescein (Na-F) among kits with TEERs of 150 to 320 Ω×cm². There were no differences in Pe of RBEC monolayer in triple co-culture tested on days 4, 5 and 6.

Result 2-1~ Drug permeability assays ~

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name	MW	CNS	transport	Recovery rate (%)
risperidone	410	+	efflux	69.8
fluvoxamine	434	+	lipophil and high protein binding	63.6
trazodone	408	+	passive lipophilic	63.1
fluoxetine	346	+	lipophil and high protein binding	62.3
hydroxyzine	448	+	passive lipophilic	53
haloperidol	376	+	passive lipophilic	52.1
vincristine	923	-	efflux	64.6
digoxin	781	-	efflux	62.7
prazosin	420	+	efflux: ABCG2	57.7
vinblastine	909	-	efflux	53.5
verapamil	491	-	efflux	51.2
nortriptyrine	300	+	Influx: NET	44.4
paroxetine	375	+	lipophil and high protein binding, Pgp inhibitor but not Pgp substrate, lipid soluble	39.7
buspirone	422	+	passive hydrophilic	39.4
chlorpromazine	355	+	efflux, Pgp substrate/inhibitor	32.3
sertraline	343	+	lipophil and high protein binding, Pgp inhibitor	18.7
paclitaxel	854	-	efflux	38.4
loperamide	514	-	efflux	38.3
loratadine	383	-	efflux	16
amiodarone	682	-	efflux	4
cyclosporin	1,203	-	efflux	1.6

Drug permeability assays were done using the BBB kit.
A set of 40 compounds and drugs with known BBB permeability has been tested.

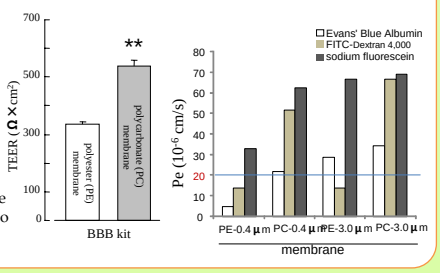
$$\text{Recovery rate (\%)} = \frac{[\text{lu minal}^{t=60}] + [\text{abluminal}^{t=60}]}{[\text{luminal}^{t=0}]} \times 100$$

The recovery rates of some compounds were very low, probably because of there adsorption to the insert membranes, Transwell®, and/or pipet tips, or water solubility of the compounds.

Transwell® Permeable Supports

#	membrane	Pore size	Pore density
3460	Polyester (PE)	0.4mm	4×10⁸ pores/cm²
3401	Polycarbonate (PC)	0.4mm	1×10⁸ pores/cm²
3462	Polyester (PE)	3.0mm	2×10⁸ pores/cm²
3402	Polycarbonate (PC)	3.0mm	2×10⁸ pores/cm²

- The BBB kit with 3.0 μm pore polycarbonate insert membrane, a candidate kit for macromolecular compounds, had also a significant high level of TEER.

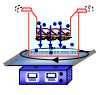


Result 2-2~ Drug permeability assays ~

8

name	MW	CNS	transport	Recovery rate (%)
sulpiride	341	±	efflux: Pgp, influx: OCTN1, OCTN2, PEPT1	91.7
phenytoin	252	+	lipophil and high protein binding	99.6
antipyrin	188	+	passive lipophilic	90
carisoprodol	260	+	passive lipophilic	89.6
indomethacin	358	+	passive hydrophilic	87.6
caffeine	212	+	passive lipophilic	87.5
carbamazepine	236	+	passive lipophilic	85.1
midazolam	326	+	passive lipophilic, highly permeable, Pgp substrate	84.2
propranolole	296	+	passive lipophilic	81.9
zolpidem	382	+	passive lipophilic	71.7
atenolol	226	-	passive hydrophilic, weak base	97.1
cimetidine	252	-	efflux	95.4
acetylsalicilic acid	180	-	organic anion, efflux (Oat1, Oat2)	94.2
epinastine	286	-	efflux	94
prednisone	358	-	efflux (Pgp)	93.2
fexofenadine	538	-	efflux: Pgp, OATP1A2	93.1
hydrocortisone	362	-	efflux (Pgp)	88.2
warfarin	346	-	lipophil and high protein binding	79.8
quinidine	783	-	efflux	72.8

We examined the reliability of *in vitro* permeability data of drugs obtained with the BBB kit.



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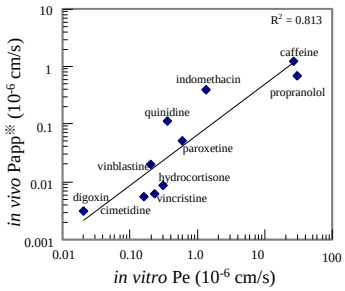

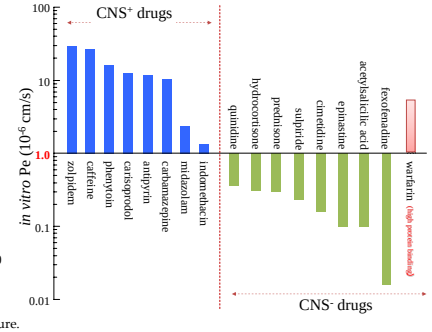

※The value of *in vivo* Papp was obtained from the literature.

- We obtained very good correlation between the BBB kit and *in vivo* permeabilities of drugs.

Conclusion

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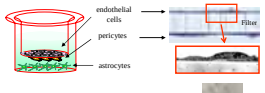
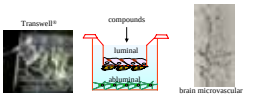
Our ready-to-use *in vitro* BBB model, the BBB kit™ is the best for investigating BBB permeability of candidate compounds of centrally acting drugs and for researches on the BBB physiology and pathology.

As the BBB kit can be frozen as a whole and stored at -80℃ for up to 6 months.





PharmaCo-Cell Company Ltd.
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URL:http://www.pharmacocell.co.jp

Nakagawa S, Deli MA, Nakao S, Honda M, Hayashi K, Nakaoke R, Kataoka Y, Niwa M: Pericytes from Brain Microvessels Strengthen the Barrier Integrity in Primary Cultures of Rat Brain Endothelial Cells. Cellular and Molecular Neurobiology, Vol. 27 No. 6 (in press) 2007
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