Quantitative Membrane Protein Expression at the Blood–Brain Barrier of Adult and Younger Cynomolgus Monkeys

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ABSTRACT: Cynomolgus monkey has been used as a model for the prediction of drug disposition in human brain. The purpose of this study was to clarify protein expression levels of membrane proteins affecting drug distribution to brain, such as transporters, receptors, and junctional proteins, in cynomolgus monkey brain microvessels by using liquid chromatography tandem mass spectrometory. In adult monkeys, three ATP-binding cassette transporters (multidrug resistance 1 (MDR1), breast cancer resistance protein (BCRP), and multidrug resistance protein 4 (MRP4)), six solute carrier transporters (glucose transporter 1 (GLUT1), GLUT3/14, monocarboxylate transporter 1 (MCT1), MCT8, organic anion transporting polypeptide 1A2, and equilibrative nucleoside tranporter 1), two junctional proteins (claudin-5 and vascular endothelial cadherin), and two receptors (insulin receptor and low-density lipoprotein receptor-related protein 1) were detected. Comparison of the expression levels with those in mouse, which we reported previously, revealed a pronounced species difference. BCRP expression in monkey was greater by 3.52-fold than that in mouse, whereas MDR1 and MRP4 expression levels in monkey were lower by 0.304- and 0.180-fold, respectively, than that in mouse. This study also investigated the developmental changes in expression of membrane proteins in neonate and child monkeys. Expression of MDR1 was similar in neonate and adult monkeys, whereas in rat, P-glycoprotein expression was reported to be significantly lower in brain microvessels of neonate as compared with adult rat. These results will be helpful to understand and predict brain concentrations of drugs in different species and at different ages of primates. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:3939–3950, 2011
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INTRODUCTION

Cynomolgus monkey has been considered as a model animal for humans because monkeys are the second nearest animal to humans in the evolutionary tree, and consequently, it has been used for the prediction of drug disposition in human brain. Substrates for P-glycoprotein (P-gp), such as verapamil and GR205171, were reported to be distributed more extensively in monkey brain than in rat brain,[1] and it was suggested that there are species differences in drug permeability across the blood–brain barrier (BBB) between primates and rodents. Such species differences would have an impact on the usefulness of animal studies to predict the brain distribution of drugs in humans, and this is considered to be a major cause of the low success rate of central nervous system (CNS)-acting drugs in clinical studies. At present, the molecular basis of species differences in the transport systems at the BBB is poorly understood.

Species differences in P-gp during brain development have also been reported. In rats, P-gp expression in neonate brain was much lower than in adult brain,[2,3] whereas in human, immunoreactivity of P-gp was detected in microvessels of fetal brain and, in some cases, the immunoreactivity was comparable to that in adult brain.[4] This implies that the BBB

Additional Supporting Information may be found in the online version of this article. Supporting Information

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function and expression of membrane proteins during development are different between primates and rodents. Therefore, it is important to consider species differences in drug distribution in infant brain between primates and rodents during the animal testing stage of pediatric drug development.

There have been several reports on mRNA expression of ATP-binding cassette (ABC) transporters in human brain microvessels. Warren et al.[5] found that mRNA expression (percent of glucose transporter 1 (GLUT1)) of multidrug resistance protein 4 (MRP4/ABCC4) in isolated brain microvessels was lower in human than in mouse, whereas no significant difference was reported in the case of multidrug resistance 1 (MDR1/mdr1a/ABCB1) mRNA expression. It was also reported that the relative mRNA expression of breast cancer resistance protein (BCRP/ABCG2) to MDR1/mdr1a/ABCB1 was higher in human brain microvessels than in rat brain microvessels.[6,7] However, mRNA expression levels do not necessarily correlate with protein expression levels. Indeed, it was reported that the protein expression of MDR1 and BCRP was downregulated by activation of estrogen receptor α-related pathways without any change of mRNA expression.[8,9] Therefore, it is necessary to determine protein expression levels, rather than mRNA, to characterize and understand the differences in transport systems that underlie species differences in drug permeability across the BBB.

Recently, we have developed multiplexed multiple reaction monitoring (MRM) analysis using liquid chromatography tandem mass spectrometory (LC–MS/MS) to quantify absolute protein expression amounts of membrane proteins, and we used this method to investigate the absolute protein expression levels of 34 transporters in mouse brain microvessels.[10] The objectives of the present study were to clarify the protein expression levels of membrane proteins affecting drug distribution, such as transporters, receptors, and junctional proteins, in cynomolgus monkey brain microvessels by means of multiplexed MRM analysis and to compare the results with those that we previously reported for mouse,[10] in order to clarify species difference between primate and rodent BBB. In addition, the expression amounts of membrane proteins in cynomolgus monkeys of different ages (neonate, child, and adult) were examined to clarify developmental changes in the expression of membrane proteins in monkey BBB.

MATERIALS AND METHODS

Reagents

All peptides listed in Supplementary Table 1 were purchased from Thermo Electron Corporation (Sedantrabe, Germany). Peptide purity (>95%) was provided by the manufacturer, using reversed-phase high-performance liquid chromatography with UV detection (RP-HPLC–UV, with a detection wavelength of 215 nm) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analyses. Other chemicals were commercial products of analytical grade.

Animals

Brains of cynomolgus monkeys, which had originated from Indonesia (Indonesian neonate, child, and adult monkeys), that is, one neonate (1 day after birth, male), one child (16 months after birth, male), and one adult (4 years 3 months after birth, male) were purchased from Shin Nippon Biomedical Laboratories, Ltd. (Kagoshima, Japan; Table 1). One-day-old and 16-month-old monkeys were categorized as neonate and child, respectively, according to the classification by Beck et al.[11] This classification categorized 0–0.5-month-old monkey as neonate and 6–36-month-old monkey as child; these ages correspond to 0–28days old and 2–12 years old in human, respectively.[11] All procedures for brain excision were performed by Shin Nippon Biomedical Laboratories, Ltd.; they were performed in accordance with the animal welfare bylaws of Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories, Ltd., and had been approved by the Institutional Animal Care and Use Committee.

Table 1. Summary of Characteristics of Monkeys of Various Ages

Monkey	Age	History of Drug Administration Experiment	Blood Removal by Perfusion Before Excising Brain	Fasting	Body Weight (kg)
Indonesian neonate monkey	1 day	None	+	None	–
Indonesian child monkey	16 months	None	+	None	1.7
Indonesian adult monkey	4 years 3 months	None	+	None	2.8
Chinese adult monkey #1	3 years 6 months	Loperamide	None	+	2.7
Chinese adult monkey #2	4 years	Paclitaxel	None	+	3.8
Chinese adult monkey #3	4 years 1 month	Quinidine	None	+	2.6
Chinese adult monkey #4	4 years 3 months	Diazepam	None	+	3.2
Chinese adult monkey #5	5 years 2 months	Indinavir	None	+	3.6

One day and 16 months after birth were categorized in neonate and child monkey, according to Beck et al.[11]