Evaluate of P-Glycoprotein Activity as an Efflux Pump in the Blood-brain Barrier of Living Human Brain

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Introduction

The Human Genome Project (HGP), a 13-year international project, completed in 2003. They identified approximately 20,000-25,000 genes in human DNA and determined the sequences of the 3 billion chemical base pairs of the human DNA. Since then, many people have expected the establishment of a system for "individualized or personalized medicine". However, it is still a long way. There are huge number of transporters and enzymes contributing to every process of drug absorption, distribution, metabolism and excretion.

In the human body, for instance, there are many drug transporters such as p-glycoprotein (P-gp), multi-drug resistance protein (MRP); organic anion transporting polypeptide (OATP), in various organs of the human body. It is not easy to determine the functional effects of various genetic mutations. The field of "pharmaco-genomics" or "pharmaco-genetics" have explored the relationship between single nucleotide polymorphisms (SNPs) and pharmaco-kinetic/-dynamic outcomes. The merit of using molecular imaging technique in this field is that we can obtain biological information regarding changes in an intermediate "endo-phenotype", lying between changes in genotype and phenotype.

As an example, P-gp, a gene product of MDR1 (Multi-drug resistance gene 1) in human, is one of the most important transporters contributing to drug transport in human body. It is working like a cellular pump equipped by a battery, transporting various xenobiotics (biologically-active substances from outside) being energized by ATP binding cassettes (ABC). The P-gp was first discovered as the cause substance of multidrug-resistant cancer cells that altered membrane permeability of the anticancer drugs. Then, it was later identified in various

normal tissues as well. Currently, it is known that the P-gp is expressed in cells of various organs such as the intestinal tract, liver, kidney, placenta, and brain [blood-brain barrier: BBB]). Most of these organs have secreting function where the cells are transporting the xenobiotics actively from the inside of endothelial cells of capillaries to the capillary lumen (Figure 1).

So far, the effect of MDR-1 polymorphism on the BBB permeability has been studied in human using PET and [^{11}C]verapamil between single nucleotide polymorphisms such as C1236T and C3435T. They demonstrated that there was no difference in the BBB permeability[1,2] where they did not find any difference among different polymorphisms. However, there have been no studies to evaluate the difference using drugs in clinical doses.

We have measured variation of histamine H1 receptor (H1R) occupancy following oral administration of various antihistamines. The variation in the cerebral H1R occupancies due to antihistamines may be in part a result of their different BBB permeabilities, as having been demonstrated as H1R occupancy using PET and [^{11}C]doxepin[2-5]. In clinical settings, however, permeability at the gut level would also affect the net antihistamine transport in to the brain.

Thus, the purpose of the present study is to examine whether the histamine H1R occupancy due to fexofenadine, a non-sedative antihistamine, varies between different genetic types or not, using a non-invasive technique such as PET. For this purpose, the authors tried to compare the amount of antihistamines transported into the brain tissue during 3 hours following medication. Fexofenadine is also a substrate of P-gp, and it is thought that BBB permeability is low even at an exceeded dose[5].

Methods

Thirty-nine healthy volunteers (24.2 +/- 4.0 years old) were studied. Informed consent was collected from each volunteer following adequate explanation, based on the permission from the ethics committee of Tohoku University Graduate School of Medicine. Blood sample was obtained from each volunteer and the polymorphism typing of MDR-1 gene was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (C3435T mutation in exon 26 and C1236T mutation in exon 12). Out of the 39 subjects, 24 volunteered consented for further PET examination.

The PET examination was conducted in the 24 subjects, using fexofenadine hydrochloride (FEX) 120 mg or lactobacteria preparation 6 mg as placebo (PLA) with the minimum washout period of 7 days. The volunteers were scanned 90 min after administration of oral tablets using an ECAT PT931 PET scanner (CTI Inc, Knoxville, TN, USA), taking 90

min for each scan (22 sequential scans: six scans for 90 seconds, seven scans for 180 seconds, six scans for 300 seconds and three scans for 600 seconds). The order of drug conditions was controlled and balanced. The basic protocol is demonstrated in the Figure 3.

After being corrected for tissue attenuation, brain images were processed by applying a Logan graphical analysis using cerebellar time activity curve as input functions to obtain binding potential (BP) images. Finally, brain BP images were created from which H1RO was calculated for the prefrontal, anterior cingulate, orbitofrontal and temporal cortices. The H1ROs following fexofenadine treatment was calculated based on the following equation: H1RO = $[(\text{mean } BP_{PLA} - \text{mean } BP_{FEX})/\text{mean } BP_{PLA}] \times 100$.

Results

Results of polymorphism analysis demonstrated that the numbers of holders of wild-type genetic pattern (CC), heterozygous mutation pattern (CT) and homozygous mutation pattern (TT), respectively were 10 (25.6%), 24 (61.5%), and 5 (12.8%) for 3435 mutation. The results for 1236 mutation were 4 (10.3%), 16 (41.0%), and 19 (48.7%), respectively. The present results were equal to previous studies for Japanese population.

Result of PET investigation is demonstrated in the Fig. 4, showing almost negligible differences between FEX and placebo conditions in some subjects. Further analysis regarding H1R occupancy following FEX treatment calculated based on the placebo data were demonstrated in the anterior cingulated gyrus, frontal cortex, occipital cortex, orbitofrontal cortex, and temporal cortex as -5.41%, -3.6%, -0.05%, -8.20%, -1.12%, respectively. In addition, an overall cortical mean value was -3.67%.

Comparison of H1R occupancy values averaged across the cortex among subgroups divided by polymorphism types (C3435T mutation) revealed the trend toward increased H1R occupancy in volunteers having mutated genes though there was no statistical significance among the subgroups (Fig. 2).

Discussions

This is a preliminary research regarding the variation in receptor occupancy of a psychoactive drug such as an antihistamine associated with genetic polymorphisms in human MDR1 gene.

First, H1R occupancy of fexofenadine in the present study was negative values in many brain regions. The values calculated in our previous study[5)] were also negative values in some

First, H1R occupancy of fexofenadine in the present study was negative values in many brain regions. The values calculated in our previous study[5] were also negative values in some brain regions such as the anterior cingulate, where the baseline data was used from different subjects. In the present study, however, the H1R occupancy was calculated using the baseline (placebo condition) data obtained from the same subjects. Then it is hard to explain the negative values by the inter-individual difference.

Secondarily, the present PET study demonstrated that relatively higher H1R occupancy in the holders of mutant genes though these results were not statistically significant. The effect of MDR polymorphisms on the BBB permeability of fexofenadine has never been studied using PET. The PET results might suggest the presence of slight decrease in efflux function of P-glycoprotein in BBB among holders of mutations in comparison to wild-type gene holders. But it is not known if there is any BBB permeability variation among other drugs.

To draw a definitive conclusion, further investigation is needed to draw a conclusion with larger sample size. The molecular imaging technique seems to be very useful for establishment of the field of individualized medicine or personalized medicine. Importantly, the molecular imaging technique enables us to access the biological information of endophenotype. Observation of the endophenotype makes possible the prediction and explanation of the possible mechanism of drug side effects.

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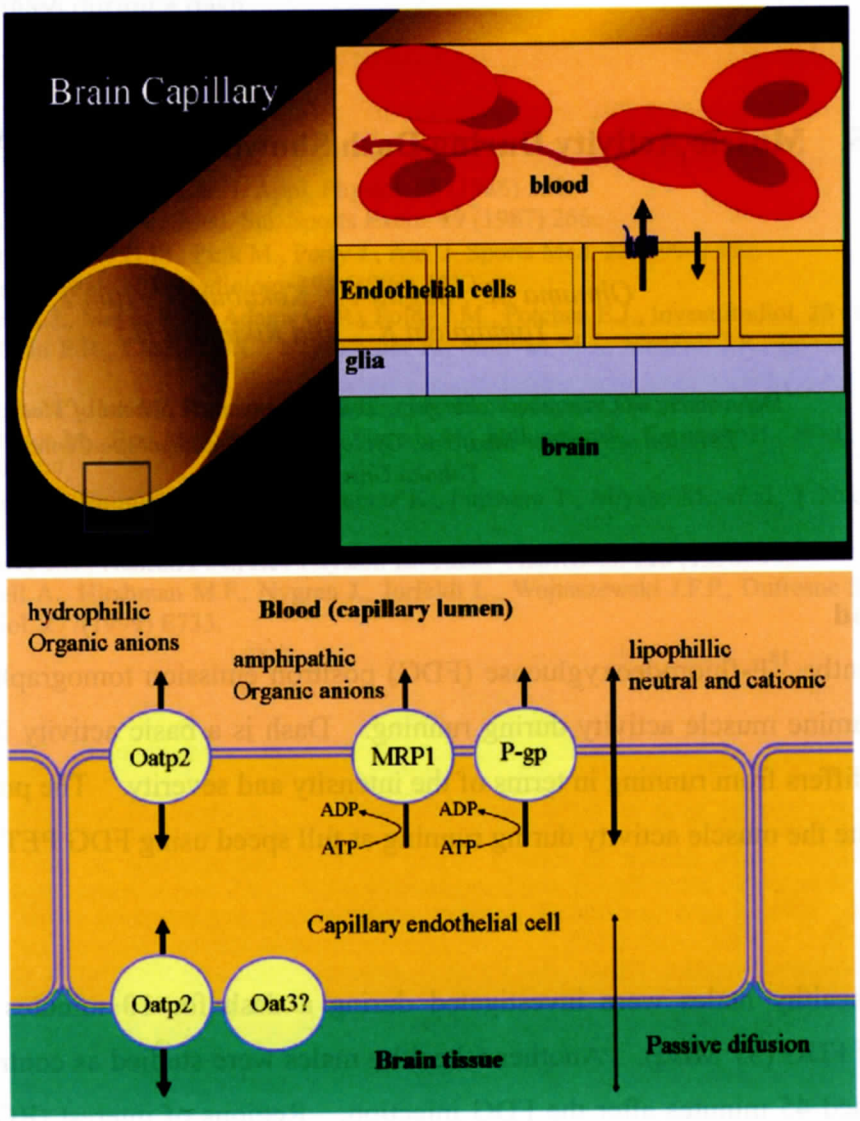

Figure 1. Basic structure of Blood-brain barrier (BBB) consisting of the capillary endothelial cells and glias. An efflux transporter (blue) is actively transporting specific substrates outward into the blood stream (TOP). Various transporters are located at the BBB. P-gp is transporting specific substrates outward using the energy supplied by ATP. Lipophillic drugs can enter the brain tissue easily. Hydorphillic drugs have difficulty to enter the brain tissue (BOTTOM).