



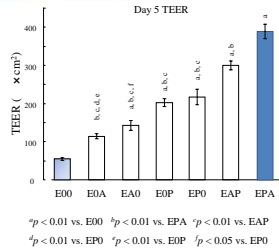
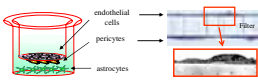
A NEW BBB MODEL: TESTING SPECIFIC FUNCTIONS

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Introduction

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.



Barrier function & drug permeability assay

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).

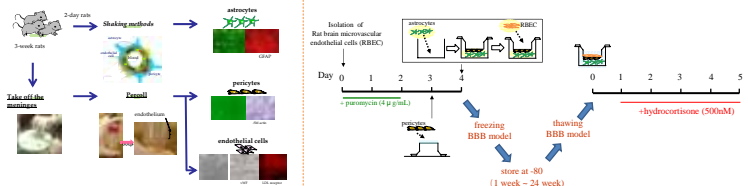


$$\frac{I}{PS_c} = \frac{I}{PS_{total}} - \frac{I}{PS_{mem}}$$

$$P_c \text{ (cm/min)} = \frac{PS_c}{A}$$

*Kis B, Deli MA, Kobayashi H, Abrakos CS, Yangita T, Katay H, Ise T, Nishi R, Gotob S, Kawaguchi K, Wada A, Greenwood J, Niwa M, Yamashita H, Ueta, Y (2001) Adrenomedullin regulates blood-brain barrier functions in vitro. Neuroreport 12:4139-4142

cell culture



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

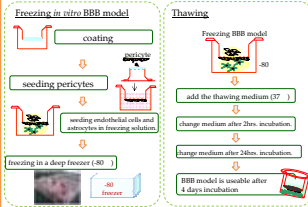
Pure cultures of rat cerebral pericytes were obtained by a prolonged, 2-week culture of isolated brain microvessel fragments, that contain pericytes beside endothelial cells.

Rat cerebral astrocytes

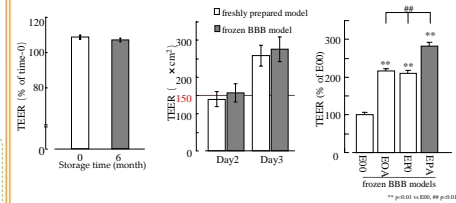
Rat cerebral astrocytes were obtained from neonatal Wistar rats. Meninges were removed and cortical pieces mechanically dissociated in astrocyte culture medium (DMEM supplemented with 10% fetal bovine serum).

Freezing & Thawing

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

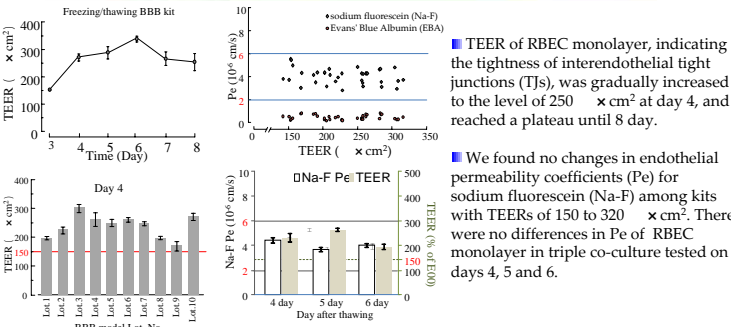


Result 1-1 -Freezing & Thawing-



- The frozen BBB kit can be stored at -80 °C 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.

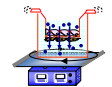
Result 1-2 ~Freezing & Thawing~



Result 2-2~ Drug permeability assays ~

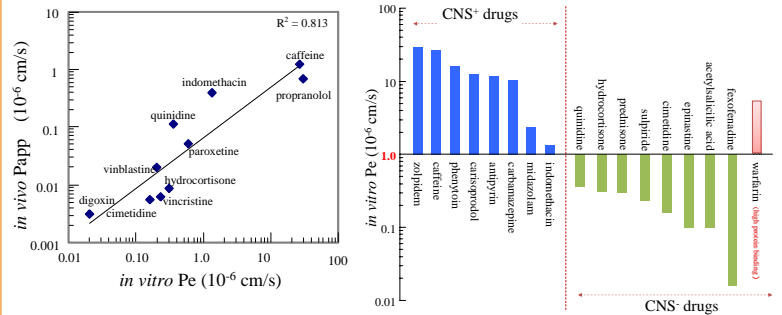
name	MW	CNS	transport	Recovery rate (%)
suripide	341	+	efflux; Pgp, influx; OCTN1, OCTN2, PEPT1	91.7
phenytoin	252	+	lipophil and high protein binding	99.6
antipyrim	188	-	passive lipophilic	90
carbamazepine	236	+	passive lipophilic	89.6
indomethacin	358	-	passive hydrophilic	87.6
caffeine	212	-	passive lipophilic	87.5
carbamazepine	236	-	passive lipophilic	85.1
midazolam	328	-	passive lipophilic, highly permeable; Pgp substrate	84.2
propofol	296	-	passive lipophilic	83.9
zolidem	382	-	passive lipophilic	71.7
atenolol	228	-	passive hydrophilic, weak base	97.1
cimetidine	252	-	efflux	95.4
acetylsalicylic acid	180	-	organic anion; efflux (OAT1, OAT2)	94.2
epinephrine	286	-	efflux	94
prednisone	358	-	efflux (P-gp)	93.2
levodopa	318	-	efflux; P-gp (OATPLA2)	93.1
hydrocortisone	362	-	efflux (P-gp)	88.2
warfarin	346	-	lipophil and high protein binding	79.8
quinidine	324	-	efflux	72.8

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



$$\frac{I}{PS_c} = \frac{I}{PS_{total}} - \frac{I}{PS_{mem}}$$

$$P_c \text{ (cm/min)} = \frac{PS_c}{A}$$



- 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.

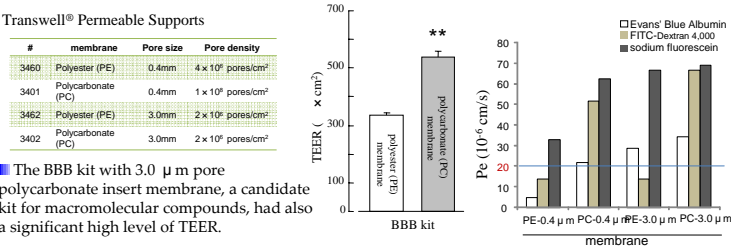
Result 2-1~ Drug permeability assays ~

name	MW	CNS	transport	Recovery rate (%)
risperidone	410	+	efflux	69.8
fluvoxamine	254	+	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	784	-	efflux	62.7
prazosin	420	-	efflux; ABCG2	57.7
vinblastine	909	-	efflux	53.5
verapamil	491	-	efflux	51.2
nortriptyline	300	-	Influx; NET	44.4
paroxetine	375	+	lipophil and high protein binding, Pgp inhibitor but not Pgp substrate, lipid soluble	39.7
bupropion	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
ipraxamide	514	-	efflux	38.3
lorazepam	383	-	efflux	16
amiodarone	682	-	efflux	4
cyclosporin	1,209	-	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.

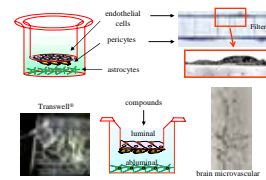
$$\text{Recovery rate (\%)} = \frac{[Du]_{\text{mem}}^{(0)} + [albumin]^{(0)}}{[albumin]^{(0)}} \times 100$$

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



Conclusion

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 °C for up to 6 months.



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Nakagawa S, Deli MA, Nakao S, Honda M, Hayashi K, Nakazoe R, Katataka 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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