論文の内容の要旨

Investigation of Blood-Brain Barrier Transport Based on the Modeling and Simulation of Receptor Occupancy of D2 Antagonists in Human Brain

（D２アンタゴニストによる受容体占有率および脳内動態解析に基づいた ヒト血液脳関門薬物透過機構の解析）

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Development of drugs for the treatment of diseases of the central nervous system (CNS) remains challenging. For drugs that act in the central nervous system (CNS), it is assumed that an unbound drug in the interstitial fluid in the brain is available to interact with the target site in the CNS. The unbound concentration is therefore a surrogate for the bound concentration at the target site. It is well known that drug concentrations in the blood are not good predictors of the pharmacological actions of CNS-directed drugs, because the blood–brain barrier (BBB), formed by a tight monolayer of brain capillary endothelial cells, limits the penetration of drugs into the brain. The purpose of this study was to investigate drug transport across the human blood brain barrier based on *in vitro* data and clinical positron emission tomography (PET) data.

PET has high sensitivity and spatial-temporal resolution. Using PET probes for the CNS targets, the interaction of drugs with their target proteins has been evaluated in vivo. Here, in this study, receptor occupancy (RO) was used as an index of the unbound concentration in the CNS to investigate the drug transport across the human BBB. A pharmacokinetics–receptor occupancy (PK–RO) model, combined with physiological and biochemical parameters, was constructed to simulate RO. Taking into account probe specificity, the available time course of RO in humans and a lack of confounding metabolites, the dopamine D2 receptor antagonists olanzapine, haloperidol, paliperidone (9-OH metabolite of risperidone), and sulpiride, were selected as test drugs in this study.

In Chapter 1, the disposition of D2 receptor antagonists was investigated in the human brain using a physiologically based pharmacokinetic model. The membrane fractions were prepared from Human Embryonic Kidney (HEK) 293 cells transfected with human D2L cDNA, which is the predominant form of two isoforms of the dopamine D2 receptor. The specific binding of [^{3}H]raclopride is saturable (K_d = 1.2 nM and B_{max} = 21.9 ± 0.7 pmol/mg

protein) and reversible. Non-specific binding accounts for less than 2% of the total binding. Replacement of [^{3}H]raclopride by D2 receptor antagonists was rapid enough to assume rapid equilibrium. The K_i values of olanzapine, haloperidol, paliperidone and sulpiride were 8.30, 1.17, 2.70 and 15.6 nM, respectively. RO of the D2 receptor by its antagonists in the human brain was predicted with the PK-RO model based on physicochemical properties of model compounds, *in vitro* parameters and *in vivo* human plasma concentration time profiles. Assuming passive diffusion across the BBB (PS_{inf} = PS_{eff}), the RO by olanzapine and haloperidol were underestimated, whereas those by paliperidone and sulpiride were slightly and markedly overestimated respectively. The sensitivity analysis showed that PS_{inf}/PS_{eff} ratio had a significant effect on the prediction of occupancy. To explain the clinical data, the influx of olanzapine and haloperidol must have been 6.9-fold and 8.2-fold greater than their efflux, whereas that of paliperidone and sulpiride must have been 1.5- and 55-fold smaller than their efflux. These results suggest that the transporters are involved in the influx or efflux process of the D2 receptor antagonists across the human BBB. This prediction was supported by a clinical study (Arakawa et al., *J Clin Psychiatry* 71:1131-7, 2010.) that reported that the RO by olanzapine, haloperidol, and risperidone were higher in the human cerebral cortex (within the BBB) than in the pituitary (outside the BBB), whereas the RO by sulpiride was lower in the cerebral cortex.

In Chapter 2, the BBB transport of model compounds was characterized in hCMEC/D3 cells, an immortalized cell line derived from human brain capillary endothelial cells. Olanzapine, haloperidol, risperidone, paliperidone and sulpiride were all transported into hCMEC/D3 cells in a time- and temperature-dependent manner. The uptake of olanzapine, haloperidol, risperidone, and paliperidone were saturable with K_m values of 6.12, 17.6, 17.1 and 46.4 μM, respectively. Digitonin treatment caused a marked reduction in the intracellular accumulation of olanzapine. The uptake of olanzapine was inhibited by cationic drugs, such as pyrilamine, quinidine, verapamil, diphenhydramine and amantadine, but not by TEA and MPP^+ (substrate/inhibitor of OCT, PMAT or MATE), ergothioneine (a specific substrate of OCTN1), L-carnitine (a substrate of OCTN2), choline (a substrate of the choline transport system) and cimetidine (substrate/inhibitor of OCT or MATE). Pyrilamine is a competitive inhibitor, and a *trans*-stimulation by pyrilamine was observed for the initial uptake of olanzapine, haloperidol, risperidone, and paliperidone: the uptake was significantly enhanced by preloading cells with pyrilamine. The transport was significantly reduced by sodium azide

(a metabolic inhibitor) or FCCP (a protonophore). The olanzapine uptake was increased at extracellular pH (8.5) or intracellular acidification, but was reduced by intracellular alkalization. Thus, the putative pyrilamine transporter likely mediates the uptake of olanzapine, haloperidol, risperidone and paliperidone in hCMEC/D3 cells. The brain uptake of olanzapine, haloperidol, risperidone, paliperidone, and sulpiride was further investigated using *in situ* mouse brain perfusion. The brain uptake of olanzapine and haloperidol was similar to that of diazepam, a flow-rate marker, whereas the brain distribution of sulpiride was similar to the vascular volume. Diphenhydramine significantly inhibited the brain uptake of olanzapine, haloperidol, and risperidone, but not paliperidone. These results suggest that the influx transporter contributes substantially to the efficient transport of olanzapine, haloperidol, and risperidone into the brain across the BBB, and thus the underestimation of RO by olanzapine and haloperidol can be explained.

To understand the efflux mechanisms involved, a transcellular transport study was performed. The BBB expresses P-gp and BCRP, which actively extrude drugs into the blood. Unlike olanzapine and haloperidol, the directional transport of risperidone and paliperidone was induced by the exogenous expression of P-gp, but not by BCRP, in MDCK II cells, indicating that these two drugs are good substrates for P-gp, but not BCRP. Furthermore, the brain distribution of olanzapine, risperidone and paliperidone in P-gp knockout mice were all significantly increased compared with those in wild type mice. Overestimation of the RO by paliperidone is attributed to its active efflux by P-gp at the BBB, whereas neither P-gp nor Bcrp accounts for the efflux of sulpiride.

The present study characterized the transport of the D2 receptor antagonists in the human BBB. A putative pyrilamine transporter contributes substantially to the efficient transport of olanzapine and haloperidol into the brain across the BBB, and to their therapeutic outcomes. On the other hand, both uptake and efflux transporters (putative pyrilamine transporter and P-gp) determine the therapeutic outcomes of risperidone and paliperidone, and unknown efflux transporter is involved in the extensive efflux of sulpiride at the BBB. Thus, PK-RO analysis of the CNS acting drugs is useful to investigate their transport mechanisms in the human BBB. This study suggests that the drug transport systems at the BBB are far more diverse than it was assumed to be. The putative pyrilamine transporter will be a target protein to improve CNS penetration of drugs. Identification of the responsible transporter will contribute to the drug development of the CNS acting drugs.