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BBB kit
(Blood-Brain Barrier in vitro Reconstruction Model)

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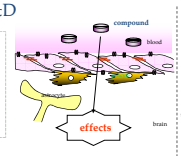
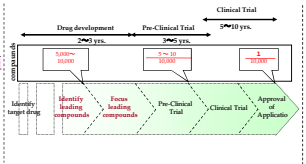
Introduction

Over 98% of drug candidates for CNS diseases do not pass through the BBB

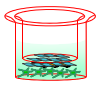
CNS drug R&D

CNS diseases
Alzheimer's disease
CJD, BSE
HIV encephalopathy
Cerebral stroke
Ataxia, Epilepsy
Mania and Depression
Insomnia
Diabetic neuropathy





BBB kit

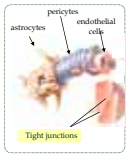


Ready – to – Use BBB kit a new tool to test BBB permeability

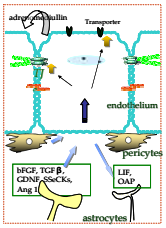
- No easy estimation of BBB targeting from physico-chemical characteristics of compounds
- Need for prediction of BBB permeability of drug candidates in CNS drug R&D

Blood-brain barrier;BBB

BBB cells
- endothelial cells
- pericytes
- astrocytes

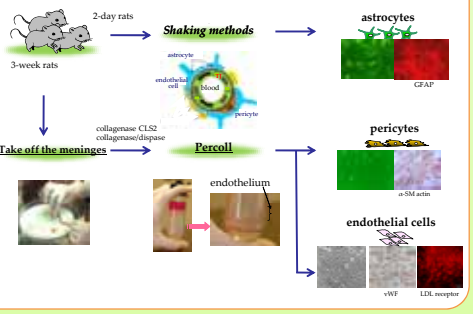


Cross-talk between BBB cells



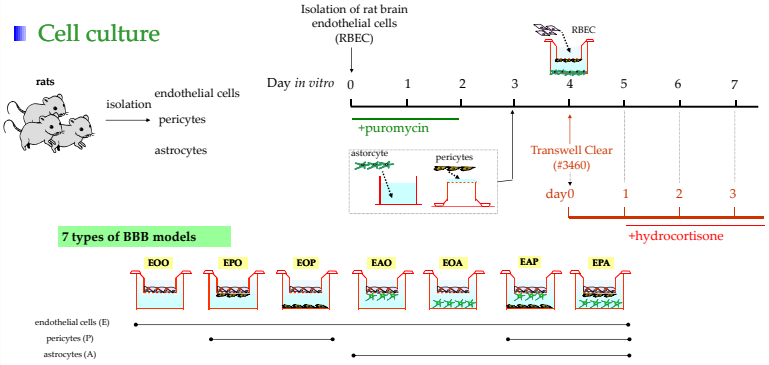
Purpose

The present study was designed to establish the in vitro BBB model, by co-culturing with BBB cells, rat brain microvascular endothelial cells (RBEC), rat pericytes and rat astrocytes. BBB cells were cultured on Transwell[11]®.



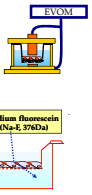
Methods

Cell culture



7 types of BBB models

Barrier functions



Transendothelial electrical resistance (TEER)

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

Transcellular transport and paracellular transport

Permeability of sodium fluorescein (Na-F: 376Da), which has small molecular weight, is regarded as paracellular transports.

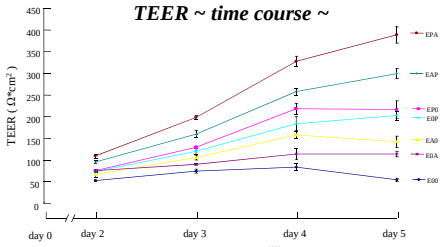
Proteins expression

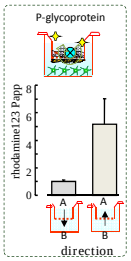
Tight junctions (TJs) -related proteins and transporter proteins were analyzed with immunohistochemical and Western-blotting techniques.

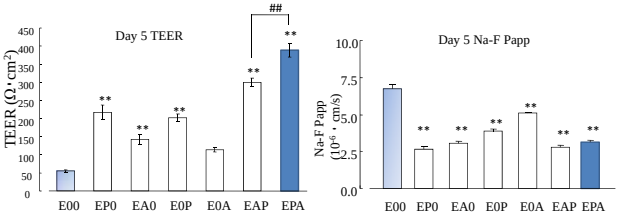
Results ~ functional analysis~

Barrier functions

TEER ~ time course ~



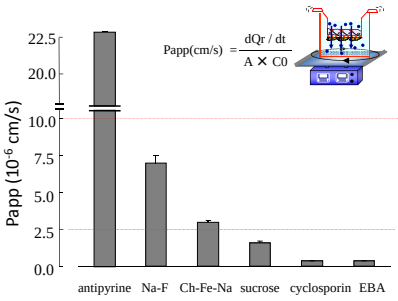




A significant increase in TEER was observed in the model of EPA which was a BBB kit composed of RBEC and pericytes co-cultured in-contact and astrocytes out-of-contact, with a reduction of Na-F permeability. $^{**}p < 0.01$ vs. E00

Compounds permeability

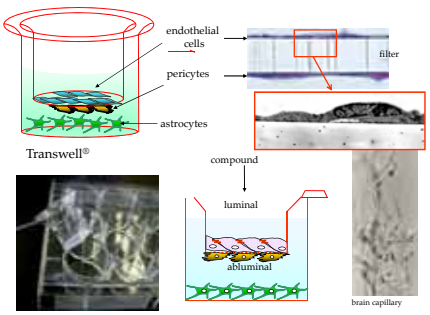
compund	MW	Transport	*in vivo*
antipyrine	188	passive lipophilic	+++
sodium fluoresceine (Na-F)	376	para-cellular	+
chlorophyllin (Ch-Fe-Na)	690	para-cellular	±
sucrose	342	para-cellular	-
cyclosporin	1,203	efflux (Pgp)	-
Evans' blue albumin (EBA)	67,000	trans-cellular	-



Good correlation was found between in vitro permeability values obtained from measurements on the BBB kit and in vivo BBB permeability data.

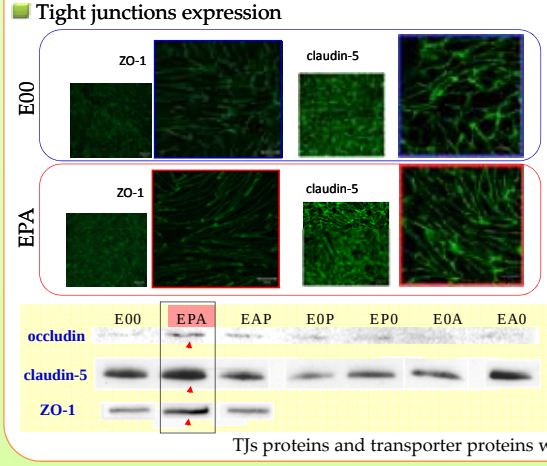
Conclusion

When co-cultured on Transwell®, RBEC, astrocytes and pericytes acquire the ability to work as the in vivo BBB functions, in harmony.
Thus, our BBB kit is suitable for research on BBB physiology or pathology and to test candidate compounds for centrally acting drugs.

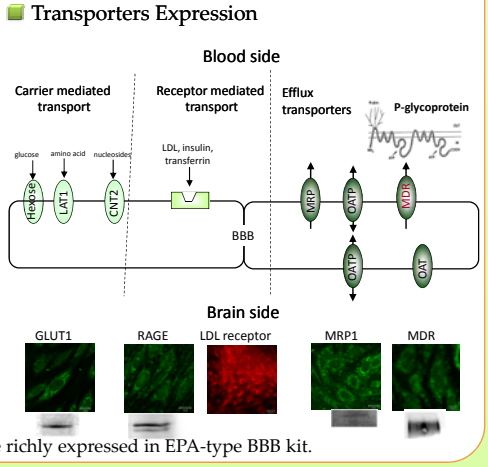


Results ~ Immunofluorescence ~

Tight junctions expression

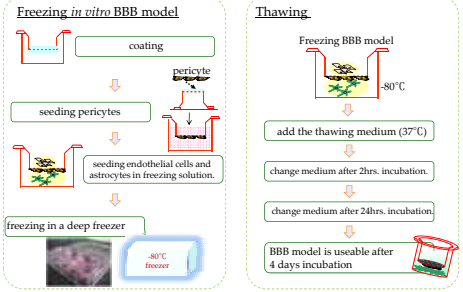


Transporters Expression



TJs proteins and transporter proteins were richly expressed in EPA-type BBB kit.

Ready-To-use BBB kit



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