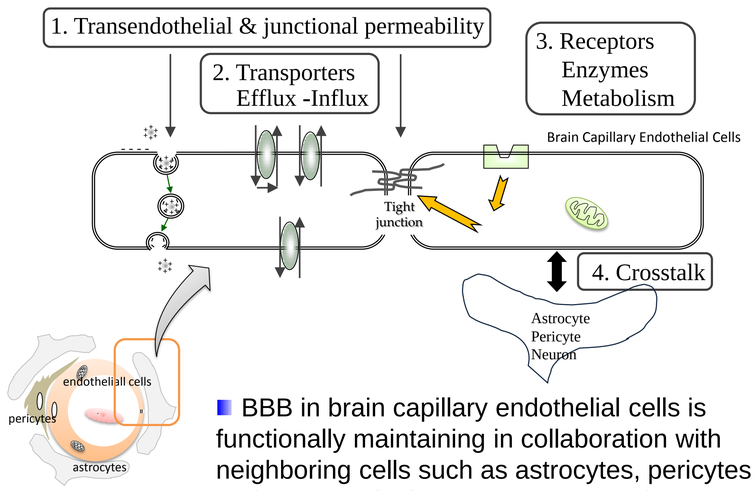
An *in vitro* Monkey BBB Model

Shinsuke Nakagawa[1,2], Mária A. Deli[2,3], Dinh Ha Duy Thuy[2], Mayumi Sagara[2],
Gohei So[1], Narumi Yamada[1], Rie Tatsumi[1,2], Kunihiko Tanaka[1], Masami Niwa[1,2]

[1] *Department of Pharmacology, Nagasaki University Graduate School of Biomedical Sciences,*
[2] *BBB Laboratory, PharmaCo-Cell Company Ltd., 1-12-4 Sakamoto, Nagasaki 852-8523, Japan,*
[3] *Institute of Biophysics, Biological Research Center of Hungarian Academy of Sciences, Szeged, Hungary*

Introduction & Methods

Blood-Brain Barrier (BBB)



- BBB in brain capillary endothelial cells is functionally maintaining in collaboration with neighboring cells such as astrocytes, pericytes and neurons, in the neurovascular unit.
- Damages of BBB are closely related to on onset and progress of neurological disorders.

In vitro BBB model

mouse	Cumbersome in handling with small size of materials Comparison with *in vivo* Data
Rat	Cumbersome in handling with small size of materials Comparison with *in vivo* Data
Bovine	Easy handling Difficult in Comparison with *in vivo* Data
Porcine	Easy handling Difficult in Comparison with *in vivo* Data
Human	The best in vitro BBB model Difficult in getting Cells
Immortalized cells	Immatured Tight Junctions

Monkey — *Macaca* fascicularis *(Macaca irus)*

Primates Simiiformes Cercopithecinae *Macaca*	
English name	Crab-eating Macaque
Scientific Name	*Macaca irus*
Distribution	Southeast Asis
Living area	Seaside, Riverside, wetland
Height	40～50cm
Tail	40～50cm
BW	5 kg
Life span	30 years

Primary culture of monkey BBB-related cells

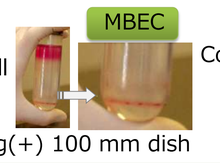
35 g (For microvessels isolation)
↓ collagenase CLS2
↓ 20% BSA
↓ collagenase /dispase
↓ Percoll
↓ Coating(+) 100 mm dish

3-year-old male 4
4-year-old female 1
13-year-old male 1

Brain : 42 g /one monkey

5 g (For pericytes)
↓ collagenase CLS2
↓ 20% BSA
↓ Percoll
↓ Coating(-) 100 mm dish (p0)
↓ 1 week
Subculture (p1)
↓ subculture(p2)

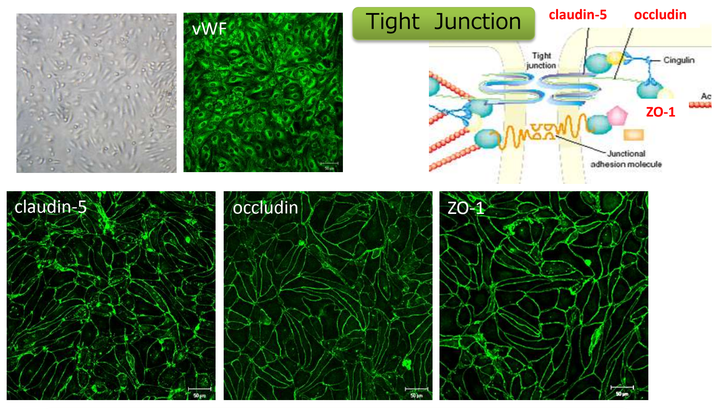
2 g astrocytes
↓ Papain
↓ Poly-L-lysin coated 75cm² flask
↓ 2~3 weeks subculture



Purpose
To elucidate the differences between rodent and primate BBB-related data, and measure permeability of novel drugs which are under research and development with human-related BBB, we need a *in vitro* monkey BBB model.

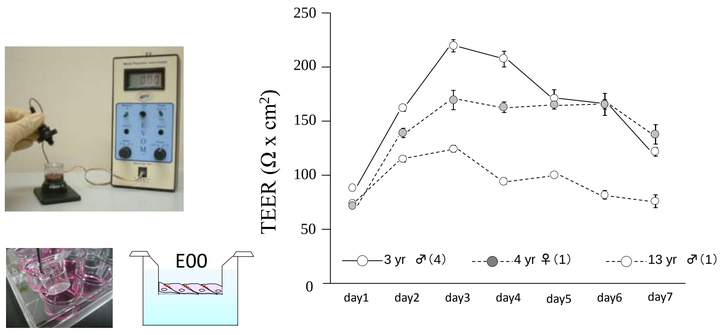
Result I

Result 1 — Target Proteins in Monkey Brain Microvascular Endothelial Cells (MBEC)



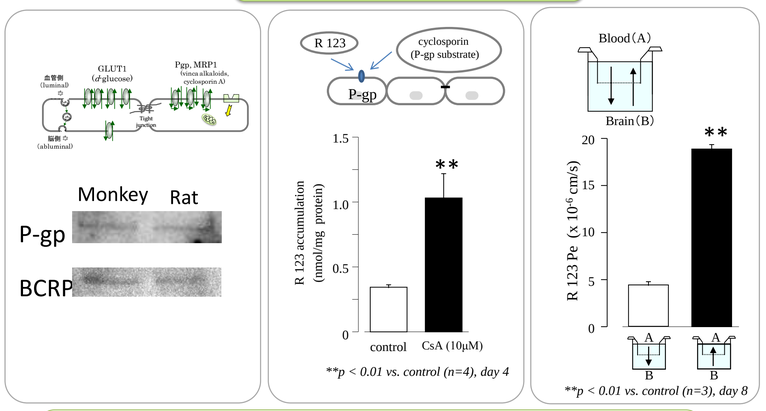
Immunopositive expressions of *von* Willebrand factor, Claudin-5, Occludin, and ZO-1

Result 2 — TEER（Trans-endothelial electrical resistance)



TEER of monolayer of MBEC increased up to over 100 Ω x cm² at Day 3, including TEER of 13 yr old Male.

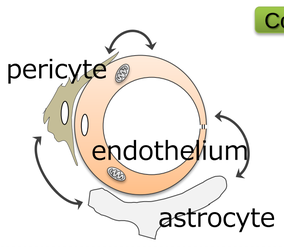
Result 3 — Transporter（P-glycoprotein, P-gp）



A significant amount of functional P-gp was detected in MBEC, with a dominant direction of brain- to blood-side.

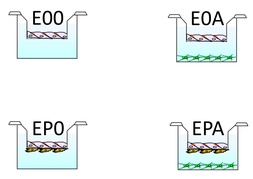
Result II

Cross-Talks between BBB-related Cells

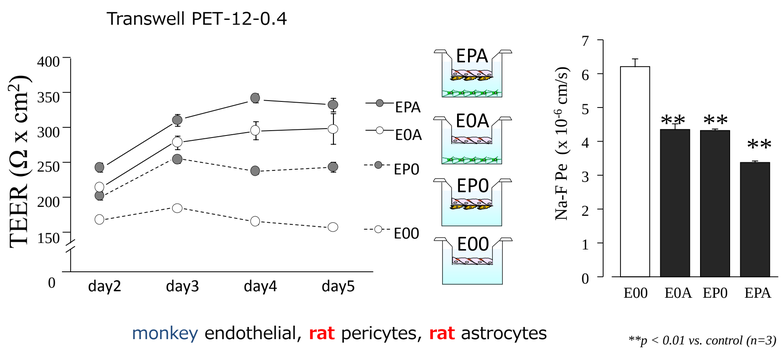


Co-Culture Effects

1. Barrier functions ⬆
   TJs protein↑ TEER↑ Pe↓…
2. Transpoters ⬆
   P-gp ↑ GLUT1↑…
3. Enzymes ⬆
   γ-GTP ↑ ALP↑…



Result 4 — Co-culture BBB Model

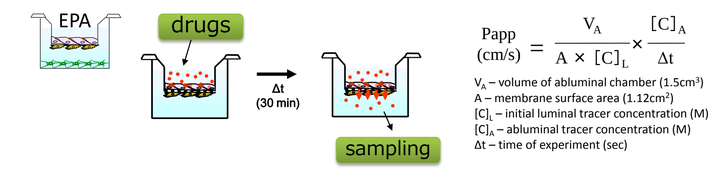


monkey endothelial, **rat** pericytes, **rat** astrocytes

**p < 0.01 vs. control (n=3)*

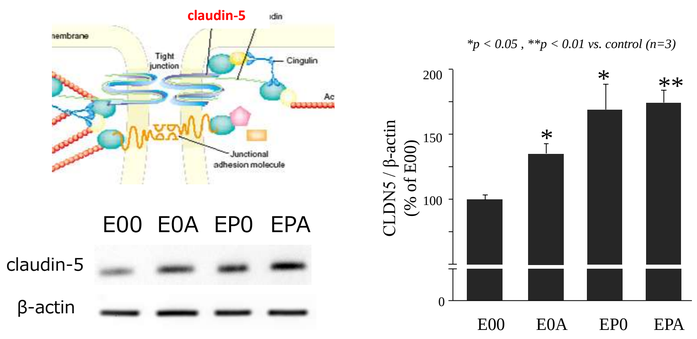
Rat pericytes (P) and astrocytes (A) drove MBEC to have the highest TEER (350 Ω x cm²) in co-culture BBB model of EPA (BBB Kit™).

BBB Transport of Drugs into Brain



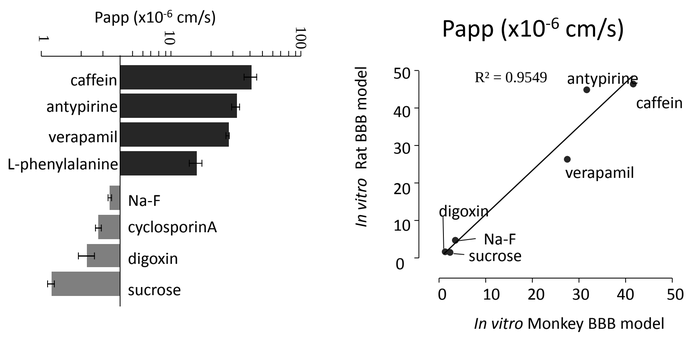
	MW	CNS	Transport
caffein	212	+	passive lipophilic
antipyrine	188	+	passive lipophilic
L-phenylalanine	165	+	influx (L-system)
verapamil	491	±	efflux
cyclosporin A	1202	-	efflux
digoxin	781	-	efflux
sucrose	342	-	passive hydrophilic
sodium fluorescein (Na-F)	376	-	passive hydrophilic

Result 5 — Co-culture BBB Model



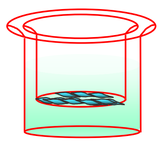
Rat pericytes (P) and astrocytes (A) collaboratively Drove MBEC to the highest expression of claudin-5 in co-culture BBB model of EPA (BBB Kit™).

Result 6 — BBB Permeability of Drugs



A very good correlation between Papp of drugs was obtained from Rat and Monkey BBB models (BBB Kit)

Summary



Monkey brain microvascular endothelial cells (MBEC)

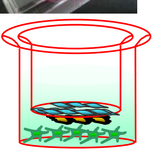
had immunopositive expressions of *von* Willebrand factor, Claudin-5, Occludin, and ZO-1.

had TEERs of over 100 Ω x cm² (monolayer, Day 3).

Had a significant amount of functional P-gp with a dominant direction of brain- to blood-side.

Monkey BBB Kit™

Rat pericytes and astrocytes collaboratively drove MBEC to the highest expression of claudin-5 in the co-culture BBB model of EPA (Monkey BBB Kit™).



Rat pericytes (P) and astrocytes (A) drove MBEC to have the highest TEER (350 Ω x cm²) in Monkey BBB Kit™.

A very good correlation between Papp of drugs was obtained from Rat and Monkey BBB Kit™.

Conclusion

Monkey BBB Kit™ is a new and useful tool for basic research and BBB permeability assays. In collaboration with rat BBB Kit™, monkey BBB Kit™ would pave the way to elucidate the differences between rodent and primate BBB-related data.