FINAL REPORT

For Japan-Korea Joint Research Project

AREA	1. Mathematics & Physics 2. Chemistry & Material Science 3. Biology 4. Informatics & Mechatronics 5. Geo-Science & Space Science 6. Medical Science 7. Humanities & Social Sciences

1. Research Title:

Regulation and its molecular mechanism of transporter protein complex at the blood-brain and placenta barrier during maturation and disease conditions.

2. Term of Research: From July 1, 2009 To June 30, 2011

3. Total Budget

a. Financial Support by JSPS: Total amount: 2,400 thousand yen

1st Year 900 thousand yen 2nd Year 1,200 thousand yen

3rd Year 300 thousand yen

b. Other Financial Support : Total amount: 0 thousand yen

4. Project Organization

a. Japanese Principal Researcher	
Name	Tetsuya Terasaki
Institution / Department Position	Tohoku University Graduate School of Pharmaceutical Sciences Professor
b. Korean Principal Researcher	
Name	Young-Sook Kang
Institution / Department Position	Sookmyung Women's University College of Pharmacy Professor

c. List of Japanese-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Sumio Ohtsuki	Tohoku University/ Graduate School of Pharmaceutical Sciences	Associate Professor
Yasuo Uchida	Tohoku University/ Graduate School of Pharmaceutical Sciences	Assistant Professor
Ken Ohmine	Tohoku University/ Graduate School of Pharmaceutical Sciences	Graduate Student
Wataru Obuchi	Tohoku University/ Graduate School of Pharmaceutical Sciences	Graduate Student
Yusuke Ochiai	Tohoku University/ Graduate School of Pharmaceutical Sciences	Graduate Student
Youhei Takizawa	Tohoku University/ Graduate School of Pharmaceutical Sciences	Graduate Student
Kengo Mutou	Tohoku University/ Graduate School of Pharmaceutical Sciences	Graduate Student
Toshihiro Yoneyama	Tohoku University/ Graduate School of Pharmaceutical Sciences	Graduate Student
Emi Nakashima	Keio University/ Faculty of Pharmacy	Professor
Masatoshi Tomi	Keio University/ Faculty of Pharmacy	Associate Professor
Tomohiro Nishimura	Keio University/ Faculty of Pharmacy	Assistant Professor
Kei Higuchi	Keio University/ Faculty of Pharmacy	Graduate Student
Yoshimichi Sai	Kanazawa University Hospital Kanazawa University	Associate Professor and Vice Director

d. List of Korean-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Na-Young Lee	Sookmyung Women's University/ College of Pharmacy	Research Professor
Chang-Sun Lee	Sookmyung Women's University/ College of Pharmacy	Graduate student
Kyung-Bok Lee	Sookmyung Women's University/ College of Pharmacy	Graduate student
Jeong-Eun Lee	Sookmyung Women's University/ College of Pharmacy	Graduate student
Hyun-Joo Park	Sookmyung Women's University/ College of Pharmacy	Graduate student
Ha-Eun Lee	Sookmyung Women's University/ College of Pharmacy	Graduate student
Hyung-Ok Choi	Sookmyung Women's University/ College of Pharmacy	Graduate student

5. Number of Exchanges during the Final Fiscal Year*

a. from Japan to Korea

*Japanese fiscal year begins April 1.

Name	Home Institution	Duration	Host Institution
None			
For Final Fiscal Year(FY2011) Total: 0 persons		For Final Fiscal Year(FY2011) Total: 0 man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total 0 persons FY2010: Total 3 persons			

b. from Korea to Japan

Name	Home Institution	Duration	Host Institution
None			
For Final Fiscal Year(FY2011) Total: 0 persons		For Final Fiscal Year(FY2011) Total: 0 man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total 1 persons FY2010: Total 0 persons			

6. Objective of Research

The Objective of this joint research is to evaluate the changes of transporter protein complex at the blood-brain barrier (BBB) and blood-placenta barrier (BPB) during maturation and disease conditions, and clarify the molecular mechanism of the regulations. The brain capillary endothelial cells and syncytiotrophoblasts express various transporters and play pivotal roles in exchange of endogenous and xenobiotic substances. Although, to date, the research has been focused mainly on normal adult brain and term placenta, to clarify the transport system at the BBB and BPB in disease conditions and during maturation is essential to understand drug permeability and safety drug usage for patients, infants and fetus.

Kang's, Ohtsuki's, Nakashima's and Sai's groups have been collaborated for this 9 years and exchanged knowledge, materials, techniques and Ph.D. students among groups. The achievements of joint research have been published in 7 international journals, and the joint symposium was held at KPS meeting in Jeju in May, 2008. By the joint research, we have clarified that transport system for taurine and nucleotides at the BBB and BPB was regulated by cytokines and hormones using in vitro cell culture model originally established by our groups. Extending the former joint research, the focus of this joint research was shifted to transporter protein complex at the BBB and BPB in disease conditions and during maturation.

Dr. Ohtsuki's group and Drs. Nakashima and Sai's group would collaborate with Dr. Kang's group for the BBB and BPB research, respectively. Dr. Ohtsuki's group would clarify protein expression profile of transporter protein complex at the BBB using original quantitative proteomic analysis, which can quantify multiple membrane proteins with high sensitivity. Dr. Kang's group would analyze the activity and regulation of transporter protein complex at BPB using in vitro cell culture model. Dr. Nakashima would clarify changes in nucleoside transport and other placental barrier function during pregnancy using rodent placenta and cell lines, and studying ezrin knockout mice to clarify mechanisms underlying decreased body weight in fetus. Dr. Sai would evaluate clinical significance of this international collaborative works from a viewpoint in the University Hospital. This joint research project will provide a new insight in understanding a molecular mechanism of BBB and BPB. It also gives very important knowledge in drug-drug interaction and a proper use of pharmaceutics for patients, children and fetus.

7. Methodology

(1) Cellular uptake study using conditionally immortalized cell lines as in vitro blood-brain and –placental barrier:

Conditionally immortalized rat brain capillary endothelial cells (TR-BBB) were used as an in vitro blood-brain barrier model. Conditionally immortalized rat syncytiotrophoblast cell line (TR-TBTs) were used as in vitro blood-placental barrier. The transport function of the cells was examined by the cellular uptake study with radio labeled compounds.

(2) Brain Efflux Index (BEI) method

Brain efflux index method (BEI) method established by Terasaki et al (1996) was used to analyze the in vivo brain-to-blood efflux transport of target compound. Briefly, radio labeled compound was micro-injected with no-permeable marker into appropriate brain region of rat or mouse. After designated time period, the brain was excised and radioactivity remaining in the brain was measured. For siRNA treatment, double-stranded siRNA (5 nmol) was dissolved in 2 ml phosphate-buffered saline and administered to mice using the hydrodynamic tail vein injection method.

(3) Quantification of transporter proteins by using LC-MS/MS

Quantification of transporter proteins was established by Kamiie et al (2008). The sets of unlabeled peptide and stable isotope labeled peptide, which were specific sequences of target transporters, were synthesized for quantitation standard. Plasma membrane fraction of the cells or tissues was isolated by sucrose density gradient centrifugation. Protein samples were denatured with lysis buffer including 7M guanidine hydrochloride, then, digested with trypsin. 50μg of digested peptides were analyzed by LC-MS/MS (ABI 4000QTRAP or API5000) in multiple reaction monitoring (MRM) mode with isotope labeled peptide mixture.

(4) Immunostaining of Placenta

Pregnant rats were perfusion fixed with formaldehyde/PBS. Frozen sections of the placenta were prepared and reacted with anti-ezrin antibody, anti-CLP36 antibody or anti-GLUT3. Cell nuclei were stained with SYTOX or DAPI. Alexa Fluor 488 or 594 labeled secondary antibodies were used for immuno-fluorescent microscopy.